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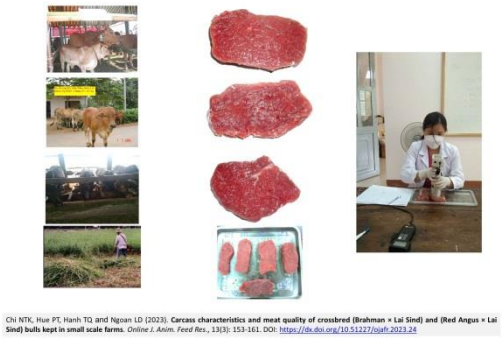
Volume 13 (3); May 27, 2023

Research Paper

Carcass characteristics and meat quality of crossbred (Brahman × Lai Sind) and (Red Angus × Lai Sind) bulls kept in small scale farms

Chi NTK, Hue PT, Hanh TQ and Ngoan LD.



Online J. Anim. Feed Res., 13(3): 153-161, 2023; pii: S222877012300024-13
DOI: https://dx.doi.org/10.51227/ojafr.2023.24

Abstract
This study aimed to evaluate carcass characteristics and meat quality of cross-bred (Brahman × Lai Sind, BL) bulls and cross-bred (Red Angus × Lai Sind, AL) bulls. A total of 30 bulls, 15 head/crossbred genotype were fattened for 90 days before slaughtering at 24 months of age. Carcass traits and meat quality were accordingly measured in 30 slaughtered animals. Results showed that the slaughter weight, carcass weight, carcass dressing, meat percentage, loin muscle area were higher for AL bulls than for BL bulls ($p<0.05$). The color of the meat was not affected by genotype with exception of L* at 48, 168 and 336 hours after slaughter, and this value was higher in AL than in BL bulls ($p<0.05$). The pH of the meat was not different between genotypes ($p>0.05$) but decreased quickly at 24 hours after slaughter ($p<0.05$), then maintained not significantly during storage times. The drip loss, cooking loss and tenderness of the meat were affected by cattle genotype and these values were lower in AL bulls than in BL bulls ($p<0.05$). In conclusion, crossbred (Red Angus × Lai Sind) bulls were higher carcass characteristics, and were better meat quality than crossbred (Brahman × Lai Sind) bulls.

Keywords: Brahman, Crossbred animals, Lai Sind cattle, Red Angus, Meat quality, Tenderness.

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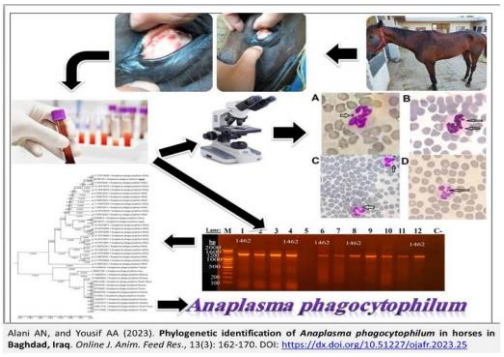
Research Paper

Phylogenetic identification of Anaplasma phagocytophilum in horses in Baghdad, Iraq

Alani AN, and Yousif AA.



Online J. Anim. Feed Res., 13(3): 162-170, 2023; pii: S222877012300025-13
DOI: https://dx.doi.org/10.51227/ojafr.2023.25

Abstract
This study aimed to detect *Anaplasma phagocytophilum* in horses through hematological and molecular tests. The 16S rRNA gene of the *Anaplasma phagocytophilum* parasite was amplified by polymerase chain reaction (PCR), then sequenced, and subjected to phylogenetic analysis to explore "Equine Granulocytic Anaplasmosis" (EGA) infection in three important gathering race horses areas in Baghdad governorate, Iraq. Blood samples were obtained from 160 horses of varying ages, three breeds, and both sexes, between January and December 2021. Prevalence and risk variables for anaplasmosis were analyzed using statistical odds ratio and chi-square tests. Results demonstrated that clinical anaplasmosis symptoms comprised jaundice, weight loss, paleness of mucus membrane with petechial hemorrhage in the third elides, and edema in extremities; There was no tick infestation. The hematological test did not significantly reveal decreases in red and white blood cells and platelet count. Microscopically found 11 from 160 smears (6.88%) had morulae within granulocytes, PCR results of *Anaplasma spp* primers was 32 positive amplicons (20%), and molecular sequencing results of "16S ribosomal RNA genes" confirmed 21 horses (13.13%) infected by *Anaplasma phagocytophilum* for the first time in Iraq horses. The results of the phylogenetic analysis revealed compatibility values similarity 98.81-99.76% with worldwide isolates. Mares occurred not significantly riskier; also age and breed were not illustrated risks of any group. This study is the first molecular detection of *Anaplasma phagocytophilum* in racehorses reared in Baghdad in Iraq. The outcomes of this study provide genetic data for early identification of *Anaplasma phagocytophilum* infection, treatment, and management of the illness in Iraq horses, as well as monitoring its transmission to the human population.

Keywords**:** *Anaplasma phagocytophilum*, Genetic, Mares, Phylogenetic analysis, Sequencing.

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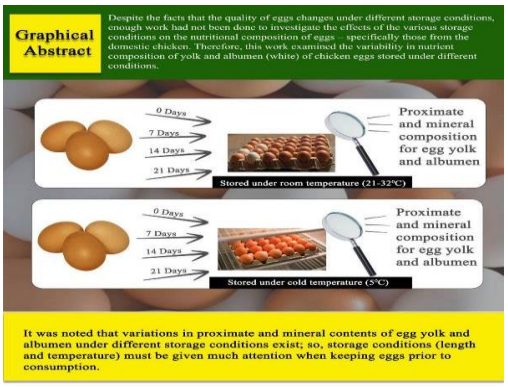
Research Paper

Variability in proximate and mineral compositions of yolk and albumen in eggs kept under different storage conditions

Kruenti F, Hagan JK, Ofori SA, and Lamptey VK.

Online J. Anim. Feed Res., 13(3): 171-176, 2023; pii: S222877012300026-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.26



Abstract
This investigation evaluated the effects of storage length and temperature on the proximate and mineral compositions of yolk and albumen (white) of chicken eggs. A total of 720 eggs were used in a 4 X 2 factorial experiment consisting of four (0, 7, 14 and 21) storage days and two (room and cold) storage temperatures. Data obtained were subjected to ANOVA. The results showed no significant effect of storage length on crude protein and ash contents of the egg parts whereas carbohydrate increased significantly with increasing storage length. Also, protein and fat contents of the yolk were largely influenced ($p<0.05$) by storage temperature but nutrients in the albumen did not differ significantly between the two storage temperatures. On the other hand, mineral compositions of the egg components did not vary noticeably by storage temperature but storage length influenced some minerals considerably. It was noted that variations in proximate and mineral contents of egg yolk and albumen under different storage conditions exist; so, storage conditions (length and temperature) must be given much attention when keeping eggs prior to consumption in order to ensure nutrient quality.

Keywords: Egg quality, Egg protein, Nutrients, Storage length, Storage temperature.

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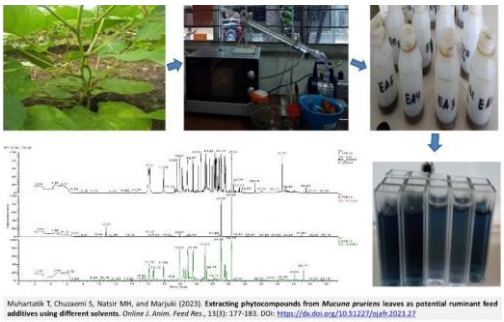
Research Paper

Extracting phytocompounds from *Mucuna pruriens* leaves as potential ruminant feed additives using different solvents

Muhartatik T, Chuzaemi S, Natsir MH, and Marjuki.

Online J. Anim. Feed Res., 13(3): 177-183, 2023; pii: S222877012300027-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.27



Abstract
Some secondary metabolites of plants could serve as ruminant feed additives. They primarily preserve protein from rumen breakdown, reduce rumen protozoa population, and decrease methane gas production. The current study aimed to identify the phytocompounds content of extracted Mucuna pruriens leaves using the Microwave-assisted extraction method using three different solvents of methanol 70% (EM), aquadest (EA), and combinations of EM and EA (EK). The phytocompounds were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Some phytocompounds identified in the Mucuna pruriens substances from GC-MS curve proportion area of EM were 10.35% inositol, 3.1% quinazoline, 4.72% anthraquinone, 3.76% Coptisine, 2.06% isoquinoline, 2.18% D-gluconic acid, 2.83% D-Fructose, 3.91% D-glucose, and 4.59% butanedioic acid. The phytocompounds for EK were 17.22% inositol, 6.36% Niclosamide, 1.4% Acetamide, 1.32% Aniline, 55.97% 4-Amino-2-(4-methoxyphenyl)-5,6,7,8-tetrahydrofuro[2,3-b] quinoline-3-carbonitrile, 17.22% inositol. Furthermore, 22.73% inositol, 6.55%, ribonoic acid, 5.58%, silanol, 21.27% butanodioic acid, 2.88% Fluoroquinoxaline, 5,31%, glycerol, 1,64%, D- gluconic acid were found in the EA. The EA had high inositol content, the EK had high quinoline content, and the EM showed moderate results for all phytobiotics. The total phenolics, flavonoids, tannins, and saponins content significantly differed among the three solvents. The EA yielded the highest concentrations of total phenolics, flavonoids, and tannins, but the lowest concentration of total saponins. In contrast, the EM yielded the lowest total phenolics, flavonoids, and tannins content, but the highest total saponins content. Meanwhile, the EK yielded modest results for all phytocompounds, with values between EA and EM. In conclusion, the methanolic extract of Mucuna pruriens substance had the highest phytocompounds and bioactive potential as ruminant feed additives.

Keywords: Feed additives, Gas chromatography, Mucuna pruriens, Phytocompounds, Solvent, Secondary metabolites

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Research Paper

Occurrence of parasites in fish marketed in the Inezgane wholesale market and the fishing port of Agadir, Morocco

Dahani S, Bouchriti N, and El Hariri O.

Online J. Anim. Feed Res., 13(3): 184-191, 2023; pii: S222877012300028-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.28

Abstract
Based on importance of animal products safety, the purpose of this work was to assess the extent of parasitism at the wholesale market level of Inezgane and the fishing port of Agadir in Morocco. For this purpose, fieldwork aimed at direct investigation of parasites involved 366 fish pieces. This study was conducted in the period between March and June 2021. The prevalence of parasitism was 20.76%. The total number of parasites collected is 2385 including 1959 nematodes, 318 xenomas, 92 cestodes, and 16 isopods. An abundance of 6.51 and an overall intensity of 31.38. These infestation parameters varied by species and location of origin. For the qualitative analysis of the parasites, the study revealed a predominance of L3 larvae of the *Anisakis* nematode with a percentage of 82.14%. Xenomas had a percentage of 13.33%. As for the cestodes of *Gymnorhynchus gigas*, the larvae were collected from the Atlantic pomfret (*Brama brama*) with a percentage of 3.86%. As a result of this study, a significant positive correlation of r=0.81 was shown between the total length of the fish and the number of anisakids. The results of this study revealed that the extent of parasitism seems to be less pronounced in some species, but there is still a presence of concern.

Keywords**:** Anisakis, Fish, Fishing port, Parasitism, Wholesale market level

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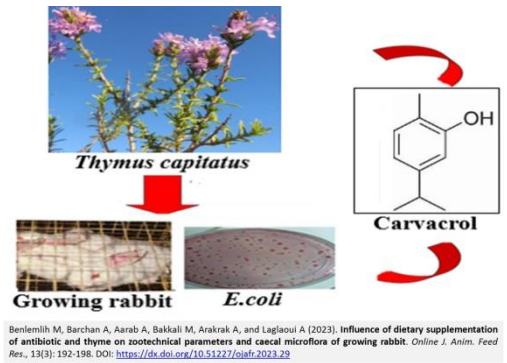
Research Paper

Influence of dietary supplementation of antibiotic and thyme on zootechnical parameters and caecal microflora of growing rabbit

Benlemlih M, Barchan A, Aarab A, Bakkali M, Arakrak A, and Laglaoui A.

Online J. Anim. Feed Res., 13(3): 192-198, 2023; pii: S222877012300029-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.29



Abstract
The objective of this study was to compare the influence of antibiotic and thyme dietary supplements on zootechnical parameters and caecal microflora of growing rabbits. One hundred and ninety eight weaned rabbits (forty days old), white New Zealand (of both sexes) were divided into three groups to submit to the following dietary treatments: Control diet, diet A (control diet + 100 ppm zinc bacitracin), and diet T (control diet + 7% *Thymus capitatus* leaves) for twenty-one days. The remaining nine days they received only the control diet. The results showed that both the live body weight and feed conversion ratio were positively affected by the antibiotic diet (P<0.05). However, the rabbits' growth performance was not influenced by dietary thyme supplements. The antimicrobial effect of thyme observed against *C. perfringens* in caecum is not determined even after 20 days of treatment. In conclusion, zootechnical parameters and mortality were not positively affected by dietary thyme supplements comparing it with the antibiotic diet, but these phytobiotics showed the antibacterial effect against *E. coli* and C*. perfringens* in caecum of rabbit.

Keywords**:** Zinc bacitracin, Dietary supplementation, Growth performance, Rabbit, Thyme**.**

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Research Paper

New growth medium for culturing lactic acid bacteria as probiotic consortium isolated from fermented fish (Budu)

Malikil Kudus S, Harnentis, Jamsari and Yetti M.

Online J. Anim. Feed Res., 13(3): 199-203, 2023; pii: S222877012300030-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.30

Abstract
This study aimed to obtain the best ratio of inoculums and types of alternative media in increasing the growth of the probiotic consortium with the observed variables consisting of viability, cell biomass and decrease in pH. Completely randomized design (CRD) factorial consisting of 2 factors with 3 replications is used; factor A was the probiotic consortium (A1: *Lactobacillus parabuchneri*: *L. buchneri*: *L. harbinensis*, *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner)* with ratio 1:1:1:1:1; A2: same consortium with ratio 1:1:1:1:2; A3: same consortium with ratio 1:1:1:2:1; A4: same consortium with ratio 1:1:2:1:1; A5: same consortium with ratio 1:2:1:1:1; A6: same consortium with ratio 2:1:1:1:1 and factor B was the type of alternative media (B1=control; B2=coconut water (90%) + cassava flour (5%) + fish waste flour (5%); B3=tofu liquid waste (90%) + flour onggok (5%) + fish waste meal (5%); B4= tofu whey (90%) + onggok flour (5%) + fish waste meal (5%). The results showed that there was an interaction between factor A and factor B which was highly significant (P<0.01) on viability, cell biomass and decrease in medium pH. In conclusion, the best ratio of probiotic consortium was 1:1:1:2:1, with growth medium coconut water (90%) + cassava flour (5%) + fish waste flour (5%) which resulted in a viability value of: 3, 02, cell biomass: 22.47 mg/ml and a decrease in the pH of the medium by 2.84.

Keywords**:** Cell biomass, Fermentation, Medium pH, Probiotic consortium, Viability.

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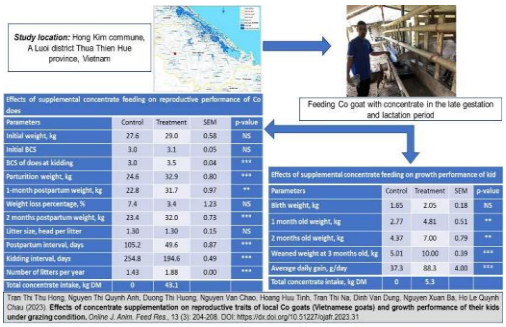
Research Paper

Effects of concentrate supplementation on reproductive traits of Co goats and growth performance of their kids under grazing condition

Tran HTT, Nguyen ATQ, Duong HT, Nguyen CV, Hoang TH, Tran NT, Dinh DV, Nguyen BX, and Ho CLQ.

Online J. Anim. Feed Res., 13(3): 204-208, 2023; pii: S222877012300031-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.31



Abstract
The experiment was conducted in A Luoi district, Thua Thien Hue province of Vietnam, to evaluate the effect of concentrate supplementation on the reproductive traits of local Co goats and growth performance of their kids. A total of 20 pregnant Co goats in the last 1.5 month of pregnancy were monitored in two reproductive cycles. Goats were randomly divided into two dietary treatments as control (CG), in which animals freely grazed, and experiment (EG), in which concentrates were supplemented at 1.0% and 1.5% BW in the late gestation and lactation periods, respectively. After kidding, kids in each treatment were kept with their mother to access milk for the whole study period. In the CG, the kids have no supplements, and in the EG, the kids of 1 to 3 months of age were supplemented the concentrate at 1.5% BW/day. The results indicated that higher body condition scores and body weights of does after kidding, 1 and 2 months postpartum were in the EG than in the CG. The supplementation of concentrate also reduced the postpartum and kidding intervals; and increased the number of litters per year of does. Furthermore, the supplementation of concentrate improved significantly the weaning weight of kids and financial benefit per doe/per litter. In conclusion, the supplementation of concentrate in late pregnancy period and lactation diets improved reproductive traits of local Co goats and also concentrate supplementation in kids' diet improved growth performance under grazing condition.

Keywords: Body condition score, Lactation, Local breeds, Pregnant, Small ruminants.

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Research Paper

Impact and prevalence of Newcastle disease and associated risk factors in village chickens in southern Niger

Ahamidou M, Essodina T, Adamou A, and Haladou G.

Online J. Anim. Feed Res., 13(3): 209-216, 2023; pii: S222877012300032-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.32

Abstract
The present study was conducted to determine the prevalence of Newcastle disease and to identify potential risk factors in village chickens in Niger. A total of 1,627 serum samples were collected using a stratified random sampling method with proportional allocation. Samples were collected from village breedings in the departments of the Maradi region (Guidan Roumdji, Madarounfa, Aguié, Gazaoua, and Tessaoua), departments of Zinder region (Takeita, Kantché, Magaria, Dungass, and Mirriah), and cities of Maradi and Zinder. Data on risk factors were collected through an interview with the herders. All collected sera were subjected to competitive enzyme-linked immunosorbent assay (cELISA) to detect Newcastle disease virus-induced antibodies. The findings were indicative of 302 positive sera, representing an overall seroprevalence of 18.6%. The Student's t-test at $p<0.05$ revealed a significant difference between regions and among some departments. Furthermore, the logistic regression test identified the agroecological zone, type of breeding, species mix, and the origin of the animals as risk factors associated with seropositivity to Newcastle disease virus. The present results confirmed the exposure of village chickens to the Newcastle disease virus, emphasizing the need to intensify vaccination campaigns and educate poultry farmers on adopting biosecurity measures.

Keywords: Newcastle disease, Risk factors, Vaccination, Village chickens, Niger.

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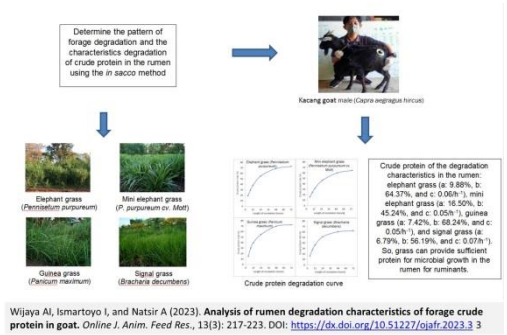
Research Paper

Analysis of rumen degradation characteristics of forage crude protein in goat

Wijaya AI, Ismartoyo I, and Natsir A.

Online J. Anim. Feed Res., 13(3): 217-223, 2023; pii: S222877012300033-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.33



Abstract
The quality of feed given to ruminants can be determined from the degradation of nutrient content in the rumen. This study aimed to determine the pattern of forage degradation and the characteristics degradation of crude protein in the rumen using the *in sacco* method. The study used 4 fistulae kacang goats with an average body weight of 14.57 kg. The forage used consisted of R1: elephant grass (*Pennisetum purpureum*), R2: mini elephant grass (*Pennisetum purpureum cv. Mott*), R3: guinea grass (*Panicum maximum*), and R4: signal grass (*Bracharia decumbens*). The nylon bag is made of polyester measuring 8x4 cm with a porosity of 40 µm. Feed samples were put into the rumen and incubated for 0, 4, 8, 12, 24, 48, and 72 hours. The parameters measured were consumption, patterns, and forage degradation characteristics by calculating the values of a, b, c, a+b, lag time, and ED. Determination characteristics of feed degradation in the rumen by *in sacco* method will be analyzed. The results showed that the characteristics of crude protein degradation had significant differences in fraction values a, b, a+b, and lag time ($P<0.05$), while c and ED did not have significant differences ($P>0.05$). In conclusion the crude protein of the degradation characteristics in the rumen were: elephant grass (a: 9.88%, b: 64.37%, and c: 0.06/h^{-1}), mini elephant grass (a: 16.50%, b: 45.24%, and c: 0.05/h^{-1}), guinea grass (a: 7.42%, b: 68.24%, and c: 0.05/h^{-1}), and signal grass (a: 6.79%, b: 56.19%, and c: 0.07/h^{-1}). So, grass can provide sufficient protein for microbial growth in the rumen for ruminants.

Keywords: Crude protein, Degradability, Forage, *in sacco*, Rumen.

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CARCASS CHARACTERISTICS AND MEAT QUALITY OF CROSSBRED (BRAHMAN × LAI SIND) AND (RED ANGUS × LAI SIND) BULLS KEPT IN SMALL SCALE FARMS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study aimed to evaluate carcass characteristics and meat quality of cross-bred (Brahman × Lai Sind, BL) bulls and cross-bred (Red Angus × Lai Sind, AL) bulls. A total of 30 bulls, 15 head/crossbred genotype were fattened for 90 days before slaughtering at 24 months of age. Carcass traits and meat quality were accordingly measured in 30 slaughtered animals. Results showed that the slaughter weight, carcass weight, carcass dressing, meat percentage, loin muscle area were higher for AL bulls than for BL bulls ($p<0.05$). The color of the meat was not affected by genotype with exception of L* at 48, 168 and 336 hours after slaughter, and this value was higher in AL than in BL bulls ($p<0.05$). The pH of the meat was not different between genotypes ($p>0.05$) but decreased quickly at 24 hours after slaughter ($p<0.05$), then maintained not significantly during storage times. The drip loss, cooking loss and tenderness of the meat were affected by cattle genotype and these values were lower in AL bulls than in BL bulls ($p<0.05$). In conclusion, crossbred (Red Angus × Lai Sind) bulls were higher carcass characteristics, and were better meat quality than crossbred (Brahman × Lai Sind) bulls.

Keywords: Brahman, Crossbred animals, Lai Sind cattle, Red Angus, Meat quality, Tenderness.

INTRODUCTION

Beef is the third most consumed meat in the world after poultry and pork at 6.4, 14.0 and 12.2 kg/person/year, respectively (OECD, 2019). Beef consumption continues to increase with population and income growth consumer input. By 2027, it is estimated that beef consumption in developed and developing countries will be 8% and 21% higher than the 2015–2017 average, respectively (OECD-FAO, 2019). Currently, consumer demand for beef products is not only concerned with the quantity but also the quality of meat and tenderness (Kim et al., 2020; Fořtová et al., 2022). The world and domestic markets are becoming more and stricter in terms of 120 meat quality standards (Hocquette and Gigli, 2005). Faced with that fact, the issue of improving beef quality is one of the main concerns of the livestock production today (Hocquette and Gigli, 2005). Factors such as breed, sex, age at slaughter, and rations affect meat quality, in which breed is considered one of the important factors affecting meat quality (Waritthitham et al., 2010). Meat quality characteristics such as tenderness, color, flavor, juiciness, water holding capacity, drip loss have impact satisfaction of consumer (Cafferky et al., 2019).

In Central Highland region of Vietnam, beef cattle production plays an important role in term of family income and sustainable development in industrial crop-livestock systems. Number of cattle in this region consisted of 13.3% of total cattle in the country (GSO, 2021) and most of animals are kept in small scale farms. However, beef cattle raising is based on the local breeds such as local Yellow cattle, and F1 (local Vietnamese yellow-*Bos indicus* and Sindh -*Bos indicus*) so called *Lai Sind*. These breeds have small body size, e.g. the mature body weight of local yellow cattle is 182.2 kg and Lai Sind of 244 kg (Van et al., 2009). Their productivity is also low and the quality of beef is poor due to slow growth and prolonged slaughter age (Karimov et al., 2016). With the aim of improving beef productivity and quality to meet the demand of beef was increasing in the country, including Central Highland region, have many policies on insemination of specialized beef breeds such as Red Angus, Droughmaster, Charolais and Brahman for crossbreeding with domestic cattle breeds in order to create a hybrid cattle with high potential yield and meat quality (Quyen et al., 2018).

In many studies, the results showed that growth performance, carcass traits and meat quality of crossbred cattle were significantly improved when compared with Vietnamese local beefs (Hue et al., 2008; Dung, 2012; La et al., 2017; Quyen et al., 2018; Vu et al., 2021; Hai et al., 2022). Many studies have been evaluating carcass traits and meat quality of crossbreds between Red Angus, Droughmaster, Charolais and Brahman with Lai Brahman (La et al., 2017; Quyen et al., 2018; Linh et al., 2022). Linh et al. (2022) studied on the meat quality of crossbred genotypes of (Charolais × Lai

Citation: Chi NTK, Hue PT, Hanh TQ and Ngoan LD (2023). Carcass characteristics and meat quality of crossbred (Brahman × Lai Sind) and (Red Angus × Lai Sind) bulls kept in small scale farms. *Online J. Anim. Feed Res.*, 13(3): 153-161. DOI: https://dx.doi.org/10.51227/ojafr.2023.24

Brahman), (Droughtmaster × Lai Brahman) and (Red Angus × Lai Brahman) and found that crossbred genotypes had no effects on some meat quality traits.

However, the studies on carcass traits and meat quality of those exotic bulls crossing with Lai Sind were limited (Hue et al., 2008; Dung, 2012). The aim of this study, therefore, was to evaluate the carcass characteristics and meat quality of two crossbred (Brahman × Lai Sind) and (Red Angus × Lai Sind) bulls kept in small scale farms in the Central Highland region, Vietnam.

MATERIALS AND METHODS

This experiment was carried out in small scale farms in Ea Kmut commune, Ea Kar district and in the Faculty of Animal Science and Vet Medicine, Tay Nguyen University, Buon Me Thuot City, Dak Lak province.

Animal experiments

Total 30 bulls of 2 genotypes: 15 BL (Brahman x Lai Sind) and 15 AL (Red Angus x Lai Sind) were raised individually in a barn with an area of 5 m^2/head. They were fattened for 3 months from 21st to 24th months of age. During fattening, animals were fed 60% ensiled VA06 grass and 40% concentrate proportionally combining rice bran, corn meal, soybean meal, urea and mineral salts for 90 days before slaughter. The research protocol was approved by the Scientific Committee of Tay Nguyen University dated 17 June'2021, Decision No: 1228-QĐ-ĐHTN

Slaughtering, meat sampling

Thirty bulls of 420-450 kg body weight were fasted for 24 hours and weighed (slaughter weight) before stunning with an electric current of 220 volts at the slaughter house. After taking out some body parts, the left half carcass was remained for meat sampling. Loin muscle (*Longissimus dorsi*) samples were taken at the 6-13th ribs and stored in a cold chamber at 2-4°C prior to meat quality measurements.

Measurements

Carcass traits: Carcass weight and dressing, meat weight and percentage, bone weight and percentage and loin muscle area were measured at the laboratory of the Faculty of Animal Husbandry - Veterinary Medicine, Tay Nguyen University.

pH of meat: pH was determined by pH meter Testo 230 (German) at 1 hours (pH_1); 24 hours (pH_{24}); 48 hours (pH_{48}); 96 hours (pH_{96}); 168 hours (pH_{168}) and 336 hours (pH_{336}) hours after slaughter with 3 replicates. The pH_1 was measured at 1h after slaughter by taking 10g of minced loin muscle into a 400 ml beaker, adding 100 ml of distilled water, homogenizing the sample and centrifuging at 7000 rpm, and measuring the pH of the solution as quickly as possible. Similarly, values of pH_{24}, pH_{48}, etc. were measured on meat samples stored at 4 ^{0}C.

Drip loss: Meat samples were cut from the loin muscle with a size of thickness of 2.5 cm, width 2 cm and length 5 cm. They were weighed, put in a storage bag, sealed and stored at 2 – 4 ^{0}C according to Brondum et al. (2000). At 24, 48, 96, 168 and 336 hours after preservation, the sample should be taken immediately from the storage bag, lightly patted dry and weighed according to Brondum et al. (2000) and Honikel (1998). Drip loss was calculated according to the formula:

$$\text{Drip loss (\%)} = \frac{P1-P2}{P1} x100$$

In which: P1(g): initial weight
P2 (g): final weight

Cooking loss: Meat samples were cut from the loin muscle with size of thickness of 2.5 cm, width 2 cm and length 5 cm, immediately weighed (initial weight), put in a polyethylene bag, heated in a water bath at 75^{0}C for 60 minutes, taken and weighed again (final weight). Cooking loss was calculated as following:

$$\text{Cooking loss (\%)} = \frac{P1-P2}{P1} x100$$

In which: P1(g): initial weight
P2(g): final weight

Meat color: Meat color was measured in the loin sample with a Minolta CR-410 colorimeter (Japan) followed to Honikel (1998) and Baublits et al. (2006). The color was expressed as L*, a* and b* readings according to standard luminance D and standard angle of view 65° (CIE, 1976 cited by Honikel, 1998; Baublits et al., 2006).

- L* = 0 (black), L* = 100 white light (white light similar to BaSO4 or MgO burnt)
- b* = - 60 (green), +60 (yellow)
- a* = - 60 (blue), + 60 (red)

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Tenderness (N/cm²): Tenderness was measured by Shear Force Warner-Bratzler method. Samples of 80 - 100 g were weighed, placed in polypropylene bags and heated in a water bath at 80 ^{0}C for 30 min, and then removed from the water. After cooling, use a steel pipe with a diameter of 1.25 cm to drill out 5-10 meat ingots, meat samples were taken along the direction of the fibers. The cutting force was determined on the meat ingots by Warner - Bratzler 2000D (USA) at the time of 12, 24, 48; 168 and 336 hours with 10 replicates/time.

Loin muscle area: The loin muscle samples were taken at the 12-13th rib and stored at 2 – 4 ^{0}C for 24 hours. Loin muscle area was measured by plastic paper and calculated according to the formula:

$$S = \frac{A2*S1}{A1} x100$$

In which: S: Loin area (cm²); S1: Plastic area before using (cm²); A1: Plastic weight before using (g); A2: Plastic weight after using (g)

Data analysis

Data were presented in the form of the mean (M), standard error of the mean (SEM). The data were statistically processed by analysis of variance (ANOVA) by General Linear Model in Minitab v. 16.2 (2010). The difference between the mean values was determined by the Tukey method at a confidence level of 95%. Statistical model:

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

Where: μ is the average value; G_i is the effect of genotype (or storage times); e_{ij} is the experimental error.

RESULTS

Carcass characteristics

Effect of genotype on carcass characteristics of crossbred bulls meat presented in Table 1. Data in Table 1 showed that cattle genotype affected the carcass traits, including the carcass dressing, the meat percentage and the loin muscle area. Those traits were higher in AL than in BL ($p < 0.05$). Values of the carcass dressing, meat percentage and loin muscle area in BL were 52.30%, 42.20% and 79.73 cm², respectively, and lower than those in AL 54.4%, 45.0% and 85.6 cm², respectively.

Changes in pH of meat during storage

Effect of storage time duration and two genotypes of cattle beef indicated in Table 2. Data showed no effect of genotype on pH value of meat at any times of storage ($p > 0.05$). Values of pH ranged 5.39-6.61 in BL meat and 5.43-6.65 in AL meat. However, pH values of meat effected by storage times ($p < 0.05$) in both meats of genotypes. pH decreased dramatically in the 1st day (24hrs) of storage ($p < 0.05$), and gradually declined from the 2nd day to 14th day after storage at 2-4^{0}C. The pH dropped 1.14 units in BL (6.61 to 5.47) and 1.15 units in AL (6.65 to 5.50) at the 1st day of storage (pH_{24}). However, after day 1st to day 14th the values of pH declined slowly 0.07 units in BL and 0.04 units in AL, but were not statistically different among them.

Changes in the color of meat during storage

The colors of two crossbred meats and their stored meat during 14 days after slaughter presented in Table 3. With exception of L* at 48, 168 and 336 hours, all color parameters were not affected by genotype ($p > 0.05$). The L* values at 48, 168 and 336 hours were higher in AL than in BL (at 48 hrs 38.91 vs 37.96; at 168 hours 39.57 vs 38.81 and 336 hrs 40.49 vs 39.72, respectively). However, the color of meat was affected by duration of storage in 2 genotypes ($p < 0.05$). In general, prolonging times of storage increased values L*, a* and b*, especially at the 1st – 2nd day of storage. The L* value of the meat increased by 2.49 units in BL and 3.97 units in AL during first 2 days of storage, and after that day to 14th day these values increased by only 1.76 units in BL and 1.88 units in AL. The values a* and b* have been changed at the same pattern of L*.

Loss of water during storage, cooking and tenderness of meat

Effect of genotype and duration of storage on the drip loss, the cooking loss and the tenderness of loin muscle presented in Table 4. The drip loss, the cooking loss and the tenderness were affected by genotype and time duration of storage ($p < 0.05$) with exception of the drip loss at 24 hours ($p > 0.05$). Generally, these values were higher in BL than in AL ($p < 0.05$) at any time of meat storage. Furthermore, prolonging storage time increased the values of drip loss and the cooking loss in both genotypes. The drip loss at 24 hours and 336 hours were 0.98% and 5.01% in BL, respectively, and 0.92% and 4.69% in AL, respectively. The cooking loss at 24 hours and 336 hours were 28.95% and 33.33% in BL, respectively, and 27.99% and 32.2% in AL, respectively.

The tenderness of meat of two genotypes increased quickly in first 2 day of storage, and then declined gradually from day 4th to day 14th. The values of the meat tenderness (WBSF - Warner-Bratzler Shear Force) at 24, 48 and 336 hours were 73.26, 93.60 and 69.09 N/cm² in BL, respectively; and 70.3, 91.0 and 67.16 N/cm² in AL, respectively.

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Table 1 - Effect of genotype on carcass characteristics of crossbred bulls

Parameters \ Genotypes	Brahman × Lai Sind (BL)	Red Angus × Lai Sind (AL)	SEM	p-value
Slaughter weight (kg)	417.3^{b}	457.3^{a}	8.694	0.003
Carcass weight (kg)	218.16^{b}	250.5^{a}	4.835	0.001
Carcass dressing (%)	52.3^{b}	54,40^{a}	0.49	0.001
Meat weight (kg)*	176.3^{b}	205,8^{a}	4.54	0.001
Meat percentage (%)	42.2^{b}	45.00^{a}	0.484	0.001
Bone weight (kg)	45.25^{b}	53.00^{a}	0.997	0.001
Bone percentage (%)	10.97	11,59	0.264	0.107
Loin muscle area (cm^{2})	79.73^{b}	85.6^{a}	0.725	0.001

*Without skin, fat and bone; ^{a,b}: Means in the same row without common letter are different at p<0.05.

Table 2 - Effect of genotype and storage times on pH of the meat

Meat pH \ Genotype	Brahman × Lai Sind (BL)	Red Angus × Lai Sind (AL)	SEM	p-value
pH_1	6.61^{A}	6.65^{A}	0.021	0.485
pH_{24}	5.47^{B}	5.50^{B}	0.017	0.205
pH_{48}	5.45^{B}	5.48^{B}	0.013	0.284
pH_{96}	5.43^{B}	5.47^{B}	0.097	0.216
pH_{168}	5.41^{B}	5.46^{B}	0.016	0.056
pH_{336}	5.39^{B}	5.43^{B}	0.016	0.098
SEM	0.016	0.017		
p-value	0.001	0.001		

^{A,B}: Means in the same column without common letter are different at p<0.05

Table 3 - Effect of genotype and storage times on meat color

Storage times (Hrs.) \ Genotype		Brahman x Lai Sind (BL)	Red Angus x Lai Sind (AL)	SEM	p-value
L* (light)	12	35.47^{D}	35.82^{B}	0.2023	0.236
	24	37.30^{C}	36.77^{B}	0.2321	0.112
	48	37.96^{B}	38.91^{A}	0.2434	0.016
	96	38.59^{AB}	39.20^{A}	0.2518	0.096
	168	38.81^{AB}	39.57^{A}	0.2526	0.044
	336	39.72^{A}	40.79^{A}	0.2971	0.017
	SEM	0.4712	0.608		
	p-value	0.008	0.044		
a* (red color)	12	18.90^{B}	18.95^{B}	0.2016	0.853
	24	18.87^{C}	19.17^{B}	0.2396	0.394
	48	20.20^{A}	20.27^{B}	0.2764	0.866
	96	20.51^{A}	20.55^{A}	0.3201	0.930
	168	20.95^{A}	20.72^{A}	0.2315	0.482
	336	20.37^{A}	20.47^{A}	0.2287	0.759
	SEM	0.339	0.241		
	p-value	0.002	0.001		
b* (yellow color)	12	5.90^{D}	6.08^{B}	0.133	0.329
	24	6.83^{C}	6.72^{B}	0.2343	0.735
	48	7.74^{B}	8.144^{A}	0.158	0.193
	96	8.57^{A}	8.38^{A}	0.160	0.087
	168	8.44^{A}	7.88^{A}	0.376	0.210
	336	8.61^{A}	8.85^{A}	0.166	0.091
	SEM	0.197	0.149		
	p-value	0.001	0.001		

^{a,b}: Means in the same row without common letter are different at p<0.05; ^{A,B,C,D}: Means in the same column within parameter without common letter are different at p<0.05

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	168	38.81^{AB}	39.57^{A}	0.2526	0.044
	336	39.72^{A}	40.79^{A}	0.2971	0.017
	SEM	0.4712	0.6080		
	p-value	0.008	0.044		
a* (red color)	12	18.90^{B}	18.95^{B}	0.2016	0.853
	24	18.87^{C}	19.17^{B}	0.2396	0.394
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	SEM	0.339	0.241		
	p-value	0.002	0.001		
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	336	8.61^{A}	8.85^{A}	0.166	0.091
	SEM	0.197	0.149		
	p-value	0.001	0.001		

^{a,b}: Means in the same row without common letter are different at $p<0.05$; ^{A,B,C,D}: Means in the same column within parameter without common letter are different at $p<0.05$

Table 4 - Effect of genotype and storage time on drip loss, cooking loss and tenderness of the meat

Storage times (Hrs.) \ Genotype		Brahman x Lai Sind (BL)	Red Angus x Lai Sind (AL)	SEM	p-value
Drip loss (%)	24	0.98^{Ea}	0.92^{Eb}	0.032	0.166
	48	2.15^{Da}	1.75^{Db}	0.113	0.019
	96	2.89^{Ca}	2.58^{Cb}	0.068	0.002
	168	4.38^{Ba}	4.13^{Bb}	0.074	0.029
	336	5.01^{Aa}	4.69^{Ab}	0.102	0.033
	SEM	0.094	0.070		
	p-value	0.001	0.001		
Cooking loss (%)	24	28.95^{Ea}	27.99^{Cb}	0.273	0.019
	48	29.79^{Da}	28.79^{Cb}	0.271	0.014
	96	30.92^{Ca}	29.91^{Bb}	0.291	0.020
	168	32.57^{Ba}	31.60^{Ab}	0.303	0.031
	336	33.33^{Aa}	32.20^{Ab}	0.358	0.034
	SEM	0.239	0.353		
	p-value	0.001	0.001		
WBSF (N/cm^2)*	24	73.26^{Ca}	70.30^{Cb}	0.401	0.039
	48	93.60^{Aa}	91.00^{Ab}	0.624	0.006
	96	85.13^{Ba}	81.37^{Bb}	1.025	0.015
	168	72.33^{Da}	69.76^{Db}	0.820	0.035
	336	69.09^{Ea}	67.16^{Eb}	0.535	0.016
	SEM	0.738	0.668		
	p-value	<0.001	<0.001		

^{a,b}: Means in the same row without common letter are different at $p<0.05$; ^{A,B,C,D}: Means in the same column within the parameter without common letter are different at $p<0.05$; * *WBSF : Warner-Bratzler Shear Force*

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DISCUSSION

Carcass characteristics

In this study, the slaughter weight, carcass dressing and meat percentage were affected by genotype, and these of crossbred (Red Angus x Lai Sind) bulls of 457.3 kg were higher than (Brahman x Lai Sind) bulls of 417.3 kg. These findings are agreement in previous results (Bartoň et al., 2006; Frederico et al., 2016; Barcellos et al., 2017; Linh et al., 2021). The authors concluded *Bos taurus* beef cattle usually have the slaughter weight and carcass dressing and meat percentage higher than *Bos indicus*. In this study, the Red Angus originated from *Bos taurus* and was popular cattle in US, Australia, etc., and the Brahman originated from *Bos indicus* cattle from India. Linh et al. (2021) studied the carcass traits of 3 cattle crossbreds between Lai Brahman cows and Charolais, Red Angus or Droughtmaster bulls of 21 months of age and found the effect of genotype on the carcass dressing and the meat percentages. The carcass dressing and the meat percentage of crossbred (Charolais × Lai Brahman - CB) were higher than crossbred (Red Angus × Lai Brahman - AB) and the crossbred (Droughtmaster × Lai Brahman - DB). The carcass dressing of CB, AB and DB were 62.1% vs 60.3% and 60.6%, respectively, and their meat percentages were 45.2% vs. 43.9% and 42.6%, respectively. Quyen et al. (2018) showed the carcass dressing in crossbred (Red Angus x Lai Sind - AL) was higher than crossbred (Brahman x Lai Sind -BL), and were 52.07% vs 48.09%. Similarly, La et al. (2017), Dat et al. (2008) and Chase et al. (2001) reported that the slaughter weight, the carcass dressing in crossbred cattle was genetically influenced by genotype. La et al. (2017) reported that the carcass dressing of crossbred (Brahman x Lai Sind) was lower than in crossbred (Limousin x Lai Sind) and (Droughmaster x Lai Sind), and were 49.7% vs 53.3% and 51.4%, respectively. In addition, Suryanto et al. (2014) showed that the carcass dressing and the meat percentage were influenced by cattle genotype and feeding diets. Vaz et al. (2002) showed that crossbred (¾ Charolais x ¼ Nelore) had a higher the slaughter weight than crossbred (¾ Nelore x ¼ Charolais), the carcass dressings were different of 53.66% and 54.62%, respectively ($p<0.05$).

In present study, the loin muscle area was affected by cattle genotype. This finding was agreement in previous studies of Bartoň et al. (2006), Quyen (2009) showed the loin muscle area between 8-9th rib of Angus of 17 months of age was lower Charolais of the same age (106.5 cm^2 vs 100.1 cm^2, respectively). Quyen et al. (2018) also indicated there were differences in the loin muscle areas of three genotypes crossbred (Droughmaster × Lai Sind), (Brahman x Lai Sind) and Lai Sind at 24 months of age. The loin muscle areas of crossbreds (Droughmaster × Laisind), (Brahman x Lai Sind) and Lai Sind were 123.68; 95.96 and 81.13 cm^2 respectively. However, Linh et al. (2021) found no effect of cattle genotype on the muscle area of three crossbreds (Charolais x Lai Brahman; Droughmaster x Lai Brahman; Red Angus x Lai Brahman). These values of the muscle area of 10-11th rib were 93.0, 85.8 and 94.2 cm^2, respectively.

pH value

The pH value of meat is related to meat quality. After slaughter, the process of anaerobic glycogenolysis produces lactic acid in the muscle, which reduces the pH of the meat. In this study, pH value of the meat was not affected by cattle genotype but affected by time storage. These results were agreement in many precent studies (Barcellos et al., 2017; Cafferky et al., 2019; Linh et al., 2022). All authors indicated that the pH of beef was not genetically influenced by cattle breeds. Linh et al. (2022) reported that pH values of the meat of three genotypes (Charolais x Lai Brahman, Droughmaster x Lai Brahman and Red Angus x Lai Brahman) were not affected by cattle genotypes. The authors showed that pH_{24}, pH_{48} of the meat ranged 5.4-5.6 and 5.3-5.5, respectively. Similarly, Li et al. (2014) indicated that pH_{48} of crossbred (Red Angus x Chinese yellow cattle) of 18 months old was 5.7. Cafferky et al. (2019) reported no difference in the pH_{48} of the meat of Angus, Charolais and Hereford and were 5.55; 5.54 and 5.53, respectively. In addition, Wu et al. (2014) classified pH of the cattle meat into low pH ≤ 5.5 (5.42 – 5.71), medium pH 6.2 (5.86 – 6.19) and high pH ≥ 6.2 (6.29–6.99) depending accordingly on the time of pH measurement. In this classification, the pH values measured in our experiment were in the average range. On the other hand, the Instituts de l'Elevage (2006) declared a final pH (5.5-5.7) as beef in a normal state and the meat was bright red (RFN), a final pH (5.2 - 5.5) was pale beef (PSE), and final pH 6.3 - 6.7 was DFD beef (dark, hard, dry beef). In this study, final pH of two meat types ranged 5.4-5.5 and the meat felled in pale beef (PSE). However, Honikel (1998) classified that if pH_{48} of the meat ranged 5.4-5.8 then the meat was normal (RFN) and pH_{48} <5.3 then the meat was PSE. In our study, pH_{48} of two genotypes ranged 5.45-5.46, and then the meat was classified to RFN.

Meat color

In this study, the color was not affected by genotype and increased gradually with the storage times. These findings were similar to previous studies (Mazzucco et al., 2016; Cafferky et al., 2019), who reported that cattle genotype did not affected the meat color when the authors have studied on Charolais, Angus and Hereford, and their crossbreds. However, Setthakul et al. (2008) indicated that the colors of crossbred (Brahman x Thai) and (Charolais × Thai) meat were different. Cuvelier et al. (2006) found that the value L* at 48hrs was highest in Blanc-Blue-Belgium beef (L* = 41.9) then Limousin beef (L* = 39.7) and lowest in Angus meat (L* = 37.4). According to Honikel (1998), the value L* ranged 35-40 then the beef was a normal, L*= 28 then the beef was dark meat. The value L* in this study ranged 35.5-41.3, therefore the meat of two genotypes crossbred (Brahman x) Lai Sind) and (Charolais x Lai Sind) was a normal. On the other hand, Muchemje et al. (2009) recommended the value L* 37-40.7 for dark meat, and then beef of two genotypes in this study was a dark. However, differences in L* at 48 hours and 336 hours in our study between two cattle genotypes did not clearly understand the reasons. According to Rooyen et al. (2017), the value a* = 12 was considered as the minimum threshold

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for meat to be accepted by consumers. The results of our study show that the values a* of meat of two genotypes crossbreds (Brahman × Lai Sind) and (Charolais × Lai Sind) at all storage times were greater than the minimum threshold value. Therefore, the meats of crossbreds (Brahman x Lai Sind) and (Charolais × Lai Sind) in our study were within the acceptable limits for consumers.

Drip loss and cooking loss

The drip loss was affected by cattle genotype and by storage times in this study. The drip loss at 48 hours in our study was 1.61-2.15% and at 336 hours 4.66-5.01%. This finding was similar in previous studies of Linh et al. (2022), who reported that the drip loss at 24 hours of crossbred (Droughmaster × Lai Brahman) meat was higher than crossbreds (Charolais × Lai Brahman) and (Angus x Lai Brahman) meat. However, Hai et al. (2022) found no difference in the drip loss of three genotypes crossbreds AB, DB and CB meat. The drip losses at 48 hours and 192 hours of AB, DB and CB were 3.78, 5.1 and 4.01% at 48 hours, and 4.99, 6.17 and 5.84% at 192 hours, respectively. According to Traore et al. (2012), the drip loss at 48 hours after slaughter could be classified as follows: low drip loss was < 2.6%, average drip loss was 2.6 to 4.0% and as high as >4.0%. According to this classification, the meat of two genotypes in our study belongs to the group of meat with a low drip loss. As the drip loss, the cooking loss also was affected by cattle genotype and storage times in this study. The cooking loss at 48 hours were higher in BL meat than in AL meat (29.79% vs 28.79%). This finding was agreement in previous study of Hue et al. (2008), who reported that the cooking losses of the meat were affected by genotype. The cooking loss at 48 hours of Lai Sind meat (31.48%) and crossbred (Brahman x Lai Sind) meat (33.49%) was higher than (Charolais × Lai Sind) meat (27.66%). However, the finding in our recent study was not similar to the findings of some previous studies (Linh et al., 2022; Hai et al., 2022). Those authors studied meat quality of some cattle crossbreds such as CB, AB, DB and concluded that the cooking loss did not affected by cattle genotype. Linh et al. (2022) reported that the cooking loss at 48 hours of CB, DB and AB meats were not different and were 28.9, 29.6 and 29.3%, respectively. Similarly, Hai et al. (2022) reported also the cooking loss at 48 hours of CB, DB and AB were 29.14, 30.42 and 28.21%, respectively.

Tenderness

Tenderness was an important parameter that determines the quality of meat. Tenderness was the human perception when biting and chewing meat. The cutting force of meat depended on many factors such as: breed, age of slaughter, feeding method, time and method of meat preservation. Tenderness was a key quality characteristic that was highly correlated with general consumer acceptance of beef.

In this study, the tenderness was genetically affected by cattle genotype and the value of WBSF of AL beef was lower than that of BL meat at all storage times ($p<0.05$). This finding was similar to the results of Hue et al. (2008), who reported that the tenderness at 48 hours of crossbred (Charolais x Lai Sind) beef was lower than that of Lai Sind meat and crossbred (Brahman x Lai Sind) meat. Some authors (Luc et al., 2009; Machado et al., 2015) found that *Bos taurus* meat often have less tenderness than *Bos indicus* meat. In this study, as above-mentioned Red Angus originated from *Bos taurus*, while Brahman was *Bos indicus*. However, some studies found no effect of cattle genotype on the tenderness of three crossbreds CB, DB and AB meats (Linh et al., 2022; Hai et al., 2022). The authors reported that the tenderness values at 48 hours of CB, DB and AB meats were 80.9-82.9, 83.77-90.0 and 79.5-81.5 N, respectively. During storage, the tenderness of beef increased gradually and reached a maximum at 48 hours after slaughter, and decreased gradually with storage times in our recent study. These findings were agreement in some present studies (Hai et al., 2022; Linh et al., 2022). Shackelford et al. (1997) classified the tenderness of beef cattle meat into 3 categories based on the value of WBSF at 40 hours: “tender” with shear force <6 kg, “medium” 6 to 9 kg and “tough” >9 kg . Thus, the meat of crossbreds (Brahman x Lai Sind) and (Red Angus x Lai Sind) in our study belongs to the beef category of medium tenderness.

CONCLUSIONS

In present study, the carcass traits such as the carcass dressing, meat percentage and loin area were affected by cattle genotype, and these values were higher in crossbred (Red Angus x Lai Sind) bulls than those in (Brahman x Lai Sind) bulls at 24 months of age. As meat quality, the values of pH and the color of the meat were not affected by cattle genotype but affected by time storage. However, the drip loss, the cooking loss and tenderness were affected by cattle genotype. In term of these indicators, the meat of crossbred (Red Angus x Lai Sind) bulls has higher quality than that of crossbred (Brahman x Lai Sind). In summary, crossbred (Red Angus x Lai Sind) bulls have better the carcass characteristics and meat quality than crossbred (Brahman x Lai Sind) bulls.

DECLARATION

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Author contributions

N.T.K. CHI, and P.T. HUE conceived and designed the experiments; P.T. HUE, N.T.K. CHI, and T.Q. HANH performed the experiments; N.T.K. CHI, P.T. HUE, and L.D. NGOAN analysed the data, N.T.K. CHI, and P.T. HUE, T.Q. HANH, L.D. NGOAN wrote the paper; all authors reviewed and approved the final manuscript.

Conflict of interest

The authors declared no conflict of interest.

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PHYLOGENETIC IDENTIFICATION OF *Anaplasma phagocytophilum* IN HORSES IN BAGHDAD, IRAQ

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study aimed to detect *Anaplasma phagocytophilum* in horses through hematological and molecular tests. The 16S rRNA gene of the *Anaplasma phagocytophilum* parasite was amplified by polymerase chain reaction (PCR), then sequenced, and subjected to phylogenetic analysis to explore "Equine Granulocytic Anaplasmosis" (EGA) infection in three important gathering race horses areas in Baghdad governorate, Iraq. Blood samples were obtained from 160 horses of varying ages, three breeds, and both sexes, between January and December 2021. Prevalence and risk variables for anaplasmosis were analyzed using statistical odds ratio and chi-square tests. Results demonstrated that clinical anaplasmosis symptoms comprised jaundice, weight loss, paleness of mucus membrane with petechial hemorrhage in the third elides, and edema in extremities; There was no tick infestation. The hematological test did not significantly reveal decreases in red and white blood cells and platelet count. Microscopically found 11 from 160 smears (6.88%) had morulae within granulocytes, PCR results of *Anaplasma spp* primers was 32 positive amplicons (20%), and molecular sequencing results of "16S ribosomal RNA genes" confirmed 21 horses (13.13%) infected by *Anaplasma phagocytophilum* for the first time in Iraq horses. The results of the phylogenetic analysis revealed compatibility values similarity 98.81-99.76% with worldwide isolates. Mares occurred not significantly riskier; also age and breed were not illustrated risks of any group. This study is the first molecular detection of *Anaplasma phagocytophilum* in racehorses reared in Baghdad in Iraq. The outcomes of this study provide genetic data for early identification of *Anaplasma phagocytophilum* infection, treatment, and management of the illness in Iraq horses, as well as monitoring its transmission to the human population.

Keywords: *Anaplasma phagocytophilum*, Genetic, Mares, Phylogenetic analysis, Sequencing.

INTRODUCTION

Horses (*Equus caballus*) are vulnerable to tick-borne infections, the most serious of which is *Anaplasma phagocytophilum*-induced "Equine Granulocytic Anaplasmosis (EGA)"(Hurtado et al., 2020; Laamari et al., 2020). It is a zoonotic illness that is responsible for "human granulocytic anaplasmosis"(Oğuz and Değer, 2021). It also infects equines, canines, and cattle (Hamidinejat et al., 2019), "A small ruminant Fever" (goats and sheep) (Aktaş et al., 2021), a wild deer (Kawahara et al., 2006), and rodents (Noaman, 2019). Many studies in Iraq diagnosed anaplasmosis by clinical, hematological and molecular methods, it has been found in sheep (Hamzah and Hasso, 2019), dogs (Al –Obaidi et al., 2020), as well as horses in Mosul city (Albadrani and Al-Iraqi, 2019), EGA in Baghdad city horses has not been confirmed via molecular or phylogenetic approaches.

Over a 28-year period, Saleem et al. (2018a) mapped the worldwide distribution of EGA and determined endemic zones. PCR technique confirmed in the united states of America (Subbiah et al., 2021; Thompson et al., 2022), in Europe (Dziegiel et al., 2013; Janzén et al., 2019), in Turkey (Oğuz, 2021), and in Iran (Noaman, 2019). Globally, ticks infest a wide variety of animal species and spread numerous illnesses, such as EGA (Narankhajid et al., 2018). Alali et al. (2022) documented distributed ticks in Iraq that transport different diseases including anaplasmosis. *Anaplasma phagocytophilum* is transmitted by Ixodidae hard ticks and proliferates in tick tissues and salivary glands (Boulanger et al., 2019). The bacterium travels transstadially but not transovarially, allowing transfer to vertebrate hosts via biting (Noaman, 2019). In animals' blood, it predominantly targets granulocytes, mainly neutrophils and eosinophils (Nelson et al., 2020), and forms morulae inside those cells, which may be utilized as a diagnostic tool for EGA identification, but "polymerase chain reaction" is the gold standard (Saleem et al., 2018b). Transplacental transmission in horses was documented (Dixon and Bedenice, 2021).

The novel species *Anaplasma phagocytophilum* comprises the old species "*Ehrlichia phagocytophila*, *E. equi*, and the pathogen of Human Granulocytic Ehrlichiosis (HGE)" (Dumler et al., 2001). It is a strict gram-negative, intracellular bacteria, and the microorganism is biologically linked to Rickettsia (Guzman et al., 2022).

"Equine Granulocytic Anaplasmosis" is generally a transitory illness (Shost et al., 2021), and Ordinarily in horses, self-limitation (Salvagni et al., 2010), Clinical symptoms differ from horse to horse, with the acute cases displaying pyrexia, depression, lethargy, inappetence (Sim et al., 2017), eyelid petechial hemorrhages, icterus, ventral edema, and

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death in severe cases (Salvagni et al., 2010), and less frequently, associated neurological manifestations are seen as ataxia, stiffness, and lying down (Gussmann et al., 2014; Nowicka et al., 2022). Hematological testing revealed thrombocytopenia, anemia (Albadrani and Al-Iraqi, 2019), and leukopenia (lymphopenia and neutropenia) can put the animal at risk of developing further infections (Saleem, et al., 2018a).

There has been a lack of research on these illnesses in horses, therefore only little is known regarding their occurrence (Tsachev et al., 2019), and the zoonotic feature of it make needs to monitoring of equestrian groups (Ebani, 2019), especially the duration of equine parasitemia averages just 129 days (Costa et al., 2021), as well as the diagnosis *Anaplasma phagocytophilum* in horses are required for the development of successful disease control strategies to avoid illness spread and possibly transfer to people (Seo et al., 2018). The purpose of this research would be to use nucleotide sequencing and phylogenetic analysis to investigate how prevalent *Anaplasma phagocytophilum* is in Baghdad horses.

MATERIALS AND METHODS

Ethical approval

Approval for this study was obtained from the committee of College of Veterinary Medicine/ University of Baghdad, Iraq. Number 39/PG on 7/1/2021.

Animal groups and clinical examination

In the period between January and December of 2021 (with the exception of May and June, due to the coronavirus situation), 160 racehorses (60 Arabian, 75 Crossbreds, and 25 Thoroughbreds) were examined at three important equestrian facilities in Baghdad city. Iraqi Equestrian School, Alzwraa Zoo, and Alameria Equestrian Club. There were approximately ninety-one mares and sixty-nine stallions in the study, with an estimated age range of 2–25 years (Khazaeel et al., 2022). The clinical signs of animals were recorded, respiratory and heart rate, and rectal temperature, with other signs, were checked on each horse.

Samples

Blood samples from studied horses were taken from the jugular vein and divided between a couple of three milliliters vacutainer tubes containing EDTA anticoagulant; one tube was utilized for Microscopic examination on slides, hematological investigation, and the other tube was stored in a freezer for PCR technique.

Microscopic examination

Microscopic observation of blood films for detection of *A. phagocytophilum* morulae inside granulocytes was done according to Kerr (2008).

Molecular genetic assay

To isolate genomic DNA from horse blood, a kit called "(Promega, USA, ReliaPrepTM Blood gDNA Miniprep System)" was used (Voytas, 2001; Badawi and Yousif, 2020). Purity and concentration of DNA measured using a NanoDrop (made by "Thermo Fisher Scientific Corporation, USA") were found to be between 1.6 and 1.9 at 260/280 nm following the manufacturer's recommendations (Desjardins and Conklin, 2010).

PCR protocol

For polymerase chain reaction, researchers employed a pair of primers derived from "the 16S ribosomal RNA genes" were used for the detection of *Anaplasma spp* in length 1469 bp that are EC9 forward "5' TACCTTGTTACGACTT 3'" and EC12 reverse "5' TGATCCTGGCTCAGAACGAACG 3'"(Kawahara et al., 2006).

Guidelines from Promega Corporation (2018) state that the volume of PCR reaction 25µl should contain 12.5µl of "(Promega, USA, 2X GoTaq® G2 Green Master Mix) that consist of Buffer (pH 8.5), 3 mM MgCl2, a mixture of 400 M each of dATP, dTTP, dCTP, and dGTP, and 50 units/mL of Taq DNA polymerase", with 7.5µl of nuclease-free water, Three microliters of template DNA, one micro-liter of each *Anaplasma spp* primers previously described in 10 pmol concentration.

A thermocycling protocol begins with initial denaturation with a ten-minute heat-up to 94°C, followed by forty cycles (denaturation via forty seconds heat-ups at 94°C and annealing at 51.5°C, extension for thirty seconds heat-ups at 72°C, final extension with a ten-minute of 72°C), and holding at 4°C for a few hours. After being electrophoresed on agarose gel (1.5% concentration) using "(Promega, USA, DiamondTM Nucleic Acid Dye)", amplified DNA fragments were most easily seen under UV light (Green and Sambrook, 2019).

Sequencing, phylogenetic analyses

The 16S rRNA gene of *Anaplasma* spp was sequenced by (Macrogen lab, Korea) using EC9 and EC12 primers; the results were analyzed using BLAST of "the NCBI database [http://www.ncbi.nlm.nih.gov/BLAST]" (Hall, 1999), and recorded in international Gene bank as Iraqi reference attainment number. The "Molecular Evolutionary Genetics Analysis (MEGA 11)" program was used to construct phylogenetic trees, It was then contrasted with information on the same gene derived from sequencing projects throughout the world (Tamura et al., 2013)

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Hematological examinations

Includes leukocytes count, the estimate of thrombocytes, and, the counting of erythrocytes, measuring hemoglobin and hematocrit, and assessing anemia morphologically (based on the Wintrobe Index) (Walton and Lawson, 2021).

Analytical statistics

Significant Results (at levels p: 0.05) suggest a possible risk of infection, as determined by statistical analysis using the application "Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Chicago, USA)" (Bluman, 2012).

RESULTS

Microscopic examination

Microscopic examination revealed *A. phagocytophilum* morulae inside granulocytes were observed microscopically in eleven horses (6.875%) mostly in neutrophils as large rounded intra-cytoplasmic inclusion bodies after Romanowsky_stained blood smears (Figure 1).

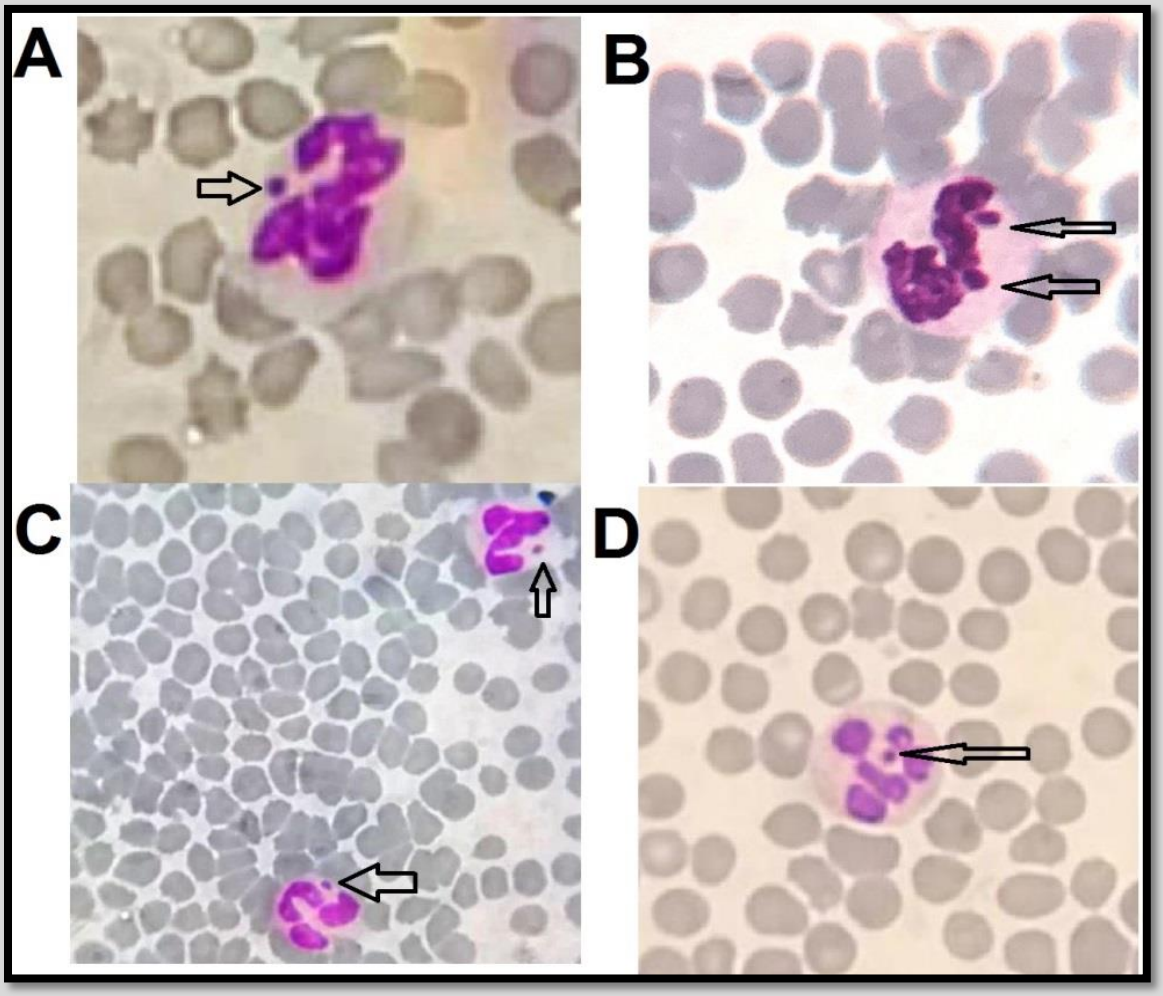

Figure 1- Horse blood with Intra-cytoplasmic inclusion bodies of *Anaplasma phagocytophilum*. A, C, and D: single large rounded (morulae) inside neutrophils, B: double large rounded (morulae) inside neutrophils. X100, Romanowsky_stained blood smears.

Molecular study

The first stage of the Molecular study depend on "the 16S ribosomal RNA genes" PCR results of *Anaplasma spp* primers, it recorded 32 positive amplicons (20%) for *Anaplasma* species from 160 studying horse blood samples (Figure 2). Then sequenced for "16S ribosomal RNA" genes and BLAST results to recorded in "the National Center for Biotechnology Information (NCBI) Gene bank" which confirmed only 21 samples (13.125%) had positive *Anaplasma phagocytophilum* for the first time in horses in Baghdad, under accessions numbers: ON872233.1 to ON872235.1, ON872238.1 to ON872241.1, ON872243.1, ON872246.1 to ON872248.1, and OP218075.1 to OP218086.1. There was no significant association between microscopic and molecular results, chi-square(X2) =3.472222; df = 1; P value = 0.062407; (P ≤ 0.05). The phylogenetic tree in **figure 3** showed these isolates had the highest similarity 98.81-99.76% sequence identity to isolates from Japan, China, Taiwan, dogs in Iraq, Belarus, Estonia, South Africa, Norway, Turkey, Austria, Russia, Sweden, South Korea, and Croatia, with 99-100% site coverage.

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Clinical signs of affected horse with *A. phagocytophilum*

The clinical symptoms of 21 affected horses were documented in Table 1 such as jaundice as the major signs occurred, Weight loss, paleness of mucus membrane with petechial hemorrhage in the third eyelids Figure 4, and edema in extremities. All horses revealed normal temperature ranged from 37.1 - 38.3Co on average 37.66Co, Nonetheless, there was a remarkable rise in the means of heart rate (40.05) (30-54 beats per minute), and respiratory rate was 17.9 (14-30 breath per minute).

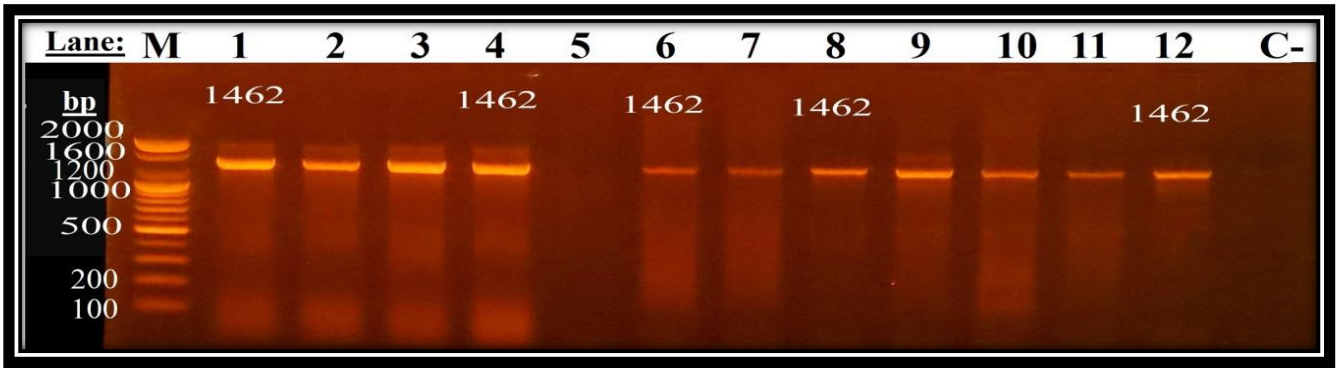

Figure 2- Agarose gel cast loaded with PCR products and screened amplicons of *Anaplasma spp* 16S ribosomal RNA genes, at 1462bp, Stained by "DiamondTM Nucleic Acid Dye", isolated from horses blood. Lane M: ladder 100 - 2000 bp, Lane 1-4 and 6-12: Positives amplicons of *Anaplasma spp*, Lane C-: control Negative, and Lane 5: Negative sample.

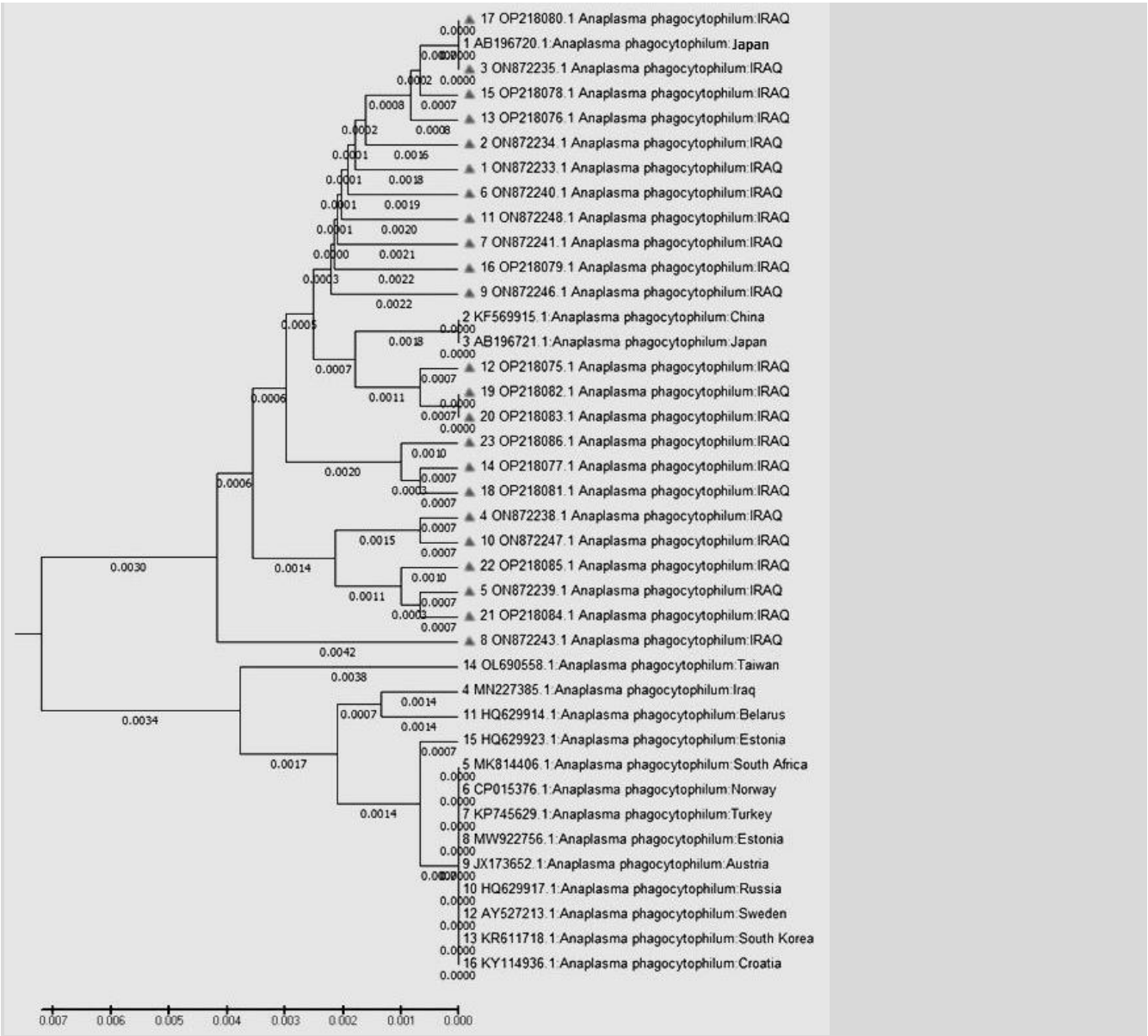

Figure 3 - Phylogenetic Scheme build with partial sequences of *Anaplasma phagocytophilum* isolates from "16S ribosomal RNA" genes. Horses Samples of Iraqi in this study marked by Gray Triangles.

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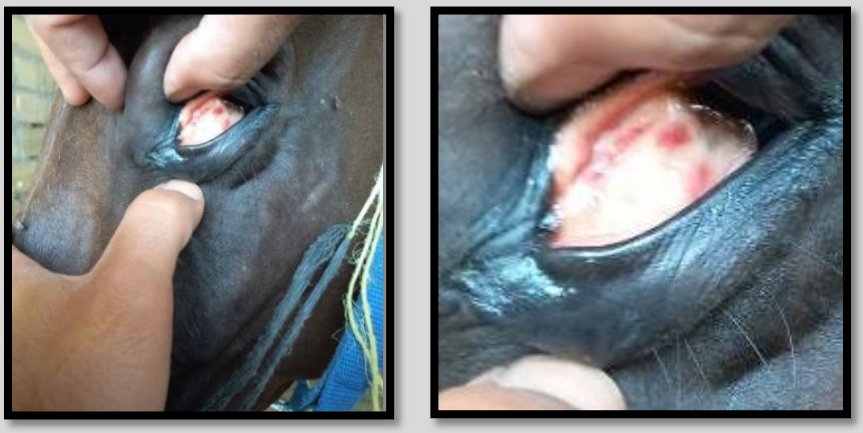

Figure 4 - Horse suffered paleness of mucus membrane with petechial hemorrhage in the third eyelids.

Heamatological study

Table 2 showed non-significant differences between three parameters red, white blood cells, and platelets count in the 21 infected horses with *A. phagocytophilum* in comparison to 139 non-affected horses. Anemia occurred in five of the 21 horses infected with *Anaplasma phagocytophilum*, and was classified as (one macrocytic hypochromic and four Normocytic normochromic), leukopenia occurred in one case, and no thrombocytopenia was discovered.

Rate of infection according to predisposing factors

Anaplasma phagocytophilum infection demonstrated predisposing factors in Baghdad racehorses Table 3. Mares are more infected but without significant differences (1.2708 odd ratios) than stallions 13 and 8, respectively. Arabian and crossbred horses had equal susceptibility (13.33%) to infection and a non-significant (1.1282 odds ratio) than Thoroughbred horses (12%). Four years old horses had the highest percentage of infection (18.75%) and four folded (4.8462 odds ratio) than young horses in their second year, who had the lowest percentage of infection (4.55%), but were statistically insignificant.

Table 1 - Clinical singes related to infection with *Anaplasma phagocytophilum*

Clinical singes	Affected horses with *Anaplasma phagocytophilum* (21) (No.) %		Clinical singes	Affected horses with *Anaplasma phagocytophilum* (21) (No.) %	
Extremities edema	(1)	4.76%	Petechial hemorrhage in 3rd eyelid	(2)	9.52%
jaundice	(6)	28.57%	Wight loss	(3)	14.29%
Pale Mucus Membrane	(2)	9.52%	Poor performance	(1)	4.76%

Table 2 - Erythrocytes, leukocytes, and thrombocytes counts in molecular positive and molecular negative horses.

Parameters / Values in	Horses affected with *Anaplasma phagocytophilum* (21)	non affected horses (139)
RBCs count (1012/L)	8.89+0.475 (4.9 – 12.9)	9.182 +0.175 (4.99 - 13.41)
Platelets count (109/L)	260.05+15.324 (145 - 389)	278.91+7.071 (128 - 642)
Total WBC count (109\L)	10.498 +0.649 (5.25 – 17.3)	11.17 +0.294 (5 – 25.4)

Table 3 - Predisposition of race horses breeds, sex, and age to infected by *Anaplasma phagocytophilum.*

Factor		Total Horses (160)	Number of infected horses (21)	%	(Odds Ratio) Confidence interval 95%
Sex	Stallions	69	8	11.59%	(1.2708) 0.4953 to 3.2608
	Mares	91	13	14.29%	
Breed	Arabian Horses	60	8	13.33%	(1.1282) 0.2844 to 4.4748
	Thoroughbred	25	3	12 %	
	Crossbreds	75	10	13.33%	
Age	2 years	22	1	4.55%	(4.8462) 0.5403 to 43.464
	3 years	24	4	16.67%	
	4 years	32	6	18.75%	
	5 years	25	3	12%	
	6 -10 years	34	5	14.71%	
	<11 years	23	2	8.7%	

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DISCUSSION

Microscopic examination of Romanowsky-stained blood smears from 11 horses (6.88%) revealed the presence of *Anaplasma phagocytophilum* morulae within granulocytes (often in neutrophils) as large, round inclusion bodies inside the cytoplasm. It was equivalent to the results of 7.26% in the Europe study and 0% morulae in Chile (Dziegiel et al., 2013; Hurtado et al., 2020), in contrast, a higher percentage of 28.9% in Mosul City Iraq (Albadrani and Al-Iraqi, 2019). First time in horses in Baghdad, Sequenced the positive 16S rRNA gene samples and BLAST it in the NCBI database to confirmed the detection of *Anaplasma phagocytophilum* in horses in Iraq, with (99-100% site coverage) 98.81-99.76% identity to (AB196721) Japan (Kawahara et al., 2006), (KF569915) China, (OL690558) Taiwan, (MN227385) dogs in Iraq (Al –Obaidi et al., 2020), (HQ629914) Belarus (Katargina et al., 2012), (MW922756) Estonia (Vikentjeva et al., 2021), (MK814406) South Africa (Kolo et al., 2020), (CP015376) Norway (Crosby et al., 2022), (KP745629) Turkey (Aktaş and Özübek, 2015), (JX173652) Austria (Dyachenko et al., 2013), (HQ629917) Russia (Katargina et al., 2012), (AY527213) Sweden (Franzén et al., 2005), (KR611718) South Korea (Oh et al., 2012), (KY114936) Croatia (Huber et al., 2017) isolates.

Microscopic findings of *Anaplasma phagocytophilum* were around half times as confirmed by molecular sequencing results (21 horses, 13.13%) but this was not statistically significant, this molecular dominant agreed with 17.9% in the Europe study and 13.6% in Chile (Dziegiel et al., 2013; Hurtado et al., 2020), Baghdad's sequencing results nearly resampled other study results as 13% in Tunisia (Mghirbi et al., 2012), 7% in Pennsylvania (Thompson et al., 2022), 6.4% in Turkey (Oğuz, 2021), but higher than 1.8% in Poland (Teodorowski et al., 2021), and lower than 45% in Brazil (Salvagni et al., 2010). This illustrated the stability of molecular technique results that make it a better tool to diagnose *Anaplasma phagocytophilum* and disparity in microscopic results may due to differences in laboratory protocols and/or the presence of morulae related to another nonpathogenic *Anaplasma spp*.

This study of 21 molecular-positive horses for *A. phagocytophilum* revealed different symptoms, jaundice, and emaciation, only two cases had pale and petechial hemorrhages in 3rd eyelids, non-significant increased heart rates, nonspecific singes or asymptomatic infections this resemble the results of (Fielding et al., 2018) and (Ebani, 2019), on other hand affected horses with Anaplasmosis located in horses in Mosul city Iraq revealed fever, depression, and ataxia in addition to the signs (Albadrani and Al-Iraqi, 2019), and the same signs in horses located in united states (Subbiah et al., 2021). Ticks infestation was recorded in most research but not detected in these molecular-positive horses, this agreed with some studies in Algeria, Chile and Turkey (Hurtado et al., 2020; Laamari et al., 2020; Oğuz, 2021), the intense care with racehorses as daily washing and grooming may lead to the disappearance of a tick, and periodic medical checks prevent the development of severe signs in this investigation.

Hematological data demonstrated that 21 Molecular Positive *Anaplasma phagocytophilum* horses had a low average of Red, White, and platelet cell counts, with no significant changes compared to 139 Molecular Negative horses, One horse had macrocytic hypochromic anemia, whereas four horses had normocytic normochromic anemia; one had leukopenia, and none had thrombocytopenia. Similar CBC count average results were recorded in horses affected with *Anaplasma phagocytophilum* in Chile and Pakistan (Saleem, et al., 2018b; Hurtado et al., 2020), in divergence Mosul horses established anemia; leukopenia and thrombocytopenia (Albadrani and Al-Iraqi, 2019), in addition, Croatia (Gotić et al., 2017), Others found leukopenia and thrombocytopenia (Deane et al., 2021) (Fielding et al., 2018), and only leukopenia was detected in other research (Razzaq et al., 2015). Most positive cases revealed no specific clinical singes or remarkable hematological abnormality with a complete absence ticks infestation that indicated asymptomatic or transient or recovered from mild infection.

Risk factors not revealed any significant differences between mares and stallions, and between Arabian and thoroughbred or crossbred horses, in addition, any horse ages group. This outcome is parallel with other's (Mghirbi et al., 2012; Razzaq et al., 2015; dos Santos et al., 2019; Laamari et al., 2020; Drážovská et al., 2021; Oğuz, 2021), the females occurred slight more susceptible that disagreed with Pakistan research showed significant increase in males (Saleem, et al., 2018b).

CONCLUSION

This study is the first molecular detection of *Anaplasma phagocytophilum* in racehorses reared in Baghdad in Iraq. The clinical signs were observed and found vital signs within reference limits and jaundice which were the major signs that occurred in 28.57% of molecular-positive horses. The results provide evidence that a large number of horses are exposed to *A. phagocytophilum* and that this bacterium is present in this area. Examined blood smears microscopically which revealed 6.875% *Anaplasma phagocytophilum* morulae inside granulocytes mostly in neutrophils as large rounded intra-cytoplasmic inclusion bodies. Hemtologically analyzed blood samples and illustrated parameters of red, white blood cells, and platelets count were non-significant differences in the 21 molecularly infected horses and anemia occurred in five cases. Susceptible to infection occurred higher in Mares at 14.29%, 4 years old at 18.75% and Arabian breed at 13.33%. PCR results were 21 samples (13.125%) had positive *Anaplasma phagocytophilum*, and the recording Phylogenetic tree had the highest similarity 98.81-99.76% to global isolates. Compatibility of our outcomes to global isolates with similar prevalence rates will facilitate applying universal protocols of control and treatment trials and using PCR techniques for early detection of infection, and management of the illness in horses in Iraq, as well as monitoring its transmission to the human population.

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DECLARATIONS

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Authors' contribution
All authors have seen and confirmed the authenticity of all the raw data and contributed equally to the details of this manuscript.

Conflict of interests
The authors have not declared any conflict of interests.

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Consent to publish
All authors agree to publish the article "Phylogenetic Identification of *Anaplasma phagocytophilum* in Horses in Baghdad, Iraq" in an online journal of animal and feed research.

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VARIABILITY IN PROXIMATE AND MINERAL COMPOSITIONS OF YOLK AND ALBUMEN IN EGGS KEPT UNDER DIFFERENT STORAGE CONDITIONS

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This investigation evaluated the effects of storage length and temperature on the proximate and mineral compositions of yolk and albumen (white) of chicken eggs. A total of 720 eggs were used in a 4 X 2 factorial experiment consisting of four (0, 7, 14 and 21) storage days and two (room and cold) storage temperatures. Data obtained were subjected to ANOVA. The results showed no significant effect of storage length on crude protein and ash contents of the egg parts whereas carbohydrate increased significantly with increasing storage length. Also, protein and fat contents of the yolk were largely influenced ($p<0.05$) by storage temperature but nutrients in the albumen did not differ significantly between the two storage temperatures. On the other hand, mineral compositions of the egg components did not vary noticeably by storage temperature but storage length influenced some minerals considerably. It was noted that variations in proximate and mineral contents of egg yolk and albumen under different storage conditions exist; so, storage conditions (length and temperature) must be given much attention when keeping eggs prior to consumption in order to ensure nutrient quality.

Keywords: Egg quality, Egg protein, Nutrients, Storage length, Storage temperature.

INTRODUCTION

Chicken eggs are superior in carbohydrates, proteins, easily digestible fats, ash (minerals) and vitamins (Huopalahti et al., 2007; International Egg Foundation, 2014); thus, eggs are one of the utmost important sources of animal protein that provide good nutrition for human and animals at the least cost (Menezes et al., 2009). They are also treasured as raw materials for making curative and beautifying (cosmetic) products per their gelling, foaming, emulsifying (Matt et al., 2009) and thickening abilities – which however are largely determined by their exterior and interior properties (Koelkebeck, 2003). Egg quality is composed of traits such as: cleanliness, weight, shell quality, freshness, yolk and albumen indexes, Haugh unit and chemical composition that influence its acceptability to consumers (Song et al., 2002).

Generally, the biological/genetic composition (Rizzi and Marangon, 2012; Aygun and Yetişir, 2014; Jahedi et al., 2020), age (Rizzi and Marangon, 2012; Aygün and Narinç, 2017) and management (King'ori, 2012; Grochowska et al., 2019) of layers in addition to the conditions of egg storage (time, temperature, humidity, air movement and handling) are common factors associated with the level of quality loss of the egg (Stadelman et al., 1995; Oliveira and Oliveira, 2013). Nys et al. (2004) reported that, eggs display very consistent composition with respect to their nutritional composition but may change at different periods and temperatures of storing them.

For instance, in a trial, eggs collected from various retail shops (which may be at different stages of storage) had crude protein contents of 8.64, 7.98, 7.60 and 7.78 g/egg (Attia et al., 2014). Ogunwole et al. (2015) also noticed highly significant effect of storage time on crude protein content in eggs from Black Bovan nera chickens; eggs stored for 0 day (11.45%) was significantly lower in protein than those stored for 7, 14, 21 and 28 days which was found to be 11.54, 11.60, 11.55 and 11.59% respectively. Comparably, Dudusola (2009) found out that quail eggs stored for day 0 (35.3%) and 4 (33.0%) were similar but significantly higher in crude protein than those stored up to day 7 (30.5%) and 21 (27.4%) and subsequently proposed that, quail eggs should be stored for 4 days at room temperature to maintain desired internal quality. Usually, eggs stored in cold condition for several months above 85% humidity or at any temperature under highly sufficient humidity and long storage time would spoil when exposed to warm temperature because conditions may be favourable for the penetration of micro-organisms (Seidler and Hilmi, 2003). However, packing eggs under modified atmosphere could increase their internal quality up to the 28th day of storage (Giampietro-Ganeco et al., 2015).

Information on the effect of storage temperature on nutritional composition of eggs is non-completed, limited or not readily accessible, whereas Dudusola (2009) suggested that refrigeration at low temperatures is the best storage condition for quail eggs. Freezing subjects microbial activities to dormancy and slows the movement of molecules to keep food safe at a constant temperature of -17.78°C even though food quality may suffer with prolonged freezer storage –

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but the freezing process per se does not destroy nutrients (USDA, 2013; Taylor, 2019). Eggs are best stored at a temperature of about −1°C and 80-85% relative humidity (Seidler and Hilmi, 2003) while cold storage at −1.5°C could keep eggs for 6-9 months (Belitz et al., 2009).

A study had shown that, seafoods frozen or kept in frozen condition certainly lose quality (Mackie, 1993). The loss in quality may be due to changes in muscle integrity, proteins and lipids (Solanki et al., 2011); as cellular disintegration during cold storage can cause acid hydrolysis of lipids to free fatty acids (Mazrouh, 2015).

In tropical countries such as Ghana, eggs collected from layer chickens are kept for several days under temperatures varying from 19-22^{0}C until they are sold out; the eggs are sold to retailers who may resell them in open markets at temperatures ranging from 28-34^{o}C or to consumers who either store them under room temperature or refrigeration at 5^{o}C prior to consumption (Hagan and Eichie, 2019). Despite the facts that the quality of eggs changes under different storage conditions, enough work had not been done to investigate the effects of the various storage conditions on the nutritional composition of eggs – specifically those from the domestic chicken (Bashir et al., 2015) compared to their physical characteristics. Therefore, this research work investigated the variability in nutrient composition of yolk and albumen of chicken eggs stored under different conditions.

MATERIALS AND METHODS

Study area

Eggs used for the study were obtained from the Teaching and Research Farm of the School of Agriculture, University of Cape Coast, Ghana and the experiment was conducted at the Nutrition Laboratory of the same University. The study area has average minimum and maximum temperatures of 21^{o}C and 32^{o}C correspondingly and an annual rainfall of 1300 mm (Kruenti et al., 2022).

Experimental design and data collection

Eggs were collected from a flock of 42 weeks old brown layer chickens fed a standard layer mash made of 18% crude protein and 3200 kcal/kg ME. All essential vaccinations and medications were duly followed. Eggs were randomly collected on the same day between 8:00 – 8:30 am from the farm, examined, cleaned with a dry cloth, packed onto paper crates in a carton and transported to the laboratory within 30 minutes. A 4 × 2 factorial experimental design involving four storage days (0 [D1], 7 [D2], 14 [D3] and 21 [D4]) and two storage temperatures (room temperature [T1]: 21-32^{o}C and cold temperature [T2]: 5^{o}C) was used. Seven hundred and twenty eggs were randomly selected and divided into eight treatment groups, with 90 eggs in each treatment; each treatment had three replicates (R1, R2 and R3) of 30 eggs. The treatment structure is as follows: treatment 1 and 2 (D1T1R1, D1T1R2, D1T1R3 and D1T2R1, D1T2R2, D1T2R3), 3 and 4 (D2T1R1, D2T1R2, D2T1R3 and D2T2R1, D2T2R2, D2T2R3), 5 and 6 (D3T1R1, D3T1R2, D3T1R3 and D3T2R1, D3T2R2, D3T2R3), 7 and 8 (D4T1R1, D4T1R2, D4T1R3 and D4T2R1, D4T2R2, D4T2R3) respectively and identified with permanent marker accordingly. Treatments 1, 3, 5 and 7 were stored under room temperature (21-32^{o}C) for the respective days whereas 2, 4, 6 and 8 were stored in a LOGIK CB BC-90 Fridge at 5^{o}C (Hagan and Eichie, 2019). Analysis was done on storage days 0, 7, 14 and 21. Before analysis was carried out, the eggs were broken onto a petri dish using a scalpel and the yolk entirely separated from the white (albumen) using a plastic yolk separator. Extra eggs stored for each treatment, replaced eggs that did not have yolk well separated from the albumen. All yolks as well as albumen per storage condition were poured into a clean and sterilized beaker each, labelled and centrifuged to homogenize. Proximate analysis was done by the methods of the Association of Official Analytical Chemists (AOAC, 2012). Mineral elements (phosphorous, calcium, potassium, sodium, iron, copper and zinc) were also determined by methods of the AOAC (2012). All equipment were sterilized before each experiment; but were also regularly and thoroughly cleaned with distilled water and tissue paper after each experimental group.

Data analysis

Data collected were subjected to ANOVA using the GLM procedure of Minitab (version 18). Differences in means were separated using the Tukey Pairwise Comparisons Method at 5% level of significance. The model used was:

$Y_{ij} = \mu + SD_i + ST_j + \varepsilon_{ij}$

Where: Y_{ij} = the dependent variable; μ = the general mean; SD_i = ith observation of storage day; ST_j = jth observation of storage temperature; ε_{ij} = the random error associated with the dependent variable.

RESULTS AND DISCUSSION

Effects of storage day on proximate composition of egg yolk and albumen

Table 1 shows effects of storage day on proximate composition of egg yolk and white (albumen). There was no significant ($p>0.05$) effect of storage length on crude protein and ash content of the egg parts. According to Réhault-Godbert et al. (2019), storage duration and conditions are associated with protein degradation, which corroborates the insignificant incessant declination in yolk and albumen protein with advancement in storage day as observed in the current work. The marginal changes detected in the protein content of the egg components are in agreement with

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Dudusola (2009) who had found significant differences in the proximate composition at various days of keeping quail eggs except for protein. The significant ($p<0.05$) reduction in yolk fat with increasing storage time and that of the albumen fat at day 14 may be due to lipid oxidation and degradation. This reduction in the fat content of yolk and albumen confirms the results reported by Ogunwole et al. (2015), Ebegbulem and Asukwo (2018) and Luo et al. (2020) who found lipid content of eggs to decrease though insignificantly as storage length increased. These findings support the idea that egg if needed, must be kept, for a short time to maintain its protein and fat at optimum level (Ogunwole et al., 2015; Ebegbulem and Asukwo, 2018). However, the continual reduction detected in yolk fat in the current work is an indication that prolonged storage could be used to reduce the high fat content of egg yolk for people who need low fat. On the other hand, ash content would not change between fresh and stored eggs which diverges from the non-substantial increases in ash content of eggs kept at the respective storage days as informed (Ebegbulem and Asukwo, 2018). The outcome of the present research indicates that carbohydrate composition of the chicken egg parts would increase with increasing storage length up to the 21st day, which could be due to the loosening/permeability of the vitelline membrane to glucose as storage days increase, thereby facilitating both in vivo and in vitro fixation of free glucose by the yolk and albumen proteins (glycoproteins) and thus increasing carbohydrate content (Réhault-Godbert et al., 2019). This makes stored eggs a better option over fresh eggs in making 'energy remedies' for people during emergencies. In addition, the variations seen in the proximate composition of the egg components during the storage periods confirm various works (Stadelman et al., 1995; Rizzi and Marangon, 2012) that as eggs age, their nutritional composition is affected. Nevertheless, the results of the current work are comparable to global standard reference values of egg proximate analysis as found in the USA (USDA, 2016) and the UK (Roe et al., 2013; McCance and Widdowson, 2021).

Table 1 - Effects of storage day on proximate composition of egg yolk and white (albumen)

Nutrient Composition (g/100g)		Storage day				p-value
		Day 0	Day 7	Day 14	Day 21	
Egg Yolk	Protein	15.5	15.3	15.3	15.2	0.67
	Fat	29.0^{a}	28.1^{b}	27.6^{c}	27.4^{c}	0.01
	Ash	1.3	1.3	1.3	1.3	0.58
	Carbohydrate	1.6^{b}	2.2^{b}	3.9^{a}	4.3^{a}	0.01
Egg White (Albumen)	Protein	10.7	10.6	10.4	10.3	0.20
	Fat	0.2^{a}	0.2^{a}	0.1^{b}	0.2^{a}	0.03
	Ash	0.8	0.8	0.7	0.8	0.50
	Carbohydrate	0.7^{c}	1.6^{b}	2.4^{ab}	3.3^{a}	0.01

Means in rows with different superscripts are significantly different; p-value: probability value ($p < 0.05$); g/100g: grams per 100 grams (%)

Effects of storage temperature on proximate composition of egg yolk and albumen

Results presented in Table 2 show that storage temperature substantially influenced crude protein and fat contents but not the ash and carbohydrate contents of the egg yolk. On the other hand, content of all studied nutrients did not significantly ($P>0.05$) differ in the egg white (albumen) between the two storage temperatures. Egg had been proclaimed a perishable food that requires cold storage to keep longer and maintain nutrient quality (Menusano, 2018). Again, earlier reports indicate that alterations of freshness criteria are accelerated at room temperature when compared to refrigerated conditions (Réhault-Godbert et al., 2019). Thus, the significantly lower values of protein and fat under the room temperature compared to the cold temperature confirm the fact that protein and lipid degradation is enhanced by room temperature rather than cold temperature. This agrees to the notion that, freezing as a common practice in the meat, fish and other animal protein-based industry, preserves quality for an extended time with minor alterations in the products' dimension (Obuz and Dikeman, 2003). The results also show that generally, there is more protein in egg yolk than the albumen as found under all storage conditions which validates the idea that many people anticipate more protein in egg yolk than the albumen (Kaewmanee et al., 2009). Therefore, consumers are advised to eat the egg yolk rather than the egg white if protein is required. The current data also suggest that people who have health challenges relating to cholesterol should not eat frozen eggs due to the high amount of lipid found in yolk from such eggs.

Table 2 - Effects of storage temperature on proximate composition of egg yolk and white (albumen)

Nutrient comp. (g/100g) / Storage Temp.	Egg Yolk				Egg White (Albumen)			
	Protein	Fat	Ash	CHO	Protein	Fat	Ash	CHO
Room Temp.	15.1^{a}	27.7^{b}	1.3	3.4	10.5	0.2	0.8	2.5
Cold Temp.	15.7^{b}	28.4^{a}	1.3	3.5	10.6	0.2	0.8	2.4
p-value	0.03	0.01	0.94	0.85	0.68	0.11	0.68	0.62

Means in a column with different superscripts are significantly different; Comp.: composition; Temp.: temperature; CHO: carbohydrate; p-value: probability ($p < 0.05$); g/100g: grams per 100 grams (%)

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Effects of storage day on mineral composition of egg yolk and albumen

Table 3 indicates effects of storage day on mineral composition of egg yolk and albumen. The results show no significant (P>0.05) effect of storage day on potassium in egg yolk and egg white as well as on calcium, sodium, copper and zinc contents of egg white. This demonstrates stability of these mineral elements during storage even though there are marginal intermittent changes in their values as storage day increases. Contrariwise, there were magnificent (P<0.05) variations in the phosphorous, calcium, sodium, iron, copper and zinc contents of the yolk as well as the phosphorus and iron composition of the egg white. Eggs are made up of significant amounts of several mineral elements (Gutierrez et al., 1996) that play different roles in the development of the human body. But the amount of the mineral elements in an egg's edible parts can vary at different egg ages (storage periods). High concentration of phosphorous and iron could be contained in the yolk from fresher eggs than stored eggs and this is an indication that, people who are deficient in these minerals should make fresh eggs their choice. On the other hand, storing eggs up to 14 days will yield higher concentrations of phosphorus and iron in egg white (albumen) and the highest concentration of calcium in the egg yolk. Consequently, people especially children who need more calcium for bone and teeth formation should be given food supplements made of yolk obtained from eggs stored up to the 14th day. The high levels of calcium and phosphorus in the egg parts at the various storage days means that, eggs whether fresh or stored up to day 14, are necessary for optimal bone development particularly in children to prevent rickets and osteomalacia (Erkan and Ozden, 2007). The current results support the substantial and irregular levels of elemental calcium in egg yolk reported by Stadelman et al. (1995). Though the amount of potassium in the chicken egg components might not be affected by storage time, eggs stored up to day 7 are most recommended for consumption if potassium is needed. On the other hand, egg storage could go up to day 21 for consumers who need high amount of sodium and copper from the yolk. Concentration of zinc in egg yolk was higher than that of the albumen; indicating that people who need more zinc should prioritize yolk from stored eggs rather than fresh ones. The opinion that potassium and sodium are the major minerals in albumen whiles calcium, potassium and phosphorus are the major elements in yolk (Stadelman et al., 1995; Roe et al., 2013; USDA, 2016; McCance and Widdowson, 2021) is completely supported by the current data. The results of this study thus confirm the fact that, the relative content of egg minerals and other nutrients may vary from one national reference to another but remains globally comparable (Roe et al., 2013).

Table 3 - Effects of storage day on mineral composition of egg yolk and egg white (albumen)

Mineral composition (µg/g)		Storage day				p-value
		Day 0	Day 7	Day 14	Day 21	
Egg Yolk	Phosphorous	1513.8^{a}	1444.3^{b}	1363.9^{c}	1376.4^{c}	0.01
	Calcium	1420.2^{b}	1984.9^{ab}	2424.3^{a}	2330.5^{a}	0.01
	Potassium	1655.3	2449.9	1604.0	1553.8	0.13
	Sodium	439.6^{ab}	455.8^{b}	511.0^{ab}	725.5^{a}	0.03
	Iron	45.1^{b}	43.6^{b}	41.7^{a}	43.9^{b}	0.02
	Copper	1.2^{a}	1.2^{a}	1.7^{a}	2.4^{b}	0.01
	Zinc	29.3^{a}	29.5^{a}	31.1^{b}	34.2^{b}	0.02
Egg White (Albumen)	Phosphorous	113.3^{a}	143.2^{b}	174.6^{b}	135.9^{ab}	0.03
	Calcium	86.3	86.2	85.9	83.5	0.16
	Potassium	1946.8	2188.4	2036.3	2121.4	0.36
	Sodium	1082.3	1209.0	1243.2	1121.7	0.52
	Iron	0.9^{a}	0.7^{a}	1.6^{b}	0.6^{a}	0.01
	Copper	0.4	0.6	0.7	0.5	0.22
	Zinc	0.3	0.2	0.2	0.5	0.11

Means in rows with different superscripts are significantly different; µg/g: microgram per gram; p-value: probability value ($p < 0.05$)

Effects of storage temperature on mineral composition of egg yolk and albumen

The results presented in Table 4 show that, the mineral composition of the domestic chickens' egg would not vary (P>0.05) under the different storage temperatures; implying that the mineral elements studied in the current research are more stable under room and cold temperatures. However, phosphorous, calcium, potassium, iron and copper contents may be numerically more in yolks from eggs stored under room temperature than those stored under cold temperature while the opposite should be expected for sodium and zinc contents of the same egg part. On the other part, phosphorus, calcium, potassium and sodium were numerically more in albumen (egg white) obtained from the refrigerated eggs than those from room temperature. In literature, information on the effects of storage temperature on mineral composition of chicken eggs are not readily available as notified by Bashir et al. (2015) and other works (Stadelman et al., 1995; Réhault-Godbert et al., 2019). Again, the results obtained in this study compares well with reference values in other parts of the world (Stadelman et al., 1995; Roe et al., 2013; USDA, 2016; McCance and Widdowson, 2021).

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Table 4 - Effects of storage temperature on the mineral composition of egg yolk and albumen

Mineral Comp. (µg/g)	P	Ca	K	Na	Fe	Cu	Zn	P	Ca	K	Na	Fe	Cu	Zn
Storage Temp.	Egg Yolk							Egg White (Albumen)						
Room Temp.	1400.6	1357.9	1248.8	531.0	37.4	1.1	30.8	155.1	78.9	1569.4	1795.0	0.8	0.3	0.2
Cold Temp.	1392.1	1255.9	1186.8	599.7	33.8	0.9	32.0	161.2	81.2	1619.3	1827.5	0.6	0.3	0.3
p-value	0.52	0.34	0.10	0.45	0.21	0.10	0.22	0.29	0.32	0.13	0.23	0.15	1.00	0.42

Comp.: composition; Temp.: temperature; P: phosphorous; Ca: calcium; K: potassium; Na: sodium; Fe: iron; Cu: copper; Zn: zinc; µg/g: microgram per gram; p-value: probability ($P < 0.05$)

CONCLUSION

Owing to the fact that storage conditions affect egg quality and the importance of yolk and albumen (white) in the egg processing industry, for products and people that need high protein, eggs should be refrigerated and not stored beyond day 14 but prolonged storage could be used to decrease fat content of eggs for products and people that need low fat. Also, the energy (carbohydrate) level of egg yolk and albumen can increase as storage length increases; therefore, food supplements used as 'energy remedies' should be made from eggs stored up to the 21st day. Potassium in egg yolk and egg white as well as calcium, sodium, copper and zinc contents of egg white are not affected by storage day whereas magnificent variations in the phosphorous, calcium, sodium, iron, copper and zinc contents of the yolk as well as the phosphorus and iron composition of the egg white are expected as storage day advances. Lastly, the aforementioned mineral elements may remain stable under both room and cold temperatures. Generally, the results of this study show that the nutritional composition of egg yolk and albumen may change when eggs are stored up to different storage days and under different temperatures. Subsequently, proper attention must be given to the period of time and temperature under which eggs are kept during distribution and sale by egg sellers or by consumers in order to ensure quality. It should however be noted that, the results of the present research are limited to 42 weeks old brown layer chickens, fed a layer mash of 18% crude protein and 3200 kcal/kg metabolizable energy and thus, the outcomes may not be generalised for other layers of diverse ages that are fed on different diet.

DECLARATIONS

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Availability of data and materials
Not applicable

Authors' contributions
All authors contributed equally to this work.

Competing interests
The authors declare that they have no competing interests.

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EXTRACTING PHYTOCOMPOUNDS FROM *Mucuna pruriens* LEAVES AS POTENTIAL RUMINANT FEED ADDITIVES USING DIFFERENT SOLVENTS

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ABSTRACT: Some secondary metabolites of plants could serve as ruminant feed additives. They primarily preserve protein from rumen breakdown, reduce rumen protozoa population, and decrease methane gas production. The current study aimed to identify the phytocompounds content of extracted *Mucuna pruriens* leaves using the Microwave-assisted extraction method using three different solvents of methanol 70% (EM), aquadest (EA), and combinations of EM and EA (EK). The phytocompounds were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Some phytocompounds identified in the *Mucuna pruriens* substances from GC-MS curve proportion area of EM were 10.35% inositol, 3.1% quinazoline, 4.72% anthraquinone, 3.76% Coptisine, 2.06% isoquinoline, 2.18% D-gluconic acid, 2.83% D-Fructose, 3.91% D-glucose, and 4.59% butanedioic acid. The phytocompounds for EK were 17.22% inositol, 6.36% Niclosamide, 1.4% Acetamide, 1.32% Aniline, 55.97% 4-Amino-2-(4-methoxyphenyl)-5,6,7,8-tetrahydrofuro[2,3-b] quinoline-3-carbonitrile, 17.22% inositol. Furthermore, 22.73% inositol, 6.55%, ribonoic acid, 5.58%, silanol, 21.27% butanodioic acid, 2.88% Fluoroquinoxaline, 5,31%, glycerol, 1,64%, D- gluconic acid were found in the EA. The EA had high inositol content, the EK had high quinoline content, and the EM showed moderate results for all phytobiotics. The total phenolics, flavonoids, tannins, and saponins content significantly differed among the three solvents. The EA yielded the highest concentrations of total phenolics, flavonoids, and tannins, but the lowest concentration of total saponins. In contrast, the EM yielded the lowest total phenolics, flavonoids, and tannins content, but the highest total saponins content. Meanwhile, the EK yielded modest results for all phytocompounds, with values between EA and EM. In conclusion, the methanolic extract of Mucuna pruriens substance had the highest phytocompounds and bioactive potential as ruminant feed additives.

Keywords: Feed additives, Gas chromatography, *Mucuna pruriens*, Phytocompounds, Solvent, Secondary metabolites

INTRODUCTION

Some plants produce bioactive compounds in the form of secondary metabolites. The secondary metabolites, such as saponin (Unnawong et al., 2021), tannins (Patra and Saxena, 2011), flavonoids (Gohlke et al., 2013), and polyphenols, are known to function as rumen modifiers. Studies have indicated that the secondary compounds possess antimicrobial properties against bacteria, fungi, and protozoa, decrease acetate and ammonia concentration, acetate to propionate ratio, and methane production, and increase propionate *in vitro* (Klevenhusen et al., 2011). *Mucuna pruriens*, also known as cowhage or velvet bean, contains active compounds commonly used as components for traditional medicine. For example, seeds of *Mucuna pruriens* contain fairly high L-Dopa, commonly used for managing and treating Parkinson's disease (Lieu et al., 2010) and as antidepressants in cases of depressive neurosis (Rana and Galani, 2014). In animal feeding, the seeds can also be used as a substitute for protein sources (Yantika et al., 2016).

Mucuna pruriens can improve soil fertility and is adapted to the humid or subhumid tropics and commonly used in maize and cotton rotations systems intercropping (Peters et al., 2001), It is also called abonera (fertilized field) and play a role in soil structure improvement, soil protection against erosion, and weed control (Buckles et al., 1998). *Mucuna pruriens* is a multi-purpose legume, particularly for smallholder farmers. In Indonesia, especially in Wonogiri, Central Java, *Mucuna pruriens* seeds are commonly used as raw materials for tempeh making. The product is popularly called koro benguk tempeh, which is a dish. Some Small and Medium Enterprises (UMKM) further process the tempeh to produce tempeh benguk chips (Winami and Dharmawan, 2017). With their nutrient contents, especially antioxidant potential, *Mucuna pruriens* can be effectively used to formulate food products for reducing factors associated with non-transmissible chronic diseases and aimed at meeting general nutritional needs (Encalada and Campos, 2021) and could affect fertility (Daramola et al., 2015).

The development of *Mucuna pruriens* cultivation provides higher access to forage. Besides, being a source of animal feed in the form of fresh or hay, *Mucuna* leaves can also be used for their secondary metabolite content. For this reason, it is necessary to identify metabolites that can be maximally used for livestock productivity, especially the content of compounds that have antimicrobial properties, so that they can maximize the nutrients provided.

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Therefore, the current study aimed to identify the phytocompounds of *Mucuna pruriens* leaves extract with different solvent and their potential for ruminant feed additives.

MATERIALS AND METHODS

Ethical approval

The current study was conducted in accordance with the Animal Care and Use Committee, University of Brawijaya Malang, East Java Indonesia, with ethical clearance number 141-KEP-UB-2022.

Collection of *Mucuna pruriens* leaves

Fresh leaves of *Mucuna pruriens* were collected from farms located in Wajak, Malang, East Java, Indonesia, in October 2021. The leaves were aerated under the shades, dried in an oven at 60°C, milled into a fine powder using a mechanical grinder, and stored in air-tight plastic for further analysis.

The extraction of *Mucuna pruriens* leaves

For extraction, microwave-assisted extraction (MAE) method was used with three different solvents, including methanol 70% (EM), aquadest (EA), and a combination of EA and EM by ratio 1:1 (EK). The MAE was performed using a modification of microwave sharp type R21D0SIN with the power 450 W, voltage 220-240 volt/50 Hz, and the dimension of 52cm × 40.7cm × 32cm in length, width, and height, respectively. The close vessel unit and temperature marker were used to ensure the temperature does not exceed 40°C (Yuan et al., 2018) with modification. As much as 33 g of *Mucuna pruriens* leaves powder was placed in a 250 ml round flask, and 200 ml of solvent based on each treatment (EM, EA, and EK) was added and homogenized. The homogenized samples were extracted in the microwave for 15 minutes with a temperature not exceeding 40°C. The chiller pump was used to flow the coolant liquid in and out the condenser then the flask was attached to a condenser. The extract was then filtered with filter paper (Whatman grade 1). The filtrate was extracted again with 200 ml solvent based on each treatment (EM, EA, and EK). After the extraction process, the volume of filtrate liquid was reduced from the original volume with three times evaporation using MAE for 15 minutes per evaporation process.

Compounds Identification using Gas Chromatography-Mass Spectrometry analysis

Gas Chromatography-Mass Spectrometry (GC-MS) was used as a qualitative identification method for extracting *Mucuna pruriens* leaves following a study by Karimi and Jaafar (2011) with some modifications. In the next step, 1 µL of samples without derivatization was injected into the GC-MS system (Thermo Scientific ISQ LT equipped with TriPlus RSH autosampler Waltham, MA, USA) with splitless mode. The A TG-5MS capillary column (30 m x 0.25 mm x 0.25 µm) was installed in the GC. The temperature was set at 50°C for two minutes, then increased at 5°C per minute, and reached up to 150°C for 10 minutes, and then it was again increased at 10°C per minute and reached up to 200°C and held for 5 minutes. Finally increase was 15°C per minute to reach 320°C and held for 5 minutes. During the compound separation carrier, hydrogen gas was generated by a hydrogen generator (Thermo Scientific Waltham, MA, USA) at a flow rate of 1 mL per minute. The injector and transfer line were set at 200°C and 320°C, respectively. The result of mass spectra obtained from the respective extracts matched the mass spectra library of the National Institute of Standard and Technology NIST version 2.2 (Yusnawan et al., 2021). The compounds that exceeded 1% level were listed.

Qualitative analysis of phytobiotics

The total phenolic contents of the *Mucuna pruriens* leaf extracts were analyzed using the Folin-Ciocalteu reagent (Singleton et al., 1999; Xu and Chang, 2007). The 100 µL of the hydrophilic extract was mixed with 2.9 mL of deionized water, 0.5 mL of Folin-Ciocalteu reagent, and 2.0 mL of 20% Na_2CO_3 solution, then stood for 90 minutes. The measurement of the absorbance was conducted using 760 nm versus reagent blank. The total phenolic content was expressed as gallic acid equivalent.

Total flavonoid content was analyzed using the aluminum chloride method (Heimler et al., 2005; Xu and Chang, 2007). The sample extract of 1 mL was placed in a 10-mL volumetric flask containing 4 mL of distilled water, 0.3 mL of 5% $NaNO_2$, and 0.3 mL of 10% $AlCl_3$, then remained for 6 minutes at room temperature. The 2 milliliters of 1 M (mol) NaOH were added, and the solution was diluted to 10 mL with distilled water. The absorbance measurement was conducted using 510 nm of the solution versus a reagent blank. The total flavonoid was expressed as catechin equivalents (CE) per gram of sample (mg CE/g sample (Heimler et al., 2005; Xu and Chang, 2007).

The total tannin contents of the *Mucuna pruriens* leaf extracts were estimated using the Folin Denish reagent (Galvao et al., 2018). Then, 2 grams of the sample extract were placed in a 500 mL boiling flask, 350 mL of distilled water was added, and refluxed for about 3 hours. Next, filtered 2 mL samples were placed in a 100 mL volumetric flask containing 2 mL of Folin Denish reagent and 5 mL Na_2CO_3. The mixture was allowed to stand for 40 minutes at room temperature. The absorbance measurement was conducted using 725 nm of the solution versus a reagent blank. The tannin content was expressed as gallic acid equivalent.

The total saponin contents of the *Mucuna pruriens* leaf extracts was analyzed based on Hiai Oura and Nakajima (1976) and the standard using Quillaja Bark (Sigma Aldrich Chemie, Steinheim, Germany). The sample extract was added

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to 0.2 mL vanillin, 0.25 mL ethanol, and 2.5 mL 72% H_2SO_4. The solution was vortexed and heated in a water bath (Watson Victor Ltd. Bw6t, Watson Victor Limited, New Zealand) at 60°C for 10 minutes. The measurement of absorbance was performed using 544 nm. Quillaja Bark equivalent was used to express the total phenolic content in *Mucuna pruriens* leaves extract.

Statistical analysis

Qualitative data of phytobiotics contained in the *Mucuna pruriens* leaf extracts were descriptively analyzed. In contrast, the quantitative ones were analyzed using SPSS software version 25. The analysis of variance (ANOVA) for a completely Randomized design with 3 treatments and 6 replications was performed on the obtained data. Duncan's Multiple Range Test was conducted to compare the mean values between treatments to determine if there was a significant effect of the treatments on phytobiotics content ($p<0.05$).

RESULTS AND DISCUSSION

Identification phytocompound in the *Mucuna pruriens* leaves extract

The identified active compounds or phytobiotics in the *Mucuna pruriens* leaves extracts and their proportion curve area (%) are presented in Table 1. As can be seen, *Mucuna pruriens* substance from EM extractions resulted in the highest number of compounds (24 compounds), followed by EA (16 compounds), and EK (8 compounds). However, EK showed a slightly lower value (90.86%) than the EM (92.89%) based on the total curve area, while the EA indicated the lowest value (84.66%). This means that EM can result in the most diverse compounds with the highest proportion of 10.35% for inositol, while EK leads to the most concentrated compound with the highest proportion of 55.77% for 4-Amino-2-(4-methoxyphenyl)-5,6,7,8-tetrahydrofuro[2,3-b] quinoline-3-carbonitrile. The second highest proportion was 17.22% for inositol, which was also found as the highest proportion compound in EM and EA solvent, with the proportion curve area of 10.35% and 22.73%, respectively. The inositol reduces plasma glucose level and can be found in the *Mucuna pruriens* seeds as d-chiro-inositol with two galacto-derivatives, O-α-d-galactopyranosil-(1→2)-d-*chiro*-inositol (FP1) and O-α-d-galactopyranosil-(1→6)-O-α-d-galactopyranosil-(1→2)-D-*chiro*-inositol (FP2). The level of 7 mg d-*chiro*-inositol has been used as an antidiabetic agent (Lampairello et al., 2012).

Niclosamide in ruminants reduces the occurrence of *Paramphistomum* infection by decreasing the number of eggs and reducing oxidative stress, as well as improving the biochemical and hematological profile of livestock (Shaheen et al., 2013). In addition, quinoline is an active substance that is commonly used as an antiprotozoal (Fournet et al., 1994). It acts as an antioxidant agent (Korrichi et al., 2009) and antiparasitic (Kadri et al., 2014).

The GC-MS analysis of *Mucuna pruriens* substance using 70% methanol solvent showed 3.1% quinazoline, 4.72% anthraquinone, 3.76% Coptisine, 2.06% isoquinoline, and several types of esters, sugar compounds, and fatty acids (Table 1). Quinazoline is an active compound as a component for the treatment of carcinoma cancer (Ardiles et al., 2020). Another broad range of quinazoline moieties are reported to have medicinal activities like antifungal, antiviral, antidiabetic, anti-inflammatory, antibacterial, and antioxidant (Karan et al., 2020). Coptisine could inhibit urease activity (Li et al., 2018). Thus, ruminants benefit from microbial fermentation (He et al., 2022), through which coptisine improves urea-N utilization efficiency for production and can decrease environmental nitrogen emission. Coptisine is an isoquinoline alkaloid (Wu et al., 2019). Anthraquinone in ruminant feed reduces ruminal sulfide production, reduces methane production, increases propionate, and decreases acetate (Kung et al., 2003). Anthraquinone contained in *Cassia Alata L* in the study of Yusiati et al. (2010) has a high potency as a methanogenesis inhibitor, thus, recommended as a feed additive to increase rumen microbial protein supply.

The research by Anosike et al. (2019) in *Mucuna pruriens* leaves using methanol extract indicated high antioxidant activity. Agbafor and Nwachukwu (2011) showed that *Mucuna pruriens* leaf extract inhibited 1,1-Diphenyl-2-picrylhydrazyl Free Radical (DPPH), indicating their antioxidant activity. Antioxidant supplementation in animal feed would enhance the cow's health in a sensitive stage, such as the transition period, and could add value to the final product (milk or meat) that benefits the consumer's health (Castillo et al., 2013).

According to Table 2, the addition of three different solvents to *Mucuna pruriens* extract resulted in significant content of total phenolic, flavonoid, tannin, and saponin contents ($p<0.05$). The extraction of *Mucuna pruriens* phytocompounds using EA showed the highest results for total phenolic, flavonoid, and tannin but the lowest for total saponin content. On the contrary, extraction of *Mucuna pruriens* leaves using EM solvent revealed the lowest results for total phenolic, flavonoid, and tannin but the highest for total saponin content. Meanwhile, the combination of aqueous and methanol solvent showed moderate results for all phytobiotics, and the values were in between EA and EM results. The saponin content of the *Mucuna pruriens* leaf extracts is lower than *Isesbania sesban* by about 10% (Tatiya et al., 2013). According to Susanti and Marhaeniyanto (2014), the saponin contents in some plant leaves are *Hibiscus rosa-sinensis* (5.89%), *Erythrina variegata L* (3.42%), *Gliricidia sepium* (8.23%), *Calliandra calothyrsus* (8.33%), *Moringa oleifera* (7.19%), *Leucaena leucocephala* (4.54%), *Swietenia macrophylla* (4.31%), *Artocarpus heterophyllus* (5.79%), *Paraserianthes faicataria* (15.04%), and *Samanea saman* (3.98%). Nevertheless, the *Mucuna pruriens* leaf extract can potentially be hepatoprotective and nephroprotective for treating liver and kidney disease in rats (Ogunmoyole et al., 2021). The incidence of liver abscesses in feedlot cattle is between 10 and 20% due to polymicrobial infections with

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gram-negative bacteria (Amachawadi and Nagaraja, 2016). The *Mucuna pruriens* had antimicrobial activity against Gram-negative bacteria (Mastan et al., 2009) and their hepatoprotective potential should protect the liver from tissue damage.

Table 1 - The compounds and curve area proportion of *Mucuna pruriens* substances using three different solvents

Identified compounds	EM (%)	EA (%)	EK (%)
(3E)-1-methyl-3-[5-(4-methylphenyl)-1,3-oxazolidin-2-ylidene]-1,3-dihydro-2H-indol-2-one	2.07	0	0
(4-Bromophenyl) bis(2,4-dibromophenyl) amine	0	1.41	0
,3-bis[(trimethylsilyl)oxy]-butanedioic acid, bis(trimethylsilyl)ester	4.59	21.27	0
(Z)-1-[Quinazolin-4(3H)-ylidene] propan-2-one	1.06	0	0
1-[3-methyl-1-(phenylsulfonyl)-thiopheno[2,3-b] carbazole]-3,5-[bis(3-methyl-2-thiophenyl)] benzene	0	1.79	0
1-cyclododecyl-3-(4-methoxy-6-methyl-5,6,7,8-tetrahydro-[1,3] dioxolo[4,5-G]isoquinolin-5-YL)-propan-2-ol	2.06	0	0
1-Methoxy-3-pentyl-6,6a,7,8-tetrahydro-6,6-dimethyl-9H-dibenzo[b,d]pyran-9-one	0	3.05	0
1-Phenyl-2-naphthol	1.48	0	0
2,3-Butanediol 2TMS PK A	0	0	5.32
2-Aziridinecarbonitrile,1-ethyl-3-phenyl-, cis-	3.04	0	0
2-Ethylthio-10-hydroxy-9-methoxy-1,4-anthraquinone	4.72	0	0
2-Fluoroquinoxaline	0	2.88	0
2-Isopropyl-4-methylpyridine-3,5-dicarbonitril	0	3.19	0
2-n-Butyl-2-[(p-hydroxymethyl)benzenesulfonyl]-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole	2.74	0	0
4-Amino-2-(4-methoxyphenyl)-5,6,7,8-tetrahydrofuro[2,3-b] quinoline-3-carbonitrile	0	0	55.97
4H,6H-Furo[3',2':3,4] naphtho[1,8-cd]pyran-4,6-dione,8,9-dihydro-3,7-dihydroxy-1,8,8,9-tetramethyl-, (S)-	4.07	0	0
5-(4-Chlorophenyl)-3,4-dihydro-2-methyl-4-oxothieno[2,3-d]-pyrimidine-6-carboxamide	1.73	0	0
5-Dimethylamino-1-ethoxy-5-ethylsulganyl-3-phenyl-1,3-cyclopentadiene	3.06	0	0
Acetamide, 2,2,2-trifluoro-	0	0	1.40
Aniline	0	0	1.32
Butanedioic acid, 2TMS derivative	0	1.80	0
Coptisine	3.76	0	0
D-Fructose, O-Methyloxim, pentakis-O-(Trimethylsilyl)-	2.83	0	0
D-Gluconic acid, 6TMS derivative	10.30	1.64	0
di(isityl)di[phosphino]silane	0	1.06	0
exo-2-oxyisoxazolidino[1,2-b]-1,3-dioxacyclopentane	0	3.72	0
Galaccititol TMS	7.06	0	0
Glycerol, 3TMS derivative	0	5.31	0
Indolo[2,1-b] quinazolin-6(12H)-one	2.04	0	0
Inositol,O,O,O,O,O,O-TMS	10.35	22.73	17.22
L-(-)-Fucose, tetrakis(trimethylsilyl)ether, methyloxime (syn)	0	1.60	0
Methyl galactoside (1S,2R,3S,4R,5R)-,4TMS derivative	4.20	0	0
N-(2'-Thiazolyl)-2,5-dimethylpyrrole-3-carbaldehyde	5.42	0	0
n-Hexadecanoic acid	0	0	1.39
Niclosamide	0	0	6.36
N-n-Butyl-2,2-bis-tert-butylacetamide	0	0	1.88
pentakis(trimethylsilyl)glucose-O-methoxime	2.59	0	0
Ribonic acid,2,3,4,5-tetrakis-O-(trimethylsilyl)-,trimethylsilyl ester	1.83	6.55	0
Silanol, trimethyl-, phosphate (3:1)	0	5.58	0
Sucrose, 8TMS derivative	2.10	0	0
Tartaric acid, 4TMS derivative	4.05	0	0
Trimethylsilyl2,3,4-tris[(Trimethylsilyl)oxy]butanoate	0	1.08	0
Xylitol, 5TMS derivative	4.80	0	0
Number of identified compounds	24	16	8
Total curve area	92.89	84.66	90.86

**Curve area proportion 0% means did not identify; EK: Combinations of aqueous and methanolic solvent, EM: Methanolic solvent, EA: Aqueous solvent

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Table 2 - The total phenolic, flavonoid, tannin, and saponin of the *Mucuna pruriens* substances using three different solvents in Indonesian

Treatment	Total phenolic (mg GAE/g)	Total flavonoid (mg CATE/g)	Total tannin (mg GAE)	Total saponin (mg QBE/g)
EA	14.55 ± 0.70^{b}	5.91 ± 0.29^{c}	0.84 ± 0.03^{c}	9.685 ± 0.18^{a}
EM	12.68 ± 0.30^{a}	3.83 ± 0.16^{a}	0.69 ± 0.02^{a}	12.41 ± 0.21^{c}
EK	13.88 ± 0.38^{b}	4.89 ± 0.25^{b}	0.74 ± 0.02^{b}	10.65 ± 0.35^{b}

^{abc}Values with different superscripts in the same column are significantly different ($p < 0.05$); EA: Aqueous solvent, EM: Methanol solvent, EK: Aqueous and methanolic combination solvent, GAE: Gallic acid equivalent, CATE: Catechin equivalent, QBE: Quillaja bark equivalent

The highest total saponin content of extracted *Mucuna pruriens* leaves using methanol solvent (12.41 mg QBE/g) indicated the potential of this extract as an additive for ruminants. The potential of saponin as an additive in ruminants has been widely studied with several benefits. As reported by Wina and Muetzel (2020), saponin plays a role in the defaunation effect by lysing protozoa cell membranes and then decreasing the protozoa. Since cholesterol is one thing avoided in meat consumption because it affects human health, saponins positively decrease blood cholesterol concentrations (Aazami et al., 2013). The administration of tea saponins showed changes in the ruminal bacterial microbiota and response metabolites (Wang et al., 2019). Another research showed that condensed tannins and saponins could reduce the proportion of methane during rumen fermentation using sugar cane top (Widiawati and Puastuti, 2016). The use of saponin also affected the rumen environment, and then could modulate the microbial community and ruminal metabolites, reducing ammonia concentrations without causing adverse effects on pH, microbial protein, and cellulase activity (Wang et al., 2019). Administration of 1% tannins and 0.6% saponins could increase microbial protein supply to the host by up to 30% and reduce methane production by up to 11% (Newbold et al., 2015) due to decreasing rumen protozoa (Wahyuni et al., 2014). An *in vivo* meta-analysis by Ridla et al. (2021) shows that dietary saponins in a low level increase dry matter, organic matter, and acid detergent. There the recommended amount is the maximum of 0.5% DM.

CONCLUSION

The extraction of the *Mucuna pruriens* leaf using methanol solvent (EM) showed the highly identified phytocompounds with the highest saponins contents (12.41 mg QBE/g). The *Mucuna pruriens* phytocompounds have the potential to reduce the occurrence of *Paramphistomum* infections, antiprotozoal, antioxidant agent, antiparasitic, antifungal, antiviral, anti-inflammatory, antibacterial, reduces methane production, increase propionate, and decreases acetate. Therefore, they can be used as feed additives for livestock productions.

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Data availability and materials
Data will be available on request.

Ethical consideration
Consent to publication and misconduct, plagiarism, data fabrication and double submission of the manuscript, and redundancy and other ethical issues were checked by the authors.

Authors' contributions
T. Muhartatik S. Chuzaemi, M.H. Natsir, Marjuki performed conceptualization, formal analysis, investigation, methodology, validation, writing original draft. Marjuki performed the supervision, review and editing of the manuscript for important academic content. All authors checked and approved the final revised article.

Conflict to interest
The authors have not declared any conflict of interest.

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OCCURRENCE OF PARASITES IN FISH MARKETED IN THE INEZGANE WHOLESALE MARKET AND THE FISHING PORT OF AGADIR, MOROCCO

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Supporting Information

RESEARCH ARTICLE

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ABSTRACT: Based on importance of animal products safety, the purpose of this work was to assess the extent of parasitism at the wholesale market level of Inezgane and the fishing port of Agadir in Morocco. For this purpose, fieldwork aimed at direct investigation of parasites involved 366 fish pieces. This study was conducted in the period between March and June 2021. The prevalence of parasitism was 20.76%. The total number of parasites collected is 2385 including 1959 nematodes, 318 xenomas, 92 cestodes, and 16 isopods. An abundance of 6.51 and an overall intensity of 31.38. These infestation parameters varied by species and location of origin. For the qualitative analysis of the parasites, the study revealed a predominance of L3 larvae of the *Anisakis* nematode with a percentage of 82.14%. Xenomas had a percentage of 13.33%. As for the cestodes of *Gymnorhynchus gigas*, the larvae were collected from the Atlantic pomfret (*Brama brama*) with a percentage of 3.86%. As a result of this study, a significant positive correlation of r=0.81 was shown between the total length of the fish and the number of anisakids. The results of this study revealed that the extent of parasitism seems to be less pronounced in some species, but there is still a presence of concern.

Keywords: Anisakis, Fish, Fishing port, Parasitism, Wholesale market level

INTRODUCTION

Morocco has a significant fishing potential, benefiting from favorable hydro-climatic conditions that give Moroccan waters diversity and a recognized marine biological richness. Morocco is a fishing-oriented country, located according to the latest FAO data from 2020 at the 17th worldwide (FAO, 2020). Parasites of fish are a potential contamination source, thus generating the main reason for discards of Moroccan fish products and a risk to public health (Youssir et al., 2017; Biary et al., 2021). Indeed, the consumption of raw fish and other almost raw or uncooked culinary fish preparations (semi-cooked fish, marinated, cold-smoked) increases the risk of contamination of consumers of seafood products by certain foodborne parasites (Klimpel et al., 2019). Among those, *Anisakis* is one of the most important nematodes in terms of public health (Klimpel et al., 2019; Fiorenza et al., 2020).

The evolutionary cycle of anisakids is an indirect cycle, characterized by 4 larval stages with a phase in the external environment, in this case in sea water. The definitive hosts are marine mammals and piscivorous birds, the intermediate hosts are crustaceans and fish are paratenic hosts which transport the larvae (Chai et al., 2005; EFSA, 2010), humans are an accidental host.

MATERIAL AND METHODS

Fieldwork

The number of pieces of fish examined in this study is 366 representing 27 species. These samples were taken at the Inezgane wholesale market (n=215) and the fishing port of Agadir (n=151), between March and June 2021 (Table 1).

Morphometric study

Each piece of fish was subjected to a freshness inspection, the determination of weight using a precision scale, and the determination of morphometric parameters, i.e, total length (TL) and fork length (LF) using an ichthyometer.

-Total length: is measured from the tip of the fish snout to the extremities of the longest rays of the caudal fin (cm);

-Fork length: is measured from the tip of the fish snout to the cartilaginous tip of the shortest ray or mid-ray of the caudal fin (in cm).

Detection of parasites

This step involves looking for visible parasites that have a dimension, color or texture that clearly distinguishes them from fish tissue. In order to collect the parasites, we first carried out a visual examination for the presence of the ectoparasites of the skin, the oral cavity and the gills. For internal parasites, the following methodology was used:

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- A first incision of the abdomen of the fish from the anus to the head;
- Visual and magnifying inspection under a light source of viscera on site and mesentery;
- Gastrointestinal tract and gonads collected and placed in petri dishes and examined;
- Existing parasites collection and counting;
- A second longitudinal incision of the musculature;
- Visual and light inspection of fish flesh;
- Existing parasites collection and counting;
- The number, type and anatomical location of each parasite are recorded.

Data processing

The results are treated by calculating the following parasitic infestation parameters: prevalence (P), intensity (I), abundance (A) for different fish species based on the three preceding parameters (Bush et al., 1997):

- Prevalence = Number of fish infested with parasites/Number of fish examined ;
- Intensity = Number of parasites detected/Number of fish infested with parasites ;
- Abundance = Number of parasites detected/Number of fish examined.

Table 1 - Fish species examined at Inezgane wholesale market level and the fishing port of Agadir

Scientific name	Species	Number of fish examined
Trachurus trachurus	Atlantic horse mackerel	50
Pagellus acarne	Axillary seabream	24
Scomber scombrus	Atlantic mackerel	32
Brama brama	Atlantic pomfret	8
Lepidopus caudatus	Silver scabbardfish	8
Merluccius merluccius	European hake	26
Phycis phycis	Forkbeard	9
Umbrina cirrosa	Shi drum	5
Zeus faber	John dory	2
Sarda sarda	Atlantic bonito	2
Engraulis encrasicolus	European anchovy	97
Sardina pilchardus	European pilchard	57
Gadus capelanus	Poor cod	8
Pomadasys incisus	Bastard grunt	1
Plectorhinchus mediterraneus	Rubberlip grunt	5
Scorpaena scrofa	Red scorpionfish	4
Cepola macrophthalma	Red bandfish	4
Scyliorhinus stellaris	Nursehound	2
Mullus barbatus	Red mullet	2
Pagellus bellottii	Red pandora	3
Spondyliosoma cantharus	Black seabream	3
Diplodus vulgaris	Common two-banded seabream	1
Diplodus sargus	White seabream	2
Dentex macrophtalmus	Large-eye dentex	5
Dentex dentex	Morocco dentex	2
Trachinus draco	Greater weever	2
Trachinus vipera	Lesser weever	2
Total		366

RESULTS AND DISCUSSION

Of the 366 fish specimens examined, 76 are infested with parasites (nematodes, cestodes and xenomas), which means an absolute prevalence of 20.76%, an abundance of 6.51 and an intensity of 31.38. Table 2 shows the repartition of parasites found in the different fish species. These results indicate a polyparasitism with a predominance of nematodes (Figures 1 and 2).

With regard to parasite associations, we detected three nematode-xenoma associations in silver scabbardfish and axillary seabream. External parasites are located primarily in the oral cavity and the gills of forkbeard. Viscera and muscle flesh are the sites frequently infested by internal parasites. Xenomas that are present with a percentage of 13.33%, was detected in the axillary seabream and in a single piece of silver scabbardfish (*Lepidopus caudatus*) is a tissue response of the parasite in response to microsporidian infestation of fish. This happens when the parasite seeks to take control of the cell's metabolism, it provides protection against the host's immune response in the form of whitish cystic structures «called xenoma» visible to the naked eye, of variable appearance, implanted in the flesh and easily detachable.

Two aspects of xenomas are retained in this study according to the form, regularly spherical cysts of whitish colour which contain a liquid substance, and cysts of elongated shape, creamy colour with a friable wall (Figure 3). In forkbeard, isopods and *Anisakis* nematodes were detected in addition from the oral cavity and gills, in the abdominal cavity and mainly in the stomach of examined fish (Figures 4-7).

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Table 2 - Repartition of parasites detected from fish specimens examined

Type of parasites	Number of parasites	%
Nematodes (*Anisakis*)	1959	82.14
Xenomas	318	13.33
Cestodes (*Gymnorhynchus gigas*)	92	3.86
Isopods	16	0.67
Total	2385	100

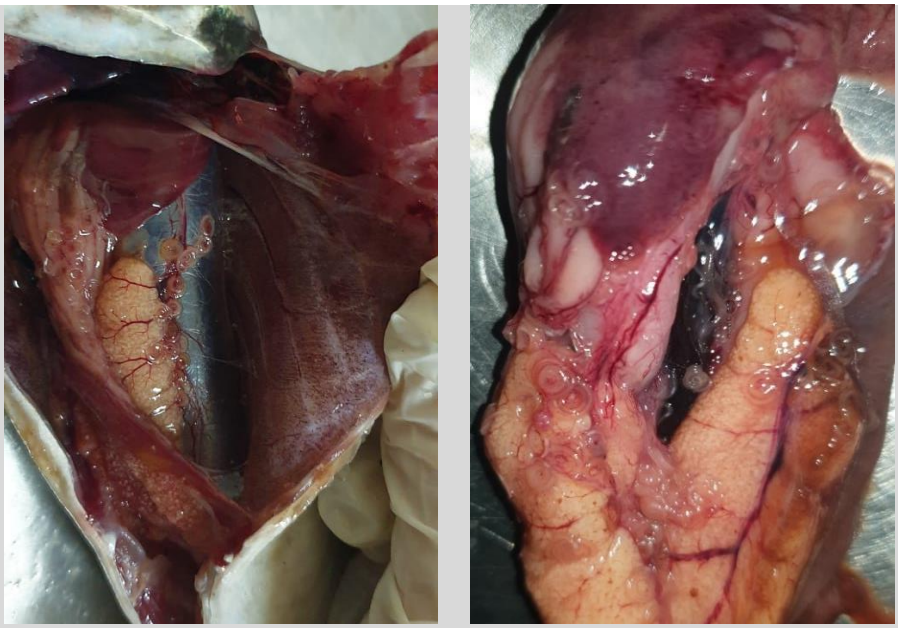

Figure 1 - Larvae of *Anisakis* in the viscera, liver and gonads of European horse mackerel (*Trachurus trachurus*)

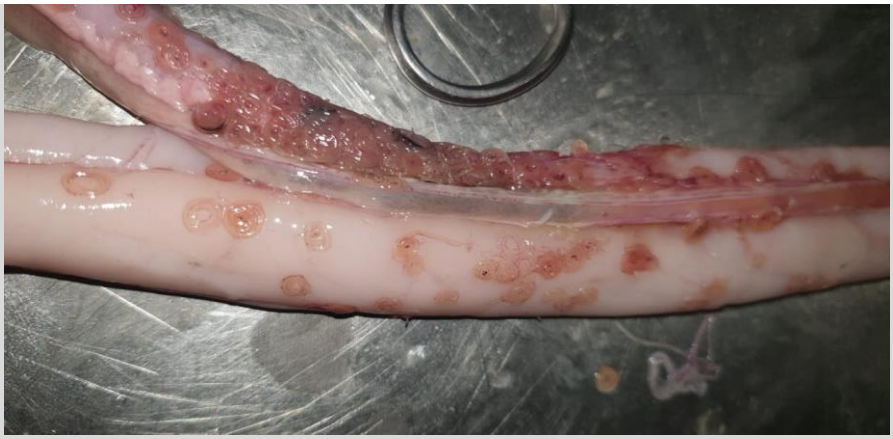

Figure 2 - Larvae of *Anisakis* in silver scabbardfish intestine (*Lepidopus caudatus*)

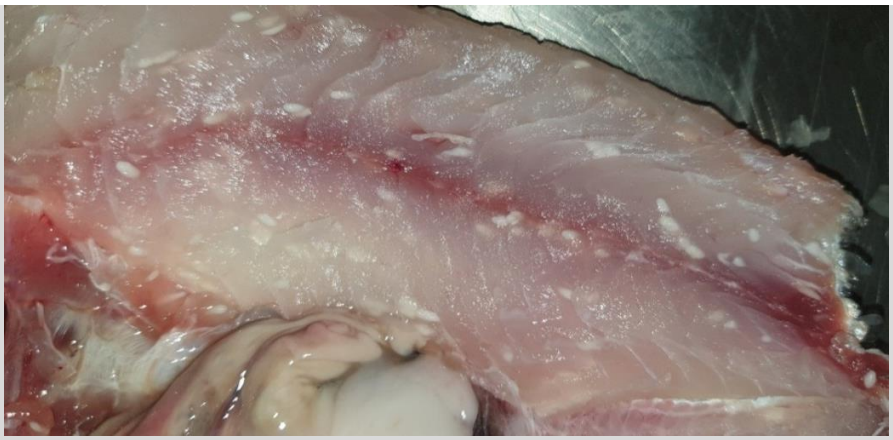

Figure 3 - Xenomas Housed in the chair of axillary seabream

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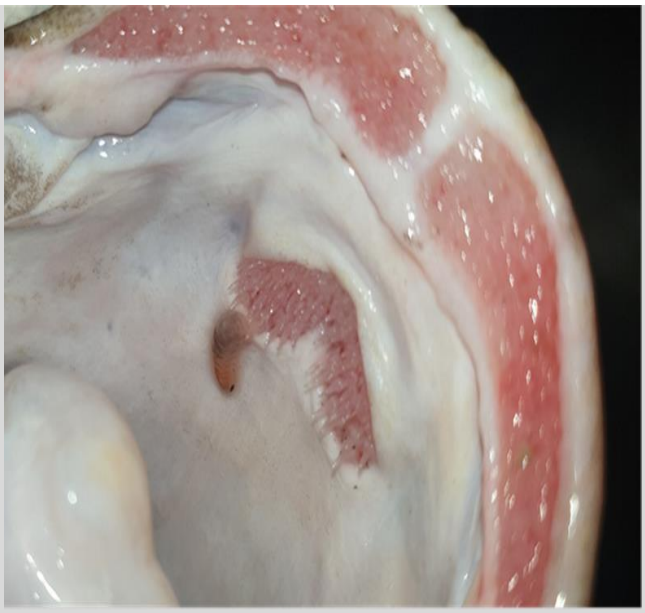

Figure 4 - Isopods in the buccal cavity of forkbeard (*Phycis phycis*)

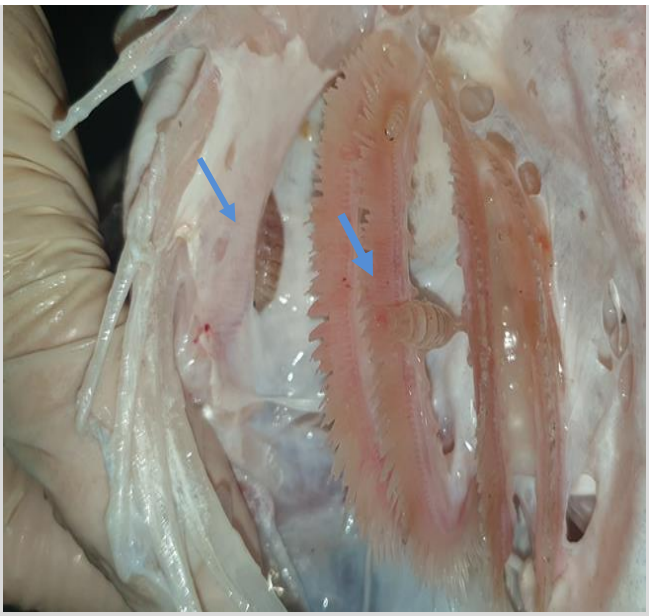

Figure 5 - Isopods in the gills of forkbeard (*Phycis phycis*)

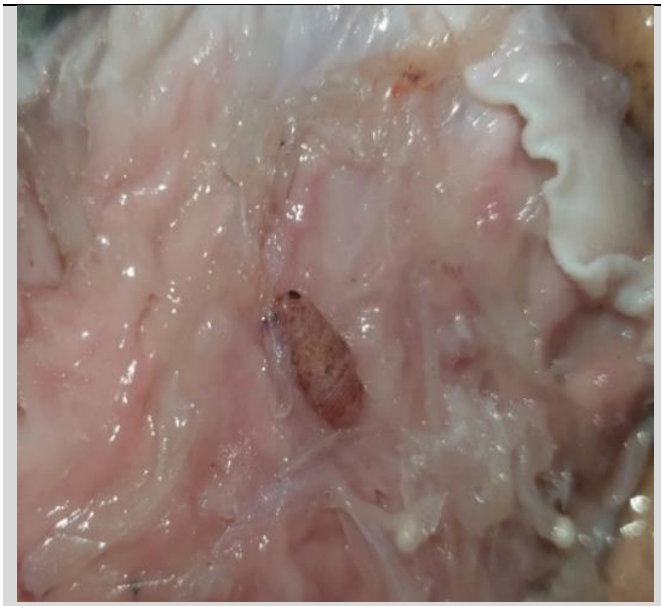

Figure 6 - Isopods in the abdominal cavity of forkbeard (*Phycis phycis*)

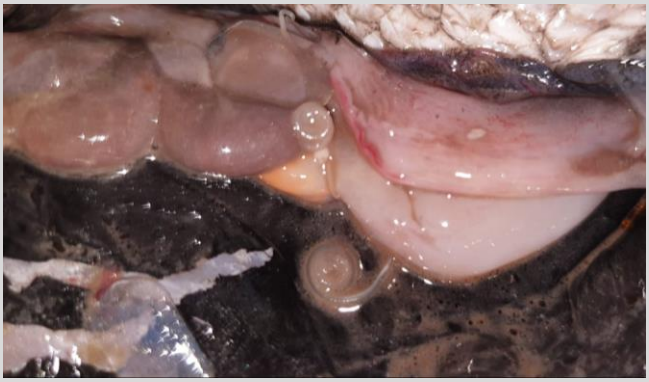

Figure 7 - Anisakis observed in the abdomen of forkbeard (*Phycis phycis*)

Study of the infestation parameters for the fish species examined

Of the 366 samples examined, 55 are infested with nematodes which mean a prevalence of 15.02%, an abundance of 5.35 and an intensity of 35.62. Fish sampled at the wholesale market level in Inezgane and the fishing port of Agadir has a maximum prevalence of nematodes of 100% recorded in silver scabbardfish. Table 3 presents the infestation parameters for the fish species examined. From the results obtained previously, we can classify fish species according to their prevalences into 6 categories (Table 4). 20 species of 27 examined (74.07%) were free of parasites.

For Atlantic mackerel, previous studies showed different prevalences, 67.9 (Abattouy et al., 2011), 60% (Lamane, 2013), and 30% (Benabbes and Boudakkou, 2019). These findings corroborate our current results, the Atlantic mackerel records a prevalence of 25% by classifying it as a low prevalence species. It can be concluded that parasitism in Atlantic mackerel has decreased on the Atlantic coast.

In this study, the European pilchard is shown to be free of parasites; this absence of parasites in this species has already been reported in previous studies on the Moroccan Atlantic coast (Lamane, 2013; Bainour, 2018; Ouakkouch, 2020). In the same context, a study by Biary et al. (2021) concluded a finding of absence of nematode parasites in European pilchard after visual inspection of samples. However, this same species is revealed in a previous study parasitized by isopods with a prevalence of 5.56% (Benabbes and Boudakkou, 2019). On the other hand, a study conducted in the northeastern Atlantic Ocean of Spain reported a prevalence of 2.5% of *anisakis* in European pilchard in the Cadiz zone and zero prevalence in the Isla Cristina zone in the same species (Molina-Fernández et al., 2015).

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Regarding anchovies, the prevalence of parasites is zero in this study, the same finding was made in the study conducted by Ouakkouch (2020), However one study reported a low prevalence in European anchovy (Bainour, 2018). These results can be explained by the fact that European pilchard and anchovies feed much more on copepods. They are only at risk of infestation after sufficient growth and feed on large plankton such as euphausiaceae (Lamane, 2013). Recently, one study reported the presence of *anisakis* larvae in commercially available anchovy oil products (Smaldone et al., 2020).

At the wholesale market level of Inezgane, the cestodes «*Gymnorhyncus gigas*» were found only in the atlantic pomfret «*Brama brama*» with a prevalence of 100% on 8 pieces of atlantic pomfret examined. The total number of parasites found in these 8 pieces of fish is 92. The 100% prevalence of cestodes in the atlantic pomfret is not a surprising result. Indeed, this result is corroborated by other results that also found a prevalence of 100% (Ouakkouch, 2020). A study reported a prevalence of 89.47% (Benabbes and Boudakkou, 2019). The 2013 study reported that Spanish researchers found that almost 100% of sea breams (*Brama brama*) were parasitized by this parasite (Lamane, 2013).

Of 24 pieces of axillary seabream examined, 13 pieces was infested with xenomas, a prevalence of 54.16%. The total number of parasites found in these 13 pieces of fish is 318.

Table 3 - Prevalence, abundance and intensity by fish species of nematodes

Species	Number of fish examined	Number of fish infested	Number of nemotodes	P (%)	A	I
Atlantic horse mackerel	50	25	1318	50	26.36	52.72
Atlantic mackerel	32	8	28	25	0.87	3.5
Silver scabbardfish	8	8	507	100	63.37	63.37
European hake	26	7	22	26.9	0.85	3.14
Forkbeard	9	5	72	55.5	8	14.4
Shi drum	5	1	8	20	1.6	8
John dory	2	1	4	50	2	4
Atlantic pomfret	8	8	0	-	-	-
Axillary seabream	24	13	0	-	-	-
Atlantic bonito	2	0	0	-	-	-
European anchovy	97	0	0	-	-	-
European pilchard	57	0	0	-	-	-
Poor cod	8	0	0	-	-	-
Bastard grunt	1	0	0	-	-	-
Rubberlip grunt	5	0	0	-	-	-
Red scorpionfish	4	0	0	-	-	-
Red bandfish	4	0	0	-	-	-
Nursehound	2	0	0	-	-	-
Red mullet	2	0	0	-	-	-
Red pandora	3	0	0	-	-	-
Black seabream	3	0	0	-	-	-
Common two-banded seabream	1	0	0	-	-	-
White seabream	2	0	0	-	-	-
Large-eye dentex	5	0	0	-	-	-
Morocco dentex	2	0	0	-	-	-
Greater weever	2	0	0	-	-	-
Lesser weever	2	0	0	-	-	-
	366	55	1959	15.02	5.35	35.62

P: Prevalence, A: Abundance, I: Intensity

Table 4 - Classification of fish species by prevalence of nematodes

Category	Prevalence (%)	Species
Very high prevalence	>80-100	Silver scabbardfish
High prevalence	>60-80	-
Moderate prevalence	>40-60	Forkbeard, atlantic horse mackerel, john dory
Low prevalence	>20-40	European hake, atlantic mackerel
Very low prevalence	>0-20	Shi drum
Zero prevalence	0	Axillary seabream, atlantic pomfret, atlantic bonito, european anchovy, european pilchard, poor cod, bastard grunt, rubberlip grunt, red scorpionfish, red bandfish, nursehound, red mullet, red pandora, black seabream, common two-banded seabream, white seabream, large-eye dentex, morocco dentex, greater weever, lesser weever

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Relationship between size and parasitism

The difference in the prevalence of parasitism in the fish examined leads us to the existence of a possible relationship between the size of fish and the number of *Anisakis* infesting the fish. Analysis of the sizes and numbers of anisakids collected from the examined fish of Atlantic horse mackerel reveals a significant correlation of R^2=0.44, which means the presence of a positive linear relationship between fish size and parasitic load (Figure 7). That is, parasitism increases with the size of the fish. This finding of increasing intensity of anisakid infestations gradually with fish size has also been reported in other studies (Bouchriti et al., 2015; Dahani et al., 2019; Ouakkouch, 2020). This is explained by the cumulative effect of parasites in the host during its lifetime (Abattouy et al., 2011). Another study reported that the direct relationship between infestation level and age or length in Atlantic horse mackerel is a widespread phenomenon in many fish species (Shawket et al., 2017).

Relationship between weight and intensity

In addition to fish size, it is interesting to review the number of parasites per kilogram of fish examined. Weight is also a factor in the variation of the parasitic risk, a positive correlation (R^2= 0.26) is shown between the weight of the fish (Atlantic mackerel) and the number of parasites isolated (Figure 8). Weight is also a risk variation factor impacting the infestation, corroborated by previous results which also reported a positive correlation between weight and parasitic intensity r=0.59 (Ouakkouch, 2020). A probability is raised of the increase in the intensity of anisakids in the muscle with the increase in weight, an explanation could be that the increase in weight is often related to the increase in fat, to which the larvae of anisakis migrate (Abattouy et al., 2011; Mo et al., 2021).

Comparative analysis with previous studies

Table 5 shows a comparison of the different work carried out on the study of the extent of coastal parasitism since 2013. The difference in infestation parameters is mainly due to the number of samples examined for each study.

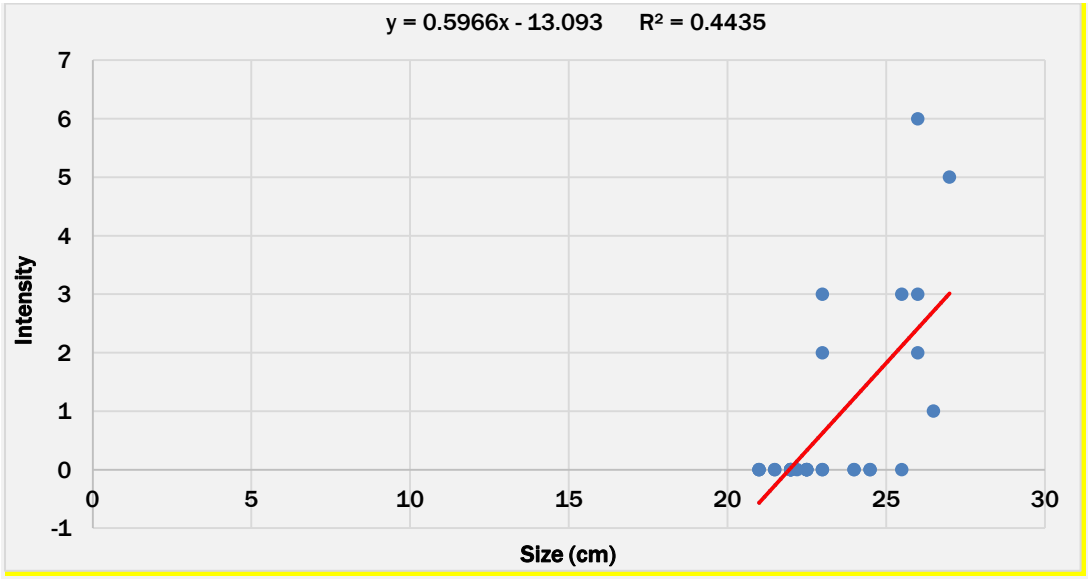

Figure 7 - Trend curve between fish size and nematode intensity.

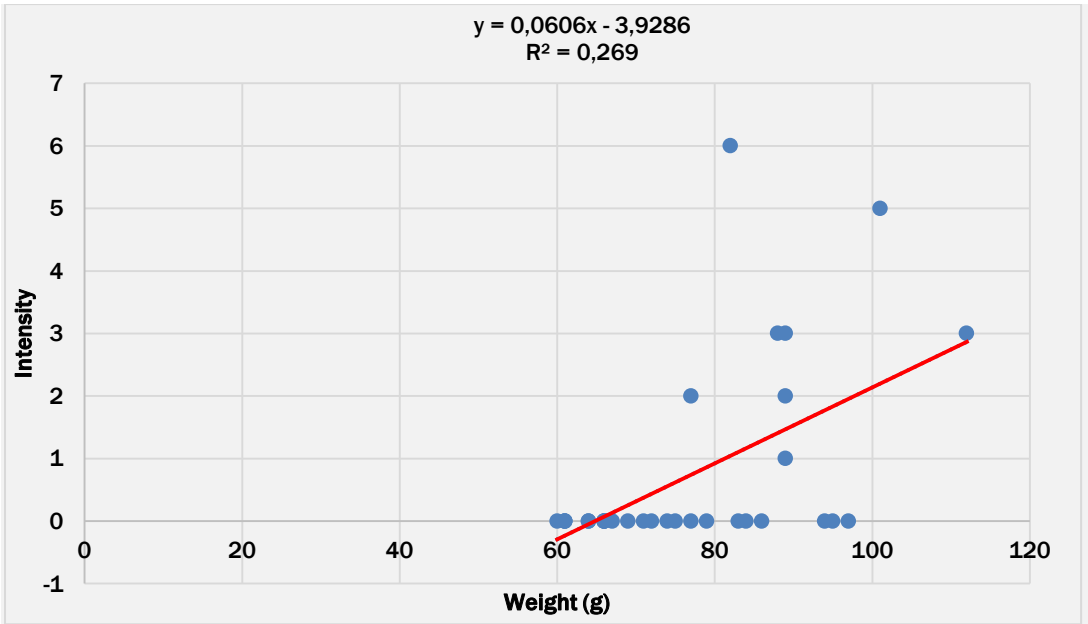

Figure 8 - Trend curve between weight and number of anisakids.

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Table 5 - Comparative analysis with previous studies

References \ Infestation parameters	Prevalence (%)	Abundance	Intensity
Present study	20.76	6.51	31.38
Ouakkouch (2020)	13.36	3.77	28.24
Benabbes and Boudakkou (2019) Atlantic	18.80	3.49	18.58
Benabbes and Boudakkou (2019) Mediterranean	24.8	0.79	3.2
Bainour (2018)	14.69	0.51	3.51
Lamane (2013)	38.16	8.67	22.73

***** The number of fish samples differs during the year according to availability.

CONCLUSION AND RECOMMENDATIONS

The study of parasitism at wholesale market of Inezgane and the fishing port of Agadir found that the markets had an absolute prevalence of 20.76% and 74.07% of the species examined were free of parasites. The species considered to be highly parasitized are the Atlantic pomfret and the silver scabbardfish, which is the most risky species by nematode infestation. The Atlantic pomfret has a prevalence of 100% of cestodes. The intensity of parasitism is correlated with the size and weight of the fish. Given the results of this study, it would seem interesting in the future to explore the following points: The xenomas detected in the axillary seabream require a more specific study in order to identify the infestation of parasites. The Atlantic pomfret requires further research as it is the species most affected by the cestodes "*Gymnorhynchus gigas*".

The results of this study are of great importance for the competent risk management authorities as well as for the processing establishments of fishery products which are invited to further strengthen the search for parasites in highly parasitized species, namely Atlantic pomfret and the silver scabbardfish. It is recommended to plan a study at the national level which will take into account several parameters, in particular the variations of parasitism according to the fishing zones and according to the seasons and the results of this study will allow the risk managers to take more management measures adapted even from a regulatory point of view, namely the prohibition of certain highly parasitized fish species in certain periods of the year or in certain fishing areas.

DECLARATIONS

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Author participations

All the authors contributed to the examination of fish, the results analysis and the writing of the final manuscript.

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Conflict of interest

The authors have declared that no competing interest exists.

Ethical regulation of Study

Not applicable. We have worked on marketed fish.

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INFLUENCE OF DIETARY SUPPLEMENTATION OF ANTIBIOTIC AND THYME ON ZOOTECHNICAL PARAMETERS AND CAECAL MICROFLORA OF GROWING RABBIT

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ABSTRACT: The objective of this study was to compare the influence of antibiotic and thyme dietary supplements on zootechnical parameters and caecal microflora of growing rabbits. One hundred and ninety eight weaned rabbits (forty days old), white New Zealand (of both sexes) were divided into three groups to submit to the following dietary treatments: Control diet, diet A (control diet + 100 ppm zinc bacitracin), and diet T (control diet + 7% *Thymus capitatus* leaves) for twenty-one days. The remaining nine days they received only the control diet. The results showed that both the live body weight and feed conversion ratio were positively affected by the antibiotic diet (P<0.05). However, the rabbits' growth performance was not influenced by dietary thyme supplements. The antimicrobial effect of thyme observed against *C. perfringens* in caecum is not determined even after 20 days of treatment. In conclusion, zootechnical parameters and mortality were not positively affected by dietary thyme supplements comparing it with the antibiotic diet, but these phytobiotics showed the antibacterial effect against *E. coli* and C*. perfringens* in caecum of rabbit.

Keywords: Zinc bacitracin, Dietary supplementation, Growth performance, Rabbit, Thyme.

INTRODUCTION

The weaning period in the rabbit life can be very critical (20-40 days of age) due to the transition from milk to solid nutrition (Gidenne et al., 2010). Also, the development of a caecal microbial activity starts to stabilize only after 7 weeks of age (Dl Meo et al., 2004). Moreover non-specific or specific enteropathy is always a major problem leading to great losses of rabbits (Abdeen et al., 2011; Conficoni et al., 2020). Furthermore, Phytobiotics are discussed as one of the promising alternatives to replace antibiotic growth promoters due to their high content of pharmacologically active compounds (Grashorn, 2010; Skoufos et al., 2020). The phytobiotics can prevent digestive disorders and optimize productivity (Krieg et al., 2009; Celia et al., 2016). They also improve health performance by increasing antioxidant activity in tissues (Settle et al. 2014; Abdel-Azeem and El-Kader, 2022) and enhancing immunity (Lee et al., 2010).

Among these phytobiotics, there are species of the Thymus genus, aromatic plants of the Lamiaceae family (Casiglia et al., 2019). Some studies have demonstrated the interesting biological properties of the Thymus (Soković et al., 2008; El-Nekeety et al., 2011; Nikolić et al., 2014). It has an antiseptic, antimicrobial, and antioxidant effect (Kaki et al., 2021; Pandey et al., 2021). Thyme improves liver function and acts as an appetite stimulant (Dauqan et al. 2017; Almanea et al., 2019).

The objective of the present study is to examine and compare the effects of antibiotic with *Thymus capitatus* as natural feed supplements on growth performance and caecal microflora in weaned rabbits.

MATERIAL AND METHODS

Ethical approval

According to Directive 2010/63/EU of 22 September 2010, and recommendation of the European Commission 2007/526/CE, the animal in the current study were used for experimental and other scientific purposes.

Medicinal plant supplementation

The areal parts of *Thymus capitatus* were collected in northern Morocco during the month of June. Identification was performed in the Laboratory by Professor Bakkali, a specialist in botany. Afterward, the leaves were separated and dried at room temperature for 2 weeks in the absence of light and then stored in sealed paper bags until their use.

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Animals and experimental procedure

A total of one hundred and ninety eight weaned rabbits (40 days old, white New Zeland of both sexes 900 ± 100 g initial weight), were divided into three groups and submitted to the following dietary treatments:

The control diet (in Table 1 formulation and chemical composition); the antibiotic diet (control diet + 100 ppm of Zinc bacitracin) and T diet (control diet + 7% *Thymus capitatus* leaves). Sixty-six rabbits per group. Rabbits were kept in in cages (L=90cm, W=40cm, H=35cm with six animals per cage) in a building with temperatures between 15 and 20°C. A total of 198 weaned rabbits were divided into three groups: 66 rabbit per group, and 66 rabbits there were in each replicate, per treatment). The length of daily illumination was 16 hours. The rabbits had access to feed and water ad libitum. The rabbits Body weight and feed intake were measured every week during the experiment as well as their mortality rates. Five animals from each group were randomly slaughtered at days 40, 50 and 60. Rabbits were weighed before and after slaughter and evisceration at days, 50, 60 and 70 to determine the yield of the carcass. The livers were weighed too.

Table 1 - Ingredients and chemical composition and nutritive value of diets in rabbits

Ingredients	Control diet (%)	T diet (%)	Chemical composition (g/100g)	Control diet	T diet
Wheat bran	28.5	26.505	Dry matter	89.9	89.7
Corn	9.5	8.835	Ash	7.7	7.8
Soybean meal	9.5	8.835	Crude protein	20.3	19.4
Sunflower meal	14.2	13.206	Ether extract	5.8	6.2
Alfalfa	33.75	31.3875	Neutral detergent fibre	30.5	30.6
Vegetable oil	2.8	2.604	Acid detergent fibre	17.5	18.25
Salt	0.5	0.465	detergent lignin	4.6	4.8
Premix *	0.6	0.558	Digestible energy (Kcal/Kg)	2522	2531
DL Methionine	0.1	0.093			
L-Lysine	0.2	0.186			
Dicalcium phosphate	0.25	0.2325			
Calcium carbonate	0.1	0.093			
Thyme leaves	0	7			

* One kilogram of Premix provides: 1000000 IU vit.A, 300000 IU vit. D, 2 g vit. E, 0.4 g vit. K, 0.075 g vit. B1, 0.4 g vit. B2, 1.218 g vit. B3, 0.099 g vit. B5, 0.083 g vit. B6, 0.190 g vit. B9, 0.030 g vit. B12, 0.005 g Biotin, 0,2 g Cuivre, 4 g Fer, 5 g Zinc, 0.012 g Iode, 0.012 g Selenium, 0.020g Cobalt, 6 g Manganese, 57 g Choline chloride and QSP calcium. Premix contained 50 ppm of Salinomycin.

Chemical analysis

Chemical analysis of diets were calculated by the Spanish foundation for the development of animal nutrition (Fundación Española para el Desarrollo de la Nutrición Animal, FEDNA) table of composition and nutritive valor of aliments (Blas et al., 2010). The essential oil of *Thymus capitatus* was analyzed with a gas chromatograph (Trace GC ULTRA; Thermo Scientific, Waltham, MA, USA) coupled to a mass spectrometer (Polaris Q MS with ion trap; Thermo Scientific) in the electron impact (EI) ionization mode (70 eV) in the 50–350 m/z range. The analysis was carried out using a VB-5 methylpolysiloxane at 5% phenyl) (Thermo Scientific) column (30m × 0.25 mm, film-thickness 0.25 µ m) using a temperature program of 40–300 ◦C at a 4 ◦C min−1. Injector temperature was set at 220 ◦C. Helium gas was used as the carrier gas at a constant flow rate of 1.4 ml min−1. Diluted samples (1% in n-hexane; Sigma–Aldrich, Steinheim, Germany) of 1.0 µL were injected in the split mode to allow better identification of compounds. The analysis was repeated twice for each sample. The constituents were identified by comparison of their retention indices and mass spectra with those in the computer library (NIST MS Library Search, v.6.0) and with literature data.

Bacteriological analysis

Bacteria from caecal samples were isolated by the standard microbiological method using the appropriate dilutions in Ringer solution. Dilutions were plated onto the following media: Mac Conkey agar for *E.coli*, incubated at 37°C for 24 h and agar Tryptose Sulphite added with antibiotic D-Cycloserine (TSC) for *C. perfringens* incubated during 48 hours at 37°C, with their enumeration determined according to the ISO 7937 standard (1997). The bacterial counts per colony were expressed in grams using this formula: (log10 CFU/g ± SD). Experiments were carried out in triplicate.

Statistical analysis

The results were quoted as mean ± standard deviation (SD), and statistical evaluation of the results was performed by the one-way ANOVA using the general linear model (GLM) procedures of SAS with the level of significance set at $p < 0.05$ and Square test for mortality.

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RESULTS

Live weight, growth rate, feed intake, feed conversion rate, carcass yield, and the mortality of rabbits during the experiment are presented in Table 2. Live body weight (BW) was positively affected by the antibiotic diet. The addition of 7% of thyme in the diet had a negative effect on the weight and growth rate of the young compared to other groups because they eat a smaller quantity of feed. Although the feed conversion rate did not differ significantly when the experimental period was considered as a whole. The rate of conversion for the antibiotic group tended to fare better during the first week of treatment.

There was a difference in the mean carcass yield of the thyme group on the first ten days of the treatment. A high mortality rate was observed in the three groups. Effects of an antibiotic and thyme dietary supplements on the caecal microbial colony-forming unit (CFU) are presented in Table 3. No significant effect was found on the caecal count of *E. coli* or *C. perfringens* due to antibiotic diet. The CFU of *E. coli* in digesta harvested from the caecum was also not influenced by dietary supplementation of medicinal plant. While it was observed the number of *C. perfringens* in the caecum of rabbits fed the thyme feed was low compared to the control and antibiotic group after 10 days of treatment and it could not even be determined after 20 days of treatment. The chemical composition of the essential oil of *Thymus capitatus* is presented in table 4. The yield of essential oil of *Thymus capitatus* was 1.96%. The *Thymus capitatus* oil was dominated by carvacrol (95.25%).

Table 2 - The Effect of dietary supplementation with thyme on rabbit growth performance and mortality.

Indices	Days	Group (mean ± SD)			
		Control diet	Antibiotic diet	Thyme diet	P value
Live weight (g)	40	910 ± 130	874 ± 125	893 ± 116	0.24 NS
	47	1183 ± 174 a	1279 ± 207 b	1171 ± 166 a	0.004**
	54	1391 ± 162 a	1514 ± 256 b	1336 ± 167 a	0.001**
	61	1623 ± 235 a	1765 ± 243 b	1550 ± 172 a	0.001**
	70	1972 ± 301 ab	2015 ± 253 b	1859 ± 202 a	0.008**
Feed intake (g/d)	40	86.8 ± 5 b	84.5 ± 6 b	78.5 ± 5 a	0.001**
	47	97 ± 12	96.5 ± 6	87.5 ± 7	0.754 NS
	54	109 ± 7	103,1 ± 13	99,2 ± 12	0.141 NS
	61	122.4 ± 13 b	110.2 ± 9a b	104.1 ± 12 a	0.002**
	70	137 ± 18	135.9 ± 18	119.8 ± 21	0.096 NS
Growth rate (g/d)	40-47	40 ± 16 a	51.7 ± 19 b	41.4 ± 13 a	0.001
	47-54	34.3 ± 12	34.8 ± 10	30.2 ± 9	0.144 NS
	54-61	36.6 ± 9	30.8 ± 9	30.3 ± 9	0.142 NS
	61-70	38.4 ± 11 b	26 ± 12 a	31.5 ± 10 b	0.002 **
Feed conversion ratio	40-47	2.56 ± 1	2.14 ± 1	2.37 ± 1	0.286 NS
	47-54	3.04 ± 0.7	2.98 ± 0.8	3.07 ± 0.7	0.915 NS
	54-61	3.45 ± 0.9	3.58 ± 0.9	3.47 ± 0.8	0.865 NS
	61-70	3.49 ± 0.7	3.9 ± 0.8	3.57 ± 0.8	0.323 NS
Carcass yield (% of BW)	50	52.1 ± 2 b	51.5 ± 1 b	48.3 ± 2 a	0.003**
	60	62 ± 7	63.4 ± 1	57.6 ± 1	0.189 NS
	70	53.4 ± 2	53.8 ± 1	51.8 ± 2	0.192 NS
Liver yield (% of BW)	50	3.1 ± 0,3	3.8 ± 0.4	3.4 ± 0.7	0.127 NS
	60	3.2 ± 0,4	3.1 ± 0.1	3.6 ± 0.4	0.182 NS
	70	4.1 ± 0.3	4 ± 0.2	4.2 ± 0.2	0.487 NS
Mortality[1] (%)	40-70	39.30%	40.90%	44.40%	> 0.05

[1] Mortality is analyzed by using a χ 2 test at P<0.05; The values with different superscript letters in a row are significantly different (p<0.05)

Table 3 - Counts of *E. coli* and *C. perfringens* in caecum of rabbits (log10 cfu/g (mean±SD)

Days	Bacteria	Group (mean ± SD)			
		Control diet	Antibiotic diet	Thyme diet	P value
40 d	*E. coli*	3.48 ± 0.2	-	-	-
	C. perfringens	2.89 ± 0.1	-	-	-
50 d	*E. coli*	4.1 ± 0.2	3.93 ± 0.5	4.2 ± 0.1	0.503 NS
	C. perfringens	2.92 ± 0.6	2.51 ± 0.4	2.24 ± 0.3	0.284 NS
60 d	*E.coli*	4.21 ± 0.7	4.2 ± 0.1	3.56 ± 0.8	0.157 NS
	C. perfringens	3.2 ± 0.3	2.71 ± 0.3	ND	0.209 NS

SD: Standard deviation; N.S: Non-significant at probability value (P>0.05). ND: Is not determined

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Table 4 - Chemical composition of *Thymus capitatus* essential oil.

No.	Component	Retention time (min)	*Thymus capitatus*
1	Myrcene	6.55	-
2	Para cymene	7.19	-
3	cis-Ocimene	7.73	-
4	α-Pinene, (-)-	7.75	-
5	γ terpinene	7.79	-
6	α-Pinene, (-)-	8.28	-
7	Linalol	8.51	-
8	Camphene	8.74	-
9	α-Phellandrene	9.70	-
10	Terpinene -4-ol	9.79	-
11	α-Pinene, (-)-	11.01	-
12	dl-Limonene	11.01	-
13	Thymol	11.38	-
14	Carvacrol	11.55	-
15	1-8 cineol	11.68	-
16	Fenchone	12.99	-
17	Fenchone	13.00	-
18	β Caryophyllene	13.08	-
19	α Campholene Aldehyde	15.63	-
20	Borneol	16.47	-
21	Isopulegyl acetate	17.81	-
22	α-Fenchyl acetate	18.23	-
23	Trans Anethol	20.07	-
24	Trans Anethol	20.10	-
25	Carvacrol	20.69	95,25
26	Caryophyllene	24.24	1,49
27	Caryophyllene	24.89	-
28	Tetradecamethylcycloheptasiloxane	27.26	-
29	3,5-Diethylphenol	37.95	0.74
31	1,15-Dihydrohexadecamethyloctasiloxane	38.85	-
32	6-Acetyl-2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-1-benzopyran	39.37	0.91

* Analyzed by authors.

DISCUSSION

The weaning age affected growth performance and the caecal fermentation was strongly stimulated by the early weaning at 21 days of age (Xiccato et al., 2003). The crude protein of the rabbit diet recommended range was 14.5 – 16.2 g for fattening rabbits and 15.4 – 16.2 g in mixed feed (Mateos and de Blas, 1998). The crude protein of the rabbit diet was high in this experience, which can explain the high mortality rate of the three groups. Mortality, clinical symptoms, and performance data were significantly improved by zinc bacitracin soluble powder in the early treatment of Epizootic Rabbit Enteropathy in rabbits reared under normal field conditions (Maertens et al., 2005). According to Benlemlih et al. (2020) dietary supplementation with 5% dried thyme improved rabbit performance. The treatment with 2.5% of fennel and thyme had also a beneficial effect and decreased the significant mortality rate (Benlemlih et al., 2014). Abdel Wareth et al. (2018) proved that thyme essential oil included up to 100 mg/kg ration with 1.50 g/kg olive oil as carrier as an supplement in the ration of growing rabbits can be used as an effective feed additive to improve performance under hot environmental conditions. However, this experiment showed that supplementing the diet with 7% of thyme increase the mortality rate. Supplementing the diet with 3% thyme did not affect rabbit mortality (Gerencsér et al., 2012). Zinc bacitracin was tested and observed a significant improvement in weight gain (Boisot et al., 2004; Letlole et al., 2021; Martínez et al., 2022). However, in agreement with our observations, Pinheiro et al. (2004) found that the growth performances were not significantly increased ($P > 0.05$) by antibiotic supply during the growing period.

Carvacrol is the main ingredient of their essential oil, is known to evoke a sense of warmth, and sensitize skin by activating the transient receptor potential channel (TRPV3) (Xu et al., 2006). So the higher quantity of thyme leaves in dietary participated in reducing the amount ingested. That makes it clear why the decrease in rabbit consumption, affects weight and growth rate.

Rabbits fed Zinc bacitracin and FormaXol diets harbored the highest percentage of non-pathogen bacteria (Cardinali et al., 2008). The concentration of different bacterial populations on caecal content decreased significantly in growing rabbits with an antibiotic growth performance diet. Coliform population was reduced in half and the total bacteria count by 26% (Pinheiro et al., 2004).

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In disagreement with our observation, rabbit feed supplemented with 3% of thyme did not affect the caecal number of *E.coli*, total anaerobic, and strictly anaerobic bacteria (Bónai et al., 2012). But essential oil extracted from thyme and anise have been reported to decrease *C. perfringens* and *E. coli* counts in both small and large intestines, accompanied by decreased intestinal lesion scores in broilers (Cho et al., 2014). The changes in intestinal microbiota might be related to the alleviation of intestinal lesions with essential oil supplementation (Du et al., 2015).

The antibacterial activity of the individual oil component was tested by the component with the widest spectrum of activity was found to be thymol followed by carvacrol (Dorman and Deans, 2000). So, the high dose of carvacrol in our phytobiotic may have reduced the number of *C. perfringens* in the caecum which has not allowed us to determine its number. Aromatherapy rabbits with thyme essential oil can be suggested to treat diarrhea and bacterial enteritis.

Carvacrol can destabilize the cytoplasmic membrane and acts as a proton exchanger. Thereby reducing the pH gradient across the cytoplasmic membrane, the resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death (Ultee et al., 2002).

CONCLUSION

The addition of a high dose of phytobiotics (7% of *Thymus capitatus*) gave negative results on different zootechnical parameters and mortality comparing it with the antibiotic. But the active compounds of phytobiotic were responsible for antibacterial effects. The presence of carvacrol in thyme essential oil can intervene in the reduction of the number of *E. coli* and *C. perfringens* in the caecum. The addition of a moderate dose of *Thymus capitatus* should be stadied.

DECLARATIONS

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Authors' contribution

All authors contributed to research conduction, analyzing and writing, equally.

Conflict of interests

The authors declare that there is no conflict of interests in this work.

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NEW GROWTH MEDIUM FOR CULTURING LACTIC ACID BACTERIA AS PROBIOTIC CONSORTIUM ISOLATED FROM FERMENTED FISH (BUDU)

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ABSTRACT: This study aimed to obtain the best ratio of inoculums and types of alternative media in increasing the growth of the probiotic consortium with the observed variables consisting of viability, cell biomass and decrease in pH. Completely randomized design (CRD) factorial consisting of 2 factors with 3 replications is used; factor A was the probiotic consortium (A1: *Lactobacillus parabuchneri*: *L. buchneri*: *L. harbinensis*, *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner)* with ratio 1:1:1:1:1; A2: same consortium with ratio 1:1:1:1:2; A3: same consortium with ratio 1:1:1:2:1; A4: same consortium with ratio 1:1:2:1:1; A5: same consortium with ratio 1:2:1:1:1; A6: same consortium with ratio 2:1:1:1:1 and factor B was the type of alternative media (B1=control; B2=coconut water (90%) + cassava flour (5%) + fish waste flour (5%); B3=tofu liquid waste (90%) + flour onggok (5%) + fish waste meal (5%); B4= tofu whey (90%) + onggok flour (5%) + fish waste meal (5%). The results showed that there was an interaction between factor A and factor B which was highly significant (P<0.01) on viability, cell biomass and decrease in medium pH. In conclusion, the best ratio of probiotic consortium was 1:1:1:2:1, with growth medium coconut water (90%) + cassava flour (5%) + fish waste flour (5%) which resulted in a viability value of: 3, 02, cell biomass: 22.47 mg/ml and a decrease in the pH of the medium by 2.84.

Keywords: Cell biomass, Fermentation, Medium pH, Probiotic consortium, Viability.

INTRODUCTION

The safe criteria for probiotics are non-toxic and non-pathogenic, have clear taxonomic identification, can live in the target species, can survive, colonize and metabolize actively in the target indicated by resistance to digestive juices and bile salts, persistence in the digestive tract, and competition, with the host microflora, produce antimicrobial compounds, antagonist against pathogens, can change the immune response, does not change and is stable during storage and field processes (Gaggia et al., 2010). The lactic acid bacteria need a source of carbon and nitrogen as well as minerals and vitamins (Mıdık et al., 2020). The MRS (de Man Ragosa and Sharpe), is a selective medium for the growth of lactic acid bacteria, but it is expensive to use commercially and on a large scale (Champagne et al., 1991).

This research was to look a good growth characteristics of the consortium probiotics which have the potential to be made on an industrial scale with a special purpose for achieve high cell concentrations and medium prices that are cheap and easy to obtain by utilizing local resources such as various agricultural and industrial wastes. The similar study was reported by Champagne et al. (1991) which the production of probiotics in large quantities and used on an industrial scale is constrained by limited costs, cell production efficiency and difficulty in harvesting. Coghetto et al. (2016) added that probiotic cells can be produced using alternative carbon and nitrogen sources, such as agro-industrial residues, such as tapioca factory waste, fish offal meal, shrimp shell meal and other waste materials.

The waste or agro-industrial residues must contain a number of food substances that can support the growth of probiotic microbes such as carbon, protein and mineral sources such as tofu liquid waste, fish waste flour, coconut water and cassava flour, and shrimp waste flour. Marlida et al. (2022) found that liquid coconut waste, tapioca waste and shrimp shell waste, the best medium for good growth and high cell concentration of mixes probiotics of *Lactobacillus plantarrum* and *Saccharomyces cereviceae*. Heenan et al. (2002) reported that growth medium for culturing probiotic bacteria for applications in vegetarian food products such as media containing 25 g/L soy peptone, yeast extract and glucose monohydrate suitable for *Lactobacillus acidophilus*, *L. paracasei* ssp. *paracasei* and *Bifidobacterium lactis* better than commonly used media in laboratory MRS and RCM. Anggraini et al. (2019) added that the best natural growing medium for lactic acid bacteria in the *Pedioccocus acidilacti* group is tofu and palm sugar liquid waste, where the best concentration is 100% tofu liquid waste and 15% palm sugar resulting in the production of gamma-aminobutyric acid

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(GABA) up to 311,485 mg/L. It has been found that 5 isolates of lactic acid bacteria have probiotic abilities, where the 5 isolates are able to live at low pH, in 0.3% bile fluid, are able to stick to the walls of the small intestine and are able to kill pathogenic bacteria such as *E.coli, S. aureus* and *Salmonella enteridis* (Susalam et al., 2022). In the future, the five isolates will be made into a probiotic consortium that is ready to be applied and commercialized. To achieve this goal, it is necessary to find an alternative medium so that the five isolates can grow together with the right balance. The purpose of this study was to find alternative medium and a ratio of the five isolates of lactic acid bacteria isolated from fish fermented (budu) a traditional fermented food from West Sumatera, Indonesia that they play an optimal role as a probiotic consortium.

MATERIALS AND METHODS

Samples

Lactobacillus parabuchneri, *L. buchneri*, *L. harbinensis*, *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner* were used as starter cultures. They were obtained from the Laboratory of Feed and Technology, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia. The cultures were stored in a 10% skim milk mixture and 1% sucrose under -20 °C. Alternative materials such as whey tofu, coconut water, tofu liquid waste, fish waste flour and cassava flour were purchased from the local market.

Chemicals

Chemicals used in this study were MRS Broth medium (de Man Rogosa and Sharpe Broth), PDA (Potatoes Dextrose Agar), and PDB (Potatoes Dextrose Broth). All media used were also purchased from Merk, Germany.

Animals and biological material

Biological materials involved in this study were *L. parabuchneri*, *L. buchneri*, *L. harbinensis*, *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner* of our own collection isolated from the previous study.

Experimental design

The completely randomized design (CRD) factorial consisting of 2 factors with 3 replications, factor A were the probiotic consortium (A1: *L. parabuchneri*: *L. buchneri*: *L. harbinensis*, *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner* with ratio 1:1:1:1:1; A2: same consortium with ratio 1:1:1:1:2; A3: same consortium with ratio 1:1:1:2:1; A4: same consortium with ratio 1:1:2:1:1; A5: same consortium with ratio 1:2:1:1:1; A6 same consortium with ratio 2:1:1:1:1 and factor B were the type of alternative media (B1=control; B2=coconut water (90%) + cassava flour (5%) + fish waste flour (5%); B3=tofu liquid waste (90%) + flour onggok (5%) + fish waste meal (5%); B4= tofu whey (90%) + onggok flour (5%) + fish waste meal (5%). The observed variables were viability, cell biomass and decrease in pH.

The measured parameters

Cell viability and cell biomass assay were measured according to Pires et al. (2013). pH was measured for each natural medium (Matouskova et al., 2021). The all founded data were analyzed using analysis of variance for a Completely Randomized Design. The significance of differences between treatments were tested with Duncan's Multiple Range Test (DMRT), with a significant value at P>0.05 (Steel and Torrie, 1995).

Sample preparation

There were three alternative media: 1) the media based on coconut water consisted of coconut water, cassava flour and fish waste flour; 2) the media based on tofu liquid waste consisted of tofu liquid waste, cassava flour, and fish waste flour; 3) the media based on whey tofu consisted of whey tofu, cassava flour and fish waste flour. The combination of *Lactobacillus Parabuchneri*, *Lactobacillus Buchneri*, *Lactobacillus Harbinensis*, *Schieferilactobacillus Harbinensis* and *Lentilactobacillus Parabuchner* (probiotic consortium) were based on the TPC (Total Plate Count) results and were divided into six ratios, namely 1:1:1:1:1; 1:1:1:1:2; 1:1:1:2:1; 1:1:2:1:1; 1:2:1:1:1 and 2:1:1:1:1, cultured on MRS-B and incubated at 37 °C for 24 hours. The experiment was triplicated.

RESULTS AND DISCUSSUON

Based on research that has been done regarding the effect of different media in probiotics consortium ratio on viability, it can be seen in Table 1. Statistical analysis showed that there was an interaction between factor A (probiotic consortium ratio) and factor B (type of media) significantly different (P<0.01) on viability. In the A3B2 treatment, the viability was higher than all treatments, namely 3.02. This value was not significantly different (P>0.05) with treatment A3B1, but significantly different (P<0.01) with A3B3 and A3B4. The stability of the viability value of the probiotic consortium is thought to be influenced by the nutritional value content in B2 media, which has an abundant carbon source due to a combination of coconut water waste, cassava flour, fish waste flour. The ratio of probiotic consortium ini A3 was dominated by *Schieferilactobacillus Harbinensis*. Colares et al. (2021) reported that *S. harbinensis* Ca12 is one of the

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probiotics that has passed resistance tests at low pH, bile salts, different candida tests, aggregation, coaggregation, has high adhesion to HT-29 cells, and inhibits HT-29 cell *Samonella* growth. Colares et al. (2021) added *S. harbinensis* Ca12 to grow optimally, supplemented with whey was marked by an increase in cell biomass of around 600%.

Table 1- Average consortium probiotic viability on different media and probiotic ratio

Different growth medium / Probiotics*	B1: (Oxoid (CM 359)	B2: (Coconut water (90%) + cassava flour (5%) + fish waste flour (5%)	B3: (Tofu liquid waste (90%) + cassava flour (5%) + fish waste flour (5%)	B4: (Whey tofu (90%) + cassava flour (5%) + fish waste flour (5%)	Average
A1: consortium probiotic* with ration 1:1:1:1:1	2.71^{ab}	2.79^{a}	1.40^{de}	1.58^{de}	2.16^{a}
A2: consortium probiotic* with ration 1:1:1:1:2	2.91^{a}	2.68^{ab}	1.38^{e}	1.30^{e}	2.07^{a}
A3: consortium probiotic* with ration 1:1:1:2:1	2.58^{ab}	3.02^{a}	1.28^{e}	1.16^{e}	2.01^{ab}
A4: consortium probiotic* with ration 1:1:2:1:1	1.68^{d}	2.76^{a}	1.20^{e}	1.27^{e}	1.73^{b}
A5: consortium probiotic* with ration 1:2:1:1:1	1.95^{cd}	2.76^{a}	1.32^{e}	1.19^{e}	1.80^{ab}
A6: consortium probiotic* with ration 2:1:1:1:1	2.00^{c}	2.76^{a}	1.32^{e}	1.20^{e}	1.82^{ab}
Average	2.31^{b}	2.80^{a}	1.34^{c}	1.28^{d}	

Note: different superscripts show highly significant different effects (P<0.01); *Consortium probiotic: *L. parabuchneri*: *L. buchneri*: *L. harbinensis*: *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner*

The results of observations on the parameter value of cell biomass can be seen in Table 2. Average probiotic consortium cell biomass. Based on the DMRT follow-up test, it showed that the A3B2 treatment was higher significantly different (P<0.01) compared to all treatments on cell biomass values. Leroy and de Vuyst (2001) found that the availability of carbon and nitrogen elements as well as the adequacy of minerals and vitamins in the media at the beginning of growth has the potential to form the final biomass. Pato et al. (2021) added, the basic material of carbon sources is the largest component in the medium and the product is in the form of cell biomass.

In the medium B2, containing coconut water, 100 grams of coconut water contains electrolytes including potassium (250 mg), phosphorus (20 mg), iron (0.29 mg), zinc (0.1 mg) and water (94.99 mg). g), as well as carbohydrates (3.71 g), sugar (2.61 g), protein (0.72 g), vitamin C (2.4 mg), Vitamin B6 (0.032 mg), Pantothenic Acid (0.043 mg), Folate (3 µg), Thiamine/vitamin B1 (0.03 mg) (USDA, 2016). In our research mineral and vitamin in the coconut water can be supported the growth of probiotic consortium faster than whey and tofu liquid, because mineral and vitamin signifcant increase in biomass. The same finding also reported by a group of researchers, whereas minerals and vitamins are very important in a microbial growth medium (Pandiyan et al., 2012; Santos et al., 2014; Chiang et al., 2015).

The pH greatly affects the growth of the probiotic consortium. As seen in Table 3, the initial pH of the medium was made the same for each growth medium, but after being inoculated with various probiotic consortium ratios, the reduction pH of the medium was very different. We can see in Tables 1 and 2, the biomass and viability also low if decreased pH lower. The results of the analysis of variance showed that there was an interaction between factor A (probiotic ratio) and factor B (type of media) which had a highly significant (P<0.01) effect on the decreased pH of the medium.

One of the uniqueness of lactic acid bacteria is the formation of acid at the end of fermentation, so that an effective method in determining the parameters of technological application is the formation of acid and the concentration of biomass. The decrease in pH is thought to be due to the active work of the probiotic consortium in carrying out the fermentation so that a lot of organic acids are produced, especially lactic acid. A group of researchers has been reported that increase acidity making increase taste and pH the medium become lower (Maslami et al., 2018: Anggraini et al., 2019; Harnentis et al., 2020; Marlida et al., 2022).

The process of decreasing the pH value in natural growth media is also caused by the release of hydrogen ions (H+) due to the breakdown of glucose which results in lactic acid which increases acidity. Febriningrum (2013) reported the pH value in a solution shows the amount of H+ concentration. The higher the pH value in a certain solution, the fewer H+ ions contained in the solution, conversely the lower the pH value, the greater the number of H+ ions.

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Table 2 - Average consortium probiotic biomass on different media and probiotic ratio

Different growth medium / Probiotics*	B1: (Oxoid (CM 359)	B2: (Coconut water (90%) + cassava flour (5%) + fish waste flour (5%)	B3: (Tofu liquid waste (90%) + cassava flour (5%) + fish waste flour (5%)	B4: (Whey tofu (90%) + cassava flour (5%) + fish waste flour (5%)	Average
A1: consortium probiotic* with ration 1:1:1:1:1	20.60^{gh}	20.62^{de}	20.82^{efgh}	20.72^{efgh}	20.69
A2: consortium probiotic* with Ration 1:1:1:1:2	20.71^{efgh}	20.30^{b}	20.87^{ef}	20.70^{fgh}	20.90
A3: consortium probiotic* with ration 1:1:1:2:1	20.67^{fgh}	22.47^{a}	20.75^{efgh}	20.68^{fgh}	21.14
A4: consortium probiotic* with ration 1:1:2:1:1	20.70^{fgh}	21.15^{cd}	20.92^{ef}	20.80^{efgh}	20.89
A5: consortium probiotic* with ration 1:2:1:1:1	20.59^{gh}	21.03^{de}	20.88^{ef}	20.88^{ef}	20.85
A6: consortium probiotic* with ration 2:1:1:1:1	20.55^{h}	21.27^{bc}	20.87^{ef}	20.83^{efg}	20.88
Average	20.64^{b}	21.31^{a}	20.85^{b}	20.77^{b}	

Note: different superscripts show highly significant different effects (P<0.01); *Consortium probiotic: *L. parabuchneri*: *L. buchneri*: *L. harbinensis*: *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner*

Table 3 - Average consortium probiotic pH decrease on different media and probiotic ratio

Different growth medium / Probiotics*	B1: (Oxoid (CM 359)	B2: (Coconut water (90%) + cassava flour (5%) + fish waste flour (5%)	B3: (Tofu liquid waste (90%) + cassava flour (5%) + fish waste flour (5%)	B4: (Whey tofu (90%) + cassava flour (5%) + fish waste flour (5%)	Average
A1: consortium probiotic* with ration 1:1:1:1:1	0.92^{g}	2.76^{b}	1.14^{f}	1.14^{f}	1.49
A2: consortium probiotic* with ration 1:1:1:1:2	0.93^{g}	2.81^{a}	1.15^{f}	1.75^{c}	1.66
A3: consortium probiotic* with ration 1:1:1:2:1	0.90^{gh}	2.84^{a}	1.17^{f}	1.62^{d}	1.63
A4: consortium probiotic* with ration 1:1:2:1:1	0.87^{h}	2.84^{a}	1.17^{f}	1.65^{d}	1.63
A5: consortium probiotic* with ration 1:2:1:1:1	0.91^{g}	2.82^{a}	1.17^{f}	1.63^{d}	1.63
A6: consortium probiotic* with ration 2:1:1:1:1	0.90^{gh}	2.81^{a}	1.16^{f}	1.56^{e}	1.60
Average	0.91^{bc}	2.81^{c}	1.16^{b}	1.56^{a}	

Note: different superscripts show highly significant different effects (P<0.01); *Consortium probiotic: *L. parabuchneri*: *L. buchneri*: *L. harbinensis*: *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner*

CONCLUSION

The conclusion of this study were the ratio of the best probiotic consortium is 1:1:1:2:1, with growth medium coconut water (90%) + cassava flour (5%) + fish waste flour (5%) which results in a viability value of: 3, 02, cell biomass: 22.47 mg/ml and a decrease in the pH of the medium by 2.84.

DECLARATIONS

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Ethical approval
This article does not contain any studies that would require an ethical statement.

Authors' contribution
M. Kudus Susalam and Y. Marlida planned all stages of the research, Harnentis assisted with research in the laboratory and Jamsari assisted in checking the final draft of the publication

Conflict of interests
All researchers involved in the research stated that there were no conflicts of interest either

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EFFECTS OF CONCENTRATE SUPPLEMENTATION ON REPRODUCTIVE TRAITS OF CO GOATS AND GROWTH PERFORMANCE OF THEIR KIDS UNDER GRAZING CONDITION

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ABSTRACT: The experiment was conducted in A Luoi district, Thua Thien Hue province of Vietnam, to evaluate the effect of concentrate supplementation on the reproductive traits of local Co goats and growth performance of their kids. A total of 20 pregnant Co goats in the last 1.5 month of pregnancy were monitored in two reproductive cycles. Goats were randomly divided into two dietary treatments as control (CG), in which animals freely grazed, and experiment (EG), in which concentrates were supplemented at 1.0% and 1.5% BW in the late gestation and lactation periods, respectively. After kidding, kids in each treatment were kept with their mother to access milk for the whole study period. In the CG, the kids have no supplements, and in the EG, the kids of 1 to 3 months of age were supplemented the concentrate at 1.5% BW/day. The results indicated that higher body condition scores and body weights of does after kidding, 1 and 2 months postpartum were in the EG than in the CG. The supplementation of concentrate also reduced the postpartum and kidding intervals; and increased the number of litters per year of does. Furthermore, the supplementation of concentrate improved significantly the weaning weight of kids and financial benefit per doe/per litter. In conclusion, the supplementation of concentrate in late pregnancy period and lactation diets improved reproductive traits of local Co goats and also concentrate supplementation in kids' diet improved growth performance under grazing conditions.

Keywords: Body condition score, Lactation, Local breeds, Pregnant, Small ruminants.

INTRODUCTION

Goat production has been developing in different regions of Vietnam, contributing to the poor alleviation and income improvement, especially for mountainous people (Abu Hatab et al., 2019; Liang and Paengkoum, 2019). In 2021, the goat population in Vietnam accounted approximately 2.7 million heads, consisting for 7.56 percent of total farm animals (GSO, 2021). The largest number of goats was observed in the Northern mountainous region and the Midland, followed by the North Central Coast and the Central coastal region (GSO, 2021).

The indiscriminate crossbreeding under natural selection or crossbreeding with imported breeds for several years were used to create the Vietnamese local goat breeds (Thuy et al., 2009; Binh et al., 2017). In recent years, goat production had been developing in Vietnam. Goat production played an important role in poverty and hunger alleviation in these rural areas (Nguyen et al., 2022). Almost of goats in A Luoi were kept by small-scale farms with an average of 5.2 heads per farm. Local Co goats were the most popular goat breed (89.84% of total) raised in A Luoi, followed by Bach Thao goats (8.02%) and crossbred goats (2.14%) (Hong et al., 2022). The animals were freely grazing in a fallow or crop harvested land areas.

The increased productivity could only be achieved through improvement of animal health, nutrition and reproduction management (Norton et al., 2009). However, these practices have been mostly restricted to the mountainous and remote areas of Vietnam. The litter size and number litter per year of local Co does were 1.5 and 1.4, respectively, lower than Bach Thao goats (Thuy et al., 2009). However, in our recent survey, the number litter per year of local Co goat in A Luoi district was lower than the data published by Thuy et al. (2009). The low pre- and post-weaning growth rates were also recorded in local Co goats in A Luoi with 1.56 kg, 6.75 kg, and 10.12-12.36 kg at birth, 3 and 6 months of age, respectively (Hong et al., 2022). The low productivity in goat farming in A Luoi was a consequence of endemic diseases, poor nutrition and a lack of reproduction management (Hong et al., 2022). Local Co goats were allowed to graze daily from 10:00 h to 16:00 h in grazing areas. They were kept in rudimentary facilities and their performance depended on grazing poor natural pastures with no supplementary feeding in rudimentary facilities. Therefore, it was difficult to meet the nutrient requirements for goats, especially in the gestation and lactation periods. These factors had all contributed to the high mortality rate, low growth performance of kids and poor reproductive efficiency of does, which in turn, resulted in severe economic losses of goat production. Furthermore, the poor management practices in farming may cause deterioration of genetic potential and productivity of local Co goat in A Luoi district (Hong et al., 2022).

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Exploitation of available local feed resource to overcome the above facts was our objective research. Many authors indicated that supplementation of feeds during late pregnancy had improved birth weight, enhanced the immune system, reduced mortality and the incidence of hypothermia (Hashemi et al., 2008; Mahboub et al., 2013). According to Sharma and Ogra (1990), supplementation of concentrate had improved significantly total DM intake, growth rate and FCR of kids. Therefore, feeding and nutrition improvement, efficient use of the feed resources were the keys to enhance goat productivity (Nampanzira et al., 2015). However, very limited information related to the performance of Co goats and their kids under grazing on poor conditions, especially in A Luoi, where has poor husbandry practices been published. In this study, the effects of supplementation of concentrate on local Co goat reproductive and growth performance of their kids under grazing condition in A Luoi district was investigated.

MATERIALS AND METHODS

This study was carried out from 2020 to 2022 with the permission from Hue University. The experiment was performed based on the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines and the international ethical regulations for farm animal studies (Hurnik and Lehman, 1988).

Location of the study

This research was conducted between the years 2021 and 2022 in A Luoi district, Thua Thien Hue province, Vietnam. A Luoi is mountainous district with 600-800 meters above the sea level. This district is the habitant of minority people.

Animal, diet and experimental design

In this study, 20 pregnant Co goats of average weight of 28.3 kg, which were both in the third or fourth parity and in the last 1.5 month of pregnancy, were monitored in two reproductive cycles. The goat body condition scores (BCS) at the beginning of the experiment were recorded by 3/5 points. The goats were balanced for parity and randomly divided into two dietary treatments as control diet (10 heads) and experiment diet (10 heads) to estimate the effect of supplemental feeding on goat reproductive efficiency and performance of their kids. All animals were allowed to graze daily from 10:00 h to 16:00 h in grazing areas as normally farmers did. The goats in CG were not supplemented concentrate. Meanwhile, the goats in EG were supplemented concentrate at 1.0% BW/day and 1.5% BW/day in the late gestation (1.5 months before giving birth) and lactation period (within 2 months postpartum), respectively. All local Co does in the EG were kept in a pen. The ingredients and chemical composition of formulated concentrate for does and kids are presented in Table 1. The animals in EG were fed concentrate twice a day (7:30 – 9:30 h and 17:00 – 19:00 h). After kidding, all the residues from the does' feeding troughs were removed at the end of their feeding time before the kids were mixed with their does. Feeds were renewed daily. Clean water was offered ad libitum, and changed before 16:00 h. The goat body condition score was recorded every 2 weeks to adjust to the BCS to 3.5/5 at kidding time. The body weight of goats was recorded monthly in the morning before feeding.

After kidding, the does and kids were weighed to record the parturition weight and birth weight, respectively, within 2 hours. Kids were kept with their mother for the whole study period. All kids had access to their dams' milk. No additional feed was provided to the kids of the does in the control group during the study. From 10 days to 1 month old, the kids of does in the treatment group were creep feeding with 69.5% maize and 29.5% roasted soybean diet. From 1 to 3 months of age, the kids in the treatment group were fed with concentrated feed at 1.5% BW/day twice a day (7:30 – 9:30 h and 17:00 – 19:00 h). The ingredients and chemical composition of concentrated feed for kids are given in Table 2. During feeding, the kids in the treatment group were separated from the does and kept in the feeding compartments adjacent to their pens. Feeds were renewed daily. Clean water was offered *ad libitum*, and changed before 16:00 h. The body weight of kid goats was recorded monthly in the morning before feeding.

Table 1 - The ingredients and chemical composition of concentrate for pregnant doe and kids

Ingredients (kg/100 kg as dry matter)	Doe	Kids
Rice bran	30.0	30.0
Maize meal	22.5	29.0
Cassava meal	25.0	15.0
Dried soybean residue	20.0	-
Roasted soybean	-	24.0
Urea	0.5	-
Vitamin-mineral premix*	1.0	1.0
Salt	1.0	1.0
Total	100	100
Chemical composition (%) and price		
Moisture	14	14
Crude protein	16	18
Crude fibre	7.8	7.5
Metabolizable energy (kcal/kg)	2,900	3,000
Price (1,000 Vietnam dong)	7.8	10.5

* 1 kg contains 3,600,000 UI vitamin A; 520,000 UI vitamin D3; 2,100 UI vitamin E; 200 mg vitamin K3; 600 mg vitamin B1; 160 mg vitamin B2; 200 mg vitamin B6; 3,200 mcg vitamin B12; 14,000 mcg biotin; 160 mcg folic acid; 3,600 mg nicotinic acid; 1,600 mg pantothenic acid; 32,400-39,600 mg Fe; 14,400-17,600 mg Cu; 46,800-57,200 mg Zn; 18,000-22,000 mg Mn; 39.6-48.4 mg Co; 540-660 mg I; 36-44 mg Se, rice hull and $CaCO_3$

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Measurements

Reproductive traits included body score condition (BSC) of does were determined based on a 5-point scale ranging from 1 (very thin) to 5 (very fat); body weights of does at the beginning of the experiment, after kidding, 1 and 2 months postpartum; litter size; postpartum interval (days); kidding interval (days); number of litters per year; and concentrate intake. Growth perfomance included body weights at birth, 1 and 2 months old of kids; and concentrate intake of kids, and estimating benefit of two dietary treatments. The feed intake was calculated based on total feed offered and residues in the feeding troughs. The weight loss percentage of does (%) was also calculated as below:

The weight loss percentage of doe (%) = (Doe parturition weight – Doe 1-month postpartum weight)/ Doe parturition weight × 100.

Statistical analysis

All the collected data were compiled, organized and analysed using SPSS software (version 15.0; SPSS Inc.). The Paired-samples T-Test was used to compare the means with a 95% confidence interval. The differences were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

Reproductive performance of Co does

The effects of concentrate supplementation on reproductive performance of Co does are presented in Table 2. There was no significant difference in body weight and BCS of goats between two groups at the beginning of study. The BCS of Co does in EG was increased, and reached 3.5 before kidding. Higher body weight of does after kidding, 1 and 2 months postpartum were observed ($p<0.05$) in EG than in CG. The supplementation of concentrate affected the weight loss percentage, postpartum interval, kidding interval, and number of litters per year of goats. The results in Table 2 indicated that feeding goats with concentrate from the 1.5 months before kidding to 2 months postpartum reduced the postpartum and kidding intervals; and increased the number of litters per year of does.

Table 2 - Effects of supplemental concentrate feeding on reproductive performance of Co does (n=10)

Parameters	CG	EG	SEM	p-value
Initial weight, kg	27.6	29.0	0.58	NS
Initial BCS	3.0	3.1	0.05	NS
BCS of does at kidding	3.0	3.5	0.04	***
Parturition weight, kg	24.6	32.9	0.80	***
1-month postpartum weight, kg	22.8	31.7	0.97	**
Weight loss percentage, %	7.4	3.4	1.23	NS
2 months postpartum weight, kg	23.4	32.0	0.73	***
Litter size, head per litter	1.30	1.30	0.15	NS
Postpartum interval, days	105.2	49.6	0.87	***
Kidding interval, days	254.8	194.6	0.49	***
Number of litters per year	1.43	1.88	0	***
Total concentrate intake, kg DM	0	43.1		

*p < 0.05; ** p < 0.01; *** p < 0.001; NS = not significant; CG: control; EG: experiment

Growth performance of Co kids

During the study, a total of 23 kids were born in each group. The effects of supplementation of concentrate on growth performance of kids are presented in Table 3. The average birth weight of kids in EG was 24.2% higher than that in CG; however, this difference was not significant. Previous results reported that the birth weight of kids from does given supplements were heavier than those from un-supplemented ones (Sumartono et al., 2016; Godah et al., 2022). According to Godah et al. (2022), the low plane of nutrition during late pregnancy of goats kept almost exclusively on the natural pasture would be unable to adequately nourish the fetus in the final stage of pregnancy and consequently birth weight will be reduced.

The body weight of goat in EG was dramatically increased and reached the average weaning weight of 10.0 kg (Table 3). Meanwhile, the slow growth rate was observed with kids in CG and was 5.0 kg per head at weaning. The average daily gain of kids at pre-weaning period in EG was higher significantly than that in CG. This result shows that supplementation of concentrate from 10-day-old to weaning enhances growth performance of kids. According to Mandal et al. (2006), the birth weight and early growth rate of animals are affected by the genetic potential, the maternal and environmental factors. The higher growth rate at pre-weaning period cannot only be sustained by milk supply from their dams (Htoo et al., 2015). Many studies also indicated that supplementation of feed for pregnant animals during late gestation period may provide adequate energy and protein so they produce more milk yield that improved growth rate of their kids (Oeak et al., 2005; Castillo-Gutierrez et al. 2022; Godah et al., 2022). Furthermore, the supplementation of

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creep feed can help improve the pre-weaning growth performance of goat kids and increase the net profit for the farmer (Machen, 2002; Htoo et al., 2015).

Economic benefit of supplementation of concentrate

The economic benefit of concentrate supplementation in diets for doe and their kids is presented in Table 4. In this calculation, the cost of labour, housing, veterinary services were not counted. Results on preliminary economic benefit showed that the one reproductive goat and her kid received supplemented concentrate in diets could get benefit of 489,41 Vietnam dong/litter as compared to the control (without concentrate supplementation), and this finding is in agreement with Abou-Elkhair et al. (2020) who studied economic impact of energy concentration of maternal diets in performance of kids. In case of twin kidding, the more benefit could be achieved. In addition, the early concentrate feeding for kids would stimulate rumen development, improve growth performance, and reduce diseases.

Table 3 - Effects of supplemental concentrate feeding on growth performance of kid

Parameters	CG	EG	SEM	p-value
Birth weight, kg	1.65	2.05	0.18	NS
1 month old weight, kg	2.77	4.81	0.51	**
2 months old weight, kg	4.37	7.00	0.79	**
Weaned weight at 3 months old, kg	5.01	10.00	0.39	***
Average daily gain, g/day	37.3	88.3	4.00	***
Total concentrate intake, kg DM	0	5.3		

*p < 0.05; ** p < 0.01; *** p < 0.001; NS = not significant; CG: control; EG: experiment

Table 4 - Estimated benefit of concentrate supplementation (1,000 VND)

Parameters	CG	EG
1. Weight gain at weaning, kg per head	3.36	7.95
2. Increased weight gain compare to the control group at weaning, kg	0	4.59
3. Amount of concentrate consumed by doe during the 105 days of experiment, kg/doe	0	43.1
4. Feed cost for doe during 105 days of experiment = (3) x 7.8*	0	336.18
5. Amount of concentrate consumed by kid from 30-90 days of age, kg/kid	0	5.3
6. Feed cost for kids from 30-90 days of age = (5) x 10.5*	0	55.65
7. Save dues reduction of kidding interval = (4)/105 x 60.2 days		192.74
8. Extra profits from increased weigh gain of kid at weaning = (2) x 150**		688.50
9. Balance or benefit per litter = (8)-(4)-(6)+(7)		489.41

*: Price of concentrate was calculated by ingredient price; **: Price at farm gate; VND : Vietnam dong; CG: control; EG: experiment

CONCLUSION

It can be concluded that supplementation of concentrate in diets for local Co doe from late pregnancy to 2 months postpartum and early concentrate supplementation for their kids improved doe reproductive and growth performance of weaning kids, and increased benefit for reproductive goat farm. It can be suggested that concentrate supplementation with local feed for does and their kids helps economizes goat production in upland areas.

DECLARATIONS

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Authors' contribution

H.T.T. TRAN contributed at designing the experiment, data collection, data analyses, giving comment on the manuscript. A.T.Q. NGUYEN contributed to implementing the experiment, data collection. H.T. DUONG contributed to implementing the experiment, data collection. C.V. NGUYEN contributed to implementing the experiment, data collection. T.H. HOANG contributed to implementing the experiment, data collection. N.T. TRAN contributed at implementing the experiment, data collection. D.V. DINH contributed by giving comments on the manuscript. B.X. NGUYEN contributed to designing the experiment, giving comments on the manuscript. C.L.Q. HO contributed at writing the manuscript, designing the experiment, data collection, data analyses. All authors read and approved the final manuscript.

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Conflict of interests

We have no conflict of interest for this article.

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IMPACT AND PREVALENCE OF NEWCASTLE DISEASE AND ASSOCIATED RISK FACTORS IN VILLAGE CHICKENS IN SOUTHERN NIGER

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ABSTRACT: The present study was conducted to determine the prevalence of Newcastle disease and to identify potential risk factors in village chickens in Niger. A total of 1,627 serum samples were collected using a stratified random sampling method with proportional allocation. Samples were collected from village breedings in the departments of the Maradi region (Guidan Roumdji, Madarounfa, Aguié, Gazaoua, and Tessaoua), departments of Zinder region (Takeita, Kantché, Magaria, Dungass, and Mirriah), and cities of Maradi and Zinder. Data on risk factors were collected through an interview with the herders. All collected sera were subjected to competitive enzyme-linked immunosorbent assay (cELISA) to detect Newcastle disease virus-induced antibodies. The findings were indicative of 302 positive sera, representing an overall seroprevalence of 18.6%. The Student's t-test at $p<0.05$ revealed a significant difference between regions and among some departments. Furthermore, the logistic regression test identified the agroecological zone, type of breeding, species mix, and the origin of the animals as risk factors associated with seropositivity to Newcastle disease virus. The present results confirmed the exposure of village chickens to the Newcastle disease virus, emphasizing the need to intensify vaccination campaigns and educate poultry farmers on adopting biosecurity measures.

Keywords: Newcastle disease, Risk factors, Vaccination, Village chickens, Niger.

INTRODUCTION

Niger is one of the countries with an agricultural vocation, so its economy is mainly based on the rural sector, including agriculture, breeding, and fishing. In 2020, the rural sector employed 80% of the active population and contributed 37.7% of the Gross Domestic Product (HCI3N, 2021). Poultry production is one of the most important economic activities, 98% of which is carried out by rural populations (HCI3N, 2021). Based on recorded poultry sales statistics, national poultry meat production was estimated at 3,592 tons of carcass equivalent in 2017 (MAG/EL, 2020). These data do not take into account the total meat production of traditional and modern poultry farming due to the lack of a reliable information system on the sector. The production of eggs for consumption was estimated at 596,717,980 eggs in 2019. In addition, the poultry capital was estimated at more than 20 billion FCFA in 2019 (MAG/EL, 2020). As in other developing countries, rural Niger residents depend significantly on poultry for their household income and dietary protein intake (Amoia et al., 2021). However, poultry practices involve few biosecurity measures and a high risk of infectious diseases (Getabalew et al., 2019; Chowdhury et al., 2020), including Newcastle disease (Otte et al., 2021). In many low-income, food-deficit countries, Newcastle disease causes significant economic losses in poultry farming. These losses are related to high poultry mortality, vaccination costs, drop in production, cost of treating animals, as well as expenses related to the implementation of biosecurity measures (Ipara et al., 2021). In traditional poultry farming, Newcastle disease kills an average of 55% of poultry per year (Antipas et al., 2012). It affects the potential source of nutrition for rural households, their source of income, and discourages them from investing in poultry breeding (de Bruyn et al., 2017; Waweru et al., 2023).

Newcastle disease is a highly contagious infectious disease of poultry caused by virulent strains of avian paramyxovirus type 1 (OIE, 2022). It is clinically characterized by respiratory impairment, nervous system impairment, gastrointestinal disorders, and reproductive impairment (Caroline, 2022). Many species of birds, both domestic and wild, are susceptible to Newcastle disease, but chickens are particularly susceptible. The pathogenicity of Newcastle disease in chickens is primarily determined by the virus strain, viral dose, and age of the chicken, environmental conditions, administration route, and transmission mode (Getabalew et al., 2019).

Newcastle disease is endemic in Niger as it is in many developing countries (Dimitrov et al., 2016; Absalón et al., 2019; Toroghi et al., 2020; Amoia et al., 2021) and negatively impacts farm household economies, dietary diversity, and consumption of animal-based foods (Knueppel et al., 2010; McElwain and Thumbi, 2017). Due to its severe nature and

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disastrous consequences, Newcastle disease is included in the list of notifiable diseases in Niger (MEL, 2011) and the World Organization for Animal Health (OIE, 2022). As such, its control represents a major challenge for national veterinary services.

The objective of the present study is to contribute to a better knowledge of the epidemiology of Newcastle disease in Niger, more specifically, to determine the frequency of Newcastle disease, its distribution, and the risk factors associated with its occurrence. The findings can provide those in charge of the national animal health policy with a guiding tool in surveillance and control.

MATERIALS AND METHODS

Ethical regulation

Due to the absence of an Animal Care Committee available at the University of Lomé at the time of this research, the research was conducted under the supervision of the research team leader following the guidelines of the Canadian Council on Animal Care (2009).

Study area

This study was conducted in the southern part of the Maradi and Zinder regions bordering the Federal Republic of Nigeria. These include the departments of Guidan Roumdji, Madarounfa, Aguié, Gazaoua, and Tessaoua in the Maradi region and the departments of Takeita, Kantché, Magaria, Dungass and Mirriah in the Zinder region, as well as the cities of Maradi and Zinder. The study area is covered by the Sahelian agroecological zone and the Sahelo-Sudanese zone. The Sahelian zone receives an average annual rainfall of 350-600 mm, and the Sahelo-Sudanese zone accumulates more than 600 mm of rain per year (Wata et al., 2012). The two study regions have an area of 197,574 km² and an estimated population of 9,584,421 in 2021, or 40.6% of the total population (INS, 2019). The investigated sites have a large poultry flock estimated at 6,205,390 birds in 2017, or 31.82% of the national flock (MAG/EL, 2020). Figure 1 illustrates the study area.

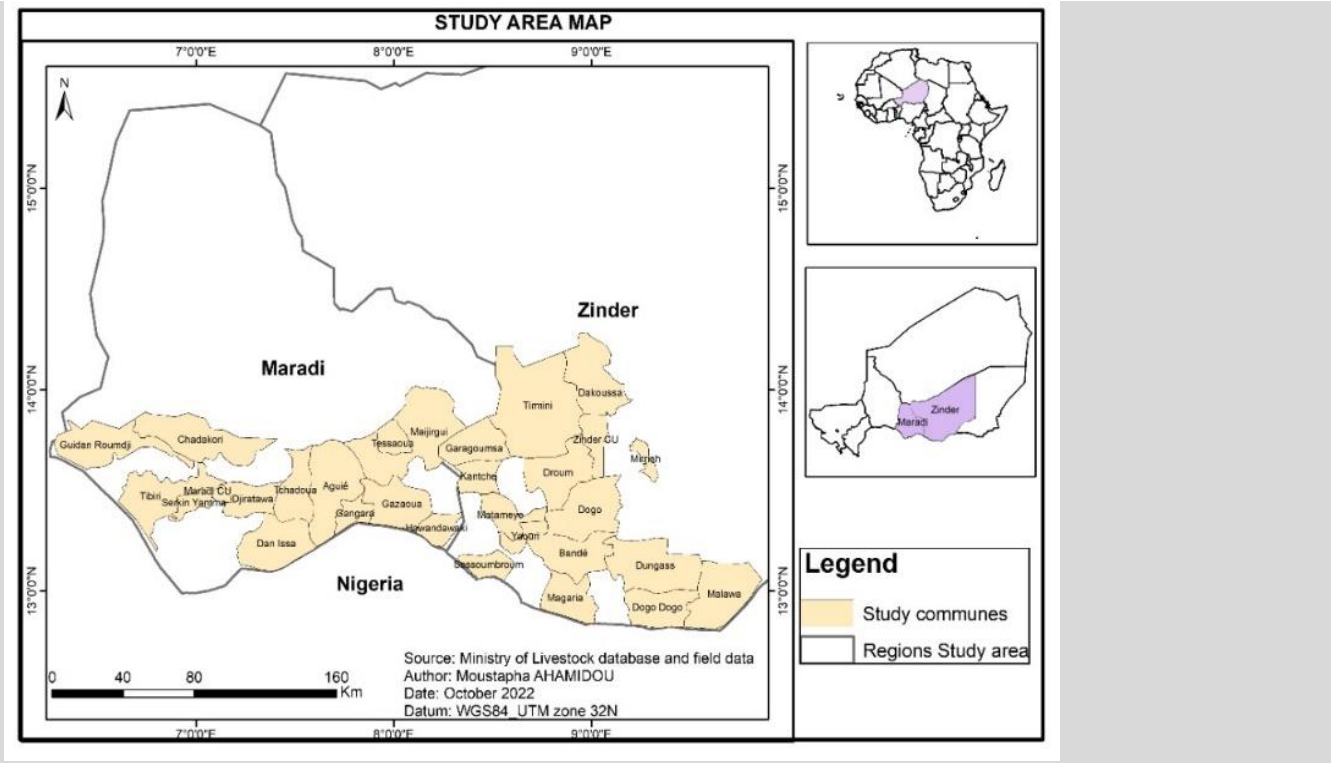

Figure 1- Map of the study area

Sampling method

Samples were selected using a stratified random sampling method with proportional allocation to the size of agricultural households. First, the agricultural departments were selected from each of the two regions along the borders with Nigeria and the cities of Maradi and Zinder. In the second step, a maximum of three communes or boroughs were selected per department or city. In the next step, a maximum of three villages were selected within each commune or city. Using the national directory of localities, the number of farm households surveyed per commune was determined in proportion to the total number of farm households. Then, the farm households to be surveyed were drawn from the list of households drawn up by the village chief. Finally, a maximum of three unvaccinated chickens were collected from each surveyed household after the owners' permission.

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Data and sample collection

Data were collected from November 2021 to January 2022 using a survey questionnaire with semi-open and closed-ended questions. The questionnaire was administered face-to-face using the KoBoCollect v2021.2.4 collection tool. Information collected included the geographic location of sites and data on poultry practices.

For sample collection, 1.5 to 2 ml of blood was collected from the chickens' wing veins in tubes without any anticoagulant. Serum samples were separated from clotted blood samples by centrifugation at 1500 rpm for 5 minutes. Serum samples were then decanted, aliquoted into microtubes, and frozen at -20°C at the Central Livestock Laboratory of Niamey, Niger, until testing.

Methods of analysis and interpretation of results

The competitive enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies to the Newcastle disease virus in the collected sera. The diagnostic kits (Reference NDVC-12P LOT K34) were obtained from the Innovative Diagnostics IDVet Laboratory in Marseille, France. The procedure used in the laboratory was conducted according to the manufacturer's instructions. The reading of the plates was done with the SkanIt Software v.3.1 at an optical density (OD) of 450 nm. The assay is validated if: the mean OD value of the negative control (ODNC) is greater than 0.6 (ODNC > 0.6) and the percentage inhibition (PI%) of the positive control (mean ODPC value) is greater than 40% (PIPC > 40%). For each sample tested, the percentage inhibition value was calculated (PI%) with the following formula: PI% = (ODNC -ODsample /ODNC)*100. Samples with a PI% greater than 40% were recorded as positive, those with a PI of 30-40% were considered doubtful, and samples with a PI less than 30% were categorized as negative.

Statistical analysis

The data obtained were analyzed using STATA 13.0 software. Student's t-test was used to compare the prevalences of Newcastle disease between departments and regions. A logistic regression model was adopted to determine the risk factors associated with Newcastle disease. The variable seropositivity to Newcastle disease was explained through the explanatory variables, including the agroecological zone, chicken sex, breeding type, species mix, and origin of the animals. Individuals (chickens) were then classified into two groups with regard to their seropositivity (those with and those without seropositivity to Newcastle disease). This variable was dichotomous or binary, and the candidate explanatory variables were all categorical.

RESULTS

Prevalence of Newcastle disease

Tables 1 and 2 tabulate the distribution of Newcastle disease prevalence in village chickens by commune and department. Of 1627 sera tested, 302 were positive for Newcastle disease virus, with an overall prevalence of 18.6%. All study communes had a prevalence of 3.3-46.7%, except for the commune of Kantché in the Zinder region, where the prevalence was 0.00%. The highest prevalence was obtained in the city of Maradi (46.7%) and the lowest in the commune of Yaouri (3.3%). Figure 2 shows the distribution map of Newcastle disease prevalence by commune and study department.

The proportion of Newcastle disease prevalence of each department or city was compared to that of all other departments or cities using the Student's t-test at a significance level of 0.05. The results of the comparison tests between the prevalence proportions of the departments or cities took two by two showed a significant difference between the departments of Takeita and Guidan Roumdji, Takeita and Madarounfa, Takeita and Aguié, Kantché and Guidan Roumdji, Kantché and Madarounfa, Kantché and Aguié, Dungass and Guidan Roumdji, Dungass and Madarounfa, Dungass and Aguié, Mirriah and Guidan Roumdji, Mirriah and Madarounfa, Mirriah and Aguié, Maradi city and Takeita department, Maradi city and Kantché department, Maradi city and Dungass department, Maradi city and Mirriah department, Zinder city and Guidan Roumdji department, Zinder city and Madarounfa department, Zinder city and Aguié department, and Maradi city and Zinder city ($p < 0.05$). However, no significant difference was found between the Gazaoua and Madarounfa departments, the Gazaoua department and Maradi city, the Magaria department and Zinder city, and the Tessaoua department and Zinder city regarding prevalence proportions ($p > 0.05$).

The Student's t-test revealed a significant difference between the Maradi and Zinder regions in terms of Newcastle disease prevalence ($p < 0.05$). The highest prevalence was recorded in the Maradi region (Table 3).

According to the results of the model, seropositivity was significantly explained by the agroecological zone, the type of breeding practised, the composition of the flock, and the origin of the poultry (Figure 3).

The risk of Newcastle disease was not the same in the two agroecological zones. It was 1.45 times higher in the Sahelo-Sudanese zone than in the Sahelian zone. Newcastle disease was more prevalent among herders who practiced divagation than those who practiced semi-divagation. The risk of a chicken being seropositive in a mid-scavenging system was 0.42 times lower than that of a scavenging system. Of note, the effect of confinement could not make a significant difference between the two systems. Poultry flocks of different species indicated different exposure rates to Newcastle disease. There was a higher risk of Newcastle disease in a breeding system composed of several avian species, compared to one containing only one particular species. The risk is 1.5 times higher for poultry in multi-species breeding than for a breeding group composed of only one avian species. Finally, when live poultry is bought on the local market, the risk of

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Newcastle disease was 2.19 times higher than when he used other sources of supply, such as the neighboring village, traditional and modern hatcheries, and imports (Tables 4 and 5).

Table 1 - Distribution of Newcastle disease prevalence in village chickens by commune or city

Regions	Departments/Cities	Communes/Cities	Samples tested	Positive samples	Prevalence (%)
Maradi	Guidan Roumdji	Chadakori	66	17	25.8
		Tibiri	79	23	29.1
		Guidan Roumdji	55	24	43.6
	Madarounfa	Serkin Yamma	23	7	30.4
		Djiratawa	48	17	35.4
		Dan Issa	37	6	16.2
	Aguié	Tchadoua	50	13	26
		Aguié	60	23	38.3
	Gazaoua	Gazaoua	50	11	22
		Gangara	32	9	28.1
	Tessaoua	Tessaoua	75	17	22.7
		Maijirgui	42	7	16.7
		Hawandawaki	35	8	22.9
	Maradi city	Maradi city	15	7	46.7
Zinder	Dakoussa	Dakoussa	53	10	18.9
		Tirmini	80	7	8.8
		Garagoumssa	36	2	5.6
	Kantché	Kantché	32	0	0
		Matamey	34	7	20.6
		Yaouri	30	1	3.3
	Magaria	Sassoumbroum	54	5	9.3
		Magaria	87	20	24.1
		Bandé	32	3	9.4
	Dungass	Malawa	87	9	10.3
		Dungass	83	12	14.5
		Dogo dogo	49	5	10.2
	Mirriah	Dogo	96	11	11.5
		Droum	97	9	9.3
		Mirriah	60	7	11.7
	Zinder city	Zinder city	50	5	12
Total			1627	302	18.6

Table 2- Distribution of Newcastle disease prevalence in village chickens by department or city

Departments/Cities	Samples tested	Positive samples	Prevalence (%)	P-Value
Guidan Roumdji	200	64	32.0a	
Madarounfa	108	30	27.8a	
Aguié	110	36	32.7a	
Gazaoua	82	20	24.4a	
Tessaoua	152	32	21.1b	
Maradi city	15	7	46.7a	< 0.05
Takeita	169	19	11.2b	
Kantché	96	8	8.3b	
Magaria	173	28	16.2b	
Dungass	219	26	11.9b	
Mirriah	253	27	10.7b	
Zinder city	50	5	10.0b	
Total	1627	302	18.6	

Table 3- Prevalence of Newcastle disease by region

Regions	Samples tested	Positive samples	Prevalence (%)	P-Value
Maradi	667	189	28.3a	< 0.001
Zinder	960	113	11.8b	
Total	1627	302	18.6	

The proportions of Newcastle disease prevalence of the departments, cities and regions with a different letter (a, b) are significantly different at $p < 0.05$.

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Table 4- Distribution of prevalence according to potential risk factors

Variables	Modalities	Samples tested	Positive samples	Prevalence (%)
Agro-ecological zone	Sahelian	638	83	13.0
	Sahelo-sudanian	989	219	22.1
Gender of chicken	Male	362	67	18.5
	Female	1265	235	18.6
Breeding methods	Scavenging system	1277	262	20.5
	Mid-scavenging system	338	38	11.2
	Claustration	11	2	18.2
Composition of the herd	Monoespecies	982	157	16.0
	Polyespecies	645	145	22.5
Origin of poultry	Local market	1033	218	21.1
	Neighboring village	501	70	14.0
	Traditional hatchery	66	10	15.2
	Modern hatchery	17	3	17.6
	Importation	10	1	10.0

Table 5- Risk factors associated with Newcastle disease in village chickens in the study area

Factor	Categories	Odd_ratio	IC (odd_ratio)	P-value
Agro-ecological zone	Sahelian		Reference	
	Sahelo-Sudanian	1.45	1.08 – 1.95	0.013*
Gender of chicken	Male		Reference	
	Female	1.01	0.75 – 1.38	0.9
Breeding methods	Scavenging system		Reference	
	Mid-scavenging system	0.42	0.28 – 5.28	< 0.001**
	Claustration	1.36	0.28 – 5.28	0.7
Composition of the herd	Monoespecies		Reference	
	Polyespecies	1.5	1.15 – 1.95	0.003**
Origin of poultry: local market	No		Reference	
	Yes	2.19	1.62 – 2.99	< 0.001**

* Significant, and ** Very significant. Reference: the comparison between the categories of the factor was made with respect to a chosen category.

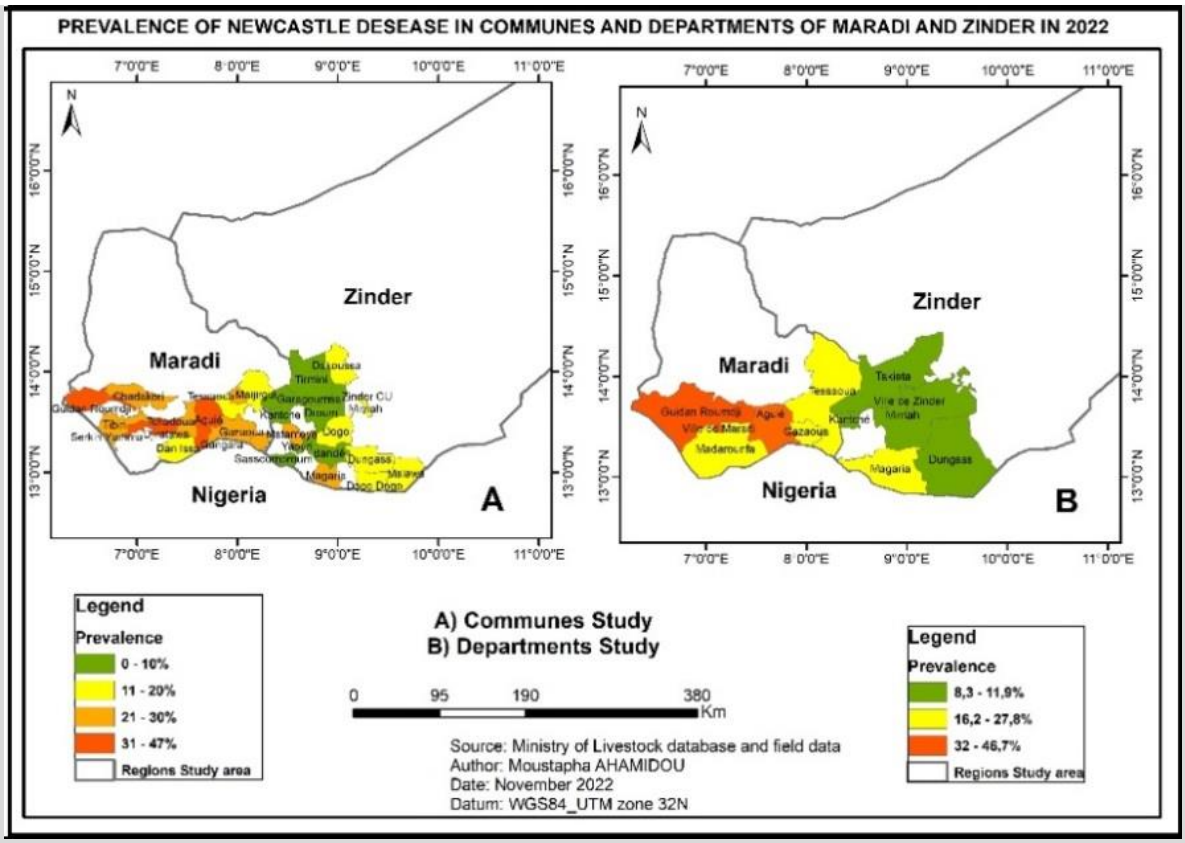

Figure 2 - Map of Newcastle disease prevalence distribution from November 2021 to January 2022 in the study area; A: Communes in the study area; B: Departments in the study area; the prevalence distribution is divided into four different colored classes: Low prevalence, medium prevalence, high prevalence, and very high prevalence.

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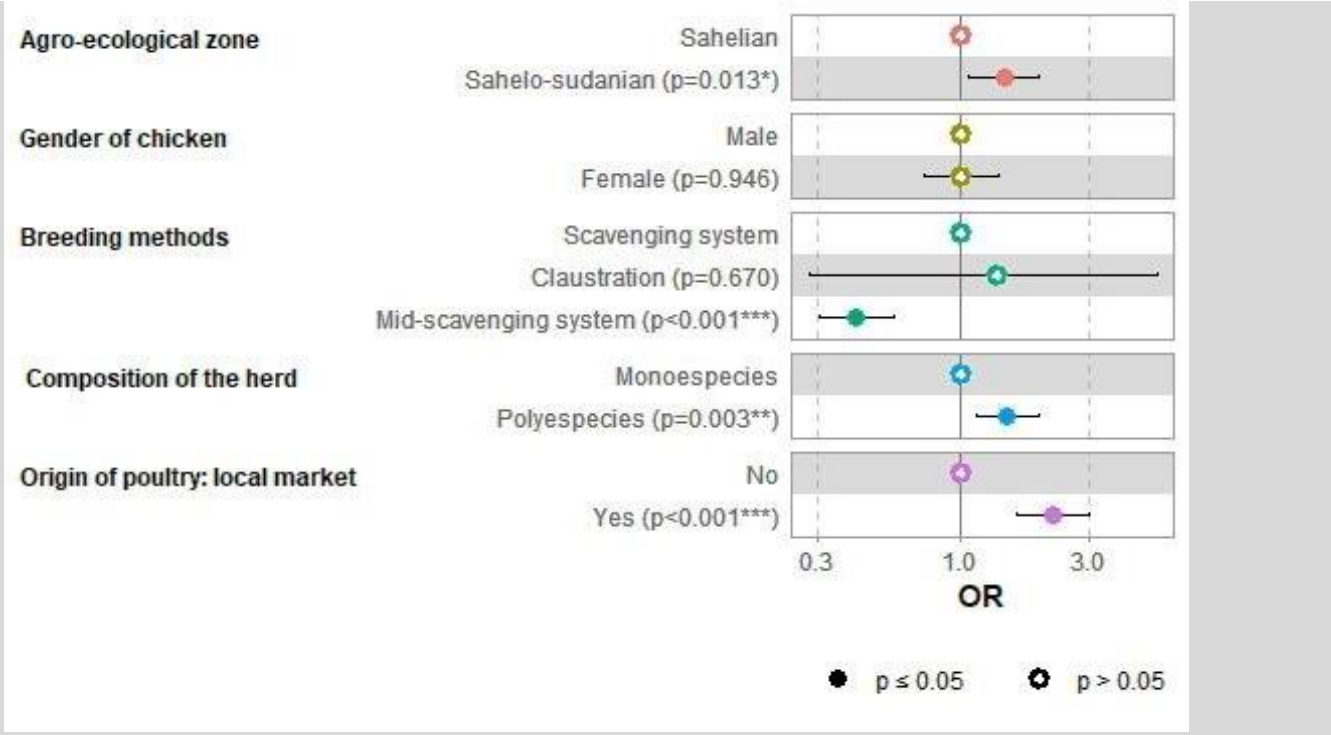

Figure 3 - Graphical presentation of potential risk factors associated with Newcastle disease

DISCUSSION

The present serological study revealed the presence of antibodies against the Newcastle disease virus in village chickens sampled in all communes of the study area except Kantché. These results consequently showed the presence of the Newcastle disease virus in village chickens in the entire study area. In both study areas, an overall seroprevalence of 18.6% was obtained using ELISA. These results are comparable to several other previous studies. Abraham-Oyiguh et al. (2014), and Meher et al. (2020) reported a prevalence of 17%, and 19.83% in their study conducted in Nigeria, and Bangladesh, respectively. The prevalence of Newcastle disease in the present study is higher than in previous reports, where Mai et al. (2014) in Cameroon and Sahoo et al. (2022) in India revealed a prevalence of 10.5% and 11.7%, respectively. However, the overall prevalence in the present study is lower, compared to those previously reported by Channa et al. (2020) and Aliye et al. (2022). These authors reported a prevalence of Newcastle disease at 48.7% and 50% in Ethiopia and Pakistan, respectively.

The logistic regression test revealed that the Newcastle prevalence was significantly higher ($p < 0.05$) in the Sahelo-Sudanian agro-ecological zone (22.1%) than in the Sahelian agro-ecological zone (13%). Therefore, the risk of seropositivity to the Newcastle disease virus was higher in the Sahelo-Sudanian zone than in the Sahelian zone. The reports of the present study are in agreement with those of a Malian study, where Molia et al. (2017) reported that Newcastle disease is more likely to be present in the Sudanian agro-ecological zone than in the Sahelian agro-ecological zone. Njagi et al. (2010) reported climate as a risk factor in the occurrence of Newcastle disease in indigenous chickens of Kenya. However, these authors found that the prevalence of Newcastle disease was significantly higher in the hot dry zone (17.8%) than in the humid cool zone (9.9%).

Poultry raised in a free-range breeding system (20.5%) were significantly different from those raised in a semi-free-range system (11.2%) regarding the prevalence of Newcastle disease virus antibodies ($p < 0.05$). The Newcastle disease seropositivity of a bird kept in a scavenging system was higher than that of a bird kept in a semi-divisional breeding system. The present observations corroborate those of a previous study conducted in Nigeria, where Lawal et al. (2015) reported exposure of animals to infectious diseases, such as Newcastle disease, in the extensive breeding system.

The prevalence of Newcastle disease virus seropositivity was significantly higher in multi-species avian breeding (22.5%) than in single-species avian breeding (16%, $p < 0.05$). The results of the present study are in agreement with those of a serological study conducted in Nigeria on Newcastle disease virus antibodies in local chickens, ducks, and pigeons, where Abah et al. (2020) revealed that the prevalence of Newcastle disease virus antibodies was higher in ducks (20.5%) than in chickens (10%) and pigeons (7.5%). These authors reported the presence of the Newcastle disease virus in the avian population, and the epidemiological role that ducks, chickens, and pigeons may play in transmitting the Newcastle disease virus to other susceptible poultry when kept in close proximity.

There was a significant difference between chickens purchased from the local live poultry market (21.1%) and those purchased from other poultry supply sources (14.1%) regarding Newcastle disease seroprevalence ($p < 0.05$). The logistic regression test showed that chickens purchased from the local live poultry market were more likely to be seropositive for

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Newcastle disease virus than those purchased from other poultry sources. These observations are consistent with a study by Ipara et al. (2019), reporting the positive effects of complex chicken trade channels on the frequency of Newcastle disease outbreaks in Kenya. Lawal et al. (2015) found that 65.5% of farmers usually took their animals to market for sale to reduce economic losses during outbreaks, which could amplify the transmission of Newcastle disease among poultry of different species present in the same market.

CONCLUSION

The present study established that Newcastle disease is endemic in the southern part of the Maradi and Zinder regions of Niger. A higher prevalence was observed in the Maradi region (28.3%), compared to the Zinder region (11.8%) with a significant difference. The agroecological zone, type of breeding practised, species mix, and source of poultry supply were statistically identified as the main risk factors for Newcastle disease seropositivity. Apart from health issues, Newcastle disease is of significant nutritional and socioeconomic importance due to the reduced production and high mortality it causes. To minimize such losses, efforts must be focused on intensifying vaccination campaigns and educating poultry farmers to adopt biosecurity measures. Finally, further studies should be considered to effectively control this disease to determine the circulating strains to match the control strategies with the epidemiological situation.

DECLARATIONS

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Authors' contribution

This work was carried out with the contribution of all authors. Ahamidou Moustapha designed the protocol, collected and analyzed data, and drafted the manuscript. Essodina Talaki and Adamou Akourki validated the protocol, supervised the data collection, and revised the manuscript. Haladou Gagara participated in the analysis of the samples. All authors read and approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no competing interests.

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ANALYSIS OF RUMEN DEGRADATION CHARACTERISTICS OF FORAGE CRUDE PROTEIN IN GOAT

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ABSTRACT: The quality of feed given to ruminants can be determined from the degradation of nutrient content in the rumen. This study aimed to determine the pattern of forage degradation and the characteristics degradation of crude protein in the rumen using the *in sacco* method. The study used 4 fistulae kacang goats with an average body weight of 14.57 kg. The forage used consisted of R1: elephant grass (*Pennisetum purpureum*), R2: mini elephant grass (*Pennisetum purpureum cv. Mott*), R3: guinea grass (*Panicum maximum*), and R4: signal grass (*Bracharia decumbens*). The nylon bag is made of polyester measuring 8x4 cm with a porosity of 40µm. Feed samples were put into the rumen and incubated for 0, 4, 8, 12, 24, 48, and 72 hours. The parameters measured were consumption, patterns, and forage degradation characteristics by calculating the values of a, b, c, a+b, lag time, and ED. Determination characteristics of feed degradation in the rumen by *in sacco* method will be analyzed. The results showed that the characteristics of crude protein degradation had significant differences in fraction values a, b, a+b, and lag time ($P<0.05$), while c and ED did not have significant differences ($P>0.05$). In conclusion the crude protein of the degradation characteristics in the rumen were: elephant grass (a: 9.88%, b: 64.37%, and c: 0.06/h^{-1}), mini elephant grass (a: 16.50%, b: 45.24%, and c: 0.05/h^{-1}), guinea grass (a: 7.42%, b: 68.24%, and c: 0.05/h^{-1}), and signal grass (a: 6.79%, b: 56.19%, and c: 0.07/h^{-1}). So, grass can provide sufficient protein for microbial growth in the rumen for ruminants.

Keywords: Crude protein, Degradability, Forage, *in sacco*, Rumen.

INTRODUCTION

Forage is the main source of energy and basic needs for ruminants, in general term (Minson, 1990). Feed costs can reach 50% - 70% of the production cost of a ruminant livestock business (Bozic et al., 2012). The forage that is generally given to ruminants is derived from the grass or graminae group. Currently the main obstacle in increasing livestock production in tropical countries is the availability and quality of feed ingredients.

Ruminant farms in Indonesia generally use grass as the main feed which can be found nearby, for example elephant grass, mini elephant grass, guinea grass, and signal grass. The four grasses can be developed in tropical climates and have good nutrition to be used as feed for ruminants.

Feed is generally assessed for its quality, one of which is based on the protein content contained therein and aspects of degradation in the rumen. Provision of protein from forage to ruminants needs to pay attention to aspects of degradation in the rumen for microbial needs and those that escape microbial degradation (by-pass) as a source of protein to be utilized by the host (Puastuti et al., 2014). Protein degradation in the rumen is necessary to provide the N source needed for microbial growth (Mutsvangwa et al., 2016). Nichols et al. (2022) added that the contribution of microbial protein in the rumen plays an important role in sustaining N requirements in ruminants.

The *in sacco* method is a method for measuring the degradation value of a feed ingredient in the rumen (Mahrez and Ørksov, 1977; Guadayo et al., 2019). The degradation value of the sample will be measured with a nylon bag containing the sample and will be incubated in the rumen of fistula cattle at certain time intervals (Ørskov, 2000). It is important to know the quality of the protein content in elephant grass, mini elephant grass, guinea grass, and signal grass. It is necessary to conduct research to analyze the patterns and characteristics of feed degradation by using forage sources in livestock *in sacco*.

MATERIALS AND METHODS

Ethical approval

The experimental procedure for *in sacco* degradation of forage feed in live animals complies with the principles of animal welfare and was approved by the Health Research Ethics Committee, Hasanuddin University (Approved Number: 645 /UN4.6.4.5.31 /PP36 /2021, Protocol UH21090601) prior to this study held.

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Experimental animals and diets

This research was conducted from September 2021 to February 2022 at Hasanuddin University, Makassar, Indonesia. This study used the Latin Square Design method 4x4 which used 4 goats with fistulas of the male kacang variety (*Capra aegragus hircus*) aged ±2 years. The average weight of the goats used as test animals was 14.57±1.219 Kg. During the observation, the animals were given the same feed consisting of elephant grass, mini elephant grass, guinea grass, signal grass, and rice bran each of the goat's dry matter requirement, namely 3% of initial body weight (BB) given twice a day every morning and evening. Drinking water is provided *ad libitum.*

Experimental design

Observation of feed degradation characteristics was carried out using the *in sacco* method, using 8x4 cm nylon bags with 40µm porosity. The feed ingredients tested were R1: elephant grass (*Pennisetum purpureum*), R2: mini elephant grass (*Pennisetum purpureum cv. Mott*), R3: guinea grass (*Panicum maximum*), and R4: signal grass (*Bracharia decumbens*) which were harvested 40 days after the last pruning. The grass will be dried in an oven (60°C) for 24 hours and ground to a size of ± 2 mm then tested for proximate analysis (AOAC, 1995) and analysis of fiber components (Van Soest, 1994) the results can be seen in table 1. Each sample of feed ingredient 3 grams was put in a nylon bag and then incubated in the rumen with an incubation period of 0, 4, 8, 12, 24, 48, and 72 hours. This study used the Latin Square Design 4×4 randomization method which consisted of four observation periods using four goats and four different forages. The incubated bag was put into the oven at 60°C for 48 hours. During this observation, each animal was given the same feed consisting of the 4 tested feed ingredients with the addition of 20% rice bran.

Calculation of crude protein content in feed before and after incubation using the Kjeldahl method (AOAC, 2001) at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The loss of crude protein in samples from nylon bags during each incubation period is assumed to be a protein that has been successfully degraded in the rumen used to calculate the degradation of crude protein feed according to the type of grass and the length of the incubation period. Determination of Crude Protein (PK) which is degraded in the sample can be known by multiplying the percentage of PK content in the initial sample. The formula for calculating the percentage loss of sample PK is presented as follows: % PK Loss = (%Initial PK x Initial Sample Weight) – (%Final PK x Final Sample Weight)

Furthermore, the crude protein lost during the incubation period is used to measure the value of Y by calculating the values a, b, c and a+b which are entered into the exponential equation according to Ørskov and McDonald (1979) as follows:

$Y = a + b\ (1\text{-}e^{-ct})$

Where:

Y= Feed degradation by rumen microbes at time t (incubation time); a= soluble fraction; b= Potentially degraded fraction; c= Potential fraction degradation rate (b); a + b= Total potential degradation, including material that escapes the codend without degradation; t_0= Incubation time at 0 hours

The effectiveness of degradation is measured by the equation according to Orksov and McDonald (1979): ED = a + [(b × c)/(c + k)], assuming the feed flow rate (k) is 0.05/h (Srakaew et al., 2021). Effectively degraded crude protein is assumed to be Rumen Degradable Protein (RDP). Rumen Undegradable Protein (RUP) from each sample is calculated by the following equation: RUP = 100% − RDP (Terefe et al. 2022). Determination of the degradation curve and feed characteristics in the rumen *in sacco* will be analyzed using the Neway program (Ismartoyo, 2011). Data analysis uses One-Way Analysis of Variance (ANOVA), and if there are differences, then continue with Duncan's test. Statistical data used Software Package for Social Sciences (SPSS) Version 25. Then used Microsoft Excel 2010 software to see the degradation curve.

RESULTS AND DISCUSSION

Feed consumption

Consume fresh ingredients in livestock is at an average of 78.52 g/Kg BW/day. According to McDonald et al. (2002) factors that influence consumption in livestock are the characteristics of feed ingredients, environment, and livestock conditions. Han et al. (2019) added that palatability greatly affects the consumption of feed in livestock. The dry matter intake in this study was around 460.59 g/day or 61.68 g/Kg$^{0.75}$/day. These results are lower than those obtained by Mbuthia and Gachuiri (2003), who obtained a value of 79 g/Kg$^{0.75}$/day, but higher than the results of Manaye et al. (2009) which was only 57.9 g/Kg$^{0.75}$/day and Han et al. (2019) who obtained a value of 369.9 g/day. The normal range of dry-meter intake per kilogram of metabolic bodyweight in goats is in the range of 34–104 g/Kg (Decandia et al., 2007). The voluntary intake of crude protein in goat poo is 56.33 g/day, or 7.55 g/Kg^{0,75}/day. These findings are similar to those obtained by Sultana et al. (2012), whose crude protein intake for goats ranged from 45.2 to 75.7 g/day, and Baete and Aregheore (2011), whose crude protein intake for grass was 7 g/Kg$^{0.75}$/day.

Crude protein degradation

Table 1 shows that the incubation period of 4, 8 and 12 hours of the four grasses had no significant difference (P<0.05). Whereas at 24, 48, and 72 hours incubation showed significant differences (P<0.05) from the various feeds

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tested. In the duncan follow-up test, 24-hour incubation of elephant grass showed a high percentage of degradation compared to other feeds and at 48 and 72 hours of incubation, degradation of crude protein in elephant grass and guinea grass showed no different results but showed significantly different results from mini elephant grass and signal grass. The loss of crude protein in the nylon bag during a certain incubation period indicates that the 72 hour incubation period is the peak of feed degradation in the rumen for forage feed. These results are in accordance with those reported by Akhirany et al. (2013) that the peak of degradation of fibrous forages in the rumen was at an incubation period of 72 hours. Based on Figure 1 which shows the curve or pattern of crude protein degradation *in sacco* on four forages. From the curve it can be seen that there is an increase in the percentage of crude protein degradation as the incubation period increases. Increased degradation of crude protein in the rumen occurs during the incubation period of 4 to 24 hours. Meanwhile, during the 48 to 72 hour incubation period, the pattern of rumen degradation of crude protein began to stabilize. After 48 to 72 hours of incubation, the degradation pattern began to stabilize because the feed substrate had decreased in the rumen, which is similar to that obtained by Guadayo et al., (2019) and Jiang et al., (2020). This is in accordance with the opinion of Zulkarnain et al. (2014) an increase in the incubation period in the rumen reduces the feed substrate due to degradation by microbes.

Table 1 - Nutritional content of feed

Chemical composition	Elephant grass	Mini elephant grass	Guinea grass	Signal grass	Bran
Dry matter (%)	28.02	26.29	23.63	33.04	90.30
Organic material (%)	82.23	82.82	88.41	90.81	85.32
Crude protein (%)	14.79	12.13	11.19	15.31	7.71
Crude fiber (%)	31.84	27.44	33.42	31.02	32.19
NDF (%)	66.22	62.71	68.14	68.24	49.65
ADF (%)	41.23	36.90	42.24	39.70	38.80
Cellulose (%)	36.17	32.80	35.36	35.36	22.13
Hemicellulose (%)	24.99	25.81	25.90	28.54	10.85
Lignine (%)	2.08	2.05	3.30	2.66	8.85

Source: Feed Chemistry Laboratory Analysis Results, Faculty of Animal Husbandry, Hasanuddin University 2022.

Characteristics degradation of forage crude protein

The degradation characteristics consist of the fraction that is easily soluble in water or degraded (a), the fraction that is not soluble in water but can be degraded (b), and the rate of degradation of the fraction b (c), the total potential for degradation (a+b), and the lag time. Each feed ingredient has different characteristics from one another

The characteristics of crude protein degradation in table 2 show that the fraction a, or the easily soluble fraction in the four grasses tested gave significantly different results ($P<0.05$). The "a" fraction in mini elephant grass (16.5% ± 0.671) was the highest compared to the other materials studied. The “a” fraction relates to the solubility of plant cell contents in water (Zulkarnain et al., 2014). This result can be attributed to the lower ADF content of mini elephant grass compared to the other three grasses. It was also reported by Katongole et al. (2021) that the high fraction of crude protein (CP) which is easily degraded by the rumen in forage feed can be affected by the low ADF content in forage. The lignin content of the forage greatly reduces the degradability of the cell wall (Hatfield & Kalscheur, 2020) because lignin cannot be broken down by microbes in the rumen (Wang et al., 2022).

The crude protein fraction that was difficult to degrade or “b” in the four grasses tested showed a significant difference ($P<0.05$). The "b" fraction in Guinea grass was the highest (68.24%±2.301) when compared to the other grasses tested. these results indicate that the value of a and fraction b are negatively related, a similar thing was reported by Larbi et al. (1996) and Bamikole et al. (2004) showed that forages with higher a values had lower b values. This fraction can be related to the tannin content in plant cells which can form tannin protein complex bonds so as to reduce protein degradation in the rumen. Min et al. (2003) and Patra and Saxena (2010) suggested that tannins can reduce the degradation of the protein fraction of feed in the rumen.

The value of c is the rate of degradation of fraction b. the results of the analysis of variance showed that the c values for the four grasses tested were not significantly different ($P>0.05$). The rate of degradation of fraction b (c) on signal grass was an average of 0.07/h^{-1} which was the highest compared to other grasses. The average degradation rate of all tested grasses was 0.05/h^{-1} which is consistent with the assumed rate of feed fraction in the rumen (Ajayi et al., 2007). From the results obtained, it was found that the fractional degradation value and the degradation rate had a negative relationship, namely a low feed degradation rate resulted in a high degradation value. This was also reported by Odedire et al. (2013) and Rasjid and Ismartoyo (2014), and Ferreira et al. (2014).

Table 2 shows the lag time of the several tested feeds showing significantly different results ($P<0.05$). From Duncan's test it can be seen that mini elephant grass has the highest lag time compared to the other three grasses, namely 4.2 hours. The longest lag time is 4.2 hours, while the fastest lag time is 1.2. These results indicate the time required for microbes to adapt to the feed substrate in the rumen. However, the results of this lag time cannot be used as an index to see the degradation of a fibrous feed ingredient because in this case the value of the fraction of the feed will

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be negative (Ørksov et al., 1980; Odedire et al., 2013).

Table 2 - Consumption of livestock voluntary feed

Goat	Intake as feed basis (g/day)	DM Intake (g/day)	CP Intake (g/day)
Goat-1	1245.14±43.29	501.29±17.43	61,31±2,13
Goat-2	1154.67±93.54	464.87±37.66	56.85±4.61
Goat-3	1049.97±76.75	422.72±30.90	51.70±3.78
Goat-4	1126.33±41.40	453.46±16.67	55.46±2.04
Average	1144.03	460.59	56.33

Description: Feed consists of 20% elephant grass + 20% mini elephant grass + 20% guinea grass + 20% signal grass + 20% rice bran

Degradation effectiveness

The effectiveness of degradation (ED) is the result of the accumulation of feed degradation characteristics such as easily degradable fraction (a), slowly degraded fraction (b), and degradation rate of b fraction (c). EDCP on the four feeds showed results that were not significantly different (P>0.05). The effectiveness of crude protein degradation in the rumen of the four treatments showed a higher EDCP value at R1, namely 40.29% ± 2.47 although according to statistical tests it does not show the difference. This can be seen from the NH3 level which is a product of feed protein content that is degraded by proteolytic enzymes in the rumen (Sari et al., 2021). In study of Mushandri (2022), which measured the concentration of NH_3 from the same four types of feed, the NH_3 level in elephant grass was the highest, 4.64 mM compared to other grasses. The effectiveness of crude protein degradation in elephant grass obtained a value of 43.20% ± 2.47 which is different from the results obtained from previous studies, namely 34.53% (da Silva et al., 2021) and 53.9% (Katongole et al., 2021). Mini elephant grass has a degradation effectiveness value of 37.88% ± 4.81, which is different from the results obtained by other researchers, namely 46.81%/d (da Silva et al., 2021), and 64% (Orsoletta et al., 2017). For Guinea grass, the degradation effectiveness value was obtained as much as 40.33% ± 2.72, this is different from other studies which obtained results of 34.18% (Ogunwole et al., 2011) and 46.67% (Bonelli et al., 2013). Meanwhile, based on the calculation of the effectiveness of degradation, Signal Grass obtained a value of 39.32% ± 5.54, this value is higher than the results obtained by Lana et al. (2007) namely 16.4% and Terefe et al. (2022) namely 27.86%. These different results were obtained because differences in the nutrient content of the samples could be caused by lighting/climate (Ballare et al., 1997), leaf/stem ratio (Lemaire et al., 2020), processing with fermentation (Ferreira et al., 2014), the addition of other materials and differences in results can also be caused by differences in the methods used.

Table 3- The average percentage of CP degradation at each incubation period

Incubation Period (Hours)	Elephant Grass (%)	Mini Elephant Grass (%)	Guinea Grass (%)	Signal Grass (%)
0	6.80 ± 2.85	4.05±1.65	6.32±1.73	2.68±0.91
4	19.58±1.70	12.75±1.79	14.04±3.22	12.50±3.68
8	27.81±1.34	23.87±0.50	27.96±5.81	26.82±4.98
12	36.47±5.57	31.07±1.67	35.60±5.24	34.78±6.24
24	$54.87±3.55^{b}$	$42.08±0.75^{a}$	$46.86±2.82^{a}$	$47.34±8.79^{a}$
48	$68.99±4.86^{b}$	$60.53±1.41^{a}$	$67.57±2.80^{b}$	$59.68±6.91^{a}$
72	$71.47±3.99^{b}$	$62.13±1.88^{a}$	$70.80±1.72^{b}$	$62.00±6.31^{a}$

a,b,c,d: Means in the same row with different superscripts differ significantly (P<0.05)

Table 4 - Characteristics of crude protein degradation

Parameters \ Feeds	R1	R2	R3	R4
A (%)	$9.88±0.38^{b}$	$16.50±0.67^{a}$	$7.42±1.09^{c}$	6.79±0.35d
B(%)	$64.37±4.85^{ab}$	$45.24±11.37^{c}$	$68.24±2.301^{a}$	$56.19±5.183^{b}$
A+B (%)	$74.25±4.85^{a}$	$61.74±11.37^{b}$	$75.66±2.31^{a}$	$62.98±5.17^{b}$
C (/H)	0.06±0.012	0.05±0.006	0.05±0.008	0.07±0.016
Lt (h^{-1})	$1.2±0.84^{b}$	$4.2±0.08^{a}$	$1.3±1.02^{b}$	$1.2±1.07^{b}$
Ed (%)	43.20±2.47	37.88±4.51	40.33±2.72	39.32±5.54
Rup (%)	56.80±2.47	62.12±4.51	59.67±2.72	60.68±5.54

R1: elephant grass (*pennisetum purpureum*), R2: mini elephant grass (*pennisetum purpureum cv. Mott*), R3: guinea grass (*panicum maximum*), R4: signal grass *(brachiaria decumbens*), a: soluble fraction, b: potential degradation fraction, a+b: total potential degradation, c: degradation rate of fraction b, lt: lag time, ed: degradation effectiveness, rup: rumen undegradable protein. Different superscripts in the same row show significant differences (p<0.05)

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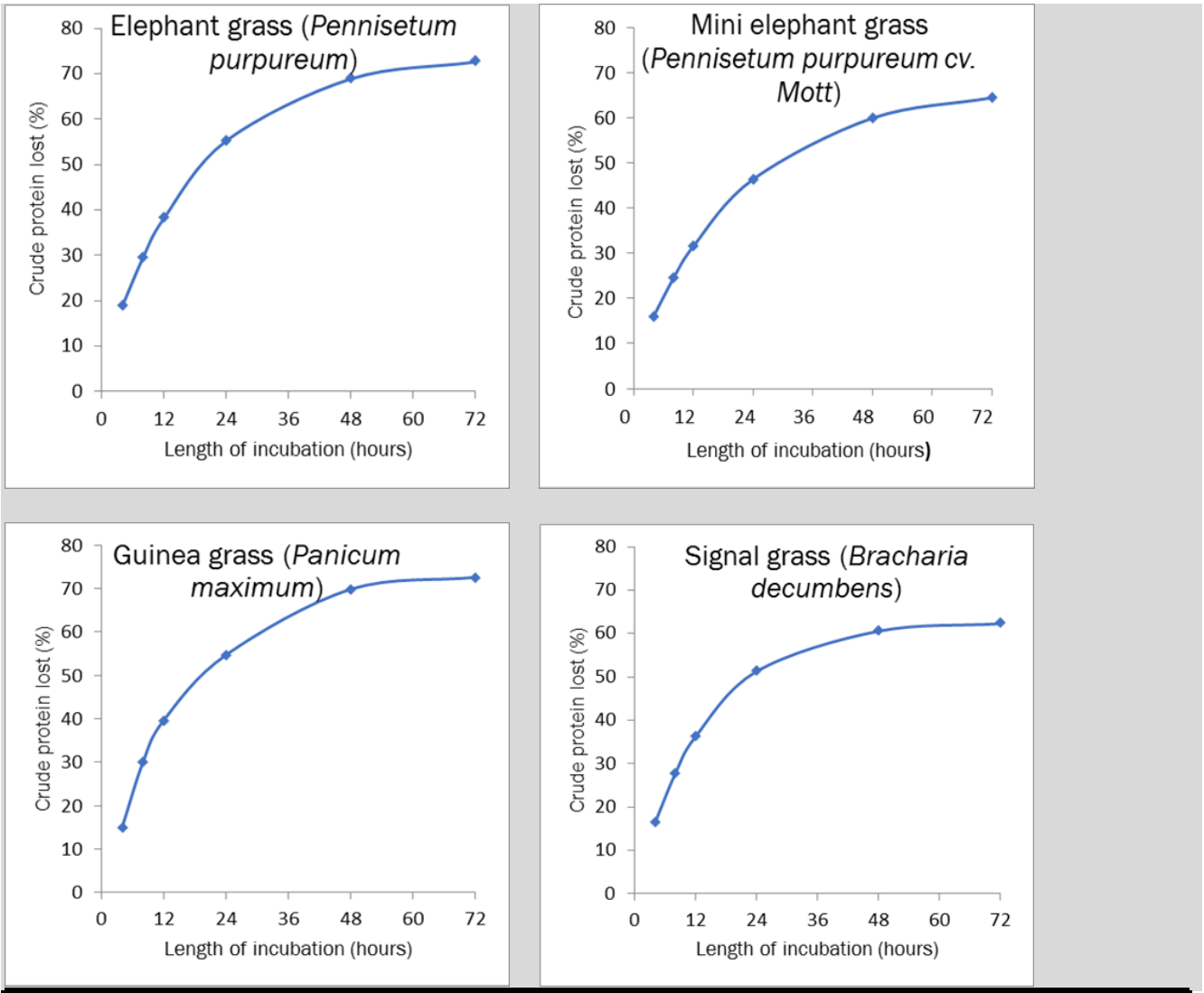

Figure 1 - Crude protein degradation curve Elephant grass (***Pennisetum purpureum***), Mini elephant grass (***Pennisetum purpureum cv. Mott***), Guinea grass (***Panicum maximum***), Signal grass (***Bracharia decumbens***)

CONCLUSION

In the results of this study, various grasses had suitable nutritional value and showed that the crude protein of the four grasses showed degradation characteristics: elephant grass (a: 9.88%, b: 64.37%, c: 0.06/h^{-1}), grass mini elephant (a: 16.50%, b: 45.24, and c: 0.05/h^{-1}), guinea grass (a: 7.42%, b: 68.24%, and c: 0.05/h^{-1}), and signal grass reached (a: 6.79%, b: 56.19%, and c: 0.07/h^{-1}). Of the four grasses, it was concluded that they were able to supply the protein requirements for microbial growth in the rumen and the protein needs of the livestock themselves.

DECLARATIONS

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Authors' contribution

Al Wijaya contributes data collection and analysis and the manuscript write up. I Ismartoyo and A. Natsir contribute on experiment, idea and research design.

Conflict of interests

The authors have not declared any conflict of interests.

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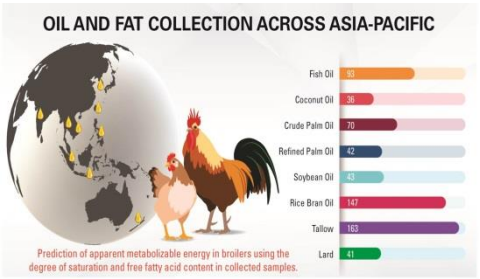

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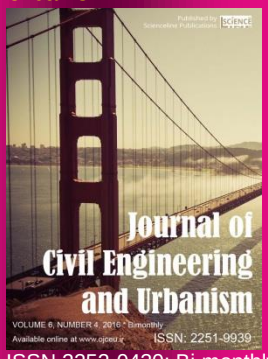

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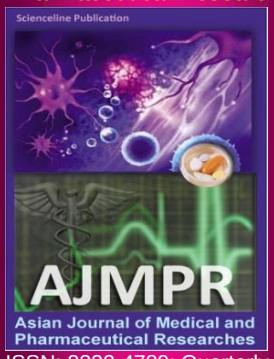

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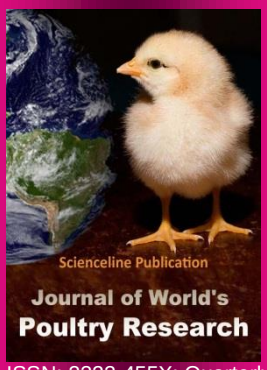

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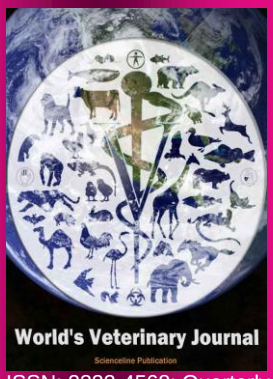

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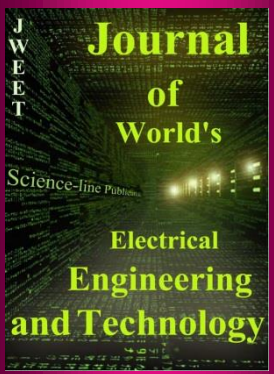

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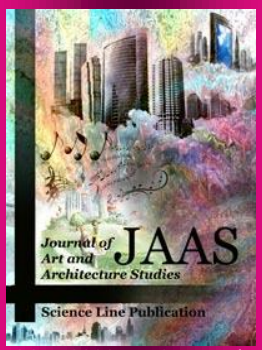

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