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Volume 13 (5); September 30, 2023

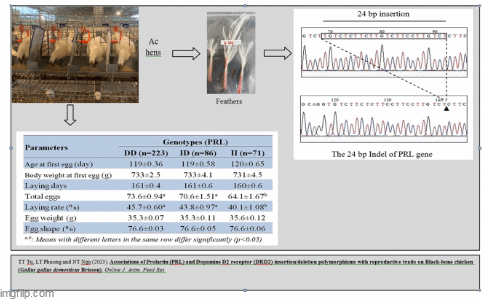
Research Paper

Associations of polymorphisms in prolactin and dopamine receptor D2 genes with reproductive traits on Silkie chicken

Tu TT, Phuong LT, and Ngu NT.

Online J. Anim. Feed Res., 13(5): 321-327, 2023; pii: S222877012300047-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.47



Abstract: The Silkie chicken (*Gallus gallus domesticus* Brisson) is one of domestic chicken breeds with commercial rearing and breeding potentials for egg production. Prolactin (PRL) and dopamine D2 receptor (DRD2) are potential genes associated with reproductive traits in chickens. This study was conducted to analyze the association of PRL and DRD2 insertion/deletion (Indel) polymorphisms with chicken reproductive traits in Silkie chickens. A total of 380 hens from 16-40 weeks of age were used, with each one being placed in a separate cage. DNA isolation was performed using feather samples, and genotypes were detected using the Indel technique. Two polymorphisms consisting of 24 base pair (bp) Indel in the promoter region of the PRL gene and 22 bp Indel in the promoter region of the DRD2 gene were identified. At both sites, the Indel polymorphisms did not follow the Hardy-Weinberg equilibrium. In addition, with the exception of total eggs over 23 weeks of laying in the PRL gene, the analysis revealed no association between these polymorphic loci and any traits collected. In conclusion, birds with the DD genotype produced the maximum egg yield (73.6 eggs/hen), whereas those with the II genotype produced approximately 9 fewer eggs (64.1 eggs/hen), resulting in laying rates of 45.7% and 40.1%, respectively. For enhancing the egg-laying capacity of Silkie chickens via selective breeding, opting for DD birds with DD genotype of PRL Indel is highly recommended.

Keywords: Egg production, Indel technique, Polymorphism, Reproductive traits, Silkie chicken.

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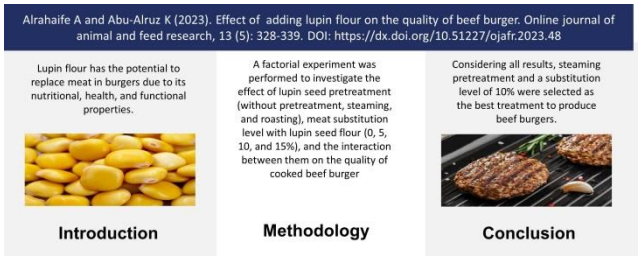
Research Paper

Effects of incorporation of lupin flour on the quality attributes of beef burger

Alrahaife AJ, and Abu-Alruz Kh.

Online J. Anim. Feed Res., 13(5): 328-339, 2023; pii: S222877012300048-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.48



Abstract: Lupin flour is known as an alternative high-ptlant protein for meat products due to its nutritional, health, and functional properties. A factorial experiment was performed to investigate the effect of lupin seed flour treatment (without, steaming, and roasting), meat substitution level with lupin seed flour (0, 5, 10, and 15%), and the interaction between them on the quality attributes of cooked beef burger by measuring CIELAB color, texture profile analysis (TPA), chemical composition (before and after cooking), and cooking properties (cooking loss, fat and moisture retention, and shrinkage). Based on the results of the factorial experiment, a completely randomized design was used to evaluate the sensory attributes of selected treatments. The different substitution levels mainly affected CIELAB color values, chemical composition, and cooking properties. On the other hand, the interaction effect between substitution level and treatment affected TPA. Considering all results, steaming treatment and a substitution level of 10% were selected as the best treatment to produce beef burgers. In comparison to the control burger, the developed burger had higher values of L* (increased by 21.26%), b* (increased by 32.94%), and moisture retention (increased by 37.85%); lower values of fat (decreased by 16.11%), protein (decreased by 6.37%), cooking loss (decreased by 43.22%), shrinkage (decreased 19.69%), and moisture content (decreased by 2.64%); and nonsignificantly different values with other tests performed. This study demonstrated that the incorporation of lupin flour in beef burgers could have the potential to substitute meat, create an alternative high-plant protein burger, and expand the application of lupin flour in the food industry.

Keywords: Beef, Chemical composition, Cooking loss, Physical properties, Sensory properties, Texture.

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Research Paper

The organoleptic, chemical and microbiological quality of maggot's frass as alternative poultry feed ingredients

Utama CS, Sulistiyanto B, Marifah B, and Cahya RI.

Online J. Anim. Feed Res., 13(5): 340-347, 2023; pii: S222877012300049-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.49

Abstract: Maggot's frass is waste from cultivating maggots (insect larvae) which consists of media from maggot cultivation mixed with feces, skin and dead body of the maggots. The aim of the study was to examine the organoleptic quality, chemistry, worm eggs, lead (Pb) as heavy metal and microbiological profile of maggot's frass as an alternative ingredient of poultry feed. A completely randomized design (CRD) with 3 treatments (T1: frass media for household waste, T2: frass media for tofu dregs, and T3: frass media for vegetable and fruit waste) and 7 replications was used. The results showed that there was no effect of different types of media treatment on the organoleptic quality, chemistry and microbiological profile of maggot's frass. The results of chemical analysis of maggot's frass revealed moisture of 26.39 - 46.26%, crude protein of 10.92 - 16.37%, worm eggs in the dregs media tofu (16 EPG), vegetable and fruit waste (32 EPG), total bacteria of 1.91-4.95 x 10^8cfu/g, and no any *Escherichia coli* and *Salmonella isolates*. Maggot's frass which comes from fruit and vegetable waste was recommended. Therefore, maggot feed using fruit and vegetable waste treatment is recommended because of its high crude protein and metabolic energy and also without any *E.coli* and *Salmonella* contamination.

Keywords: Black Soldier fly; Feed; Maggot's frass; Larva; Waste.

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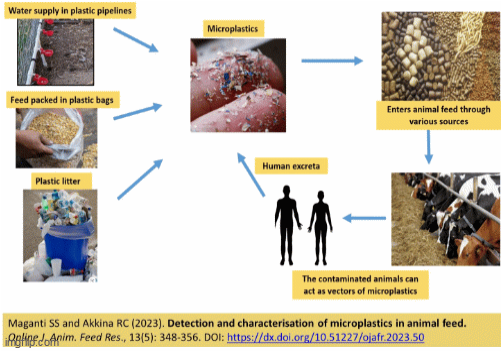
Research Paper

Detection and characterisation of microplastics in animal feed

Maganti SS and Akkina RC.

Online J. Anim. Feed Res., 13(5): 348-356, 2023; pii: S222877012300050-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.50



Abstract: Microplastics (MPs) the products of plastic breakdown, are entering the environment as a result of plastic abuse, which are of size less than 5mm. Due to their ubiquitous nature, MPs have become a significant environmental concern. One alarming area of MPs contamination is their potential presence in the feed of edible animal species. Growing research suggests that MPs can enter food products and subsequently move to various trophic levels of food chains. Hence, assessing the threat of MPs contamination in animal feed is important for food security and human health. In this investigation, 36 livestock and poultry feed samples were collected from 12 different farms, MPs were detected using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimeter (DSC). The Nano particle analyser was used to determine the size distribution, and Pyrolysis-GC/MS was used to quantify MPs. According to the findings, all the feed samples contained a significant amount of Polyethylene terephthalate (PET), Polypropylene (PP), and Polyvinyl chloride (PVC) and the particle size ranged from 2.02 to 10.7 µm. Present study has given detailed information on the size distribution of MPs in animal feed, which is thought to enable them to pass through membrane barriers. From the findings it is evident that there are high chances of MPs entering animal feed due to the continuous contact of the feed with plastic-based materials. These MPs can accumulate in the tissues of animals and potentially be transferred to humans through the consumption of meat, milk, and other animal-derived products. Subsequently these MPs can finally bio-accumulate in humans and cause serious health issues.

Keywords: Feedstuff, Membrane barriers, Nanoparticles, Pyrolysis-GC/MS, Size distribution.

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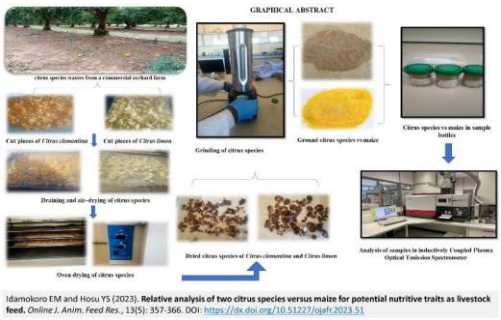
Research Paper

Relative analysis of two citrus species versus maize for potential nutritive traits as livestock feed

Idamokoro EM and Hosu YS.

Online J. Anim. Feed Res., 13(5): 357-366, 2023; pii: S222877012300051-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.51



Abstract: The present study seek to assess the nutritional qualities and the mineral composition of citrus fruits (pulps + peels) of two different species (*Citrus clementina* and *Citrus limon*), while comparing its nutritive perspective with *Zea mays L* (yellow maize) commonly used as livestock feed. Proximate evaluation was done via the method of the association of official analytical chemists (AOAC). Elemental components of citrus species were measured by means of a standard spectrometer. The proximate evaluation of the sample indicated that *Citrus limon* fruit contained comparable amounts of protein, fibre and lipid, but significantly higher ash contents than yellow maize. While the *Citrus clementina* was higher in protein and ash content, but comparable moisture content to *Zea mays L*. Meanwhile, minerals including Ca, Mg, K, Na, Cu, Mn and Fe were significantly higher in the two citrus species than in *Zea mays L*. Therefore the manuscript revealed that the *Citrus clementina* and *Citrus limon* species possess the potentials to be utilized as livestock feed ingredients.

Keywords: Citrus fruit, Elemental composition, Maize, Nutritional quality, Proximate analysis.

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Research Paper

Evaluation of oxidative stress parameters of horses housed with goats in individual boxes

Yildirim F and Apaydin Yildirim B.

Online J. Anim. Feed Res., 13(5): 367-372, 2023; pii: S222877012300052-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.52

Abstract: Horses are animals that are affected very quickly by the warnings coming from the environment. In this study, it was aimed to evaluate oxidative stress parameters of horses obtained from saliva analysis related to animal welfare as a result of keeping horses together with goats. While the research was being prepared, three groups were developed based on the time spent sheltering goats and horses. The horses were housed alone in the first and last 15-day groups, and along with the goats in the second 15-day group. In these stages, the levels of malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities and ischemia-modified albumin (IMA) level in saliva were examined. Results showed that MDA and IMA levels decreased, but CAT, GPx, and SOD activities increased. It was concluded that goats had a positive effect on horses according to the oxidative stress parameters examined in terms of animal welfare. However, there is still a need for research that will house horses with various animals in acceptable animal welfare circumstances, analyse their stress metrics, and maintain a high level of welfare.

Keywords: Animal welfare, Equus caballus, Oxidative stress, Single stall housing, horse, goat.

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Research Paper

Physico-chemical and sensory quality of Pekin duck jerky sonicated with coconut shell liquid smoke and stored for different periods

Salsabila N, Rosyidi D, and Susilo A.

Online J. Anim. Feed Res., 13(5): 373-383, 2023; pii: S222877012300053-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.53

Salsabila N, Rosyidi D, and Susilo A (2023). **Physico-chemical and sensory quality of Pekin duck jerky sonicated with coconut shell liquid smoke and stored for different periods.** *Online J. Anim. Feed Res.*, 13(5): 373-383. DOI: https://dx.doi.org/10.51227/ojafr.2023.53

Abstract: This study aimed to determine the effect of adding sonicated coconut shell liquid smoke to pekin duck jerky with different storage times at room temperature and vacuum packed. Ground duck jerky is made from Pekin duck meat (*Anas platyrhynchos domesticus*) soaked in coconut shell liquid smoke (CSLS) which has been sonicated for 20 minutes and seasoned with spices such as garlic, galangal, coriander, tamarind, salt, and coconut sugar. A laboratory experiment was done using a completely randomized design (CRD) consisting of 5 treatments (control: 0 day storage period, T1: 7 days, T2: 14 days, T3: 21 days, and T4: 28 days) and 4 replications. The results showed that the addition of sonicated CSLS with differences in the shelf life of pekin ground duck jerky had a significant effect ($P<0.01$) on pH, texture, color L, a*, b*, Aw, water content, fat, carbohydrates by difference, thiobarbituric acid (TBA), and iodine number. Had a significant effect ($P<0.05$) on ash content, and had no significant effect on Water Holding Capacity (WHC), protein content, and organoleptic quality. It was concluded that storing ground duck jerky for 14 days at room temperature and vacuum packed did not show any damage to pH, water activity, water content, fat, protein, TBA and iodine number, and did not occur rancidity.

Keywords: Liquid smoke, Jerky, Pekin duck, Shelf life, Sonication.

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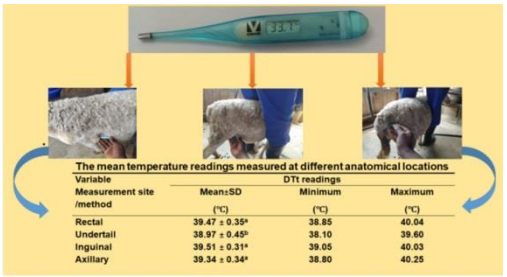
Research Paper

Comparison of rectal thermometry with the alternative undertail, axillary, and inguinal temperature measurements in sheep

Abigaba R, Sianangama PC, Chibinga O, Gulaita N, Muloongo S, and Mwanga ES.

Online J. Anim. Feed Res., 13(5): 384-390, 2023; pii: S222877012300054-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.54



The mean temperature readings measured at different anatomical locations

Variable	DTt readings		
Measurement site /method	Mean±SD	Minimum	Maximum
	(℃)	(℃)	(℃)
Rectal	39.47 ± 0.35[a]	38.85	40.04
Undertail	38.97 ± 0.45[b]	38.10	39.60
Inguinal	39.51 ± 0.31[a]	39.05	40.03
Axillary	39.34 ± 0.34[a]	38.80	40.25

Abigaba R, Sianangama PC, Chibinga O, Gulaita N, Muloongo S, and Mwanga ES (2023). **Comparison of rectal thermometry with the alternative undertail, axillary, and inguinal temperature measurements in sheep.** *Online J. Anim. Feed Res.*, 13(5): 384-390. DOI: https://dx.doi.org/10.51227/ojafr.2023.54

Abstract: This study was conducted to ascertain the suitability of alternative locations for temperature measurement, with reference to rectal thermometry in sheep, using a digital thermometer (DT). The study employed a single-factor multilevel design, considering anatomical location (site) as the main factor. This anatomical location factor had four conditions, including rectal ($_{rectal}$DTt), undertail ($_{undertail}$DTt), inguinal ($_{inguinal}$DTt), and axillary ($_{axillary}$DTt) locations. A total of 16 sheep were recruited for the study, and each treatment had eight replicates. The data obtained were descriptively analyzed using means and standard deviations, while inferential statistics included analysis of variance (ANOVA), Pearson's correlation, Tukey's test, t-test, and Bland-Altman plot. The mean $_{inguinal}$DTt was the highest (39.51 ± 0.31℃), while the lowest was the mean $_{undertail}$DTt (38.97±0.45). The effect of anatomical location on temperature readings was statistically significant. The difference between mean $_{rectal}$DTt and $_{inguinal}$DTt, or $_{axillary}$DTt was not significant. The $_{rectal}$DTt measurements were significantly correlated with those of each treatment. Equivalence analysis revealed a non-significant bias between the $_{rectal}$DTt and $_{inguinal}$DTt pair. The Bland-Altman plot showed a good level of correlation and considerable agreement between $_{rectal}$DTt and $_{inguinal}$DTt measurements. In conclusion, temperature measurement at the inguinal location results in readings that are similar to those of rectal thermometry and thus may be of clinical importance in the future, particularly with digital thermometer application in sheep.

Keywords: Anatomical location, Body temperature, Digital thermometer, Sheep, Rectal thermometry.

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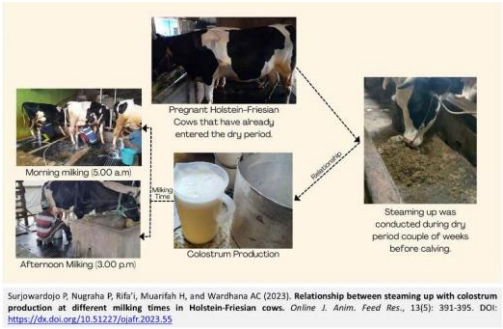
Research Paper

Relationship between steaming up with colostrum production at different milking times in Holstein-Friesian cows

Surjowardojo P, Nugraha P, Rifa'i, Muarifah H, and Wardhana AC.

Online J. Anim. Feed Res., 13(5): 391-395, 2023; pii: S222877012300055-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.55



Abstract: Aims of study was to determine the relationship between steaming up with colostrum production at different milking times. The animals used in this research were 36 pregnant Holstein-Friesian (HF) cows. The method used in this research was a case study. Samples were determined with purposive sampling. The selected animal was divided into two groups, T_1 (control) and T_2 (steaming up). The steaming up was done two weeks prior to calving. The total average of colostrum production from HF cows that were in T_2 group was 11.96±2.40 liter/cow/day, while the mean value of colostrum production from HF cows that were in T_1 group was 8.05±1.80 liter/cow/day. The average colostrum production that was collected at morning milking from cows in T_2 group was 6.38±1.36 liter/cow/day and at afternoon milking was 5.58±1.11 liter/cow/day, significantly higher than T_1 group which was 4.22±0.92 liter/cow/day at morning milking and 3.83±0.90 liter/cow/day. The result of the regression equation on morning milking is Y = 2.059 + 2.159x. This means that steaming up treatment can increase colostrum production by as much as 2.159 liters at morning milking. While the result of the regression equation on afternoon milking is Y = 1.753 + 2.078x. This means steaming up treatment can escalate the colostrum production as much as 2.078 at afternoon milking. That equation is used as the basis for estimating the relationship between steaming up with colostrum production at both milking times, with a correlation coefficient (r) between steaming up and colostrum quantity at morning milking is 0.692, which means the relationship is in the strong category. Meanwhile, the relationship between steaming up and colostrum yield at afternoon milking is 0.666, which means the relationship is also in the strong category. It was concluded that steaming up had a very significant effect at both milking times in Holstein-Friesian cows.

Keywords: Calving, Colostrum production, Holstein-Friesian Cows, Milking time, Steaming up.

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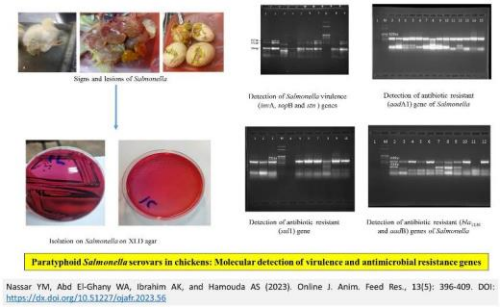
Research Paper

Paratyphoid Salmonella serovars in chickens: molecular detection of virulence and antimicrobial resistance genes

Nassar YM, Abd El-Ghany WA, Ibrahim AK, and Hamouda AS.

Online J. Anim. Feed Res., 13(5): 396-409, 2023; pii: S222877012300056-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.56



Abstract: Paratyphoid salmonellosis is a serious disease threatens the poultry industry worldwide, besides its public health hazard. The aims of this study were characterization of paratyphoid *Salmonella* spp. in chicken flocks of some Egyptian governorates, demonstration of the antimicrobial susceptibility of the isolated *Salmonella* spp., and detection of some virulence genes and antibiotic resistance genes using recent molecular techniques. A total of 238 organ samples were collected from 52 broiler, layer, and breeder chicken flocks, representing 9 Egyptian governorates. Conventional characterization of *Salmonella* isolates revealed a total isolation rate of 56.3% (134/238). Moreover, the isolation rates of *Salmonella* spp. were (49/79; 62%), (47/81; 58%), (10/18; 55.5%), (9/20; 45%), (2/6; 33.3%), (2/3; 66.7%), and (15/82; 53.6%) from liver, yolk sac, heart, spleen, caecum, ovary, and dead-in-shell embryos, respectively. A total of 32/238 (13.44%) isolates of *Salmonella* were found. Serological identification revealed presence of *S. enteritidis* (21.9%), *S. kentucky* (15.6%), *S. typhimurium* (12.5%), *S. molade* (12.5%), *S. takoradi* (9.4%), *S. wingrove* (6.3%), *S. infantis* (6.3%), *S. tsevie* (6.3), *S. shangani* (3.1%), *S. bargny* (3.1%), and *S. papuana* (3.1%). All *Salmonella* strains (32/32; 100%) were resistant to streptomycin, while almost all of them (31/32; 96.9%) were susceptible to meropenem. The amplification of *16S* rRNA gene of *Salmonella* isolates using uniplex polymerase chain reaction (PCR) generated a specific *Salmonella* product of approximate 550 base pair. The multiplex PCR revealed presence of *inv*A (100%), *stn* (65.6%), and *sop*B (40.6 %) virulence-associated genes as well as *aad*A1 (100%), *bla*TEM (59.4%), *aad*B (18.75%), and *sul*1 (28.1%) antibiotic resistance genes. In conclusion, virulent paratyphoid *Salmonella* spp. are circulating in the Egyptian flocks, causing economic loses. Additionally, they

became resistant to the most commonly used field antibiotics. Therefore, regular molecular surveillance studies on the circulating *Salmonella* spp. and their resistance to the used antibiotics are of significant importance.

Keywords: Antibiotic resistance genes, Chicken, Paratyphoid *Salmonella*, PCR, Serology, Virulence

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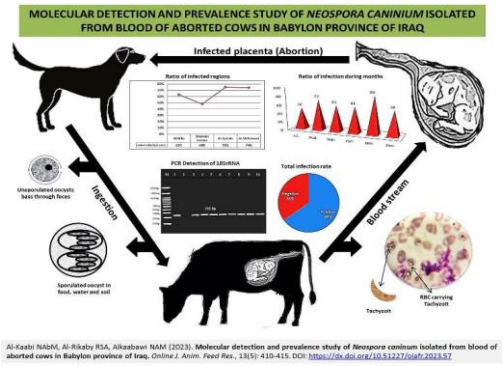
Research Paper

Molecular detection and prevalence study of Neospora caninum isolated from blood of aborted cows in Babylon province of Iraq

Al-Kaabi NAbM, Al-Rikaby RSA, Alkaabawi NAM.

Online J. Anim. Feed Res., 13(5): 410-415, 2023; pii: S222877012300057-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.57



Abstract: Neosporosis is internationally documented as one of the most popular diseases in cattle that cause economic losses due to high levels of abortion cases. Although *Neospora caninum* has been recently classified as a new species, it is still sharing many features with *Toxoplasma gondii*. This study aimed to detect and Imaging *N. caninum* in the blood of aborted cows, and prevalence study of *N. caninum* infection based on age, region and month. Blood samples from 106 aborted cows were collected using the appropriate method. First, these samples were examined microscopically via blood smears using Giemsa dye to diagnose the *N. caninum* within RBC. A qPCR technique was carried out to detect accurately *18S rRNA* gene accurately. The results revealed that 65% of total aborted cases were positive for *18S rRNA* detection of *N.caninum*, although this parasite was found microscopically in 15% of blood smear samples. According to PCR results, the prevalence study showed that the highest rate of infection was signed in the Al-Qassim district (75%) followed by the Al-Mahaweel district (74%) and decreased in Western Hamza district (48%). According to study months, November recorded the peak of infection (88%), then August (71%), whereas July recorded the lowest percentage (50%). The statistical analysis revealed there was no significant difference between the subjected regions and study months based on (P<0.05). On the other hand, it was found that cows less than 3 years old were more susceptible to infection than those over 3 years old. the results revealed that 71% of infected cows were less than 3 years old, while 29% were at age over 3 years old with a significant difference (P .(0.005>In conclusion ,*N .caninum* can be detected through blood within RBC. Age and regional factors in cows play an important role in resisting infection with this pathogen.

Keywords: Cow, Neosporosis, N. caninum, Prevalence, 18S rRNA

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ASSOCIATIONS OF POLYMORPHISMS IN PROLACTIN AND DOPAMINE RECEPTOR D2 GENES WITH REPRODUCTIVE TRAITS ON SILKIE CHICKEN

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[2]*Vietswan Poultry Breeding Joint Stock Company, Binh Duong 75000, Vietnam*
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RESEARCH ARTICLE

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ABSTRACT: The Silkie chicken (*Gallus gallus domesticus* Brisson) is one of domestic chicken breeds with commercial rearing and breeding potentials for egg production. Prolactin (PRL) and dopamine D2 receptor (DRD2) are potential genes associated with reproductive traits in chickens. This study was conducted to analyze the association of PRL and DRD2 insertion/deletion (Indel) polymorphisms with chicken reproductive traits in Silkie chickens. A total of 380 hens from 16-40 weeks of age were used, with each one being placed in a separate cage. DNA isolation was performed using feather samples, and genotypes were detected using the Indel technique. Two polymorphisms consisting of 24 base pair (bp) Indel in the promoter region of the PRL gene and 22 bp Indel in the promoter region of the DRD2 gene were identified. At both sites, the Indel polymorphisms did not follow the Hardy-Weinberg equilibrium. In addition, with the exception of total eggs over 23 weeks of laying in the PRL gene, the analysis revealed no association between these polymorphic loci and any traits collected. In conclusion, birds with the DD genotype produced the maximum egg yield (73.6 eggs/hen), whereas those with the II genotype produced approximately 9 fewer eggs (64.1 eggs/hen), resulting in laying rates of 45.7% and 40.1%, respectively. For enhancing the egg-laying capacity of Silkie chickens via selective breeding, opting for DD birds with DD genotype of PRL Indel is highly recommended.

Keywords: Egg production, Indel technique, Polymorphism, Reproductive traits, Silkie chicken.

INTRODUCTION

The productive value of animals is determined by their ability to meet certain production demands and this is typically measured by the quality and quantity of the product obtained during a given period (Rodina et al., 2019; Martinelli et al., 2020). In poultry, the process of egg production involves various synchronized metabolic and physiological processes that determine the number of produced eggs, rate of laying, and egg hatchability (Mench et al., 2011). Typically, the average egg production of most indigenous or native breeds/fowls is low (Nguyen et al., 2020), and this low productivity in backyard farming is primarily due to the limited production potential of the existing indigenous germ plasm, as noted by Haunshi et al. (2009). Many factors, particularly environmental and genetic ones, have a significant impact on these traits (Niknafs et al., 2012; Tongsiri et al., 2019; Loengbudnark et al., 2023). When considering the genetic effects on egg production in chickens, prolactin (PRL) (Mohamed et al., 2016) and dopamine D2 receptor (DRD2) (Xu et al., 2010) have been identified as potential candidate genes. Prolactin, a hormone generated by the pituitary gland, is important in the regulation of egg formation since it increases broodiness, which temporarily suppresses ovarian activity and prevents egg laying (Sharp et al., 1984). In the promotor region of PRL gene, the mutation at -358 position with 24 base pair (bp) insertion/deletion (Indel) influenced egg productivity in different chicken breeds (Jiang et al., 2005; Cui et al., 2006; Begli et al., 2010; Yousefi et al., 2012; Lotfi et al., 2013). Additionally, in birds, dopamine was found to have a crucial function in the secretion of PRL as it binds to its specific receptors, namely, dopamine D1 receptor and DRD2 (Youngren et al., 1998). It has been reported that the DRD2 gene governs the reproductive traits of poultry and is associated with the number of chicken eggs produced after 300 days (Xu et al., 2011b) as well as the age at which the first egg is laid (Xu et al., 2011a).

Among indigenous chickens in Vietnam, black-boned chicken, named “Ac” or Silkie chicken with white feathers, black bone and skin, is a famous breed. Silkie chicken brings many benefits to Vietnamese because of its therapeutic potential from meat and is used as a medical food for improving human health. In terms of nutritional value, chicken meat contains essential amino acids and iron. The Silkie eggs were also chosen by consumers because they have no fishy smell, are fatty, fragrant, high in white protein, have a high percentage of yolks, and a very attractive dark color (Phuong

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et al., 2023). The eggs of Silkie chicken were of small size (31.3-36.2 g/egg) and hen-day-egg production rate was 52.3-58.1% at 23-37 weeks old (Thuy and Ha, 2022). Because of their small size and low growth and egg production rate, Silkie chickens were mainly kept on a small scale and easy to access with a low investment. They were a major source of income and nutritionally rich food for households. Recently, the Silkie chickens have been raised industrially for egg production in Tien Giang and Long An provinces (Vietnam) on a large scale. However, breeding stock is still a major obstacle due to poor breeder quality and unstable yield; besides, egg production has not reached its potential. In Vietnam, some recent studies focused on nutrition to improve the reproductive performance of Silkie chickens (Phuoc et al., 2019; Thuy and Ha, 2022), but there have been no publications investigating and selecting this breed to improve egg production.

Therefore, this study aimed to investigate the associations of two polymorphisms of PRL and DRD2 genes with reproduction traits in Silkie hens.

MATERIALS AND METHODS

Experimental chickens

A total of 380 Silkie chicken hens, ranging in age from 16 to 40 weeks, were individually housed in cages and fed a diet containing 17% crude protein and 2,850 Kcal/kg metabolized energy for the entire duration of the experiment. Clean water was available to the chickens at all times. All chickens were vaccinated against common diseases before the 40-week experimental period of laying.

Phenotypic data

For laying hens, age at first egg (AFE) and body weight at first egg (BWFE) were recorded. In addition, eggs were collected daily at 5 P.M. and numbered to monitor individual yield. To evaluate the chickens' reproductive performance, the following parameters were recorded and calculated: total egg yield (per bird basis) and laying rate. The weight of the eggs and their shape index (the ratio of the short diameter of the egg to the long diameter) were measured every week (one egg/hen) throughout the entire experiment.

DNA extraction and genotyping

Genomic DNA isolated from chicken feathers (Bello et al., 2001) was subjected to amplification in a thermal cycler. The polymerase chain reaction (PCR) was performed in a 25 µl reaction containing 12.5 µM PCR Master Mix 2X (Phu Sa Genomics Joint Stock Company, Vietnam), 20 pM each primer, and 100 ng genomic DNA. The reaction was carried out with the following conditions: denaturation (95^{o}C for 5 minutes), 35 cycles of 95^{o}C for 30 seconds, annealing for 45 seconds, extension (72^{o}C for 45 seconds), and final extension (72^{o}C for 10 minutes). The PCR products of each gene were split on 3.5% agarose gel for 45 minutes at 80V for the identification of genotypes. The polymorphic site was also confirmed by sequencing with Sanger's method (Sanger et al., 1977). Information regarding primers and polymorphisms is provided in Table 1.

Table 1 - Information for primers and polymorphisms

Locus	Sequence (5'-3')	GenBank Acc. No.	PCR size (bp)	Tm (°C)	References
PRL Indel	F: TTTAATATTGGTGGGTGAAGAGACA R: ATGCCACTGATCCTCGAAAACTC	AF288765	154/130	54	Cui et al. (2006)
DRD2 Indel	F: TGCACTTCAATCCTTCCCAGCTT R: TTGCGCTGCCCATTGACCA	EU313425.1	187/165	62	Xu et al. (2010)

F: Forward primer; R: Reverse primer; Tm: Annealing temperature

Data analysis

The frequencies of alleles were calculated through allele counting in accordance with the Hardy-Weinberg equilibrium and the potential deviations from the expected genotype frequencies were tested using a Chi-square test. Moreover, the General Linear Model of Minitab software version 16.2.1 was used to analyze the association between genotype and egg yield and egg traits using the model of $Yij = \mu + Gi + \xi ij$ (where Yij: traits observed; µ: general mean, Gi: influence of genotype; ξij: random error). Data are presented as Least square mean ± Standard error.

Ethical considerations

The study was performed by authorized, qualified, and trained veterinarians, scientists, and technicians, in compliance with the guidelines of the Institutional Animal Ethics Committee (IAEC).

RESULTS AND DISCUSSION

Allele and genotype frequencies in the Silkie chicken population

Figures 1 and 2 display the genotype analysis of two genes. The bands depicted in the agarose gel electrophoresis allowed for the differentiation of genotypes for each polymorphism. The study detected three genotypes, which also

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resulted from a mutation in the promoter region of the PRL gene. The 24 bp Indel polymorphism presented a DNA fragment size of 154 bp for the I allele and 130 bp for the D allele (Figure 1a, b). Moreover, the promoter region of the PRL gene from Silkie chicken had 24 inserted nucleotides (TGTCTCTTCTTGTCTTCCTTGTCT) (Figure 3a,b).

A 22-base pair insertion/deletion (Indel) was found in the DRD2 gene of Silkie chickens. Figure 2a illustrates two alleles, the I allele (187 bp) and the D allele (165 bp). The promoter region of the DRD2 gene in Silkie chickens had a 22 bp (GTTGCTACCCTTAGCAAAGGCT) insertion, as shown in Figure 2b. Figure 4 presents a partial DNA sequence with 22 nucleotides inserted in the I allele of the DRD2 gene.

Table 2 present the allele and genotype frequencies of two genes. The PRL Indel locus had a lower I allele frequency (0.30) compared to the D allele frequency (0.70) in the population. However, the frequency ratio of the I allele (0.54) was higher than the D allele frequency (0.46) in the DRD2 Indel locus. Additionally, the results indicate that the genotypic frequencies of the DRD2 and PRL Indel loci in the Silkie chicken population did not adhere to the Hardy-Weinberg principle ($p<0.001$).

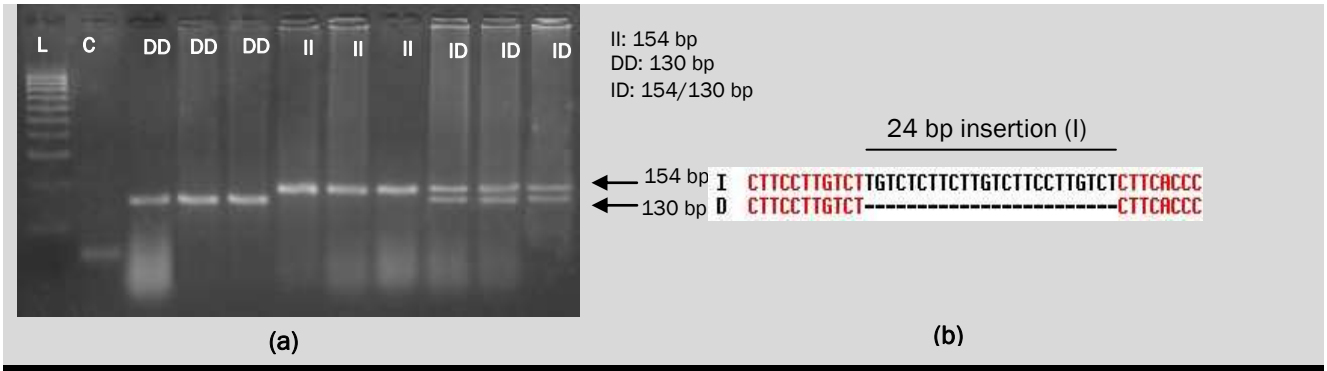

Figure 1 - Genotypes of PRL Indel. a) PCR products displayed on 3.5% agarose gel electrophoresis; b) 24 bp insertion sequences; Lane L: DNA marker (100-1,000 bp); lane C: negative control; lane 3, 4, 5: DD genotype; lane 6, 7, 8: II genotype; lane 9, 10, 11: ID genotype

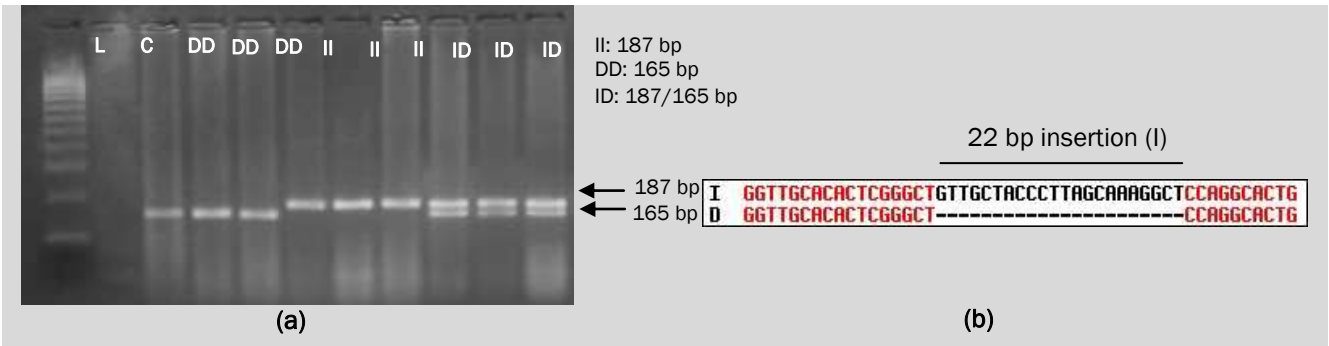

Figure 2 - Genotypes of DRD2 Indel. a) PCR products presentation on 3.5% agarose gel electrophoresis; b) 22 bp insertion sequences; Lane L: DNA marker (100-1,000 bp); lane C: negative control; lane 3, 4, 5: DD genotype; lane 6, 7, 8: II genotype; lane 9, 10, 11: ID genotype

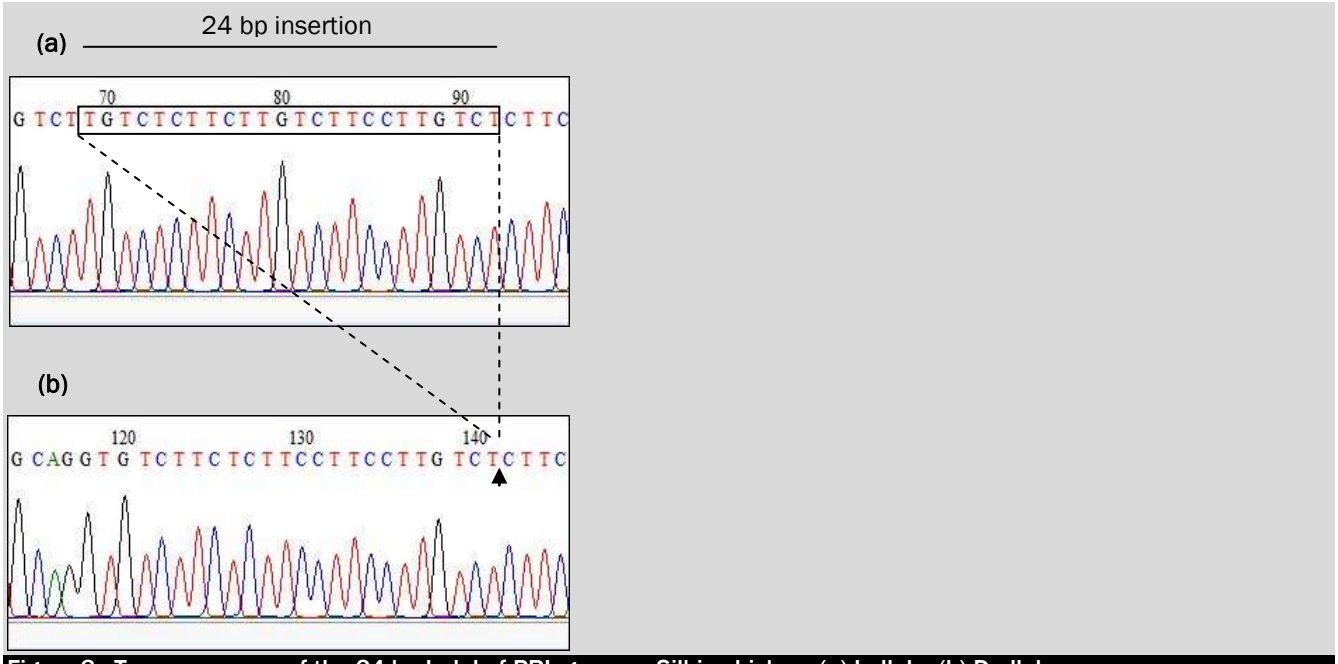

Figure 3 - Two sequences of the 24 bp Indel of PRL gene on Silkie chicken. (a) I allele; (b) D allele

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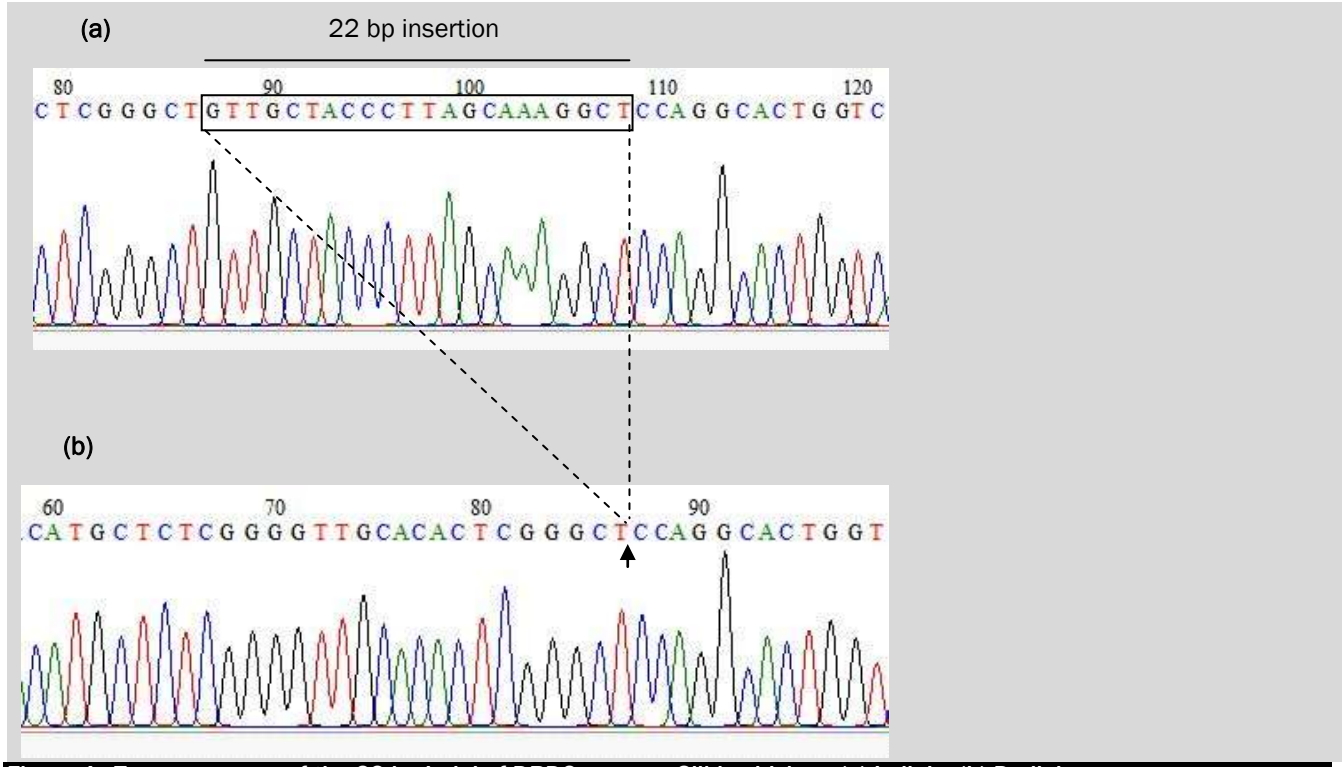

Figure 4 - Two sequences of the 22 bp Indel of DRD2 gene on Silkie chicken. (a) I allele; (b) D allele

Table 2 - Distribution of allele and genotype frequencies of two genes in the Silkie hen population

Locus	Genotype frequency			Allele frequency		HWE (χ2)
PRL Indel	DD	ID	II	D	I	
	0.59 (223)	0.22 (86)	0.19 (71)	0.70	0.30	80.8***
DRD2 Indel	DD	ID	II	D	I	
	0.26 (101)	0.38 (144)	0.36 (135)	0.46	0.54	21.1***

HWE: Hardy-Weinberg Equilibrium; (n): hen number; ***: p<0.001

Association of two polymorphisms on reproductive traits in Silkie chicken

During the 23 weeks of laying, among the PRL Indel genotypes, chickens bearing the DD genotype produced a higher number of eggs than those with the II genotype (p<0.001). As a result, the laying rate was also the highest in DD birds. For the polymorphic site of the DRD2 gene, no association was found for all the examined parameters (Table 3).

Table 3 - Association of INDEL polymorphisms with reproductive traits in laying chickens

Parameters	Genotypes (PRL)			Genotypes (DRD2)		
	DD (n=223)	ID (n=86)	II (n=71)	DD (n=101)	ID (n=144)	II (n=135)
AFE (day)	119±0.36	119±0.58	120±0.65	120±0.56	119±0.47	119±0.48
BWFE (g)	733±2.5	733±4.1	731±4.5	734±3.9	730±3.3	733±3.4
Laying days	161±0.4	161±0.6	160±0.6	161±0.6	162±0.5	161±0.5
Total eggs	73.6±0.94^{a}	70.6±1.51^{a}	64.1±1.67^{b}	69.1±1.45	70.6±1.22	68.5±1.25
Laying rate (%)	45.7±0.60^{a}	43.8±0.97^{a}	40.1±1.08^{b}	43.1±0.93	43.8±0.79	42.7±0.80
Egg weight (g)	35.3±0.07	35.3±0.11	35.6±0.12	35.4±0.10	35.3±0.09	35.3±0.09
Egg shape (%)	76.6±0.03	76.6±0.05	76.6±0.06	76.6±0.05	76.6±0.04	76.6±0.04

AFE: Age at first egg; BWFE: Body weight at first egg.^{a,b}: Means with different letters in the same row differ significantly (p<0.05).

The I allele frequency of the PRL Indel in this study differs from that of Chinese chicken breeds. Cui et al. (2006) reported I allele frequencies of the PRL Indel as follows: 0.02 in Taihe Silkie F0 generation, 0.2 in Taihe Silkie F1 generation, 0.05 in Yangshan, 0.17 in Nongdahe, 0.22 in White Rock, and 1.00 in White Leghorn chickens. Furthermore, the I allele was identified in native Iranian chickens with a frequency of 0.72 (Begli et al., 2010), in Mazandaran chickens with a frequency of 0.59 (Rashidi et al., 2012), and in Poltava clay chickens with a frequency of 0.00 (Kulibaba, 2015).

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DRD2 and PRL Indel polymorphisms did not conform to the Hardy-Weinberg equilibrium, possibly due to small sample sizes or the exclusive use of Silkie hens in the population. On the other hand, in the Pushkin chicken breed, Mitrofanova et al. (2017) discovered a 22 bp Indel in the DRD2 gene, with an I allele frequency of 0.41, lower than the D allele frequency of 0.59. Similarly, Datumada and Thongsaiklaing (2020) found that the I allele frequency of the DRD2 Indel in Thai native chickens was 0.23, lower than the D allele frequency of 0.77. For the PRL Indel, Lumatauw and Mu'in (2016) detected I allele frequency of 0.31 in Papua local chickens. Moreover, several previous studies reported similar results, the I allele frequency was 0.13 in Lien Minh chickens (Nguyen et al., 2018), 0.19 in Ri chickens, and 0.12 in Mia chickens (Vinh et al., 2021). In a study on Ningdu Sanhuang chickens, Xu et al. (2011b) analyzed the PRL Indel and found that hens with the ID genotype produced an average of 97.3 eggs, which was higher than the 94.0 eggs produced by chickens with the DD genotype. Additionally, hens with the ID genotype had an average first egg age of 191.38 ± 14.09 days, slightly earlier than the 195.71 ± 16.15 days for the DD genotype (Xu et al., 2011a). Furthermore, the ID genotype in PRL Indel exhibited a higher average egg weight (47.6 g/egg) compared to other genotypes in Lien Minh chickens (Nguyen et al., 2018).

The egg weights in the present study were much smaller than other local chickens in Vietnam such as Ri chickens (41.7 g/egg) and Mia chickens (44.7 g/egg) (Moula et al., 2012), Bang Troi chickens (48.4 g/egg) (Thinh et al., 2020), black Noi hens (48.3 g/egg), and dark brown Noi hens (49.7 g/egg) (Hoa et al., 2021). In addition, Silkie chickens had egg weight lower than indigenous chickens in southern Ethiopia (46.6 g/egg in lowland; 48.6 g/egg in midland and 45.4 g/egg in highland) (Berhanu et al., 2022) and Sidama region (Ethiopia) (44.9 g/egg in lowland; 49.5 g/egg in midland; and 42.9 g/egg in highland) (Legesse and Kefyalew, 2023). This study identified a 22-bp Indel in the DRD2 gene of Silkie chickens. The researchers analyzed the genetic diversity of this DRD2 Indel polymorphism in Silkie chickens and found that the frequency of the Indel allele was relatively low in the population (Xu et al., 2011b). The study provides insight into the genetic variation present in the DRD2 gene of Silkie chickens and highlights the potential implications of this variation for their dopamine signaling and related functions.

It's worth noting that genetic variation in the DRD2 gene has been studied in various species, including humans, and has been associated with a range of behavioral and physiological traits, such as addiction, impulsivity, and obesity (Switala et al., 2022). However, the specific effects of the DRD2 gene Indel polymorphism on reproductive traits in chickens are not well-established in the scientific literature, and further research would be needed to investigate any potential associations.

In the works of various authors, it was found that the presence of a 24-bp insertion in the promoter region of the avian prolactin gene is positively correlated with the intensity of egg-laying activity in birds and broody behavior (Jiang et al., 2009; Kulibaba and Podstreshnyi, 2012). The results of Jiang et al. (2005) have shown that PRL could be a genetic marker in breeding against broodiness in chickens. Prolactin gene in chicken (cPRL) specifically on the promoter region is a candidate gene for brooding behavior (Shimada et al., 1991; Dunn et al., 1998), and egg production (Cui et al., 2006). The cPRL promoter gene is an important part that responsible for the expression or the function of cPRL. The position of promoter in cPRL gene is located at the starting point (Lewin, 1997) and has its role in activating the early transcription for the gene expression. If a mutation occurs in this promoter region, the cPRL gene will not function and fail to express. Therefore, the promoter region is a crucial part of the gene as it controls the initiation of gene expression by binding to RNA polymerase and other transcription factors. Mutations in the promoter region can affect the binding of these factors and thus alter the expression of the gene. In the case of cPRL, a mutation in the promoter region can disrupt the early transcription process and prevent the gene from being expressed, leading to a loss of function. This can have significant effects on the chicken's behavior and physiology, as demonstrated by the association of cPRL promoter gene with brooding behavior and egg production.

Recently, it was found that the Tellicherry native chicken population has a high frequency of the I allele, which is associated with a 24bp Indel polymorphism in the promoter region of the prolactin gene (Manoharan et al., 2021). The study suggests that this polymorphism could be used as a potential molecular marker for selection and breeding in native chickens. Furthermore, the presence of an association between the 24bp insertion polymorphism and egg production was observed, indicating that this polymorphism could also be used for improving egg production in Tellicherry native chicken population (Manoharan et al., 2021). In prior research (Ngu et al., 2015), a point mutation (DRD2/*Bse*GI) was reported in Noi chicken, another indigenous Vietnamese breed. However, this polymorphism exhibited no impact on the birds' egg production. The DD genotype of the 24-bp Indel polymorphism in the prolactin gene has been found to be associated with a higher laying rate in chickens.

CONCLUSION

In the local Silkie chicken, a 24-bp Indel genetic variation was discovered in the promoter region of the prolactin gene. The D allele, which carries this Indel, was observed at a high frequency of 0.70. This indicates that by carefully managing breeding practices to manipulate the frequency of the D allele, it may be possible to improve egg production.

DECLARATIONS

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Authors' contribution

This work was conducted with contribution of all authors. T.T. Tu and N.T. Ngu designed the experimental procedures. T.T. Tu and L.T. Phuong performed the experiments. T.T. Tu, L.T. Phuong and N.T. Ngu interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Begli HE, Zerehdaran S, Hassani S, Abbasi MA and Ahmadi AK (2010). Polymorphism in prolactin and PEPCK-C genes and its association with economic traits in native fowl of Yazd province. Iranian Journal of Biotechnology, 8: 172-177. http://www.ijbiotech.com/article_7114.html

Bello N, Olga F and Armand S (2001). Isolation of genomic DNA from feathers. Journal of Veterinary Diagnostic Investigation, 13: 162-164. DOI: https://doi.org/10.1177/104063870101300212

Berhanu B, Aberra M, Wondmeneh E and Tadelle D (2022). Production performance and egg quality evaluation of indigenous chickens across different agro-ecologies of Southern Ethiopia. Veterinary Integrative Sciences, 20(1): 133-145. DOI: https://doi.org/10.12982/VIS.2022.012

Cui JX, Du HL, Liang Y, Deng XM, Li N and Zhang XQ (2006). Association of polymorphisms in the promoter region of chicken prolactin with egg production. Poultry Science, 85: 26-31. DOI: https://doi.org/10.1093/ps/85.1.26

Datumada H and Thongsaiklaing T (2020). 22 bp Indel mutation polymorphism of dopamine receptor D2 (DRD2) gene of Thai native chicken in Narathiwat province. Journal of Mahanakorn Veterinary Medicine, 15(2): 189-197. https://li01.tci-thaijo.org/index.php/jmvm/article/view/242102

Dunn IC, McEwan G, Ohkubo T, Sharp PJ, Paton IR and Burt DW (1998). Genetic mapping of the chicken prolactin receptor gene: a candidate gene for the control of broodiness. British Poultry Science, 39: 23-24. DOI: https://doi.org/10.1080/00071669888160

Haunshi S, Doley S and Shakuntala I (2009). Production performance of indigenous chicken of northeastern region and improved varieties developed for backyard farming. Indian Journal of Animal Sciences, 79(9): 901-905. https://epubs.icar.org.in/index.php/IJAnS/article/view/2683

Hoa DV, Diep DV, Huong NT, Khanh DN, Hue LT, Hieu NM, et al. (2021). Growing and laying performances of two varieties of Noi chickens raised in an intensive farming system. Vietnam Journal of Science, Technology and Engineering, 64(2): 54-58. DOI: https://doi.org/10.31276/VJSTE.64(2).54-58

Jiang RS, Xu GY, Zhang XQ and Yang N (2005). Association of polymorphisms for prolactin and receptor genes with broody traits in chickens. Poultry Science, 84: 839-845. DOI: https://doi.org/10.1093/ps/84.6.839

Jiang RS, Zhang LL, Geng ZY, Yang T and Zhang SS (2009). Single nucleotide polymorphisms in the 5'-flanking region of the prolactin gene and the association with reproduction traits in geese. South African Journal of Animal Science, 39(1): 83-87. DOI: https://doi.org/10.4314/sajas.v39i1.43550

Kulibaba RA (2015). Polymorphism of growth hormone, growth hormone receptor, prolactin and prolactin receptor genes in connection with egg production in Poltava clay chicken. Agricultural Biology, 50(2): 198-207. DOI: https://doi.org/10.15389/agrobiology.2015.2.198eng

Kulibaba RA and Podstreshnyi AP (2012). Prolactin and growth hormone gene polymorphisms in chicken lines of Ukrainian selection. Cytology and Genetics, 46: 390-395. DOI: https://doi.org/10.3103/S0095452712060060

Legesse YT and Kefyalew BR (2023). Evaluation of fertility, hatchability and egg quality of indigenous chickens in different agro-ecologies of the Sidama Region, Ethiopia. Veterinary Integrative Sciences, 21(1): 201-219. DOI: https://doi.org/10.12982/VIS.2023.016

Lewin B (1997). Trancription is catalysed by RNA polymerase. In: Genes VI. Oxford University Press, Inc, New York.

Lotfi E, Zerehdaran S, Ahani M and Dehnavi E (2013). Genetic polymorphism in prolactin gene and its association with reproductive traits in Japanese quail (*Coturnix-coturnix*). Poultry Science Journal, 1(1): 29-35. DOI: https://doi.org/10.22069/PSJ.2013.1484

Loengbudnark W, Chankitisakul V, and Boonkum W (2023). The genetic impact of heat stress on the egg production of Thai native chickens (*Pradu Hang dum*). PlOS One, 18(2):e0281328. DOI: https://doi.org/10.1371/journal.pone.0281328

Lumatauw S and Mu'in MA (2016). A 24-bp Indel (Insertion-Deletion) polymorphism in promoter prolactin gene of Papua local chickens. Animal Production, 18(1): 1-7. DOI: https://doi.org/10.20884/1.anprod.2016.18.1.526

Manoharan A, Sankaralingam S, Binoj Chacko P and Aravindakshan TV (2021). Identification of 24bp indel(s) polymorphism in the promoter region of prolactin gene and its association with broodiness in Tellicherry native chicken. Indian Journal of Animal Research, 55(10): 1137-1140. DOI: https://doi.org/10.18805/ijar.B-4158

Martinelli G, Vogel E, Decian M, Farinha MJ, Bernardo LV, Borges JA, and et al. (2020). Assessing the eco-efficiency of different poultry production systems: An approach using life cycle assessment and economic value added. Sustainable Production and Consumption, 24:181-193. DOI: https://doi.org/10.1016/j.spc.2020.07.007

Mench JA, Sumner DA and Rosen-Molina JT (2011). Sustainability of egg production in the United States-The policy and market context. Poultry Science, 90(1): 229-240. DOI: https://doi.org/10.3382/ps.2010-00844

Mitrofanova OV, Dementeva NV, Krutikova AA, Yurchenko OP, Vakhrameev AB and Terletskiy VP (2017). Association of polymorphic variants in MSTN, PRL, and DRD2 genes with intensity of young animal growth in Pushkin breed chickens. Cytology and Genetics, 51(3): 179-184. DOI: https://doi.org/10.3103/S0095452717030082

Mohamed MMO, Shaaban AH, Abeer AIH and Walaa AH (2016). Polymorphism of prolactin gene and its association with egg oroduction trait in four commercial chicken lines. Journal of the Hellenic Veterinary Medical Society, 68(3): 391-404. DOI: https://doi.org/10.12681/jhvms.15502

Moula N, Antoine-Moussiaux N, Do LD, Nguyen TC, Pham DK, Vu TD, and et al. (2012). Egg quality comparison of two Vietnamese chicken breeds (Ri and Mia). Proc. The 1st Poultry international Symposium, pp. 379-383. https://orbi.uliege.be/bitstream/2268/133429/1/58

Niknafs S, Nejati-Javaremi A, Mehrabani-Yeganeh H, and Fatemi SA (2012). Estimation of genetic parameters for body weight and egg production traits in Mazandaran native chicken. Tropical animal health and production, 44:1437-1443. DOI: https://doi.org/10.1007/s11250-012-0084-6

Ngu NT, Xuan NH, Vu CT, An NT, Dung TN and Nhan NTH (2015). Effects of genetic polymorphisms on egg production in indigenous Noi chicken. Journal of Experimental Biology and Agricultural Science, 3(4):487-493. DOI: https://doi.org/10.18006/2015.3(6).487.493

Nguyen TTB, Duc NH, Quy VC, Yen HT, Loan TT, Thuy DTN, and et al. (2018). Effect of nucleotide polymorphism of candidate genes on egg production traits in native Lien Minh chicken. Livestock Research for Rural Development, 30(6): Article #103. http://www.lrrd.org/lrrd30/6/ntdt30103.html

Nguyen VD, Moula N, Moyse E, Do Duc L, Vu Dinh T, and Farnir F (2020). Productive performance and egg and meat quality of two indigenous poultry breeds in Vietnam, Ho and Dong Tao, fed on commercial feed. Animals, 10(3):408. DOI: https://doi.org/10.3390/ani10030408

Phuoc TV, Dung NNX, Manh LH and Tu NNX (2019). Effect of dietary turmeric (*Curcuma longa*) extract powder on productive performance and egg quality of black-bone chicken (Ac chicken). Livestock Research for Rural Development, 31(23): Article #23. http://www.lrrd.org/lrrd31/2/truong31023.html

Phuong LT, Tu TT and Ngu NT (2023). Genetic variants of INHA/PstI and VIPR1/HhaI and their relationship with reproductive traits in Silkie chicken (*Gallus gallus domesticus* Brisson). Veterinary Integrative Sciences, 21(3): 831–841. DOI: https://doi.org/10.12982/VIS.2023.059

Rashidi H, Rahimi-Mianji G, Farhadi A and Gholizadeh M (2012). Association of prolactin and prolactin receptor gene polymorphisms with economic traits in breeder hens of indigenous chickens of Mazandaran province. Iranian Journal of Biotechnology, 10(2): 129-135. http://www.ijbiotech.com/article_7185.html

Rodina O, Savchenko T, Basiurkina N, and Kwilinski A (2019). Improvement of the assessment methods of product competitiveness of the specialized poultry enterprises. Management Theory and Studies for Rural Business and Infrastructure Development, 41(1):43-61. DOI: https://doi.org/10.15544/mts.2019.05

Sanger F, Nicklen S and Coulson AR (1977). DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America, 74(12): 5463–5467. DOI: https://doi.org/10.1073%2Fpnas.74.12.5463

Sharp PJ, Macnamee MC, Talbot RT, Sterling RJ and Hall TR (1984). Aspects of the neuroendocrine control of ovulation and broodiness in the domestic hen. Journal of Experimental Zoology, 232(3): 475-483. DOI: https://doi.org/10.1002/jez.1402320314

Shimada K, Ishida H, Sato K, Seo H and Matsui N (1991). Expression of PRL gene in incubation hens. Journal of Reproduction and Fertility, 91(1): 147-154. DOI: https://doi.org/10.1530/jrf.0.0910147

Switala K, Bojarczuk A, Hajto J, Piechota M, Buryta M and Leońska-Duniec A (2022). Impact of the DRD2 Polymorphisms on the Effectiveness of the Training Program. International Journal of Environmental Research and Public Health, 19(9): 4942. DOI: https://doi.org/10.3390/ijerph19094942

Thinh NH, Vinh NT, Lam NT, Nga MTT and Doan BH (2020). External characteristics and reproductive performance of Bang Troi chicken. Vietnam Journal of Agricultural Sciences, 18(10): 823-830. http://tapchi.vnua.edu.vn/wp-content/uploads/2020/09/tap-chi-so-10.1.2sua.pdf

Thuy NT and Ha NC (2022). Effect of *Moringa oleifera* and *Curcuma longa* powders in diets on laying performances and hatchability of local hens in the south of Vietnam. Livestock Research for Rural Development, 34(52): Article #52. http://www.lrrd.org/lrrd34/6/3452nthi.html

Tongsiri S, Jeyaruban GM, Hermesch S, van der Werf JH, Li L, and Chormai T (2019). Genetic parameters and inbreeding effects for production traits of Thai native chickens. Asian-Australasian Journal of Animal Sciences, 32(7): 930. DOI: https://doi.org/10.5713%2Fajas.18.0690

Vinh NT, Giang NTP, Linh NV, Dang PK, Cahn NX, Giang NTC, et al. (2021). Single nucleotide polymorphisms of candidate gens related to egg production traits in Vietnamese indigenous chickens. Brazilian Journal of Poultry Science, 23(2): 1-6. DOI: https://doi.org/10.1590/1806-9061-2020-1298

Xu HP, Shen X, Zhou M, Luo CL, Kang L, Liang Y, et al. (2010). The dopamine D2 receptor gene polymorphisms associated with chicken broodiness. Poultry Science, 89: 428-438. DOI: https://doi.org/10.3382/ps.2009-00428

Xu H, Zeng H, Luo C, Zhang D, Wang Q, Sun L, et al. (2011a). Genetic effects of polymorphisms in candidate genes and the QTL region on chicken age at first egg. BMC Genetics, 12: 33-42. DOI: https://doi.org/10.1186/1471-2156-12-33

Xu H, Zeng H, Zhang D, Jia X, Luo C, Fang M, et al. (2011b). Polymorphisms associated with egg number at 300 days of age in chickens. Genetics and Molecular Research, 10: 2279-2289. DOI: https://doi.org/10.4238/2011.October.3.5

Youngren O, Chaiseha Y and El-Halawani ME (1998). Regulation of prolactin secretion by dopamine and vasoactive intestinal peptide at the level of the pituitary in the turkey. Neuroendocrinology, 68(5): 319-325. DOI: https://doi.org/10.1159/000054380

Yousefi S, Raoufi Z, Rasouli Z and Zerehdaran S (2012). Investigation of prolactin gene polymorphism in Japanese quail. Iranian Journal of Biotechnology, 45(1): 289-292. https://profdoc.um.ac.ir/paper-abstract-1059079.html

Citation: Tu TT, Phuong LT, and Ngu NT (2023). Associations of polymorphisms in prolactin and dopamine receptor D2 genes with reproductive traits on Silkie chicken. *Online J. Anim. Feed Res.*, 13(5): 321-327. DOI: https://dx.doi.org/10.51227/ojafr.2023.47

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EFFECTS OF INCORPORATION OF LUPIN FLOUR ON THE QUALITY ATTRIBUTES OF BEEF BURGER

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ABSTRACT: Lupin flour could have the potential to be an alternative to meat products due to its nutritional, health, and functional properties. A factorial experiment was performed to investigate the effect of lupin seed flour treatment (without, steaming, and roasting), meat substitution level with lupin seed flour (0, 5, 10, and 15%), and the interaction between them on the quality attributes of cooked beef burger by measuring CIELAB color, texture profile analysis (TPA), chemical composition (before and after cooking), and cooking properties (cooking loss, fat and moisture retention, and shrinkage). Based on the results of the factorial experiment, a completely randomized design was used to evaluate the sensory attributes of selected treatments. The different substitution levels mainly affected CIELAB color values, chemical composition, and cooking properties. On the other hand, the interaction effect between substitution level and treatment affected TPA. Considering all results, steaming treatment and a substitution level of 10% were selected as the best treatment to produce beef burgers. In comparison to the control burger, the developed burger had higher values of L* (increased by 21.26%), b* (increased by 32.94%), and moisture retention (increased by 37.85%); lower values of fat (decreased by 16.11%), protein (decreased by 6.37%), cooking loss (decreased by 43.22%), shrinkage (decreased 19.69%), and moisture content (decreased by 2.64%); and nonsignificantly different values with other tests performed. This study demonstrated that the incorporation of lupin flour in beef burgers could have the potential to substitute meat, create an alternative burger with a high percentage of plant protein, and expand the application of lupin flour in the food industry.

Keywords: Beef, Chemical composition, Cooking loss, Physical properties, Sensory properties, Texture.

INTRODUCTION

There is an increasing trend globally for replacing meat with plant-based products (Estell et al., 2021; Smetana et al., 2021; Bryant et al., 2022); it is among the top three trends (Estell et al., 2021). This trend comes as a response to the increased problems encountered in meat production. Pollution, high carbon footprint, the spread of animal diseases (Zhang et al., 2022), negative impacts on the environment (Smetana et al., 2021), increased social cost, animal welfare (Siegrist and Hartmann, 2023), and adverse health effects (Marinova and Bogueva, 2019) are examples of problems of meat production.

Different meat alternatives are investigated in the literature, such as cultured meat, microbial proteins, insect proteins, and plant-based proteins (Zhang et al., 2022), where the last type is the most promising one in terms of acceptability by consumers (Siegrist and Hartmann, 2023). Plant-based meat alternatives are mostly centered on pulses. Lupin is one of the candidates to replace meat due to its nutritional and health benefits. Lupin contains 30-42% proteins, 30-41% fibers (mainly insoluble), fat in the form of mono poly-unsaturated fatty acids, minerals, vitamins, and antioxidants. Another nutritional benefit of lupin compared to other legumes is the low antinutritional factors (Abreu et al., 2023). Several review papers reviewed lupin's health benefits, with the most recent being conducted by Bryant et al. (2022), which reported strong indicators that lupin consumption improves satiety, reduces blood pressure, and lower degree indicators of decreasing serum lipids and improves glycemic index. Lupin addition was investigated in different food products; Abreu et al. (2023) recently reviewed papers that investigated adding lupin to different food products. However, it has been reported that lupin protein lacks gelling and thickening properties, which may limit its use as a food ingredient (Abreu et al., 2023).

One of the most popular meat products is the beef burger, whose market size in 2020 was 862 billion USD, representing 36% of the global fast food market (Petrat-Melin and Dam, 2023). Therefore, reducing the meat in burgers by replacing it with plant protein will participate in the global reduction of meat production. To the best of our knowledge, limited studies evaluated lupin seed flour as a meat alternative in burgers. These studies investigated the final products using chemical and sensory methods with no information regarding the effect of using lupin on instrumental color and texture values (El-Sayed, 2009; Dalain et al., 2023). In addition, little information is available about the use of different treatments on the functionality of lupin flour. Therefore, the current study aimed to investigate the effects of different treatments of lupin seed flour and different meat substitution levels on the quality attributes of cooked beef burgers.

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MATERIALS AND METHODS

Materials

To conduct the current study, the following ingredients were used: frozen beef meat (18.34% protein, 10.66% fat, and 67.67% moisture as tested by Food scan) was imported from Brazil and obtained from the national poultry company; sweet lupin seeds (Egypt) obtained from Al-Sufara'a bakery, Refined salt (Amra, Jordan), and burger spices were obtained from Al-jada'el company (Amman, Jordan).

Experiment design

A 3*4 factorial experiment with two replicates was performed to study the effects of lupin seed treatment (without, steamed, and roasted) and different levels of lupin seed flour used to replace meat (0, 5, 10, and 15%) on the quality attributes of beef burgers. Based on the results of the factorial experiment, selected samples from different treatments were sensory evaluated using a completely randomized design.

The basic formula for the preparation of the meat burger

A commercial beef burger recipe was adopted and modified from one of the local meat suppliers (national poultry company, meat processing plant, Al Karak-Jordan). The components of the formula were as follows: Brazilian beef meat (89%), salt (0.40%), spices (0.1%), and water (10.5%).

Treatment of lupin seeds and preparation of lupin flour

The roasting and steaming treatments were used to modify the functional properties of lupin seeds. 200 g of lupin seeds were utilized, with two replicates per treatment. In roasting treatment, the lupin seeds were milled (high-speed multifunction comminutor wm-500, China), and then the flour passed a sieve with a diameter of 1 micron. Flour was roasted in an oven at 160 °C for 10 mins (JE IO TECH (OV12), Korea). The steaming treatment was done on lupin seeds before grinding using a flow cook (CFS, Denmark) with 500 rpm fan speed with 40% wet steam (160 °C). Steamed seeds were dried in a drier for 24 hours at 50 °C. Finally, lupin was ground on a mill (high-speed multifunction comminutor wm-500, China) and allowed to pass through a 1-micron sieve.

Preparation of burgers with different levels of treated lupin flour to replace meat

This experiment used ten meat burgers' formulas (Table 1). The first formula was the original formula -without lupin- (control), which was described previously in the section entitled "the basic formula for the preparation of the meat burger." In the other formula, different levels of treated lupin flour were used to replace meat.

The first step was weighing all the needed ingredients. After that, the frozen beef meat (-7 to - 9 ℃) was ground using a commercial frozen meat cutter (auto-grind machine, CFS, Denmark) equipped with a 20 mm grinding plate. After grinding, the meat temperature rose to -4 ℃. The next step was mincing the meat, which was done with a meat mincer (K&G Watter, 419/E130, Germany) equipped with a 3 mm mince plate. Minced meat was portioned according to the required amount for each formula. At this step, other ingredients in the formula were added and mixed manually for three minutes. A detailed description of treated lupin seed flour preparation was described in the section entitled "treatment of lupin seeds and preparation of lupin flour." A portion of the meat mixture (70g) was formed using a manual circular plastic mold (9.5 cm in diameter). The temperature of the meat mixture was monitored to be at 0 ± 1°C during the forming step. Burger pieces were placed in a shock freezer (Blitzer, S6F-30.2Y-40P, Germany) at a temperature of -33 to -36 °C for 30 mins until the product's core temperature reached (-15 to -18 ℃). The burgers were then vacuum-sealed in plastic bags, placed in cardboard boxes, and frozen at -18 ℃ for 18 days before being grilled and evaluated.

Table 1 - Developed formulas used in the study to prepare beef burgers

Ingredients	Different levels of lupin flour used to replace meat			
	0% (control)	5%	10%	15%
Beef Meat (10% Fat)	89	84.55	80.1	75.65
Salt	0.40	0.40	0.40	0.40
Spices	0.1	0.1	0.1	0.1
Water	10.50	10.50	10.50	10.50
Lupin flour*	0	5% of meat (4.45% of total mix)	10% of meat (8.9% of total mix)	15% of meat (13.35% of total mix)
Total	100%	100%	100%	100%

*untreated or steamed or roasted

Cooking of burger pieces

Frozen burger pieces were removed from the freezer and grilled directly using a commercial grill (Electric Grill, Sonifer, China). The grilling was performed at 185 to 215 °C and continued for 10.5 mins. The burger was placed for two minutes on the first side and two minutes on the other side. After that, the burger piece was flipped every minute until the core temperature of the bean reached 75 °C, knowing that the diameter and weight measurements were taken for each

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burger piece before and after grilling. After that, the burger pieces were cooled to room temperature and packed in plastic bags for further evaluation.

Color evaluation

The color of cooked samples was assessed using a non-contact spectrophotometer (X-rite VS-450, UK) and Oncolor software (CyberSoft, UK). The International Commission on Illumination (CIE) Lab color values and differences were determined for each sample. Three burgers were evaluated for each treatment, and the average was calculated to perform the statistical analysis.

Cooking measurements

Cooking loss

Burger pieces were weighed before and after cooking. After cooking, the burger pieces were allowed to cool at room temperature for 15 mins. Cooking loss was determined according to the following formula:

$$\text{Cooking loss (\%)} = \frac{Raw\ burger\ weight - Cooked\ burger\ weight}{Raw\ burger\ weight} \times 100$$

Moisture retention and fat retention

According to the formulas presented by Romero et al. (2019), the moisture retention and fat retention values were determined.

$$\text{Fat retention (\%)} = \frac{Cooked\ weight \times Percent\ fat\ in\ cooked\ samples}{Raw\ weight \times percent\ fat\ in\ raw\ samples} \times 100$$

$$\text{Moisture retention (\%)} = \frac{Cooked\ weight \times percent\ moisture\ in\ cooked\ samples}{Raw\ weight \times percent\ moisture\ in\ raw\ samples} \times 100$$

Shrinkage

The dimensional shrinkage was calculated according to Ismail et al. (2021) as follows:

$$\text{Diameter shrinkage (\%)} = \frac{(Raw\ diameter - Cooked\ diameter)}{Raw\ diameter} \times 100$$

Chemical analysis

Before and after cooking, the protein, fat, and moisture percentages of beef meat burgers were measured using FOSS FoodScan™ Meat Analyzer (Scanco, Costa Rica, San José).

TPA

TPA parameters (hardness, resilience, springiness, cohesiveness, and chewiness) were determined using a texture analyzer (TVT, Perten, Sweden) according to the method described by Alrawashdeh and Abu-Alruz (2022).

Sensory evaluation

Based on the results of the previous test, five burger mixes - with different levels of meat substitution with lupin flour - were selected (control "without lupin," 5% and 10% with untreated lupin flour, 5% and 10% with steamed lupin flour). Roasted lupin flour was excluded in this part due to its negative impact on the cooked beef burger's TPA compared to the steamed lupin seed flour. From the National Poultry Company, ten skilled panelists were chosen after asserting that they usually consume lupin without being allergic. Members of the committee were requested to assess the samples and report their findings on a sensory evaluation form. The grilled samples were evaluated on a nine-point hedonic scale, with one signifying severe hate and nine denoting intensity. Three sensory parameters (color, taste, and texture) were assessed for each sample. Three-digit codes were used to number each sample. Burgers were grilled according to the procedure described in the section "cooking of burger pieces."

Statistical analysis

Using Minitab® 19.20.20, the data were analyzed using a fully randomized factorial design (CRD) with two replicates. For sensory evaluation, data was analyzed according to the completely randomized design. All the data are presented as mean values with their standard deviations. The statistically significant differences between the means were determined using Tukey's test, and the significance level was set at $p \leq 0.05$.

RESULTS

A 3*4 factorial design was used to assess the main effects of two factors (Type of treatment of lupin seed flour and substitution level of meat with lupin flour) and their interaction on the quality of a developed meat burger. Three levels of treatment type (Lupin without treatment, steamed lupin, and roasted lupin) and four substitution levels (0, 5, 10, and

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15%) were applied. In the following sections, only significant results were reported. Only the results of the interaction effects were reported when they were significant; if not, the significant main effects were only reported.

CIELAB color values of cooked beef burger

L* values

L* values of cooked beef burgers were significantly affected (P≤0.05) only by substitution levels; therefore, only the results of the effect of substitution level were presented. Substitution levels above 5% significantly increased the L* values of meat burgers compared to 0 and 5% substitution levels (Figure 1).

a* values

a* values of meat burgers were not significantly affected by substitution levels, treatment type, and interaction between them. The a* values for different samples of burger meat ranged between 7.96 and 8.71 (Table 2).

b* value

b* values of meat burgers were significantly affected (P≤0.05) only by substitution levels. Up to a 10% substitution level, there was a significant increase in b* values with every increase in substitution level (Figure 2).

ΔE*ab values

ΔE*ab values of meat burgers were significantly affected (P≤0.05) only by substitution levels. There was a significant increase in ΔE*ab with every increment in the substitution level (Figure 3).

Table 2 - Effect of substitution levels on CIELAB color values of cooked burger

Substitution level	L*	a*	b*	ΔE*ab
0%	35.52±0.11^{b}	8.51±0.035	16.21±0.61^{c}	2.43±0.76^{d}
5%	36.82±4.41^{b}	8.71±0.49	19.13±1.05^{b}	5.85±1.57^{c}
10%	43.07±1.55^{a}	8.40±0.44	21.55±0.67^{a}	10.08±1.29^{b}
15%	45.72±1.73^{a}	7.96±0.85	22.35±0.75^{a}	12.39±1.51^{a}

*M±Std.D, the values in the same column followed by the same letter are not significantly different at the 0.05 probability level as determined by Tukey's test.

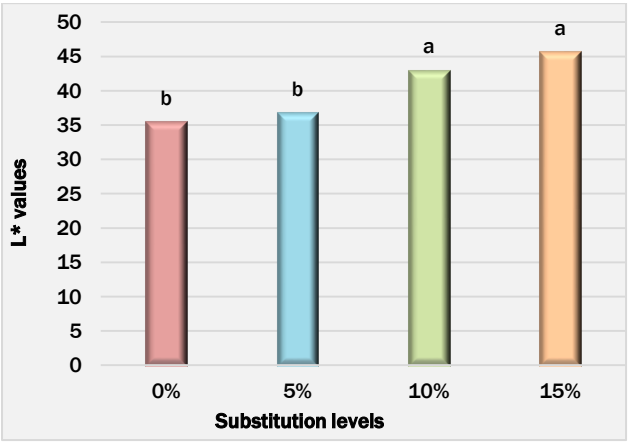

Figure 1 - Effect of using different substitution levels of lupin flour on the L*values of cooked meat burgers. Values followed by the same letter are not significantly different

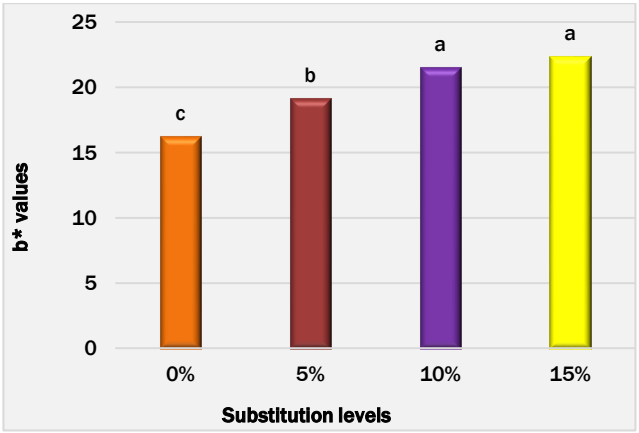

Figure 2 - Effect of using different substitution levels with lupin flour on the b* values of cooked meat burgers. Values followed by the same letter are not significantly different

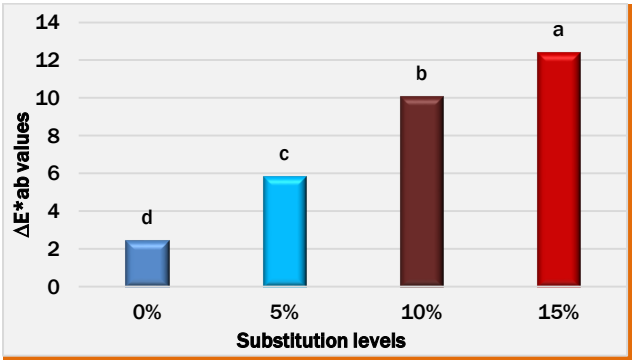

Figure 3 - Effect of using different substitution levels with lupin flour on the ΔE*ab values of cooked meat burgers. Values followed by the same letter are not significantly different

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Texture profile analysis (TPA)

Hardness values were significantly affected by the treatment type of lupin seed flour, substitution levels, and their interaction; accordingly, only the results of the interaction effect were presented (Figure 4). The hardness values were not significantly affected in the roasting and steaming treatments with varying lupin flour substitution levels compared to the control sample (0% substitution level). In the lupin without treatment, the hardness values were significantly affected by increased substitution levels of more than 5%, and the meat burger with a 10% substitution level had the lowest hardness value.

Cohesiveness values were significantly affected by the treatment type of lupin seed flour, the substitution levels of lupin flour, and the interaction between them; accordingly, only the results of the interaction effect will be presented (Figure 5). In roasting treatment with increased substitution levels of more than 5%, the cohesiveness significantly decreased compared to a 0% substitution level. The control treatment significantly decreased the cohesiveness values at a 15% substitution level, but it had no significant effect at the different levels of substitution. In contrast, the cohesiveness values were not significantly affected in the steaming treatment with varying substitution levels.

Resilience values of meat burgers were significantly affected ($P \leq 0.05$) by substitution levels, treatment type of lupin seed flour, and interaction between them; consequently, only the results of the interaction effect were displayed (Figure 6). The resilience values significantly decreased as the substitution level increased to 15% in the lupin without treatment and steamed lupin. In roasting treatment, the resilience values significantly decreased with increased substitution levels of lupin flour of more than 5% compared to a 0% substitution level.

Springiness values of meat burgers were not significantly affected by the treatment type of lupin seed flour, substitution levels, and interaction between them. The results of interaction effects are shown in Table 3.

The treatment type of lupin seed flour, the substitution levels, and the interaction between them significantly affected chewiness values; therefore, only the results of the interaction effect were shown (Figure 7). Steaming and roasting treatments with different levels of substitution of lupin flour did not significantly affect the chewiness values. In contrast, as the substitution level increased from 5% to 15% in lupin without treatment, the values of chewiness significantly decreased compared to a 0% substitution level, with no significant differences between substitution levels above 0%.

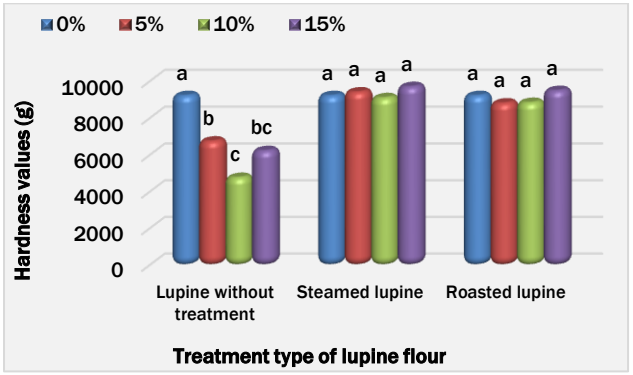

Figure 4 - Effect of the interaction between treatment type and substitution levels on hardness values on cooked meat burgers. Values followed by the same letter are not significantly different

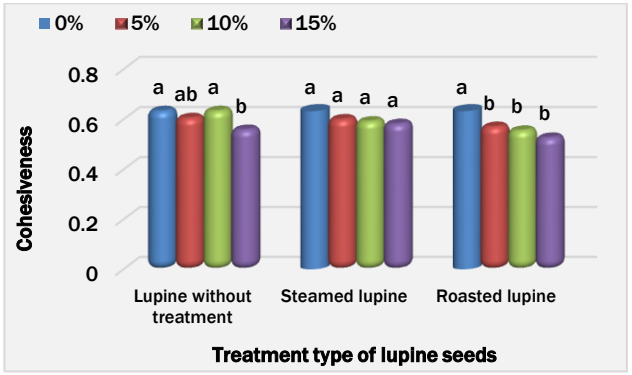

Figure 5 - Effect of the interaction between treatment type and substitution levels on cohesiveness values of cooked meat burger. Values followed by the same letter are not significantly different

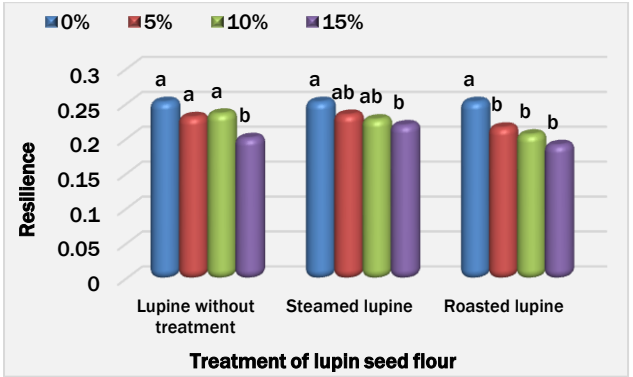

Figure 6 - Effect of the interaction between treatment type and substitution levels on resilience values of cooked meat burger. Values followed by the same letter are not significantly different

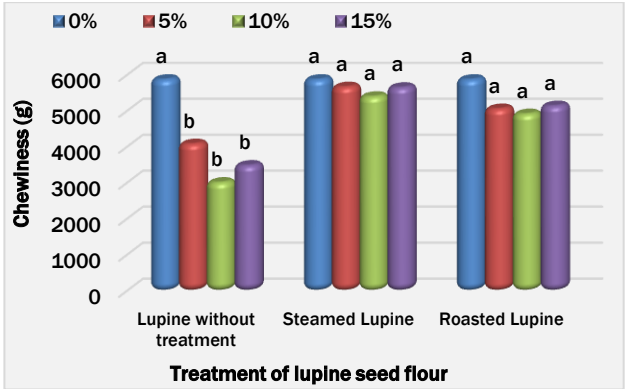

Figure 7 - Effect of the interaction between treatment type and substitution levels on chewiness values of the cooked meat burger. Values followed by the same letter are not significantly different

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Table 3 - The effect of the interaction between treatment type of lupin seed flour and substitution on TPA of cooked meat burger

Texture Profile Analysis TPA*						
Interaction effect		Hardness (g)	Cohesiveness	Resilience	Springiness	Chewiness (g)
Treatment type	Substitution level					
Lupin without treatment	0% (control)	9239±310^{a}	0.633±0.02^{a}	0.2533±0.001^{a}	0.7483±0.007	5881±85^{a}
	5%	6771±297^{b}	0.605±0.00^{ab}	0.2317±0.002^{abc}	0.7267±0.028	4088±185^{bc}
	10%	4795±212^{c}	0.633±0.02^{a}	0.2367±0.005^{ab}	0.7650±0.026	3015±21^{c}
	15%	6253±323^{bc}	0.558±0.01^{bc}	0.2017±0.002^{de}	0.7517±0.049	3490±267^{c}
Steaming treatment	0% (control)	9239±310^{a}	0.633±0.02^{a}	0.2533±0.001^{a}	0.7483±0.007	5881±85^{a}
	5%	9432±198^{a}	0.600±0.01^{ab}	0.2350±0.012^{ab}	0.7167±0.014	5674±246^{a}
	10%	9123±270^{a}	0.592±0.01^{ab}	0.2283±0.012^{abc}	0.6933±0.019	5406±258^{a}
	15%	9724±608^{a}	0.582±0.01^{abc}	0.2200±0.001^{bcd}	0.7150±0.026	5658±270^{a}
Roasting treatment	0% (control)	9239±310^{a}	0.633±0.02^{a}	0.2533±0.001^{a}	0.7483±0.007	5881±85^{a}
	5%	8831±528^{a}	0.570±0.02^{bc}	0.2167±0.009b^{cde}	0.7067±0.028	5064±487^{ab}
	10%	8862±754^{a}	0.555±0.01^{bc}	0.2067±0.009^{cde}	0.7083±0.049	4923±641^{ab}
	15%	9511±183^{a}	0.527±0.04^{c}	0.1917±0.007^{e}	0.7050±0.035	5146±341^{ab}

*M±Std.D, the values in the same column followed by the same letter are not statistically significantly different at the 0.05 probability level as determined by Tukey's test.

Chemical analysis

The moisture content of cooked and uncooked burgers was significantly affected only by substitution levels (Figure 8). Only cooked burgers made with 10% and 15% substitution levels had significantly less moisture content than the control treatment, while the burgers made with a 5% substitution level did not significantly differ from the control. In contrast, the moisture content of uncooked burgers significantly decreased with each 5% increase in the substitution level, and its values ranged between 62.55 and 71.07%, with significant differences between them.

The fat content of cooked burgers was significantly affected only by substitution levels, but the fat content of uncooked burgers was not significantly affected by substitution levels, treatment type, and interactions between them. The fat content of cooked burgers significantly decreased with increasing substitution levels above 5%, and the lowest fat content (10.78%) was recorded for burgers formulated with a 15% substitution level (Figure 9).

The protein content of uncooked burgers was significantly affected by substitution levels, treatment type, and interactions between them; therefore, the results of the interaction effect will be presented. The protein content of cooked burgers was significantly affected by the substitution level; therefore, the results of the substitution level will be presented (Figure 10). Regarding the effect of flour substitution level, the protein content of uncooked burgers increased significantly with increasing substitution levels. All the treatments of uncooked burgers made with a 15% substitution level had the highest effect in increasing the protein content. Samples of cooked burgers formulated with substitution levels of 5, 10, and 15% had the highest effect in decreasing the protein content with no significant differences between them, which was significantly different from the control treatment.

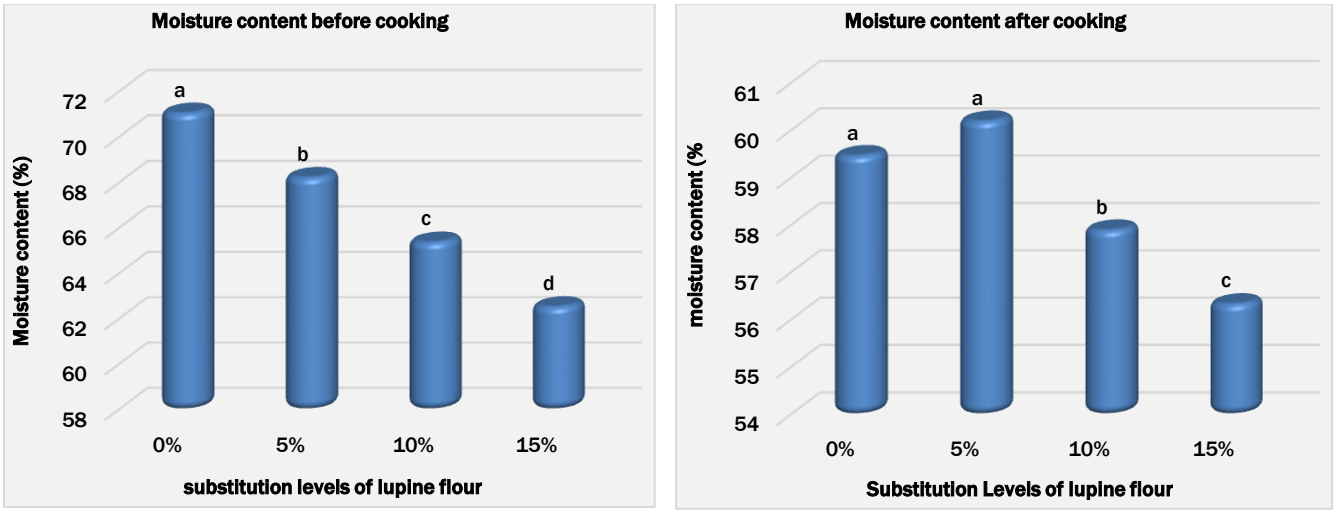

Figure 8 - Effect of using different levels of substitution of lupin flour on the moisture content of meat burgers before and after cooking. Values followed by the same letter are not significantly different

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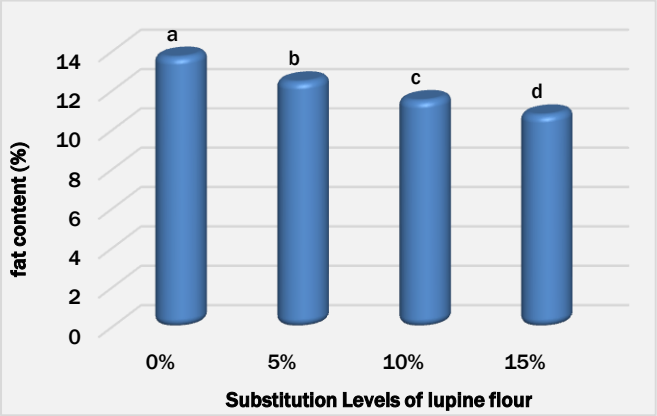
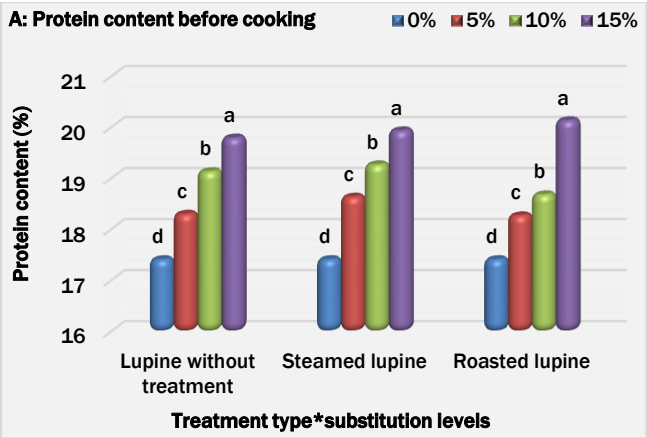


Figure 9 - Effect of using different levels of substitution of lupin flour on the fat content of burgers after cooking. Values followed by the same letter are not significantly different



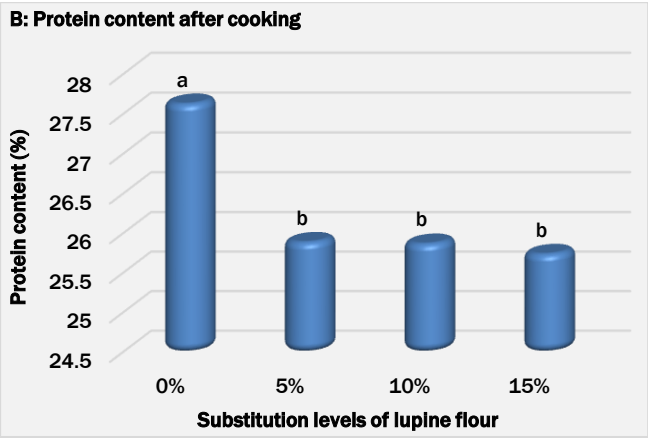


Figure 10 - Factors affecting protein content in burgers: (A) before cooking and (B) after cooking. Values followed by the same letter are not significantly different.

Cooking measurements

The values of cooking loss were significantly affected only by substitution levels. The values of cooking loss significantly decreased with each 5% increase in substitution level, with significant differences between them. On the other hand, as substitution levels increased from 0% to 15%, cooking loss values decreased significantly from 32.14% to 13.09% (Figure 11). Fat retention was not significantly affected by substitution levels, treatment type, and interaction between them. The substitution levels significantly affected moisture retention (Figure 12). A significant increase in moisture retention values was obtained when substitution levels were increased, with significant differences between them. The burgers made with a 15% substitution level had the highest effect in increasing moisture retention values. The values of shrinkage were significantly affected by substitution levels of lupin flour (Figure 13). The results showed significant differences between values of shrinkage when increasing substitution levels. The lowest significant shrinkage values were for burgers with a 15% substitution level, which was followed by a 10% substitution level. The impact of using selected different treatments and different levels of lupin seed flour on sensory analysis is shown in Table 4. The steaming treatment with 10% lupin seed flour had the highest sensory score, whereas the control treatment with 0% lupin seed flour received the lowest sensory score. However, these differences were not significantly different.

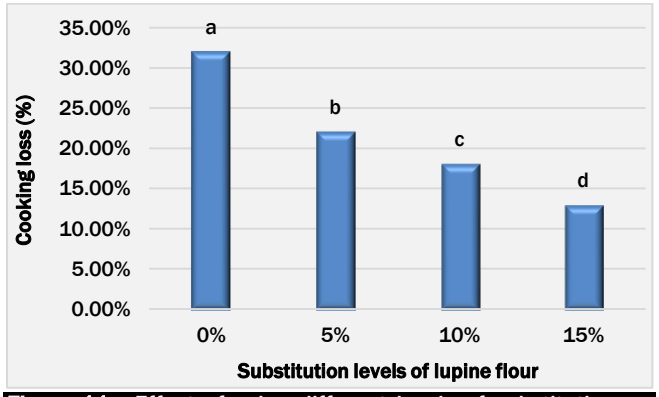


Figure 11 - Effect of using different levels of substitution on values of cooking loss. Fat retention. Values followed by the same letter are not significantly different.

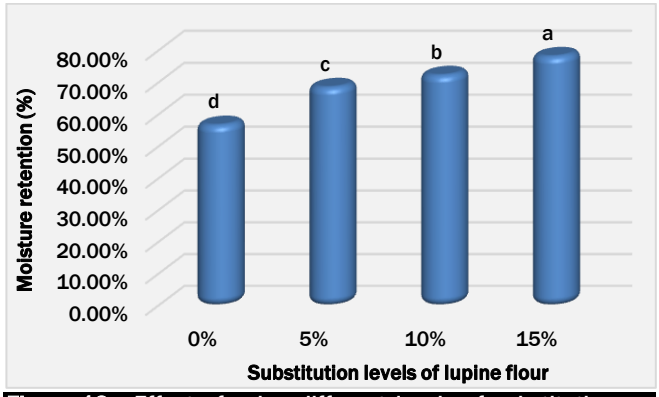


Figure 12 - Effect of using different levels of substitution on values of moisture retention. Values followed by the same letter are not significantly different.

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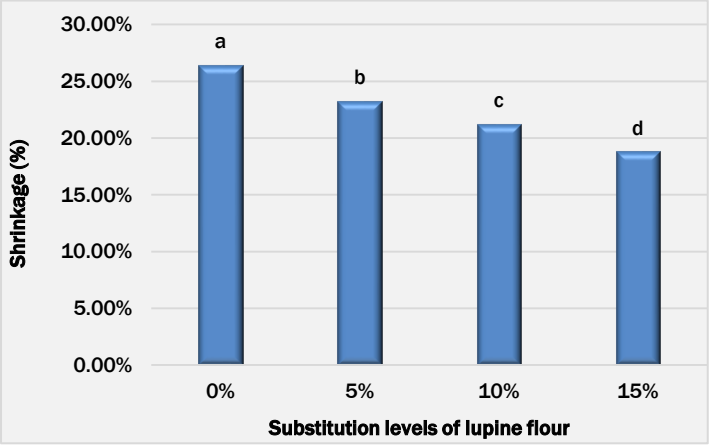

Figure 13 - Effect of using different levels of substitution on values of shrinkage. Values followed by the same letter are not significantly different

Table 4 - Effect of using selected treatments of lupin seed flour and substitution levels on sensory analysis scores*

Treatment	% Substitution	Texture	Color	Taste
Without	0 (control)	6.70±1.95[a]	7.20±1.23[a]	5.80±2.62[a]
	5	7.60±0.84[a]	6.70±1.77[a]	7.10±2.03[a]
	10	7.20±1.48[a]	6.20±1.55[a]	6.20±1.69[a]
Steaming	5	7.60±2.37[a]	6.60±2.55[a]	6.80±2.15[a]
	10	7.30±1.64[a]	7.50±1.27[a]	7.80±0.79[a]

*The values are expressed as M±Std.D, and the values in the same column that are followed by the same letter are not significantly different at the 0.05 probability level.

DISCUSSION

Formulation of beef burgers

High meat consumption worldwide is a growing danger to human health (Caputo et al., 2023). Red and processed meat intake has been associated with elevated risks of (CVD) (Bechthold et al., 2017) and a high prevalence of malignancies (Bouvard et al., 2015). Due to their high protein content (Sujak et al., 2006), low-fat content (Annicchiarico et al., 2014), and significant beneficial effects on the physiological state of the human body, in particular for those suffering from diabetes, hypertension, obesity, and (CVD) (Ahmed, 2014; Prusinski, 2017), this study sought to use lupin flour as a meat alternative in the preparation of beef burgers by substituting a portion of the meat with lupin flour. To achieve this purpose, the effects of the treatment of lupin seeds (without treatment, steaming, and roasting) and the substitution level of meat with lupin flour (0, 5, 10, and 15%). To the best of our knowledge, limited studies investigated the incorporation of lupin flour into meat products; for instance, El-Sayed (2013) studied the effect of replacing meat with 5 and 7.5% of lupin seed flour on the quality characteristics of beef burger patties. Dalain et al. (2023) studied the impact of three different levels of sweet lupin flour (10, 20, and 30%) on the quality of chicken burgers. Regarding previous studies on the effect of the treatments of lupin flour on the quality of beef sausage, Leonard et al. (2019) studied the impact of roasted lupin flour on beef sausage's physicochemical and sensory characteristics.

Instrumental color analysis

The CIELAB color parameters (L*, b*, and ΔE*ab) were significantly affected (P≤0.05) only by substitution levels. In general, the developed beef burgers had higher L*, b*, and ΔE*ab with increasing substitution levels regardless of the treatment type. It is expected for the color of beef burgers to become lighter when using vegetables as an alternative to meat. This result is consistent with the results of Lee et al. (2021), who found that by replacing meat with lupin flour, the concentration of this pigment decreased, resulting in a product with a lighter color. In another study, L* values of chicken patties increased due to replacing chicken meat with oat flour (Serdaroğlu, 2006). Shokry (2016) found that as the meat substitution with quinoa flour increased, the meat burger's L* and b* values increased. Similarly, Al-Juhaimi et al. (2017) reported that as the Moringa oleifera seed flour level increased, the beef burger patties had high L* and b* values.

TPA

Textural properties of meat burgers are among the most influential factors in consumer acceptance Fiorentini et al. (2020). The hardness, cohesiveness, resilience, and chewiness values were significantly affected by the interaction

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between the substitution levels of lupin flour and the type of treatment, whereas different treatments did not affect springiness. This means that the texture profile parameters changed in a manner dependent on the type of treatment and the substitution level used. It is evident from the results the importance of the treatment of lupin flour in maintaining the texture integrity of cooked beef burgers – compared to the control with zero substitution level – with increasing the substitution level. From the results, using steamed lupin seeds flour with a 10% substitution level resulted in a texture profile that did not significantly differ from the control beef burger (0% substitution level); this indicates the role of lupin seeds treatment with steaming in improving the functional properties of lupin flour. Using untreated lupin seed flour to substitute meat in beef burgers significantly decreased hardness, chewiness, cohesiveness, and resilience. The results of the untreated lupin seeds flour are consistent with the results of the previous study; for instance, Vu et al. (2022) reported that the cooked plant-based patties had softer (lower hardness, cohesiveness, resilience, and chewiness) than the cooked beef patties. The changes in the structural properties might be attributed to the changes in the chemical composition and structure of different formulas; it has been reported that animal protein's heterogeneity with vegetable protein adversely affects its structural properties (Godschalk-Broers et al., 2022). Bakhsh et al. (2021) reported significant differences in the textural properties of meat and meat substitutes after cooking.

Steamed lupin flour is an excellent choice for incorporating lupin flour into beef burgers. A previous study highlighted that protein isolates derived from legume grains subjected to steaming exhibited improved foaming properties (Naiker et al., 2020). This indicates that using steamed lupin flour can enhance the foaming capacity and stability of the burger mixture. Proteins with good foaming properties, characterized by flexible surfactant molecules that form cohesive visco-elastic films at the air-water interface, can contribute to the desired texture of the burgers. The steaming treatment enhances the ability of lupin proteins to rapidly adsorb at the air-water interface, leading to increased foamability and improved cohesion in the burger mixture. Additionally, the higher ratio of acidic to basic amino acids in steamed lupin flour enhances protein solubility and flexibility, allowing for better spreading on the air-water interface and improved foam formation. Therefore, steamed lupin flour in beef burgers can enhance their textural properties by improving foaming capacity, stability, and cohesion (Adebowale and Maliki, 2011; Naiker et al., 2020).

Chemical analysis

The chemical parameters tested were mainly affected by the substitution level. This result is consistent with the findings of Serdaroğlu et al. (2018), who found that the incorporation of various levels of dried pumpkin pulp and seed mixture (0, 2, 3, and 5%) led to significant alterations in the majority of chemical parameters (protein, moisture, and fat) for both raw and cooked beef patties. The moisture content of uncooked (62.55 to 71.07%) and cooked burgers (56.34 – 60.2%) was significantly affected only by substitution levels. Logically, the moisture content decreased with an increased substitution level because we removed beef meat and added dry lupin flour instead. The moisture content range in uncooked burgers was 8.52%, whereas in cooked burgers, it was 3.68%. The decreased range in cooked burgers was related to the improved water retention properties of burgers made with lupin flour, which increased with increasing substitution levels. Because the moisture contents of cooked burgers ranged from (50.78–60.86%), this result is close to what Abbas (2009) discovered in his study on the concurrent manufacturing of burgers from veal and legumes (peas and beans). Results were in contrast to the findings of Devatkal et al. (2011), who reported that the moisture content of cooked nuggets made with 5% sorghum flour instead of wheat flour was greater than that of the control group.

The fat content of uncooked burgers was not significantly affected by substitution levels, treatment type, and interactions between them. However, the fat content of cooked burgers ranged between 10.78 and 13.72, significantly decreasing with each increment of lupin flour addition (Figure 9). This can be explained by the improved water retention with increasing the substitution level, considering that the fat content was expressed as a wet matter basis. The protein content of uncooked burgers increased significantly with increasing substitution levels due to the higher amount of protein in lupin flour (30–40%) Prusinski (2017) compared to that in beef meat used in this study (18.34%). However, the protein values after cooking were significantly lower in samples containing lupin flour (25.74-25.89%) compared to the control sample (27.63%). The justification of the contradictory results between protein content in uncooked and cooked might be explained by the increased water-holding capacity of burgers with the increased substitution levels and the low gelling power of lupin proteins (Abreu et al., 2023), which could cause loss of protein upon cooking.

Cooking measurements

The meat industry relies heavily on cooking properties such as cooking yield, cooking loss, and shrinkage to predict the behavior of products during cooking (Ahmed and Abdel-Rahman, 2022). It was evident from the results that the cooking loss values were significantly decreased by increasing the substitution levels. Regarding cooking shrinkage, it could be noticed that the values decreased as the level of substitution lupin flour increased from 5 to 15%. The lowest significant cooking loss and shrinkage values were for burgers with a 15% substitution level. These results may be attributed to the content of total fibers in lupin seeds, averaging 101 g/kg (Tizazu and Emire, 2010), and illustrated the role of high fiber content in the enhancement of cooking yield, water holding capacity, cooking loss, and shrinkage. This result was in line with a previous study by Shokry (2016) on the cooking yield in meat burgers formulated with quinoa flour, who illustrated that the cooking yield increased with increasing quinoa flour incorporated in beef burgers. Tabarestani and Tehrani (2014) found that combining soy flour with starch increased cooking yield, and splitting pea flour

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in the mixed formula improved textural properties. As the levels of substitution of lupin seed flour increased, the moisture retention values were significantly affected and increased. This result is in accordance with those found by El-Sayed (2013), in which the moisture retention values were increased with increasing levels of lupin flour in beef burger samples. Tabarestani and Tehrani (2014) also documented improved moisture retention in low-fat hamburger patties with starch added.

Sensory Evaluation

A sensory evaluation test was conducted to evaluate consumers' acceptability and satisfaction toward beef burgers treated by steaming compared to the control sample (Table 4). The results showed no significant difference between steaming treatments with varying substitution levels compared to the control. This result is in agreement with the work of Devatkal et al. (2011), who reported that adding 5% sorghum flour to gluten-free chicken nuggets had the same flavor and texture as the control group (5% wheat flour), regarding all the sensorial attributes. Ramadan et al. (2016) found no significant differences in color, flavor, odor, appearance, or general acceptability between chicken burgers made with other grains such as wheat, sorghum, and maize. Dalain et al. (2023) studied the formulation of chicken burgers with three various levels of substitution of sweet lupin (10, 20, and 30%) and reported that chicken burgers containing 20% sweet lupin flour had the greatest sensory qualities.

CONCLUSION

Due to what we have reviewed of the results, the use of lupin flour had an apparent effect on improving the properties of beef burgers, as the most important results were reducing the shrinkage rate and the percentage of losses after cooking, noting that the shrinkage rate was in the control sample at (26.45%) and reached (18.8%), and the cooking loss percentage was in the control sample at (32.14%) and reached (13.09%). However, the results demonstrated the important effect of treatment, particularly steaming, in improving the functional properties of lupin seed flour, which were evident in maintaining the texture properties of the cooked beef burger while increasing the substitution levels. Lupin flour can be recommended as a potential new functional material for meat manufacturers that can replace soy proteins, which will be reflected in the quality of the final product.

DECLARATIONS

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Authors' contribution

The first author performed the experiments and analysis and participated in writing. The second author designed the experiments, statistically analyzed the results, and participated in writing.

Conflict of interests

The authors have not declared any conflict of interest.

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REFERENCES

Abreu B, Lima J, and Rocha A (2023). Consumer Perception and Acceptability of Lupin-Derived Products: A Systematic Review. Foods, 12(6): 1241. https://doi.org/10.3390/foods12061241

Abbas QS (2009). Manufacturing of beef parallel by veal meat and some legumes. Tikrit University Magazine of Agricultural Sciences, 9(1). Google Scholar

Adebowale, OJ and Maliki K (2011). Effect of fermentation period on the chemical composition and functional properties of Pigeon pea (Cajanus cajan) seed flour. International Food Research Journal, 18(4): 1329-1333. Google Scholar

Ahmed AR (2014). Influence of chemical properties of wheat-lupine flour blends on cake quality. American Journal of Food Science and Technology, 2(2): 67-75. https://doi.org/10.12691/ajfst-2-2-4

Ahmed R and Abdel-Rahman A (2022). Effect of Papaya Wastes on Quality Characteristics of Meat Burger. New Valley Journal of Agricultural Science, 2(6): 483-511. https://doi.org/10.21608/nvjas.2022.172933.1110

Dalain SYA, Haddad MA and Al-Qaraleh SY (2023). Using sweet lupine powder as a healthy supplement for the production of functional chicken burger in Jordan. Current Research in Nutrition and Food Science, 11(1): 164–

Citation: Alrahaife AJ, and Abu-Alruz K (2023). Effects of incorporation of lupin flour on the quality attributes of beef burger. *Online J. Anim. Feed Res*., 13(5): 328-339. DOI: https://dx.doi.org/10.51227/ojafr.2023.48

173. https://doi.org/10.12944/crnfsj.11.1.11

Al-Juhaimi F, Ghafoor K, Babiker EE, Matthäus B and Özcan MM (2017). The biochemical composition of the leaves and seeds meals of moringa species as non-conventional sources of nutrients. Journal of Food Biochemistry, 41(1): 12322.https://doi.org/10.1111/jfbc.12322

Alrawashdeh H and Abu-Alruz K (2022). Development of High-Fiber, Low Fat Chicken Nuggets. International Journal of Food Studies, 11(2): 354–373. https://doi.org/10.7455/ijfs/11.2.2022.a8

Annicchiarico P, Manunza P, Arnoldi A and Boschin G (2014). Quality of Lupinus albus L. (white lupin) seed: extent of genotypic and environmental effects. Journal of Agricultural and Food Chemistry, 62(28): 6539-6545.https://doi.org/10.1021/jf405615k

Bakhsh A, Lee SJ, Lee EY, Hwang YH and Joo ST (2021). Traditional plant-based meat alternatives, current, and future perspective: a review. J. Agric. Life Sci, 55(1): 1-11. https://doi.org/10.14397/jals.2021.55.1.1

Bechthold A, Boeing H, Schwedhelm C, Hoffmann G, Knüppel S, Iqbal K, De Henauw S, Michels N, Devleesschauwer B, Schlesinger S and Schwingshackl L (2017). Food groups and risk of coronary heart disease, stroke and heart failure: A systematic review and dose-response meta-analysis of prospective studies. Critical Reviews in Food Science and Nutrition, 59(7): 1071–1090. https://doi.org/10.1080/10408398.2017.1392288

Bouvard V, Loomis D, Guyton KZ, Grosse Y, Ghissassi FE, Benbrahim-Tallaa L, Guha N, Mattock H and Straif K (2015). Carcinogenicity of consumption of red and processed meat. Lancet Oncology, 16(16): 1599–1600. https://doi.org/10.1016/s1470-2045(15)00444-1

Bryant L, Rangan A and Grafenauer S (2022). Lupins and health outcomes: A systematic literature review. Nutrients, 14(2): 327. https://doi.org/10.3390/nu14020327

Caputo V, Sogari G and Van Loo E J (2023). Do plant-based and blend meat alternatives taste like meat? A combined sensory and choice experiment study. Applied Economic Perspectives and Policy, 45(1): 86-105. https://doi.org/10.1002/aepp.13247

Devatkal SK, Kadam DM, Naik PK and Sahoo J (2011). Quality characteristics of gluten-free chicken nuggets extended with sorghum flour. Journal of Food Quality, 34(2): 88-92. https://doi.org/10.1111/j.1745-4557.2010.00367.x

Estell ML, Hughes J and Grafenauer, S (2021). Plant Protein and Plant-Based Meat Alternatives: Consumer and Nutrition Professional Attitudes and Perceptions. Sustainability, 13(3): 1478. https://doi.org/10.3390/su13031478

El-Sayed SM (2013). Influence of using lupine flour as binder on quality characteristics of beef burger patties. Journal of Applied Sciences Research, 9(6): 3809-3819.

Fiorentini M, Kinchla AJ and Nolden AA (2020). Role of Sensory Evaluation in Consumer Acceptance of Plant-Based Meat Analogs and Meat Extenders: A Scoping review. Foods, 9(9): 1334. https://doi.org/10.3390/foods9091334

Godschalk-Broers L, Sala G and Scholten E (2022). Meat Analogues: Relating structure to texture and sensory perception. Foods, 11(15): 2227. https://doi.org/10.3390/foods11152227

Ismail MA, Chong GH and Ismail-Fitry MR (2021). Comparison of the microstructural, physicochemical and sensorial properties of buffalo meat patties produced using bowl cutter, universal mixer and meat mixer. Journal of Food Science and Technology, 58(12): 4703–4710. https://doi.org/10.1007/s13197-020-04960-y

Leonard W, Hutchings SC, Warner RD and Fang Z (2019). Effects of incorporating roasted lupin (Lupinus angustifolius) flour on the physicochemical and sensory attributes of beef sausage. International Journal of Food Science and Technology, 54(5): 1849–1857. https://doi.org/10.1111/ijfs.14088

Marinova D and Bogueva D (2019). Planetary health and reduction in meat consumption. Sustainable Earth, 2(1): 1-12. https://doi.org/10.1186/s42055-019-0010-0

Serdaroğlu M, Kavuşan HS, İpek G and Öztürk B (2018). Evaluation of the Quality of Beef Patties Formulated with Dried Pumpkin Pulp and Seed. PubMed, 38(1): 1–13. https://doi.org/10.5851/kosfa.2018.38.1.001

Naiker TS, Baijnath H, Amonsou EO and Mellem JJ (2020). Effect of steaming and dehydration on the nutritional quality and functional properties of protein isolates produced from Lablab purpureus (L.) Sweet (hyacinth bean). Journal of Food Processing and Preservation, 44(2). https://doi.org/10.1111/jfpp.14334

Petrat-Melin B and Dam S (2023). Textural and Consumer-Aided characterisation and acceptability of a hybrid meat and Plant-Based burger patty. Foods, 12(11): 2246. https://doi.org/10.3390/foods12112246

Prusinski J (2017). White lupin (Lupinus albus L.) - nutritional and health values in human nutrition - a review. Czech Journal of Food Sciences, 35(2): 95–105. https://doi.org/10.17221/114/2016-cjfs

Ahmed A (2014). Influence of chemical properties of Wheat-Lupine flour blends on cake quality. American Journal of Food Science and Technology, 2(2): 67–75. https://doi.org/10.12691/ajfst-2-2-4

Ramadan BR, Sorour MAH and Kelany MA (2016). Improving oxidative stability of beef burgers under chilled storage using cereal grain fractions. University of Agricultural Sciences and Veterinary Medicine Iasi, 56(12): 183-178.

Romero MC, Fogar RA, Doval MM, Romero A and Judis MA (2019). Optimisation of cooking properties of healthier beef patties and quality evaluation during frozen storage. Journal of Food Measurement and Characterization, 13(3): 1907–1916. https://doi.org/10.1007/s11694-019-00109-4

Serdaroğlu M (2006). The characteristics of beef patties containing different levels of fat and oat flour. International Journal of Food Science and Technology, 41(2): 147–153. https://doi.org/10.1111/j.1365-2621.2005.01041.x

Siegrist M and Hartmann C (2023). Why alternative proteins will not disrupt the meat industry. Meat Science, 203, 109223. https://doi.org/10.1016/j.meatsci.2023.109223

Shokry AM (2016). The usage of quinoa flour as a potential ingredient in production of meat burger with functional

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properties. Middle East Journal of Applied Sciences, 6(4): 1128-1137.

Sujak A, Kotlarz A and Strobel W (2006). Compositional and nutritional evaluation of several lupin seeds. Food chemistry, 98(4): 711-719. https://doi.org/10.1016/j.foodchem.2005.06.036

Smetana S, Profeta A, Voigt R, Kircher C and Heinz V (2021). Meat substitution in burgers: nutritional scoring, sensorial testing, and Life Cycle Assessment. Future Foods, 4, 100042. https://doi.org/10.1016/j.fufo.2021.100042

Tabarestani S and Tehrani M (2014). Optimization of physicochemical properties of low-fat hamburger formulation using blend of soy flour, split-pea flour and wheat starch as part of fat replacer system. Journal of Food Processing and Preservation, 38(1): 278-288. https://doi.org/10.1111/j.1745-4549.2012.00774.x

Tizazu H and Emire SA (2010). Chemical composition, physicochemical and functional properties of lupin (Lupinus albus) seeds grown in Ethiopia. African Journal of Food, Agriculture, Nutrition and Development, 10(8). https://doi.org/10.4314/ajfand.v10i8.60895

Vu G, Zhou H and McClements DJ (2022). Impact of cooking method on properties of beef and plant-based burgers: Appearance, texture, thermal properties, and shrinkage. Journal of Agriculture and Food Research, 9, 100355. https://doi.org/10.1016/j.jafr.2022.100355

Zhang C, Guan XY, Yu S, Zhou J and Chen J (2022). Production of meat alternatives using live cells, cultures and plant proteins. Current Opinion in Food Science, 43: 43-52. https://doi.org/10.1016/j.cofs.2021.11.002

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THE ORGANOLEPTIC, CHEMICAL AND MICROBIOLOGICAL QUALITY OF MAGGOT'S FRASS AS ALTERNATIVE POULTRY FEED INGREDIENTS

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ABSTRACT: Maggot's frass is waste from cultivating maggots (insect larvae) which consists of media from maggot cultivation mixed with feces, skin and dead body of the maggots. The aim of the study was to examine the organoleptic quality, chemistry, worm eggs, lead (Pb) as heavy metal and microbiological profile of maggot's frass as an alternative ingredient of poultry feed. A completely randomized design (CRD) with 3 treatments (T1: frass media for household waste, T2: frass media for tofu dregs, and T3: frass media for vegetable and fruit waste) and 7 replications was used. The results showed that there was no effect of different types of media treatment on the organoleptic quality, chemistry and microbiological profile of maggot's frass. The results of chemical analysis of maggot's frass revealed moisture of 26.39 - 46.26%, crude protein of 10.92 - 16.37%, worm eggs in the dregs media tofu (16 EPG), vegetable and fruit waste (32 EPG), total bacteria of 1.91-4.95 x 10^8cfu/g, and no any *Escherichia coli* and *Salmonella isolates*. Maggot's frass which comes from fruit and vegetable waste was recommended. Therefore, maggot feed using fruit and vegetable waste treatment is recommended because of its high crude protein and metabolic energy and also without any *E.coli* and *Salmonella* contamination.

Keywords: Black Soldier fly; Feed; Maggot's frass; Larva; Waste.

INTRODUCTION

New feed resources are solution to farmers regarded to the problem of cost and efficacy of feed. Alternative feed ingredients usually come from waste, such as agricultural waste, market waste, household waste and industrial waste (Boumans et al., 2022; Siddiqui et al., 2022). Population growth causes an increase in organic waste which has a negative effect on the environment (Henault-Ethier et al., 2017). Maggot or larvae of the Black Soldier Fly (BSF; *Hermetia illucens*) is bioconversion agents that able to convert organic waste into protein biomass (Chiam et al., 2021). Maggot has been used as an alternative feed ingredient to replace fish meal, because maggot's crude protein content is high (Luthada-Raswiswi et al., 2022). Makkar et al. (2014) reported that the use of maggot as much as 19% in poultry feed showed increased the digestibility and had no negative effects. Furthermore, utilization 10-15% maggot flour into poultry feed can improve performance and quality of carcasses (Hwangbo et al., 2009). However, intensive maggot cultivation has resulted in the emergence of new problems, namely by-products in the form of frass which has a fairly good nutritional content, it has the potential to be used as a poultry feed ingredient.

Frass is leftover feed media from maggot cultivation mixed with fecal, skin and dead body of the maggot. Feed media directly affected the physical and chemical properties of frass, because the residue of undigested feed remained in the frass (Garttling and Schulz, 2022). The Maggot feed media is based on the organic waste, such as vegetable waste, fruit waste, restaurant waste, sausage industrial waste, tofu dregs, cassava and cassava peels. That was reported the quality of frass produced from different maggot feed media. Song et al. (2021) noted that frass from a mixture of tofu dregs and wheat flour contained 4.78% nitrogen (N), or 29.87% protein. Frass from a mixture of radish and carrot waste contains 2.04% N or 12.75% protein, from restaurant waste contains 3.15% N or 19.69% protein (Chiam et al., 2021), from a mixture of fruit and vegetable waste contains 1.83% N or 11.44% protein (Lopes et al., 2022). Klüber et al. (2022) stated that frass from maggot cultivation with Palm Kernel Meal (PKM) feed had a crude protein content of 19.32%, 0.36% crude fat and 21.11% crude fiber. Taking into the results of the research above, it appears that frass is worthy of consideration for use as poultry feed.

This study aimed to examine the quality of frass as an alternative feed ingredient in terms of organoleptic, chemical, and microbiological quality. The benefit of research is to become a reference for breeders to try alternative feed

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ingredients that are easy to get, inexpensive, of good quality, and meet the needs of animals. The research hypothesizes are the different types of maggot feed affect the organoleptic, chemical, and microbiological quality of maggot's frass.

MATERIALS AND METHODS

Materials

Materials used in the present study included frass, 0.3 N H_2SO_4, 1.5 N NaOH, aquadest, salt, lead colorimetric test kit, 0.85% physiological NaCl, crystal violet solution, 96% alchohol, Lugol's solution, pH paper test (Merck®, Germany), silica gel, filter paper and safranin O (C.I. 50240). The tools used were analytical balance kern ABJ-220 with an accuracy of 0.01 gram, porcelain cup, sistered glass, Universal Drying Oven UN 55, 50 ml glass beaker, desicator, vacuum pomp, soxhlet, thermo furnace F48010-26, glass jar, glass handle loop - W4, object class 25,4x76.2 mm, 22×22 ml cover glass, and an Olympus CX-33 microscope.

Methods

The research design used a completely randomized design (CRD) with 3 treatments and 7 replications. Treatment T1: using feed from household waste, T2: using feed from tofu dregs, T3: using feed from vegetable and fruit waste. Parameters observed included frass organoleptic tests, nutritional quality of frass, worm eggs, heavy metal (Pb) and maggot's frass microbiological profile.

Organoleptic test

Organoleptic tests measured include color, odor, texture and contamination. Data collection was carried out with 20 semi-trained panelists. The test is carried out using a scoring method with 7 levels of a comparison scale (Utama and Christiyanto, 2021).

Pollution assessment

Score 1: 0 level contamination; Score 2: 1 level contamination; Score 3: 2 level contamination; Score 4: 3 level contamination; Score 5: 4 level contamination; Score 6: 5 level contamination; Score 7: 6 level contamination.

Odor assessment

Score 1: The odor of ammonia is very strong; Score 2: A strong odor of ammonia; Score 3: Ammonia odor is a bit overpowering; Score 4: Typical odor of ammonia; Score 5: Slight odor of ammonia; Score 6: Very slight odor of ammonia; Score 7: Ammonia has no odor.

Texture assessment

Score 1: No lumps; Score 2: Very few lumps; Score 3: Few lumps; Score 4: Moderate; Score 5: more lumps; Score 6: Very many lumps; Score 7: Combine everything.

Color rating

Score 1: Deep Black	Score 2: Black	Score 3: Dark Dark Brown	Score 4: Dark Brown	Score 5: Chocolate	Score 6: Light Brown	Score 7: Yellow Brown.

Proximate test

Proximate analysis consisting of moisture, ash content, crude fat, crude protein and crude fiber was carried out using the AOAC (2005).

Worm contamination test

Worm egg contamination testing was carried out using the Mc Master method (Foka et al., 2021). The number of eggs observed in each column is calculated using the formula: EPG = 2n × 50

Which, n= The number of worm eggs counted in the counting chamber; EPG= Eggs per gram.

Lead (Pb) heavy metal contamination test

Testing for the Pb metal content in the samples was carried out using the atomic absorption spectrophotometer (AAS) method (Oloo and Awuor, 2019). Calculation of the concentration of lead (Pb) uses the equation for the concentration of Pb as follows (AOAC, 2005); (µg/g) = "C x VC x V" / "W W"

Which, C = concentration measured in mg/L (ppm) converted to µg/L (ppb); V = total sample volume (mL) converted to liters (L); W = sample weight (g).

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Microorganism contamination test

Determination of the number of bacterial colonies for each sample was measured using the total plate count (TPC) method. Bacterial population is calculated as follows: Bacterial population (cfu/g) = Number of Colonies × Dilution

Data analysis

The research data obtained was tested using analysis of variance (ANOVA) to determine the effect of treatment with a significant level of 5%. If there is a real effect, it be continued with the Duncan multiple range test.

RESULTS AND DISCUSSIONS

Organoleptic quality of Maggot's frass

Organoleptic tests including data on color, odor, texture and contamination of frass did not show any differences in the characteristics shown in table 1.

The results of organoleptic parameters showed that among the treatment of different types of maggot feed did not show a significant difference (P>0.05) to frass color. The average value of frass color was 1.34. The value of 1.34 showed that frass has a deep black color. Van Looveren et al., (2022) stated that frass has a black color. Frass color is influenced by moisture, contaminants in feed and maggot feed ingredients (Basri et al., 2022).

The results of showed that the treatment of different types of maggot feed did not affect the odor of frass (P>0.05). The average odor rating is a score of 4, which means it has a distinctive odor of ammonia. The odor of ammonia comes from the decomposition of organic matter by microorganisms. Jayanegara et al. (2017) stated that the odor of ammonia is due to the evaporation of gases resulting from the decomposition of bacteria. Factors that can affect the odor of frass are moisture, fat and protein content and the type of feed (Tschirner and Simon, 2015).

The average result of the texture value of frass is 2.70. The value of 2.70 indicates that the texture of the frass has a few lumps. A slightly lumpy texture indicates that the moisture of Maggot's frass is quite high. Jayanegara et al. (2017) stated that the moisture affected the texture, because high moisture facilitates the appearance of lumps and damage caused by the emergence of bacteria, mold and fungi. The texture of a feed ingredient may affected feed consumption and digestibility (Pazoki et al. 2017).

The results of frass contaminates showed that the treatment did not affect frass contamination (P>0.05). In the assessment of contamination, the average score is 2.53. This shows that there are 2 types of contamination in frass. Contaminants that are often found in frass are yarn and plastic. Beale et al. (2022) stated that contamination of frass in the form of plastic material could be minimized by separating it before giving it to maggot. Maggot's frass contamination comes from a mixture in feed that cannot be digested and will remain in frass. Lopes et al. (2022) stated that materials that cannot be digested by maggot will remain in frass.

The organoleptic conditions of maggot's frass in this study showed the appropriate color and odor. The presence of contaminants in maggot's frass needs to be cleaned before being used as a poultry feed ingredient.

Table 1 - Frass organoleptic test

Parameter \ Treatments	T1	T2	T3	P-Values
Color	1.25±0.26	1.37±0.31	1.64±0.45	NS
Odor	4.75±1.49	4.08±1.51	4.02±1.10	NS
Texture	2.53±0.79	2.56±0.92	3.02±0.77	NS
Contamination	2.35±0.29	2.60±0.40	2.64±0.53	NS

NS: non-significant (P>0.05).

Chemical quality of maggot's frass

The chemical quality of frass includes water content, organic matter, crude protein, ether extract, crude fiber, nitrogen-free extract (NFE), pH, metabolic energy, the presence of worm eggs and heavy metals (Pb) shown in table 2. Different types of maggot feed influence characteristics and contents of maggot frass.

The types of maggot feed showed significant differences (P<0.05) in frass moisture. The highest moisture was at T3 with 46.29% followed by T1 35.41% and T2 26.39%. The difference in moisture in Frass is caused by the moisture of the type of feed given. Pham et al. (2015) stated that vegetable waste has a high moisture, namely around 91.56%. The moisture of maggot's frass from the three treatments is high because the moisture is more than 14%. Sharif et al. (2021) stated that moisture that is too high can reduce the quality of feed ingredients, because it will become a medium for growing microorganisms. Different treatments showed significant results (P<0.05) on frass ash content. Ash content showed the amount of inorganic material content in a material. Elechi et al. (2021) stated that during the maggot maintenance phase, frass mixed with maggot manure which has a lot of inorganic substances. The treatment 1 had a high ash content indicating that frass contained a lot of inorganic substances which affected the organic matter content. Yildirim-Aksoy et al. (2020) stated that the ash component in maggot's frass consists of mineral content, salts and inorganic compounds in the form of oxides in organic waste provided and not consumed by maggot.

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Table 2 - Chemical Quality of Maggot's frass

Parameter \ Treatment	T1	T2	T3	P-Values
Moisture (%)	35.41±13.27[a]	26.39±12.02[b]	46.26±9.13[c]	*
Dry matter (%)	95.70±1.61	96.79±1.46	95.20±1.31	NS
Ash (%)	23.50±9.67[a]	19.05±5.83[a]	13.16±5.43[b]	*
Crude protein (%)	10.92±3.01[b]	14.72±6.75[a]	16.37±3.98[a]	*
Extract ether (%)	2.04±0.97	3.40±3.63	2.70±1.87	NS
Crude fiber (%)	16.62±5.53	18.95±7.64	17.14±6.01	NS
Nitrogen free extract (NFE; %)	46.92±4.71[b]	43.88±4.88[b]	50.63±5.40[a]	*
Metabolic energy (Kkal/Kg)	2318.52±438.00[b]	2454.48±452.36[b]	2617.45±291.89[a]	*
pH	6.98±0.45	6.75±0.77	6.88±1.31	NS
Worm eggs (*Egg per Gram*)	0[c]	16.00±5.77[b]	32.00±3.47[a]	*
Pb (lead) (mg/ kg)	0	0	0	NS

Different superscripts in the same line show a significant difference (P<0.05), NS: non-significant (P>0.05); * : P<0,05.

The results of crude protein levels in table 2 show that there are differences in the type of treatment feed (P<0.05). Frass crude protein levels in the study were highest at T3, namely 16.37%. Arabzadeh et al. (2022) stated that frass has a crude protein content of around 19.32%. The T3 treatment has a higher protein content which is possible because fruit and vegetable waste has a better nutritional content. The protein content in maggot's frass comes from leftover feed, maggot droppings, loose maggot skin and dead maggot during the cultivation period (Chiam et al. 2021).

Treatment of did not show significant differences in extract ether content of frass. Maggot's frass extract ether content ranges from 2.04 – 3.40%. Arabzadeh et al. (2022) stated that maggot's frass contains extract ether of around 0.36%. Maggot frass's fat content comes from maggot's ability to convert fiber in organic waste into fat in maggot's body which comes out with feces. According to Zheng et al. (2012), the black soldier fly has cellulose bacteria in its digestion to help convert organic waste fiber into fat and protein in its body biomass.

The results of the statistical analysis showed that the treatment of different types of feed did not affect the crude fiber content (P>0.05). The crude fiber content in frass ranges from 16.62 - 18.95%. Arabzadeh et al. (2022) stated that maggot's frass has a crude fiber content of 21.11%. The low crude fiber content in frass is because the crude fiber will be hydrolyzed by microbes into an energy source in the form of carbon for BSF maggot. Frass crude fiber content is affected by the type of feed given during the cultivation period. Garttling and Schulz (2022) stated that the content of the type of feed given will affect frass crude fiber.

The value of extract material without maggot's frass nitrogen showed significant differences (P<0.05) from the treatment of different types of feed. Significantly different NFE values were due to significantly different protein values. Barroso et al. (2014) stated that the content of extract ingredients without nitrogen (NFE) of feed ingredients is very dependent on other components such as crude protein, crude fiber, crude fat, ash. Nitrogen free extract is a soluble carbohydrate including monosaccharides, disaccharides and polysaccharides which are easily soluble in acid and alkaline solutions and have high digestibility (Chiang and Lai, 2018).

The results of the analysis of variance showed that the treatment of different types of feed affected the metabolic energy content (P<0.05). The T3 treatment had the highest energy content with 2617.45 Kcal/kg. This can be caused because fruit and vegetable waste has a fairly high energy content and there is starch that is not digested by maggot so that it is excreted with feces and mixed into frass. Barzegar et al. (2019) stated that high metabolic energy is due to the high starch content in feed ingredients. The value of the metabolic energy content is related to the nutritional content of the material (Banavar et al. 2022).

Maggot's frass pH values of the three treatments ranged from 6.75 to 6.98. Song et al. (2021) stated that frass has a pH value of around 6-7. Frass pH will affect the content of microorganisms and the activity of microorganisms contained in frass. According to Jiang et al. (2019) the pH of a material also affects the activity of microorganisms.

In the observation of heavy metals, no lead (Pb) contamination was found in all frass samples. Heavy metal contamination cannot be degraded naturally and can accumulate in the bodies of animals and humans, affecting the work of enzymes, causing allergies and being mutagenic, teratogenic or carcinogenic (Geng et al., 2022). The limit for heavy metal contamination in feed is a maximum of 10 ppm. According to Elechi et al. (2021) the threshold for heavy metals content in feed is 10 mg/kg.

There was worm contamination in the T2 treatment with a total of 16.00 eggs per gram and T3 32.00 eggs per gram (EPG). The content of worm eggs in maggot's frass is still relatively safe. Utama et al. (2021) stated that the worm egg content in the mild class ranges from 0-500 EPG. The types of worm eggs detected were Nematoda (*Trichostrongylus Sp*), Nematode *Trichostrongylus Sp* and Nematoda *Ascaridia Sp.* The content of these worm eggs is thought to come from the feed given, because most of the feed comes from vegetable waste which comes from markets (Van der Spiegel, 2013).

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Frass microbiology profile

The results of the analysis of total bacteria, total lactic acid bacteria (LAB), total fungi, *Escherecia coli* contamination, and *Salmonella sp* can be seen in Table 3. The type of feed treatment did show a significant difference (P<0.05) to the total bacteria. The highest total content of maggot frass bacteria at T2 ranged from 4.95 x 10^6 Cfu/g. The T2 treatment may contain substrates that affect the survival and activity of a microorganism. Paritosh et al. (2017) stated that the pH value, temperature and substrate content in a medium will affect the activity of microorganisms. Total bacteria include gram positive and negative bacteria that live in a material. The total content of good bacteria in feed poultry is around 3 x 10^6 CFU/g (Cegielska-Radziejewska et al., 2013).

The total LAB value in maggot's frass is around 1.30 x 10^6 - 2.52 x 10^6 Cfu/g. The insignificant LAB content was due to relatively the same pH value. Astuti et al. (2022) stated that the increase and decrease in total LAB was influenced by several factors such as nutrient availability, temperature and pH value. LAB can survive in frass made possible because of the availability of protein and carbohydrates as a source of energy for LAB to develop. Hadj Saadoun et al. (2020) stated that LAB requires easily soluble carbohydrates to be a source of energy and protein to grow. The LAB population for poultry digestion that does not have a negative effect is around 10^8 CFU (Prado-Rebolledo et al., 2016).

The results of the analysis showed that different feed treatments did not show significant differences in the total number of fungi (P>0.05). The total content of fungi in maggot's frass ranged from 0.20 - 0.70 x 10^6 Cfu/g. The appearance of mushrooms is due to the high moisture in maggot's frass. Astuti and Komalasari (2020) stated that fungi will grow more easily in environments with high moisture content or humid conditions. Fungi can appear and develop on materials that have a moisture of more than 13% and a pH of 3 - 8 (Dewi et al., 2014).

Frass samples were not found to be contaminated with E.coli bacteria. This is possible because of the antibacterial content contained in the maggot body. Kar et al. (2021) stated that the advantage of black soldier fly is that it has anti-microbial content. The anti-bacterials contained in maggot include methanol, lauric acid and saturated fatty acids. Ardiansyah et al. (2021) stated that BSF larvae have natural antibacterial and antiviral properties that can prevent the emergence of pathogenic bacteria. The content of pathogenic bacteria in maggot's frass will be harmful when given as feed (Alonso-Hernando et al., 2013).

Table 3 - Frass microbiological profile

Parameter \ Treatment (10^6 Cfu/g)	T1	T2	T3	P-Values
Total Bacteria	2.08±2.27^b	4.95±3.80^a	1.91±2.71^b	*
Total LAB	1.30±1.20	2.00±1.41	2.52±3.54	NS
Total Fungi	0.60±0.65	0.70±1.09	0.20±0.27	NS
E.Coli	0	0	0	NS
Salmonela	0	0	0	NS

Different superscripts in the same line show a significant difference (P<0.05), NS: non-significant (P>0.05); * : P<0,05.

Table 4 - Bacterial culture results from maggot's frass

Type bacteria	Bacterial morphology	Total	(%)
Gram positive	Solitary	18	25.35
	Stem	13	18.31
	Duplo	11	15.49
	Coccus	11	15.49
	Yeast	7	9.86
	Oval	7	9.86
	Row	2	2.82
	Spores	2	2.82
Total		71	100
Gram negative	Solitary	13	50
	Stem	12	46.15
	Cocobasil	1	3.85
Total		26	100

Frass samples were not found to be contaminated with *Salmonella sp. Salmonella* bacteria are included in the category of pathogenic bacteria. Tariq et al. (2022) stated that *Salmonella* is a gram negative that is harmful to livestock. The impact of *Salmonella* bacterial infection in livestock can cause *Salmonellosis*. According to Ferarri et al. (2019) *salmonella* contamination can cause *salmonellosis* with symptoms of diarrhea, in poultry the contamination can reach the eggs. One way to reduce the content of *Salmonella* in a material is by heating it to a temperature of around 90^0C (Santos Dalolio et al., 2017).

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Table 4 shows that the growth of bacteria, 97 bacteria where 9 types of bacterial morphology consist of 8 types of gram positive and 3 types of gram negative. 9 types of gram-positive bacteria morphology, namely solitary (25.35%), stem (18.31%), duplo (15.49%), coccus (15.49%), yeast (9.86%), oval (9 .86%), lined (2.82%), spore (2.82%) and 3 types of gram-negative morphology, namely solitary (50%), stem (46.15%) and cocobasil (3.85%).

Gram positive bacteria have a cell wall structure with a thick peptidoglycan content. The total weight of the cell wall of gram-positive bacteria is a multiplayer peptidoglycan network bound by the inner membrane (Gangwal et al., 2022). Gram positive bacteria are dominated by bacteria with rod and solitary morphology. Examples of bacteria with solitary morphology are staphylococcus and bacteria with rod morphology are lactic acid bacteria. The presence of gram-positive bacteria such as lactic acid bacteria can minimize the risk of digestive disorders caused by negative bacteria such as *Escherichia coli* (Utama, et al. 2021).

Gram-negative bacteria with rod and solitary morphology are namely *Shigella sp.* and *Ralstonia solanacearum* (Komariah et al., 2013). The content of *Shigella* sp in feed ingredients will be dangerous because they are pathogenic. *Shigella sp.* produces exotoxins that interfere with the digestive tract. The content of the *Ralstonia solanacearum* bacteria is possible because it was carried along with vegetable waste because its presence is often found in tomato plants. The presence of lactic acid bacteria can minimize the presence of gram-negative bacteria such as *Shigella* sp. Fanin et al. (2018) stated that the number of gram-negative bacteria will decrease as the number of gram-positive bacteria increases.

The results of the microbiological content of maggot's frass with different types of feed media showed no difference. The microbiological content of maggot's frass in the study showed safe results, seen from the content of total bacteria, total LAB and total fungi and there was no *E.coli* and *Salmonella* content.

CONCLUSION

Treatment of different types of feed did not affect the organoleptic quality and microbiological profile of maggot's frass but affected the chemical quality maggot's frass. Therefore, maggot feed using fruit and vegetable waste treatment is recommended because of its high crude protein (16.37%) and metabolic energy (2617.45 Kcal/kg) and also without any *E.coli* and *Salmonella* contamination.

DECLARATIONS

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Author's contribution

C.S. Utama and B. Sulistiyanto provided recommendations and suggestions on research topics, article preparation and finalization of scientific articles; B. Marifah and R.I. Cahya conducted research, data processing and article preparation.

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Conflict of interests

The authors declare that they have no competing interest.

REFERENCES

Alonso-Hernando A, Alonso-Calleja C and Capita R (2013). Growth kinetic parameters of Gram-positive and Gram-negative bacteria on poultry treated with various chemical decontaminants. Food Control, 33(2): 429–432. DOI https://doi.org/10.1016/j.foodcont.2013.03.009

AOAC (2005). Official Methods of Analysis, 18th edn. Association of Official Analytical Chemists, Arlington, Vrginia, USA.

Arabzadeh G, Delisle-Houde M, Tweddell RJ, Deschamps M-H, Dorais M, Lebeuf Y, Derome N and Vandenberg G (2022). Diet Composition Influences Growth Performance, Bioconversion of Black Soldier Fly Larvae: Agronomic Value and In Vitro Biofungicidal Activity of Derived Frass. Agronomy. 12 (8): 1765. DOI: https://doi.org/10.3390/agronomy12081765

Ardiansyah F, Susanto and Wahyuni (2021). Use of water hyacinth and fermented fruit waste as BSF (black soldier fly) media on the quality of BSF maggot flour. Journal of Animal Production and Processing Technology. 9 (1): 1-4. DOI: https://doi.org/10.29244/jipthp.9.1.1-6

Astuti DA and Komalasari K (2020). Feed and Animak Nutrition: insect as animal feed. International Conference: Increasing Tropical Animal Production for Food Security. In IOP Conference Series: Earth and Environmental Science (Vol. 465: 1 – 10. DOI: https://doi.org/10.1088/1755-1315/465/1/012002

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Astuti DA and Wiryawan KG (2022). Black soldier fly as feed ingredient for ruminants. Animal Bioscience, 35 (2):356 – 363. DOI : https://doi.org/10.5713/ab.21.0460

Banavar A, Amirkolaei SK, Duscher L, Khairunisa BH, Mukhopadhyay B, Schwarz M and Urick S (2022). Nutritional evaluation of black soldier fly frass as an ingredient in florida pompano (*Trachinotus carolinus L.*) diets. Animals, 12 (18): 2407. DOI : https://doi.org/10.3390/ani12182407

Barroso FG, de Haro C, Sánchez-Muros MJ, Venegas E, Martínez-Sánchez A and Pérez-Bañón C (2014). The potential of various insect species for use as food for fish. Aquaculture, 422-423:193–201. DOI: https://doi.org/10.1016/j.aquaculture.2013.12.024

Basri NEA, Azman NA, Ahmad IK, Suja F, Jalil NAA and Amrul NF (2022). Potential Applications of Frass Derived from Black Soldier Fly Larvae Treatment of Food Waste: A Review. Foods, 11(17):2664. DOI: https://doi.org/10.3390/foods11172664

Beale DJ, Shah RM, Marcora A, Hulthen A, Karpe AV, Pham K and Paull C (2022). Is there any biological insight (or respite) for insects exposed to plastics? Measuring the impact on an insect's central carbon metabolism when exposed to a plastic feed substrate. Science of the Total Environment, 831: 154840. DOI: https://doi.org/10.1016/j.scitotenv.2022.154840

Barzegar S, Wu, SB, Noblet J, Choct M and Swick RA (2019). Energy efficiency and net energy prediction of feed in laying hens. Poultry Science, 98(11):5746-5758. DOI : https://doi.org/10.3382/ps/pez362

Boumans IJ, Schop M, Bracke MB, de Boer IJ, Gerrits WJ and Bokkers EA (2022). Feeding food losses and waste to pigs and poultry: Implications for feed quality and production. Journal of Cleaner Production, 378: 134623. DOI: https://doi.org/10.1016/j.jclepro.2022.134623

Cegielska-Radziejewska R, Stuper K and Szablewski T (2013). Microflora and mycotoxin contamination in poultry feed mixtures from western Poland. Annals of agricultural and environmental medicine, 20 (1): 30-35. https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-4c0fa4a4-8974-4e49-b58e-1a2011281a80/c/fulltext516.pdf

Chiam Z, Lee JTE, Tan JKN, Song S, Arora S, Tong YW and Tan HTW (2021). Evaluating the potential of Okara-derived black soldier fly larval frass as a soil amendment. Journal of Environmental Management, 286 (112163): 1-10. DOI: https://doi.org/10.1016/j.jenvman.2021.112163

Chiang CF and Lai LS (2018). Effect of enzyme-assisted extraction on the physicochemical properties of mucilage from the fronds of Asplenium australasicum (J. Sm.) Hook. International Journal of Biological Macromolecules. DOI: https://doi.org/10.1016/j.ijbiomac.2018.11.181

Elechi MC, Kemabonta KA and Ogbogu SS (2021). Heavy metal bioaccumulation in prepupae of black soldier fly Hermetia Illucens (Diptera: Stratiomyidae) cultured with organic wastes and chicken feed. International Journal Tropical Insect Science, 41: 2125–2131. DOI: https://doi.org/10.1007/s42690-021-00427-5

Fanin N, Kardol P, Farrell M, Nilsson MC, Gundale MJ and Wardle DA (2018). The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biology and Biochemistry. DOI : https://doi.org/10.1016/j.soilbio.2018.10.010

Foka ISP, Yamssi C, Enyetornye B, Anangmo CN, Mbida M and Mayaka TB (2021). Reduction of Ascaridia galli Pathology by Salmonella typhimurium in Broiler Chicken. Journal of Parasitology Research, 2021: Article ID 5386575. DOI: https://doi.org/10.1155/2021/5386575

Gangwal A, Sangwan N, Dhasmana N, Kumar N, Keshavam CC and Singh LK (2022). Role of serine/threonine protein phosphatase PrpN in the life cycle of Bacillus anthracis. PLoS Pathogens, 18(8): e1010729. https://doi.org/10.1371/journal.ppat.1010729

Garttling D and Schulz H (2022). Compilation of black soldier fly frass analyses. Journal of Soil Science and Plant Nutrition, 22: 937–943. DOI: https://10.1007/s42729-021-00703-w

Geng W, Zhao Y and Mao Z (2022). The Effects of combined use of black soldier fly larvae frass fertilizer with exogenous selenium on rice growth and accumulation of heavy metals. Journal of Soil Science and Plant Nutrition, 22: 5133–5143. DOI: https://10.1007/s42729-022-00989-4

Gonzalez A, Nobre C, Simões LS, Cruz M, Loredo A and Rodríguez-Jasso RM (2021). Evaluation of functional and nutritional potential of a protein concentrate from Pleurotus ostreatus mushroom. Food Chemistry, 346:128884. DOI: https://doi.org/10.1016/j.foodchem.2020.128884

Hadj Saadoun J, Luparelli AV, Caligiani A, Macavei LI, Maistrello L and Neviani E (2020). Antimicrobial biomasses from lactic acid fermentation of black soldier fly Prepupae and related by-products. Microorganisms. 8(11): 1785. DOI: https://doi.org/10.3390/microorganisms8111785

Henault-Ethier L, Martin JP and Housset J (2017). A dynamic model for organic waste management in Quebec (D-MOWIQ) as a tool to review environmental, societal and economic perspectives of a waste management policy. Waste Management. 66 : 196–209. DOI: https://doi.org/10.1016/j.wasman.2017.04.021

Hwangbo J, Hong EC, Jang A, Kang HK, Oh JS, Kim BW and Park BS (2009). Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. Journal Environment Biology. 30 (4): 609–614. http://www.jeb.co.in/journal_issues/200907_jul09/Paper_24.pdf

Jayanegara A, Novandri B, Yantina N and Ridla M (2017). Use of black soldier fly larvae (*Hermetia illucens*) to substitute soybean meal in ruminant diet: An in vitro rumen fermentation study. Veterinary World. 10(12):1439-1446. DOI : https://doi.org/10.14202/vetworld.2017.1439-1446

Jiang C, Jin W, Tao X, Zhang Q, Zhu J, Feng S and Zhang Z (2019) Black soldier fly larvae (*Hermetia illucens*) strengthen the metabolic function of food waste biodegradation by gut microbiome. Microbial Biotechnology. 12 (3); 528 – 543. DOI : https://doi.org/10.1111/1751-7915.13393

Kar SK, Schokker D and Harms AC (2021). Local intestinal microbiota response and systemic effects of feeding black soldier fly larvae to replace soybean meal in growing pigs. Scientific Reports, 11: 15088. DOI: https://10.1038/s41598-021-94604-8

Klüber P, Tegtmeier D, Hurka S, Pfeiffer J, Vilcinskas A, Rühl M and Zorn H (2022). Diet fermentation leads to microbial adaptation in black soldier fly (Hermetia illucens) larvae reared on palm oil side streams. Sustainability, 14(9), 5626. DOI : https://doi.org/10.3390/su14095626

Lopes IG, Yong JWH and Lalander C (2022) Frass derived from black soldier fly larvae treatment of biodegradable wastes. Waste Management. 142: 65-76. DOI: https://doi.org/10.1016/j.wasman.2022.02.007.

Luthada-Raswiswi RW, O'Brien G and Mukaratirwa S (2022). Fishmeal replacement with animal protein source (*Crocodylus niloticus* meat meal) in diets of mozambique tilapia (*Oreochromis mossambicus*) of Different size groups. Applied Sciences, 12: 7211. DOI : https://doi.org/10.3390/app12147211

Citation: Utama CS, Sulistiyanto B, Marifah B, and Cahya RI (2023). The organoleptic, chemical and microbiological quality of maggot's frass as alternative poultry feed ingredients. *Online J. Anim. Feed Res*., 13(5): 340-347. DOI: https://dx.doi.org/10.51227/ojafr.2023.49

Makkar HP, Tran G, Heuzé V and Ankers P (2014). State-of-the-art on use of insects as animal feed. Animal feed science and technology, 197: 1-33. DOI : https://doi.org/10.1016/j.anifeedsci.2014.07.008

Oloo JO and Awuor FO (2019). Suitability of Kibuye market organic waste for composting as a means of solid waste management for Kisumu city. International Journal Waste Resources. 9(2): 1-3. DOI: https://doi.org/10.35248/2252-5211.19.9.370

Paritosh K, Kushwaha SK, Yadav M, Pareek N, Chawade A and Vivekanand V (2017). Food Waste to Energy: An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling. BioMed Research International, 2017: 1–19. DOI: https://doi.org/10.1155/2017/2370927

Pazoki A, Ghorbani GR, Kargar S, Sadeghi-Sefidmazgi A, Drackley JK and Ghaffari MH (2017). Growth performance, nutrient digestibility, ruminal fermentation, and rumen development of calves during transition from liquid to solid feed: Effects of physical form of starter feed and forage provision. Animal Feed Science and Technology, 234, 173–185. DOI: https://doi.org/10.1016/j.anifeedsci.2017.06.004

Pham TPT, Kaushik R, Parshetti GK, Mahmood R and Balasubramanian R (2015). Food waste-to-energy conversion technologies: Current status and future directions. Waste Management. 38: 399–408. DOI: https://doi.org/10.1016/j.wasman.2014.12.004

Prado-Rebolledo OF, Delgado-Machuca J de J, Macedo-Barragan RJ, Garcia-Márquez LJ, Morales-Barrera JE, Latorre JD and Tellez G (2016). Evaluation of a selected lactic acid bacteria-based probiotic on Salmonella enterica serovar Enteritidis colonization and intestinal permeability in broiler chickens. Avian Pathology, 46(1): 90–94. DOI: https://doi.org/10.1080/03079457.2016.1222808

Santos Dalólio F, da Silva JN, Carneiro de Oliveira AC, Ferreira Tinôco Ide F, Christiam Barbosa R, Resende Mde O and Teixeira Coelho S (2017). Poultry litter as biomass energy: A review and future perspectives. Renewable and Sustainable Energy Reviews, 76: 941–949. DOI: https://doi.org/10.1016/j.rser.2017.03.104

Siddiqui SA, Ristow B, Rahayu T, Putra NS, Yuwono NW and Mategeko B (2022). Black soldier fly larvae (BSFL) and their affinity for organic waste processing. Waste Management, 140:1-3. DOI: https://doi.org/10.1016/j.wasman.2021.12.044

Sharif M, Zafar MH, Aqib AI, Saeed M, Farag MR and Alagawany M (2021). Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition. Aquaculture, 531: 735885. DOI: https://doi.org/10.1016/j.aquaculture.2020.735885

Song S, Liang AW, Tan JKN, Cheong JC, Chiam Z, Arora S, Lam WN dan Wan THT (2021). Upcycling food waste using black soldier fly larvae: Effects of Futher Composting on Frass quality, fertilising effect and its global warming potensial. Journal of Cleaner Production, 288: 125664. DOI: https://doi.org/10.1016/j.jclepro.2020.125664

Tariq S, Samad A, Hamza M, Ahmer A, Muazzam A, Ahmad S and Amhabj AMA (2022). Salmonella in Poultry; An Overview. International Journal of Multidisciplinary Sciences and Arts, 1(1): 80-84. DOI: https://doi.org/10.47709/ijmdsa.v1i1.1706

Tschirner M and Simon A (2015). Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. Journal of Insects as Food and Feed, 1 (4): 249–259. DOI:https://doi.org/10.3920/jiff2014.0008

Utama CS and Christiyanto M (2021). The Feasibility of fermented litter as a feed ingredient for ruminant livestock. Journal of Advanced Veterinary and Animal Research, 8 (2): 312–322. DOI: https://doi.org/10.5455/javar.2021.h517

Utama CS, Sulistiyanto B and Sumarsih S (2021). Chemical and microbiological quality of phytobiotics from blends plant with different fermentation ripening. Online Journal of Animal and Feed Research, 11 (5): 171 – 179. DOI: https://dx.doi.org/10.51227/ojafr.2021.26

Van der Spiegel M, Noordam MY and Van der Fels-Klerx HJ (2013). Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Comprehensive Reviews in Food Science and Food Safety, 12(6): 662-678. DOI: https://doi.org/10.1111/1541-4337.12032

Van Looveren N, Vandeweyer D and Van Campenhout L (2022). Impact of Heat Treatment on the Microbiological Quality of Frass Originating from Black Soldier Fly Larvae (*Hermetia illucens*). Insects, 13(1): 1-15. DOI: https://doi.org/10.3390/insects13010022

Yildirim-Aksoy M, Eljack R and Beck BH (2020). Nutritional value of frass from black soldier fly larvae, Hermetia illucens, in a channel catfish, Ictalurus punctatus, diet. Aquaculture nutrition, 26(3): 812-819. DOI: https://doi.org/10.1111/anu.13040

Zheng L, Li Q, Zhang J and Yu Z (2012). Double the biodiesel yield: Rearing black soldier fly larvae, Hermetia illucens, on solid residual fraction of restaurant waste after grease extraction for biodiesel production. Elsevier. Renewable Energy, 41: 75 -79. DOI: https://doi.org/10.1016/j.renene.2011.10.004

Citation: Utama CS, Sulistiyanto B, Marifah B, and Cahya RI (2023). The organoleptic, chemical and microbiological quality of maggot's frass as alternative poultry feed ingredients. *Online J. Anim. Feed Res*., 13(5): 340-347. DOI: https://dx.doi.org/10.51227/ojafr.2023.49

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DETECTION AND CHARACTERISATION OF MICROPLASTICS IN ANIMAL FEED

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ABSTRACT: Microplastics (MPs) the products of plastic breakdown, are entering the environment as a result of plastic abuse, which are of size less than 5mm. Due to their ubiquitous nature, MPs have become a significant environmental concern. One alarming area of MPs contamination is their potential presence in the feed of edible animal species. Growing research suggests that MPs can enter food products and subsequently move to various trophic levels of food chains. Hence, assessing the threat of MPs contamination in animal feed is important for food security and human health. In this investigation, 36 livestock and poultry feed samples were collected from 12 different farms, MPs were detected using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimeter (DSC). The Nano particle analyser was used to determine the size distribution, and Pyrolysis-GC/MS was used to quantify MPs. According to the findings, all the feed samples contained a significant amount of Polyethylene terephthalate (PET), Polypropylene (PP), and Polyvinyl chloride (PVC) and the particle size ranged from 2.02 to 10.7 µm. Present study has given detailed information on the size distribution of MPs in animal feed, which is thought to enable them to pass through membrane barriers. From the findings it is evident that there are high chances of MPs entering animal feed due to the continuous contact of the feed with plastic-based materials. These MPs can accumulate in the tissues of animals and potentially be transferred to humans through the consumption of meat, milk, and other animal-derived products. Subsequently these MPs can finally bio-accumulate in humans and cause serious health issues.

Keywords: Feedstuff, Membrane barriers, Nanoparticles, Pyrolysis-GC/MS, Size distribution.

INTRODUCTION

The industrial revolution has escalated the economic growth of the country while depleting the social and health standards of the poverty line. The ever-racing production of daily products also creates equal and sometimes more pollutants that are discharged relentlessly into the ecosystem (Pan et al., 2022). The lagging pace between the pollutant generation rate and their degradation rate has necessitated the introduction of new and logistic methods to deal with it. The most important and serious problem in the present day is microplastics (MPs; Fadare et al., 2020). The use of various kinds of plastics as raw materials, packaging material and ingredients in many industrial sectors due to their cheaper price has made it inevitable to be avoided. The flushed MPs without any primary treatment in the environment can cause many natural imbalances thus disrupting the ecosystem (Reeves et al., 2022). Many new scientific researchers are trying to find alternatives and techniques that help in minimising and eliminating microplastic entry into our daily lives.

The pervasive nature of MPs has made it a hit spot for many scientists. MPs can be classified into two major groups depending on their origin (1) Primary MPs (2) secondary MPs (Du et al., 2020). Primary microplastics are commercially synthesised as such in small size for many industrial applications such as cosmetics, textile industries, for making fishing nets or any other filtering materials, while the secondary MPs are the result of fragmentation, decomposition or recycling of macro plastic materials. The surface characteristics of MPs encourage both heavy metal adsorption and desorption. The heavy metals trapped on to these microplastics surfaces cause additional stubbornness to them by creating complexes that are hard to decompose (Hale et al., 2020).

The contamination of water and soil with these MPs had led to a channel for them to enter the food chain (Picó and Barceló, 2019). These MPs, being in nano-micro scale can easily enter the plant and animal biological system through osmosis. The MP content gets stagnated in the food storage bodies of plants such as fruits, roots and leaves. When an animal or a human consumes these crops the amount of microplastic content multiplies depending on the quantity of intake. There they enter the bio-chain naturally (Lehel and Murphy, 2021). Moreover, the presence of MPs in animal feed is becoming prevalent these days. Fish meals are used to feed farm livestock like pigs, poultry, and fish because of their high-calorie count, good amino acid profile, and affordability. Fish contaminated with MPs can act as a source of MPs (Thiele, 2021). In addition, pig feed samples obtained from China, have shown the presence of polycarbonate (PC) and

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polyethylene (PET) (Xu, 2022). By the consumption of water contaminated with MPs, they directly enter the blood stream of any organism leading to its accumulation in the body and later creating macro lumps that obstruct the natural flow of fluids in the bio-system (Norberg-Hodge, 2006). MPs were identified in the excreta of farm animals like sheep which is a clear evidence of MP contamination in animals (Beriot, 2021). As there are very few methods and techniques available to recognise the presence of MPs a lot more research is needed in this area.

The major victims of this microplastic pollution are the aquatic plants and animals. Especially, fish that consume MPs through water have major respiratory and reproductive issues (Wang et al., 2020). The fish have special respiratory routes that involves transportation of water through the canals of their gills allowing exchange of gases. The accumulation of these MPs in these narrow canalised system makes it hard for the water flow (Koongolla et al., 2020). Apart from the obstruction of the respiratory gills, the consumption of these MPs effects the reproductive capacity of many microorganisms and fishes in the aquatic system that effects the overall balance in the ecosystem (Jin et al., 2022).

MATERIALS AND METHODS

Sample collection

All the animal feeds (cow, pig, chicken, and fish) samples were collected from different livestock farms located in and nearby locations of Visakhapatnam. A total of 36 feed samples (100g) were collected from 12 different livestock farms (cow feed A,B,C, pig feed D,E,F, chicken feed G,H,I, fish feed J,K,L). All the samples were carefully weighed and gathered in sterile glass bottles which were pre-cleaned and stored in a dry place until further analysis. The details of farms and the composition of feed was given in Table 1.

Table 1 - Composition of various animal feeds used in the study.

Animal	Farm	Composition
COW	A	Corn, straw, whole cotton seed, vitamins and minerals mix
	B	Basal grain, chopped hay, soybean hulls, vitamins
	C	Corn silage, crushed maize, wheat bran, mineral mixture
PIG	D	Soybean meal, meat and bone meal, wheat, and barley.
	E	Wheat, maize, wheat bran, peas
	F	Palm kernel meal, rice bran, maize cassava
CHICKEN	G	Soya, maize, bone meal fish meal, growth premixes
	H	Corn gluten meal, soybean oil, limestone mineral mixes
	I	Soy protein concentrate, crude fibre, mineral, and vitamin premix
FISH	J	Palm oil, groundnut cake and maize powder, bone meal, premix
	K	Mustard oil cake, poultry by-products, bone meal, vitamin mixes
	L	Oyster shell meal, soybean grits, mustard oil cake, mineral, and vitamin mixes

Processing feed samples

At the time of collection, the feed samples were in various forms (pellets, powder). The pellet form samples were finely ground and homogenized using a mortar and pestle. All the ground feed samples were oven dried to remove any moisture that might have been present. Stainless steel sieves (mesh size 5mm) were used for primary filtration to remove large-sized coarse particles which might obstruct the vacuum filtration process. In secondary filtration, the samples were passed through a glass fibre filter with a 20 mm diameter and 2µm mesh size. The MPs were separated from the samples using Accelerated solvent extraction (van der Veen et al., 2022) with tetrahydrofuran and methanol. Prior to transferring the filter papers to a pyrolysis cup with residue they were placed in clean petri dishes, and dried at 45^{o}C in an oven for 4hrs.

Characterisation of MPs

The chemical composition of the particles was determined using FT-IR (Fourier transform infrared) in the region of 4000 to 400 cm^{-1} by Bruker and the thermal characteristics (melting points) of the polymers were assessed using DSC (Differential scanning colorimeter) STA7300 Thermal Analysis System. A technique that included heating from 30 to 300°C, cooling to 30°C, and then heating again to 300°C with nitrogen flow rate of 100 mL/min^{-1} was used for the analysis.

Surface Morphology and size analysis

The topography of the MPs was determined using Field Emission Scanning Electron Microscopy (FESEM, Carlzeiss Ultra 55). The average size of the extracted particles was obtained using Nano Particle Size Analyser (NPA), HORIBA SZ-100.

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Quantification using Pyrolysis - GC/MS

According to a previously described and approved procedure (Leslie et al. 2022 and van der Veen et al. 2022), the microplastic content of each sample was examined. The multi shot pyrolysis unit EGA/PY-3030D was used for the analysis in "double shot" mode. The sample was initially put into the pyrolyzer unit at 100°C and heated to 300°C at a rate of 50°C/min. The GC/MS measurement for any volatile substances on the filter began after the sample was withdrawn since they thermally desorb between 100° and 300°C. A 30-meter long, 0.25 millimetre thick, 0.25-micrometer Ultra Alloy-5 column was installed in the GC/MS (Agilent 6890 GC and 5975C MS). Measurements were carried out in split mode (1:50 split ratio) and full scan mode (m/z 33–500). On the GC column, the chemicals were recovered. The column was designed to operate for a total of 20 minutes, rising from 40°C (2 min) at a rate of 20°C/min to 360°C, then holding at 360°C for 2 min. The pyrolyzer was heated to 600°C following the thermal desorption stage, and the filter was reintroduced (1min) for the following measurement (pyrolysis). The column was configured to run for 20 min, going from 40°C (2 min) at a rate of 40°C/min to 360 °C (2 min). The components that are volatilized at 300°C are the substances that are desorbed in the first run (or "shot") and may comprise unpolymerized monomers, additives, and other sorbed chemicals. With the exception of PET, where the derivatization product already forms at 300°C and the results from both the first and second shots were combined, any monomers (such as benzene or styrene) that may have been present during this run were not taken into consideration when calculating the concentrations of plastic particles. The other polymer concentrations linked to the polymers were determined using the pyrolysis second "shot" chromatograms.

RESULTS AND DISCUSSION

In this investigation, a total of 36 animal feed samples, including nine samples each of cow, pig, chicken, and fish feed, were gathered. All the tested feed samples were found to contain MPs, which was confirmed using FTIR and DSC, but the concentration and type of polymer identified varied among different feed samples and farms which can be seen in fig.4. While some of the feed were found to contain plastic particles which are visible to naked eyes, these macroplastics were separated during primary filtration process. Ten procedural blank samples were collected, analysed, and compared to the feed samples in the same series and all the experiments were performed in triplicates.

Characterisation of MPs

Identification of the MPs is essential, hence FTIR seems to be a preferable method for examining their properties with respect to functional groups. From the FTIR spectra (figure 2), functional groups specific to each polymer were observed. Polymers with peaks 1860, 1766, 1240 and 720 were confirmed as PET, samples which showed peaks 2898, 1415, 1258, 736 and 600 were identified as PVC, and polymers with peaks 2920, 1719, 1170, 1454, 970 were detected as PP. The outcomes of DSC (figure1) were in accordance with the results of FTIR. The endothermic peaks represented in the graph indicate the melting points of the respective polymers, while the ascending points indicate the exothermic reactions. A slight shift can be seen in the melting points of PP and PET with peak temperatures 160±3°C and 260±3°C, respectively, but the endothermic peaks acquired in this study were in congruence with the data available in the literature (Guaita et al., 1985). The thermolytic behaviours of plastics are reportedly affected by thermolysis conditions and the status of plastic particles such as additives, particle size, degree of polymerization, and crystallinity (Guaita et al., 1985; Choi et al., 2021). These findings confirm that PP and PET were the most widely utilised and discarded polymers, which is incompetence with other recent studies that demonstrated their pervasive prevalence in cattle and poultry farms globally (Geyer et al., 2017). Plastic bottles, polythene bags and disposable plastic items can be a possible source of PET in the feed samples because PET was found in significant amounts in all the samples (Peez et al., 2019).

Quantification, size analysis and surface morphology

The results of Py-GC/MS show that each sample had a different distribution of polymer types and concentrations. The highest concentration of PET was 693µg while PP and PVC were in the range of 597µg and 553µg respectively (table 2a-2b). In a single sample, up to three different types of polymers were discovered. When compared among all the farms (cow, pig, chicken and fish) the highest concentration of microplastics was extracted from the samples of chicken farms (G, H, I) then followed by the fish farms (J, K, L) which can be observed from Figure 4. Microplastic particles ranged in size from 2.02 to 10.7 µm, with a higher proportion of those in the 3-6 µm size range compared to other ranges. Based on the outcomes these particles can have the ability to enter the blood stream by crossing the intestinal membrane barrier (Luo et al., 2019). According to literature MPs can occur in different shapes like fibres, fragments, pellets etc., among which MP fibres were found to be more toxic and damaging compared to other shapes of MPs (Thornton Hampton, 2022). In the present study, to know the physical form and shape of extracted MPs, they were observed under the Scanning electron microscope. Few Scanning electron microscopic images of the extracted MPs from various farms (Figure 3) exhibited fibre-like MPs with surface fractures and disrupted structures. The disintegration of the fibres suggests that these plastics might have undergone mechanical weathering. The surface properties of a polymer play a key role in determining its adsorption abilities. The surface morphology of the extracted MPs may facilitate the adsorption of heavy metals, toxic chemicals and microorganisms from their surrounding environment onto their surface further increasing the risk of these contaminants.

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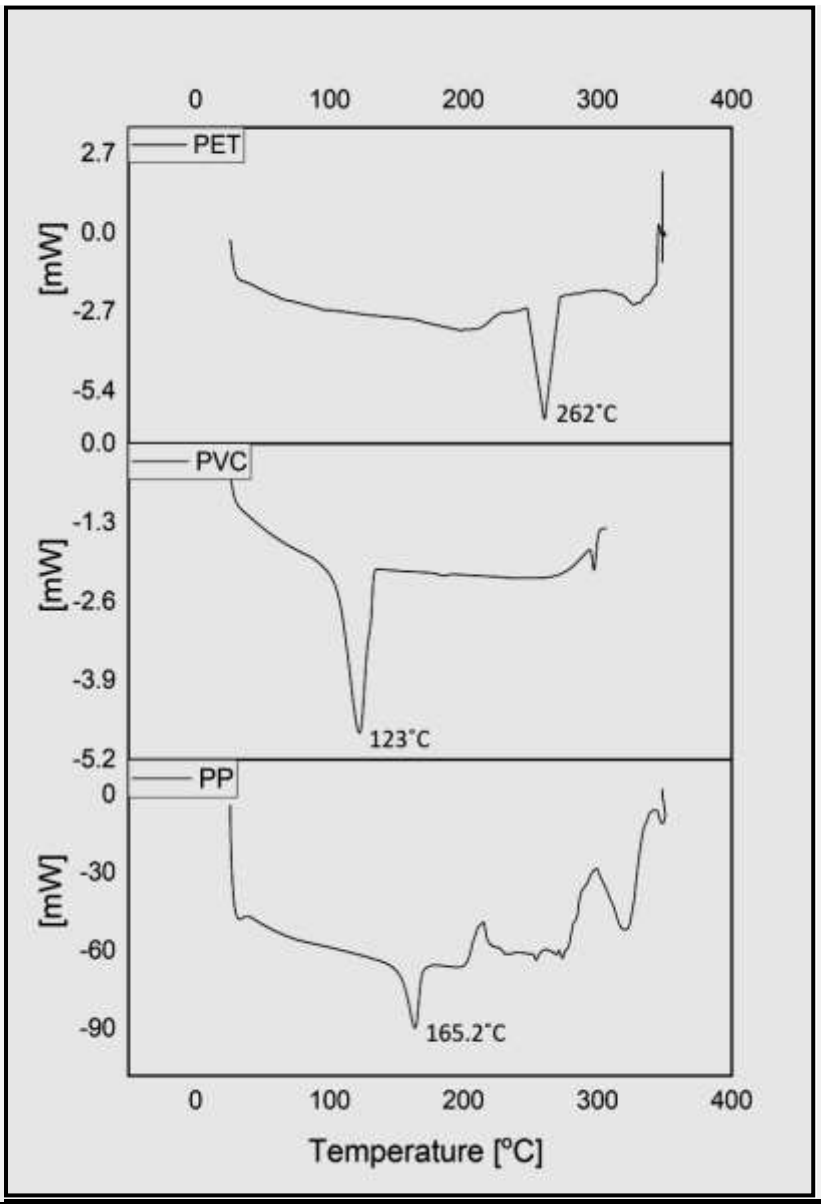

Figure 1 - DSC curves of polymers illustrating the melting points of MPs detected in the feed samples.

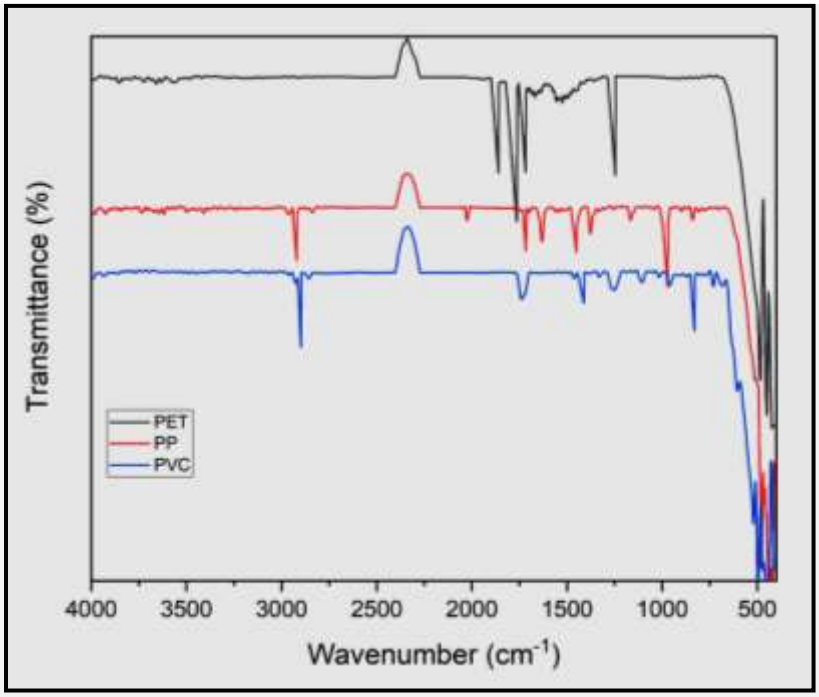

Figure 2 - FTIR Spectra of polymers identified in the feed samples

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Table 2a - Concentration (µg/g) and size of MPs in feed samples cow feed (A-C) and pig feed (D-F)

Sample No.	Feed type	Farm	PP	PET	PVC	Size (µm)
1	Powder	A1	163	173	–	2.1
2	Powder	A2	164.7	171.9	–	2.0
3	Powder	A3	165.9	172	–	2.02
4	Powder	B1	–	89	601.3	9.24
5	Powder	B2	–	88.6	600.6	9.0
6	Powder	B3	–	87.8	602	8.9
7	Powder	C1	245	325	482.6	11.0
8	Powder	C2	246.6	324.4	483	10.70
9	Powder	C3	247.2	325.8	483.8	10.1
10	Pellet	D1	98	237.3	95.9	7.0
11	Pellet	D2	97	236	96.7	7.1
12	Pellet	D3	99.6	236.7	97.1	6.93
13	Pellet	E1	322	156	238	6.0
14	Pellet	E2	320.9	157.5	238	6.44
15	Pellet	E3	321.5	156.7	238	5.44
16	Pellet	F1	–	586.8	–	8.93
17	Pellet	F2	–	587.6	–	8.02
18	Pellet	F3	–	588.1	–	7.8

PP- Polypropylene; PET- Polyethylene terephthalate; PVC- Polyvinyl Chloride

Table 2b - Concentration (µg/g) and size of MPs in feed samples chicken feed (G-I) and fish feed (J-L)

Sample No.	Feed type	Farm	PP	PET	PVC	Size (µm)
19	Powder	G1	84.9	693	366	10.61
20	Powder	G2	83.5	691	367.8	9.6
21	Powder	G3	84	693.5	367	10.0
22	Powder	H1	–	348	420	9.43
23	Powder	H2	–	348.5	421	9.0
24	Powder	H3	–	347	420.8	8.93
25	Powder	I1	351	161	340	7.65
26	Powder	I2	351.9	163	341	7.0
27	Powder	I3	350	162	341.6	8.0
28	Grain	J1	157.3	93	257	9.84
29	Grain	J2	158.3	93	2576.9	8.84
30	Grain	J3	158	92.1	258.2	9.0
31	Grain	K1	597	519	–	7.67
32	Grain	K2	596	519	–	7.09
33	Grain	K3	596.4	518	–	6.67
34	Grain	L1	–	319	158	8.02
35	Grain	L2	–	565	158.4	8.9
36	Grain	L3	–	288	157	9.0

PP- Polypropylene; PET- Polyethylene terephthalate; PVC- Polyvinyl Chloride

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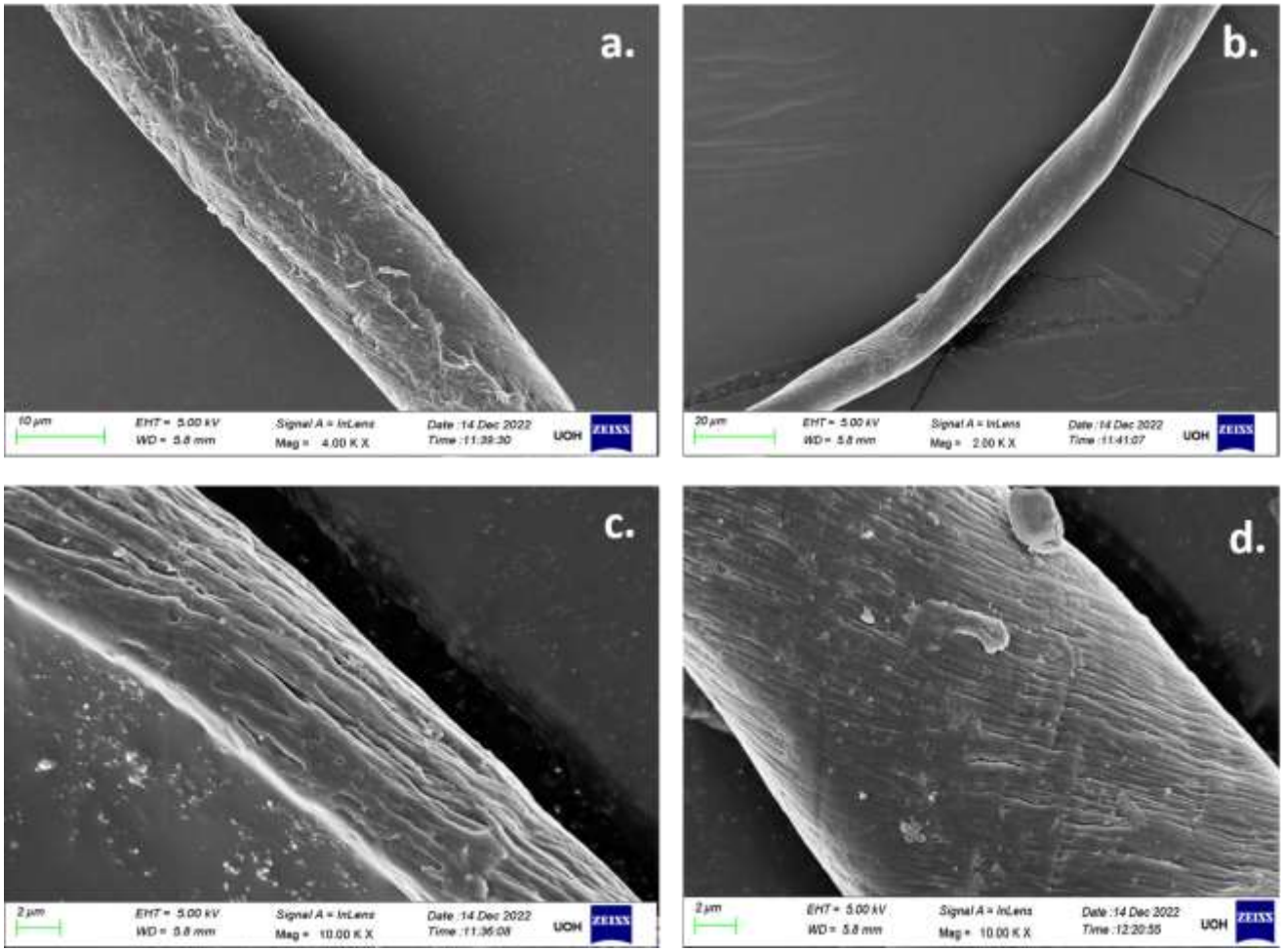

Figure 3 - SEM images of microplastics detected in the feed samples from various animal farms, a) Cow farms b) Pig farms c) Chicken farms and d) Fish farms.

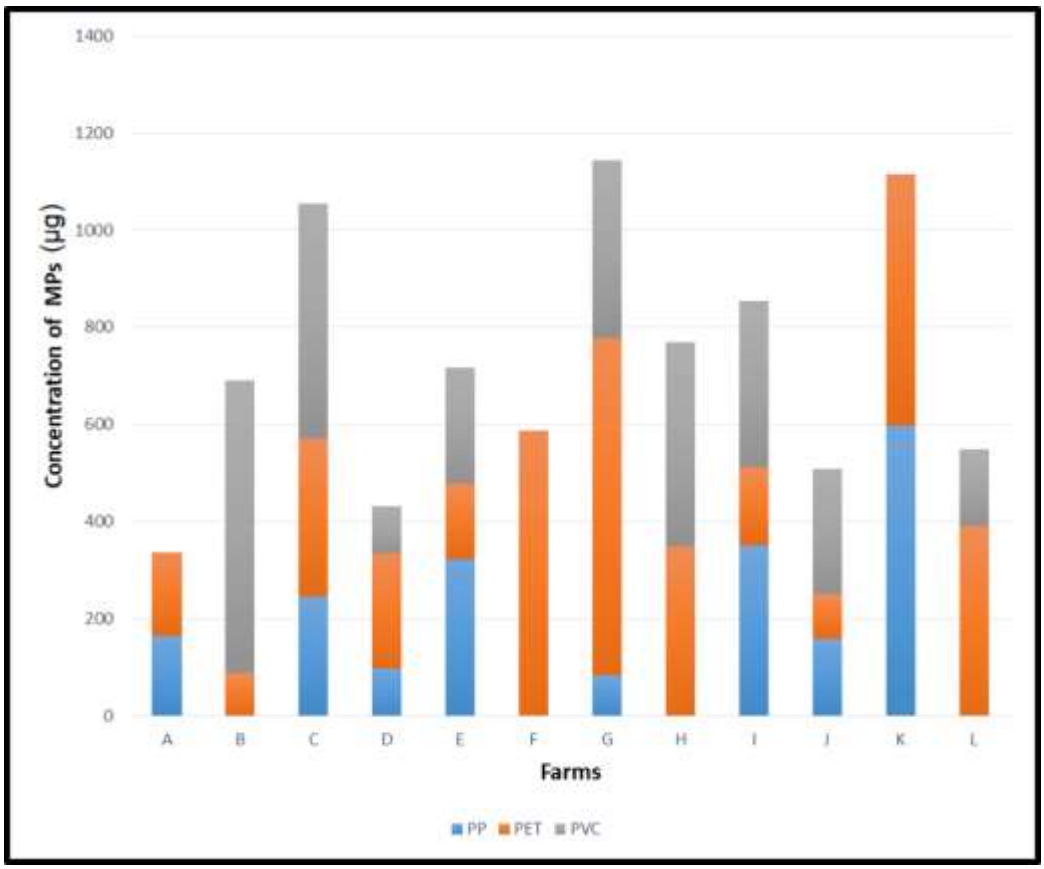

Figure 4 - Graphical representation of concentration of different types of polymers detected in all the farms (A-L)

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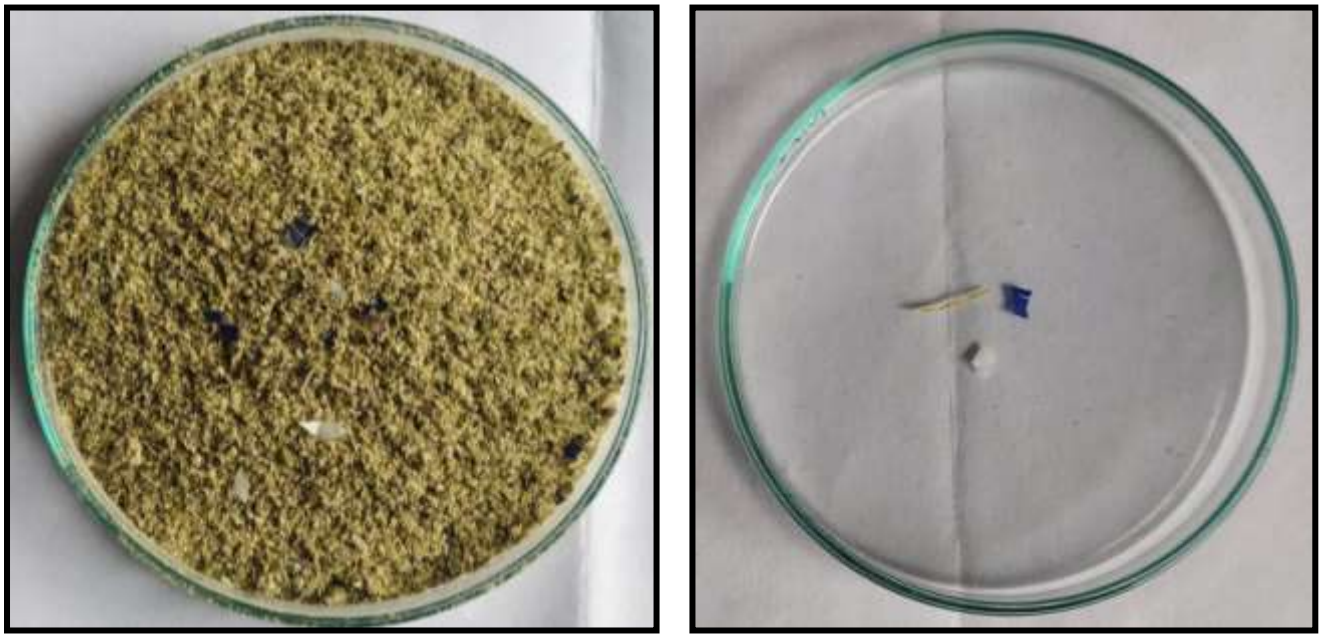

Figure 5 - Macroplastic particles were observed in the few samples, which were separated during the process of sieving

Sources of MPs into feed and their health effects

There can be numerous ways in which MPs enter the livestock feed. The main entry pathways for MPs into grazing-based and mixed livestock production systems have been identified as plastic mulching, fragmentation of plastic wastes, and stream water discharge (Ramachandraiah et al., 2022). MP pollution in farmyards was extensive, which may be related to a lack of trained cleaning personnel. Additionally, the inner surface of feedbags is made of PET, while their exterior is constructed of PP. Some animal farms use liquid feeding systems, which deliver the feed through PVC troughs or pipelines (De Lange et al., 2006). On the other hand, feed mixers, transporting vehicles, feed storage tanks, and other anthropogenic activities can contribute to the MP contamination of feed. A recent study suggested that MPs might also reside in the digestive system, and given the co-occurrence of PP, PE, and PR MPs in animal manure and feed, it is plausible that these MPs could be passed out by the cattle when they consume contaminated feed thereby polluting the soil (Stock et al., 2020). There have been several irreversible, long-term health impacts linked to MPs. The digestive gland's oxidative balance was altered in the mussel *Mytilus spp.* after exposure to polystyrene micro beads (2 and 6 m, 32 g/L, for seven days; decreased catalase and glutathione reductase activities, as well as lowered lipid peroxidation (Paul-Pont et al., 2016). Furthermore, numerous animal studies indicate that ingesting microplastics impairs crucial intestinal processes like the maintenance of the gut barrier and the maintenance of the gut microbiota. These plastic-associated abnormalities may increase immunological, inflammatory, and metabolic ailments, given the multifunctional nature of the intestinal system.

Microplastic exposure has been associated with immune system dysfunction in a number of investigations involving invertebrates. The intestinal lamina propria and the draining mesenteric lymph node are home to myeloid cells, innate lymphoid cells, and T cells, which are crucial fundamental units of the immune system. On exposure to MPs serious damage to these cells was observed. When exposed to polystyrene particles (500 nm and 30 µm), hemocytes showed several abnormalities, including a significant drop in cell count and phagocytosis activity, as well as various alterations in immunological markers related to oxidative stress, apoptosis, and inflammatory response (Shi et al., 2020 and Tang et al., 2020). The dominance of plastics in our daily lives is attributed to chronic, continuous exposure to MPs, according to the growing body of research.

CONCLUSION

One of the potential absorption pathways is through food or feeding. Based on the outcomes of the present study it was understood that severe MP contamination occurred in cattle and poultry farms, mirroring the situation in the land and aquatic environment. Among all the feed samples PET was very predominant. MPs were discovered in all 36 feed samples with an average size ranging between 2.02-10.7 µm. The consumption of feed contaminated with MPs is not only harmful to animals but also to humans (tertiary consumers), thereby leading to bioaccumulation of MPs in the food chains. Thus there is an immediate need to implement modern strategies to prevent the MPs issue from getting worse.

DECLARATIONS

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Authors' contribution

Sharon Sushma carried out major part of experiments, analysis, and assisted in data curation. Akkina Rajani chowdary is involved in data curation, analysis, overall supervision of the entire research, and prepared the manuscript.

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Competing interest

The authors declare that there is no competing interest to this research publication.

Ethics committee approval

Not applicable.

REFERENCES

Beriot N, Peek J, Zornoza R, Geissen V, Lwanga EH. (2021) Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain. Science of the Total Environment. 755:142653. DOI: https://doi.org/10.1016/j.scitotenv.2020.142653

Choi D, Jung S, Lee SS, Lin KY, Park YK, Kim H, and et al. (2021). Leveraging carbon dioxide to control the H2/CO ratio in catalytic pyrolysis of fishing net waste. Renewable and Sustainable Energy Reviews, 138:110559. DOI: https://doi.org/10.1016/j.rser.2020.110559

De Lange CF, Zhu CH, Niven S, Columbus D, Woods D (2006). Swine liquid feeding: nutritional considerations. In 27th Western Nutrition Conference, Winnipeg, Manitoba, Canada. Western Nutrition Conference Committee. Available at: https://www.cabdirect.org/cabdirect/abstract/20073057259

Du F, Cai H, Zhang Q, Chen Q, and Shi H (2020). Microplastics in take-out food containers. Journal of Hazardous Materials, 399:122969. DOI: https://doi.org/10.1016/j.jhazmat.2020.122969

Fadare OO, Wan B, Guo LH, and Zhao L (2020). Microplastics from consumer plastic food containers: Are we consumingit? Chemosphere, 253:126787. DOI: https://doi.org/10.1016/j.chemosphere.2020.126787

Geyer R, Jambeck JR, and Law KL (2017). Production, use, and fate of all plastics ever made. Science advances, 3(7):e1700782. DOI: https://doi.org/10.1126/sciadv.1700782

Guaita M, Chiantore O, and Costa L (1985). Changes in degree of polymerization in the thermal degradation of polystyrene. Polymer degradation and stability, 12(4):315-32. DOI: https://doi.org/10.1016/01413910(85)90123-5

Hale RC, Seeley ME, La Guardia MJ, Mai L, Zeng EY (2020). A global perspective on microplastics. Journal of Geophysical Research: Oceans, 125(1):e2018JC014719. DOI: https://doi.org/10.1029/2018JC014719

Jin T, Tang J, Lyu H, Wang L, Gillmore AB, Schaeffer SM (2022). Activities of microplastics (MPs) in agricultural soil: a review of MPs pollution from the perspective of agricultural ecosystems. Journal of Agricultural and Food Chemistry, 70(14):4182-4201. DOI: https://doi.org/10.1021/acs.jafc.1c07849

Koongolla JB, Lin L, Pan YF, Yang CP, Sun DR, Liu S, Xu XR, Maharana D, Huang JS, Li HX (2020). Occurrence of microplastics in gastrointestinal tracts and gills of fish from Beibu Gulf, South China Sea. Environmental Pollution. 258:113734. DOI: https://doi.org/10.1016/j.envpol.2019.113734

Lehel J, Murphy S. (2021). Microplastics in the food chain: food safety and environmental aspects. Reviews of Environmental Contamination and Toxicology, 259 7:1-49. DOI: https://doi.org/10.1007/398_2021_77

Leslie HA, Van Velzen MJ, Brandsma SH, Vethaak AD, Garcia-Vallejo JJ, Lamoree MH (2022). Discovery and quantification of plastic particle pollution in human blood. Environment International, 163:107199. DOI: https://doi.org/10.1016/j.envint.2022.107199

Luo T, Wang C, Pan Z, Jin C, Fu Z, Jin Y (2019). Maternal polystyrene microplastic exposure during gestation and lactation altered metabolic homeostasis in the dams and their F1 and F2 offspring. Environmental Science & Technology, 53(18):10978-92. DOI: https://doi.org/10.1021/acs.est.9b03191

Norberg-Hodge H (2006). Sustainable economies—local or global? The future of sustainability, 99-115. DOI: https://doi.org/10.1007/1-4020-4908-0

Pan Z, Liu Q, Xu J, Li W, Lin H (2022). Microplastic contamination in seafood from Dongshan Bay in southeastern China and its health risk implication for human consumption. Environmental Pollution, 303:119163. DOI: https://doi.org/10.1016/j.envpol.2022.119163

Paul-Pont I, Lacroix C, Fernández CG, Hégaret H, Lambert C, Le Goïc Nand et al. (2016). Exposure of marine mussels Mytilus spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. Environmental pollution, 216:724-37. DOI: https://doi.org/10.1016/j.envpol.2016.06.039

Peez N, Janiska MC, Imhof W (2019). The first application of quantitative 1 H NMR spectroscopy as a simple and fast method of identification and quantification of microplastic particles (PE, PET, and PS). Analytical and bioanalytical chemistry, 411:823-33. DOI: https://doi.org/10.1007/s00216-018-1510-z

Picó Y, and Barceló D (2019). Analysis and prevention of microplastics pollution in water: current perspectives and future directions. ACS omega, 4(4):6709-19. DOI: https://doi.org/10.1021/acsomega.9b00222

Reeves A, Chaudhuri P, Chakraborty S (2022). Fate and consequences of microplastics in the environment and their impact on biological organisms. In Microbial Biodegradation and Bioremediation. Elsevier Inc., pp. 69-79. DOI: doi.org/10.1016/B978-0-323-85455-9.00010-2

Ramachandraiah K, Ameer K, Jiang G (2022). Micro-and nanoplastic contamination in livestock production: Entry pathways, potential effects and analytical challenges. Science of the Total Environment, 8:157234. DOI: https://doi.org/10.1016/j.scitotenv.2022.157234

Shi W, Han Y, Sun S, Tang Y, Zhou W, Du X, Liu G (2020). Immunotoxicities of microplastics and sertraline, alone and in combination, to a bivalve species: size-dependent interaction and potential toxication mechanism. Journal of hazardous materials, 396:122603. DOI:https://doi.org/10.1016/j.jhazmat.2020.122603

Stock V, Fahrenson C, Thuenemann A, Dönmez MH, Voss L, Böhmert L, Braeuning A, Lampen A, Sieg H (2020). Impact of artificial digestion on the sizes and shapes of microplastic particles. Food and chemical toxicology, 135:111010. DOI: https://doi.org/10.1016/j.fct.2019.111010

Tang Y, Rong J, Guan X, Zha S, Shi W, Han Y, Du X, Wu F, Huang W, Liu G (2020). Immunotoxicity of microplastics and two persistent organic pollutants alone or in combination to a bivalve species. Environmental Pollution, 258:113845. DOI: https://doi.org/10.1016/j.envpol.2019.113845

Thiele CJ, Hudson MD, Russell AE, Saluveer M, Sidaoui-Haddad G (2021). Microplastics in fish and fishmeal: an emerging environmental challenge? Scientific reports, 11(1):2045. DOI: https://doi.org/10.5258/SOTON/D1400

Thornton Hampton LM, Brander SM, Coffin S, Cole M, Hermabessiere L, Koelmans AA, Rochman CM (2022). Characterizing microplastic hazards: which concentration metrics and particle characteristics are most informative for understanding toxicity in aquatic organisms?. Microplastics and Nanoplastics, 2(1):1-6. DOI: https://doi.org/10.1186/s43591-022-00040-4

Van der Veen I, van Mourik LM, van Velzen MJ, Groenewoud QR, Leslie HA (2022).Plastic Particles in Livestock Feed, Milk, Meat and Blood: A Pilot Study. Department of Environment & Health, Faculty of Science, Vrije Universiteit Amsterdam, The Netherlands. Pp. 1-48. Article link

Wang W, Ge J, Yu X (2020). Bioavailability and toxicity of microplastics to fish species: A review. Ecotoxicology and environmental safety, 189:109913. DOI: https://doi.org/10.1016/j.ecoenv.2019.10991

Xu J, Bi W, Hua L, Cheng Z, Wang Y, Li D, Liu W, Wang L, Sun H. (2022) Wide occurrence of seven phthalate plasticizers and two typical microplastics in pig feed. Chemosphere. 307:135847. DOI: https://doi.org/10.1016/j.chemosphere.2022.135847

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RELATIVE ANALYSIS OF TWO CITRUS SPECIES VERSUS MAIZE FOR POTENTIAL NUTRITIVE TRAITS AS LIVESTOCK FEED

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ABSTRACT: The present study seek to assess the nutritional qualities and the mineral composition of citrus fruits (pulps + peels) of two different species (*Citrus clementina* and *Citrus limon*), while comparing its nutritive perspective with *Zea mays L* (yellow maize) commonly used as livestock feed. Proximate evaluation was done via the method of the association of official analytical chemists (AOAC). Elemental components of citrus species were measured by means of a standard spectrometer. The proximate evaluation of the sample indicated that *Citrus limon* fruit contained comparable amounts of protein, fibre and lipid, but significantly higher ash contents than yellow maize. While the *Citrus clementina* was higher in protein and ash content, but comparable moisture content to *Zea mays L*. Meanwhile, minerals including Ca, Mg, K, Na, Cu, Mn and Fe were significantly higher in the two citrus species than in *Zea mays L*. Therefore the manuscript revealed that the *Citrus clementina* and *Citrus limon* species possess the potentials to be utilized as livestock feed ingredients.

Keywords: Citrus fruit, Elemental composition, Maize, Nutritional quality, Proximate analysis.

INTRODUCTION

A large chunk of food produced worldwide is wasted, causing about 1.3 billion tons of food to go into waste (Morshedy et al., 2022). Among these recorded food wastes, 45 % of them are generated from fruits and vegetables (FAO, 2016). These food wastes are known to be produced by agro-industrial processing, agricultural production, and damage during storage and transportation (Torres-Leon et al., 2018). However, information on the optimum utilization of fruit and vegetable wastes is scarce, leading to grave impacts on the immediate surroundings, economic, and social sectors (Morshedy et al., 2022). Due to this menace, different research works have been reported in an effort to assess ways of lowering food wastage, and reducing their economic and societal menace (Castro et al., 2020).

Several bioactive compounds in citrus fruits varies from one species to the other and in their different parts (Karasawa and Mohan, 2018; Maheshwari et al., 2022). For instance, citrus waste peels possess an extensive diversity of minor compounds with high antioxidant reactions in comparison to the other parts of citrus fruit (Czech et al., 2020). Peels are also fingered as a source of pectin, molasses, and limonene (Manthey and Grohmann, 2001; Rafiq et al., 2018). The rind (outer portion) of citrus fruits is often processed and used as diet for cattle (Zema et al., 2018). Meanwhile, at other times, the rind of citrus is disposed into society and treated as fruit waste, which contains 50 % of the whole weight of the fruit (Anagnostopoulou et al., 2006; Marin et al., 2007). These wastes are seen as possible source of environmental pollution (Barros et al., 2012). Peels from citrus fruit also possesses several natural compounds, such as heraclenin, auraptene, imperatorin, and bergamottin, and bio-active elements, with advanced dietary and therapeutic worth (Genovese et al., 2014; Sunagawa et al., 2022). The relatively insignificant monetary value and widely available citrus materials such as their peel and pulp, which appears to be waste of citrus, can be seen as a prospective source of animal feed and nutraceuticals (Rafiq et al., 2018; Zema et al., 2018). Whole citrus (pulps and peels) fruits, being rich in bioactive molecules can be used as dietary additives, dietary fiber, antioxidants, and mineral ingredients (Czech et al., 2020). Citrus fruits, from their various species such as lemon, limes, soft citrus, and mandarins are among the broadly grown fruits globally, due to its wide public demands. Several nations including Spain (leading in highest amount of production), Italy, Turkey, Portugal, Greece, Morocco, Tunisia, and Algeria are foremost producers of clementine fruits with an estimate of about 25 % (about 4,795,000 tons) of the whole citrus species in the regions around the Mediterranean basin (Fabroni et al., 2016). In other continent like South America, the production of Clementine citrus is projected at about 2 million tons, with nations such as Brazil, Peru, Uruguay, and Argentina taking the leading in terms of production (Fabroni et al., 2016). In the continent of Asia, China is known (contributing about 14 million tons) to be the highest producer of Clementine citrus from its overall production of 19 million tons (Fabroni et al., 2016).

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Citrus fruit, is an essential crop in South Africa (Ladaniya, 2008). About 1.6 million metric tons of fruits are reported harvested and processed in South Africa yearly. They are processed into juice drinks, leaving their peels and pulps as unwanted products and wastes. South Africa is a key exporter of citrus fruits. This invariably suggests that tons of waste are generated in the process, and are often discarded into the surrounding.

More than 50 % of citrus fruit produced in South Africa goes into waste with Eastern Cape Province leading the park (Van Dyk et al., 2013), and these wastes are regularly thrown away into the environment (Botha, 2003). Likewise, in Egypt for instance, it was reported that more than 1.9 million tons of crop wastes are discarded on a yearly basis, among which citrus fruits contribute a large proportion (Alnaimy et al., 2017). Unwanted waste generated from citrus fruit processing is a potential economic and societal nuisance if they are not effectively transformed or biodegraded for value addition/useful materials (Tripodo et al., 2004).

There is currently an increased quest for alternative feedstuffs for improving livestock farming globally. This is because of the constant rise in the regular animal feedstuffs which most animal farmers may not be able to afford. The current bulk of citrus fruits as by-products from citrus farms could be useful in bridging the feed gap in livestock farming and reducing the recurring animal-human competition on grains, especially maize. Therefore, investigating the potential of different citrus fruits as a prospective livestock feedstuff is worth the while.

According to Ülger et al. (2020), citrus wastes used as feedstuffs lowered the price of feed, thereby increased profit. Judging from this perspective, citrus fruits (labelled as wastes) are seen to be an important feed alternatives in livestock dietary scheme (Ülger et al., 2020). According to Steyn et al. (2017), citrus fruits can be harvested, processed and utilized as animal feed to boost livestock husbandry. Equally, the attempt to evaluate the nutritive prospect of citrus fruits (pulp and peels) of South African origin is calculative, as this could be an insightful venture in the right direction with respect to its use as an alternative feed to yellow maize for farmers judging from the constant hike in the price of grains including yellow maize. According to the research by Steyn et al. (2017), they observed that citrus fruit waste may be fed to livestock as sole-feed, or/and as a substitute, thus citrus fruit could form an essential in their feeding scheme. The goal of the current manuscript is therefore to assess the proximate and elemental values of whole (peel and pulp) *Citrus clementina* and *Citrus limon* fruits as a potential livestock feed resource, and to compare their nutritional parameters to that of *Zea mays L* (yellow maize), a commonly used livestock feed (chicken, goats, sheep etc.) by indigenous farmers in South Africa. The nutritive characteristics of these dried whole citrus fruits were compared to that of *Zea mays L* (yellow maize) obtained from an agro-allied store. *Citrus limon* and *Citrus clementina* fruits were adopted for this work because they are abundantly available in the study area with several commercial citrus farms located in the area.

MATERIALS AND METHOD

Procurement of yellow maize

The *Zea mays* L (yellow maize) that was used in this study was purchased from a well-known farm store precisely from the town of Alice where most indigenous and commercial livestock farmers purchase yellow maize for animal feed. Alice town is a small town in the Eastern Cape region of South Africa. The variety of the yellow maize which is a well-known grain in the area is the Okavango flint maize. The geographical location of Alice town is situated at 32° 43028.66" and 26° 3405.88" in latitude and longitude.

Citrus waste collection

The complete citrus fruit of *Citrus limon* and *Citrus clementina* fruits which were meant to be waste (since they have lost their market values in terms of appearance by the owners) were gotten from a well-known commercial citrus farm (Nandeshook farm) and kept in clean plastic bags before further processing in the laboratory. The *Citrus* fruits gotten from the farm site were Eureka Lemon and Clementine (*C. limon* and *C. clementina*), all from the Eastern Cape region of South Africa with geographical co-ordinates of -33° 44'43.04".

Sample preparation of citrus fruit (pulps + peels)

The citrus fruits of *C. clementina* and *C. limon* were cut into pieces and spread on a flat clean surfaced platform (wooden materials) for air-drying and drained of their liquid. After drying, the citrus fruits were afterward oven-dried at a temperature of 50 °C for two days (48 h) to get the desired dried matter. The samples were blended into small particles using a blending machine. The blended samplers were neatly gathered into clean plastics, labeled and kept in cool conditions, for further evaluation.

Analysis of proximal composition of citrus fruit

Determination of moisture component

The moisture constituent of the prepared citrus fruit was done via standard practice (AOAC, 2000). With the aid of a drained and empty weighing container weighed (W_i) in an oven for measurement of its initial weight. The sample was then kept in the previously measured container and then weighed for the second time (W_j), and oven-dried for three days (72 h) at 40 °C. The sample was afterward cooled and reweighed (W_k). The value of the water content was obtained using following formula:

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Moisture content (%) = $\frac{W_j - W_k}{W_j - W_i} \times 100$

Where; W_i represents the weight of the empty container; W_j represents the weight of the container + drained citrus fruit before drying; W_k represents the weight of container + drained citrus fruit sample.

Ash content determination

The content of ash in the drained citrus fruit was determined via the procedure of the dry ashing method (Agrilasa, 2007). The container was dried at 105°C for one hour, thereafter the container was weighed on cooling (W_i). Following that, 2 g of the samples were kept in the weighed vessel and re-weighed (W_j). The container, together with the sample was then ashed at 250 °C for an hour. The ashed samples were then left to cool down and later weighed again (W_k). The citrus ash was evaluated as follows:

Ash content (%) = $\frac{W_j - W_k}{W_j - W_i} \times 100$

Where; W_i is referred to the weight of the dried container; W_j is referred to the weight of container + whole *Citrus* fruit (pulps + peels) sample; W_k is the weight of container + ashed *Citrus* fruit (pulps + peels) sample.

Crude protein

Crude protein was estimated from the total nitrogen (TN) constituent in the sample using the micro Kjeldahl technique (Hussain et al., 2011).

Percentage nitrogen content (%) =

$$\frac{[(\text{mL standard acid} \times \text{N of acid}) - (\text{mL blank} \times \text{N of base})] - (\text{mL std base} \times \text{N of base}) \times 1.4007}{\text{Weight of sample (g)}}$$

Where; N represents the normality i.e. 1.4007 known as the single factor of nitrogen molecular weight;

Calculation of Crude protein = Nitrogen content (sample) × 6.25

Crude fibre determination

Dietary fibre of sample was determined using the technique of the modified acid-base digestion procedure (Aina et al., 2012). The crude fibre was calculated below as;

Crude fibre (%) = $\frac{C_2 - C_1}{\text{Weight of Sample}} \times 100$

Where; C_1 = is referred to the weight of container + whole Citrus fruit (pulps + peels) sample; C_2 is the weight of container + ashed Citrus fruit (pulps + peels) sample.

Crude Lipid determination

This was determined using the Soxhlet extraction method (Al-Harrasi et al., 2012). The estimation of lipid was determined using the formula:

Crude lipid (%) = $\frac{W_2 - W_1}{\text{Weight of original sample}} \times 100$

Where; W_1 is referred to the weight of container + whole Citrus fruit (pulps + peels) sample; W_2 is the weight of container + ashed Citrus fruit (pulps + peels) sample.

Determination of total carbohydrate content

This was done by deducting crude fibre, total protein, ash content, crude fibre, and lipid from the total dry matter of the sample: Total carbohydrate (%) = 100 − (crude fibre + % moisture content + total ash + crude protein + crude lipid).

Determination of energy value

The aggregate of energy value of citrus fruit was determined using the Atwater factors as follows: 4 kcal, 9 kcal, and 4 kcal to determine the caloric figure. The sum of the multiplied lipid, crude protein, and carbohydrate, is explained from the formula below:

Energy value (kcal/100 g) = (total carbohydrate × 4) + (crude fat × 9) + (crude protein × 4) (Idamokoro et al., 2022).

Mineral determination

The mineral evaluation was done to quantitatively analyze mineral attributes in the citrus fruit (pulps + peels) segments. The elements that were determined including iron, manganese, phosphorus, potassium, copper, calcium, magnesium, sodium, nitrogen and zinc were analyzed via the Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Sample preparation of yellow maize

The purchased yellow maize was crushed into fine particles using a blending machine. The blended citrus fruits were neatly assembled into clean plastics, labeled, and kept in cool conditions, before carrying out further analysis. The same

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procedure that was used for analysing the elemental and proximate composition of the citrus fruits, was also employed to analyse the yellow maize. The yellow maize and the whole citrus were analysed in triplicates.

Data analysis

Analysed citrus fruit and yellow maize samples were given as mean ± standard deviation before being estimated in a one-way analysis of variance (ANOVA) using the MINITAB 19 statistical software. ANOVA was pegged at 95 % using the Fisher LSD confidence level. The measurement of statistical differences was placed at a probability of $p < 0.05$.

RESULTS

Nutritional component

The proximate contents of citrus from *Citrus limon* Pulp + Peel (CLPP); and *Citrus clementina* Pulp + Peel (CCPP) gotten from the commercial farm representing the nutritive contents of their pulps + peels and *yellow maize* are shown in Table 1.

The moisture content (MC) values of the citrus CLPP and CCPP were given as 11.87±0.41 % and 9.71±0.60 %, respectively, while it was 9.22 ±0.12 % in yellow maize. Of the three samples, highest content of ash was recorded in CLPP (5.91 ± 0.27 %) significantly, followed by CCPP (5.08±0.03 %) and yellow maize (1.47±0.04 %).

The content of lipid was highest in CLPP (4.27±0.02 %), which was statistically at par with maize (4.13±0.17%), but was significantly higher than those of the CCPP (2.92±0.12 %). The crude fibre content for yellow maize (30.66±3.36 %) had comparable mean value to the CCLP (27.63±3.23 %), but had significantly higher mean value CCPP (19.67± 0.36 %) accordingly. The crude protein content of CCPP fruit had a significantly higher mean value of 6.65± 0.20 % compared to maize (5.06±0.10 %), and CLPP (4.92±0.06 %). Total carbohydrate content mean value for yellow maize (68.24±4.49 %) was significantly higher compared to those of the CLPP fruit (4.84±0.93 %) and CCPP (3.13± 0.35 %). The aggregate energy mean value for yellow maize was 330.39±16.26 % which was significantly higher than those of CLPP fruit (70.72±6.60 %), and CCPP fruit (65.46±2.57 %).

Table 1 - Nutritional components (%) of *Citrus limon* and *Citrus clementina* from a commercial farm as compared to *Zea mays L*

Parameters (%)	CLPP	CCPP	*Zea mays L*
Moisture	11.87±0.41^a	9.71±0.60^b	9.22±0.12^b
Ash	5.91±0.27^a	5.08±0.03^b	1.47±0.04^c
Lipid (Fat)	4.27±0.02^a	2.92±0.12^b	4.13±0.17^a
Carbohydrate	4.84±0.93^b	3.13±0.35^b	68.27±4.49^a
Protein	4.92±0.06^b	6.65±0.20^a	5.06±0.10^b
Fibre	27.63±3.23^a	19.67±0.36^b	30.66±3.36^a
Caloric value	70.72±6.60^b	65.46±2.57^b	330.39±16.26^a

The letter variants along a row indicates significant differences at $p < 0.05$ among samples of *Citrus limon, Citrus clementina* and *Zea mays L*. CLPP= Citrus Limon Pulp + Peel; CCPP = Citrus Clementina Pulp + Peel. The values of components reported are mean ± standard deviation. NB: Caloric value: (kCal/100 g). Data is based on dry matter.

Mineral Composition

The citrus fruit and maize samples showed significant variation in their mineral composition (Table 2). Calcium content was significantly higher in the CLPP (603.33±20.55 mg/100 g) than CCPP (396.67 ± 4.71 mg/100 g) with maize (10.00 ± 4.71 mg/100 g) samples. Magnesium was also significantly higher in CLPP fruit (130.00 ± 0.00 mg/100 g) than CCPP (113.33 ± 4.71 mg/100 g) and maize (100.00 ± 0.00 mg/100 g) samples. The peak value of potassium was recorded in CLPP fruit (1503.33±16.99 mg/100 g) which had a statistical difference from that CCPP fruit (1453.33±16.99 mg/100 g), and maize sample (350.00±8.16 mg/ 100 g).

The CLPP excelled statistically for sodium content (73.33 ± 4.71 mg/100 g) over CCPP (43.33 ± 4.71 mg/100 g) and maze (20.00 ± 0.00 mg/100 g) samples. Likewise, zinc mean value was also highest in the CLPP fruit (4.63 ± 4.43 mg/100 g), followed by CCPP fruit (1.70 ± 0.21 mg/100 g) and maize (0.65±0.56 mg/100g) with no significant difference. Meanwhile, maize proved best significantly for phosphorus content (233.33 ± 4.71 mg/100 g) over CLPP (173.33 ± 4.71 mg/100 g) and CCPP (156.67 ± 4.71 mg/100 g). The CLPP had significantly higher manganese content (1.00 ± 0.08 mg/100 g) followed by CCPP (0.80 ± 1.11 mg/100 g), while it was lowest in maize (0.50 ± 0.00 mg/100 g). Copper was generally low in content, being the highest in CCPP fruit (0.27 ± 0.05 mg/100 g) statistically, followed by CLPP (0.20 ± 2.77 mg/100 g); no copper was found in the maize sample. The CLPP tended to show the highest content of iron (5.23 ± 0.23 mg/100 g) significantly over CCPP (3.77 ± 0.05 mg/100 g) and maize sample (2.90 ± 0.59 mg/100 g).

The sodium-potassium ratio (Na^+/K^+) was registered significantly highest in CCPP fruit (1.29 ± 0.01 mg/100 g) when compared to maize (1.07 ± 0.02 mg/100 g) and CLPP (0.94 ± 0.02 mg/100 g) samples. It was largely noticed that, manganese, copper, with sodium-potassium ratio (Na^+/K^+) was low in all the analysed samples, while potassium, calcium,

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zinc, magnesium, and iron components were considerable. Meanwhile, it was observed that samples of *C. limon* proved to be important stores of potassium, calcium, magnesium, manganese and sodium in comparison to whole clementine fruit and maize. Furthermore, clementine fruit had a significantly higher content of copper and sodium-potassium ratio (Na^+/K^+), as compared to the *Citrus limon* fruit and maize.

Table 2 - Mineral components (mg/100 g) of *Citrus limon* and *Citrus clementina* fruit from a commercial farm as compared to *Zea mays L*.

Parameters (%)	CLPP	CCPP	*Zea mays L*
Calcium	603.33±20.55^a	396.67±4.71^b	10.00±4.71^c
Magnesium	130.00±0.00^a	113.33±4.71^b	100.00±0.00
Potassium	1503.33±16.99^a	1453.33±16.99^b	350.00±8.16^c
Sodium	73.33±4.71^a	43.33±4.71^b	20.00± 0.00^c
Phosphorus	173.33±4.71^b	156.67±4.71^c	233.33±4.71^a
Zinc	4.63±4.43^a	1.70±0.21^a	0.65±0.56^a
Manganese	1.00±0.08^a	0.80±1.11^b	0.50±0.00^c
Copper	0.20±2.77^b	0.27±0.05^a	0.00±0.00^c
Iron	5.23±0.23^a	3.77±0.05^b	2.90±0.59^b
Na^+/K^+	0.94±0.02^c	1.29±0.01^a	1.07±0.02^b

The letter variants along a row indicates significant differences at $p < 0.05$ among samples of *Citrus limon, Citrus clementina* and *Zea mays L*. CLPP= Citrus Limon Pulp + Peel; CCPP = Citrus Clementina Pulp + Peel. The values of components reported are mean ± standard deviation. Data is based on dry matter.

DISCUSSION

Fruits or their parts generally are known to be vital nutritional sources of nutrients for livestock, as they contain useful metabolites and bio- active compounds necessary for their growth and development (Wadhwa, 2015; Wang et al., 2019).

Moisture content has an inverse relationship with the shelf life of individual produce (Nwofia et al., 2012). Low water (moisture) content in citrus fruit (*Citrus limon* and *Citrus clementina*) and maize as recorded in present study is an indication of long storability (Ogbonna et al., 2016). Similar observation was observed by Sharif et al. (2018) in feed for lamb, however, the moisture composition of samples was higher than that of the study of Alnaimy et al. (2017), who reported only 3.5 % moisture content.

Ash content is essential in livestock nutrition since the help in the building of mineral formation. Ash was noticeably higher in content for both citrus fruit species, but lesser than yellow maize in the present study, which is in consonance with the study of Alnaimy et al. (2017). Low ash content in livestock nutrition may indicate of the presence of higher mineral elements in the feed (Adebowale and Bayer, 2002).

With regards to dietary fibre in food, their presence depicts a significant indicator, as it supports the digestive system, intestinal gut health and quickens bowel movement. Dietary fibre also lowers the amount of cholesterol when utilized by livestock (Omokore and Alagbe, 2019) suggesting that dried lemon and orange fruits as handy roughage resource materials which can be ideal as feed materials. The fibre content in the maize and both citrus fruits were comparable to the maize based animal feed (26.15 %) of Ülger et al. (2020). However, our result for crude fibre in both whole citrus fruit and maize was higher in comparison to the crude fibre of lemon pulp (11.52 %) prepared by Ülger et al. (2020), and that of citrus pulp (15.6 %) observed in the study of Wang et al (2017).

Differences in crude fibre that was observed, may be the result of the tree age of *Citrus* species, method of processing, location and individual citrus species (Audu et al., 2018). Crude fibre in the *Citrus limon* and *Citrus clementina* were less when compared to those obtained in the *Zea mays L* which is an indication of their prospect as a livestock feed resource. Dried citrus possess nutritionally significant fibre by-products, and have been utilized as feed ingredients, because they provide an environmentally stable gut biome in livestock, thereby promoting better overall animal production and performance (Steyn et al., 2017). Proteins in feed are important elements known for repairing damaged cells and they are also helpful in body-building and growth. In addition, dietary proteins assist in lowering glucoregulatory mechanism in animals (Comerford and Pasin, 2016). Dietary protein in the citrus fruit is commendable. Our result showed that the protein value in the citrus fruit and maize were in line those (6.00 % – 8. 68 %) reported by several other authors (Allam et al., 2011; Santos et al., 2014; Sharif et al., 2018; Allam et al., 2020), but they were lesser in value (14.90 %) compared to the protein content as observed in the study of Luzardo et al. (2021). Citrus can be utilized in steer's diet, because of its positive effect in improving livestock performance (Luzardo et al., 2021). Citrus fruit have also advanced the milk production of ruminants (Bampidis and Robinson, 2006; Bakr, 2020).

With respect to dietary carbohydrates, they are accountable for energy in the metabolic process in livestock (Olanipekun et al., 2016). The carbohydrate values were significantly low in citrus fruit compared to maize. However, the carbohydrate content in the citrus fruits were higher than other fruit and vegetable ingredients such as cucumber (2.17

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%), coriander (2.16 %), courgette (1.99 %), and tomatoes (2.93 %; Kamau et al., 2020). The low carbohydrate content in the citrus fruits is indicative to be a good dietary supplement (important nutrients) for animal utilization instead of the main energy source.

High starch content in grains have been reported to have a negative effect on the microbial activity in the rumen of ruminants resulting from the production of lactic acid, reducing pH in the rumen (Poulsen et al., 2012; Jacobs, 2014). The effect of high starch component in grains affects fibre degradation in a negative way which in turn also affects the performance of animals in terms of production. Meanwhile, despite the negative effect of feeding high starchy contents to livestock, it is still practiced broadly due to the high energy content that they possess, because scientist still presumes that they promote good production performance such as milk yield in animals (Steyn et al., 2017).

Dietary fats perform several significant roles in feed such as energy production, supports in cells and organs structure formation, and increase palatability of feeds (Alagbe, 2020). Ingestion of dietary fat in modest quantity is helpful to animals, but consumption in excess, negatively hampers animal health. However, some research work have attempted to use fruits and vegetable intake to cushion the effect of the high level of fats that may be consumed in the system (Djuric et al., 2002). The amount of fat in the citrus fruits and maize ranged from 2.92-4.27% in our study was in line with the findings of Alnaimy et al. (2017), and was higher than the wastes of most of the fruit and vegetable wastes such as coriander (0.09 %), courgette (0.25 %), cucumber (0.21 %), spinach (0.17 %), tomatoes (0.12 %), banana (0.50 %), mango (0.68 %), and pawpaw (0.34 %) (Kamau et al., 2020).

The mean of energy (feed ingredient) in the citrus fruit was very much lower, than in maize (330.39 %). However, the energy content of citrus fruit observed was higher than those (3.06 to 40.00 %) of other fruits and vegetables (Kamau et al., 2020). Conversely, Isong et al. (1999) reported higher energy levels of some vegetables (248.8 % to 307.1 %) compared to citrus fruit in this study.

Mineral nutrients play a vital part in supplying bio-elements in the feed of animals as a balanced diet. Dietary minerals perform numerous functions (such as metabolic and physiological processes) in animals. The mineral content of citrus fruit was significantly higher than maize, except for phosphorus. Howbeit, all the mineral contents is in the accepted amount needed in feed inclusion (McDowell, 1996). The amount of calcium in the citrus fruit was higher than the values observed for maize. The calcium content recorded in the citrus fruit was lower (1600 mg/100 g) compared to another study observed by Bampidis and Robinson (2006). The calcium content in the citrus fruit from the present finding, however, proved superior over the range (30.1 and 25.9 mg/100 g) of mandarin and lemon (Czech et al. 2020). Possible reasons for the contradictory findings could be because of the different region of study, different method of processing of the citrus, soil types, or difference in the stage of harvest of citrus fruits. Judging from the amount of calcium content present in the citrus fruit observed in our result, the citrus fruits may be ideal if added to feed for livestock utilization.

Magnesium plays multiple parts in the body functions of animals. It is important in enzyme activation. Magnesium is important in bone fortification (Kartika et al., 2011). The amount Mg present in the citrus fruit and maize samples are line to the findings by ADAS (1992) and Bath et al. (1980) in citrus, but higher in compared to the findings of Czech et al. (2020) for lemon (9.86 mg/100 g) and mandarin (11.1 mg/100 g).

Potassium helps in the physiological development of organisms (Hounsome et al., 2018). They also help to regulate water and acid base balance in the body (Indrayan et al., 2009). The potassium content for the citrus fruit was in line (620 – 1100 mg/ 100 g) with the recommendations for standard feed formulation (NRC, 1988; 2001). However, the potassium content was higher than those observed in citrus by other authors (Bampidis and Robinson, 2006; Czech et al., 2020).

Sodium plays an important role in the coordination of nerves and muscles of organisms (Akpanyung, 2005). The standard amount of Na in feed should fall within 60 – 90 mg/ 100 g for citrus (NRC, 1988; 2001), and this was in consonance with the whole lemon fruit, but higher than those of the *Citrus clementine* in our finding. Conversely, the amount of Na for *C. limon* and *C. clementina* fruits and *Z. mays* L was lower than those (100 mg/ 100 g) for citrus fruit in the study by Ensminger and Olentine (1978). However, we recorded a higher sodium value of citrus fruit than the orange fruit (30 mg/ 100 g) in another study (Bampidis and Robinson, 2006). Factors such as the type of fruits, citrus variety among others affect the amount of sodium in citrus (Wadhwa et al., 2015).

Upholding the ionic equilibrium in feeds assists the utilization of K and Na in animal nutrition. From the current study, the K and Na values could be attributed to the low Na+/K+ ratio. How it, the Na+ /K+ ratio in feed is significant most importantly if they have a ratio of less amount, which is indicative of the right Na^+/K^+ amount to advance the equilibrium of ions needed in the animal body.

With respect to phosphorus in feed, they advance calcium absorption and they also function in bone strengthening. Likewise, phosphorus performs numerous roles in the creation of several important bio-molecules required in animals including phosphoproteins (casein), phospholipids, phosphate esters (ATP), nucleic acids, hexose phosphates, as well as creatine phosphate (Alagbe, 2019). According to the standard amount of P in livestock feed by the National Research Council (NRC) which ranged between 110 to 120 mg/100 g for citrus (NRC, 1988; 2001), are in consonance with the ones in our finding. However, the values of phosphorus (17.9 and 21.8 mg/100 g) were lower to those reported in another study (Czech et al., 2020).

Zinc as an important nutritional micro-element, acts as a cofactor for several vital enzymes activity. It functions in the building formation of lipid and starch, and in the synthesis of amino acids and proteins (Bashir et al., 2020). Zinc plays a significant function in tissue repairs of the body (Miltan et al., 2014). The amount of zinc in citrus ranges between 1100

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to 1600 mg/ 100 g (NRC, 1988; 2001). The amount of zinc in the citrus fruits and maize in this study was less than (3410 mg/ 100g) those observed in another study (Alnaimy et al., 2017). However, on the contrary, the amount of Zn for citrus fruits and maize our finding was higher, than those (0.26 and 0.22 mg/100 g) reported in another study (Czech et al., 2020).

The value of Mg in diets cannot be over emphasized. Manganese plays the role of a catalyst in the synthesis of glycoproteins and lipids (Shomar, 2012). Furthermore, manganese advances the synthesis of vitamin K, and helps in skeletal development in the animal. The amount of Mg in citrus fruits and maize in our findings was in consonance with the values of manganese (0.7- 0.9 mg/100 g) as those approved for livestock (NRC, 1988; 2001). The amount of Zn in our findings was also similar to those (0.5 – 1.4 mg/ 100 g) reported in another study (ADAS, 1992). Conversely, the amount of Zn (0.07 and 0.05 mg/100 g) in citrus as observed by Czech et al (2020) was lower than the values observed in the present study.

Copper is another micro-nutrient important for pro-oxidant and they function as unsaturated fats. Copper also helps in the moderation of red blood cells. The body utilization of Cu in animals is normally very low for normal body functioning, as high copper consumption in the body can be damaging and could probably lead to liver organ damage. The amount of Cu reported in citrus fruits in our findings was similar to those (0.3 to 0.6 mg/ 100 g) observed in another study (ADAS, 1992). On the contrary, the amount of Cu reported for citrus fruits was higher compared to the amount (0.05 and 0.07 mg/ 100 g) observed by Czech et al (2020). The low amount of Cu reported for citrus fruits in our findings is commendable because they are needed in lower quantity in the body. Bampidis and Robinson (2006) also reported a low copper content for citrus from compiled results of previous studies which is in line with copper content recorded in the present study.

The body requires iron for the formation of enzymes (Lieu et al., 2001). Iron is required for transporting oxygen to all parts of the body where they are needed during respiration (Gupta et al., 2014). They also function in haemoglobin synthesis. The amount of Fe for the citrus fruits and maize were higher compared to the ones (0.29 and 0.31 mg/100g) reported in another study (Czech et al., 2020), but were lower to the ones (15.1 - 37.7 mg/ 100 g) observed by the National Research Council (NRC, 1988; 2001).

CONCLUSION

The *Citrus limon* and *Citrus clementina* fruits were found to have a reasonable amount of lipids and fibre, but with high protein contents in comparison to *Zea mays L* (yellow maize). More visibly, is the fact that the *Citrus limon* and *Citrus clementina* had rich dietary bio-active such as Na, K, Cu, Zn, Mg, Ca, Fe, and Mn, with the exception of phosphorus (P) when compared to *Zea mays L* and should therefore be considered as a rich naturally available nutrient for animal feed. Our findings also revealed the potential *Citrus limon* and *Citrus clementina* fruits as possible livestock ingredients. Since citrus fruits are relatively free of charge raw materials that is widely available in the studied area, its feeding worth as alternative feedstuff should be further researched for livestock feeding scheme which is the common source of income for most indigenous people in the region.

DECLARATIONS

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Authors' contribution
E.M. Idamokoro and Y.S. Hosu conceptualize the study.
Y.S. Hosu collected the citrus fruit.
E.M. Idamokoro did the experiment on citrus sample.
E.M. Idamokoro did data collection and analysis of citrus result.
E.M. Idamokoro wrote and edited the manuscript.

Conflict of interests
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REFERENCES

ADAS. (1992). Dried citrus pulp. ADAS Nutrition Chemistry Feed Evaluation Unit. Technical Bulletin Number 92/8: 1– 22. https://www.feedipedia.org/node/1098.

Adebowale KO and Bayer E (2002). Active carbons for low temperature conversion chars. Electronic Journal of Environmental, Agricultural and Food Chemistry, 7:3304-3315. https://inis.iaea.org/search/search.aspx?orig_q=RN:33050383.

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Agrilasa. (2007). Method 6.1.1. Dry ashing. In Handbook of Feeds and Plant Analysis, 2nd ed.; Palic, P., Claasens, A.S., Collier, J., Loock, A., Hattingh, D., Eds.; Agricultural Laboratory Association of South Africa: Pretoria, South Africa.

Aina VO, Sambo B, Zakari A, Haruna MSH, Umar H, Akinboboye RM and Mohammed A (2012). Determination of nutritional and anti-nutrient content of Vitis vinifera (Grapes) grown in Bomo (Area C) Zaria, Nigeria. Advance Journal of Food Science and Technology, 4: 445 – 448. https://maxwellsci.com/print/ajfst/v4-445-448.pdf.

Akpanyung EO (2005). Proximate and Mineral Element Composition of Bouillon Cubes Produced in Nigeria. Pakistan Journal of Nutrition, 4:327-339. DOI: http://dx.doi.org/10.3923/pjn.2005.327.329

Alagbe JO (2019). Haematology, serum biochemistry, relative organ weight and bacteria count of broiler chicken given different levels of Luffa aegyptiaca leaf extracts. International Journal of Biomedical and Advance Research, 7:382-392. DOI: http://dx.doi.org/10.33945/SAMI/IJABBR.2019.4.8

Alagbe JO (2020). Performance, hematology and serum biochemical parameters of weaner rabbits fed different levels of fermented Lagenaria brevifora whole fruit extract. Advances in Research Reviews, 1:5-15.

Al-Harrasi A, Al-Rawahi A, Hussain J, Rehman NU, Ali L and Hussain H (2012). Proximate analysis of the resins and leaves of Boswellia sacra. Journal of Medicinal Plants Research, 6: 3098 – 3104. DOI: http://dx.doi.org/10.5897/JMPR11.1264

Allam SM, Abou-ward GA, Tawila MA, Ali MA, Al-Hosseiny HM, Sayed SK and El-Naggar S. (2011). Nutritional and economical impact of using dried orange pulp as energy source in growing lambs rations. Egyptian Journal of Nutrition and Feeds, 14: 337 – 347.

Allam SM, El-Bedawy TM, Bakr MH and Mahmoud AEM (2020). Effect of feeding dried orange pulp to lactating dairy cows on nutrients digestibility, blood constituents, plasma antioxidant biomarker and pathogenic fecal bacteria. Pakistan Journal of Zoology, 52:79-86. DOI: http://dx.doi.org/10.17582/journal.pjz/2020.52.1.79.86

Alnaimy A, Gad AE, Mustafa MM, Atta MAA and Basuony HAM (2017). Using of citrus by-products in farm animals feeding. Open Access Journal of Science,1:58–67. DOI: http://dx.doi.org/10.15406/oajs.2017.01.00014

Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN and Boskou D (2006). Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). Food Chemistry, 94:19 –25. https://doi.org/10.1016/j.foodchem.2004.09.047.

Association of Official Analytical Chemists (AOAC). Official Methods of Analysis of AOAC, 17th ed; AOAC. International: Gaithersburg, MD, USA, 2000.

Audu, I.W, Audu, B.S. and Suleiman Y. 2018. Phytochemistry and proximate composition of root, stem bark, leaf and fruit of desert date, Balanites aegyptiaca. Journal of Phytopharmacology, 7:464-470. DOI: http://dx.doi.org/10.31254/phyto.2018.7602

Bakr MH (2020). Citrus pulp as an innovative feed ingredient in ruminant nutrition. A review. Egyptian Journal of Animal Production, 57:73-80. DOI: http://dx.doi.org/10.21608/EJAP.2020.98258

Bampidis VA and Robinson PH (2006). Citrus by-products as ruminant feeds: A review. Animal Feed Science and Technology, 128:175–217. DOI: https://doi.org/10.1016/j.anifeedsci.2005.12.002.

Barros HR de M, Ferreira TAP de C and Genovese MI (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. Food Chemistry, 134:1892–1898. DOI: http://dx.doi.org/10.1016/j.foodchem.2012.03.090.

Bashir M, Alagbe JO, Adegbite, MB and Omokore EA (2020). Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. International Journal of Zoology and Animal Biology, 3:1-8. DOI: https://doi.org/10.31579/2692-9406/022.

Bath DL, Dunbar JR, King JM, Berry SL, Leonard RO and Olbrich SE (1980). By-products and unsusual feedstuffs in livestock rations. Western Regional Extension Publication No. 39. USDA-ARS, Washington, DC, USA.

Botha Z (2003). Citrus waste-to-wealth project approved. Creamer Media's Engineering News. https://www.engineeringnews.co.za/article/citrus-wastetowealth-project-approved-2003-04-04 (Accessed, July 2021).

Castro LAd, Lizi JM, Chagas EGLd, Carvalho RAd, Vanin FM (2020). From orange juice by-product in the food industry to a functional ingredient: application in the circular economy. Foods, 9:593-611. DOI: https://doi.org/10.3390/foods9050593.

Comerford KB and Pasin G (2016). Emerging evidence for the importance of dietary protein source on glucoregulatory markers and type 2 diabetes: Different effects of dairy, meat, fish, egg, and plant protein foods. Nutrients, 8: 446-464. DOI: https://doi.org/10.3390/nu8080446.

Czech A, Zarycka E, Yanovych D, Zasadna Z, Grzegorczyk I and Kłys S (2020). Mineral Content of the Pulp and Peel of Various Citrus Fruit Cultivars. Biological Trace Element Research, 193:555–563. DOI: 10.1007/s12011-019-01727-1.

Djuric Z, DiLaura NM, Jenkins I, Darga L, Jen CK, Mood D, Bradley E and Hryniuk WM (2002). Combining weight-loss counselling with the weight watchers plan for obese breast cancer survivors. Obesity Research, 10: 657– 665. DOI: DOI: http://dx.doi.org/10.1038/oby.2002.89

Ensminger ME, Olentine Jr., CG (1978). Feeds and Nutrition, 1st ed. The Ensminger Publishing Company, Clovis, CA, USA.

Fabroni S, Romeo FV, Rapisarda, P (2016). Nutritional Composition of Clementine (Citrus x clementina) Cultivars. Nutritional Composition of Fruit Cultivars, 2016: 149-172. DOI: https://doi.org/10.1016/B978-0-12-408117-8.00007-6

FAO, Global initiative on food loss and waste reduction- Save food (2016).

Genovese S, Fiorito S, Locatelli M, Carlucci G and Epifano F (2014). Analysis of biologically active oxyprenylated ferulic acid derivatives in citrus fruits. Plant Foods for Human Nutrition, 69:255–260.

Gupta I, Clauder-Munster S, Klaus B, Jarvelin AI, Aiyar RS, Benes V, Wilkening S, Huber W, Pelechano V and Steinmetz, LM (2014). Alternative polyadenylation diversifies post-transcriptional regulation by selective RNA-protein interactions. Molecular Systems Biology, 10: 1–11. DOI: http://dx.doi.org/10.1002/msb.135068

Hounsome N, Hounsome B and Lobo MG (2018). Chapter 2: Biochemistry of Vegetables—Major classes of Primary metabolites (Carbohydrates, Amino acids, Vitamins, Organic acids and Fatty acids). In Handbook of Vegetables and Vegetable Processing; Siddiq, M., Uebersax, M.A., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2018; pp. 25–46.

Hussain J, ur Rehman N, Al-Harrasi A, Ali L, Ullah R, Mabood F, Hussain H, Ismail M (2011). Nutritional prospects and mineral compositions of selected vegetables from Dhoda sharif Kohat. Journal of Medicinal Plants Research, 5: 6509 – 6514. DOI: http://dx.doi.org/10.5897/JMPR11.478

Idamokoro EM, Hosu YS, Oyedeji OO, Miya GM, Kuria SK and Oyedeji AO (2022) A comparative analysis of the proximate and mineral composition of whole Citrus limon and Citrus clementina as a prospective alternative feed resource for livestock farming in South Africa. Frontiers in Sustainable Food Systems 6:1021175. DOI: http://dx.doi.org/10.3389/fsufs.2022.1021175

Citation: Idamokoro EM and Hosu YS (2023). Relative analysis of two citrus species versus maize for potential nutritive traits as livestock feed. *Online J. Anim. Feed Res.*, 13(5): 357-366. DOI: https://dx.doi.org/10.51227/ojafr.2023.51

Indrayan AK, Sharma S, Durgapa ID, Kumar N and Kumar K (2009). Nutritive value of some indigenous plant rhizome resembling ginger. Natural Product Radiance, 8:507-513. https://www.semanticscholar.org/paper/Nutritive-value-of-some-indigenous-plant-rhizomes-Indrayan-Agrawal/2f071493954e328ed79fa64cbc73e796292a435d.

Isong EU, Adewusi SAR, Nkanga EU, Umoh EE and Offiong EE (1999). Nutritional and phytogeriatological studies of three varieties of Gnetum africanum ('afang'). Food chemistry, 64: 489 - 493. DOI: https://doi.org/10.1016/S0308-8146(98)00139-3.

Jacobs JL (2014). Challenges in ration formulation in pasture-based milk production systems. Animal Production Science, 54: 1130-1140. DOI: https://doi.org/10.1071/AN14463.

Kamau JM, Mbui DN, Mwaniki JM and Mwaura FB (2020). Proximate analysis of fruits and vegetables wastes from Nairobi County, Kenya. Research Journal of Food Science and Nutrition, 5: 9-15. DOI: https://doi.org/10.31248/RJFSN2019.088.

Karasawa MM, and Mohan C (2018). Fruits as prospective reserves of bioactive compounds: a review. Natural products and bioprospecting, 8:335-346. DOI: https://doi.org/10.1007/s13659-018-0186-6

Kartika H, Shido J, Nakamoto ST, Li QX and Iwaoka WT (2011). Nutrient and mineral composition of dried mamaki leaves (*Pipturus albidus*) and infusions. Journal of Food Composition and Analysis, 24: 44–48. DOI: https://doi.org/10.1016/j.jfca.2010.03.027.

Ladaniya M (2008). Citrus fruit. Biology Technology and evaluation, Academic Press, Elsevier Inc. https://doi.org/10.1016/C2012-0-06022-X

Lieu PT, Heiskala M, Peterson PA and Yang Y (2001). The roles of iron in health and disease. Mol. Aspects Med. 22, 1–87. doi: DOI: http://dx.doi.org/10.1016/S0098-2997(00)00006-6

Luzardo S, Banchero G, Ferrari V, Ibáñez F, Roig G, Aznárez V, Clariget J, La Manna A (2021). Effect of Fresh Citrus Pulp Supplementation on Animal Performance and Meat Quality of Feedlot Steers. Animals, 11: 3338-3341. http://dx.doi.org/10.3390/ani11123338

Maheshwari S, Kumar V, Bhadauria G, and Mishra A (2022). Immunomodulatory potential of phytochemicals and other bioactive compounds of fruits: A review. Food Frontiers, 3(2):221-238. https://doi.org/10.1002/fft2.129

Manthey JA and Grohmann K (2001). Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. Journal of Agriculture and Food Chemistry, 49:3268–3273. https://doi.org/10.1021/jf010011r.

Marín FR, Soler-Rivas C, Benavente-García O, Castillo J and PérezAlvarez JA (2007). By-products from different citrus processes as a source of customized functional fibres. Food Chemistry, 100:736–741. https://doi.org/10.1016/j.foodchem.2005.04.040.

McDowell LR (1996). Feeding minerals to cattle on pasture. Animal feed science and technology, 60(3-4):247-71. https://doi.org/10.1016/0377-8401(96)00983-2

Morshedy SA, Zahran SM, Sabir SA and El-Gindy YM (2022). Effects of increasing levels of orange peel extract on kit growth, feed utilization, and some blood metabolites in the doe rabbits under heat stress conditions. Animal Biotechnology, DOI: http://dx.doi.org/10.1080/10495398.2022.2038615

National Research Council (NRC) (1988). Nutrient Requirements of Dairy Cattle, 6th rev. ed. National Academy Press, Washington, DC, USA.

National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle, 7th rev. ed. National Academy Press, Washington, DC, USA.

Nwofia GE, Ojimelukwe P and Eji C (2012). Chemical composition of leaves, fruit pulp and seeds in some *Carica papaya* (L) morphotypes. International Journal of Medicinal and Aromatic Plants, 2: 200 –206. https://www.academia.edu/47921749/Chemical_composition_of_leaves_fruit_pulp_and_seeds_in_some_Carica_papaya_L_morphotypes.

Ogbonna OA, Izund, AI, Okoye NH, Ikeyi AP (2016). Phytochemical compositions of fruits of three Musa species at three stages of development. IOSR Journal of Pharmacy and Biological Sciences, 11: 48 – 59. DOI: http://dx.doi.org/10.9790/3008-1103044859

Olanipekun MK, Adewuyi D and Adedeji DE (2016). Ethnobotanical importance and phytochemical analyses of some selected medicinal plants in Ado-Ekiti Local Govt. Journal of Herbal Medicinal Research, 1:7-16. https://www.iosrjournals.org/iosr-javs/papers/vol5-issue6/E0562831.pdf.

Omokore EO and Alagbe JO (2019). Efficacy of dried *Phyllantus amarus* leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits. International Journal of Academic Research and Development. 4: 97–104. http://dx.doi.org/10.55014/pij.v1i4.52

Poulsen M, Jensen BB and Engberg RM (2012). The effect of pectin, corn and wheat starch, inulin and pH on the in vitro production of methane, short chain fatty acids and on the microbial community composition in rumen fluid. Anaerobe, 18: 83-90. https://doi.org/10.1016/j.anaerobe.2011.12.009.

Rafiq S, Kaul R, Sofi SA, Bashir N, Nazir F, Ahmad Nayik G (2018). Citrus peel as a source of functional ingredient: a review. Journal of the Saudi Society of Agricultural Sciences, 17:351–358. https://doi.org/10.1016/j.jssas.2016.07.006.

Santos GT, Lima LS, Schogor ALB, Romero JV, De Marchi FE, Grande PA, et al. (2014). Citrus pulp as a dietary source of antioxidants for lactating holstein cows fed highly polyunsaturated fatty acid diets. Asian Australasian Journal of Animal Science, 27: 1104 -1113. http://dx.doi.org/10.5713/ajas.2013.13836

Sharif M, Ashraf MS, Mushtaq N, Nawaz H, Mustafa MI, Ahmad F, Younas M and Javaid A (2018). Influence of varying levels of dried citrus pulp on nutrient intake, growth performance and economic efficiency in lambs. Journal of Applied Animal Research, 46: 264-268. https://doi.org/10.1080/09712119.2017.1294540.

Shomar B (2012). Zamzam water: Concentration of trace elements and other characteristics. Chemosphere, 86: 600–605. https://doi.org/10.1016/j.chemosphere.2011.10.025.

Steyn L, Meeske R and Cruywagen CW (2017). Replacing maize grain with dried citrus pulp in a concentrate feed for Jersey cows grazing ryegrass pasture. South African Journal of Animal Science, 47: 553-564. http://dx.doi.org/10.4314/sajas.v47i4.14.

Sunagawa Y, Kawaguchi S, Miyazaki Y, Katanasaka Y, Funamoto M, Shimizu K, and et al. (2022). Auraptene, a citrus peel-derived natural product, prevents myocardial infarction-induced heart failure by activating PPARα in rats. Phytomedicine. ;107:154457. https://doi.org/10.1016/j.phymed.2022.154457

Torres-Leon C, Ramırez-Guzman N, Londono-Hernandez L, et al. (2018). Food waste and by products: An opportunity to minimize malnutrition and hunger in developing countries. Frontiers in Sustainable Food Systems, 2: 1-17. https://doi.org/10.3389/fsufs.2018.00052.

Tripodo MM, Lanuzza F, Micali G, Coppolino R, Nucita F (2004). Citrus waste recovery: a new environmentally friendly procedure to obtain animal feed. Bio-resource Technology, 91:111-155. https://doi.org/10.1016/S0960-8524(03)00183-4.

Citation: Idamokoro EM and Hosu YS (2023). Relative analysis of two citrus species versus maize for potential nutritive traits as livestock feed. *Online J. Anim. Feed Res.*, 13(5): 357-366. DOI: https://dx.doi.org/10.51227/ojafr.2023.51

Ülger I, Beyzi SB, Kaliber M and Konca Y (2020). Chemical, nutritive, fermentation profile and gas production of citrus pulp silages, alone or combined with maize silage. South African Journal of Animal Science, 50:161-169. http://dx.doi.org/10.4314/sajas.v50i1.17.

Van Dyk JS, Gama R, Morrison D, Swart S and Pletschke BI (2013). Food processing waste: Problems, current management and prospects for utilization of the lignocellulose component through enzyme synergistic degradation. Renewable and Sustainable Energy Reviews, 26:521-531. http://dx.doi.org/10.1016/j.rser.2013.06.016.

Wadhwa MWM (2015). Waste to worth: Fruit wastes and by-products as animal feed. CAB Reviews, 10: 1–26. https://doi.org/10.1079/PAVSNNR201510031.

Wang C, Gao GL, Huang JX, Zhang KS, Zhong H, Wang HW, Su J, Xie M and Wang QG (2017). Nutritive value of dry citrus pulp and its effect on performance in geese from 35 to 70 days of age. The Journal of Applied Poultry Research, 26: 253–259. https://doi.org/10.3382/japr/pfw069.

Wang S, Alseekh S, Fernie AR and Luo J (2019). The structure and function of major plant metabolite modifications. Molecular Plant, 12: 899 – 919. https://doi.org/10.1016/j.molp.2019.06.001.

Zema DA, Calabrò PS, Folino A, Tamburino V, Zappia G and Zimbone SM (2018). Valorisation of citrus processing waste: a review. Waste Management, 80:252–273. https://doi.org/10.1016/j.wasman.2018.09.024

Citation: Idamokoro EM and Hosu YS (2023). Relative analysis of two citrus species versus maize for potential nutritive traits as livestock feed. *Online J. Anim. Feed Res.*, 13(5): 357-366. DOI: https://dx.doi.org/10.51227/ojafr.2023.51

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EVALUATION OF OXIDATIVE STRESS PARAMETERS OF HORSES HOUSED WITH GOATS IN INDIVIDUAL BOXES

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Horses are animals that are affected very quickly by the warnings coming from the environment. In this study, it was aimed to evaluate oxidative stress parameters of horses obtained from saliva analysis related to animal welfare as a result of keeping horses together with goats. While the research was being prepared, three groups were developed based on the time spent sheltering goats and horses. The horses were housed alone in the first and last 15-day groups, and along with the goats in the second 15-day group. In these stages, the levels of malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities and ischemia-modified albumin (IMA) level in saliva were examined. Results showed that MDA and IMA levels decreased, but CAT, GPx, and SOD activities increased. It was concluded that goats had a positive effect on horses according to the oxidative stress parameters examined in terms of animal welfare. However, there is still a need for research that will house horses with various animals in acceptable animal welfare circumstances, analyse their stress metrics, and maintain a high level of welfare.

Keywords: Animal welfare, Equus caballus, Oxidative stress, Single stall housing, horse, goat.

INTRODUCTION

Behavior is described as a change in the condition of an animal's body in all or part of it, or in other words, the animal's reaction to its environment while it is in direct contact with it (Özbeyaz and Akçapınar, 2006). Most animals use behavior to adapt to and regulate their surroundings (Kappeler, 2010). Unsuitable care and feeding circumstances in animal husbandry might result in aberrant behavior in animals (Mellor, 2015; Arndt et al., 2022). Horse abnormal behaviors are characterized by a succession of unwanted, ineffective, and recurrent negative habits (McAfee et al., 2002; Gill et al., 2005).

Most research believes that abnormal behaviors are induced by the suppression of friendship, i.e., loneliness in individual compartments. As a result, offering friendship to other animals or establishing conditions that are not harmful to horses will benefit their welfare.

As with most animals, horses need someone to live their daily lives and social interactions and care. Isolating horses completely from their surroundings or from their social environment might result in the development of undesired disorders (Landsberg and Denenberg, 2019). Therefore, the primary goal of this research was to see how the enhanced environment created by the breeders for the goats influenced the horses, and the horses were allowed to interact with the goats.

It is known that unstimulated saliva plays an important role in oral immunity, enamel integrity and keeping the oral mucosa wet. Insufficient saliva flow causes dental caries, mucosal deterioration, and dry mouth (Ono et al., 2006). In the last 10 years, the use of saliva as a diagnostic fluid has become increasingly important. The use of saliva as a diagnostic material in the monitoring of drug metabolisms in autoimmune disorders, pharmacology, endocrinology, nephrology, infectious diseases, cardiovascular diseases, psychiatry, oncology, and many other fields is becoming widespread (Streckfus and Bigler, 2002). There are many defenses in the body against oxidative stress caused by free radicals, and the first line of these defenses is saliva. It has been stated that the attacks of free radicals on the oral mucosa can cause different results from infection to cancer. Saliva is the first defense step against free radicals with the antioxidant molecules (a rich source of antioxidants) and enzymes it contains (the salivary peroxidase system) (Maciejczyk et al., 2021).

Various antioxidant defense systems have been developed in biological systems to limit the formation of free radicals and the damage they cause. Glutathione peroxidase (GPx) is an antioxidant enzyme that contains the amino acid selenocysteine in its active site and is effective on H_2O_2, steroid and lipid hydroperoxides. The primary scavenging enzyme in scavenging oxygen free radicals (ROS) is superoxide dismutase, which converts O_2- to H_2O_2. GPx, on the other hand, is

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the key enzyme responsible for the detoxification of cellular H_2O_2. It transforms H_2O_2 into molecular oxygen and water (McCord, 2000; Wei et al., 2004). Saliva is the first biological agent to encounter foreign substances that enter our body through food, drink or inhalation and is the first defense step against free radicals (Streckfus and Bigler, 2002).

There is no scientific research in the literature that shows the change in some oxidative stress values that are considered important for animal welfare when goats and horses are housed together. For this reason, this research aims to show the changes in some oxidative stress parameters MDA, IMA, SOD, GPx and CAT activities that occur when keeping goats and horses in a paddock. In accordance with all of the observations, literature, and reviews, the hypothesis of this study is that goats will contribute positively to some oxidative stress parameters of horses that are regarded as important in terms of animal welfare.

MATERIALS AND METHODS

Research plan

While forming the study groups of this research, three groups were formed considering the residence times of goats and horses together and separately. During the first and last 15 days of these groups, horses were housed alone, and in the second 15-day group, horses were housed together with goats. At the end of each 15-day stage, saliva samples were taken from horses, and the materials for the research were created.

Ethical approval

Experimental procedures adopted in this study were complied with the ARRIVE guidelines (Percie du sert et al., 2020).

The test area, animals and management

Figures 1 and 2 show the research areas in the barn where the horses stay alone or with the goats. In these areas, horses and goats were housed together (19:00 to 08:00 h) while the horses were in the stables.

The horse barn where the research was conducted had a length of 19.5 meters, a width of 9.5 meters, and a middle roof height of 6 meters. The horses in the stable used in the research were sheltered in six individual boxes surrounded by iron fences over the walls. The individual compartment of each horse, where the study was carried out, had an area of 3.5 x 3 m2. The service road in the middle of the barn is 2.8 meters wide.

The breeds of six horses used in the study were 3 Thoroughbred (1 gelding, and 2 mare) and 3 Haflinger (1 stallion, 1 gelding, and 1 mare), and their ages ranged from 9 to 20. Horses and goats were given both concentrate and roughage twice a day at 08:00 and 19:00 while they were in the barn. The nutritional needs of the animals used in the study were given daily by the breeders. The needs of these nutrients were calculated by the enterprise and presented as roughage and concentrate. Goats used in the research—breed-hair goats—were used to be 6 months old and female.

To meet the daily water needs of horses and goats, they always had clean water in front of them. The cleaning and maintenance of their litter material was done daily at 08:00. The breeders and observers at the shelter constantly checked the general condition of the horses and goats. Everything needed by horses and goats in the animal shelter was met as much as possible. In addition, horses and goats were grazed and irrigated in an outdoor paddock area during daylight hours convenient for observers.

Figure 1 - The horse's box stalls.

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Figure 2 - Housing horse and goat in the box stall.

Accustoming goats to horses

Initially, for a certain period of time, horses and goats eventually become acquainted with one another. The goats were permitted to stay with the horses for 15 days (16-30 days) after becoming acclimated to them during the second groups days of the research. One horse stayed with a goat during the research while the goats and horses were together. The same goat was placed next to the same horse each time.

Saliva analysis

Saliva samples were taken from the mouths of all Horses in the morning on an empty stomach by means of swaps. A total of 18 (6x3) saliva samples were collected on the 15th, 30th and 45th days of the study. In saliva samples taken; MDA level (Yoshioka et al., 1979), CAT (Goth, 1991), GPx (Matkovics et al., 1988), SOD activities (Sun et al., 1988) and IMA (Bar-Or et al., 2000) levels were measured with Biotek ELISA Reader (Bio Tek µQuant MQX200 Elisa reader/USA).

Statistical analysis

To examine the outcomes of salivary data from horses, a statistical program was used. (SPSS, Version 25, IBM Corp., Armonk, NY, USA). With this program, oxidative stress parameter variables (MDA, SOD, CAT, GPx, and IMA) obtained from horse saliva analysis were analyzed using the GLM procedure. Tukey multiple range tests were performed on unique groups according to the results. If the level of difference was $P<0.05$ in the whole data analysis, it was considered statistically significant.

RESULTS AND DISCUSSION

The results obtained between the study groups formed at 15-day intervals are shown in Table 1. As a consequence of the research groups, the investigated parameters of horses, MDA level ($P<0.05$), SOD ($P<0.01$), CAT ($P<0.01$), GPx activities ($P<0.01$) and IMA level ($P<0.001$) were significantly affected by goats. According to the evaluations made between the observation groups (1-3), it was determined that MDA and IMA levels decreased, but CAT, GPx, and SOD activities increased after the goats were placed next to the horses.

While Figure 3 shows that a goat even rides a horse, and the horse did not showed any attitude towards it, the results of the saliva analysis were also an indication that the goats have a beneficial impact on the horses in the same direction. Therefore, it can be concluded that keeping the goat with the horse does not prevent the welfare of the horse but is also positive for the health of the animal and reduces stress. However, as with every animal, it should not be forgotten that horses can unintentionally harm goats due to their large body size, and precautions should be taken to prevent this.

The current study is the first to report on the effect of goats on animal welfare-related oxidative stress parameters (MDA, CAT, GPx, SOD and IMA) in box stall horses. Individual stable boxes are normal practice for many racehorse breeders when it comes to performing horses like racehorses (Munoz et al., 2014; Munoz et al., 2018). Similarly to prior research, the six box stalls used for this study kept their horses in separate stable boxes (Normando et al., 2002; Hockenhull and Creighton, 2010; Leme et al., 2014; Hanis et al., 2020).

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Horses are still mostly confined in single stalls, despite mounting scientific evidence that single-stall living is detrimental to horse wellbeing. As a result, social relationships are critical, and they are a requirement for ensuring excellent horse welfare (Lesimple et al., 2020). In this research, we used goat, a live material, for horse socialization, and we discovered positive responses in certain oxidative stress measures related to animal wellbeing.

As with many creatures, horses are creatures that need social contact or communication with other living things during the day. Isolating horses from social life and communication may cause undesirable problems for them. The communication between goats and horses was ensured in this study, and their idle time was reduced. Also, they were observed less as a consequence of these regulations of undesired blood results related to animal welfare.

Table 1 - Oxidative stress parameter values observed at 15-day intervals in the research group consisting of Thoroughbred and Haflinger horses (Mean ± SD).

Variable	Group 1 (0-15 days)	Group 2 (16-30 days)	Group 3 (31-45 days)	*P* value
MDA (nmol/mL)	1.90 ± 0.06 ^{ab}	1.86 ± 0.50 ^{b}	1.92 ± 0.06 ^{a}	0.022
SOD (U/mL)	15.59 ± 0.75 ^{ab}	16.05 ± 0.52 ^{a}	15.13 ± 0.92 ^{b}	0.018
CAT (IU/mL)	16.39 ± 1.88 ^{b}	18.45 ± 1.68 ^{a}	16.00 ± 2.31 ^{b}	0.010
GPx (U/L)	0.19 ± 0.03 ^{ab}	0.21 ± 0.03 ^{a}	0.18 ± 0.02 ^{b}	0.008
IMA (ΔABSU)	0.18 ± 0.02 ^{a}	0.12 ± 0.01 ^{b}	0.17 ± 0.03 ^{a}	<0.001

The influence of differences between observation periods is shown by the P values; different superscript letters within each row indicate differences between groups (P < 0.05). MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, IMA: Ischemia-modified albumin

Figure 3 - A goat has ridden a horse.

CONCLUSION

When the data collected after the goats were placed near to the horses was analyzed, it was discovered that MDA and IMA levels fell while CAT, GPx, and SOD activities increased. These oxidative stress parameter results show that goats have a positive effect on the animal welfare of horses. Because horses are sensitive to their surroundings, their

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environment might induce stress parameter values that produce significantly diverse effects for them. So, there is a need for research that will house horses with various animals in acceptable animal welfare circumstances, analyze their stress metrics, and maintain a high level of welfare.

DECLARATIONS

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Ethical statement for publication

All authors have reviewed the ethical issues.

Authors' contribution

Both authors contributed to the field studies, data collection, and experiment design stages of the research. While B. Apaydin Yildirim did their saliva analysis, F. Yildirim analyzed the data statistically. Both authors contributed to the article's writing and read and approved the final article.

Data availability

The data used and analyzed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors have not declared any conflict of interest.

REFERENCES

Arndt SS, Goerlich VC and Van Der Staay FJ (2022). A dynamic concept of animal welfare: the role of appetitive and adverse internal and external factors and the animal's ability to adapt to them. Frontiers in Animal Science, 3: 908513. https://doi.org/10.3389/fanim.2022.908513

Bar–Or D, Lau E and Winkler JV (2000). A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia—a preliminary report. The Journal of Emergency Medicine, 19: 311-315. https://doi.org/10.1016/s0736-4679(00)00255-9

Gill W, Meadows DG and Neel VB (2005). Understanding horse behaviour, Agricultural Extension service, Institute of Agriculture, 4-H Member Guide, Unit 8, Grade 12, Tennessee. https://www.shelbycountytn.gov/DocumentCenter/View/1177/Understanding-Horse-Behavior?bidId=

Goth L (1991). A simple method for determenation of serum catalase activity and revision of serum catalase activity and revision of reference range. Clinica Chimica Acta, 196: 143–152. https://doi.org/10.1016/0009-8981(91)90067-m

Hanis F, Chung ELT, Kamalludin MH and Idrus Z (2020). The influence of stable management and feeding practices on the abnormal behaviors among stabled horses in Malaysia. Journal of Equine Veterinary Science, 94: 103-230. https://doi.org/10.1016/j.jevs.2020.103230

Hockenhull J and Creighton E (2010). Unwanted oral investigative behaviour in horses: A note on the relationship between mugging behaviour, hand-feeding titbits and clicker training. Applied Animal Behaviour Science, 127(3-4): 104-107. https://doi.org/10.1016/j.applanim.2010.08.008

Kappeler P and Kraus C (2010). Levels and mechanisms of behavioural variability. Animal Behaviour: Evolution and Mechanisms, Springer-Verlag Berlin Heidelberg, 655-684. https://doi.org/10.1007/978-3-642-02624-9_21

Landsberg GM and Denenberg S (2019). Behavior problems in horses, MSD Veterinary Manual, Merck Sharp, and Dohme Corp., a subsidiary of Merck and Co., Inc., Kenilworth, NJ, USA. https://www.msdvetmanual.com/horse-owners/behavior-of-horses/behavior-problems-in-horses

Leme DP, Beatriz A, Parsekian H, Kanaan V and Jose M (2014). Management, health, and abnormal behaviors of horses: A survey in small equestrian centers in Brazil. Journal of Veterinary Behavior, 9: 114-118, http://dx.doi.org/10.1016/j.jveb.2014.01.004

Lesimple C, Reverchon-Billot L, Galloux P, Stomp M, Boichot L, Coste C and Hausberger M (2020). Free movement: A key for welfare improvement in sport horses?. Applied Animal Behaviour Science, 225: 104972. https://doi.org/10.1016/j.applanim.2020.104972

Maciejczyk M, Bielas M, Zalewska A and Gerreth K (2021). Salivary biomarkers of oxidative stress and inflammation in stroke patients: from basic research to clinical practice. Oxidative Medicine and Cellular Longevity, 1-22. https://doi.org/10.1155/2021/5545330

Matkovics B, Szabo L and Varga IS (1988). Determination of enzyme activities in lipid peroxidation and glutathione pathways. Laboratoriumi Diagnosztika, 15: 248–250. https://cir.nii.ac.jp/crid/1572824500566167680

McAfee LM, Mills DS and Cooper JJ (2002). The use of mirrors for the control of stereotypic weaving behaviour in the stabled horse. Applied Animal Behaviour Science, 78: 159-173. https://doi.org/10.1016/S0168-1591(02)00086-2

McCord JM (2000). The evalution of free ardicals and oxidative stres. The American Journal of Medicine, 108 (8), 652-659. https://doi.org/10.1016/S0002-9343(00)00412-5

Mellor DJ (2015). Positive animal welfare states and encouraging environment-focused and animal-to-animal interactive behaviours. New Zealand Veterinary Journal, 63(1): 9-16. https://doi.org/10.1080/00480169.2014.926800

Munoz AL, Ainardi CF, Rehhof VC, Cruces LJ, Ortiz RR and Briones LM (2014). Prevalence of stereotypies in thoroughbred race horses at Club Hípico Concepcion, Chile. Revista MVZ Córdoba, 19: 4259-4268. http://www.scielo.org.co/pdf/mvz/v19n3/v19n3a07.pdf

Munoz L, Leon C, Cruces J, Ortiz R and Briones M (2018). Locomotor stereotypies and racing performance in thoroughbred horses. Journal of Veterinary Behavior, 25: 24-27. https://doi.org/10.1016/j.jveb.2018.03.006

Normando S, Canali E, Ferrante V and Verga M (2002). Behavioral problems in Italian saddle horses. Journal of Equine Veterinary Science, 22: 117-120. https://doi.org/10.1016/S0737-0806(02)70123-8

Ono K, Morimoto Y, Inoue H, Masuda W, Tanaka T and Inenaga K (2006). Relationship of the unstimulated whole saliva flow rate and salivary gland size estimated by magnetic resonance image in healthy young humans. Archives of Oral Biology, 51(4): 345-349. https://doi.org/10.1016/j.archoralbio.2005.09.001

Özbeyaz C and Akçapınar H (2006). Horse breeding [English], Ankara University, Faculty of Veterinary Medicine, Animal Science, Ankara, 2006. https://acikders.ankara.edu.tr/course/

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M and et al. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Journal of Cerebral Blood Flow & Metabolism, 40(9): 1769-1777. https://doi.org/10.1177/0271678X2094382

Streckfus CF and Bigler LR (2002). Saliva as a diagnostic fluid. Oral Diseases, 8(2): 69-76. https://doi.org/10.1034/j.1601-0825.2002.1o834.x

Sun Y, Oberley LW and Li Y (1988). A simple method for clinical assay of superoxide dismutase. Clinical Chemistry, 34: 497-500. https://doi.org/10.1093/clinchem/34.3.497

Wei PF, Ho KY, Ho YP, Wu YM, Yang YH and Tsai CC (2004). The investigation of glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1β in gingival crevicular fluid: implications for oxidative stress in human periodontal diseases. Journal of Periodontal Research, 39: 287-293. https://doi.org/10.1111/j.1600-0765.2004.00744.x

Yoshioka T, Kawada K and Shimada T (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. American Journal of Obstetrics and Gynecology, 135(3): 372-376. https://doi.org/10.1016/0002-9378(79)90708-7

Citation: Yildirim F and Apaydin Yildirim B (2023). Evaluation of oxidative stress parameters of horses housed with goats in individual boxes. *Online J. Anim. Feed Res.*, 13(5): 367-372. DOI: https://dx.doi.org/10.51227/ojafr.2023.52

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PHYSICO-CHEMICAL AND SENSORY QUALITY OF PEKIN DUCK JERKY SONICATED WITH COCONUT SHELL LIQUID SMOKE AND STORED FOR DIFFERENT PERIODS

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ABSTRACT: This study aimed to determine the effect of adding sonicated coconut shell liquid smoke to pekin duck jerky with different storage times at room temperature and vacuum packed. Ground duck jerky is made from Pekin duck meat (*Anas platyrhynchos domesticus*) soaked in coconut shell liquid smoke (CSLS) which has been sonicated for 20 minutes and seasoned with spices such as garlic, galangal, coriander, tamarind, salt, and coconut sugar. A laboratory experiment was done using a completely randomized design (CRD) consisting of 5 treatments (control: 0 day storage period, T1: 7 days, T2: 14 days, T3: 21 days, and T4: 28 days) and 4 replications. The results showed that the addition of sonicated CSLS with differences in the shelf life of pekin ground duck jerky had a significant effect ($P<0.01$) on pH, texture, color L, a*, b*, Aw, water content, fat, carbohydrates by difference, thiobarbituric acid (TBA), and iodine number. Had a significant effect ($P<0.05$) on ash content, and had no significant effect on Water Holding Capacity (WHC), protein content, and organoleptic quality. It was concluded that storing ground duck jerky for 14 days at room temperature and vacuum packed did not show any damage to pH, water activity, water content, fat, protein, TBA and iodine number, and did not occur rancidity.

Keywords: Liquid smoke, Jerky, Pekin duck, Shelf life, Sonication.

INTRODUCTION

The livestock sector in Indonesia needs to be developed more broadly to meet food needs and the community's nutritional needs. Duck is a type of poultry that fulfills animal protein because a fast growth rate supports it (Inayat et al., 2023). The Pekin duck (*Anas platyrhynchos domesticus*) is a bird species in the Anatidae family. Nutrient content such as high levels of water, protein, and fat can accelerate the process of decay in meat. Fresh meat that is not immediately processed will quickly rot due to infection and putrefaction by bacteria and fungi (Febrianta, 2022). The thick layer of fat in duck is found under the skin, making duck meat have thick skin. Duck meat has a high-fat content compared to other poultry, such as chicken and turkey. Poultry fat generally consists primarily of unsaturated fatty acids (Banaszak et al., 2020). Duck meat contains unsaturated fatty acids that reach more than 60% of the total fatty acids and is high in essential amino acids, which makes the oxidation process easier which can cause rancidity and reduce nutritional quality (Jin et al., 2021). Fresh duck meat is easily damaged if not processed immediately or given special handling and has a distinctive fishy smell. Therefore, preservation and processing of meat are carried out to prevent the decline in quality and nutrition and increase the taste consumers can accept. High fat in food affects sensory values such as increased textural and flavor qualities, but fat makes the food vulnerable to rancid smell. Duck meat not immediately processed will quickly become rancid due to oxidation. The oxidation process occurs due to damage by enzymes due to the presence of microbes in fat, thus affecting the shelf life and causing damage to sensory characteristics, which can reduce nutritional value.

Processing jerky is a food diversification to increase the taste that consumers can accept and improve quality. Jerky is a processed product of fresh meat added with spices and dried. The process of making jerky can be done by two methods, namely, the method of slice and grind. The drying method for making jerky can be done by drying with the help of sun heat and using an oven. The advantage of the sun heat method is that it is more economical but has the disadvantage that it takes a long time, depends on weather conditions, and sanitation is not maintained so that the quality of the jerky is not good. The advantage of using an oven is that the temperature can be adjusted, but it can result in case hardening, namely the condition of the outside (surface) of the jerky becoming dry while the inside is still wet (Kim et al., 2022). Ground jerky is a traditionally processed meat product made from ground beef added with coconut sugar, table salt, and spices, then printed into thin sheets with a thickness of approximately 3 mm and dried (Sorapukdee et al., 2016). Dried jerky is brown because of the maillard reaction, a non-enzymatic browning reaction between sugar and protein. The maillard reaction is a reaction that occurs between carbohydrates and primary amino acid groups, which produce a brown color in jerky (Dewi et al., 2020).

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Coconut shell liquid smoke can extend the storage period and improves sensory quality in color, texture, and jerky flavor. Liquid smoke has functional properties as an antioxidant, antibacterial and forms a distinctive color and taste. Liquid smoke can preserve food ingredients due to the presence of acid compounds, phenolic derivatives, and carbonyls (Himawati et al., 2018). Liquid smoke has antimicrobial properties and antioxidant compounds such as phenols and organic acids, so that it can be a safe preservative. The advantages of using liquid smoke are that it is easy to apply, fast, even product uniformity, produces good food characteristics, and is environmentally friendly. The component components of liquid smoke consist of the three most significant constituent compounds, namely acids which can affect the product's taste, pH, and shelf life. Carbonyl, which reacts with protein, will form a brown color in food, and phenol is the main constituent of aroma and shows antioxidant activity (Desvita et al., 2020).

The sonication method on CSLS is used to reduce the particle size of the liquid smoke to make it easier for compound components to absorb into duck meat during the soaking process so that more compound components are produced. The sonication method aims to reduce the sample particle size so that it can absorb more optimally. The non-thermal sonication method breaks down sample particles, enhances mass transfer, and homogenizes without harming molecular structures, while low temperatures prevent the loss of compounds with low boiling points (Franca-Oliveira et al., 2021). In addition, sonication or ultrasonic processes can reduce the sample size to nano size so that the texture of the sample is softer and smoother because the particle size is getting smaller (Agustini et al., 2021). The advantage of the sonication method is that it does not result in significant changes in the chemical structure of the particles and compounds of the raw materials used.

The addition of sonicated CSLS to the production of ground duck jerky has not been widely studied. Therefore, it is necessary to examine the effect of adding sonicated CSLS on the storage period of ground duck jerky in terms of physical, chemical, and organoleptic quality.

Ethical regulation of study

Research materials

a) The ingredients used to make jerky consisted of male pekin duck (*Anas platyrhynchos domesticus*) meat (breast, thigh, and wings) aged 50 days. Lubna brand coconut shell liquid smoke was purchased online for as much as 1.5%, while other ingredients used were table salt, coconut sugar, garlic, galangal, coriander, and tamarind.

b) Equipment for the production of duck jerky includes chopper Philips brand, knife, digital scale i2000 scale, label, glass mould, beaker glass, stirrer, dropper pipette, PE plastic (polyethylene), food dehydrator brand Tiross LT-06, ultrasonic processor brand Biomaisen model UCD-250, spoon, cutting board, slicer, machine, and plastic vacuum.

Research methods

A laboratory experimental method using a Completely Randomized Design (CRD) has been used. The study consisted of 5 treatments with a shelf life of control: 0 days, T1: 7 days, T2: 14 days, T3: 21 days, and T4: 28 days with each treatment consisting of 4 replications, so there were 20 experimental units.

Procedure for making ground duck jerky

Thoroughly wash the duck breast, thighs, and wings, then grind the meat until smooth. The ground meat is soaked for 30 minutes in liquid smoke with a concentration of 1.5% of distilled water which has been sonicated for 20 minutes, and then the meat is drained. After that, the meat is mashed and mixed thoroughly with spices such as garlic, galangal, coriander, tamarind, salt, and coconut sugar. The meat is printed with a glass mold measuring (20×18) cm with a thickness of 3 mm and placed on polyethylene plastic, then dried in a food dehydrator for 3 hours 15 minutes at 50^{0}C and turned every hour so that it dries evenly. Jerky was removed from the food dehydrator and cooled to room temperature. Jerky packaging was carried out in a vacuum and stored at room temperature for 0, 7, 14, 21, and 28 days.

Research analysis variables

Testing pH using a pH meter (Bishnoi et al., 2006). Water Holding Capacity (WHC) testing uses centrifugation (Chan et al., 2022). Texture testing uses a texture analyzer (Lis et al., 2021). L, a*, b* color testing using a color reader (Kulapichitr et al., 2022). Testing Water Activity (Aw) using an Aw meter (Husna et al., 2020). Testing for water, fat, and protein content uses the FOSS Food Scan method (Anderson and Collaborators:, 2007). Ash content testing and testing carbohydrate Levels by difference using the total carbohydrate method by difference (Yirmaga, 2013). Thiobarbituric acid (TBA) testing uses spectrophotometry (Zeb and Ullah, 2016). Testing iodine numbers using titrimetric (Rahmah et al., 2019). The organoleptic quality test used the descriptive test method using a score range of 1-5 and using 5 trained panelists and compared it with commercial beef jerky.

The organoleptic quality rating scale is: Color 5 = dark brown, 4 = brown, 3 = brownish red, 2 = red, and 1 = blackish brown. Texture 5 = very soft, 4 = soft, 3 = slightly soft, 2 = hard, and 1 = very hard. Aroma 5 = strongly smells of spices and smoke, 4 = smells of spices and smoke, 3 = slightly smells of spices and smoke, 2 = only smells of spices, and 1 = only smells of smoke. Taste 5 = strongly tastes meat and smoke, 4 = tastes meat and smoke, 3 = slightly tastes meat and smoke, 2 = only tastes meat, and 1 = only tastes smoke. Overall acceptance 5 = very acceptable, 4 = acceptable, 3 = somewhat acceptable, 2 = not accepted, and 1 = very not accepted.

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Data analysis

The data obtained in this study were analyzed using a variety of Analyses of Variance (ANOVA) using Complete Random Design (CRD). If the results obtained significantly or very significantly different data, then it was continued with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The average pH value, water holding capacity, and texture are shown in Table 1.

Table 1 – Average value pH, water holding capacity, and texture

Treatments	pH	Water holding capacity (%)	Texture (N)
Control	5.64 ± 0.04[a]	67.75 ± 3.86	7.69 ± 0.24[a]
T1	5.69 ± 0.03[a]	69.50 ± 2.89	8.39 ± 0.27[ab]
T2	5.75 ± 0.04[ab]	71.00 ± 4.24	9.22 ± 0.21[b]
T3	6.43 ± 0.05[b]	68.25 ± 3.10	9.47 ± 0.30[bc]
T4	6.54 ± 0.06[b]	65.50 ± 2.65	9.89 ± 0.18[bc]

[a,b,c,d:] Means different superscripts in the same column show a very significant effect (P<0.01). T1: storage period for 7 days, T2: 14 days, T3: 21 days, T4: 28 days

Effect of shelf life of ground duck jerky on pH value

The analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the pH value of ground duck jerky. Table 1 shows that the average pH value of ground duck jerky has increased with increasing storage time. The average pH value ranged from 5.64 to 6.54. The increase in pH value and the length of storage in ground duck jerky is due to the activity of microorganisms. Microbial activity is responsible for the rise in pH and prolonged storage of ground duck jerky (Fadlilah et al., 2020). The pH level significantly impacts the microbial population, with higher pH values promoting optimal microbial growth (Domínguez et al., 2022). During storage, bacteria can break down chemical compounds in meat, particularly proteins, into simpler compounds, leading to the production of substances like NH_3 and H_2S (Domínguez et al., 2022). The length of storage of jerky will be followed by an increase in pH due to the penetration of free water in the air. Penetration of free water from the air into the beef jerky makes the jerky moist and a suitable medium for the growth of bacteria. Based on the previous result, pH will increase with the more extended storage due to food undergoing chemical changes caused by a decrease in protein levels by microorganisms so that it can reduce the shelf-life of the resulting product (Amit et al., 2017). Proteolytic bacteria can enzymatically hydrolyze free amino acids, which cause an increase in the pH of food products. Foods with a pH close to neutral pH contain more numbers and types of bacteria. Microbial degradation of amino acids in meat leads to an increase in pH, as microbes utilize the remaining molecules as an energy source, resulting in elevated levels of NH_3 and H_2S (Diether and Willing, 2019).

The effect of ground duck jerky shelf life on water holding capacity

The results of the analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had no significant effect (P>0.05) on the water holding capacity (WHC) of ground duck jerky. Table 1 showed that the average WHC value ranges from 65.50% to 71.00%. The lowest average is found on T5, or a storage period of 28 days, and the highest average is on T2, with a storage period of 14 days. The water holding capacity (WHC) of ground duck jerky decreased to 68.25% and 65.50% during the 21-day and 28-day shelf life, respectively. This decline can be attributed to microbial activity, which damages proteins and reduces their ability to bind to water. According to Domínguez et al. (2022), the reduction in WHC is a consequence of microbial activity during storage, leading to protein damage and a subsequent decrease in their water-binding capacity. Decreased protein levels in meat during storage can weaken the meat's ability to bind water so that the WHC value of the meat decreases. The decrease in water binding capacity is caused by the increasing amount of lactic acid, which results in many myofibril proteins being damaged, so that the protein's ability to bind with water becomes low. Phenolic compounds in liquid smoke can bind water by loosening the bonds of meat fibers so that some of the freely bound water will enter the inner space of the meat so that the WHC increases (Indiarto et al., 2019). The WHC value will affect the level of meat tenderness during processing. The higher the WHC value, the better the meat quality. The tenderness of jerky is related to consumer palatability. The high WHC value is caused by the high content of intramuscular fat that has not been oxidized and high protein content. High pH conditions can also cause an increase in the water holding capacity value.

Effect of shelf life of ground duck jerky on texture

The analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the texture value of ground duck jerky. Table 1 show that the average texture value of ground duck jerky ranges from 7.69 to 9.89 N. Ground duck jerky has a texture value that increases with the length of storage. Increasing the texture value of the ground duck jerky indicates that the

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texture of the jerky is getting harder during storage. The rise in the texture of ground duck jerky is associated with a reduced protein content in the product, as proteins have a lower capacity to bind water. This is consistent with a prior study that also indicated that protein content can impact the hardness of a product (Arandini et al., 2022). Products that are stored for a long time can reduce protein levels so that the protein's ability to bind water becomes low and will cause the product texture to become harder. Phenolic compounds in liquid smoke can form hydrogen bonds with water, thereby affecting the ability of meat to retain water. The WHC value will affect the tenderness, elasticity and texture of the meat. A low WHC value will make meat products tough. Changes in the tenderness of the jerky during storage are due to changes in the protein in the jerky, the higher the tenderness value, the harder the texture of the jerky.

The influence of the shelf life of ground duck jerky on color L, a*, b*

Data and color analysis results for lightness (L), redness (a*), and yellowness (b*) are presented in Table 2.

Table 2 – Average value of lightness (L), redness (a*), and yellowness (b*)

Treatments	Lightness (L)	Redness (a*)	Yellowness (b*)
Control	29.41 ± 0.10^{ab}	11.36 ± 0.54^{a}	4.80 ± 0.47^{a}
T1	29.39 ± 0.53^{a}	12.38 ± 0.85^{ab}	5.58 ± 0.49^{ab}
T2	30.90 ± 0.98^{bc}	15.93 ± 0.62^{b}	8.25 ± 0.26^{b}
T3	31.15 ± 0.62^{c}	17.11 ± 0.74^{bc}	8.44 ± 0.55^{bc}
T4	30.88 ± 0.26^{b}	18.09 ± 0.61^{c}	8.83 ± 0.22^{bc}

$^{a,b,c,d;}$ Means different superscripts in the same column show a very significant effect (P<0.01). T1: storage period for 7 days, T2: 14 days, T3: 21 days, T4: 28 days

Lightness (L)

The results of the analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant (P<0.01) effect on the lightness (L) of ground duck jerky. Table 2 shows that the average lightness value (L) ranges from 29.39 to 31.15. Pekin duck jerky tended to increase lightness during storage, with the highest average lightness at 21 days and the lowest at 7 days. The lightness increase in ground duck jerky is due to the liquid smoke that can function as an antimicrobial and antioxidant to slow down microbial growth and inhibit fat oxidation which can affect the light color of the duck jerky. Meat that has decreased color during storage is due to fat oxidation. Adding liquid smoke to meat not only helps maintain the meat's color during storage but also acts as an antimicrobial agent, inhibiting fat oxidation (Akilie et al., 2021).

Lightness level of a product is indicated by the lightness (L) value using a colorimeter. The lightness level ranges from 0-100. The closer to 100, the lightness it is, while the closer to 0, the darker it is. Changes in the color of the meat during storage are caused by microbial growth in the meat, which can cause discoloration to darken, and the lightness of the meat can decrease. The color change is caused by a change or destruction of the meat pigment, namely myoglobin which is oxidized to brown metmyoglobin (Domínguez et al., 2022). The lightness of the jerky produced can be caused by coconut sugar, Maillard reaction, and sugar caramelization. The browning reaction in foods containing sugar can be accelerated by heating so that the reducing sugar component will form a brown compound (Montazeri et al., 2013). In the Maillard reaction or browning reaction, the carbonyl group of reducing sugar reacts with the amino group of meat protein and amino acids non-enzymatically to produce a dark brown color in meat (Kim et al., 2022).

Redness (a*)

The results of the analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the redness (a*) of ground duck jerky. Table 2 shows that the average redness value (a*) ranges from 11.36 to 18.09. Duck jerky shows a redder color. The redness of duck jerky is affected by a non-enzymatic browning reaction. The presence of reducing sugars and heat-triggered proteins will result in a non-enzymatic browning reaction (Maillard reaction). One of the results of this reaction is the presence of a brownish-red product. The red hue of duck jerky is influenced by coconut sugar, imparting a caramelizing effect that results in a brownish-red color. The organic acid compounds in coconut shell liquid smoke contribute to the red color, while phenols and carbonyls are responsible for the brown coloration in the smoke product (Himawati et al., 2018).

Yellowness (b*)

The results of the analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the yellowness (b*) of ground duck jerky. Table 2 shows that the average yellowness value (b*) ranges from 4.80 to 8.83. Yellowness color is produced due to a chemical reaction between phenol and oxygen and between protein and carbonyl in liquid smoke. The higher the oxygen, phenol, and carbonyl levels in liquid smoke, the more golden or brownish the color of the meat will be. The characteristic brownish-yellow color of smoked products is attributed to phenolic compounds present in liquid smoke, such as guaiacol, syringol, cresol, phenols, and ethers (Mathew et al., 2015). Montazeri et al. (2013) stated that the golden to brownish yellow color arises due to the interaction between phenol and oxygen, which produces a brownish

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yellow color on the surface of the food being smoked. The average values of water activity (Aw), water content, fat, protein, ash, carbohydrates by difference, TBA, and iodine number are shown in Table 3.

Table 3 – Average value of water activity, moisture content, fat, protein, ash, carbohydrate by difference, TBA., and iod number

Variables \ Treatments	Control	T1	T2	T3	T4
Water activity (Aw)	0.64 ± 0.04^{a}	0.68 ± 0.03^{ab}	0.71 ± 0.02^{ab}	0.74 ± 0.04^{b}	0.78 ± 0.02^{b}
Water content (%)	39.94 ± 0.43^{a}	42.77 ± 0.27^{b}	43.90 ± 0.98^{bc}	44.84 ± 0.43^{c}	47.06 ± 0.65^{d}
Fat level (%)	9.05 ± 0.42^{a}	10.09 ± 0.30^{b}	10.20 ± 0.20^{bc}	10.29 ± 0.47^{c}	10.72 ± 0.24^{c}
Protein level (%)	29.17 ± 0.22	29.24 ± 0.28	29.26 ± 0.50	29.13 ± 0.17	28.82 ± 0.41
Ash content (%)	0.39 ± 0.06^{bc}	0.41 ± 0.08^{c}	0.36 ± 0.05^{bc}	0.31 ± 0.02^{b}	0.26 ± 0.05^{a}
Carbohydrate by difference (%)	21.46 ± 0.18^{e}	17.49 ± 0.61^{d}	16.28 ± 0.74^{c}	15.44 ± 0.86^{b}	13.25 ± 0.44^{a}
TBA. (µM/g)	0.53 ± 0.06^{a}	0.56 ± 0.03^{ab}	0.59 ± 0.02^{b}	0.62 ± 0.02^{bc}	0.63 ± 0.02^{c}
Iodine number (g)	83.73 ± 2.59^{d}	79.87 ± 1.68^{c}	77.93 ± 2.01^{bc}	77.06 ± 1.40^{b}	76.95 ± 1.33^{a}

$^{a,b,c,d;}$ Means different in the same line show a very significant (P<0.01) effect on Aw, water content, fat, carbohydrates by difference, TBA, and iodine number, and have a significant (P<0.05) effect on ash content. T1: storage period for 7 days, T2: 14 days, T3: 21 days, T4: 28 days

Effect of shelf life of ground duck jerky on water activity (Aw)

The results of the analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the water activity (Aw) of ground duck jerky. Table 3 shows that the average Aw ranges from 0.64 to 0.78. Ground duck jerky experienced an increase in Aw with longer storage time. The increase in Aw was related to the increase in water content in duck jerky, with increasing storage time and humidity during storage also affecting the Aw value of jerky. Humidity in the environment during the storage period at room temperature is related to the water content value. The higher the water content, the higher the water activity value (Aw). An increase in water activity (Aw) is associated with humidity, which represents an equilibrium relationship between the moisture content in the air (Meko et al., 2016). The high-water content will absorb water and vice versa to achieve equilibrium. The increase in Aw during storage was also caused by microorganisms' degradation of molecules in foodstuffs, namely by releasing bound water, which resulted in free water formation.

Water activity is directly linked to water content, meaning that a high-water content will elevate the water activity (Aw) and create favorable conditions for microbial growth (Pujaningsih et al., 2021). Food products with high Aw values are more prone to spoilage. The high Aw will make it easy for microbes to grow and cause food spoilage. The water activity in jerky products has considerable potential for contamination by microorganisms. A high Aw value will experience degradation caused by natural enzymatic or microbial damage. Water activity is closely tied to both water content and microbial proliferation. The rise in water activity (Aw) is a consequence of microorganism metabolism, leading to increased Aw levels in food products (Sharma et al., 2020). Factors like storage temperature, humidity in the storage area, and microbial activity contribute to this elevation in Aw values.

The effect of ground duck jerky shelf life on water content

The analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the water content of pekin duck ground jerky. Table 3 shows that the average water content of ground duck jerky ranges from 39.94% to 47.06%. The lowest average was found at 0 days of storage, and the highest average was at 28 days of storage. Jerky with a high-water content can make the jerky not last long because the high-water content can accelerate the damage to the duck jerky due to the presence of microbes in the product. The increase in water content in ground duck jerky during storage was due to the high humidity at room temperature, reaching 75%. The moisture content in meat had increased during room temperature storage, and the humidity influenced this in the surrounding environment during the room temperature storage period (Lekahena and Jamin, 2018). The humidity in the storage area affects the increase in moisture content because the product will absorb water from the surrounding air during storage at room temperature. High water content makes it easy for bacteria, mold, and yeast to multiply, which will cause changes in food ingredients (Pujaningsih et al., 2021). Increased water content was caused by air and water vapor entering the plastic vacuum packaging, so it increased during storage. If the humidity in the storage room is high, then the moisture content of the material can increase, and vice versa. The increase in water content is also due to ground duck jerky experiencing a decrease in quality caused by the oxidation of fat, which contains various unsaturated fatty acids and mineral content, which can accelerate fat oxidation. High water content can cause the product to be more easily damaged due to spoilage microbes that use water in the product as a growth medium (Arandini et al., 2022). High water content can cause microbes to multiply quickly, affecting product quality. Referring to the previous research, the breakdown of protein during storage into components such as ammonia and H_2S (hydrogen sulfide) can cause rancid odors and is followed by microorganism activity which causes the release of bound water to become free water, increasing water content (Budaraga et al., 2021). The increase in water content is influenced by humidity at room temperature because room temperature humidity can reach 86% (Alp and

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Bulantekin, 2021). Humidity at room temperature affects the increase in the resulting moisture content. The higher the humidity value, the more water vapor it contains, so the product's moisture content increases. High water content can cause the product to be damaged more quickly due to destructive microorganisms taking advantage of the water content contained in the product for their growth.

The effect of ground duck jerky shelf life on fat content

The analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect ($P<0.01$) on the fat content of ground duck jerky. The data in Table 3 shows that the average fat content of ground duck jerky ranges from 9.05% to 10.72%. The longer the storage, the fat content of the beef jerky increases. High levels of fat microorganisms cause them to multiply quickly and undergo oxidation, making the beef jerky turn rancid. The high fat content of duck jerky during storage is caused by increased water content which can reduce the quality of the jerky. A rapid increase in fat content can lead to the swift oxidation of food items, resulting in the development of an unpleasant rancid odor (Liu et al., 2023). As mentioned by Arandini et al. (2022), a high fat content creates favorable conditions for microbial growth. If stored for a long period of time, there is a risk of rancidity due to the increase in microorganisms, so the storage period is reduced. Meat that contains higher fat will produce a higher TBA value. This shows that the oxidation rate is influenced by the fat and fatty acid content. The breakdown of fat causes food to become rancid, thereby reducing its quality and nutritional value.

The effect of ground duck jerky shelf life on protein content

The results of analysis of variance showed that the difference in shelf life of ground duck jerky with room temperature storage and vacuum packaging did not have a significant effect ($P>0.05$) on the protein content of ground duck jerky. Data in Table 3 shows the average protein content of duck jerky ranges from 28.82% to 29.26%. The highest average was in T2 with a shelf life of 14 days, and the lowest was in T4 with a shelf life of 28 days. Storing duck jerky for 0 to 14 days experienced an increase in protein levels, this was due to the presence of phenolic compounds and organic acids in coconut shell liquid smoke which act as antioxidants and antibacterials so that they are able to inhibit pathogenic bacteria which can hydrolyze amino acids. Phenolic compounds have antimicrobial properties that can inhibit the growth of microbes in food. Ground duck jerky with a storage period of 21 to 28 days experienced a decrease in protein content because increasing water content would increase the number of microorganisms that could degrade the protein in the jerky. Arandini et al. (2014) stated that longer storage can cause a decrease in protein levels because the increasing number of microorganisms causes degradation of the protein contained in food. The growth of microorganisms causes a reduction in protein content because microorganisms require a source of nutrition for their growth, thereby causing a decrease in protein levels. The decrease in protein levels during storage is also caused by decomposition by proteolytic enzymes and the help of bacteria into carboxylic acids.

Effect of duck jerky shelf life on ash content

The analysis of variance showed that differences in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a significant effect ($P<0.05$) on the ash content. Table 3 shows that the average ranges from 0.26 to 0.41%. The longer the storage of ground duck jerky, the lower the ash content. The highest average ash content was in T1, with 7 days of storage, and the lowest average was in T4, with 28 days of storage. The ash content decreased during storage due to increased microorganisms with increasing water content in duck jerky. The ash content is related to minerals in food (Anggarani et al., 2019). The more minerals, the ash content in the food will increase. Extended room temperature food storage reduces ash content as microbes, reliant on minerals for growth and sustenance, consume these minerals over time (Arandini et al., 2022). During storage, there is an increase in Aw and water content, which microbial growth, bacteria, and mold will generally follow. Ash content is an inorganic substance left over from the combustion of organic material. Ash content is a mixture of inorganic or mineral components contained in a food. Foodstuffs comprise 96% organic matter and water; the remainder comprises mineral elements.

Effect of store period of ground duck jerky on carbohydrates by difference

The analysis of variance showed that the difference in the shelf life of ground duck jerky stored at room temperature and vacuum packed had a very significant effect ($P<0.01$) on the carbohydrate content difference in ground duck jerky. Table 3 shows that the average carbohydrate content ranges from 13.25% to 21.46%. Ground duck jerky experienced a decrease in carbohydrate content with increasing storage time. The elevated water content during storage influenced the chemical composition and quality of duck jerky, causing a decrease in carbohydrates. This decrease was attributed to chemical interactions that led to the formation of new compounds, ultimately impacting the overall food quality (Oessoe et al., 2014). Calculation of carbohydrates by difference is the determination of carbohydrates in foodstuffs roughly, and the results are usually listed in the list of ingredients. The decrease in carbohydrate content can be caused by an increase and decrease in other nutrient content, such as water, fat, protein, and ash, during storage because the carbohydrate content is highly dependent on the reduction factor. Carbohydrates in jerky are used to improve the texture of jerky to make it more tender. Carbohydrate content is needed as a binder and filler for jerky products, thus helping stability and increasing water absorption, making the texture soft in jerky (Chan et al., 2022). Carbohydrates have an essential role in determining the characteristics of food in taste, colour, and texture (Handayani et al., 2023).

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Effect of shelf life of ground duck jerky on thiobarbituric acid (TBA)

The results of the analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the levels of Thiobarbituric acid (TBA)/tranquillity of ground duck jerky. The data in Table 3 shows that the average TBA content of ground duck jerky ranges from 0.53 to 0.63 µM/g. Pekin duck jerky experienced an increase in TBA value every week. This indicated an increase in the rancidity of ground duck jerky. Ground duck jerky experienced increased TBA value due to increased fat content during storage, making it easier for jerky to undergo oxidation. Rahman et al. (2015) stated that increasing the fat content in food can potentially increase the fat oxidation rate, thereby affecting the value of thiobarbituric acid (TBA), and the food will cause rancidity (Liu et al., 2023). Meat that contains high fat will affect the increasing TBA value. This shows that the oxidation rate is affected by the fat and fatty acid content. Himawati et al. (2018) stated that liquid smoke is antioxidant and antibacterial and can preserve food ingredients due to the presence of phenol, carbamic, and carbonyl compounds. The results showed that the duck jerky had a slightly rancid smell on the 21st day or the 3rd week due to the higher TBA value of 0.62 µM/g. An increase in the TBA value causes a more pungent rancid odor and an increase in TBA during storage is caused by the breakdown of fat which causes a rancid smell and taste due to oxidation reactions (Liu et al., 2023).

Effect of shelf life of ground duck jerky on iodine number

The analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect (P<0.01) on the iodine number of ground duck jerky. The data in Table 3 shows that the mean value of ground duck jerky's iodine number ranges from 76.95 to 83.73 g, with the highest average being control which is 0 days of storage, and the lowest average being T4, which is 28 days of storage. Ground duck jerky during storage showed a decrease in iodine number. A decrease in iodine number indicates the development of rancidity in the product due to damage to the double bond by oxidation and the formation of secondary oxidation products during storage (Mahmud, 2023). The iodine number measures the amount of unsaturated fatty acids in fat. The decrease in iodine number is caused by the decomposition of fats and the saturation of double bonds through the degradation of hydroperoxides forming secondary products in the form of carboxylic acids, carbonyls, and other degradation products. The level of unsaturation will decrease because the double bond has been broken, so the iodine number decreases. A low iodine number indicates that not many unsaturated fatty acids are contained in the fat. These unsaturated fatty acids can bind to iodine and form unsaturated bonds. The number of bound iodine indicates the number of double bonds. The iodine number shows the number of iodine molecules that can form double bonds in fat, expressed in grams of iodine per 100 g of sample. The iodine number is used to determine the unsaturation and rancidity of a product. The higher the iodine number, the more advanced the rancidity process will occur in the product. Rancidity is damage or change in the smell and taste of a product. Unsaturated fatty acids can bind iodine and form saturated compounds. The amount of iodine bound indicates the number of double bonds contained in the product, the higher the iodine number, the better the quality of the product. The greater the iodine number, the more double bonds there are in the fatty acids of a product. A decrease in iodine number occurs when hydrogen molecules migrate to carbon, causing the fatty acid to become saturated. The double bonds of unsaturated fatty acids can bind oxygen to form peroxide which causes rancidity (Mahmud, 2023).

Effect of shelf life of ground duck jerky on organoleptic quality

Organoleptic testing was carried out by 5 trained panelists using a descriptive test. Organoleptic tests include colour, texture, aroma, taste, and overall acceptance. A descriptive scoring test assesses product intensity with increasing or decreasing order. The score used in the study ranged from 1 to 5, the higher the score given by the panelists, the higher the preference of the panelists for the product being tested, and vice versa. Testing the organoleptic quality of ground duck jerky using a comparison, namely commercial ground beef jerky. The average values for colour, texture, aroma, taste, and overall acceptance are in Table 4.

Table 4 – Average value of colour, texture, and overall acceptance of organoleptic quality

Treatments	Colour	Texture	Aroma	Taste	Overall acceptance
Control	4.15 ± 0.96	4.10 ± 1.29	4.30 ± 1.29	4.30 ± 0.58	4.40 ± 0.82^{d}
T1	4.10 ± 0.58	4.05 ± 1.50	4.15 ± 0.96	4.15 ± 0.50	4.20 ± 0.82^{cd}
T2	4.00 ± 0.82	3.50 ± 1.29	4.10 ± 1.29	4.20 ± 0.82	4.15 ± 0.96^{c}
T3	3.90 ± 0.58	3.70 ± 1.29	3.70 ± 1.29	3.80 ± 0.82	3.35 ± 0.50^{b}
T4	3.70 ± 0.58	3.45 ± 1.71	2.95 ± 0.96	3.10 ± 0.58	2.45 ± 0.50^{a}

$^{a,b,c,d;}$ Means different superscripts in the same column show a very significant effect (P<0.01). T1: storage period for 7 days, T2: 14 days, T3: 21 days, T4: 28 days

Effect of shelf life of ground duck jerky on organoleptic color

The analysis of variance showed that the difference in the shelf life of ground duck jerky when stored at room temperature and vacuum packed did not have a significant effect (P>0.05) on the color value of ground duck jerky based

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on organoleptic quality. The data in Table 4 shows that the average color score of ground duck jerky ranges from 3.70 to 4.15. The 0 to 14 days showed the brown duck jerky color criterion, and the shelf life of 21 and 28 days showed brownish-red jerky. The oxidation of proteins and fats causes color changes during shelf life. Commercial beef jerky, a comparison stored at freezer temperature, had the same color until the 28th day, namely brownish red. With the addition of liquid smoke, ground duck jerky can maintain its color due to the presence of phenolic compounds in the liquid smoke. Besides that, vacuum packaging can also preserve the color of the jerky. The brown color of beef jerky is produced due to the Maillard reaction during drying (Dewi et al., 2020). Compound components in CSLS, such as organic acids, carbonyls, and phenols, function to form a reddish brown color (Montazeri et al., 2013). The color of the jerky produced affects the consumers liking.

Effect of shelf life of ground duck jerky on organoleptic texture

The analysis of variance showed that the difference in the shelf life of ground duck jerky when stored at room temperature and vacuum packed did not have a significant effect (P>0.05) on the texture value of ground duck jerky based on organoleptic quality. The data in Table 4 shows that the average texture score of ground duck jerky ranges from 3.45 to 4.10. Ground duck jerky has the criteria for being soft with a shelf life of 0 to 7 days; on days 14 to 28 the beef jerky has a slightly soft texture. The texture of duck jerky does not change significantly during storage. This shows that liquid smoke is able to maintain the texture of ground duck jerky during the storage period. The commercial ground beef jerky used as a comparison material until the 28th day stored in the freezer had a soft texture. Phenolic compounds in liquid smoke can form hydrogen bonds with water, thereby affecting the binding capacity, elasticity and density of water.

The texture of duck jerky during storage is still acceptable because the beef jerky still has a relatively soft and chewy texture, the texture of the beef jerky is also influenced by the water content. The water content in duck jerky increases during storage so it doesn't change the texture of the jerky too much. The decrease in the texture of the jerky to become somewhat soft is due to the low protein value, so that the ability of the protein to bind water is reduced so that the WHC value of the jerky during storage decreases. The WHC value will affect tenderness. The texture will change along with changes in the water content of the food product (Akilie et al., 2021). Water content is one of the characteristics that greatly influences the appearance, texture and taste of a food product.

Effect of shelf life of ground duck jerky on organoleptic aroma

The analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had no significant effect (P>0.05) on the aroma value of ground duck jerky. Table 4 shows that the average value of duck jerky aroma ranges from 2.95 to 4.30. Jerky stored for 0 to 14 days has the criteria for smelling like spices and smoke. On the 21st day the beef jerky smelled slightly of spices and smoke, and the jerky smelled slightly rancid. On the 28th day the aroma of the jerky changed to just smelling of spices and smelled rancid. Commercial jerky during storage in the freezer until the 28th day is still acceptable because it does not smell rancid. The difference in the aroma of ground duck jerky and commercial jerky during storage is caused by differences in storage temperature. Ground duck jerky is stored at room temperature, making it easier for bacteria to grow. This may affect the sensory quality of the jerky. Aroma scores tend to decrease during product storage as a result of chemical component decomposition, resulting in an unpleasant rotten and rancid odor (Budaraga et al., 2021). The presence of enzymes and microorganisms causes deviations in the smell and aroma of the product. The foul smell occurs due to the activity of proteolytic bacteria which break down proteins into simple compounds such as polypeptides, amino acids, H_2S and the rancid smell is caused by lipolytic enzymes and protein breakdown.

Montazeri et al. (2014) stated that liquid smoke compounds such as guaiacol, eugenol, and syringol provide aroma to smoked products. Other components that also play a role in aroma and taste are p-cresol, o-cresol, guaiacol, 4-memethyguaiacol, 4-ethyl guaiacol, eugenol, 4-proppyguaiacol, and isoeugenol. Foodstuffs that contain high protein, when damaged by microbes, will produce a rancid odor. The stage of protein breakdown begins with the presence of microbes in a food ingredient, and proteins are broken down into small molecules in the form of free amino acids, dipeptides, and sugars. Microbes will use these small molecular food ingredients, and then the microbial population will overgrow along with the production of even smaller fraction compounds, such as cadaverine, putreceine, organic acids, CO_2, H_2S, and NH_3. Rancid odor in meat during storage occurs due to oxidation of unsaturated fatty acids and protein degradation. Rancidity is a process of lipid oxidation (Othón-Díaz et al., 2023). The smell of fat will change to an unpleasant odor and if the rancidity has reached its limit, it will taste bitter with longer storage time. The increasing growth of microorganisms can cause the aroma to become increasingly rancid and sour.

Effect of shelf life of ground duck jerky on organoleptic taste

The analysis of variance showed that differences in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had no significant effect (P>0.05) on the taste value of ground duck jerky. Table 4 shows that the average value of jerky flavor ranges from 3.10 to 4.30. Storage of duck jerky for 0 to 14 days has the criteria of meaty and smoked taste. Storage for 21 and 28 days of duck jerky has slightly tasted of meat and smoke. Storage for 21 days of duck jerky tastes slightly rancid, and commercial beef jerky during storage until 28 is still acceptable. Components in smoke that affect the taste of smoked products are phenols, carbonyls, and organic acids, and they function as antioxidants, which can inhibit the oxidation of protein and fats during storage so that the rate of

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decline in the flavor score can be hampered. The fat oxidation process in a product will affect the taste, color, texture and nutritional content of the product (Shahidi and Hossain, 2022). Changes in taste occur due to oxidation of unsaturated fats and fat decomposition. Phenolic compounds actively contribute to shaping the product's taste, enhancing its flavor, and promoting the presence of antioxidant components such as phenol aldehydes, 2,6-dimethoxyphenol, 2,6-dimethoxy phenolic acids, and 4-ethylphenol. Additionally, these compounds also play a key role in creating the taste and aroma of the final product (Montazeri et al., 2013). Components in smoke that affect the taste of smoked products are phenols, carbonyls, and organic acids, and they function as antioxidants, which can inhibit the oxidation of protein and fats during storage so that the rate of decline in the flavor score can be hampered.

Effect of ground duck jerky shelf life on overall acceptance

The analysis of variance showed that the difference in the shelf life of ground duck jerky by being stored at room temperature and vacuum packed had a very significant effect ($P<0.01$) on the overall acceptance value of ground duck jerky. Table 4 shows that the average overall acceptance score ranges from 2.45 to 4.40. Storage of ground duck jerky for 0 to 14 days has an acceptable overall acceptance value. Storage of jerky on the 21st day has a somewhat acceptable overall value because the jerky has started to smell and taste rancid. On the 28th day, ground duck jerky was not accepted because it was already flavorful and tasted rancid. Commercial beef jerky used as a comparison for up to 28 days of storage had overall acceptability that was still acceptable. This is due to the difference in storage temperature so that commercial ground beef jerky stored in the freezer can maintain its nutritional quality so that there are no deviations based on organoleptic quality, and ground duck jerky stored at room temperature shows an increase in water and fat content, which can reduce the nutritional quality so that it can affect other chemical components. The longer the storage, the lower the panelists' assessment of overall acceptability. This is caused by increasing levels of water, fat and rancidity, thereby reducing the acceptance of the aroma and taste of ground duck jerky, thereby reducing the overall product acceptance score.

CONCLUSION

The results showed that ground duck jerky stored at room temperature and vacuum packed had a very significant effect on pH, texture, color L, a*,b*, Aw, moisture content, fat, carbohydrates based on difference, TBA, and number iodine, significant effect on ash content, and no significant effect on WHC, protein content, and organoleptic quality. Pekin duck jerky with the addition of sonicated coconut shell liquid smoke and stored for different periods of time was able to survive until the 14th day because more than that the jerky experienced an increase in pH of 6.43-6.54; Aw of 0.74-0.78; water content of 44.84-47.06%; fat of 10.29-10.72%; TBA of 0.62-0.63 µM/g and decreased protein content by 29.13-28.82% and iodine number 77.06-76.95 g which can reduce the quality of duck jerky.

DECLARATIONS

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Authors' contribution

N. Salsabila research and developed, made samples, analyzed parameters, analyzed data, and compiled and revised the manuscript. A. Susilo and D. Rosyidi developed the research design, provided input, and directed and approved the final manuscript.

Conflict of interests

The authors have declared no conflict of interest.

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REFERENCES

Agustini R, Amyliana NA, Herdiyastuti N, and Sanjaya IGM (2021). Nano-encapsulation of black rice yeast extract with poloxamer supporting matrix. Presented at the International Joint Conference on Science and Engineering 2021 (IJCSE 2021), Atlantis Press, pp. 375–381. https://doi.org/10.2991/aer.k.211215.065

Akilie MS, Xyzquolyna D, and Gobel MR (2021). The effect of commercial liquid smoke immersion on tofu storage period. Anjoro: International Journal of Agriculture and Business, 2(1): 34–40. https://doi.org/10.31605/anjoro.v2i1.971

Alp D, and Bulantekin Ö (2021). The microbiological quality of various foods dried by applying different drying methods: a review. European Food Research and Technology, 247(6): 1333–1343. https://doi.org/10.1007/s00217-021-03731-z

Amit SK, Uddin MdM, Rahman R, Islam SMR, and Khan MS (2017). A review on mechanisms and commercial aspects of food preservation and processing. Agriculture & Food Security, 6(1): 51. https://doi.org/10.1186/s40066-017-0130-8

Anderson S and Collaborators: (2007). Determination of fat, moisture, and protein in meat and meat products by using the FOSS FoodScan Near-Infrared spectrophotometer with FOSS artificial neural network calibration model and associated database: Collaborative Study. Journal of AOAC international, 90(4): 1073–1083. https://doi.org/10.1093/jaoac/90.4.1073

Anggarani MA, Ayuningsih AD, Yudianto E, and Prasodi BA (2019). The composition of water and ash of Secang wood's Simplicia and Secang wood herbal drink powder. Journal of Physics: Conference Series, 1417(1): 012033. https://doi.org/10.1088/1742-6596/1417/1/012033

Arandini NPCA, Singapurwa NMAS, and Semariyani AAM (2022). Preservation of tofu with liquid smoke from coconut shell. SEAS (Sustainable Environment Agricultural Science), 6(2): 102–111. https://doi.org/10.22225/seas.6.2.5377.102-111

Banaszak M, Kuźniacka J, Biesek J, Maiorano G, and Adamski M (2020). Meat quality traits and fatty acid composition of breast muscles from ducks fed with yellow lupin. Animal, 14(9): 1969–1975. https://doi.org/10.1017/S1751731120000610

Bishnoi SW, Rozell CJ, Levin CS, Gheith MK, Johnson BR, Johnson DH, et al. (2006). All-Optical Nanoscale pH Meter. Nano Letters, 6(8): 1687–1692. https://doi.org/10.1021/nl060865w

Budaraga IK, Saibuma V, and Hermalena L (2021). Quality of red tuna (Yellowfin tuna) fishball, white oyster mushroom (*Pleurotus ostreatus*) on different types of packaging and storage time.IOP Conference Series: Earth and Environmental Science, 715(1): 012068. https://doi.org/10.1088/1755-1315/715/1/012068

Chan SS, Roth B, Jessen F, Jakobsen AN, and Lerfall J (2022). Water holding properties of Atlantic salmon. Comprehensive Reviews in Food Science and Food Safety, 21(1): 477–498. https://doi.org/10.1111/1541-4337.12871

Desvita H, Faisal M, Mahidin, and Suhendrayatna (2020). Preservation of meatballs with edible coating of chitosan dissolved in rice hull-based liquid smoke. Heliyon, 6(10). https://doi.org/10.1016/j.heliyon.2020.e05228

Dewi EN, Nurbaiti AA, and Purnamayati L (2020). Chemical changes of shredded catfish (*Clarias gariepinus*) added with different concentration of sucrose during storage at room temperature. E3S Web of Conferences, 147: 03001. https://doi.org/10.1051/e3sconf/202014703001

Diether NE, and Willing BP (2019). Microbial Fermentation of Dietary Protein: An Important Factor in Diet–Microbe–Host Interaction.Microorganisms, 7(1): 19. https://doi.org/10.3390/microorganisms7010019

Domínguez R, Pateiro M, Munekata PES, Zhang W, Garcia-Oliveira P, Carpena M, et al. (2022). Protein oxidation in muscle foods: a comprehensive review. Antioxidants, 11(1): 60. https://doi.org/10.3390/antiox11010060

Fadlilah A, Rosyidi D, and Susilo A (2020). Microbial qualities of rabbit meat fermented with Lactobacillus plantarum. IOP Conference Series: Earth and Environmental Science, 492(1): 012058. https://doi.org/10.1088/1755-1315/492/1/012058

Febrianta H (2022). The Effectiveness of Turmeric Extract (Curcuma longa) to Increase The Shelf Life of Duck Meat.Social Economics and Ecology International Journal (SEEIJ), 6(2): 49–52. https://doi.org/10.21512/seeij.v6i2.9702

Franca-Oliveira G, Fornari T, and Hernández-Ledesma B (2021). A review on the extraction and processing of natural source-derived proteins through eco-innovative approaches. Processes, 9(9): 1626. https://doi.org/10.3390/pr9091626

Handayani BR, Werdiningsih W, Rahayu TI, and Fajri AZ (2023). Properties of ready to eat ground beef jerky with the addition of tapioca flour. AIP Conference Proceedings, 2586(1): 060008. https://doi.org/10.1063/5.0111168

Himawati E, Riyadi NH, and Manuhara GJ (2018). The effect of adding coconut shell liquid smoke by distillation and redistillation on the chemical, microbiological, and sensory properties of pindang layang fish (*Decapterus* spp.) during storage. Asian Journal of Tropical Biotechnology, 15(2). https://www.smujo.id/bbs/article/view/9051

Husna SS, Hintono A, and Rizqiati H (2020). Texture, water absorption, aw and hedonic quality flakes white millet (*Panicum miliaceum*) with addition of pumpkin flour (*Cucurbita moschata*). Journal of Applied Food Technology, 7(2). https://doi.org/10.14710/alj.v%vi%i.215

Inayat M, Abbas F, Rehman MH, and Mahmud A (2023). Physico-Chemical Parameters, Oxidative Stress, and Fatty Acid Profile of American Pekin Ducks (Anas Platyrhynchos Domesticus) Raised under Different Production Systems.Brazilian Journal of Poultry Science, 25: eRBCA. https://doi.org/10.1590/1806-9061-2022-1724

Indiarto R, Nurhadi B, Tensiska T, Subroto E, and Istiqamah YK (2019). Effect of liquid smoke on microbiological and physico-chemical properties of beef meatballs during storage. Food Research, 4: 522–531. https://doi.org/10.26656/fr.2017.4(2).341

Jin S, Yang H, Liu F, Pang Q, Shan A, and Feng X (2021). Effect of Dietary Curcumin Supplementation on Duck Growth Performance, Antioxidant Capacity and Breast Meat Quality. Foods, 10(12): 2981. https://doi.org/10.3390/foods10122981

Kim DH, Shin D-M, Lee JH, Kim YJ, and Han SG (2022). Effect of Different Brine Injection Levels on the Drying Characteristics and Physicochemical Properties of Beef Jerky. Food Science of Animal Resources, 42(1): 98–110. https://doi.org/10.5851/kosfa.2021.e66

Kulapichitr F, Borompichaichartkul C, Fang M, Suppavorasatit I, and Cadwallader KR (2022). Effect of post-harvest drying process on chlorogenic acids, antioxidant activities and CIE-Lab color of Thai Arabica green coffee beans. Food Chemistry, 366: 130504. https://doi.org/10.1016/j.foodchem.2021.130504

Lekahena VNJ, and Jamin R (2018). The quality of smoked skipjack during storage time at room temperature. IOP Conference Series: Earth and Environmental Science, 175(1): 012003. https://doi.org/10.1088/1755-1315/175/1/012003

Lis A, Staniewski B, and Ziajka J (2021). A comparison of butter texture measurements with the AP 4/2 penetrometer and TA.XT. Plus texture analyzer.International Journal of Food Properties, 24(1): 1744–1757. https://doi.org/10.1080/10942912.2021.1999262

Liu Y, Cao Y, Yohannes Woldemariam K, Zhong S, Yu Q, and Wang J (2023). Antioxidant effect of yeast on lipid oxidation in salami sausage.Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.1113848

Mahmud SK (2023). Animal and vegetable lipid solution test in identifying fatty acid hydrolysis reactions. Jurnal Ilmiah dr. Aloei Saboe, 10(1): 44–54. https://doi.org/10.47918/jias.v10i1.259

Citation: Salsabila N, Rosyidi D, and Susilo A (2023). Physico-chemical and sensory quality of Pekin duck jerky sonicated with coconut shell liquid smoke and stored for different periods. *Online J. Anim. Feed Res*., 13(5): 373-383. DOI: https://dx.doi.org/10.51227/ojafr.2023.53

Mathew S, Zakaria ZA, and Musa NF (2015). Antioxidant property and chemical profile of pyroligneous acid from pineapple plant waste biomass.Process Biochemistry, 50(11): 1985–1992. https://doi.org/10.1016/j.procbio.2015.07.007

Meko AUI, Berhimpon S, Suwetja IK, and Ijong FG (2016). Moisture sorption isotherm of Tuna Sei, smoked with liquid smoke. International Journal of Fisheries and Aquaculture, 8(10): 98–104. https://doi.org/10.5897/IJFA2016.0573

Montazeri N, Oliveira ACM, Himelbloom BH, Leigh MB, and Crapo CA (2013). Chemical characterization of commercial liquid smoke products.Food Science & Nutrition, 1(1): 102–115. https://doi.org/10.1002/fsn3.9

Oessoe YYE, Maramis R, Warouw OOJ, and Mandey L (2014). Changes on carbohydrates and protein content in North Sulawesi local rice during storage. IOSR Journal of Environmental Science, Toxicology and Food Technology, 8: 16–21. https://doi.org/10.9790/2402-08231621

Othón-Díaz ED, Fimbres-García JO, Flores-Sauceda M, Silva-Espinoza BA, López-Martínez LX, Bernal-Mercado AT, et al. (2023). Antioxidants in Oak (Quercus sp.): Potential Application to Reduce Oxidative Rancidity in Foods. Antioxidants, 12(4): 861. https://doi.org/10.3390/antiox12040861

Pujaningsih RI, Widiyanto, Baginda IMT, Sri M, and Hilmiyatun N (2021). Total bacteria, total fungus, and water activity content of multinutrient block with green betle leaf levels added as feed supplements for goat. IOP Conference Series: Earth and Environmental Science, 667(1): 012027. https://doi.org/10.1088/1755-1315/667/1/012027

Rahmah KS, Ramdani SS, Kurniasih N, Meilawati A, Wahlanto P, and Yusuf AL (2019). Quality test of bulk palm cooking oil in local market, Banjar, West Java, Indonesia base on Perokside value, iodin value and number of free fatty acid. Journal of Physics: Conference Series, 1179(1): 012187. https://doi.org/10.1088/1742-6596/1179/1/012187

Shahidi F, and Hossain A (2022). Role of Lipids in Food Flavor Generation. Molecules, 27(15): 5014. https://doi.org/10.3390/molecules27155014

Sharma R, Garg P, Kumar P, Bhatia SK, and Kulshrestha S (2020). Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods. Fermentation, 6(4): 106. https://doi.org/10.3390/fermentation6040106

Sorapukdee S, Uesakulrungrueng C, and Pilasombut K (2016). Effects of Humectant and Roasting on Physicochemical and Sensory Properties of Jerky Made from Spent Hen Meat. Food Science of Animal Resources, 36(3): 326–334. https://doi.org/10.5851/kosfa.2016.36.3.326

Yirmaga MT (2013). Improving the Indigenous Processing of Kocho, an Ethiopian Tradit.Journal of Nutrition & Food Sciences, 3(1): 182. https://doi.org/10.4172/2155-9600.1000182

Zeb A, and Ullah F (2016). A Simple Spectrophotometric Method for the Determination of Thiobarbituric Acid Reactive Substances in Fried Fast Foods.Journal of Analytical Methods in Chemistry, 2016: e9412767. https://doi.org/10.1155/2016/9412767

Citation: Salsabila N, Rosyidi D, and Susilo A (2023). Physico-chemical and sensory quality of Pekin duck jerky sonicated with coconut shell liquid smoke and stored for different periods. *Online J. Anim. Feed Res*., 13(5): 373-383. DOI: https://dx.doi.org/10.51227/ojafr.2023.53

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COMPARISON OF RECTAL THERMOMETRY WITH THE ALTERNATIVE UNDERTAIL, AXILLARY, AND INGUINAL TEMPERATURE MEASUREMENTS IN SHEEP

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ABSTRACT: This study was conducted to ascertain the suitability of alternative locations for temperature measurement, with reference to rectal thermometry in sheep, using a digital thermometer (DT). The study employed a single-factor multilevel design, considering anatomical location (site) as the main factor. This anatomical location factor had four conditions, including rectal ($_{rectal}$DTt), undertail ($_{undertail}$DTt), inguinal ($_{inguinal}$DTt), and axillary ($_{axillary}$DTt) locations. A total of 16 sheep were recruited for the study, and each treatment had eight replicates. The data obtained were descriptively analyzed using means and standard deviations, while inferential statistics included analysis of variance (ANOVA), Pearson's correlation, Tukey's test, t-test, and Bland-Altman plot. The mean $_{inguinal}$DTt was the highest (39.51 ± 0.31℃), while the lowest was the mean $_{undertail}$DTt (38.97±0.45). The effect of anatomical location on temperature readings was statistically significant. The difference between mean $_{rectal}$DTt and $_{inguinal}$DTt, or $_{axillary}$DTt was not significant. The $_{rectal}$DTt measurements were significantly correlated with those of each treatment. Equivalence analysis revealed a non-significant bias between the $_{rectal}$DTt and $_{inguinal}$DTt pair. The Bland-Altman plot showed a good level of correlation and considerable agreement between $_{rectal}$DTt and $_{inguinal}$DTt measurements. In conclusion, temperature measurement at the inguinal location results in readings that are similar to those of rectal thermometry and thus may be of clinical importance in the future, particularly with digital thermometer application in sheep.

Keywords: Anatomical location, Body temperature, Digital thermometer, Sheep, Rectal thermometry.

INTRODUCTION

The core body temperature, also known as animal body temperature, is the average temperature of an animal's deep body core (Godyń et al., 2019). Additionally, it is closely related to the animal's metabolism and life activities, which can reflect the physiological and health status of animals (Cai et al., 2023). There are many conditions that cause temperature changes, namely infectious diseases, thermal stress, synchronization and estrus status, onset of lambing, among others (Underwood et al., 2015; Fischer-Tenhagen and Arlt, 2020). It is possible to have a better understanding of the physiological changes that occur in an animal and in-depth analysis of its health by way of body temperature measurement (Cai et al., 2023). Body temperature assessment should be the first procedure to be done when examining sheep, and the results interpreted in conjunction with other clinical signs (Stockler et al., 2021). In view of this, early measurement of this physiological marker would result in timeous decision-making or management of many conditions, and minimizes undue reproductive and economic losses (Godyń et al., 2019; Abigaba and Sianangama, 2023).

The are many body temperature sensors that have been explored for application in sheep, for example, temperature loggers, transponders, clinical digital thermometers (digital thermometer; DT), clinical mercury thermometers (mercury thermometer; MT), and non-contact infrared devices like infrared thermometers and thermal infrared cameras (Pourjafar et al., 2012; Abecia et al., 2015). However, most of these devices are either sophisticated to use, less accurate, expensive, not readily available, or potentially hazardous (George et al., 2014). These drawbacks are of great concern to the farmers, particularly those who live in the rural setting or practice smallholder farming system. Accordingly, the MTs and DTs have been used by many clinicians and some farmers to measure the body temperature of various livestock species, including sheep, for many years. This notwithstanding, the MTs have been sidelined in some countries like USA because of the following drawbacks: generally time consuming, susceptible to breakage, and may cause environmental toxicity (Katsoulos et al., 2016). Recently, the DT device has gained popularity among many users worldwide. This popularity is

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attributed to many factors, namely accuracy and relative rapidity of measurement, nontoxic to the environment, user friendly, less expensive, and availability (Cadioli et al., 2010; Hine et al., 2015).

The DT device is traditionally applied in sheep per rectum, hence it is among the temperature sensors under the group known as rectal thermometers. Temperature measurement per rectum is referred to as rectal thermometry; this method remains the gold standard for the body temperature assessment in sheep (Katsoulos et al., 2016). This notwithstanding, rectal thermometry has been associated with potential drawbacks, such as stress, fomite for disease spread, rectal injuries to animal, inter alia (Katsoulos et al., 2016; Yadav et al., 2017). Notably, some of these disadvantages also apply to the DT device when it is used to measure temperature per rectum (Muhammed et al., 2019). Hence the ability to measure body temperature at the peripheral locations may be helpful and less invasive (Kearton et al., 2020). In view of the foregoing, there is an urgent need to search for an alternative anatomical location that is safer, non-invasive, easy to use, and robust to external variations, particularly with a digital thermometer. This study was conducted to compare rectal thermometry with the alternative temperature that is measured at the minimally-invasive skin locations among adult sheep of both genders.

MATERIALS AND METHODS

Ethical consideration

This study used animals that were physically healthy; the procedures conducted on them were non-lethal and inflicted little or no distress to the study animals. The animal handling, including restraint, experimentation, inter alia, was done with strict supervision by the institutional committee on the animal research. The procedures were done in accordance with the guide for the care and use of agricultural animals in research and teaching (ASAS, 2020).

Study area

This study was conducted during the month of July, 2023, at the farm station that is owned by the School of Veterinary Medicine, The University of Zambia, located in Lusaka, Zambia. Zambia is located at a latitude S 14° 20' 0" and longitude E 28° 30' 0" according to the GeoNames geographical database Google Earth-2023. The country lies, in the tropics, within the Southern-African region. In terms of its weather conditions, Zambia receives an average annual precipitation that ranges from 800 to 1400 mm, while the temperature ranges from 10 to 20℃ during winter and 20 to 30℃ in the hot-dry seasons (Bailey et al., 2021). The average ambient temperature and humidity conditions at the field station ranged from 21.5 to 22.5℃ and 41 to 51%, respectively, during the study period.

Experimental animals

This study included physically-healthy sheep that belonged to the School of Veterinary Medicine, the University of Zambia. The weight of these sheep ranged from 43 to 74 kg, with an average of 56 kg. Their age ranged from 1 to 3.5 years, with an average of 2.4 years. All the animals were kept under a semi-intensive rearing mode; they were mostly grazing within the paddocks with minimal supplementation using a commercial concentrate. The concentrate was supplied by National Milling Limited, Lusaka, Zambia. Water was provided to the sheep *ad libitum*.

Experiment design

The study employed a single-factor multilevel design to determine differences between temperature measurement methods in sheep. According to the design, anatomical location was the main factor considered. This factor had four levels (conditions), namely rectal, axillary, undertail, and inguinal locations, and the measurement conducted at each location was regarded as a measurement method. In this case the measurements were performed at the rectal (rectal temperature; $_{rectal}$DTt), undertail (undertail temperature; $_{undertail}$DTt), inguinal (inguinal temperature; $_{inguinal}$DTt), and axillary (axillary temperature; $_{axillary}$DTt) locations. Additionally, the $_{rectal}$DTt was considered as the reference measurement method. A total of 16 adult sheep were used for this study, and each condition had 8 replicates. In this study, each animal was assessed for all the measurement locations.

Temperature measurement

Prior to the temperature measurement, each sheep was physically restrained according to the procedures by Stockler *et al.* (2021). Then, temperature readings (DTt) were taken from each sheep after 20-minutes lapse; this was done to minimize the potential effects of psychogenic fever on the study results. Temperature measurement was conducted on the sheep in a semi-temperature controlled indoor facility, which aimed to minimize variations in the environmental conditions like temperature changes. Furthermore, the measurement of temperature was done using a functional veterinary digital thermometer (DT; GB Kruuse digital thermometer, Taipei, Taiwan). The measuring range of this thermometer was 30.0 - 43.9℃, and its resolution was 0.1℃. Temperature measurement, at the various anatomical locations considered, was done following an order that was determined using a simple random selection. In this case, selection was done using folded papers that bore the name of each anatomical location; these were tossed followed by randomly picking one at time without replacing it. This selection procedure was intended to minimize the bias; hence it was repeated for each sheep under study. Additionally, all the temperature data were obtained at the different anatomical locations on the same day.

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The $_{rectal}$DTt measurement was performed based on the previous procedure (Pourjafar et al., 2012). In the case of the $_{inguinal}$DTt and $_{axillary}$DTt measurements, the procedures were based on another study (Levy et al., 2020), with a minor modification. Briefly, $_{axillary}$DTt measurement was conducted by carefully inserting a DT probe deep into the left axilla, approaching from the caudal aspect, and aiming towards the dorsum. This procedure was done on a study sheep standing with both forelimbs close to its body. Again, a similar standing posture, with both hindlimbs close to the body, was considered during the $_{inguinal}$DTt measurement. The $_{inguinal}$DTt readings were obtained by inserting a DT probe deep in the left inguinal area, approaching from the cranial aspect, and aiming towards the dorsum. With regard to the $_{udertail}$DTt measurement, the DT probe was introduced in between the ano-triangular surface and tail base, approaching from the lateral aspect, and aiming towards the cranial direction. For each measurement location, the DTt readings were recorded whenever an alarm went off and the degree sign stopped flashing. The duration between the DT placement at the site and temperature recording ranged from 15 to 65 seconds. Additionally, each anatomical location was measured twice and an average of the two (DTt) readings considered as a single datum.

Data analysis

In the Statistical Package for Social Scientists (SPSS® IBM 26 version, USA), data were analyzed using descriptive statistics, including means and standard deviations (SD). The Shapiro-Wilk and Lavene's tests were used to check for normality and homogeneity of the data, respectively. For inferential analysis, selected statistical tests were conducted. A One-way analysis of variance (ANOVA test) between subjects was conducted using Generalized Linear Model, a univariate analysis procedure, to determine the main effect of location factor. The following statistical model was considered;

$Y_{ij} = \mu + \delta_i + e_{ij}$

Where, Y_{ij} is the dependent variable denoting the trait measured (DTt reading), μ represents the overall mean, δ_i signifies the fixed effect of the i^{th} location (i = rectal, undertail, inguinal, and axillary location), and e_{ij} is the random error term. Tukey test was used to determine the pairs whose means differed. The correlation between the different temperature measurements was determined using a Pearson's correlation test. A one-sample t-test was employed to establish the level of bias between the reference and alternative measurements (locations), while a Bland-Altman analysis was employed to compare selected DTt measurement methods. In all cases, significance was taken at a level of $p < 0.05$.

RESULTS

The mean temperature at different anatomical locations

The results of ANOVA that was conducted to determine the effect of location factor on the temperature readings are presented in this subsection. The Lavene's test showed equality of the groups' variance ($F(3,60) = 1.212$, $p > 0.05$). The main effect of location factor on temperature readings (DTt) was statistically significant ($F(3,60) = 7.241$, $p > 0.05$, $\eta_p^2 = 0.266$). The observed effective size (η_p^2) indicated that 26.6% of the variance in the DTt was explained by the location factor. The mean DTt, including $_{rectal}$DTt, $_{undertail}$DTt, $_{inguinal}$DTt, and $_{axillary}$DTt, that was measured at the rectal, inguinal, axillary, and undertail locations, respectively, are presented in Table 1. The mean $_{inguinal}$DTt was the highest (39.51 ± 0.31℃), while the lowest mean value was $_{undertail}$DTt (38.97 ± 0.45℃). There was no significant difference between measurements at the rectal ($_{rectal}$DTt) and inguinal ($_{inguinal}$DTt) area ($p > 0.05$), or axillary location ($p > 0.05$). The mean $_{rectal}$DTt was significantly higher than that of the $_{undertail}$DTt measurement ($p < 0.05$).

The correlation between temperature readings at different anatomical locations

The results from a correlation analysis of the various DTt readings, including $_{rectal}$DTt, $_{undertail}$DTt, $_{inguinal}$DTt, and $_{axillary}$DTt, are shown below (Table 2). Considering $_{rectal}$DTt as the standard measurement, the correlation between $_{rectal}$DTt and $_{axillary}$DTt readings was the stronged ($r = 0.928$, $p < 0.05$), while the lowest was observed with the $_{undetail}$DTt ($r = 0.782$, $p < 0.05$). When 'stardard method factor' is not considered, the correlation between $_{inguinal}$DTt and $_{axillary}$DTt readings ($r = 0.943$, $p < 0.05$) and that of the $_{undetail}$DTt and $_{inguinal}$DTt ($r = 0.582$, $p < 0.05$) was the strongest and weakest, respectively.

Reliability and comparison of the DTt measurements (methods) in sheep

The results of a reliability analysis that quantitatively analyzed for the potential significance in the mean of differences (bias) between paired DTt readings or measurements, viz. $_{rectal}$DTt measurement (standard method) with each of the alternative anatomical locations (methods), are presented below (Table 3). The $_{rectal}$DTt-$_{undertail}$DTt pair had the largest mean of differences (bias) (0.5±0.28℃), while the $_{rectal}$DTt-$_{inguinal}$DTt pair had the lowest bias (-0.03±0.15℃). The equivalence analysis revealed a significant bias between $_{rectal}$DTt and $_{undertail}$DTt measurements ($p < 0.05$), as well as $_{rectal}$DTt and $_{axillary}$DTt ($p < 0.05$). The bias between $_{rectal}$DTt and $_{inguinal}$DTt measurements was not significantly different ($p > 0.05$); moreover, the data for the difference values met the normality assumption. The relationship between $_{rectal}$DTt and $_{inguinal}$DTt measurements or methods is shown in Figure 1. From the plot, most of the data points are close to the zero line, a similar distribution of the points is observed around the bias (mean of difference) line. Most of the data points are within the agreement limits and not significantly different ($p > 0.05$, with a 95% confidence interval).

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Table 1 - The mean temperature readings measured at the different anatomical locations

Variable	DTt readings		
Measurement site/method	Mean±SD (℃)	Minimum (℃)	Maximum (℃)
Rectal	39.47 ± 0.35[a]	38.85	40.04
Undertail	38.97 ± 0.45[b]	38.10	39.60
Inguinal	39.51 ± 0.31[a]	39.05	40.03
Axillary	39.34 ± 0.34[a]	38.80	40.25
Total	39.32±0.41	-	-

[a,b] Different letters (superscript) within the same column denote a significant difference ($p < 0.05$), SD: standard deviation, ℃: degrees Celsius, DTt: temperature reading by a digital thermometer

Table 2 - Correlation between DTt readings obtained from the different anatomical locations

	$_{rectal}$DTt	$_{undertail}$DTt	$_{inguinal}$DTt	$_{axillary}$DTt
$_{rectal}$DTt	1			
$_{undertail}$DTt	0.782*	1		
$_{inguinal}$DTt	0.898*	0.582*	1	
$_{axillary}$DTt	0.928*	0.640*	0.943*	1

DTt: temperature readings by a digital thermometer, correlation coefficient 0.00-0.10: negligible, 0.10-0.39: weak, 0.4-0.69: moderate, 0.7-0.89: strong, 0.9-1.0: very strong correlation, *significant correlation at $p < 0.05$

Table 3 - Shows results of equivalence analysis for the different DTt measurement pairs

Paired sites/methods	DTt measurements (difference)					
	Mean±SD (℃)	df	t-value	*p*-value	95% CI	
					Lower	Upper
Rectal - undertail	0.5±0.28	15	7.214	<0.05	0.35	0.65
Rectal - inguinal	-0.03±0.15	15	-0.8	>0.05	-0.11	0.05
Rectal - axillary	-0.13±0.13	15	4.134	<0.05	0.07	0.20

DTt: temperature readings by digital thermometer, CI: confidence interval, SD: standard deviation, df: degrees of freedom, ℃: degrees Celsius, <: less than, >: greater than

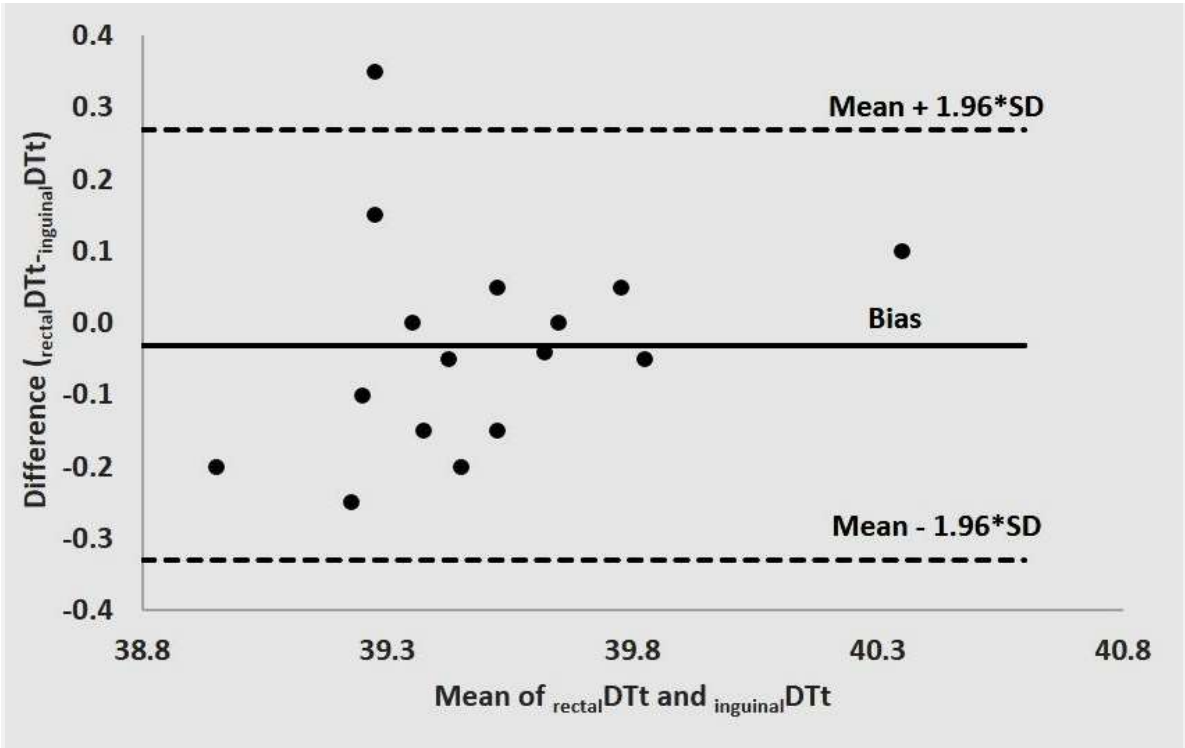

Figure 1 - A Bland-Altman plot showing the rectal-inguinal temperature relationship

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DISCUSSION

The measurement of body core temperature is among the means employed to monitor the health and reproductive status of animals, including sheep (George et al., 2014; Godyń et al., 2019). The notion behind this is that when the measured temperature falls outside the range of temperatures covered by the normal circadian rhythm (Weinert and Waterhouse, 2007), it is regarded as an abnormal body temperature and thus signals changes in the animal status. For this reason, its monitoring provides an early signal regarding the status of the animal in terms of morbidity, thermal stress, synchronization and estrus status, calving onset, and other factors that may impact on rhythmicity of normal body temperature (Fischer-Tenhagen and Arlt, 2020; Kearton et al., 2020). However, many of the temperature sensors fall short of the needed basic qualities, viz. non-invasive, rapid, safer, easy to use, and robust to external variations. Rectal thermometers, including the clinical digital thermometer, are generally robust to external variations and acceptably accurate. Moreover, rectal thermometry, a standard method for the body temperature measurement, relies on the use of these clinical thermometers. Since rectal thermometry is associated with many drawbacks (Abecia et al., 2015; Yadav et al., 2017), the current study has discovered an alternative location on sheep at which temperature measurement may be conducted using a digital thermometer. This measurement location is less invasive and allows manipulation with ease.

This study revealed that mean temperature at the inguinal location, as well as axillary temperature, was similar to rectal temperature. The observed mean temperature values, particularly at the inguinal and indeed rectal locations, were consistent with the earlier reported rectal temperature (39.48±0.09℃) (Katsoulos et al., 2016). Moreover, the mean temperature values for both inguinal and rectal measurements fell within the established body temperature ranges for a normal or healthy sheep (39.0-39.75℃) (Stockler et al., 2021). The consistency between the observed mean inguinal temperature and the reference normal body temperature for sheep points to the potential utility of inguinal thermometry. Currently, rectal temperature is mostly used to estimate the body core temperature of animals, including sheep (Katsoulos et al., 2016). However, the temperature acquired by this method can be affected by digestion, peristaltic movements, fecal masses, muscle tone, and physical activity (Abecia et al., 2015). In terms of the numerical comparisons, the inguinal temperature was generally closer to the body core temperature compared to its rectal counterpart. It is plausible that the closely apposed thigh and abdominal wall (inguinal) was responsible for the aforementioned, since this anatomical predisposition may have minimized heat loss to the environment. It is noteworthy that variations in temperature readings, numerical and or statistical, were observed at different locations. This was consistent with the previous study reports for many animals species, including sheep (Kearton et al., 2020), cattle (George et al., 2014), and chickens (Abigaba and Sianangama, 2023).

The strong correlation observed between rectal and inguinal temperature was consistent with the previous findings in sheep (Katsoulos et al., 2016) and chickens (Abigaba and Sianangama, 2023), but disagreed with those of cattle (unpublished data). Moreover, the mean of difference between the rectal-inguinal temperature pair was not statistically significant ($p > 0.05$). This points to the reliability of inguinal temperature measurement (method) for body core temperature estimation in sheep. With regard to the rectal-axillary temperature pair, the observed bias was significant despite the similarity in their means and a strong correlation. The current correlation results are consistent with the previous study findings in a related species, which reported a strong correlation between the rectal and axillary temperature ($r=0.95$, $p < 0.01$) (Chaturvedi et al., 2004). Discussing about deductions from the foregoing, it can be stated that all odds favour the inguinal location for temperature measurement in sheep compared to axillary location. However, further studies utilizing larger sample sizes, under a more controlled environment, may be needed to validate the current findings.

It should be mentioned that correlation coefficients reveal a relationship between variables or methods but do not determine their agreement (Doğan, 2018). The observed significant bias from the zero-point limit, in the case of rectal-axillary temperature pair, supports this notion. For this reason, the Bland-Altman analysis was conducted for the rectal-inguinal temperature pair only, since their mean of differences did not show a significant bias. The results indicated a good level of correlation and or agreement because most of the observed data points were close to the bias and zero lines, respectively, although the observed limits were considerably outside the previously suggested difference of ± 0.2℃ (Fulbrook, 1993). The current findings are similar with those of the previous study in a related species (Abigaba and Sianangama, 2023), although the limits of agreement was generally higher than the case of an earlier study. It is plausible that this disparity in the limits of agreement was attributed to the smaller sample size, species studied, and environmental conditions under the current study. Moreover, this study did not factor in the contested lustiness of rectal thermometry to external variations (Abecia et al., 2015). Hence future studies intended to validate the current findings must factor in these issues. Additionally, a consideration of the potential hyperthermia or fevers that are attributed to localized causes, for example lactation and mastitis (Stockler et al., 2021) will also be crucial. A proper positioning of the thermometer probe at the inguinal location is also suggested to minimize the environmental effects on temperature readings. These may confound the actual estimates of the body core temperature.

CONCLUSION

Body temperature monitoring contributes to the early detection and management of febrile conditions and changes in the physiological state of animals, including sheep. In an effort to search for a suitable thermometry method, it was observed

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that variations in temperature readings existed among the different anatomical locations under study. However, it was revealed that the rectal and inguinal temperature measurements in sheep had similar means, strong correlation, with a non-significant bias between them, particularly when a digital thermometer is employed. Additionally, the observed higher numerical value of the inguinal temperature could suggest a better reflection of the body core temperature than the case is with the standard rectal thermometry. Further studies are needed to validate the current findings, particularly on the inguinal location, for generalization and future application.

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Authors' contribution

R. Abigaba conceived and designed study, collected and analyzed data, and wrote the manuscript, Ph. C. Sianangama designed, supervised study, and reviewed the manuscript, O. Chibinga Supervised study and reviewed manuscript, N. Gulaita designed study and reviewed manuscript, M. C. Sitali supervised study and reviewed manuscript, E.S. Mwaanga conceived and supervised study and reviewed manuscript. All authors read and approved the final manuscript for publication.

Availability of data and materials

The additional data from the present study may be provided on request from the corresponding authors

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This study did not receive any funding.

Ethical consideration

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues including consent to publish, misconduct, fabrication of data, and redundancy have been checked by the authors.

Conflict of interests

The authors declare no conflict of interest regarding this publication

REFERENCES

Abecia JA, Pascual-Alonso M, Aguayo-Ulloa LA and Maria GA (2015). Comparison of several devices to measure body temperature in sheep. Paper presented at the 7th European Conference on Precision Livestock Farming (EC-PLF). Millan, Italy. p 221-229. Available at: file:///C:/Users/User/Downloads/MILAN2015PLF-1.pdf.

Abigaba R and Sianangama PC (2023). Suitability of inguinal and axillary sites for temperature measurement using digital thermometers: A comparison with rectal thermometry in Broiler chickens. Journal of World's Poultry Research, 13(2): 191-198. DOI: https://dx.doi.org/10.36380/jwpr.2023.21.

Bailey M, Heinrich D and Kruczkiewicz A (2021). Climate profiles of countries in Southern Africa: Zambia. Climate factsheet (pp. 1-5). Red Cross Crescent Climate Centre, The Hague, Netherlands. Available at: https://www.climatecentre.org/wp-content/uploads/Climate-Profiles-of-Countries-in-Southern-Africa-Zambia.pdf

American Society of Animal Science (ASAS) (2020). Guide for the care and use of agricultural animals in research and teaching, 4th Edition. American Dairy Science Association, Illinois, USA. p 225. Available at: https://www.asas.org/docs/default-source/default-document-library/agguide_4th.pdf?sfvrsn=56b44ed1_2

Cadioli F, Fidélis Jr O, Garcia SD, Battaglia CT, Tomazzela D, Barra BG and et al. (2010). Comparison between mercury and non-contact infrared thermometers in lambs' rectal and ocular temperatures measurements. Paper presented at the Proceedings of the 26th Congress of the World Association for Buiatrics. Santiago de Chile, Chile. p 1-2. Available at: file:///C:/Users/User/Downloads/367-3.pdf

Cai Z, Cui J, Yuan H and Cheng M (2023). Application and research progress of infrared thermography in temperature measurement of livestock and poultry animals: A review. Computers and Electronocs in Agriculture, 205: 107586. DOI: https://doi.org/10.1016/j.compag.2022.107586.

Chaturvedi D, Vilhekar KY, Chaturvedi P and Bharambe MS (2004). Comparison of axillary temperature with rectal or oral temperature and determination of optimum placement time in children. Indian Pediatrics, 41: 600-603. Available at: https://www.indianpediatrics.net/june2004/600.pdf.

Doğan ÖN (2018). Bland-Altman analysis: A paradigm to understand correlation and agreement. Turkish Journal of Emergency Medicine, 18: 139–141. DOI: https://www.doi.org/10.1016/j.tjem.2018.09.001.

Fischer-Tenhagen C and Arlt SP (2020). Taking body temperature in cattle - critial evaluation of an established diagnostic test.Tierarztl Prax Ausg G Grosstiere Nutztiere, 48(4): 262-267. DOI: https://www.doi.org/10.1055/a-1197-5339

Fulbrook P (1993). Core temperature measurement in adults: A literature review. Journal of Advanced Nursing, 18(9): 1451-1460. DOI: https://www.doi.org/10.1046/j.1365-2648.1993.18091451.x

George WD, Godfrey RW, Ketring RC, Vinson MC and Willard ST (2014). Relationship among eye and muzzle temperatures measured using digital infrared thermal imaging and vaginal and rectal temperatures in hair sheep and cattle. Journal of Animal Science, 92: 4949–4955. DOI: https://www.doi.org/10.2527/jas.2014-8087.

Godyń D, Herbut P and Angrecka S (2019). Measurements of peripheral and deep body temperature in cattle – A review. Journal of Thermal Biology, 79: 42-49. DOI: https://www.doi.org/10.1016/j.jtherbio.2018.11.011.

Hine L, Laven RA and Sahu SK (2015). An analysis of the effect of thermometer type and make on rectal temperature measurements of cattle, horses and sheep. NewZealand Veterinary Journal, 63(3): 171–173. DOI: http://dx.doi.org/10.1080/00480169.2014.967325.

Katsoulos PD, Athanasiou LV, Karatzia MA, Valasi I, Boscos C and Karatzias H (2016). Comparison of a non-contact infrared thermometer with a rectal digital thermometer for use in ewes. Small Ruminant Research, 143: 84-88. DOI: https://doi.org/10.1016/j.smallrumres.2016.09.004.

Kearton TR, Doughty AK, Morton CL, Hinch GN, Godwin IR and Cowley FC (2020). Core and peripheral site measurement of body temperature in short wool sheep. Journal of Thermobiology, 90: 102606. DOI: https://doi.org/10.1016/j.jtherbio.2020.102606.

Levy I, Allender MC and Keller KA (2020). Comparison of axillary and inguinal body temperature to rectal temperature in healthy guinea pigs (Cavia porcellus). Journal of Exotic Pet Medicine, 34: 1–5. DOI: http://www.doi.org/10.1053/j.jepm.2020.03.016.

Muhammed MU, Musa MA and Abdullahi GA (2019). Comparison between rectal and body surface temperatures obtained by digital and non-contact infrared thermometer in some large animal species. International Journal of Research - GRANTHAALAYAH, 7(8): 62-68. DOI: https://wwww.doi/10.5281/zenodo.3379836.

Pourjafar M, Badiei K, Chalmeh AA, Rahmani Shahraki AR and Naghib M (2012). Body temperature in horses, cattle, sheep and goats measured by mercury, digital and non-contact infrared thermometers. Online Journal of Veterinary Research, 16(4): 195-203. Available at: http://onljvetres.com/temperatureabs2012.htm

Stockler RM, Stockler JW, Shipley FC and Pugh DG. (2021). Physical Examination, handling, and restraint of sheep, goats, and cervids. In Sheep, Goat, and Cervid Medicine (3rd edition). Elsevier, Amesterdam, pp. 1-14. DOI: https://www.doi/10.1016/B978-0-323-62463-3.00010-4

Underwood WJ, Blauwiekel R, Delano ML, Gillesby R, Mischler SA and Schoell A (2015). Biology and diseases of ruminants (sheep, goats, and cattle). In Laboratory animal medicine, Academic Press, Elsevier Inc. pp. 623-694. DOI: https://doi.org/10.1016/B978-0-12-409527-4.00015-8

Weinert D and Waterhouse J (2007). The circadian rhythm of core temperature: effects of physical activity and aging. Physiology & Behavior, 90(2-3):246-256. DOI: https://doi.org/10.1016/j.physbeh.2006.09.003

Yadav B, Singh G and Wankar A (2017). The use of infrared skin temperature measurements for monitoring heat stress and welfare of crossbred cattle. Indian Journal of Dairy Science, 70(1): 1-5. Available at: file:///C:/Users/User/Downloads/IJDS-3.pdf

Citation: Abigaba R, Sianangama PC, Chibinga O, Gulaita N, Muloongo S, and Mwanga ES (2023). Comparison of rectal thermometry with the alternative undertail, axillary, and inguinal temperature measurements in sheep. *Online J. Anim. Feed Res*., 13(5): 384-390. DOI: https://dx.doi.org/10.51227/ojafr.2023.54

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RELATIONSHIP BETWEEN STEAMING UP WITH COLOSTRUM PRODUCTION AT DIFFERENT MILKING TIMES IN HOLSTEIN-FRIESIAN COWS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Aims of study was to determine the relationship between steaming up with colostrum production at different milking times. The animals used in this research were 36 pregnant Holstein-Friesian (HF) cows. The method used in this research was a case study. Samples were determined with purposive sampling. The selected animal was divided into two groups, T_1 (control) and T_2 (steaming up). The steaming up was done two weeks prior to calving. The total average of colostrum production from HF cows that were in T_2 group was 11.96±2.40 liter/cow/day, while the mean value of colostrum production from HF cows that were in T_1 group was 8.05±1.80 liter/cow/day. The average colostrum production that was collected at morning milking from cows in T_2 group was 6.38±1.36 liter/cow/day and at afternoon milking was 5.58±1.11 liter/cow/day, significantly higher than T_1 group which was 4.22±0.92 liter/cow/day at morning milking and 3.83±0.90 liter/cow/day. The result of the regression equation on morning milking is Y = 2.059 + 2.159x. This means that steaming up treatment can increase colostrum production by as much as 2.159 liters at morning milking. While the result of the regression equation on afternoon milking is Y = 1.753 + 2.078x. This means steaming up treatment can escalate the colostrum production as much as 2.078 at afternoon milking. That equation is used as the basis for estimating the relationship between steaming up with colostrum production at both milking times, with a correlation coefficient (r) between steaming up and colostrum quantity at morning milking is 0.692, which means the relationship is in the strong category. Meanwhile, the relationship between steaming up and colostrum yield at afternoon milking is 0.666, which means the relationship is also in the strong category. It was concluded that steaming up had a very significant effect at both milking times in Holstein-Friesian cows.

Keywords: Calving, Colostrum production, Holstein-Friesian Cows, Milking time, Steaming up.

INTRODUCTION

In general terms, dairy cows make a major contribution to the national milk needs for human nutrition (Graulet, 2014). There are two most common dairy cow breeds in Indonesia, which are Holstein-Friesian (HF) and Jersey, whilst HF has the highest milk production than any other breed that can reach 4500-5000 liter in one lactation period (305 days) (Sembada, 2018). Milk production in Indonesia is relatively low, with production in 2022 as much as 968.980,14 tons (Statistics Indonesia, 2023). Dairy milk production depends on various factors such as the genetic, environmental, genetic, and environmental interaction, how milking increases milk production and milking interval (Beerda et al., 2007; Abdelsayed et al., 2014; Garantjang et al., 2020). Many local farmers are looking for a way to increase their cow's milk production (Kant and Yadav, 2016). One of the ways is steaming up treatment for cross-bred cattle (Sirohi et al., 2014; Kant and Yadav, 2016).

Steaming up or extra feeding of nutrients helps enhance the cow's milk production (Das et al., 2007). Steaming up was conducted during a dry period, approximately 2-3 weeks before calving (Arfuso et al., 2016). The purpose of steaming up is to give better production performance of the dairy cow in the next lactation period because it allows the udder secretory cells to develop maximally before the start of the lactation period (Nugraha and Surjowardojo, 2022). High-energy feeding consumption during late gestation will high-energy feed consumption required during late gestation ensures adequate nutrient supply and produce good growth of the mother and fetus (Dharmawan et al., 2019). Feeding extra nutrients to cows before calving is claimed to increase milk production and make cows ready for high intakes of concentrates that should be fed in early lactation (Pradhan et al., 2011). Moreover, increasing the quantity and quality of the feed is an effort to counteract NEB (Negative Energy Balance) (Bünemann et al., 2020). Cows are unable to adapt to

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the metabolic demands during the pre- and post-partum transition period and are more prone to subsequent negative events (Fiore et al., 2017). In addition, steaming up also has a role in bovine colostrum production, which is important to maintain dairy cow production performance (Surjowardojo et al., 2021).

Colostrum is the first milk that comes out from the udder after calving, which contains antibodies and nutrition for the calf (Surjowardojo et al., 2021) and it is produced for the next five days (Quinn et al., 2020). Colostrum has a yellowish color with a thick texture that is given to the calf as long as the colostrum still produce and has not transitioned into fresh milk (Surjowardojo et al., 2022). Colostrum has unreplaceable contents such as macro- and micro-nutrient, immunoglobulins, antimicrobial peptides, and growth factors. More specifically, the defense mechanism from colostrum can counter bacteria and virus contamination (Surjowardojo et al., 2020) and it is vital for calf since a passive immune system of the calf was obtained from consuming colostrum (Susilorini et al., 2023). There is evidence that colostrum is essential for nutritional and immunological support, growth, and development of the newborn calf (Playford and Weiser, 2021). The contributing factors in colostrum production are dry period length, dry period nutrition, parity, season, and previous 305 days of milk production (Gavin et al., 2018). Nugraha and Surjowardojo (2022) in their research stated that steaming up treatment can increase the colostrum yield. Therefore, the main objective of this study was to examine the relationship between steaming up with the colostrum production at different milking times.

MATERIALS AND METHODS

The research method used in this research was a case study at Koperasi Peternakan Sapi Perah (KPSP) Setia Kawan, Nongkojajar, Tutur Sub District, Pasuruan Regency. The material used in this research was 36 HF cows, which had already entered the dry period. The samples were determined by purposive sampling. These cows were either in their 2nd or in their 3rd lactation period. The selected animals were in their late stage of pregnancy and divided into two groups, the control (T_1) group and steaming up (T_2) group. The animals in group T_1 were provided with 30 kg grass per day and 4 kg concentrate per day whilst, in group T_2 were given 35 kg grass per day and 8 kg concentrate per day, two weeks before calving. Concentrate consists of a mix of pollard, corn DDGS (Distiller Dried Grains with Soluble), wheat DDGS, CGF (Corn Gluten Feed) and PKE (Palm Kernel Expeller), which contains vitamins, minerals, and antioxidants as feed additives. Field observation was held to obtain primary data and identify colostrum production. The first colostrum was measured with a measuring container at the exact moment after the cows gave birth or no later than 30 minutes after. Afterwards, colostrum production was observed until the next five days. Morning milking was carried out at 5.00 a.m., while afternoon milking was at 3.00 p.m. Colostrum production from both treatment groups was analyzed statistically by linear regression. Statistical analysis was conducted using Microsoft Excel 365 and IBM SPSS Statistics 26 software.

Ethical regulations

This study was conducted in accordance with the Animal Care and Use Committee, Universitas Brawijaya, Malang, East Java, Indonesia, with ethical clearance number 062-KEP-UB-2023.

RESULTS AND DISCUSSION

According to the data in Table 1, the total colostrum production in T_2 (steaming up) was 11.96±2.40 liter/cow/day, dramatically higher than that produced in the treatment T_1 (control) group which was 8.05±1.80 liter/cow/day. This is in accordance with (Nugraha and Surjowardojo, 2022) which stated that steaming up treatment on dairy cows that was carried out by farmers gives a better performance of colostrum production compared to cows that were not treated with the treatment. The cows in T_2 (steaming-up) group produced 6.38±1.36 liter/cow/day at morning milking and 5.53 liter/cow/day in the afternoon, which was significantly higher than that produced in the treatment T_1 (control) group which was 4.22±0.92 liter/cow/day at morning milking and 3.83±0.90 liter/cow/day in the afternoon (Jurkiewicz et al., 2005) stated that increasing the nutrient value of diets during the final week before calving and one week after will improve the milk production and the blood metabolite, as indicated by the improved protein and energy balance of periparturient cows and lower susceptibility of the cow's metabolic order. Steaming up can also have a positive effect on calf birth weight (Das et al., 2007).

Table 1 - Colostrum production at morning milking and afternoon milking

Mean ± Std. Dev / Treatment	N	Colostrum Production at Morning Milking (5.00 a.m.) (liter/cow/day)	Colostrum Production at Afternoon Milking (3.00 p.m.) (liter/cow/day)	Total Colostrum Production (liter/cow/day)
T_1 (control) group	18	4.22±0.92[a]	3.83±0.90[a]	8.05±1.80[a]
T_2 (steaming up) group	18	6.38±1.36[b]	5.58±1.11[b]	11.96±2.40[b]
Total	36	5.30±1.58	4.70±1.34	10.01±2.88

Steaming up treatment showed a very highly significant different effect ($P < 0.001$) on colostrum production at both milking times.

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This research also showed colostrum that was collected in the morning milking had higher average production compared to colostrum production in afternoon milking on either treatment. Milk production collected at morning milking tends to be higher than that harvested at afternoon milking because the interval time for milking in the morning was longer (14 hours) than the interval time for milking in the afternoon (10 hours). Milking interval is one of the factors that affect milk quality and quantity, longer interval tends to have higher milk production (Garantjang et al., 2020).

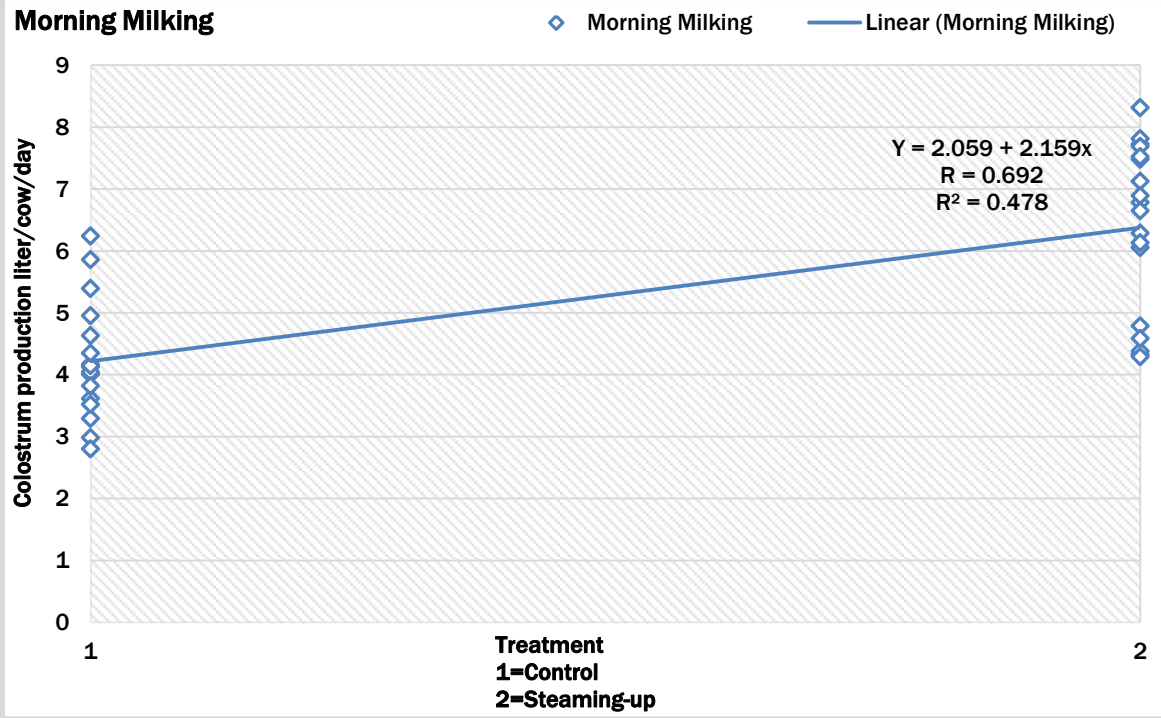

Figure 1 - Colostrum production at morning milking

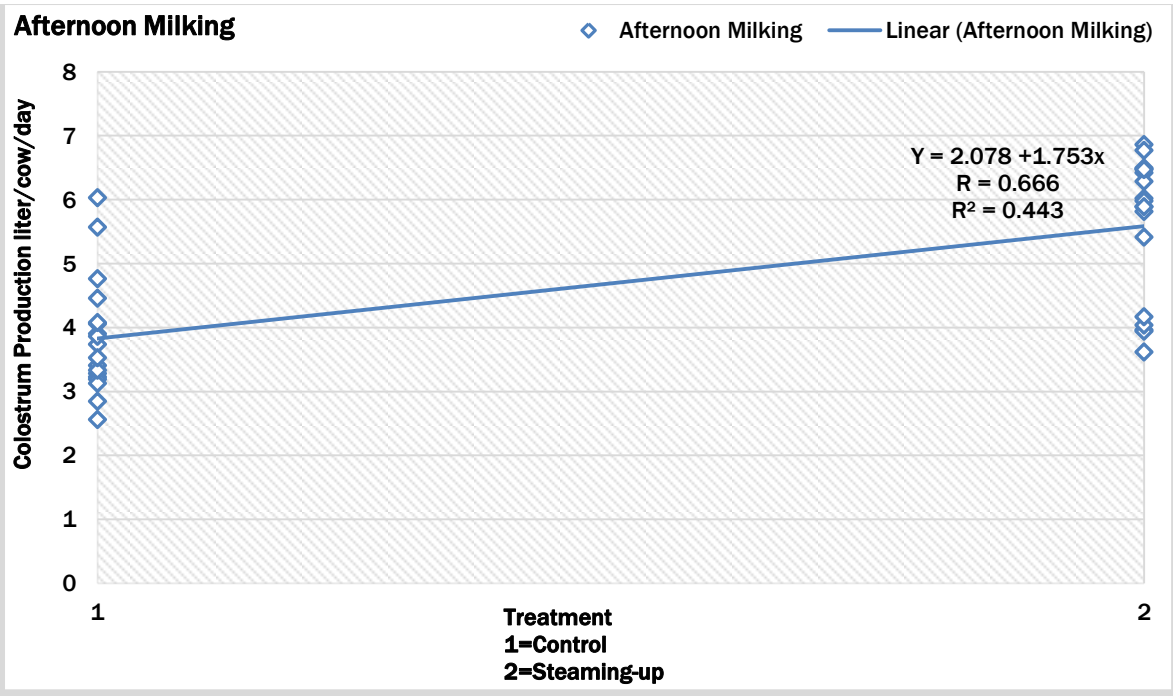

Figure 2 - Colostrum production at afternoon milking

As reported in Figure 1, the result of the regression and correlation analysis between steaming up with colostrum production at morning milking shows that the correlation coefficient (R) value is 0.692 which means both variables have a positive strong relationship. While in Figure 2, shows that the R-value of the data analysis between steaming up with colostrum production afternoon milking is 0.666 which means both variables also have a strong relationship. Figure 1 also shows that the value of the coefficient of determination (R2) is 0.478. which implies that the effect of steaming up

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on colostrum production at morning milking is 47.8%. While the remaining 52.2% is influenced by other factors. Soufleri et al. (2021) stated that colostrum production is affected by several factors like lactation period, season, parity, dry period length, previous lactation milk yield, milking interval and BCS. Surjowardojo et al. (2020) also stated that harvest time can also be a factor that can be affecting colostrum production. Whilst in Figure 2 depicts that the value of the R^2 is 0.443 which means that steaming up treatment affects 44.3% of the colostrum production at afternoon milking and the remaining 55.7% is determined by other factors.

Moreover, in Figure 1, the result of regression and correlation analysis shows that the value of the regression is Y = 2.059 + 2.159x. It means that excessive feeding both in quality and quantity before calving can increase the colostrum yield at morning milking as big as 2.159 liter/cow/day. Kant and Yadav (2016) in their research stated that cows that were fed with 4 kg of concentrate at each meal had higher colostrum production and milk production compared to cows that were fed with 2 kg of concentrate. Colostrum production experienced a peak in the production when the cows were in their 3rd lactation period and age at first gestation also affects the amount of colostrum production (Putri and Surjowadojo, 2022). Meanwhile, in Figure 2, the value of the regression is Y = 2,078 + 1.753x. This implies that steaming up treatment performed on HF cows could escalate the production of colostrum at afternoon milking as much as 1.753 liter/cow/day. According to Surjowardojo et al. (2022) the quality of the colostrum that is harvested in the afternoon is higher, but the colostrum production is lower. It is caused by the increasing temperature in the afternoon which affects cow physiology. On the other hand, colostrum production that is collected in the morning has a higher production, yet the quality is lesser.

CONCLUSION

Steaming up treatment had a strong relationship with production colostrum and had a very highly significant effect ($P < 0.001$) on colostrum production in both milking times (morning and afternoon) on Holstein-Friesian cows. It suggested that farmer need to carry out steaming up treatment to their HF cows because it has been proven to have a positive effect on colostrum production in both morning and afternoon milking which are suitable for fulfilling the calf need and bodes well for milk production in the next lactation.

DECLARATIONS

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Authors' contribution

P. Surjowardojo and P. Nugraha designed the study and manuscript writing. Rifa'i and A. C. Wardhana Collecting samples and data. H. Muarifah analysis data and manuscript writing. All authors drafted and revised the manuscript as read, evaluation and approved the final manuscript.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Abdelsayed M, Thomson PC, and Raadsma HW (2014). A review of the genetic and non-genetic factors affecting extended lactation in pasture-based dairy systems. Animal Production Science, 55(8): 949-966. https://doi.org/10.1071/AN13300

Arfuso F, Fazio F, Panzera M, Gianneto C, Di Pietro S, Giudice E and Piccione G (2017). Lipid and lipoprotein profile changes in newborn calves in response to the perinatal period. Acta Veterinaria-Beograd, 67(1):25-32. https://doi.org/10.5194/aab-59-429-201

Beerda B, Ouweltjes W, Šebek LB, Windig JJ, and Veerkamp RF (2007). Effects of genotype by environment interactions on milk yield, energy balance, and protein balance. Journal of Dairy Science, 90(1):219-228. https://doi.org/10.3168/jds.S0022-0302(07)72623-1

Bünemann K, Frahm J, Kersten S, Hüther L, Meyer U, Sauerwein H, Hummel J, Zeyner A, and Dänicke S (2020). Effects of pre-calving body condition and different post partum concentrate feed proportions on immune-associated and hematological parameters in pluriparous dairy cows. Animals, 10(12):1-18. https://doi.org/10.3390/ani10122251

Dharmawan R, Surjowardojo P, and Susilorini TE (2019). Effect of steaming up by *gliricidia sepium* to dairy goats during dry period on goat kid growth of etawah crossbred. Indonesian Journal of Animal Science, 29(2): 101-108. https://doi.org/10.21776/ub.jiip.2019.029.02.02

Citation: Surjowardojo P, Nugraha P, Rifa'i, Muarifah H, and Wardhana AC (2023). Relationship between steaming up with colostrum production at different milking times in Holstein-Friesian cows. *Online J. Anim. Feed Res.*, 13(5): 391-395. DOI: https://dx.doi.org/10.51227/ojafr.2023.55

Das KS, Das N, and Ravikumar GVPP (2007). Effect of 'steaming-up' on subsequent production in dairy cows. Indian Journal of Animal Sciences, 77(7): 583-585. https://epubs.icar.org.in/ejournal/index.php/IJAnS/article/view/5500

Fiore E, Piccione G, Arfuso F, Zumbo A, and Gianesella M (2017). Metabolic changes in dairy cows at different lactation class during the transition period. Large Animal Review, 23(23):45-48. https://vetjournal.it/images/archive/LAR%202017/Fiore_imp.pdf

Garantjang S, Rusdy M, Hatta M, Nohong B, and Sema (2020). Effect of milking time on milk production and milk quality of dairy cow fed with fermented corn cob. IOP Conference Series: Earth and Environmental Science, 492: 012054 https://doi.org/10.1088/1755-1315/492/1/012054

Gavin K, Neibergs H, Hoffman A, Kiser JN, Cornmesser MA, Haredasht SA, et al. (2018). Low colostrum yield in jersey cattle and potential risk factors. Journal of Dairy Science, 101(7):6388-6398. https://doi.org/10.3168/jds.2017-14308

Graulet B (2014). Ruminant milk: A source of vitamins in human nutrition. Animal Frontiers, 4(2):24-30. https://doi.org/10.2527/af.2014-0011

Jurkiewicz, A, Strzetelski JA, Kowalczyk J, and Bilik K (2005). Concentrate-enriched diets for calving heifers in the periparturient period increase milk yield and improve blood metabolite profile. Journal of Animal and Feed Sciences. 14(1):259-262. https://doi.org/10.22358/jafs/70533/2005

Kant S, and Yadav RK (2016). Effects of steaming up upon milk production and reproductive performance in crossbreed cows. Agriways, (2):138-144. http://agriwaysjournal.com/wp-content/uploads/journals/volume4.2/4.pdf

Manosalva DG, Grispoldi L, Spagnolo M, and Crociati M (2022). Delayed first milking in unassisted overnight calving did not affect the quality of colostrum but influenced serum brix refractometry in Holstein calves at two days of life. Animals, 12 1665. https://doi.org/10.3390/ani12131665

Nugraha P and Surjowardojo P (2022). Effect of steaming up on old pregnant parent weights, calf birth weight and colostrum production in PFH cattle in sumber rejeki group, Princi, Dau, Malang District. Bantara Journal of Animal Science, 4(2):52-60. https://doi.org/10.32585/bjas.v4i2.2825

Playford RJ and Weiser MJ (2021). Bovine colostrum: its constituents and uses. Nutrients, 13(1):265. https://doi.org/10.3390/nu13010265

Pradhan SM, Khanal B, and Hamal N (2011). Effect of steaming up practice in late pregnant stage in dairy cows. Proceedings of the 8th National Workshop on Livestock and Fisheries Research, 5(8):241-244.

Putri Z and Surjowardojo P (2022). Differences of colostrum production in fore and rear teat of FHC dairy cow. Jurnal Ternak, 13(2):53-57. https://doi.org/10.30736/jt.v13i2.151

Quinn EM, O'Callaghan TF, Tobin JT, Murphy JP, Sugrue K, Slaterry H, et al. (2020). Changes to the oligosaccharide profile of bovine milk at the onset of lactation. Dairy, 1(3):284-296. https://doi.org/10.3390/dairy1030019

Sembada P (2018). Transformation of dairy production systems in Indonesia: assessing sustainability and long term trajectories of farms, Doctoral dissertation, Montpellier SupAgro, Spécialité: EFSA - Écologie Fonctionnelle et Sciences Agronomiques Discipline: Sciences Agronomiques, Rabat. https://theses.hal.science/tel-02159387/

Sirohi AS, Patel AK, Mathur BK, Misra AK, and Singh M (2014). Effects of steaming-up on the performance of grazing does and their kids in arid region. Indian Journal of Animal Research, 48(1):71-74. https://www.indianjournals.com/ijor.aspx?target=ijor:ijar1andvolume=48andissue=1andarticle=015

Soufleri A, Banos G, Panousis N, Fletouris D, Arsenos G, Kougioumtzis A, and Valergakis GE (2021). Evaluation of factors affecting colostrum quality and quantity in holstein dairy cattle. Animals, 11 2005. https://doi.org/10.3390/ani11072005c

Statistics Indonesia. 2023. Fresh milk production by province. Accessed March 30th, 2023. https://www.bps.go.id/indicator/24/493/1/produksi-susu-segar-menurut%20provinsi.htmlr

Surjowardojo P, Muarifah H, Rifa'i, and Handayani IM (2022). Colostrum quality of friesian holstein (FH) cow with different age of colostrum based on fat content. Agriovet Journal, 5(1):125-132. https://doi.org/10.51158/agriovet.v5i1

Surjowardojo P, Noegroho VAK, Syahputra FT, and Yulinasari AP (2020). Colostrum quality of friesian holstein crossbreed from different milking day. IOP Conference Series: Earth and Environmental Science, 478: 012071. https://doi.org/10.1088/1755-1315/478/1/012071

Surjowardojo P, Susilorini TE, and Rifa'i (2021). Production colostrum of dairy cows Friesian Holstein (FH) in different lactation periods. Agriovet Journal, 4(1):31-36. https://doi.org/10.51158/agriovet.v4i1

Susilorini TE, Surjowardojo P, Sikone HY, Wardhana AC, and Rifa'i (2023). The performance of IgM colostrum of Friesian Holstein dairy cows at different lactation periods. International Journal of Veterinary Science, 12(1):136-138. https://doi.org/10.47278/journal.ijvs/2022.158

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PARATYPHOID *SALMONELLA* SEROVARS IN CHICKENS: MOLECULAR DETECTION OF VIRULENCE AND ANTIMICROBIAL RESISTANCE GENES

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ABSTRACT: Paratyphoid salmonellosis is a serious disease that threatens the poultry industry worldwide, besides its public health hazard. The aims of this study were characterization of paratyphoid *Salmonella* spp. in chicken flocks of some Egyptian governorates, demonstration of the antimicrobial susceptibility of the isolated *Salmonella* spp., and detection of some virulence genes and antibiotic resistance genes using recent molecular techniques. A total of 238 organ samples were collected from 52 broiler, layer, and breeder chicken flocks, representing 9 Egyptian governorates. Conventional characterization of *Salmonella* isolates revealed a total isolation rate of 56.3% (134/238). Moreover, the isolation rates of *Salmonella* spp. were (49/79; 62%), (47/81; 58%), (10/18; 55.5%), (9/20; 45%), (2/6; 33.3%), (2/3; 66.7%), and (15/82; 53.6%) from liver, yolk sac, heart, spleen, caecum, ovary, and dead-in-shell embryos, respectively. A total of 32/238 (13.44%) isolates of *Salmonella* were found. Serological identification revealed presence of *S. enteritidis* (21.9%), *S. kentucky* (15.6%), *S. typhimurium* (12.5%), *S. molade* (12.5%), *S. takoradi* (9.4%), *S. wingrove* (6.3%), *S. infantis* (6.3%), *S. tsevie* (6.3), *S. shangani* (3.1%), *S. bargny* (3.1%), and *S. papuana* (3.1%). All *Salmonella* strains (32/32; 100%) were resistant to streptomycin, while almost all of them (31/32; 96.9%) were susceptible to meropenem. The amplification of *16S* rRNA gene of *Salmonella* isolates using uniplex polymerase chain reaction (PCR) generated a specific *Salmonella* product of approximately 550 base pair. The multiplex PCR revealed presence of *inv*A (100%), *stn* (65.6%), and *sop*B (40.6 %) virulence-associated genes as well as *aad*A1 (100%), *bla*TEM (59.4%), *aad*B (18.75%), and *sul*1 (28.1%) antibiotic resistance genes. In conclusion, virulent paratyphoid *Salmonella* spp. are circulating in the Egyptian flocks, causing economic losses. Additionally, they became resistant to the most commonly used field antibiotics. Therefore, regular molecular surveillance studies on the circulating *Salmonella* spp. and their resistance to the used antibiotics are of significant importance.

Keywords: Antibiotic resistance genes, Chicken, Paratyphoid *Salmonella*, PCR, Serology, Virulence

INTRODUCTION

The poultry industry has grown dramatically around the world accounting for approximately 45% of global trade (Mottet and Tempio, 2017). This industry is considered as a significant source of animal protein for the Egyptians. Paratyphoid salmonellosis is one of the most important bacterial diseases affecting poultry and regarded a major source of food-borne infection in humans (Menghistu et al., 2011; Abd El-Ghany, 2020). Foodborne diseases are frequently associated with the consumption of animal-derived foods, primarily poultry products such as eggs and undercooked chicken (Castro-Vargas et al., 2020). These diseases have negative economic impacts on poultry industry as a result of the costs of investigation, surveillance, prevention, and treatment (Kuria, 2023). *Salmonella* spp. are rod-shape Gram-negative bacteria belonging to family *Enterobacteriaceae* and are known to infect many hosts (Xu et al., 2020).

Despite the European Union in 2006 prohibited using of some antibiotics as growth promotors feed additives, few countries are still using them (Gyles, 2008; Diab et al., 2019). Besides, many other antimicrobials are used haphazardly to control *Salmonella* infections either in poultry field or for human infection (Xu et al., 2020). As a result of indiscriminate use of these antibiotics, the emergence of multiple drug-resistant (MDR) *Salmonella* serotypes have significantly risen (Ammar et al., 2019). *Salmonella* spp. have developed resistance to a wide range of antimicrobials (Azizpour, 2018). The mechanisms by which the bacteria can acquire antibiotic resistance are variable and it can occur via mutations or horizontal transfer of resistance genes (Kapoor et al., 2017). However, MDR *Salmonella* spp. can be transmitted from poultry to humans through the food chain or via the direct contact (Firoozeh et al., 2012). The main threats affecting

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humans after exposure to these antibiotic-resistant bacteria are inability to treat patients successfully, along with the high risk of transmission of such resistant bacteria (Roca et al., 2015). Thus, the existence of antibiotic resistance *Salmonella* strains has become a serious global issue. In addition, presence of both antimicrobial resistance-associated genes as well as virulence associated genes in *Salmonella* strains are important indicators of pathogenicity degree (Capuano et al., 2013; Lamas et al., 2016). Among *Salmonella* spp., there are many virulence and antibiotic associated resistance genes that have been detected molecularly using polymerase chain reaction (PCR) assays. For instance, the virulence associated genes including *inv*A, *sop*B, *stn*, and *pef* are used for genetic characterization of *Salmonella* strains (Badr et al., 2021; Elshebrawy et al., 2021; Ghetas et al., 2021; Hassan et al., 2021). Moreover, the antibiotic resistance genes such as *aad*A1 (streptomycin), *aad*B (gentamicin), *sul*1 (sulphonamides), and *bla*TEM (β lactams) have been detected in many isolates of *Salmonella* spp. (Ammar et al., 2019; Alam et al., 2020; Herrera-Sánchez et al., 2020).

Therefore, the aims of this study were characterization of paratyphoid *Salmonella* spp. in chicken flocks of some Egyptian governorates, demonstration of the antibiotic sensitivity of the isolated *Salmonella* spp., and detection of some virulence genes and antibiotic resistance genes using molecular techniques.

MATERIALS AND METHODS

Sampling

From November 2020 to April 2021, a total of 238 samples were collected from 52 broiler, layer, and breeder chickens' flocks in El-Mansoura, El-Sharqia, El-Qalyubia, El-Beheira, El-Minya, El-Fayoum, El-Gharbeya, Damietta, and Giza Egyptian governorates (Table 1). Samples were taken from diseased chicks showing whitish diarrhea, pasty vent, and omphalitis, dead-in-shell embryos, and breeders with low fertility and hatchability. The post-mortem lesions of both freshly dead and scarified diseased chickens were un-absorbed yolk sac, congested liver and spleen, typhlitis, and misshapen or discolored ova. Tissue samples were collected under aseptic conditions from liver (79), yolk sac (81), cecum (6), heart (18), spleen (20), ovary (3), gallbladders (3), and organs of dead-in-shell embryos (28), and then rapidly transferred in ice box to the laboratory for further processing.

Ethical regulations

The study design and methodology were approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University, Egypt, with an approval number (01122022543).

Isolation and identification

Isolation and identification of *Salmonella* spp. were done according to ISO 6579:2002 guidelines. The collected samples were inoculated in buffered peptone water and incubated at 37°C for 18-20 hr. Then, 1 ml of pre-enriched broth was transferred into tubes containing 10 ml of Selenite F broth and incubated at 37±1°C for 24 hr. A loopful from each broth culture was inoculated onto selective plating medium such as xylose lysine deoxycholate agar (XLD), brilliant green agar (BGA), and MacConkey agar media, incubated at 37°C for 24 hr, and then checked for the of growth of typical *Salmonella* colonies. Suspected colonies were stained with Gram's and examined microscopically (Quinn et al., 2011). The biochemical identification of suspected *Salmonella* colonies was performed according to ISO 6579:2002 guidelines using triple sugar iron agar (TSI), indole, methyl red, vogues proskauer (VP), citrate utilization, urea hydrolysis, and H_2S production tests. The serological identification of *Salmonella* was carried out according to Kauffman-White Scheme (Kauffman, 1974). Both somatic (O) and flagellar (H) antigens (DENKA SEIKEN Co., Japan) were demonstrated using polyvalent and monovalent O antisera according to a slide agglutination test and H antisera according to a tube agglutination test.

The antimicrobial susceptibility test

The antimicrobial susceptibility test of the isolated *Salmonella* strains was done using disc diffusion method, according to the guidelines stipulated by National Committee for Clinical Laboratory Standards (NCCLS, 2020). The used antibiotic discs (Oxoid Limited, Basingstoke, Hampshire, UK) were ciprofloxacin (CP) (5µg), tetracycline (T) (30µg), ampicillin (AM) (10µg), cefotaxime (CF) (30µg), meropenem (M) (10µg), nalidixic acid (NA) (30µg), colistin (CO) (25µg), streptomycin (S) (10µg), levofloxacin (5µg), kanamycin (K) (30µg), clindamycin (CL) (10µg), amikacin (AK) (30µg), gentamicin (G) (10µg), and sulphamethoxazole (25µg). The tested strains were evaluated as susceptible, intermediate, and resistant by measuring the inhibitory zones. Moreover, the multiple antibiotic resistance (MAR) index for each *Salmonella* strain was determined (Singh et al., 2010) as follow;

MAR index= Number of antibiotics to which an isolate was resistant / total number of the tested antibiotics.

Each strain was classified as MDR if it exhibited resistance to three or more antimicrobial classes. Besides, the strain was considered to be resistant if it expressed MAR index > 0.2 (Shehata et al., 2019).

Molecular detection of *Salmonella* spp. virulence-associated and antibiotic resistance genes

The detection of *16S* rRNA of *Salmonella* isolates genes were carried out using uniplex PCR (Borges et al., 2017). The primers sequences are listed in Table 2, and the cycling conditions of primers during PCR are shown in Table 3. The

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PCR reactions were adjusted in 25 µL reaction mixture containing 5 µL of DNA template, 12.5 µL of 2x PCR master mix, 1.25 µL each of forward and reverse primers (10 pmol/µL), and 5 µL nuclease free water. The PCR products were run through 1.5% agarose gel electrophoresis and a 100 bp DNA ladder was used as a size marker.

The multiplex PCR was used for testing the presence of both virulence associated genes and antibiotic resistance genes in the isolated *Salmonella* spp. The tested virulence associated genes were *inv*A, *sop*B, *stn*, and *pef*, while the antibiotic resistance genes were *aadA1* (streptomycin), *aad*B (gentamicin), *sul*1 (sulphonamides), and *bla*TEM (β lactams). The primers sequences of virulence associated genes and the antibiotic resistance genes are presented in Tables 4 and 5, respectively.

RESULTS

Suspected *Salmonella* isolates appeared as small, non-lactose fermenter, colorless, and transparent colonies on MacConkey agar plates. However, isolates appeared as pink colonies with black centers on XLD and pinkish white or red colonies surrounded by a red halo on BGA agar. Gram staining revealed presence of Gram-negative, medium size, and rod-shaped bacilli under the microscope. The isolates fermented glucose, but did not ferment lactose and sucrose on TSI slants, and appeared as red slant with yellow butt, with or without H_2S production. Moreover, they were negative for indole, VP, and urea hydrolysis, whereas positive for methyl red and citrate utilization.

The conventional bacteriological characterization revealed that the isolation rates of *Salmonellae* were (49/79; 62%), (47/81; 58%), (10/18; 55.5%), (9/20; 45%), (2/6; 33.3%), (2/3; 66.7%), and (15/82; 53.6%) from liver, yolk sac, heart, spleen, caecum, ovaries, and dead-in-shell embryos, respectively. However, no isolation (0/3; 0%) was obtained from gallbladder (Table 6). The total isolation rate was 134/238 (56.3%). The number of the total strains of *Salmonella* was 32/238 (13.44%). Moreover, positive *Salmonella* isolates were detected in 49 out of 76 in broilers, 4 out of 10 in layers, and 2 out of 3 in breeder chicken flocks.

By using polyvalent and monovalent O and H antiserum, the slide and tube agglutination tests confirmed that the tested *Salmonella* isolates (n= 32) belonged to 11 paratyphoid *Salmonella* serovars. Table 7 shows the types and percentages of the isolated *Salmonella* serovars. A total of 11 serovars of paratyphoid *Salmonella* were identified as *S. enteritidis* (7, 21.9%), *S. kentucky* (5, 15.6%), *S. typhimurium* (4, 12.5%), *S. molade* (4, 12.5%), *S. takoradi* (3, 9.4%), *S. wingrove* (2, 6.3%), *S. infantis* (2, 6.3%), *S. tsevie* (2, 6.3%), *S. shangani* (1, 3.1%), *S. bargny* (1, 3.1%), and *S. papuana* (1, 3.1%).

Paratyphoid *Salmonella* serovars isolated from the liver (n= 49) were *S. enteritidis* (12), *S. typhimurium* (6), *S. kentucky* (8), *S. takoradi* (7), *S. molade* (4), *S. wingrove* (3) , *S. bargny* (3) , *S. shangani* (1), *S. infantis* (2), and *S. tsevie* (3), while those isolated from the yolk sac (n= 47) were *S. enteritidis* (9), *S. typhimurium* (4), *S. kentucky* (9), *S. takoradi* (6), *S. molade* (7), *S. wingrove* (2), *S. bargny*(3), *S. shangani* (1), *S. infantis* (2), and *S. tsevie* (4). Moreover, strains of the heart (n= 10) were *S. enteritidis* (2), *S. typhimurium* (3), *S. wingrove* (1), *S. shangani* (1), *S. molade* (2), and *S. infantis* (1), but those of spleen (n= 9) were *S. enteritidis* (1), *S. typhimurium* (5), *S. kentucky* (1), *S. papuana* (1), and *S. wingrove* (1). Caecal serovars (n= 2) were *S. takoradi* (2), while ovarian serovars (n= 2) were *S. enteritidis* (1) and *S. molade* (1). Regarding serovars of the dead-in-shell embryos (n= 15), they were *S. kentucky* (3), *S. takoradi* (6), *S. molade* (3), and *S. bargny* (3).

Regarding the distribution of *Salmonella* serovars in the surveyed Egyptian governorates, the highest isolation rates were from El-Dakhlia, El-Sharqia, Damietta and El-Qalubia, followed by El-Fayoum, El-Minya, Giza, and El-Beheira. Nevertheless, there was no isolation from El-Gharbeya. The data in Table 8 reveals detection of *S. enteritidis* from El-Qalubia, El-Dakhlia, El-Beheira, El-Minya, El-Fayoum, and Damietta governorates, while *S. typhimurium* was found in El-Dakhlia, El-Fayoum, El- Sharkqia, and Giza. Moreover, *S. kentucky* was isolated from El-Dakhlia, El-Minya, and Damietta and *S. molade* from El-Beheira, El-Fayoum, and Damietta. *S. takoradi* was isolated from El-Dakhlia, El- Sharqia, and Damietta, though *S. wingrove* was demonstrated in El-Dakhlia and El-Qalubia. Both *S. shangani* and *S. bargny* were detected in El-Dakhlia, then *S. tsevie* was isolated from El-Dakhlia and El-Sharqia. *S. infantis* was demonstrated in El-Fayoum and El- Sharqia, whereas S. Papuana was found in El-Qalubia

As shown in Table 9, all strains of *Salmonella* were completely resistant to streptomycin 100% (32/32), followed by clindamycin 93.8% (30/32), nalidixic acid 75% (24/32), amikacin 65.6% (21/32), tetracycline 50% (16/32), cefotaxime 43.7% (14/32),, kanamycin 34.4% (11/32), sulphamethoxazole 28.1% (9/32), ampicillin 18.8% (6/32), ciprofloxacin 12.5% (4/32), and colistin 12.5% (4/32). However, almost all strains of *Salmonella* were susceptible to meropenem 96.9% (31/32), followed by levofloxacin 90.6% (29/32), gentamicin 87.5% (28/32), colistin 84.4% (27/32), ciprofloxacin 81.3% (26/32), ampicillin 68.8% (22/32), sulphamethoxazole 71.9% (23/32), kanamycin 59.3% (19/32), and cefotaxime 56.3% (18/32).

Additionally, a total of 24 out of 32 *Salmonella* strains were resistant to 3 or more of the tested antimicrobials with an average MAR index 0.350. Surprisingly, out of the tested (n= 14) antimicrobials, two strains of *S. enteritidis* were resistant to 14, one strain of *S. kentucky* was resistant to 13 antimicrobials, and one strain of *S. typhimurium* was resistant to 10 antimicrobials.

The results of uniplex PCR confirmed that the amplification of *16S* rRNA gene of all *Salmonella* isolates generated a product of approximate molecular size of 550 base pair (bp) (Figure 1). Concerning the virulence genes, the amplified

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products for *inv*A, *sop*B, *stn*, and *pef* genes were 284, 517, 617, and 700 bp, respectively, while those of the antibiotic resistance genes *aad*A1, *aad*B, *sul*1, and *bla*TEM were 629, 300, 591, and 608 bp, respectively.

All *Salmonella* strains (n= 32) were screened by multiplex PCR for identification of the virulence associated genes (*inv*A, *stn*, *sop*B, and *pef*) and the antibiotic resistance genes (*aad*A1, *aad*B, *sul*1, and *bla*TEM). The results indicated that *inv*A, *sop*B, and *stn* genes were harbored by 100%, 40.6%, and 65.6% of *Salmonella* strains, respectively, while none of them had *pef* gene (Table 10 and Figure 2). Further, all strains (100%) harbored *aad*A1 associated with streptomycin resistance, but 59.4%, 18.75%, and 6.3% of strains possessed *bla*TEM (ampicillin), *aad*B (gentamicin), and *sul*1 (sulphamethoxazole) resistance genes, respectively (Table 11 and Figures 3-5).

Table 1 - The source and number of the samples examined for detection of *Salmonella*

Governorates	Chickens			Dead-in-shell embryonic organs	No. of samples	No. of flocks
	Broiler	Layer	Breeder			
A	39	6	3	28	145	28
B	18	2	0	0	49	14
C	19	2	0	0	44	10
Total	76	10	3	28	238	52

A= El-Mansoura, El-Gharbeya, El-Beheira & Damietta; B= El-Qalyubia, El-Sharqia & Giza; C= El-Minya & El-Fayoum

Table 2 - Primer sequences for detection of *16S* rRNA gene of *Salmonellae*

Target gene	Primer Sequence	Gene	Reference
16S rRNA	GCA ACG CGA AGA ACC TTA CC (Forward) GGT TAC CTT GTT ACG ACT T (Reverse)	*16S* rRNA	Gopinath et al. (1998)

Table 3 - Cycling conditions of the used primers during PCR

Gene	Primary Denaturation	Secondary Denaturation	Annealing	Extension	No. of Cycles	Final Extension
16S rRNA gene and other genes	90°C for 5 min.	90°C for 1 min.	52°C for 1 min.	72°C for 1 min.	39	72°C for 7 min.

Table 4 - Primers sequences of virulence associated genes of *Salmonella* serovars

Target gene	Primer sequence	Virulence factor	Reference
*inv*A	GTGAA ATT ATC GCC ACG TTC GGG CAA (Forward) TCAT CGC ACC GTC AAA GGA ACC (Reverse)	*Salmonella* species/SPI-1	Oliveira et al. (2003)
stn	TTG TGT CGC TAT CAC TGG CAA CC (Forward) ATT CGT AAC CCG CTC TCG TCC (Reverse)	Enterotoxin/Chromosome	Murugkar et al. (2003)
*sop*B	TCA GAA GRC GTC TAA CCA CTC (Forward) TAC CGT CCT CAT GCA CAC TC (Reverse)	Translocated effector protein/SPI-5	Huehn et al. (2010)
pef	TGT TTC CGG GCT TGT GCT (Forward) CAG GGC ATT TGC TGA TTC C (Reverse)	Plasmid encoded fimbriae/Plasmid	Murugkar et al. (2003)

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Table 5 - Primers sequences of antiiotic resistance genes of *Salmonella* serovars

The antibiotic	Target gene	Primer sequence	Reference
Streptomycin	*aad*A1	CTC CGC AGT GGA TGG CGG (Forward)	Chuanchuen and Padungtod (2009)
		GAT CTG CGC GCG AGG CCA (Reverse)	
Gentamicin	*aad*B	CTAGCTGCGGCAGATGAGC (Forward)	
		CTCAGCCGCCTCTGGGCA (Reverse)	
Sulfamethoxazole	*sul*1	CGGACGCGAGGCCTGTATC (Forward)	
		GGGTGCGGACGTAGTCAGC (Reverse)	
Ampicillin	*bla*TEM	ATCAGTTGGGTGCACGAGTG (Forward)	
		ACGCTCACCGGCTCCAGA (Reverse)	

Table **6** - The number and percentage of the positive *Salmonella* samples

Group	Liver		Yolk sac		Cecum		Heart		Spleen		Gallbladder		Ovary		Dead-in-shell embryos		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A	26/47	55.3	26/51	50.9	2/5	40	1/1	100	3/10	30	0	0	2/3	66.6	15/28	53.6	75/145	51.7
B	12/16	75	10/14	71.4	0/1	0	5/9	55.5	5/6	83.3	0/3	0	0	0	0	0	32/49	56.3
C	11/16	68.8	11/16	68.8	0	0	4/8	50	1/4	25	0	0	0	0	0	0	27/44	61.4
Total	49/79	62	47/81	58	2/6	33.3	10/18	55.5	9/20	45	0/3	0	2/3	66.7	15/28	53.6	134/238	56.3

A= El-Mansoura, El-Gharbeya, El-Beheira & Damietta; B= El-Qalyubia, El-Sharqia & Giza; C= El-Minya & El-Fayoum

Table 7 - The types and percentages of the isolated *Salmonella* serovars

Identified strains	Number	%	Antigenic structure		
			Group	O	H
S. enteritidis	7	21.9	D1	1,9,12	g,m
S. kentucky	5	15.6	C3	8,20	i : Z6
S. typhimurium	4	12.5	B	1,4,5,12	i : 1,2
S. molade	4	12.5	C2	8,20	Z10 : Z6
S. takoradi	3	9.4	C2	8,20	i : 1,5
S. wingrove	2	6.3	C2	6,8	c : 1,2
S. infantis	2	6.3	C1	6,7	r : 1,5
S. tsevie	2	6.3	B	4,5	i : e,n,z15
S. shangani	1	3.1	E1	3,10	d : 1,5
S. bargny	1	3.1	C3	8,20	i : 1,5
S. papuana	1	3.1	C1	6,7	r : e,n,Z15

Table 8 - Types of *Salmonella* serovars isolated from the Egyptian governorates

Salmonella serovar	Frequency of each isolate		Governorate
	No.	%	
S. enteritidis	7	21.9	El-Qalubia, El-Dakhlia, El-Beheira, El-Minya, El-Fayoum, & Damietta
S. kentucky	5	15.6	El-Dakhlia, El-Minya, & Damietta
S. typhimurium	4	12.5	El-Dakhlia, E-Fayoum, El-Sharqia, & Giza
S. molade	4	12.5	El-Beheira, El-Fayoum, & Damietta
S. takoradi	3	9.4	El-Dakhlia, El-Sharqia, & Damietta
S. wingrove	2	6.3	El-Dakhlia and El-Qalubia.
S. infantis	2	6.3	El-Fayoum & El-Sharqia.
S. tsevie	2	6.3	El-Dakhlia & El-Sharqia
S. shangani	1	3.1	El-Dakhlia
S. bargny	1	3.1	El-Dakhlia
S. papuana	1	3.1	El-Qalubia

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Table 9 - The antimicrobial susceptibility test of the isolated *Salmonella* strains

Salmonella serovars	No.	Antibiotic resistance													
		S	CL	NA	AK	T	CF	K	SXT	AM	CP	CO	G	L	M
S. enteritidis	7	100	100	85.7	85.7	71.4	57.1	42.9	42.9	28.5	28.5	28.5	14.3	4.3	4.3
S. kentucky	5	100	80	80	60	60	60	60	40	40	20	20	20	20	0
S.typhimurm	4	100	100	75	50	50	25	50	25	25	0	25	0	0	0
S. molade	4	100	100	75	75	50	50	25	25	25	25	0	0	0	0
S. takoradi	3	100	100	66.6	66.6	33.3	33.3	33.3	33.3	0	0	0	0	0	0
S. wingrove	2	100	100	100	50	50	50	50	50	0	0	0	0	0	0
S. infantis	2	100	100	50	50	50	50	0	0	0	0	0	0	0	0
S. tsevie	2	100	50	50	50	50	50	0	0	0	0	0	0	0	0
S. shangani	1	100	100	100	100	0	0	0	0	0	0	0	0	0	0
S. bargny	1	100	100	100	100	0	0	0	0	0	0	0	0	0	0
S. papuana	1	100	100	0	0	0	0	0	0	0	0	0	0	0	0
Total	32	32	30	24	21	16	14	11	9	6	5	4	2	2	0
Resistant %		100	93.8	75	65.6	50	43.7	34.4	28.1	18.8	12.5	12.5	6.3	6.3	3.1
Intermediate %		0	3.1	9.4	9.4	6.3	0	6.3	3.1	12.5	6.3	3.1	6.3	3.1	0
Susceptible %		0	3.1	15.6	25	43.7	56.3	59.3	68.8	68.8	78.1	81.3	87.5	90.6	96.9

S: Streptomycin; CL: Clindamycin; NA: Nalidixic acid; AK: Amikacin; T: Tetracycline; CF: Cefotaxim; K: Kanamycin; SXT: Sulphamethoxazol; AM: Ampicillin; CP: Ciprofloxacin; CO: Colistin; G: Gentamicin; L: Levofloxacin; M: Meropenem

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Table 10 - Percentages of virulence-associated genes of *Salmonella* serovars

Salmonella serovar \ Virulence genes	No.	*16S* rRNA		*invA*		*stn*		*sopB*		*pef*	
		No.	%	No.	%	No.	%	No.	%	No.	%
S. enteritidis	7	7	100	7	100	6	85.7	5	71.5	0	0
S. kentucky	5	5	100	5	100	4	80	2	40	0	0
S. typhimurm	4	4	100	4	100	4	100	1	25	0	0
S. molade	4	4	100	4	100	1	25	0	0	0	0
S. takoradi	3	3	100	3	100	1	33.3	1	33.3	0	0
S. wingrove	2	2	100	2	100	2	100	2	100	0	0
S. infantis	2	2	100	2	100	1	50	1	50	0	0
S. tsevie	2	2	100	2	100	1	50	1	50	0	0
S. shangani	1	1	100	1	100	1	100	0	0	0	0
S. bargny	1	1	100	1	100	0	0	0	0	0	0
S. papuana	1	1	100	1	100	0	0	0	0	0	0
Total	32	32	100	32	100	21	65.6	13	40.6	0	0

Table 11 - Percentages of antibiotic associated genes of *Salmonella* serovars

Salmonella serovar	No.	The tested antibiotic resistance genes							
		aadA1		*sul1*		*bla*TEM		*aadB*	
		No.	%	No.	%	No.	%	No.	%
S. enteritidis	7	7	100	3	42.8	3	42.8	0	0
S. kentucky	5	5	100	2	40	3	60	2	40
S. typhimurm	4	4	100	1	25	2	50	0	0
S. molade	4	4	100	1	25	2	50	1	25
S. takoradi	3	3	100	1	33.3	1	33.3	1	33.3
S. wingrove	2	2	100	1	33.3	2	100	0	0
S. infantis	2	2	100	0	0	2	100	1	50
S. tsevie	2	2	100	0	0	1	50	1	50
S. shangani	1	1	100	0	0	1	100	0	0
S. bargny	1	1	100	0	0	1	100	0	0
S. papuana	1	1	100	0	0	1	100	0	0
Total	32	32	100	9	28.1	19	59.4	6	18.75

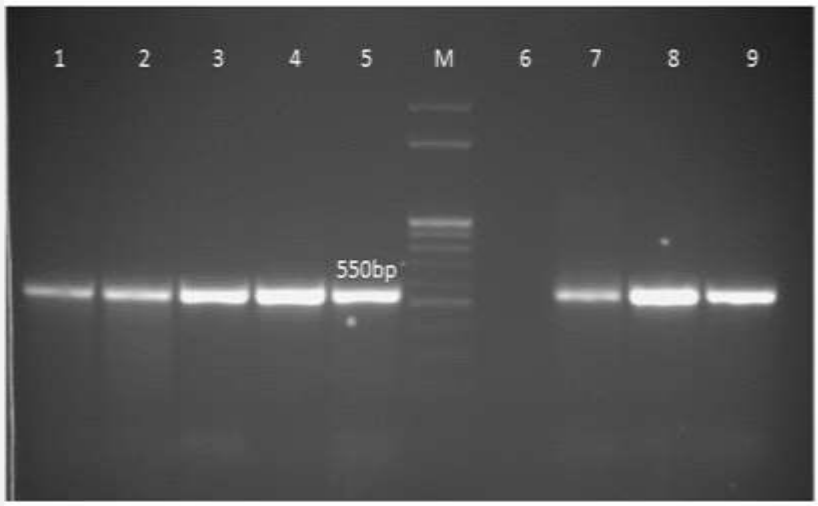

Figure 1 - Agarose gel electrophoresis for amplified samples, where Lanes 1, 2, 3, 4, 7, 8, and 9 represent the positive *Salmonella* strains, Lane 5: Positive control showing 550 bp for the amplified *16S* rRNA gene, Lane M: 100-bp DNA Marker and Lane 6: Negative control

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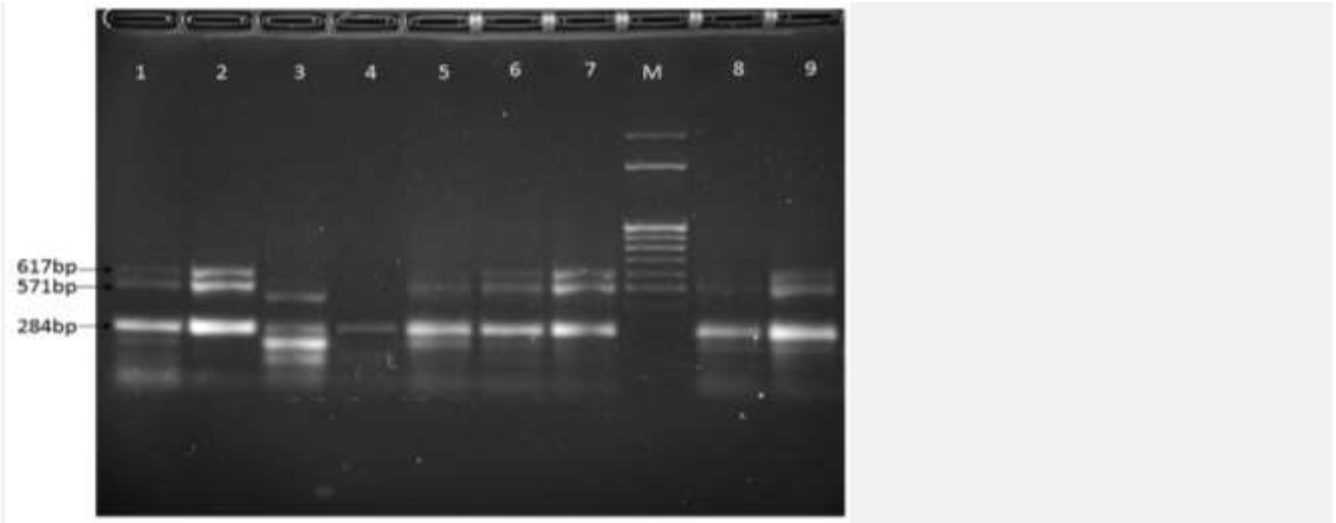

Figure 2 - Multiplex PCR products of *Salmonella* virulence genes. Lane 1: Positive control, Lanes 2, 5, 6, 7, and 8 showing amplicons of 284 bp, 517 bp, and 671 bp of *inv*A, *sop*B and *stn* genes respectively, Lanes 3 and 4 showing amplicons of 284 bp of *inv*A gene and Lane M: 100-bp DNA marker

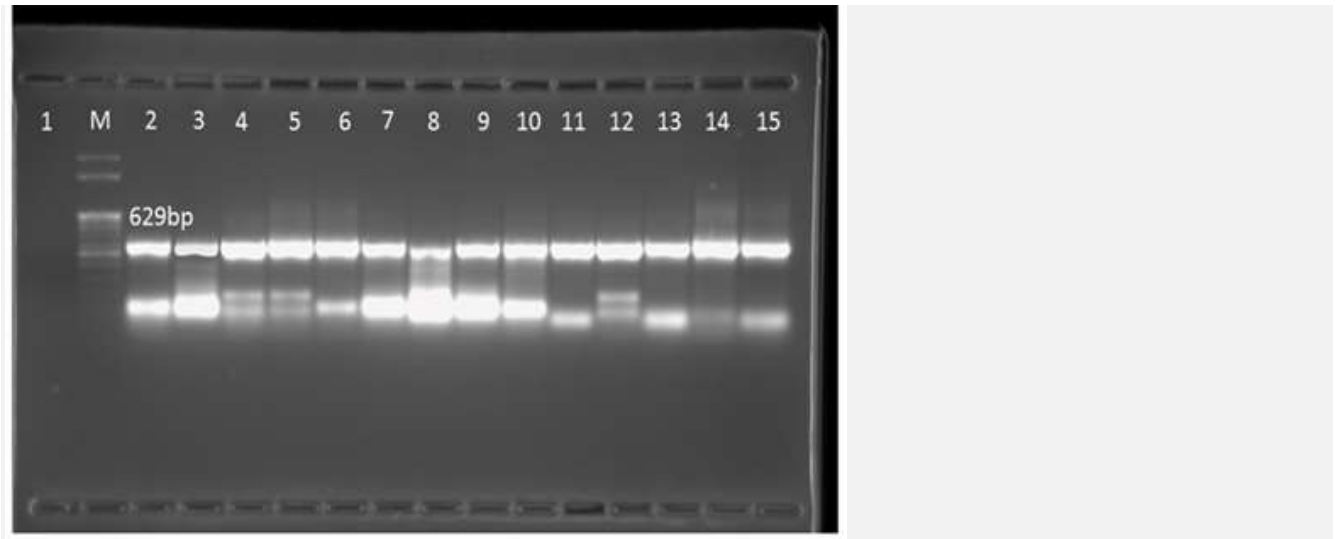

Figure 3 - Agarose gel electrophoresis for PCR products of antibiotic resistance gene (streptomycin) of *Salmonella* showing amplicon of 629 bp of *aad*A1 gene. Lane 1: Negative Control, Lane M: 100-bp DNA marker, and Lane 2: Positive control

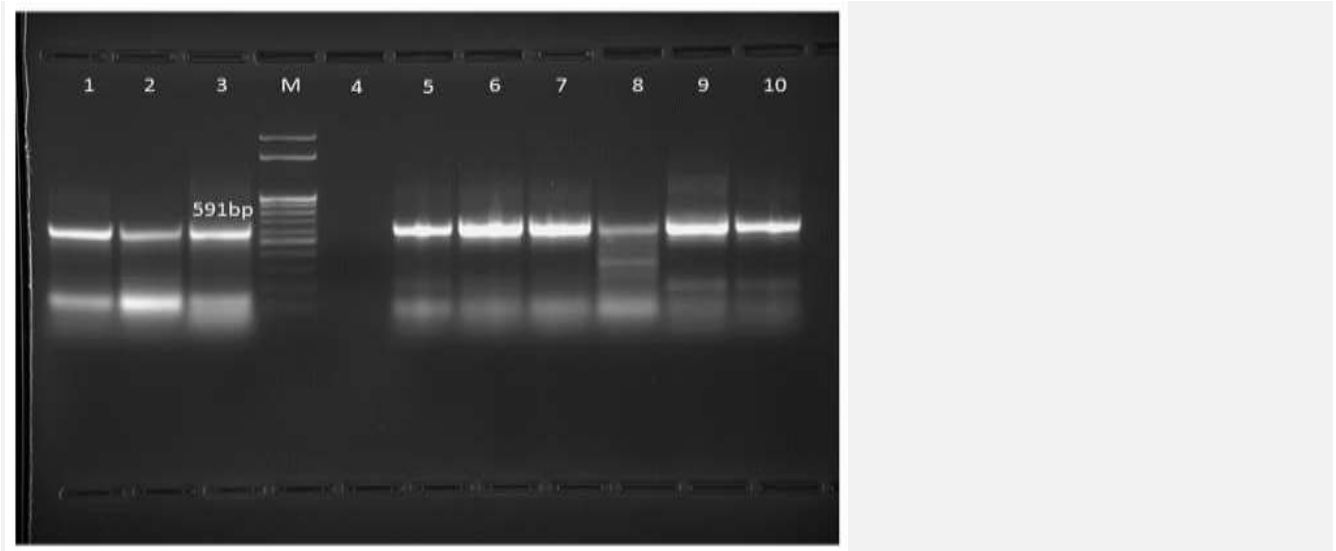

Figure 4 - Agarose gel electrophoresis for PCR products of antibiotic resistance gene (sulfamethoxazole) of *Salmonella* showing amplicon of 591 bp of *sul*1 gene. Lane M: 100-bp DNA marker, Lane 3: Positive control and Lane 4: Negative Control

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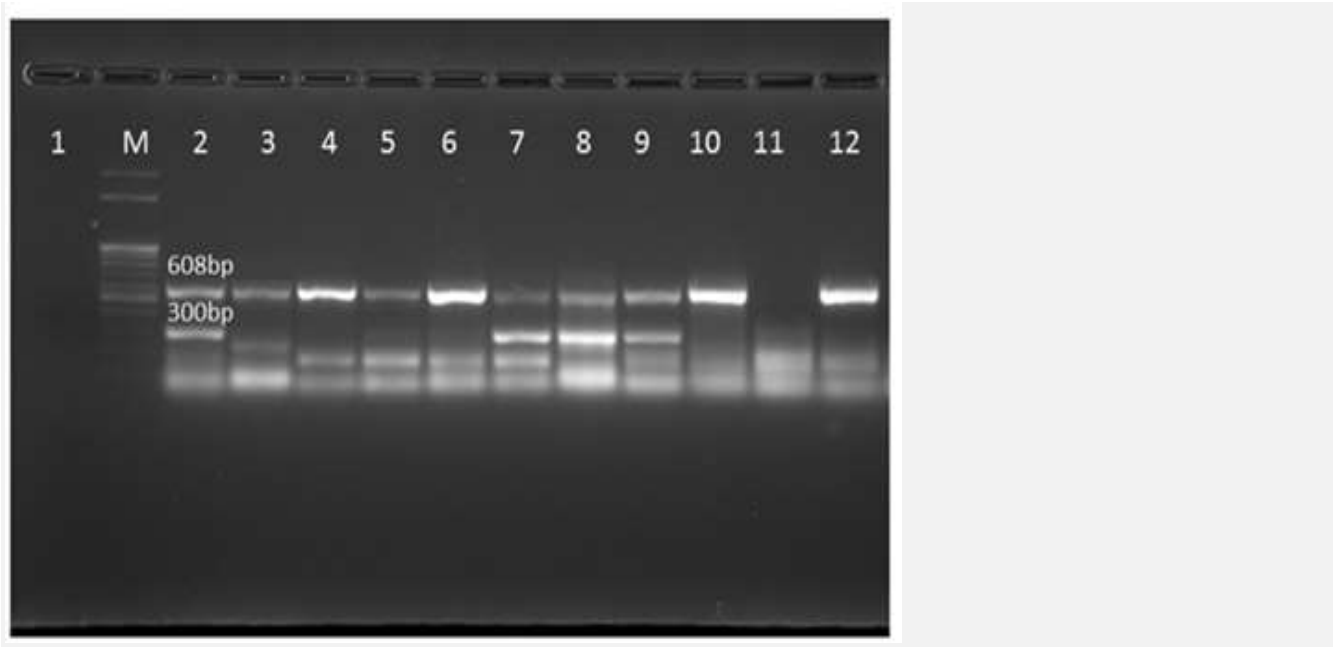

Figure 5 - Multiplex PCR products of antibiotic resistance genes (ampicillin and gentamicin) of *Salmonella* showing amplicon of 608 bp of bla_{TEM} gene and 300bp of *aad*B gene, respectively. Lane 1: Negative Control, Lane M: 100-bp DNA marker and Lane 2: Positive control.

DISCUSSION

Paratyphoid salmonellosis is a prevalent foodborne disease which has a global economic and public health concern (Aziz et al., 2018). Therefore, this study characterized paratyphoid *Salmonella* spp. in chicken flocks of some Egyptian governorates. Our results indicated that *S. enteritidis* was the most prevalent serovar (21.8%), followed by *S. kentucky* (15.6%) and *S. typhimurium* (12.5 %). These results are in agreement with the findings of Elsayed et al. (2019) who detected *S. enteritidis* (38.8%), *S. kentucky* (23.3%), and *S. typhimurium* (23.3%). Further, Elshebrawy et al. (2021) detected similar observation. Many reports indicated that *S. enteritidis* is one of the predominant circulating serovars in poultry flocks, as it has been isolated in rates of 58.33% (Rabie et al., 2012), 55.6% (Moussa et al., 2010), and 37.25% (Abd El-Ghany et al., 2012). Additionally, *S. typhimurium* is another most frequently reported and prevalent serovar worldwide (WHO, 2006). The study of Ammar et al. (2016) indicated that the isolation rates of *S. typhimurium* and *S. enteritidis* were 52.94% and 11.76%, respectively (Ammar et al., 2016) and 86.6% and 9%, respectively (El-Sharkawy et al., 2017). Regarding the low isolation rates of *S. tsevie* (6.3%) and *S. papuana* (3.1%) in this study, nearly similar results were obtained by Al-baqir et al. (2019) and Abd El-Tawab et al. (2015) who reported that the isolation rate of *S. tsevie* was (2%) and *S. papuana* was 2.3%, respectively. Other serovars of *Salmonella* such as *S. wingrove* (6.3%) and *S. shangani* (3.1%) were detected here, which may be due to improper biosecurity measures within farms and the possibility of disease transmission via various reservoirs and farm workers (Elshebrawy et al., 2021).

The highest isolation rate of *Salmonella* serovars was from the liver (62%), rather than the other tissues examined. The previous reports of Menghistu et al. (2011), Abdel-Aziz (2016), Al-baqir et al. (2019), and Saleem et al. (2022) mentioned similar finding. It is important to note that the high detection of *Salmonella* in the liver live may indicate the potential of the pathogen to cause a systemic infection.

It has been noticed that the highest isolation of *Salmonella* serovars was from El-Dakhlia, El-Sharqia Damietta, and El-Qalubia followed by El-Fayoum, El-Minya, Giza, and El-Beheira governorate. These results are in accordance with the fact that the highest percentages of chicken farms allocated in El-Dakhlia, El-Sharqia, Damietta and El-Qalubia rather than other Egyptian governorates.

The development of MDR *Salmonella* strains has emerged as a major public health concern around the world (Marshall and Levy, 2011). This resistance poses a direct threat to human health when treatment is hindered without a complete course and when there are interactions with both animals and humans pathogens (Frye and Jackson, 2013).

Concerning the antimicrobial susceptibility test, the results of the current study indicated that all of the isolated *Salmonellae* strains were resistant to streptomycin (100%), which may be due to the misuse of this antibiotic in the poultry field. However, other studies reported that 100% (Habrun et al., 2012) or 89.7% (El-Sharkawy et al., 2017) of isolates were sensitive to streptomycin.

On the other side site, 96.9% of the isolated *Salmonella* strains were susceptible to meropenem. The same result was obtained by Abou Elez et al. (2021). Furthermore, Elshebrawy et al. (2021) detected a low resistance rate (7.6%) of *Salmonella enterica* serovars against meropenem. It has been reported that carbapenems could be used in the treatment

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of salmonellosis in MDR cases (Calayag et al., 2017). The low resistance rate against meropenem could be attributed to the underuse of carbapenems in veterinary medicine particularly for chicken infections.

Here, the resistance of the isolates to ciprofloxacin was 15.6%, which agrees with that of Donado-Godoy et al. (2012) (15%). In comparison, the resistance to ciprofloxacin was 50% (Abdel Rahman et al., 2014), 35% (Al-baqir et al., 2019), 33% (Badr et al., 2021), or 27.8% (Elshebrawy et al., 2021). As well, the resistance to ampicillin was 18.8%, which was much less than Diab et al. (2019) (68.2%), Ghetas et al. (2021) (78.5%), Alam et al. (2020) (82.85%), Nabil and Yonis, (2019) (94.1%), and Raji et al. (2021) (100%). Further, 50% of the isolated *Salmonellae* showed resistance to tetracycline, which nearly agreed, but lower than those stated by Raji et al. (2021) (75%) and Ibrahim et al. (2021) (62%). The maximum resistance to tetracycline (97.14%) was reported by Alam et al. (2020).

Despite conventional cultural methods offer the advantage of being able to detect live cells and evaluate large numbers of samples, they are time-consuming and laboring (Maciorowski et al., 2006; Margot et al., 2013). On the other hand, molecular techniques such as PCR are rapid, specific, and sensitive, and could replace traditional detection methods (Siddique et al., 2009; Ibrahim et al., 2014). Moreover, multiplex PCR platform provides a greater detection efficiency, allows simultaneous detection of several diseases at the same time, and saves both money and time (Lee et al., 2014; Li et al., 2020).

In the current study, the amplification of *16S* rRNA gene of all *Salmonella* isolates gave a unique specific product at 550 bp. Also, the amplified products of the virulence genes (*inv*A, *sop*B, *stn*, and *pef*) were 284, 517, 617, and 700 bp, respectively. The *inv*A protein is an inner membrane component of the *Salmonella* pathogenicity island 1 type 3 secretion system (Shah et al., 2011). The *inv*A gene allows bacteria to invade the host cells (Cha et al., 2013). This gene is only found in *Salmonella* spp. and is therefore a valuable diagnostic tool for genetic identification (O'Regan et al., 2008). Several studies have noted a high frequency of *inv*A virulence gene in *Salmonella* serovars (Karmi, 2013). All examined *Salmonella* serovars (100%) contained *inv*A gene. Many other studies agreed with ours (Shabnam and Kwai, 2010; Campioni et al., 2012; Samanta et al., 2014; Radwan et al., 2016; Amini et al., 2018; Hassan et al., 2018; ElSheikh et al., 2019; Ramatla et al., 2020; Elshebrawy et al., 2021).

The *Salmonella* enterotoxin (*stn*) gene encodes the Stn protein which causes gastroenteritis and regulates the integrity of bacterial cell membranes (Huehn et al., 2010). This gene was found in 65.6% of the strains in this study, which is similar to Elshebrawy et al. (2021) (65.8%). Other researchers have also noted high rates of *stn* gene (Zou et al., 2012; Osman et al., 2014; Ammar et al., 2019; Sabry et al., 2020; Hassan et al., 2021).

Fimbriae are essential for *Salmonella* pathogenicity, as they facilitate the attachment of the pathogen to epithelial cells. The *pef* gene encodes *pef* fimbria (Murugkar et al., 2003; Ammar et al., 2016). The examined isolates in the present study had no *pef* gene, which is similar to the results reported by Elkenany et al. (2019), Elhariri et al. (2020), and Hassan et al. (2021). Also, a low frequency of the *pef* gene was detected by Ahmed et al. (2016) (6.7%) and Ghetas et al. (2021) (21%). This could be explained by the fact that bacteria use other fimbriae to attach to the host cells. However, in other studies, various findings were noted; for example, Awad et al. (2020) identified *pef* gene in 54.8% of the isolates and highlighted the role of fimbriae in the infection.

By triggering secretory pathways, promoting inflammation or changing ion balances within cells, the *sop*B gene contributes to the development of diarrhea (Ahmed et al. 2016). The prevalence of the *sopB* gene is 40.6% which is lower than Awad et al. (2020) (87.1%), Mohamed et al. (2021) (91.3%), and Badr et al. (2021) (100%).

The amplified products of the antibiotic resistance genes (*aad*A1, *aad*B, *sul*1, and *bla*TEM) were 629, 300, 591, and 608 bp, respectively. In this study, we observed that the phenotypical resistance to ampicillin is 18.8%, but 59.4% of the isolates tested positive for the *bla*TEM gene by PCR, a gene encoding for resistance to β-lactams. This assumes that some antimicrobial resistance genes in some bacteria are inactive or "silent" *in-vitro*. However, these silent genes can spread to other bacteria or can become active *in-vivo* under antimicrobial pressure (Ma et al., 2007). In contrast, El-Sharkawy et al. (2017) found that all isolated strains of *S. enteritidis* were ampicillin-susceptible and *bla*TEM negative, suggesting that these strains had a different ampicillin resistance mechanism. In a previous work, according to Aslam et al. (2012), the *bla*TEM gene was present in 17% of *Salmonella* spp. isolated from retail meats in Canada and was the most frequently found resistance gene. According to Lu et al. (2011), 81.2 % of the 108 *S. indiana* isolates tested positive for the *bla*TEM gene. Ammar et al. (2019) noticed the *bla*TEM gene in 100% of the isolates. The genes *bla*PSE-1 and *bla*TEM, which encode β-lactamases conferring resistance to ampicillin, were found in 69.4% of the strains, according to a report by Herrera-Sánchez et al. (2020). In the present study, we found out that all isolates of *Salmonella* were completely resistant to streptomycin and the gene for streptomycin resistance, *aad*A1 was found in all the isolated strains. This rate is higher than those reported by Chuanchuen and Padungtod, (2009), who detected the resistance gene *aad*A1 in (17%), Doosti et al. (2017) (45.6%), Alam et al. (2020) (77.1%), and Herrera-Sánchez et al. (2020) (87.8%).

6.3% of the isolated *Salmonella* strains were resistant to gentamycin, however the *aad*B gene that confers resistance to gentamycin was found in 18.75% of the isolated strains, as the gene was silenced. In contrast, the *aad*B gene was not found in any of the strains in the study conducted by Herrera-Sánchez et al. (2020).

A percentage of 28.1 of the isolated strains were phenotypically resistant to sulphamethoxazole and 28.1% of isolates being positive for *sul*1 which confer sulphamethoxazole resistance. This result is lower than those reported by Adesiji et al. (2014) (100%), Mohamed and Suelam (2010) (97.3%), Aziz et al. (2018) (83.3%), and El-Sharkawy et al. (2017) (57%).

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CONCLUSION

Paratyphoid *Salmonella* spp. found in poultry flocks at high rates pose a zoonotic danger. Additionally, MDR *Salmonella* serovars with a diverse range of virulence genes have been detected in the existed *Salmonella* spp. These results indicate the importance of the constant surveillance of antibiotic resistant *Salmonella* strains, the use of alternatives instead of antimicrobials in poultry, and adoption of strong public health and food safety protocols to reduce the human health risk associated with salmonellosis.

DECLARATIONS

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Author's contribution

This work was carried out with the contribution of all authors. Nassar YM conducted the practical part of the research and helped in data collection, analysis, and writing. Ibrahim AA conducted the molecular work and validated it. Hamouda AS, Abd El-Ghany WA, and Ibrahim AA designed the protocol, supervise the work, and approved it. Abd El-Ghany WA helped in data collection and write, formatted, and submit the draft of the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

REFERENCES

Abd El-Ghany W A (2020). Salmonellosis: A food borne zoonotic and public health disease in Egypt. Journal of infection in the developing Countries, 14(7): 674-678. DOI: https://doi.org/10.3855/jidc.12739

Abd El-Ghany WA, El-Shafii SS, and Hatem ME (2012). A survey on *Salmonella* species isolated from chicken flocks in Egypt. Asian Journal of Animal and Veterinary Advances, 7(6): 489-501. DOI: https://doi.org/10.3923/ajava.2012.489.501

Abd El-Tawab A, Ashraf A, El-Hofy FI, Ammar AM, Nasef SA, and Nabil NM (2015). Studies on different *Salmonella* serotypes isolated from poultry in different governorates in Egypt. Benha Veterinary Medical Journal, 28(2): 169-175. DOI: https://doi.org/10.21608/bvmj.2015.32498

Abdel Rahman M, Hamed E, Roshdy H, Sorour H, and AlAtfeehy N (2014). Bacteriological studies on native and imported apparently healthy one day old chicks. Suez Canal Veterinary Medical Journal, 19(2): 263-276. DOI: https://dx.doi.org/10.21608/scvmj.2014.65854

Abdel-Aziz NM (2016). Detection of *Salmonella* species in chicken carcasses using genus specific primer belong to *invA* gene in Sohag city, Egypt. Veterinary World, 9(10): 1125-1128. DOI: https://doi.org/10.14202/vetworld.2016.1125-1128

Abou Elez RM, Elsohaby I, El-Gazzar N, Tolba HM, Abdelfatah EN, Abdellatif SS, et al. (2021). Antimicrobial resistance of *Salmonella enteritidis* and *Salmonella typhimurium* isolated from laying hens, table eggs, and humans with respect to antimicrobial activity of biosynthesized silver nanoparticles. Animals, 11(12): 3554. DOI: https://doi.org/10.3390%2Fani11123554

Adesiji YO, Deekshit VK, and Karunasagar I (2014). Antimicrobial-resistant genes associated with *Salmonella* spp. isolated from human, poultry, and seafood sources. Food Science & Nutrition, 2(4): 436-442. DOI: https://doi.org/10.1002/fsn3.119

Ahmed HA, El-Hofy FI, Shafik SM, Abdelrahman MA, and Elsaid GA (2016). Characterization of virulence-associated genes, antimicrobial resistance genes, and class 1 integrons in *Salmonella* enterica serovar Typhimurium isolates from chicken meat and humans in Egypt. Foodborne Pathogens and Disease, 13(6): 281-288. DOI: https://doi.org/10.1089/fpd.2015.2097

Alam SB, Mahmud M, Akter R, Hasan M, Sobur A, Nazir KNH, et al. (2020). Molecular detection of multidrug resistant *Salmonella* species isolated from broiler farm in Bangladesh. Pathogens, 9(3): 201. DOI: https://doi.org/10.3390/pathogens9030201

Al-baqir A, Hussein A, Ghanem I, and Megahed M (2019). Characterization of paratyphoid *Salmonellae* isolated from broiler chickens at Sharkia governorate, Egypt. Zagazig Veterinary Journal, 47(2): 183-192. DOI: https://doi.org/10.21608/zvjz.2019.10544.1028

Amini K, Salehi TZ, Nazari Z, Nikbakht G, Ranjbar R, Amini J, et al. (2018). Molecular detection of *invA* and *spv* virulence genes in *Salmonella typhimurium* isolated from human and animals in Iran. Advanced Journal of Microbiology Research, 12 (3): 001-009. Available at: https://www.internationalscholarsjournals.com/articles/molecular-detection-of-inva-and-spv-virulence-genes-in-salmonella-enteritidis-isolated-from-human-and-animals-in-iran.pdf

Ammar AM, Abdeen EE, Abo-Shama UH, Fekry E, and Kotb Elmahallawy E (2019). Molecular characterization of virulence and antibiotic resistance genes among *Salmonella* serovars isolated from broilers in Egypt. Letters in Applied Microbiology, 68(2): 188-195. DOI: https://doi.org/10.1111/lam.13106

Ammar AM, Mohamed AA, Abd El-Hamid MI, and El-Azzouny MM (2016). Virulence genotypes of clinical *Salmonella* serovars from broilers in Egypt. Journal of Infection in Developing Countries, 10(04): 337-346. DOI: https://doi.org/10.3855/jidc.7437

Aslam M, Checkley S, Avery B, Chalmers G, Bohaychuk V, Gensler G, et al. (2012). Phenotypic and genetic characterization of antimicrobial resistance in *Salmonella* serovars isolated from retail meats in Alberta, Canada. Food Microbiology, 32(1): 110-117. DOI: https://doi.org/10.1016/j.fm.2012.04.017

Awad A, Gwida M, Khalifa E, and Sadat A (2020). Phenotypes, antibacterial-resistant profile, and virulence-associated genes of *Salmonella* serovars isolated from retail chicken meat in Egypt. Veterinary World, 13(3): 440-445. DOI: https://doi.org/10.14202%2Fvetworld.2020.440-445

Aziz SAA, Abdel-Latef GK, Shany SA, and Rouby SR (2018). Molecular detection of integron and antimicrobial resistance genes in multidrug resistant *Salmonella* isolated from poultry, calves and human in Beni-Suef governorate, Egypt. Beni-Suef University Journal of Basic and Applied Sciences, 7(4): 535-542. DOI: https://doi.org/10.1016/j.bjbas.2018.06.005

Azizpour A (2018). Determining the antibiotic resistance patterns of isolated *Salmonella* from broiler flocks to 28 antimicrobial agents used in Iran. Journal of Comparative Pathobiology, 15(1): 2411-2420. Available at: https://jcp.srbiau.ac.ir/article_11831.html?lang=en

Badr H, Roshdy H, Sorour HK, AbdelRahman MAA, Erfan AM, Salem N, et al. (2021). Phenotypic and genotypic characterization of *Salmonella* enterica serovars isolated from imported poultry. Advances in Animals and Veterinary Sciences, 9(6): 823-834. DOI: http://dx.doi.org/10.17582/journal.aavs/2021/9.6.823.834

Borges KA , Furian TQ, De Souza SN, Tondo EC, Streck AF, Salle CTP, et al. (2017). Spread of a major clone of *Salmonella* enterica serotype Enteritidis in poultry and in salmonellosis outbreaks in Southern Brazil. Journal of Food Protection, 80(1): 158-163. DOI: https://doi.org/10.4315/0362-028x.jfp-16-299

Calayag AMB, Paclibare PAP, Santos PDM, Bautista CAC, and Rivera W (2017). Molecular characterization and antimicrobial resistance of *Salmonella enterica* from swine slaughtered in two different types of Philippine abattoir. Food Microbiology, 65: 51-56. DOI: https://doi.org/10.1016/j.fm.2017.01.016

Campioni F, Bergamini AMM, and Falcão JP (2012). Genetic diversity, virulence genes and antimicrobial resistance of Salmonella Enteritidis isolated from food and humans over a 24-year period in Brazil. Food Microbiology, 32(2): 254-264. DOI: https://doi.org/10.1016/j.fm.2012.06.008

Capuano F, Mancusi A, Capparelli R, Esposito S, and Proroga YT (2013). Characterization of drug resistance and virulotypes of *Salmonella* strains isolated from food and humans. Foodborne Pathogens and Disease, 10(11): 963-968. DOI: https://doi.org/10.1089/fpd.2013.1511

Castro-Vargas RE, Herrera-Sánchez MP, Rodríguez-Hernández R, and Rondón-Barragán IS (2020). Antibiotic resistance in *Salmonella* spp. isolated from poultry: A global overview. Veterinary World, 13(10): 2070 -2084. DOI: https://doi.org/10.14202%2Fvetworld.2020.2070-2084

Cha SY, Kang M, Yoon RH, Park CK, Moon OK, and Jang HK (2013). Prevalence and antimicrobial susceptibility of *Salmonella* isolates in Pekin ducks from South Korea. Comparative Immunology, Microbiology and Infectious Diseases, 36(5): 473-479. DOI: https://doi.org/10.1016/j.cimid.2013.03.004

Chuanchuen R, and Padungtod P (2009). Antimicrobial resistance genes in *Salmonella enterica* isolates from poultry and swine in Thailand. Journal of Veterinary Medical Science, 71(10): 1349-1355. DOI: https://doi.org/10.1292/jvms.001349

Diab MS, Zaki RS, Ibrahim NA, and Abd El Hafez MS (2019). Prevalence of multidrug resistance non-typhoidal *Salmonellae* isolated from layer farms and humans in Egypt. World Veterinary Journal, 9(4): 280-288. DOI: https://dx.doi.org/10.36380/scil.2019.wvj35

Donado-Godoy P, Clavijo V, León M, Tafur MA, Gonzales S, Hume M, et al. (2012). Prevalence of *Salmonella* on retail broiler chicken meat carcasses in Colombia. Journal of Food Protection, 75(6): 1134-1138. DOI: https://doi.org/10.4315/0362-028x.jfp-11-513

Doosti A, Mahmoudi E, Jami MS, and Mokhtari-Farsani A (2017). Prevalence of *aad*A1, *aad*A2, *aad*B, *str*A and *str*B genes and their associations with multidrug resistance phenotype in *Salmonella Typhimurium* isolated from poultry carcasses. The Thai Journal of Veterinary Medicine, 46(4): 691-697. DOI: https://he01.tci-thaijo.org/index.php/tjvm/article/view/73827

Elhariri M, Elhelw R, Selim S, Ibrahim M, Hamza D, and Hamza E (2020). Virulence and antibiotic resistance patterns of extended-spectrum beta-lactamase-producing *Salmonella enterica* serovar Heidelberg isolated from broiler chickens and poultry workers: a potential hazard. Foodborne Pathogens and Disease, 17(6): 373-381. DOI: https://doi.org/10.1089/fpd.2019.2719

Elkenany R, Elsayed MM, Zakaria AI, El-Sayed SAES, and Rizk MA (2019). Antimicrobial resistance profiles and virulence genotyping of *Salmonella enterica* serovars recovered from broiler chickens and chicken carcasses in Egypt. BMC Veterinary Research, 15: 124. DOI: https://doi.org/10.1186/s12917-019-1867-z

Elsayed M, El-Gohary F, Zakaria A, and Gwida M (2019). Tracing of *Salmonella* contaminations throughout an integrated broiler production chain in Dakahlia governorate, Egypt. Pakistan Veterinary Journal, 39(4): 558-562. DOI: https://doi.org/10.29261/pakvetj/2019.038

El-Sharkawy H, Tahoun A, El-Gohary AEGA, El-Abasy M, El-Khayat F, Gillespie T, et al. (2017). Epidemiological, molecular characterization and antibiotic resistance of *Salmonella enterica* serovars isolated from chicken farms in Egypt. Gut Pathogens, 9: 8. DOI: https://doi.org/10.1186%2Fs13099-017-0157-1

Elshebrawy HA, Mahros MA, Abd-Elghany SM, Elgazzar MM, Hayashidani H, and Sallam KI (2021). Prevalence and molecular characterization of multidrug-resistant and β-lactamase producing *Salmonella* enterica serovars isolated from duck, pigeon, and quail carcasses in Mansoura, Egypt. LWT, 149: 111834. DOI: https://doi.org/10.1016/j.lwt.2021.111834

ElSheikh M, Abdeen E, and Ammar A (2019). Molecular detection of some virulence genes of *Salmonella* serotypes isolated from poultry in Egypt. Journal of Current Veterinary Research, 1(1): 86-93. DOI: https://doi.org/10.21608/jcvr.2019.36570

Firoozeh F, Zahraei-Salehi T, Shahcheraghi F, Karimi V, and Aslani MM (2012). Characterization of class I integrons among *Salmonella enterica* serovar Enteritidis isolated from humans and poultry. FEMS Immunology & Medical Microbiology, 64(2): 237-243. DOI: https://doi.org/10.1111/j.1574-695X.2011.00883.x

Frye JG, and Jackson CR (2013). Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica*, *Escherichia coli*, and *Enteroccocus* spp. isolated from US food animals. Frontiers in Microbiology, 4: 135. DOI: https://doi.org/10.3389/fmicb.2013.00135

Ghetas A, Abdelbaki M, Fedawy H, Sedeek DM, Bosila MA, Samy AA, et al. (2021). Antimicrobial resistance and virulence genotyping of different *Salmonella* serovars isolated from chickens in Egypt. Advances in Animal and Veterinary Sciences, 9(12): 2124-2131. DOI: http://dx.doi.org/10.17582/journal.aavs/2021/9.12.2124.2131

Gopinath RS, Singh VP, Bhattacharya A, and Sharma B (1998). Molecular subtyping of the Indian isolates of *Salmonella gallinarum* using 16S rRNA gene loci PCR-Amplicon as a probe in Banglore. Proceedings of the Second Pan Commonwealth Veterinary Conference, pp .893-899.

Gyles CL (2008). Antimicrobial resistance in selected bacteria from poultry. Animal Health Research Reviews, 9(2): 149-158. DOI: https://doi.org/10.1017/s1466252308001552

Habrun B, Šimpraga B, Kompes G, and Krstulović F (2012). Antimicrobial resistance and serotyping of *Salmonella* enterica subsp. enterica isolated from poultry in Croatia. Veterinarski Archive, 82(4): 371-381. Available at: https://hrcak.srce.hr/file/125117

Hassan WH, Abed AH, Thabet A, and El Nady AM (2018). Genetic analysis of multidrug resistant *Salmonella* isolated from broiler chickens. Journal of Veterinary Medical Research, 25(1): 121-131. DOI: https://doi.org/10.21608/jvmr.2018.43309

Citation: Nassar YM, Abd El-Ghany WA, Ibrahim AK, and Hamouda AS (2023). Paratyphoid *Salmonella* serovars in chickens: molecular detection of virulence and antimicrobial resistance genes. *Online J. Anim. Feed Res.*, 13(5): 396-409. DOI: https://dx.doi.org/10.51227/ojafr.2023.56

Hassan WH, Hassan HS, Hassan WM, Shany SA, and Osman GS (2021). Identification and characterization of *Salmonella* species isolated from broiler chickens. Journal of Veterinary Medical Research, 28(1): 21-29. DOI: https://dx.doi.org/10.21608/JVMR.2021.89244.1041

Herrera-Sánchez MP, Rodríguez-Hernández R, and Rondón-Barragán IS (2020). Molecular characterization of antimicrobial resistance and enterobacterial repetitive intergenic consensus-PCR as a molecular typing tool for *Salmonella* spp. isolated from poultry and humans. Veterinary World, 13(9): 1771. DOI: https://doi.org/10.14202/vetworld.2020.1771-1779

Huehn S, La Ragione RM, Anjum M, Saunders M, Woodward MJ, Bunge C, et al. (2010). Virulotyping and antimicrobial resistance typing of *Salmonella* enterica serovars relevant to human health in Europe. Foodborne Pathogens and Disease, 7(5): 523-535. DOI: https://doi.org/10.1089/fpd.2009.0447

Ibrahim WA., Abd El-Ghany WA, Nasef SA, and Hatem ME (2014). A comparative study on the use of real time polymerase chain reaction (RT-PCR) and standard isolation techniques for the detection of *Salmonella* in broiler chicks. International Journal of Veterinary Science and Medicine, 2 (1): 67-71. DOI: https://doi.org/10.1016/j.ijvsm.2013.11.001

Ibrahim S, Wei Hoong L, Lai Siong Y, Mustapha Z, CW Zalati CS, Aklilu E, et al. (2021). Prevalence of antimicrobial resistance (AMR) *Salmonella* spp. and *Escherichia coli* isolated from broilers in the East Coast of Peninsular Malaysia. Antibiotics, 10(5): 579. DOI: https://doi.org/10.3390/antibiotics10050579

International Organization for Standardization (ISO) (2002). Microbiology of food and animal feeding stuffs-horizontal method for the detection of *Salmonella* spp., 4th edition. Geneva. Available at: https://www.iso.org/obp/ui/#iso:std:iso:6579:ed-4:v1:en

Kapoor G, Saigal S, Elongavan A (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. Journal of Anaesthesiology, Clinical Pharmacology, 33(3): 300-305. DOI: https://doi.org/10.4103%2Fjoacp.JOACP_349_15

Karmi M (2013). Detection of virulence gene (*invA*) in *Salmonella* isolated from meat and poultry products. International Journal of Genetics, 3(2): 7-12. DOI: https://doi.org/10.5829/idosi.ijg.2013.3.2.82204

Kauffman G (1974). Kauffmann White Scheme. Acta Pathologica et Microbiologica Scandinavica, 61: 385.

Kuria KNJ (2023). Salmonellosis in Food and Companion Animals and Its Public Health Importance. Salmonella - Past, Present and Future [Working Title]. IntechOpen. http://dx.doi.org/10.5772/intechopen.109324

Lamas A, Fernandez-No IC, Miranda JM, Vázquez B, Cepeda A, and Franco CM (2016). Prevalence, molecular characterization and antimicrobial resistance of *Salmonella* serovars isolated from northwestern Spanish broiler flocks (2011–2015). Poultry Science, 95(9): 2097-2105. DOI: https://doi.org/10.3382/ps/pew150

Lee N, Kwon KY, Oh SK, Chang HJ, Chun HS, and Choi SW (2014). A multiplex PCR assay for simultaneous detection of *Escherichia coli* O157: H7, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* in Korean ready-to-eat food. Foodborne Pathogens and Disease, 11(7): 574-580. DOI: https://doi.org/10.1089/fpd.2013.1638

Li P, Zhang D, Li H, Pang J, Guo H, and Qiu J (2020). Establishment and application of multiplex PCR for simultaneously detecting *Escherichia coli*, *Salmonella*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* in minks. Frontiers in Veterinary Science, 7: 588173. DOI: https://doi.org/10.3389/fvets.2020.588173

Lu Y, Wu CM, Wu GJ, Zhao HY, He T, Cao XY, et al. (2011). Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. Foodborne Pathogens and Disease, 8(1): 45-53. DOI: https://doi.org/10.1089/fpd.2010.0605

Ma M, Wang H, Yu Y, Zhang D, and Liu S (2007). Detection of antimicrobial resistance genes of pathogenic *Salmonella* from swine with DNA microarray. Journal of Veterinary Diagnostic Investigation, 19(2): 161-167. DOI: https://doi.org/10.1177/104063870701900204

Maciorowski KG, Herrera P, Jones FT, Pillai SD, and Ricke SC (2006). Cultural and immunological detection methods for *Salmonella* spp. in animal feeds–a review. Veterinary Research Communications, 30(2): 127-137. DOI: https://doi.org/10.1007/s11259-006-3221-8

Margot H, Stephan R, O'Mahony E, and Iversen C (2013). Comparison of rapid cultural methods for the detection of *Salmonella* species. International Journal of Food Microbiology, 163(2-3): 47-50. DOI: https://doi.org/10.1016/j.ijfoodmicro.2013.02.013

Marshall BM, and Levy SB (2011). Food animals and antimicrobials: impacts on human health. Clinical Microbiology Reviews, 24(4): 718-733. DOI: https://doi.org/10.1128/cmr.00002-11

Menghistu HT, Rathore R, Dhama K, and Agarwal RK (2011). Isolation, identification and polymerase chain reaction (PCR) detection of *Salmonella* species from field materials of poultry origin. International Journal of Microbial Research, 2(2): 135-142. Available at: https://www.idosi.org/ijmr/ijmr2(2)11/7.pdf

Mohamed M, Mohamed R, Gharieb R, Amin M, and Ahmed H (2021). Antimicrobial resistance, virulence associated genes and biofilm formation of *Salmonella* species isolated from different sources. Zagazig Veterinary Journal, 49(2): 208-221. DOI: https://doi.org/10.21608/zvjz.2021.81722.1144

Mohamed MEM, and Suelam IIA (2010). Isolation of non-typhoid *Salmonella* from humans and camels with reference to its survival in abattoir effluents. Global Veterineria, 5(6): 356-361. Available at: https://www.idosi.org/gv/gv5(6)10/10.pdf

Mottet, A., and Tempio, G. (2017). Global poultry production: Current state and future outlook and challenges. World's Poultry Science Journal, *73*(2): 245-256. https://doi.org/10.1017/S0043933917000071

Moussa IM, Gassem MA, Al-Doss AA, Sadik WAM, and Mawgood AA (2010). Using molecular techniques for rapid detection of *Salmonella* serovars in frozen chicken and chicken products collected from Riyadh, Saudi Arabia. African Journal of Biotechnology, 9(5): 612-619. DOI: https://doi.org/10.5897/AJB09.1761

Murugkar HV, Rahman H, and Dutta PK (2003). Distribution of virulence genes in *Salmonella* serovars isolated from man & animals. Indian Journal of Medical Research, 117: 66-70. Avaiable at: https://pubmed.ncbi.nlm.nih.gov/12931840/

Nabil NM, and Yonis AE (2019). Isolation of *Salmonella* characterized by biofilm formation and disinfectant resistance from broiler chickens. Alexandria Journal of Veterinary Sciences, 62(2): 26-36. DOI: http://dx.doi.org/10.5455/ajvs.57274

National Committee for Clinical Laboratory Standards (NCCLS) (2020). Performance standards for antimicrobial susceptibility testing: eleventh informational supplement. M100- S11. 30th edition, Wayne. PA. USA. Available at: chrome-https://clsi.org/media/3481/m100ed30_sample.pdf

Oliveira SD, Rodenbusch CR, Ce MC, Rocha SLS, and Canal CW (2003). Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. Letters in Applied Microbiology, 36(4): 217-221. DOI: https://doi.org/10.1046/j.1472-765x.2003.01294.x

O'Regan E, McCabe E, Burgess C, McGuinness S, Barry T, Duffy G, et al. (2008). Development of a real-time multiplex PCR assay for the detection of multiple *Salmonella* serotypes in chicken samples. BMC Microbiology, 8: 165. DOI: https://doi.org/10.1186/1471-2180-8-156

Citation: Nassar YM, Abd El-Ghany WA, Ibrahim AK, and Hamouda AS (2023). Paratyphoid *Salmonella* serovars in chickens: molecular detection of virulence and antimicrobial resistance genes. *Online J. Anim. Feed Res.*, 13(5): 396-409. DOI: https://dx.doi.org/10.51227/ojafr.2023.56

Osman KM, Marouf SH, Zolnikov TR, and AlAtfeehy N 2014. Isolation and characterization of *Salmonella enterica* in day-old ducklings in Egypt. Pathogens and Global Health, 108(1): 37-48. DOI: https://doi.org/10.1179%2F2047773213Y.0000000118

Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, and Fitzpatrick ES (2011). Veterinary Microbiology and Microbial Disease. 2nd Edition, Wiley-Blackwell Science. Available at: https://www.wiley.com/enbe/Veterinary+Microbiology+and+Microbial+Disease%2C+2nd+Edition-p-9781405158237

Rabie NS, Khalifa NO, Radwan ME, and Afify JS (2012). Epidemiological and molecular studies of *Salmonella* isolates from chicken, chicken meat and human in Toukh, Egypt. Global Veterineria, 8(2): 128-132. Available at: https://fvtm.stafpu.bu.edu.eg/Zoonotic%20Diseases/957/publications/Nashwa%20Osman%20Khalefa_GLOBAL%20VET.pdf

Radwan IA, Abed AH, Abd Al-Wanis SA, Abd El-Aziz GG, and El-Shemy A (2016). Antibacterial effect of cinnamon and oreganium oils on multidrug resistant *Escherichia coli* and *Salmonellae* isolated from broiler chickens. Journal of the Egyptian Veterinary Medical Association, 76(2): 169-186.

Raji MA, Kazeem HM, Magyigbe KA, Ahmed AO, Lawal DN, and Raufu IA (2021). *Salmonella* serovars, antibiotic resistance, and virulence factors isolated from intestinal content of slaughtered chickens and ready-to-eat chicken gizzards in the Ilorin Metropolis, Kwara State, Nigeria. International Journal of Food Science, 2021: 8872137. DOI: https://doi.org/10.1155/2021/8872137

Ramatla TA, Mphuthi N, Ramaili T, Taioe MO, Thekisoe OM, and Syakalima M (2020). Molecular detection of virulence genes in *Salmonella* spp. isolated from chicken faeces in Mafikeng, South Africa. Journal of the South African Veterinary Association, 91(0): 1-7. DOI: https://doi.org/10.4102%2Fjsava.v91i0.1994

Roca I, Akova M, Baquero F, Carlet J, Cavaleri M, Coenen S, et al. (2015). The global threat of antimicrobial resistance: science for intervention. New Microbes and New Infections, 6: 22-29. DOI: https://doi.org/10.1016/j.nmni.2015.02.007

Sabry MA, Abdel-Moein KA, Abdel-Kader F, and Hamza E (2020). Extended-spectrum β-lactamase-producing *Salmonella* serovars among healthy and diseased chickens and their public health implication. Journal of Global Antimicrobial Resistance, 22: 742-748. DOI: https://doi.org/10.1016/j.jgar.2020.06.019

Saleem G, Farooq U, Javed MT, Naseer R, Aslam HB, Mustafa G, et al. (2022). Pathobiological and immunohistochemical findings in broiler chickens naturally infected with Salmonella enterica serotype *Gallinarum* biotype *Gallinarum*. Pakistan Veterinary Journal, 42(1): 88-94. DOI: https://doi.org/10.29261/pakvetj/2021.083

Samanta I, Joardar SN, Das PK, Sar TK, Bandyopadhyay S, Dutta TK, et al. (2014). Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India. Journal of Applied Poultry Research, 23(3): 536-545. DOI: https://doi.org/10.3382/japr.2013-00929

Shabnam M, and Kwai Lin T (2010). Isolation and molecular sub typing of *Salmonella enterica* from chicken, beef and street foods in Malaysia. Scientific Research and Essays, 5(18): 2713-2720. Available at: https://eprints.um.edu.my/18919/

Shah DH, Zhou X, Addwebi T, Davis MA, Orfe L, Call DR, et al. (2011). Cell invasion of poultry-associated *Salmonella enterica* serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system. Microbiology, 157(5): 1428-1445. DOI: https://doi.org/10.1099/mic.0.044461-0

Shehata AA, Basiouni S, Elrazek AA, Sultan H, Tarabees R, Abd MS, et al. (2019). Characterization of *Salmonella* enterica isolated from poultry hatcheries and commercial broiler chickens. Pakistan Veterinary Journal, 39(4): 515-520. DOI: https://doi.org/10.29261/pakvetj/2019.033

Siddique RA, Saxena M, and Lakhchaura BD (2009). PCR based rapid detection of *Salmonella* from poultry samples. Indian Journal of Public Health, 53(4): 226-228. Available at: https://pubmed.ncbi.nlm.nih.gov/20469760/

Singh S, Yadav A, Singh S, and Bharti P (2010). Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. Food Research International, 43(8): 2027-2030. DOI: https://doi.org/10.1016/j.foodres.2010.06.001

World Health Organization (WHO) (2006). Progress report (2000-2005): building capacity for laboratory-based foodborne disease surveillance and outbreak detection and response. World Health Organization. DOI: https://apps.who.int/iris/handle/10665/43424

Xu Y, Zhou X, Jiang Z, Qi Y, Ed-Dra A, Yue M (2020). Epidemiological investigation and antimicrobial resistance profiles of *Salmonella* isolated from breeder chicken hatcheries in Henan, China. Frontiers in Cellular and Infection Microbiology, 10: 497. DOI: https://doi.org/10.3389/fcimb.2020.00497

Zou M, Keelara S, and Thakur S (2012). Molecular characterization of *Salmonella enterica* serotype Enteritidis isolates from humans by antimicrobial resistance, virulence genes, and pulsed-field gel electrophoresis. Foodborne Pathogens and Disease, 9(3): 232-238. DOI: https://doi.org/10.1089/fpd.2011.1012

Citation: Nassar YM, Abd El-Ghany WA, Ibrahim AK, and Hamouda AS (2023). Paratyphoid *Salmonella* serovars in chickens: molecular detection of virulence and antimicrobial resistance genes. *Online J. Anim. Feed Res*., 13(5): 396-409. DOI: https://dx.doi.org/10.51227/ojafr.2023.56

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MOLECULAR DETECTION AND PREVALENCE STUDY OF *Neospora Caninum* ISOLATED FROM BLOOD OF ABORTED COWS IN BABYLON PROVINCE OF IRAQ

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Supporting Information

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ABSTRACT: Neosporosis is internationally documented as one of the most popular diseases in cattle that cause economic losses due to high levels of abortion cases. Although *Neospora caninum* has been recently classified as a new species, it is still sharing many features with *Toxoplasma gondii*. This study aimed to detect and Imaging *N. caninum* in the blood of aborted cows, and prevalence study of *N. caninum* infection based on age, region and month. Blood samples from 106 aborted cows were collected using the appropriate method. First, these samples were examined microscopically via blood smears using Giemsa dye to diagnose the *N. caninum* within RBC. A qPCR technique was carried out to detect accurately *18S rRNA* gene accurately. The results revealed that 65% of total aborted cases were positive for *18S rRNA* detection of *N. caninum*, although this parasite was found microscopically in 15% of blood smear samples. According to PCR results, the prevalence study showed that the highest rate of infection was signed in the Al-Qassim district (75%) followed by the Al-Mahaweel district (74%) and decreased in Western Hamza district (48%). According to study months, November recorded the peak of infection (88%), then August (71%), whereas July recorded the lowest percentage (50%). The statistical analysis revealed there was no significant difference between the subjected regions and study months based on (P<0.05). On the other hand, it was found that cows less than 3 years old were more susceptible to infection than those over 3 years old. the results revealed that 71% of infected cows were less than 3 years old, while 29% were at age over 3 years old with a significant difference (P<0.005). In conclusion, *N. caninum* can be detected through blood within RBC. Age and regional factors in cows play an important role in resisting infection with this pathogen.

Keywords: Cow, Neosporosis, N. caninum, Prevalence, 18S rRNA

INTRODUCTION

Neosporosis is a parasitic disease caused by *N. caninum*. It is recognised as an intracellular protozoan parasite of livestock distributed worldwide. In cattle, it is considered one of the main causes of abortion (Wei et al., 2022). Neosporosis has been related to epizootic and sporadic abortion in dairy herds worldwide. Since the discovery of Neosporosis, some studies have been conducted to assess the prevalence and to identify factors related to the disease, Prevalence's has been estimated in ranges between 16.8% and 70% (Waldner et al., 2004). In intermediate hosts, tachyzoites and tissue cysts are the infective stages (Mahajan et al., 2019). It has been reported in many countries with different prevalence rates since the disease was recognized in 1988 (Noori et al., 2019).

Morphology for parasite tachyzoites are ovoid, crescent or spherical in shape according to the phase of division and measures 3-7 × 1-5 µm in size (Speer et al., 1999), bradyzoites are slightly longer, slenderer than the tachyzoites, measuring 8.1 × 2 µm. parasite, capable of forming tissue cysts containing up to 100 parasites and Oocysts almost rounded measuring about 11.7 × 11.3 µm in diameter, surrounded by a smooth, colorless, 0.6-0.8 µm thick wall (Lindsay et al., 1999). Definitive hosts (dog, coyote, dingo) shed unsporulated oocysts in their feces, which sporulate within several days to become infectious to the intermediate host such as cow, sheep, goats or deer, water buffaloes, horse, etc. when they consume foods or water contaminated by them (Lindsay and Dubey, 2020; Venturoso et al., 2021). García et al. (2015) found that many cases of abortion in both dairy and beef cattle were caused by *N. caninum*. Cattle get infected due to feeding mostly on mixed, drinking from rivers, and existing in contact with other animals at the same farm. As observed in many reports, the second way to spread the infection is by consuming parasite oocysts or eggs contaminated food or water or grazing on contaminated pastures (Claude et al., 2017). In addition, the transmission of infection could be transplanted during pregnancy to the fetus from the infected mother (Jiménez-Pelayo, et al., 2019). Previous specific

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studies showed that the higher anti-*N. caninum* antibodies were found in cattle living on farms with dogs in comparison with those without dogs, suggesting the active involvement of dogs in the transmission of parasites to cattle (Gharekhani et al., 2020). The release of oocysts of *N. caninum* through dog faces could contaminate the food and water which become the source of infection (Ahmed et al., 2021). Billions of dollars were lost annually as reported in New Zealand and the US by the infection of Neosporosis, due to loss of fetus, milk yield, gaining weight, and time for rebreeding (Reichel et al., 2013).

The aim of our study is to detect *N. caninum* in the blood of aborted cows using the PCR technique and to study the prevalence of this infection in the Babylon province. Furthermore, the presence of *N. caninum* in red blood cells was also observed.

MATERIALS AND METHODS

Collection and examination processes for 106 blood samples of aborted cattle were carried out from different ages and regions of the Babylon province: (Al. Qassime, Al-Hilla, and Mahaweel Districts) as well as different periods from July 2022 to January 2023. The blood samples were collected especially from aborted cows, and 3 ml of venous blood (jugular vein) was taken in a 5 ml EDTA tube (AMEER et al., 2019).

Microscopic examination

Direct microscopic examination was performed to diagnose this parasite and to study the morphology of it. A small drop of blood was applied to the slide approximately 20 mm from one end. A spreader of another slide was placed on the blood drop at an angle of 20-30° and drawn back to another end to blood smear. After air drying, the sample was fixed with methyl alcohol for a minute and then stained with Giemsa stain for a minute again, wishing and drying were carried on after that, the samples were ready for microscopic examination (Fraser et al., 2010)

Molecular detection

Genomic DNA extraction from 400 µl blood was done using manufacturer instructions (QIA GENE KIT). The concentration of DNA was measured using a Nanodrop device at an absorbance of (260 /280) nm. PCR technique was performed on the collected samples, all PCR samples were prepared following the manufacturer protocol (1µl of 10 pmol forward and reverse primers, 8 µl genomic DNA, 12.5 Mastermix, and complete volume to 25 µl of nuclease-free water. Primers for 18S rRNA were self-designed with a predicted size (236 bp) to be used in this stage based on the NCBI database of Genbank: MT860359. The foreword primer sequence (NeoF) was (gtgtacggcgaagggactc), while the reverse primer sequence (NeoR) was (gccaagacatccattgctga). PCR programs were set according to the annealing temperature of each primer (94 ^{o}C for 5 minutes, for 40 cycles of 94 ^{o}C for 30 seconds, 50 ^{o}C for 25 seconds, 72 ^{o}C for 45 seconds, and a final extension at 72 ^{o}C for 8 minutes, with a final hold at 4 ^{o}C (THERMO-CYCLER; FISHER, GERMANY). A 1.5% agarose gel electrophoresis was prepared for checking PCR products using 1X Tris-Borate- EDTA (TBE buffer), and then the components were dissolved into 1 L of distilled H2O. They were run for 60 min/ 80 volts (Hube et al., 2005). DNA bands were compared with a 1500 bp DNA ladder to confirm the specific size of the 18S rRNA gene for the subjected parasite. Imaging was carried out using a UV Trans-illuminator for DNA detection.

Ethical regulation

It was obtained from the Faculty Scientific Committee (College of Veterinary Medicine, Al-Muthanna University, Iraq) numbered 202205 – Naer Abdulbari Madlool Alkaabawi.

RESULTS

Direct microscopic examination

All the collected blood samples of aborted cows were examined by direct smear method; it appeared that 15% of blood samples were suspected Neospora at the tachyzoite stage. These tachyzoites were ovoid and crescent in shape according to the phase of division and measurements of size (3-7 × 1-5 µm) using an ocular micrometer lance as shown in (Figure 1).

Detection of 18S rRNA of *N. caninum*

According to the results of the microscopic examination, all samples were run on PCR for 18S rRNA detection of *N. caninum* with PCR product size (236 bp). The results show that 65% of the total samples are infected with *N. caninum* (Figure 2).

Prevalence of *N. caninum* infection in aborted cows based geographical areas using PCR is at the highest rate in Al-Qassim and Al-Mahaweel districts which are 75% and 74%, respectively. Al-Hilla District records 63%. The lowest rate is in Western Hamza district which archives 48%. The statistical analysis (P>0.05) reveals that there is no significant difference (P=0.154) in the prevalence of parasites among geographical areas. These statistics are similar with no significant difference (P=0.239) to the prevalence of *N. caninum* infection according to the time of infection, although the

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infection rate was at the highest level during November (88%) (Figure 3). On the other hand, it is found that the *N. caninum* parasite is detected in (71%) of aborted cows aged 1-3 years group. Meanwhile, a ratio of 29% is identified in the aborted cows at age over 3 years group. This refers to a significant difference between these two groups based on statistical analysis at P<0.05 that recorded P<0.005.

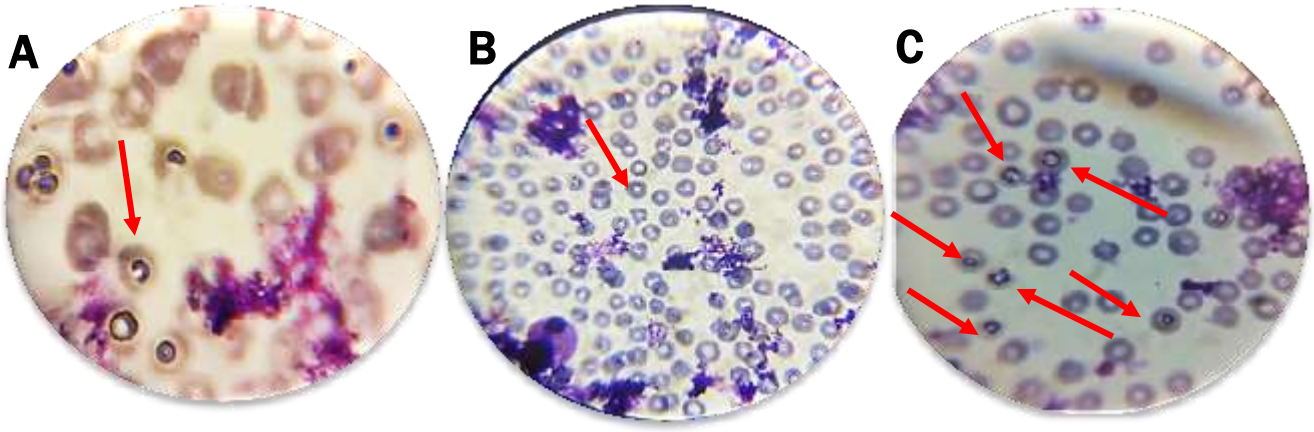

Figure 1 - Shows different stages of infection with *Neospora* caninum parasite inside the red blood cells at (40x) after dying by Giemsa stain. (A and B) pictures refer to tachyzoite phase, while (C) refers to heavy infection with *N. caninum* in red blood cells (40x)

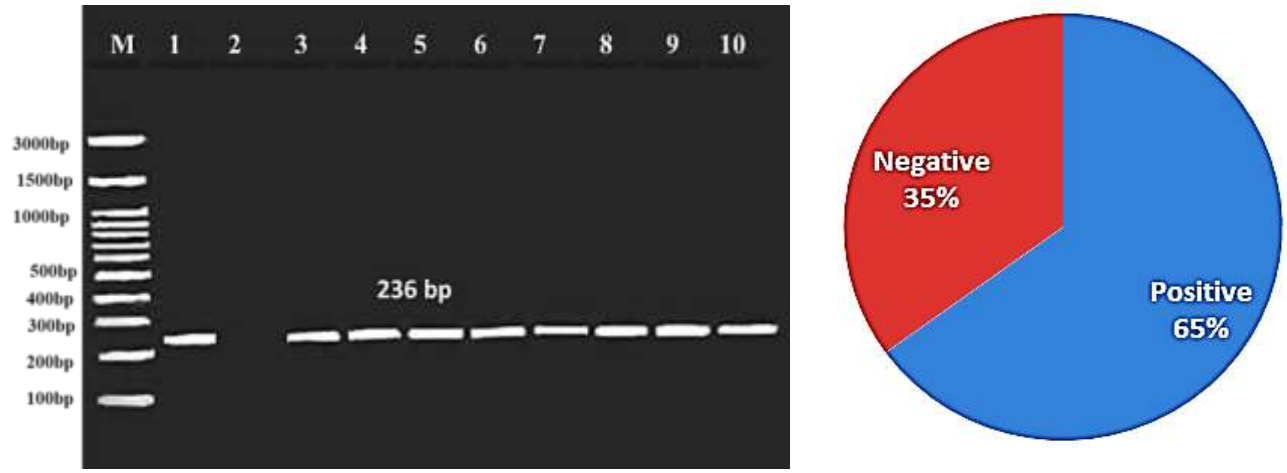

Figure 2 - A) It shows the PCR amplification results of the 18s rRNA gene of *N. caninum* isolated from aborted cow. The agarose gel picture shows the PCR product bands with a molecular weight of 236 bp. (M) refers to (3 Kbp) DNA ladder; 1) positive control; 2) Negative control. (1-9) PCR results of blood samples. B) shows the infection ratio with *N. caninum* over 106 samples.

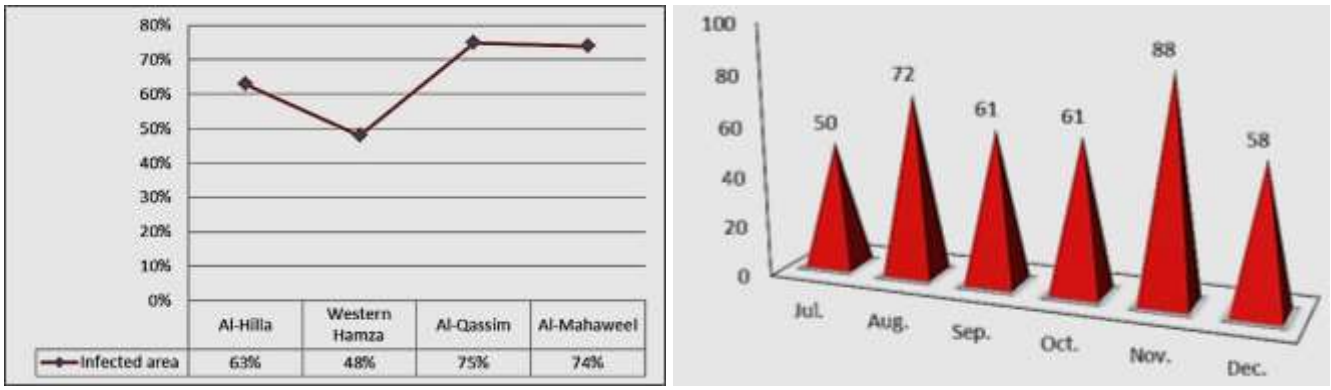

Figure 3 - Refers to the prevalence of *N. caninum* infection according to the region and the time of infection. It is clear to see that the highest ratio of infection is in Al-Qassim district, which records (75%) while November is the critical time of infection (88%).

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DISCUSSION

Neospora caninum is the main cause of neosporosis as a polysystemic disease that is an obligated intracellular parasite that belongs to the phylum Apicomplexa (Dubey and Schares, 2011). Neosporosis was misdiagnosed as toxoplasmosis due to sharing several morphological and biological features that are closely related to *Toxoplasma gondii* (Dubey et al., 2017). In 1984 was the first recognition of *N. caninum* in Norwegian dogs (Bjerkas et al., 1984), then a significant classification was done to consider it as a separate species from *Toxoplasma gondii* since 1988 (Dubey et al., 1988; Seltmann et al., 2020).

Among diagnostic techniques, the PCR is more sensitive and specific than other tests and less likely to be affected by autolysis or postmortem changes. In addition, it can be applied for the identification of *N. caninum* DNA in blood, semen, brain, spinal cord, different fetal fluids, embryonic tissues, and even oocysts in the faeces of the final host (Kamali et al., 2014).

The prevalence of *N. caninum* infections was the highest in the Al-Qassim and Al-Mahaweel districts which were 75% and 74.07% respectively, while in the Al-Hilla District was 63.33% and the lowest rate was in Western Hamza district which was 48%. The statistical analysis reveals that there was no significant difference in the prevalence of parasites among geographical areas (P>0.05). Globally, several various studies were carried out to detect the prevalence of bovine *N. caninum*, using different diagnostic tests that revealed significant variation in their prevalence between countries, regions as well as between herds as Semango et al. (2019) discuss in their paper. The results of this study were higher than those reported by Al-Gharban et al. (2017) that were (12.36%). However, it was nearly close to the findings of Japa et al. (2019), they found the diversity of *N. caninum* infection rates (16-50 %) in different districts of Phayao province in Thailand using PCR for placental samples of beef cattle. This success in the detection of DNA was expected because the blood was confirmed to be a transport factor for Neospora tachyzoites between body tissues (Okeoma et al., 2004). Neosporosis has a wide range of infection rates with the presence of contrast between the study's districts. This finding might be attributed to the inequality of applied techniques and/or their cut-off origin of evaluated herds and the probability of frequent exposure to sources of infection (Moura et al., 2011). As well as, the increase in *N. caninum* prevalence could occur because discrepancy of animal housing or poor hygienic management, herds that are involved in a study, increasing exposure to definitive host or intermediate hosts, and the contact, directly or indirectly, to adjacent endemic areas (Celik et al., 2013; Llano et al., 2018).

The prevalence of *N. caninum* infections was at a high rate within 1-3 year's old group which was 71%, while the lower rate of infection was at the age over 3 years old group, which was 29%. The statistical analysis using the T-test parameter showed that there was a significant difference between age groups (P<0.05). Furthermore, the results of the present study showed that the lowest per cent was in the age group years old which may be due to the fact that cows in this age (>3 years) have good resistance and immunity against *N.caninum*. These results agreed with Noori et al., (2019), who discussed that the vertical transmission of *N. caninum* might be the reason for increasing the infection in cows under 3 years old more than those increasing over 3 years based on his seroprevalence study. However, the results of Metwally et al. (2023) revealed that those aged (3-5) years old were more exposed to the infection with seropositive version, due to horizontal transmission. Moreover, the results of Razmi et al. (2006) and Mallah et al. (2012) also disagreed with the present data, when they announced that there is no significant difference between age groups (P>0.05). The age effect might be influenced by management practices such as replacement rate, and the cattle may be exposed to horizontal transmission, or by selective culling of seropositive animals (Bartels et al., 2006).

On the other hand, the results of this study revealed that the prevalence of *N. caninum* infection based on the time recorded that the highest rate of infection was in November (88 %) followed by 72% in August, while the lower prevalence was in July which was 50% (figure 3). The statistical analysis reveals no significant difference at (P>0.05) in aborted cows among studied months (figure 3). Distribution of *N. caninum* infection takes place all year round, the peak of infection was observed in Nov. with a percentage of 88%, this result was in agreement with (Ibrahim et al. 2012) who found the peak of Neospora infection in autumn and winter California. However, this result was in disagreement with Pitel et al. (2001) in France, who found the peak of Neospora infection in March-June. These differences in infection may be due to seasonal differences in parasite exposure and/or oocyst survival by providing suitable environmental conditions (temperature and humidity) Because of its close relationship with *T. gondii* it is assumed that the environmental resistance of *N. caninum* oocysts is similar to *T. gondii* oocysts that the degree of suitable temperatures for sporulation of oocysts was ranged between (22-30) C˚ (Dubey, 2007).

CONCLUSION

Overall, despite, that there are many sources to isolate *N. caninum* from the infected body, blood is the main linker to spread the pathogens into other body organs, and apparently, it is the best source for early diagnosis of infection. We can also conclude that the age factor plays a crucial role in resisting the infection, especially over 3 years old cows. However, the region and time factors do not make any difference in spreading the infection of *N. caninum* in the Babylon province of Iraq.

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DECLARATIONS

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Authors' contribution

Nawras Abdul bari Madlol Alkabi did the study conception, design, and monitored the experiments. Ra'afat Sabbar Abbas Al-Rikaby did all the preparations for sample collection, and the laboratory works involving the molecular part. Naer Abdulbari Alkaabawi prepared and read the molecular part of the laboratory work, interpreted the data and revised the final version of this paper.

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Conflict of interest

The authors have not declared any conflict of interest.

Consent to publish

We, give our consent for the publication of identifiable details, which can include photograph(s) and details within the text to be published in the OJAFR.

REFERENCES

Ahmed F, Cappai MG, Morrone S, Cavallo L, Berlinguer F, Dessì G, et al. (2021). Raw meat based diet (RMBD) for household pets as potential door opener to parasitic load of domestic and urban environment. Revival of understated zoonotic hazards? A review. One health, 13: 100327. https://doi.org/10.1016/j.onehlt.2021.100327

Al-Gharban HA, Al-Eodawee EM and Al-Shabbani AH (2017). Seroepidemiological and molecular identification of *Neospora caninum* in cattle in Wasit province. Basrah Journal of Veterinary Research, 16(2): 172-183. https://www.cabdirect.org/cabdirect/abstract/20183043305

Ameer QJ. Madlol NA, and Kaabawi, NA (2019). Detection of Theileriaannulata isolated from cattle using Real-Time PCR. International Journal of Pharmaceutical Research (09752366), 11(2). Google Scholar

Bartels CJ, van Schaik G, Veldhuisen JP, van den Borne BH, Wouda W, and Dijkstra T (2006). Effect of Neospora caninum-serostatus on culling, reproductive performance and milk production in Dutch dairy herds with and without a history of *Neospora caninum*-associated abortion epidemics. Preventive Veterinary Medicine, 77(3-4): 186-198. https://doi.org/10.1016/j.prevetmed.2006.07.003

Bjerkas I. Mohn SF, and Presthus J (1984). Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Zeitschrift für Parasitenkunde, 70(2): 271-274. Google Scholar

Celik HA, Kozan E, Mustafa Eser, Yilmaz O, Birdane MK, and Sarimehmetoğlu HO (2013). A research on seroprevalence of *Neospora caninum* in cattle. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 60(2): 99-102. http://vetjournal.ankara.edu.tr/en/download/article-file/659795

Dubey JP, and Schares G (2011). Neosporosis in animals—the last five years. Veterinary parasitology, 180(1-2): 90-108. https://doi.org/10.1016/j.vetpar.2011.05.031

Dubey JP, Carpenter JL, Speer CA, Topper MJ, and Uggla, ANDA (1988). Newly recognized fatal protozoan disease of dogs. Journal of the American Veterinary Medical Association, 192(9): 1269-1285. https://europepmc.org/article/med/3391851

Dubey JP, Hemphill A, Calero-Bernal R, and Schares G (2017). Neosporosis in Animals (1st ed.). CRC Press. https://doi.org/10.1201/9781315152561

Dubey JP, Schares G, and Ortega-Mora L (2007). Epidemiology and control of neosporosis and *Neospora caninum*. Clinical microbiology reviews, 20(2): 323-367. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1865591/

Fraser ST, Isern J, and Baron MH (2010). Use of transgenic fluorescent reporter mouse lines to monitor hematopoietic and erythroid development during embryogenesis. In Methods in enzymology. 476: 403-427. Academic Press, Elsevier Inc. https://doi.org/10.1016/S0076-6879(10)76022-5

García-Ispierto I, Serrano-Pérez B, Almería S, Martínez-Bello D, Tchimbou AF, et al. (2015). Effects of crossbreeding on endocrine patterns determined in pregnant beef/dairy cows naturally infected with *Neospora caninum*. Theriogenology, 83(4): 491-496. https://doi.org/10.1016/j.theriogenology.2014.10.013

Gharekhani J, Yakhchali, M, and Berahmat R (2020). Neospora caninum infection in Iran (2004–2020): A review. Journal of Parasitic Diseases, 44: 671-686. https://doi.org/10.1007/s12639-020-01266-w

Claude LH, Lavado A, Rivera D, Talloni MN, West CH (2017). Seroprevalence and risk factors for *Neospora caninum* in small dairy farms in central Chile. Revista MVZ Córdoba, 22(1): 5666-5673. http://www.scielo.org.co/scielo.php?pid=S0122-02682017000105666&andscript=sci_arttext

Hube F, Reverdiau P, Iochmann S, and Gruel Y (2005). Improved PCR method for amplification of GC-rich DNA sequences. Molecular biotechnology, 31: 81-84. https://doi.org/10.1385/MB:31:1:081

Ibrahim AME, Elfahal AM, and El Hussein ARM (2012). First report of *Neospora caninum* infection in cattle in Sudan. Tropical Animal Health and Production, 44(4): 769-772. https://doi.org/10.1007/s11250-011-9963-5

Citation: Al-Kaabi NAbM, Al-Rikaby RSA, Alkaabawi NAM (2023). Molecular detection and prevalence study of *Neospora caninum* isolated from blood of aborted cows in Babylon province of Iraq. *Online J. Anim. Feed Res.*, 13(5): 410-415. DOI: https://dx.doi.org/10.51227/ojafr.2023.57

Japa O, Nuangmek A, Prakhammin K, and Flynn RJ (2019). Prevalence of vertically transmitted *Neospora caninum* amongst beef cattle in Phayao, Thailand. Parasitology international, 70: 98-101. https://doi.org/10.1016/j.parint.2019.02.008

Jiménez-Pelayo L, García-Sánchez M, Vázquez P, Regidor-Cerrillo J, Horcajo P, et al. (2019). Early *Neospora caninum* infection dynamics in cattle after inoculation at mid-gestation with high (Nc-Spain7)-or low (Nc-Spain1H)-virulence isolates. Veterinary research, 50: 1-14. https://doi.org/10.1186/s13567-019-0691-6

Kamali A, Seifi HA, Movassaghi AR, Razmi GR, and Naseri Z (2014). Histopathological and molecular study of *Neospora caninum* infection in bovine aborted fetuses. Asian Pacific Journal of Tropical Biomedicine, 4(12): 990-994. https://doi.org/10.12980/APJTB.4.201414B378

Lindsay DS, and Dubey JP (2020). Neosporosis, toxoplasmosis, and sarcocystosis in ruminants: an update. Veterinary Clinics: Food Animal Practice, 36(1): 205-222. https://doi.org/10.1016/j.cvfa.2019.11.004

Lindsay DS, Upton SJ, and Dubey JP (1999). A structural study of the *Neospora caninum* oocyst. International journal for parasitology, 29(10): 1521-1523. https://doi.org/10.1016/s0020-7519(99)00121-6

Llano HAB, Guimarães MS, Soares RM, Polo G, and da Silva AC (2018). Seroprevalence and risk factors for *Neospora caninum* infection in cattle from the eastern Antioquia, Colombia. Veterinary and Animal Science, 6: 69-74. https://doi.org/10.1016/j.vas.2018.03.001

Mahajan V, Banga HS, and Filia G (2019). Patho-epidemiological and risk factor studies for detection of Neospora-associated abortion in cattle and buffaloes in Punjab, India. Revue Scientifique et Technique (International Office of Epizootics), 38(3): 801-808. https://europepmc.org/article/med/32286566

Mallah MO, Dawood KA, and Alrodhan MA (2012). Seroepidemiological study for the prevalence of *Neospora caninum* in Dairy and Beef cattle in some Iraqi provinces. Al-Qadisiya Journal of Veterinary Medical Science, 11(1): 103-110. https://www.iasj.net/iasj/download/67f498f704052c5b

Metwally S, Hamada R, Sobhy K, Frey CF, and Fereig RM (2023). Seroprevalence and risk factors analysis of *Neospora caninum* and Toxoplasma gondii in cattle of Beheira, Egypt. Frontiers in veterinary science, 10: 1122092. https://doi.org/10.3389/fvets.2023.1122092

Moura AB, Souza AP, Sartor AA, Bellato V, Pisetta GM, Teixeira EB, and Heusser Junior A (2011). *Neospora caninum* antibodies and risk factors in dogs from Lages and Balneário Camboriú, SC. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 63: 262-265. https://www.scielo.br/j/abmvz/a/ch8NS5ymf9Wx3Pxcqq5zDMw/?lang=en&andformat=pdf

Noori M, Rasekh M, Ganjali M, and Nourollahi Fard SR (2019). Seroprevalence of *Neospora caninum* Infection and Associated Risk Factors in Cattle of Sistan Areas, Southeastern Iran in 2016. Iranian journal of parasitology, 14(2): 340–346. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6737369/

Okeoma CM, Williamson NB, Pomroy WE, Stowell KM, and Gillespie L (2004). The use of PCR to detect *Neospora caninum* DNA in the blood of naturally infected cows. Veterinary parasitology, 122(4): 307-315. https://doi.org/10.1016/j.vetpar.2004.06.001

Pitel PH, Pronost S, Chatagnon G, Tainturier D, Fortier G, and Ballet JJ (2001). Neosporosis in bovine dairy herds from the west of France: detection of Neospora caninum DNA in aborted fetuses, seroepidemiology of *N. caninum* in cattle and dogs. Veterinary Parasitology, 102(4): 269-277. https://doi.org/10.1016/S0304-4017(01)00544-1

Reichel MP, Ayanegui-Alcérreca MA, Gondim LF, and Ellis JT (2013). What is the global economic impact of *Neospora caninum* in cattle–the billion dollar question? International journal for parasitology, 43(2): 133-142. https://doi.org/10.1016/j.ijpara.2012.10.022

Razmi GR, Mohammadi GR, Garrosi T, Farzaneh N, Fallah AH, and Maleki M (2006). Seroepidemiology of *Neospora caninum* infection in dairy cattle herds in Mashhad area, Iran. Veterinary Parasitology, 135(2): 187-189. https://doi.org/10.1016/j.vetpar.2005.09.004

Seltmann A, Schares G, Aschenborn OH, Heinrich SK, Thalwitzer S, Wachter B, and Czirják GÁ (2020). Species-specific differences in Toxoplasma gondii, *Neospora caninum* and *Besnoitia besnoiti* seroprevalence in Namibian wildlife. Parasites & vectors, 13(1): 1-12. https://doi.org/10.1186/s13071-019-3871-3

Semango G, Hamilton CM, Kreppel K, Katzer F, Kibona T, Lankester F, et al. (2019). The Sero-epidemiology of *Neospora caninum* in Cattle in Northern Tanzania. Frontiers in veterinary science, 6: 327. https://doi.org/10.3389/fvets.2019.00327

Speer CA, Dubey JP, McAllister MM, and Blixt JA (1999). Comparative ultrastructure of tachyzoites, bradyzoites, and tissue cysts of *Neospora caninum* and Toxoplasma gondii. International journal for parasitology, 29(10): 1509-1519. https://doi.org/10.1016/s0020-7519(99)00132-0

Venturoso PD, Venturoso OJ, Silva GG, Maia MO, Witter R, Aguiar DM, et al. (2021). Risk factor analysis associated with *Neospora caninum* in dairy cattle in Western Brazilian Amazon. Revista Brasileira de Parasitologia Veterinária, 30: e023020-e023020. https://doi.org/10.1590/S1984-296120201088

Waldner C, Wildman BK, Hill BW, Fenton RK, Pittman TJ, Schunicht OC, et al. (2004). Determination of the seroprevalence of *Neospora caninum* in feedlot steers in Alberta. The Canadian Veterinary Journal, 45(3): 218. https://europepmc.org/article/med/9731264

Wei XY, An Q, Xue NY, Chen Y, Chen YY, Zhang Y, et al. (2022). Seroprevalence and risk factors of *Neospora caninum* infection in cattle in China from 2011 to 2020: A systematic review and meta-analysis. Preventive Veterinary Medicine, 203: 105620. https://doi.org/10.1016/j.prevetmed.2022.105620

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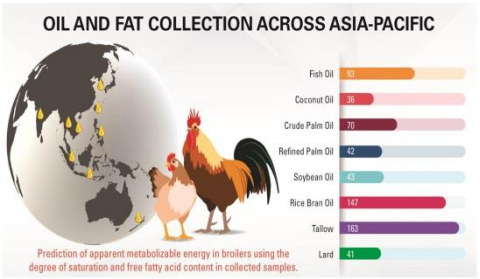

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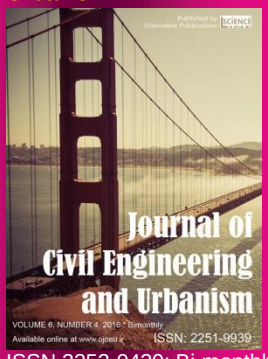

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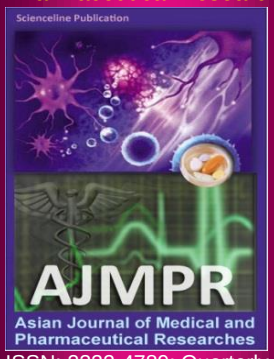

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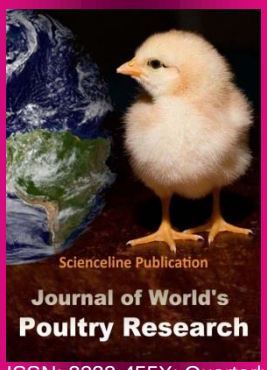

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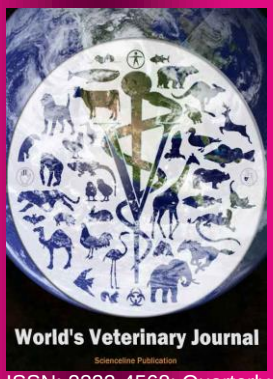

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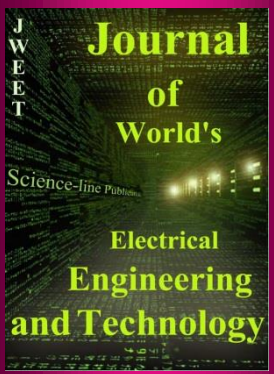

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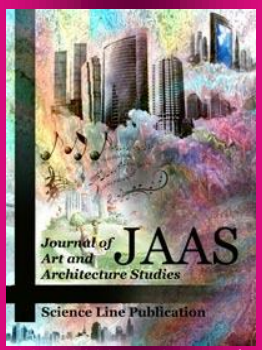

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