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Online J. Anim. Feed Res., 13 (2): 79-152; March 30, 2023

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Volume 13 (2); March 30, 2023 [EndNote XML for Agris]

Review

Use of fish waste to silage preparation and its application in animal nutrition

Raziye R, Bahareh SH, and Parastoo P.

Online J. Anim. Feed Res., 13(2): 79-88, 2023; pii: S222877012300013-13

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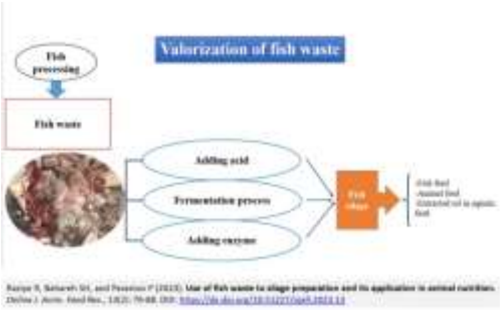

Abstract
In recent years, global aquaculture production has increased, leading to an increase in fish waste. These wastes, which in many cases are disposed directly without trying to take advantage of them, are a major environmental and economic problem that may affect the sustainability of the fishing and aquaculture industry. Therefore, their use seems necessary to reduce pollution and make the aquatic industry more efficient. Most of well-known technologies for using fish waste are not economically attractive due to the need for high initial investment. But an easy and inexpensive way to use these wastes is to convert them into silage. Fish silage is a product of good nutritional quality included in animal diets as part of the feed. Fish silage is a liquid product made from whole fish or parts of it to which lactic acid-producing acids, enzymes or bacteria are added, and the liquefaction of the material indicates the action of enzymes present in the fish. Therefore, the purpose of this review is to investigate the use of aquatic waste for preparing silage and the possibility of using it in animal nutrition.

Keywords: Fermentation, Fish by-product, Fish silage, Protein hydrolysis, Silage.

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Research Paper

Impact of phytogenic formulation on performance and fatty liver disease of broiler chickens

Kaninathan A, Subramaniyam S, Marimuthu S, and D'souza P.

Online J. Anim. Feed Res., 13(2): 89-96, 2023; pii: S222877012300014-13

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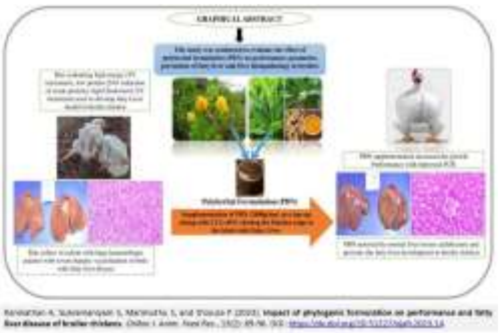

Abstract
This study was conducted to evaluate the effect of polyherbal (phytogenic) formulation (PHF: containing *Acacia nilotica* and *Curcuma longa*) on performance parameters, liver histopathology and prevention of fatty liver in broilers. 700 day-old chicks were randomly distributed to seven groups (10 replicates / group; 10 birds each), namely positive control (T1) fed with basal diet + choline chloride (CCL) 60% (1000g), negative control (T2) fed with high energy (5% increment), low protein (24% reduction), high cholesterol (2% increment) diet, T3 (T2 + PHF; 1000g-full cycle), T4 (T2 + PHF; 2000g-full cycle), T5 (T2 + CCL 60% (1000g-full cycle)), T6 (T5 + PHF; 1000g-grower and finisher stage), T7 (T5 + PHF; 2000g-finisher stage). Average daily gain (ADG; g), average daily feed intake (ADFI; g) and feed conversion ratio (FCR) were calculated at 1-14 days, 15-28 days, 29-42 days, and 1-42 days. Serum triglycerides analysis, gross and histopathological observations of liver morphology were performed for the samples of control and experimental groups on day 42. The performance parameters; ADG, ADFI, FCR, and liveability were found to be improved in all the groups as compared to the negative control group. However, better performance was observed in PHF (2000g) top-up group (during the finisher stage) as compared to the negative control group. Serum triglyceride levels were increased non-significantly as compared to the negative control indicating that more fat is mobilized from liver to serum. In addition, PHF supplementation at 2000g during the finisher phase had restored the liver tissue architecture as well as improved the liver score when compared to the negative control group. It is concluded that PHF (2000g/ton) during the finisher stage can be used as a top-up to improve the performance parameters as well as to prevent the fatty liver condition in broiler chickens.

Keywords: Basal diet, Broiler chicken, Choline chloride, Herbal formulation, Triglycerides.

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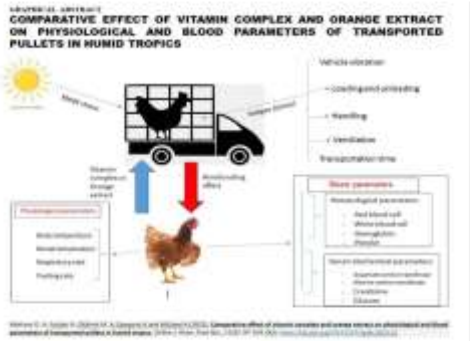
Research Paper

Comparative effect of vitamin complex and orange extract on physiological and blood parameters of transported pullets in humid tropics

Mathew O. A, Foluke A, Olufemi M. A, Opeyemi A and Micheal A.

Online J. Anim. Feed Res., 13(2): 97-104, 2023; pii: S222877012300015-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.15



Abstract

The comparative modulating effects of synthetic and natural source of ascorbic acid (AA) were investigated on transporting pullet birds in the hot-dry season of humid tropics. Ninety-six 16 weeks Isa-brown pullets were randomly allotted in a completely randomized design into four treatments of oral vitamin supplementation 5 days before transportation i.e; T_1 (ordinary water), T_2 (synthetic vitamin), T_3 (30% citrus-sweet orange), T_4 (50% citrus-sweet orange). Birds were crated and transported for 3 hrs covering 135km at 45km/hr. Meteorological values were monitored during the journey and no mortality was recorded. The results revealed that treatments had a significant effect ($p < 0.05$) on measured physiological parameters [body temperature (BTC), rectal temperature (RTC), respiratory rate (RR) and panting rate (PR)], hematological parameters and measured serum biochemical parameters as compared to the control group (T_1). The treatments group of orange at different inclusion ratios (T_3 and T_4) compared well with pullet birds on oral supplementation of synthetic vitamin (T_2) and were significantly different ($p < 0.05$) from birds on control water treatment (T_1). Birds on control (T_1) had the highest values for all measured physiological parameters which were significantly different from other groups ($p<0.05$). Birds in the control treatment (T_1) were more stressed as compared to other treatment groups, indicated by increased hematological and serum biochemical parameters except for a decrease in hemoglobin (Hb) as compared to other treatments. It can be deduced from this study that the oral supplementation of natural source of ascorbic acid (*Citrus sinensis)* and synthetic vitamin supplement helps to ameliorate the effect of transportation stress. *Citrus sinensis* extract can be a suitable alternative that is readily available for farmers and stakeholders.

Keywords: Ascorbic acid, Physiological measures, Pullets, Synthetic vitamin, Transportation stress.

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Research Paper

Physico-chemical properties and digestibility of ammoniated Bambara groundnut (*Vigna subterranea*) shell for ruminants

Putri R, Dewi SP, Kurniawan FA, Ridla M and Retnani Y.

Online J. Anim. Feed Res., 13(2): 105-110, 2023; pii: S222877012300016-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.16

Abstract

This experiment was conducted to evaluate the physical properties, chemical quality, and digestibility of the ammoniated Bambara groundnut (*Vigna subterranea*) shell as ruminant feed. Bambara groundnut shell (BGS) were collected, ground with a grinder machine, afterward added 0, 3 and 5 (% DM) urea levels into 500 g of sample. Samples were mixed until homogeneous, then put into plastic bottles, after that stored for 7 and 14 days. Opened, dried in the oven at 650C for 48 hours and ground. A completely randomized design (CRD) was used with 5 treatments of BGS ammoniation (T0= control, T1= BGS + 3% urea and 7 days storage, T2= BGS +5% urea and 7 days storage, T3= BGS + 3% urea and 14 days storage, and T4= BGS + 5% urea and 14 days storage time), 4 replications each. The result of this study showed that the increase of urea level and days storage time, can decrease crude fiber, neutral detergent fiber, acid detergent fiber, and hemicellulose contents of all samples ($P<0.05$) and increase the value of bulk density, tapped density, in vitro dry matter digestibility and in vitro organic matter digestibility in comparison to untreated samples ($P<0.05$). It was concluded that the T4 was the best treatment. The BGS ammoniated with 5% urea for a period 14 days of storage causes the lowest value of crude fiber, neutral detergent fiber, acid detergent fiber, and hemicellulose, and also causes the highest value of bulk density, tapped density, crude protein, in vitro dry matter digestibility and in vitro organic matter.

Keywords: Ammoniation, Fibrous feed, Bambara groundnut shell, Ruminant, Urea.

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Research Paper

Impact of phase-feeding programs on performance of broiler chickens in Nigeria

Ebegbulem VN, Archibong EE, Kperun ThN, Izuki ED and Udayi MA.

Online J. Anim. Feed Res., 13(2): 111-115, 2023; pii: S222877012300017-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.17

Abstract
Phase-feeding is the feeding of several diets for a relatively short period of time to specifically meet an animal's nutrient requirements. The study evaluated the effect of different phase feeding methods on growth and carcass characteristics of broiler chickens. A total of 120-day-old chicks of the FIDAN strain were assigned to four dietary treatments of 30 birds each, 15 birds per replicate. Birds were fed at different phases: Phase 1 were fed broiler starter diet alone for 8 weeks; Phase 2 birds were fed starter diet from 0-4 weeks and 1st finisher diet from 5-8 weeks. Phase 3 birds were fed starter diet from 0-3 weeks, 1st finisher diet from 4-6 weeks and 2nd finisher diet from 6-8 weeks. Phase 4 birds were fed starter diet from 0-2 weeks, 1st finisher diet from 2-4 weeks, 2nd finisher diet from 4-6 weeks and 3rd finisher diet from 6-8 weeks of age. Result no significant differences (p>0.05) between the groups in body weight gain (2.91–2.47 kg/bird) and feed conversion ratio (2.03–2.34). Total feed intake was highest in phase 1 (6.70 kg/bird) followed by phase 2 birds (6.41 kg). Dressed weight in Phase 1 was significantly (p<0.05) higher than others, followed by Phase 2. Dressing percentage did not differ significantly (p>0.05) between the groups. Feed cost between treatments was however significantly (p<0.05) different, Phase 1 diet being costliest. Phase-feeding using phase 4 regime elicited reduced dietary cost without compromising optimal performance of the birds.

Keywords: Diet; Feed cost; Feed efficiency; Nutrition; Phase-feeding.

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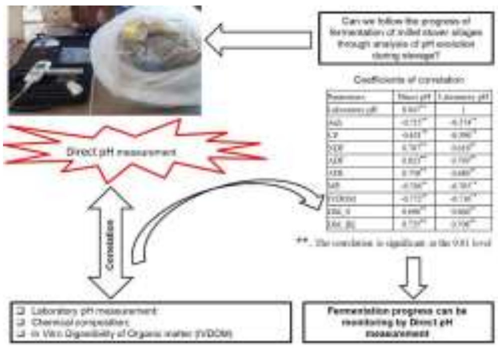
Research Paper

Evaluation of fermentation progress during storage of millet stovers silage based on pH-indicators

Korombé HS, Bado VB, Abdou N, Umutoni C, Ibrahima A, Gouro AS.

Online J. Anim. Feed Res., 13(2): 116-126, 2023; pii: S222877012300018-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.18



Abstract
This study aimed at evaluating the fermention levels of pearl millet [*Pennisetum Glaucum* (L.) R. Br] stovers silage during storage based on pH evolution. A completely randomized experimental design in a 6×2×2 factorial scheme with three replications for each treatment was used to evaluate three factors (6 cultivars, 2 different cutting stages, and with or without salt addition to the cultivars). The silages were prepared in plastic bags and stored for 60 days at room temperature. The results revealed that the pH values of the treatments were significantly (P < 0.05) higher on the first day than in the other periods and a rapid drop in pH, with significant differences (P < 0.05), to levels below 4 was obtained on the third day of storage for the majority of local Sadoré and Siaka Millet silages (Niger). Four types of pH evolution were recorded and the variation was statistical significant among cultivars. Also, analysis of the relationships between pH, chemical composition parameters and In Vitro Digestibiliy of Organic Matter (IVDOM) showed that increasing pH values were associated with increasing Dry Matter content of stovers before silage (DM_BE), Dry Matter content of silages (DM_S), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) values and decreasing Crude Protein (CP), Metabolizable Energy (ME), IVOMD, and Ash values. However, the pH values obtained for all silages showed that all the millet stovers used were suitable for silage. At the maturity stage, it is thus possible to use the grain for human consumption and to ensile the stovers for animal feed. This study also shows that monitoring the pH in the silo makes it possible to evaluate the quality of the fermentations to avoid losses on the farms.

Keywords: Dual-purpose varieties; Harvesting stage; Monitoring of pH; Silage; Stovers conservation.

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Research Paper

Effect of three different processing techniques of soybean on nutritional and growth performance of Japanese quail (*Coturnix japonica*)

Ekeocha A, Aganga A, Okiki P, Olubiyo P, Oluwadele J.

Online J. Anim. Feed Res., 13(2): 127-131, 2023; pii: S222877012300019-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.19

Abstract
The experiment investigated the effects of various soya bean groups (boiled, fermented, and roasted) on Japanese quail at 3 weeks old. 160 Japanese quail were randomly assigned to four treatments (control, boiling soya beans, fermented soya beans, roasted soya beans) with four duplicates each. The 12-week trial lasted. Data on weekly body weights and feed conversion ratio were analyzed using analysis of variance (ANOVA) and Tukey's honestly significant at 5% probability test. The result shows there are significant differences in weekly weights of Japanese quail at weeks 1(828.12-1083.24g), 2(1026.47-1362.02g), and 3(1325.69-1528.20g) with the highest observed in birds in treatment 2(boiled soya beans). The maximum FCR was in week 1 for all treatments, while the lowest was in treatment 3 for weeks 5 and 9 (0.83; P<0.005). Week 1 to week 12 feed conversion ratio decreases. The birds' feed conversion ratios varied significantly (P<0.05). The quails in treatment 4 (roasted soybeans) had the greatest weekly weight after the trial (1742.34g). Thus, quails in treatment 3 (roasted soybean) had the best development performance than the control, boiled and fermented. So it advised that roasted soybean can be an efficient diet for Japanese quails for maximum performance.

Keywords: Growth performance, Feed conversion ratio, Nutrient; Processing techniques, Roasted soybean.

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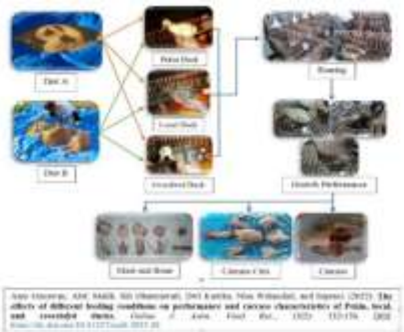
Research Paper

The effects of different feeding conditions on performance and carcass characteristics of pekin, local, and crossbred ducks

Aam G, A. Malik, Siti D, Dwi K, Nisa W, and S.

Online J. Anim. Feed Res., 13(2): 132-136, 2023; pii: S222877012300020-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.20



Abstract
The objective of the study was to investigate performance and carcass quality of Pekin, local, and cross-breed ducks raised under different feeding (varied in protein and fiber levels). A total of 180 male ducks aged 14 days, consisting of 60 Pekin ducks, 60 local ducks (Mojosari), and 60 cross-breed (Mojosari + Alabio) ducks were used in the study. Each type of duck was randomly divided into 36 units of cages, each of which was filled with 5 ducks. The data obtained from the study were analyzed according to a completely randomized design with 2×3 factorial pattern. Initial body weight of local ducks at 14 days was significantly (P<0.01) lower than that of Pekin and cross-breed ducks, while, Pekin ducks were higher than the other two types of ducks. On the other hand, feed consumption was significantly (P<0.01) influenced by the type of diet and breed. While carcass percentage was significantly (P<0.01) influenced by breed and diet types. The percentage of Pekin duck carcasses that received diet A (low protein and fiber) was significantly different (P<0.05) from cross-breed ducks, in comparison to local ducks. In conclusion the feed conversion rate and final body weight for diet A showed better results than diet B (high protein and high fiber) on the Pekin duck. Meanwhile, the carcass characteristics like abdominal fat of diet B (for pekin, local duck, and cross breed) were better than diet A (for Pekin and local duck). It's suggested to use Pekin ducks with low protein and low fiber diet to get the better performance, in compared with local ducks which needs high protein and high fiber content in diet.

Keywords: Carcass characteristics, Cross-bred birds, Nutrients, Pekin Duck, Performance.

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Research Paper

Antioxidant activity of raw and cooked onions in rabbit doe nutrition

Tawfeeq AA, Shallal EN, Abdulwahid AM and Aldahham BJM.

Online J. Anim. Feed Res., 13(2): 137-142, 2023; pii: S222877012300021-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.21

Abstract
The aim of this research is to examine how raw and cooked onions affect some antioxidant enzymes and some tissues in female rabbits. Twenty-four female albino rabbits weighing (1-1.5 Kg), (5-6 months age), non-pregnant, were used for the experiment, and they were divided into three groups for a duration of 28 days. A 20 g/kg raw onion and same amount of cooked onion were added to the second and third groups' diets respectively for comparison of results with control groups without any addition of onion. Results showed that diets supplemented with raw and cooked onion significantly increased the superoxide dismutase (SOD) activity. The cooked onion group showed normal and no pathological changes in liver, kidney, and heart tissues, while liver tissues of both control and raw onion groups suffered extreme congestion in the central veins of the liver lobules and in kidney tissues of only control rabbits, developed hemorrhagic foci was observed. In the control and raw onion groups, the heart tissue showed the development of hemorrhagic foci and necrosis in the heart muscle fibers. In conclusion, both raw and cooked onions boosted the activity of SOD enzyme, but the cooked onions showed to be more effective than raw at protecting liver, kidney, and heart tissues against cell necrosis caused by oxidative processes.

Keywords: Cooked onion, Oxidative stress, Rabbits, Superoxide dismutase, Tissue.

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Research Paper

The quality of fermented rice straw with *Trichoderma viride* inoculum

Badat M, Umi K, Hilarius Yosef S, Rifa'I.

Online J. Anim. Feed Res., 13(2): 143-147, 2023; pii: S222877012300022-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.22

Abstract
The Rice straw has several nutritional weaknesses, namely its high silica and lignin content, and its low level of protein, minerals and vitamins, so the impact on digestibility is also low. Aim of present study was to evaluating nutritional efficacy of rice straw after fermentation with *Trichoderma viride*. The study was conducted by using complete random design. There were three different treatments with four replicates for each treatment. Fermented rice straws were treated with varying concentrations of Trichoderma viride inoculum as follows; 0.5% (T1), 1% (T2), and 1.5% (T3). Fermented rice straw's nutrients, including dry ingredients, organic material, crude fiber, crude protein, dry matter digestibility coefficients, and organic matter digestibility coefficients were measured in this study. P3 (1.5% of *T. viride*) treatment performed a proper nutrient, with 80.02% dry ingredients, 80.03% organic materials, 31.68% crude fiber, 5.72% protein, 38.46% dry matter digestibility coefficient, and 61.05% organic matter digestibility coefficient. In conclusion, using 1.5% *Trichoderma viride* to improve the quality of rice straw, as stimulator of fermentation process can be efficient in ruminant or non-ruminant nutrition.

Keywords: Agricultural by-product; Crude fiber; Digestibility; Ruminants; *Trichoderma viride*.

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Research Paper

Effects of substitution of fermented chicken litter with concentrate on nutrient digestibility and performance of sheep

Christiyanto M, Pangestu E, Nuswantara LK, Surono, and Utama CS.

Online J. Anim. Feed Res., 13(2): 148-152, 2023; pii: S222877012300023-13

DOI: https://dx.doi.org/10.51227/ojafr.2023.23

Abstract
The study aimed to investigate the effects of supplementing fermented chicken litter on feed consumption, nutrient digestibility (dry matter/DM, organic matter/OM, crude fiber/CF, extract ether/EE, crude protein/CP), total digestible nutrients (TDN), and average daily gain (ADG) in sheep. A completely randomized design with 4 treatments and 3 replications, namely T0 = concentrate without the addition of fermented litter, T1 = 90% concentrate + 10% fermented litter, T2 = 80% concentrate + 20% fermented litter, T3 = 70% concentrate + 30% fermented litter and T4 = 60% concentrate + 40% fermented litter was used. The parameters studied were dry matter digestibility (DMD), organic matter digestibility (OMD), extract ether digestibility (EED), crude fiber digestibility (CFD), crude protein digestibility (CPD), TDN, feed consumption and average daily gain. The results revealed that sheep fed different levels of fermented litter did not affect OMD, DMD, EED, CPD, CFD, TDN, dry matter consumption, and average daily gain (ADG). It was concluded that fermented chicken litter can be incorporated in sheep diet, without considerable negative effects.

Keywords: Digestibility, Feed, Fermentation, Litter, Sheep.

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USE OF FISH WASTE TO SILAGE PREPARATION AND ITS APPLICATION IN ANIMAL NUTRITION

Raziye RAEESI, Bahareh SHABANPOUR, and Parastoo POURASHOURI

Gorgan University of Agricultural Sciences and Natural Resources, Faculty of Fisheries and Environmental Sciences, Department of Seafood Processing, Gorgan, Iran

Email: r.reisi2012@yahoo.com

Supporting Information

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ABSTRACT: In recent years, global aquaculture production has increased, leading to an increase in fish waste. These wastes, which in many cases are disposed directly without trying to take advantage of them, are a major environmental and economic problem that may affect the sustainability of the fishing and aquaculture industry. Therefore, their use seems necessary to reduce pollution and make the aquatic industry more efficient. Most of well-known technologies for using fish waste are not economically attractive due to the need for high initial investment. But an easy and inexpensive way to use these wastes is to convert them into silage. Fish silage is a product of good nutritional quality included in animal diets as part of the feed. Fish silage is a liquid product made from whole fish or parts of it to which lactic acid-producing acids, enzymes or bacteria are added, and the liquefaction of the material indicates the action of enzymes present in the fish. Therefore, the purpose of this review is to investigate the use of aquatic waste for preparing silage and the possibility of using it in animal nutrition.

Keywords: Fermentation, Fish by-product, Fish silage, Protein hydrolysis, Silage.

INTRODUCTION

Seafood processing leads to significant amounts of waste (Adeoti and Hawboldt, 2014; Bruno et al., 2019; Nikoo et al., 2019; Ozogul et al., 2021). In some cases, these wastes may be removed from the processing and consumption cycle at no cost (Knuckey et al., 2014). But aquaculture plants will usually have to pay to remove them (Knuckey et al., 2014; Martínez-Alvarez et al., 2015; Etemadian et al., 2021). Therefore, using these wastes and producing new products, in addition to helping to protect the environment, also increases their revenue (Arruda et al., 2007). In most cases, these wastes are used to make fish meal (Shabani et al., 2019), which requires significant amounts of energy to cook, dry and evaporate (Arvanitoyannis and Kassaveti, 2008), and is a costly process (Yamamoto et al., 2005). But fish silage can be produced with a simple process, with the need for simple equipment and machinery (meat grinder, mixer and plastic containers), on a small scale, with low energy consumption and without the need for skilled workers (Vidotti et al., 2002); therefore, the cost of silage production is low (Arruda et al., 2007). Fish silage is a liquid product made from whole fish or parts of it to which lactic acid-producing acids, enzymes or bacteria are added, and the liquefaction of the material indicates the action of enzymes present in the fish (FAO, 2003).

In aquaculture (like any animal husbandry), feed accounts for a high percentage of costs (between 30 and 60% of the total) (Arruda et al., 2007). Currently, the most abundant source of animal protein for the production of animal diets is fish meal (Vidotti et al., 2002), which has no anti-nutritional factors and is a very good source of protein in aquatic feed compared to soybean powder (Martínez-Alvarez et al., 2015); while the global market has always been looking for a suitable alternative, researchers around the world have greatly attempted to identify substitute protein sources in order to reduce nutritional costs (Arruda et al., 2007). On the other hand, increasing demand for fish meal and fluctuations in wild pelagic fish catch (mostly Anchovy) have pushed up fish meal prices over the years. In addition, the effects of thermal damage caused by the drying process on the protein quality and overall protein digestibility, as well as running costs, has increased interest in fish meal substitutes in aquatic feed (such as fish silage, protein hydrolysis and fermentation products) (Martínez-Alvarez et al., 2015). Soybean powder is currently used as a source of protein to reduce the cost of producing aquatic feed (Urán et al., 2009), which can affect growth and feed efficiency due to its anti-nutritional properties (such as phytic acid or lectins; Martínez-Alvarez et al., 2015). The use of fish silage as a protein component in aquatic feed can reduce feed costs and thus improve the profitability of fish farming (Borghesi et al., 2008).

The greatest advantage of fish silage is attributed to its nutritional value; because fish waste (which accounts for half of the processing industry raw materials) is a low-cost source of nutrients. In addition, the production of fish silage does not have the odor and wastewater problems occurring during the production of fish meal (Arruda et al., 2007). The presence of formic acid in the product (if formic acid is used to prepare silage) can promote aquatic growth and health,

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especially under adverse microbial conditions (Olsen and Toppe, 2017). The process of producing silage in tropical climates is fast and it is possible to use the product on site (Arruda et al., 2007), finally it does not attract insects due to its acidic odor and does not contain any pathogens (such as Salmonella) (Vidotti et al., 2002). The purpose of this study is to discuss the use of aquatic waste for silage and to investigate the possibility of using it in aquatic and livestock feed.

History of silage

Fish silage was common in some Scandinavian countries for animal feed (Backhoff, 1976). After World War II, the use of formic acid to protect wastes began to be used in animal diets. Acid silage was developed in the 1920s by A. I. Virtanen using sulfuric and hydrochloric acids to preserve forage (Arruda et al., 2002). Experiments with fish in Sweden began around 1936, using sulfuric acid and molasses, and formic acid. Experiments were performed by Edin in 1940 and a formula based on pH, protein and ash measurements was proposed to calculate the content of acid required by the raw materials ground by him. Olsen set up a table based on this formula, providing the content of acid needed for various Scandinavian ingredients (Tatterson and Windsor, 1974). Production of industrial-scale silage began in Denmark in 1948, and three years later it reached 15,000 tons a year (Backhoff, 1976), and there is still a thriving industry despite a slight modification in techniques (Tatterson and Windsor, 1974). Fish silage has been produced on a commercial scale in Poland and Denmark since the 1960s to produce pig and poultry feed or as a protein supplement in livestock and aquaculture feed (Arruda et al., 2007). In the tropics, although the potential for the use of fish silage has been known, it is less used, which is probably due to the failure of optimal methods of production, use and storage in environmental conditions. Commercial use of fish silage is largely limited in northern Europe, where it is mainly used in the wet feed of pigs, fur-bearing animals and fish (Goddard and AL-Yahyaio, 2001).

Various sources used in the production of silage

Silage can be produced from a variety of raw materials including maize (Hu et al., 2009; Santos et al., 2013; Moloney et al., 2013; Weiss et al., 2016), sugarcane (Ávila et al., 2014; Gandra et al., 2016), casein (Åsgård and Austreng, 1985), silkworm pupae (Rangacharyulu et al., 2003), oats (Gomes et al., 2019), seaweed (Herrmann et al., 2015), poultry slaughterhouse waste (Ashayerizadeh et al., 2017) and aquatic waste. In the following, the use of aquatic waste for silage production is examined.

Use of fish waste

Given the increasing trend of global fish production in recent years (for example, an increase from about 20 million tons in 1950 to 171 million tons in 2016) and an increase in aquatic consumption (from 9 kg in 1961 to 20/2 kg per in 2015), the production of aquatic waste has also increased. By-products are obtained by processing aquatic products on an industrial scale (from fishing or aquaculture) (Marti-Quijal et al., 2020), the amount of which can include 20 to 60% of the total mass, depending on the type of aquatic products (Choi, 2020). Sustainable management of waste from aquatic processing is a global problem (Ivanovs et al., 2018). An important waste reduction strategy for the industry is the recovery of marketable by-products from fish waste (Arvanitoyannis and Kassaveti, 2008). Using these wastes to produce new products, in addition to reducing the environmental and health problems caused by improper use of industrial aquatic wastes, can also increase their revenue (Arruda et al., 2007).

Fish by-products contain large contents of fats, proteins and organic matter that can cause problems such as odor and pest-breeding during their excretion (Choi, 2020). A wide range of high quality compounds can be recovered from these by-products and used for human consumption. Some of these compounds such as proteins, amino acids, oils, hydroxyapatite, enzymes, collagen and gelatin have high added value. Therefore, their study is very important for valuing fish waste (Marti-Quijal et al., 2020). The three most common methods of using fish waste are to produce fish meal/oil, to produce silage, or to use waste in the production of organic fertilizers (Arvanitoyannis and Kassaveti, 2008).

Fish silage and its types

Fish silage is a liquid product obtained from the liquefaction of all or part of fish (Tatterson and Windsor, 1974) and has been proposed as a simple and inexpensive substitute for the production of fish meal (van‘t Land and Raes, 2019). Since this product can be produced using relatively small amounts of raw materials in the isolated spaces, the need for expensive processing and maintenance equipment is eliminated (Goddard and AL-Yahyaio, 2001). In general, fish silage is a product with high biological value and its composition is almost similar to the raw material used (Vidotti et al., 2002). Therefore, the composition and nutritional quality of the product is determined primarily by the composition and freshness of the raw materials. Although processing parameters (such as acid type, pH, additives, storage time and temperature) are more controlled during production, these parameters can also affect the nutritional quality and final composition of fish silage. It is more difficult to control the composition and freshness of raw materials, as it strongly depends on the composition of the catch, the time of transport and the method of processing (Van’t Land et al., 2017). Fish silage is divided into three phases during storage, with fish oil at the top, highly soluble proteins, minerals in the middle layer, and semi-soluble materials and bones at the bottom. Proper mixing and stirring is essential to reduce lipid fraction oxidation and maintain product homogeneity (Fagbenro, 1994). Fish silage can be produced in different ways, which are discussed below.

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Acidic (chemical) silage: In making this type of silage, the enzymes in the fish mass are spread throughout it by crushing and mixing, and the acidity is regulated in such a way as to benefit the rapid action of these enzymes and inhibit bacterial function (Tatterson and Windsor, 1974). In fact, liquefaction, which is an autolytic process, is done by enzymes in fish. This process is accelerated by an acid creating the right conditions for decomposing the tissues by enzymes and also limits the growth of spoilage bacteria (Arvanitoyannis and Kassaveti, 2008). Through this process, whole biomolecules are broken down into smaller components (such as proteins into peptides and lipids into fatty acids) (van't Land and Raes, 2019). The substrate is mixed with acid (strong mineral acids or organic acids) to adjust the pH of the mixture to less than 4. At this pH, serine proteases are completely inactive, but the septic enzymes and pepsin are highly active. Primarily, pepsin is responsible for the production of fish silage, the content value of which can be very high in the visceral part of fish (Fagbenro, 1996). In one study, during silage of cod in the presence of 3% (volumetric/volumetric) formic acid, enzymes mainly responsible for liquefaction, enzymes in the intestine, skin and other parts of fish (other than meat) were reported (Backhoff, 1976). In the acidic method, the raw materials should preferably be ground or crushed into small pieces and occasionally stirred to create the desired uniformity and greater contact of the material with the acid; because untreated parts can cause problems. Room temperature is typically used for maintenance, which stimulates the desired biochemical changes (Arruda et al., 2007).

Different acids or combinations of acids can be used. Although mineral acids (such as hydrochloric acid and sulfuric acid) are relatively inexpensive, they are detrimental to the feed component, which must be neutralised before the animals are fed. Formic acid has the advantage of being stored at a higher pH, in which case it does not need to be neutralized for use in feed (Tatterson and Windsor, 1974). However, silages produced with formic acid are more expensive than silages produced with inorganic acids. The liquefaction process with formic acid can be performed in the pH range of 4 to 4/5, which is due to the antiseptic properties of formic acid. But while using mineral acids, the pH of the final product should be around 2 to prevent the growth of bacteria (Tatterson and Windsor, 1974).

Fermented (biological) silage: Fermentation for fish has been used for many years and is a low-level, cost-effective technology for tropical developing countries (Fagbenro, 1996). Fish fermentation is traditionally used to increase the shelf life of fish, which leads to the formation of the desired bacterial metabolites. Fermentation of by-products increases the quality of hydrolysis of proteins, oils and the production of antioxidant compounds. It is a safe, environmentally friendly, low-energy technology (Marti-Quijal et al., 2020). This is a bio-fermentation process performed with the addition of lactic acid bacteria and fermentable carbohydrates to the substrate (Shabani et al., 2019). However, fermentation is also performed without the addition of primer culture, which is called natural fermentation, which has been studied in some studies (Zahar et al., 2002). For proper fermentation, the material must contain lactic acid bacteria and the nutritional needs of these bacteria (including fermentable carbohydrates, amino acids, nucleotides, vitamins, and growth factors) enabling optimal fermentation. Also, the storage temperature should be within the required range for fermenting bacteria (Lindgren and Pleje, 1983).

Since aquatic wastes are high in unsaturated fatty acids, they are very sensitive to fat oxidation and spoilage, which reduces the quality of fish meal during storage (Shabani et al., 2019). Yano et al. (2008) stated that the fermentation of aquatic waste by microorganisms can improve their quality by reducing their fat content. Biologically prepared silage may have improved nutritional value (compared to fish meal) due to increased digestibility and biological activity and the removal of undesirable and anti-nutritional compounds. This product can contain some functional compounds (such as lactic acid bacteria) and organic acids (mainly lactic acid) preventing pathogen contamination by creating acidic conditions in the silage causing a longer shelf life (Shabani et al., 2019).

Enzymatic silage: Liquid fish products (such as silage) can be produced by adding other enzymes (Tatterson and Windsor, 1974; Borghesi et al., 2008; Borghesi et al., 2008; Hevrøy et al., 2005; Khosravi et al., 2015). These exogenous enzymes used can be obtained from various animal, plant or microbial sources (Ovissipour et al., 2009; Hathwar et al., 2011). In general, various studies have shown that the use of these hydrolyses can be used successfully in feed. In some cases, improved cellular immunity and growth (Goosen et al., 2014), increased growth performance, feed utilization, digestibility, innate immunity, and resistance to fish disease (Khosravi et al., 2015) have been reported.

Problems related to silage

Fish silage is usually produced and stored as a liquid close to where it is used (Goddard and AL-Yahyaio, 2001). But the high water content of this product makes its transportation for long distances uneconomical. Therefore, the production of fish silage on an industrial scale is limited (Backhoff, 1976). Drying liquid fish products reduces storage and transportation costs, facilitates the inclusion of fish silage in the diet, and also limits microbial growth (Van't Land and Raes, 2019). Drying, however, means an additional cost (Arruda et al., 2007); because conventional drying methods are often expensive, energy-consuming and tedious, and require harsh conditions (Van't Land and Raes, 2019). Drying can be done by adding agricultural by-products commonly used in animal feed (Vidotti et al., 2002). Freeze-drying and spray drying of protein products are also recommended, as bioactive compounds maintain functional properties (such as emulsification and water holding capacity) and nutritional quality. However, considering the long time spent in freeze-drying and the application of high temperatures in spray drying, both of these processes can cause unwanted structural changes in the product (Van't Land and Raes, 2019).

V'ant Land and Raes (2019) conducted a study to investigate the drying potential of silage using refractance window drying. In this method, the products are dried by transferring them in a thin layer, on a polyester film, on a hot water bath,

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and convection, conduction and radiation heat transfer occurs (Nindo et al., 2003). Due to the application of low drying temperatures and short storage times, this method was introduced with high potential to maintain quality when concentrating silage. Mild conditions also indicate the potential for sustainable yield and biological activity of hydrolyzed fish products (Van't Land and Raes, 2019). This method also has the advantages of improved energy efficiency and lower costs than spray and freeze drying methods (Nindo et al., 2003; Baeghbali et al., 2016).

Due to its high content of unsaturated lipids, fish silage is prone to oxidation and the formation of toxic products (Fagbenro, 1996) that can jeopardize the nutritional value of the diet (Arruda et al., 2007), reduce product quality, especially during storage, and may reduce the nutritional performance of animals (Fagbenro, 1996). The lipid oxidation process also changes the taste, color and texture of the silage, and the oxidation process can be accelerated if the fish silage comes in contact with light and air. Therefore, in order to achieve a more uniform and stable product, it is recommended to remove the fat part of the silage during its preparation (Arruda et al., 2007). In addition, lipid oxidation can be prevented by adding a variety of antioxidants, including ethoxyquinone, butylated hydroxytoluene (BHT), and butylated hydroxy anisole (BHA). However, since these synthetic antioxidants are expensive and are slowly metabolized in animal muscle, they are banned from many meat and fish products (Fagbenro, 1996).

The antioxidant properties of spices and plant extracts on fish and meat products have been proven to be generally available at low cost. Therefore, they are offered as cheaper substitutes to synthetic antioxidants (Fagbenro and Jauncery, 1998). In some studies, onion extract has been used as a natural antioxidant in fermented silages of shrimp head (Fagbenro, 1996) and tilapia (Fagbenro and Jauncery, 1998), which has acted effectively as a fat antioxidant.

The use of acids to produce fish silage can potentially eliminate essential amino acids (especially tryptophan) and reduce the nutritional value of silage (Martínez-Alvarez et al., 2015). In addition, total deamination can be caused by prolonged hydrolysis, which is reflected by a decrease in essential amino acids and an increase in volatile nitrogen. Therefore, there is a possibility of many changes in the final product (Van't Land et al., 2017).

Processing fish silage with organic acid and storing the product may, due to hydrolysis, change proteins into smaller protein fragments, more peptides, and free amino acids (Nørgaard et al., 2015). High contents of free amino acids can interfere with the mechanisms of amino acid and polypeptide uptake in fish (Goddard and AL-Yahyaio, 2001). Optimal maintenance can be achieved by inhibiting the activity of endogenous proteolytic enzymes by limiting the proteolysis rate (Fagbenro, 1996). Salt can be used to inhibit protein hydrolysis in fish silage (Fagbenro and Jauncey, 1993). Inhibition of protein hydrolysis by the addition of trona (Sodium sesquicarbonate) has also been reported (Fagbenro, 1996). In addition, the process of silage autolysis can be stopped by cooking raw fish before fermentation (Fagbenro and Jauncey, 1993) or before adding acid (Espe et al., 1992), pasteurizing (Hardy et al., 1984) or subsequently lowering the pH (Stone and Hardy, 1986). The use of fish waste silage in feed formulations may be significantly limited due to its unpleasant odor (Arvanitoyannis and Kassaveti, 2008). In addition, the problems associated with bitterness in hydrolyzed silage can make the product extremely unpleasant for animals fed with fish-enriched silage (Kristinsson and Rasco, 2000).

Use of fish silage

Fish silage can have various applications, including substituting fish meal in animal and aquatic feed, and using the oil extracted from it to be included in aquatic diets, which are addressed below.

Use of fish silage in fish feed

Aquaculture is the biggest contributor to increasing fish production, which is one of the fastest growing agricultural activities in the world (Arruda et al., 2007). Today, more than a quarter of the world's seafood is sourced from aquaculture, and the FAO forecasts that by 2030 it will be closer to 50 percent (Arvanitoyannis and Kassaveti, 2008). Given this rapid growth of global aquaculture, this industry needs high quality food (Olsen and Toppe, 2017), and future demand for fish farming largely depends on the availability of suitable, inexpensive, and sufficient quantities of feed (Martínez-Alvarez et al., 2015). Fish meal is often used as a source of protein in animal feed, which reduces the stocks of wild fish (as a raw material) leading to stiff competition and rising prices (FAO, 2016). Access to it is also seasonal (Vidotti et al., 2002). Therefore, there is a growing concern to identify substitute protein sources in order to minimize the use of fish meal in feed formulations (Samaddar et al., 2015). Fish silage is known as a simple and inexpensive substitute to fish meal (van't Land and Raes, 2019). Fish silage can be fed directly into wet diets or dried or squeezed as part of animal feed (Goddard and AL-Yahyaio, 2001). However, the use of fish silage in aquatic feed depends on its apparent digestibility coefficients (Borghesi et al., 2008). It is important to know that international feed law in Europe does not allow fish products or components to be used in the same feed (Borghesi et al., 2008). In different studies, fish silage has been studied in the diets of different aquatic species, which has been presented in the table below with a summary of the results of each of them.

Application of oil extracted from fish silage in aquatic feed

Oil extracted from fish silage can also be used in aquatic feed, which was examined in some studies, and benefits such as improved cellular immune function in South African abalone (*Haliotis midae*) (Goosen et al., 2014) and antimicrobial properties in the diet and digestive tract of Mozambique tilapia (*Oreochromis mossambicus*) have been reported. It was also introduced as a good source of polyunsaturated fatty acids and a cost-effective substitute to some

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common fish oils (Goosen et al., 2014). However, it was stated that the optimal content of oil in the diet should be determined in order to avoid negative effects on production efficiency (Goosen et al., 2014).

Use of fish silage in feed of other animals

Fish meal has been considered a source of high quality protein in diets for many years. In recent years, in northern Europe, fish silage has gradually replaced fish meal in pig diets, as it has as good an effect on as fish meal (Nørgaard et al., 2015). Other studies have shown that fish silage, as a protein component, can be replaced with fish meal in the diet of various animals such as Omani sheep (Al-Abri et al., 2014), rats (Espe et al., 1992), broilers (Vizcarra-Magaña et al., 1999; Santana-Delgado et al., 2008), and Japanese quail (Panda et al., 2017).

Table 1 - Summary of some reported studies on silage processing, use it in diets and it outcomes.

Raw material	Treatment	Fish specie	Percentage of silage in diet	Outcome	References
Whole Pacific whiting (*Merluccius productus*) and its wastes	2% sulfuric and 0/75% propionic acid	Rainbow Trout (*Salmo gairdneri*)	50%	Apparent digestibility values were higher for the fish silages than for fish meal.	(Stoneand Hardy, 1989)
Whole Pacific whiting	2% sulfuric and 0/75% propionic acid	Rainbow Trout	50%	Growth and feed conversion were significantly affected by feeding diets containing silage.	(Hardy et al., 1983)
By-products of shelled shrimp (*Pandalus borealis*)	4/8% sulphuric and 1/2% propionic acid	Rainbow Trout (*Salmo Gairdneri*)	10/5%	The digestion of the astaxanthin was improved by ensiling. The rate of accumulation of the pigment in the fish muscle was markedly higher in fish fed the silage diet than those given fresh or dried shrimp waste.	(Torrissen et al., 1981)
Enriched poultry by-product meal with silage of tuna by-products	2/5% citric and 2/5% phosphoric acid	Rainbow Trout (*Oncorhynchus mykiss*)	21, 40 and 62%	Growth of rainbow has been improved.	(Barreto-Curiel et al., 2016)
Marine fish processing by-products	3% formic acid	Rainbow Trout (*Oncorhynchus mykiss*)	20, 40 and 60%	Silage had potential to replace fish meal up to 20% in diets (without adverse effects on growth performance, fatty acid composition and serum biochemical variables).	(Güllü et al., 2014)
Fish processing waste	-	Rainbow Trout (*Oncorhynchus mykiss*)	25, 50 and 100%	Replacing the fish meal with 50% fish silage had a positive effect on growth.	(Guzel et al., 2011)
Spiny Atlantic dogfish *(Squalus acanthias)* offal Herring	3/5% formic acid	Atlantic Salmon (*Salmo salar*)	24/6% 22%	The palatability of both silage diets fed to the smaller fish was decreased.	(Heras et al., 1994)
-Freshwater and saltwater commercial fish waste -Tilapia filleting residue	-15% sugar cane molasses, 5% *Lactobacillus plantarum* -Acid silage: 2% formic and 2% sulfuric acid	Pacu (*Piaractus mesopotamicus*) Fingerlings	The different ratios	Apparent protein digestibility and protein productive values were significantly different.	(Vidotti et al., 2002)
Fish by-products	60% fish by-products, 30% rice bran, 5% dried molasses and 5% of *Lactobacillus plantarum*	-Nile Tilapia (*Oreochromis niloticus*) -African Catfish (*Clarias gariepinus*)	25, 50,75 and 100%	Dried fish silage successfully replaced up to 25 and 50% of fish meal in diets. While, the higher levels of replacement reduced growth performance, feed utilization parameters as well as significant effect on fish body composition of fishes.	(Soltan and Tharwat, 2006)
-Seeds of Jatropha curcas kernel meal (JCK) and tilapia waste silage (FS) -Tilapia byproducts	1/5% of formic acid	White Shrimp (*Litopenaeus vannamei*)	The different ratios	A combination of 18/75% fish silage and 39/75% JCK meals is a potential alternative to fish meal in practical diets for *L.vannamei*.	(Rodríguez-González et al., 2018)
Raw heads of the river prawn (*Macrobrachium vollenhovenii*)	*Lactobacillus plantarum* at 30°C using molasses or cassava starch	Fingerlings Catfish (*Clarias gariepinus*)	15%	Apparent digestibility coefficients of dry matter, crude protein, gross energy and essential amino acids in the silage by catfish fingerlings was high (>70%).	(Fagbenro and Bello-Olusoji, 1997)
Shrimp heads	17% acetic acid	Tilapia (*Oreochromis niloticus* Linnaeus)	10, 20 and 30%	Proximate composition of the feeds (except for a higher protein content than the commercial feed) did not differ statistically.	(Cavalheiro et al., 2007)
Filleting by-products and 80% whole Nile tilapias	AS: 1/5% formic and 1/5% propionic acid BS: 1/4% *Lactobacillus plantarum*, 18% sugarcane molasses were added to AS ES:10 g of protease to AS	Nile Tilapia (*Oreochromis niloticus*)	30%	The highest amount of apparent digestibility coefficient (ADS) and the highest average ADC of amino acids were related to ES.	(Borghesi et al., 2008)
Shrimp (*Penaeus* spp) head waste	10% refined sugar cane and 5% *lactobacillus* spp.	Nile Tilapia (*Oreochromis niloticus L*)	10, 15, 20, 25 and 30%	Growth ratio was improved at dietary inclusion levels as high as 15%. The diets containing shrimp silage were well accepted by the fish.	(Plascencia-Jatomea et al., 2002)
-Heated and unheated mackerel, S. *juponicus* -Heated abalone viscera	2/6% phosphoric and 2/6% citric acid	Juvenile Abalone (*Haliotis fulgens*)	31/8 31/8 20	Significantly higher growth rates occurred when abalone were fed artificial diets containing both fish silages (compared with the kelp, *Mucrocystis pyrifera*).	(Viana et al., 1996)
Nile tilapia (*Oreochromis niloticus*) filleting residue	2% Formic and 3% Phosphoric acid	Pacific White Shrimp (*Litopenaeus vannamei*)	1/5, 3/0, 4/5 and 6/0%	Shrimp final weight was statistically influenced by biofloc system ($P<0.05$) but not by the diet.	(Neto et al., 2019)
Nile tilapia (*Oreochromis niloticus*) processing waste	The acid silage was produced	Pacific White Shrimp (*Litopenaeus vannamei*)	1/5, 3/0, 4/5 and 6/0%	After 45 days, water quality parameters remained within the recommended range for *L. vannamei* culture. Regarding to the production performance survival was above	(Lobato et al., 2019)

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				70% in all treatments.	
Rainbow trout viscera	2/5% formic acid	Mozambique Tilapia (*Oreochromis mossambicus*)	16%, 28/5%	Low silage inclusion improved phagocytic activity of leucocytes (compared with the reference). High silage inclusion significantly decreased growth and led to higher mortality. Formic acid had no effect on growth.	(Goosen et al., 2016)
Ungutted tilapia	5% sugar beet molasses and 2% *Lactobacillus plantarum*	-Juvenile *Oreochromis niloticus* -*Clanas gariepinus*	25, 50 and 75%	Apparent protein digestibility decreased with increasing dietary level of CO-dried fish silage: soybean meal blend	(Fagbenro et al., 1994)
Indian oil sardines (*Sardinella longiceps*)	1/5% mixture of formic and propionic acid (1:1)	Tilapia (*Orenchromis aureus*)	30%	Essential amino acids were present at levels exceeding the requirements for tilapia. The apparent digestibility coefficients of crude protein, dry matter and gross energy for silage were equivalent to those of fishmeal.	(Goddard and AL-Yahyaio, 2001)
Discards from snapper (*Lutjanus* spp.), grunt (*Haemulon plumieri*) and grouper (*Epinephelus* spp.) filleting	1/5% formic acid	Juvenile *Litopenaeus vannamei*	294/6 and 604/0 (g/kg)	Waste fish silage was beneficial for the white shrimp *L. vannamei*. It sustained reasonable weight gain combined with soybean meal in practical diets.	(Gallardo et al., 2012)
Tilapia (*Oreochromis* spp.) by-products	4% formic acid	Hybrid Red Tilapia *(Oreochromis mossambicus×Oreochromis niloticus×Oreochromis aureus*)	25, 50 and 75%	Less expensive dried fish silage with rice bran is an alternative protein source for tilapia feed up to 50% of fishmeal replacement.	(Madage et al., 2015)
-	-	Eel fingerlings (1.7 g)	10, 15 and 20%	The food conversion efficiency, the protein efficiency ratio and the specific growth rate have been Increased.	(Gonçalves et al., 1989)
Residue from the processing of Nile tilapia fillets (*Oreochromis niloticus*)	2% Formic and 3% Phosphoric acid	Pacific White Shrimp (*Litopenaeus vannamei*)	1/5, 3/0, 4/5 and 6/0%	The combination of the biofloc system and tilapia silage feed-based were reported as good options to increase the sustainability of intensive shrimp culture and the overall shrimp quality and shelf life.	(Gonçalves et al., 2019)
Whole whiting (*Merlangius meriangus*)	3% Formic acid	Mirror Carp (*Cyprinus Carpio*)	43/4%	Intake of the fish silage diet was slow compared with diets containing cooked fish and fish meal whilst mortality reached 40% in one replicate.	(Wood et al., 1985)
Stunted tilapia (*O reochromis niloticus*) brooders culled	15% Sugar beet molasses and 5% *Lactobacillus plantarum*	Tilapia (*Oreochromis niloticus*)	66%	Moist fish silage pellets were physically stable and highly digestible to *O. niloticus*, and suitable as farm-made feeds for fish.	(Fagbenro and Jauncery, 1998)
-Fresh hake -Hake stored for 48 h at 17°C	Eight different *Lactobacillus* cultures and whey powder, molasses and sugar	-Calves -Cattle -Pigs	65/4% 90%	The final quality and stability of fermented silage relates to the choice of *Lactobacillus*, the quality and age of the fish material.	(Van Wyk and Heydenrych, 1985)
Fish body viscera	Lacto bacillus bacteria 5% Molasses, 5% yogurt, 30% orange peels and 60% minced body viscera	*Labeo rohita*	50, 75 and 100%	Fish silage had reasonable concentration of nutrients. Fermented fish silage contains high concentrations of mono-unsaturated fatty acids which has positive impact upon growth.	(Haider et al., 2017)
-Muscle of Pacific creole-fish (*Paranthias colonus*) -Humboldt squid (*Dosidicus gigas*)	10% carbohydrate source and 5% an overnight-wet pellet of *L. sakei* strain 5-4	Pacific Red Snapper (*Lutjanus peru*)	44/733% 43/361%	The marine silages with squid enriched or combined with *L. sakei* 5-4 increased the body weight and stimulated the physiological and humoral immune parameters in fish infected with *A. veronii*.	(Reyes-Becerril et al., 2012)

CONCLUSION

Using fish waste to prepare silage, in addition to solving the major problem of disposing of this waste and reducing the environmental effects caused by them, can improve the overall productivity of animal protein consumption, add nutritional benefits to diets prepared from these materials and also reduce the cost of diets. If fish silage can be a substitute to fish meal in the formulation of diets, the effect of fat content on the taste of the final product should be investigated. There is also a need to standardize and maintain the nutritional quality of silage. Further studies are needed to evaluate the appropriate amount of silage input in diets.

DECLARATIONS

Corresponding author

E-mail: r.reisi2012@yahoo.com

Authors' contribution

R. Raeesi had the idea for the article and drafted it, and performed the literature search.
B. Shabanpour performed Supervision and editing.
P. Pourashouri critically revised the work.

Conflict of interests

The authors declare that they have no conflict of interest.

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IMPACT OF PHYTOGENIC FORMULATION ON PERFORMANCE AND FATTY LIVER DISEASE OF BROILER CHICKENS

Arigesavan KANINATHAN, Suresh SUBRAMANIYAM, Saravanakumar MARIMUTHU, and Prashanth D'SOUZA

R&D Centre, Natural Remedies Private Limited, Veerasandra Industrial Area, Electronic City (PO), Hosur Road, Bangalore 560100, Karnataka, India

Email: saravana.k@naturalremedy.com

Supporting Information

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ABSTRACT: This study was conducted to evaluate the effect of polyherbal (phytogenic) formulation (PHF: containing *Acacia nilotica* and *Curcuma longa*) on performance parameters, liver histopathology and prevention of fatty liver in broilers. 700 day-old chicks were randomly distributed to seven groups (10 replicates / group; 10 birds each), namely positive control (T1) fed with basal diet + choline chloride (CCL) 60% (1000g), negative control (T2) fed with high energy (5% increment), low protein (24% reduction), high cholesterol (2% increment) diet, T3 (T2 + PHF; 1000g-full cycle), T4 (T2 + PHF; 2000g-full cycle), T5 (T2 + CCL 60% (1000g-full cycle)), T6 (T5 + PHF; 1000g-grower and finisher stage), T7 (T5 + PHF; 2000g-finisher stage). Average daily gain (ADG; g), average daily feed intake (ADFI; g) and feed conversion ratio (FCR) were calculated at 1-14 days, 15-28 days, 29-42 days, and 1-42 days. Serum triglycerides analysis, gross and histopathological observations of liver morphology were performed for the samples of control and experimental groups on day 42. The performance parameters; ADG, ADFI, FCR, and liveability were found to be improved in all the groups as compared to the negative control group. However, better performance was observed in PHF (2000g) top-up group (during the finisher stage) as compared to the negative control group. Serum triglyceride levels were increased non-significantly as compared to the negative control indicating that more fat is mobilized from liver to serum. In addition, PHF supplementation at 2000g during the finisher phase had restored the liver tissue architecture as well as improved the liver score when compared to the negative control group. It is concluded that PHF (2000g/ton) during the finisher stage can be used as a top-up to improve the performance parameters as well as to prevent the fatty liver condition in broiler chickens.

Keywords: Basal diet, Broiler chicken, Choline chloride, Herbal formulation, Triglycerides.

Abbreviation: ADG: Average daily gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio; CCL: Choline chloride; PHF: Polyherbal formulation; HELP: High energy low protein

INTRODUCTION

Domesticated chickens are the most important source of protein to be used for the human consumption worldwide, and consequently it would fetch an enormous economic added value in near future (Padhi, 2016). Generally, modern broilers would grow very fast and gain excessive weight due to the genetic modification and the standardized diet composition (Petracci et al., 2015). Owing to the rapid development of the meat-type chickens in a very short time would result in a high level of production performance but with some welfare issues that include skeletal leg deformity leading to defective movement (Julian, 1998; Çapar Akyüz and Onbaşılar, 2020). Therefore, society is mostly interested in animal–welfare friendly farmed meat-type chicken that would be reared using diet with high energy and low protein specifications (Bona et al., 2018). However, when the birds with restricted activity (occurs mostly in caged birds) are allowed to consume high energy diet, exhibits a disturbance in the metabolism of fat especially in the liver tissues, which results in accumulation of fat in the hepatocytes and thus causes a sudden death (Lin et al., 2021). The fatty liver syndrome is a non-infectious metabolic disorder mostly encountered in the poultry sector, as the 90% of *de novo* fatty acid is synthesized in the liver and its mobilization towards non-hepatic tissues depends upon the availability of lipoproteins in the liver. However, high energy and low protein diet increased the hepatic fatty acid without affecting the lipoprotein synthesis. As a result, the lipids cannot be completely mobilized which leads to the deposition of excess lipids in the hepatocytes (Zhang et al., 2017). The pathophysiology of the fatty liver associated haemorrhage syndrome is yet to be clearly identified. Nevertheless, the haemorrhage may result from the rupture of hepatic reticulin and capsule due to the abnormal liver swelling caused by deposition of fat (Trott et al., 2014). Hence, the better understanding of metabolic process and dietary factors that can interfere with the liver function is crucial to raise the commercial broiler flocks with exceptional zootechnical performance (Zaefarian et al., 2019).

Fatty liver is induced by various factors such as nutritional, hormonal, environmental and metabolic changes (Juanola et al., 2021). Many nutrients such as choline, phospholipids, vitamin B and vitamin E, demonstrate a vital role in maintaining the lipid homeostasis in the liver. Lack of these nutrients in the diet causes a disturbance in the metabolic functions such as lipid absorption, β-oxidation and lipoprotein synthesis that leads to excessive fat accumulation in the liver (Zeisel and Da Costa, 2009). More specifically, choline, a vitamin like water-soluble micronutrient and a lipotropic

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agent, plays a preventive role by enabling the fat utilization and transportation from the hepatic to the extrahepatic tissues (Biswas and Giri, 2015). It was reported that a diet with low choline content causes fatty liver syndrome due to low availability of carrier lipoproteins (Fon Tacer and Rozman, 2011), growth retardation and perosis in fast-growing broiler strains. To avoid the detrimental responses associated with choline deficiency, synthetic choline chloride (CCL) has been supplemented to the poultry diet to improve the growth performance and carcass quality (Gregg et al., 2022). However, due to its hygroscopicity, it would accelerate the oxidative loss of other vitamins which is already present in the feed when it is included in the feed formulation (Rath et al., 2017). To counteract the negative impacts, an herbal feed additive, Kolin Plus™, is used in the place of synthetic choline chloride in broiler diet. It is already reported to have choline like function in broilers (Selvam et al., 2018). On the other hand, a recent finding suggested that a high energy and low protein along with a high cholesterol diet caused the fat accumulation in the liver which leads to fatty liver disorder in chickens (Lin et al., 2021).

Hence in the current study, we have employed high energy (5% increment), low protein (24% reduction), high Cholesterol (2% increment) formulated diet to induce fatty liver model in broiler chicken. Besides, we have evaluated the effect of PHF alone or in combination with CCL on prevention of fatty liver associated problems in broiler chickens.

MATERIAL AND METHODS

Polyherbal formulation (PHF)

Kolin Plus™ is a PHF developed by M/s. Natural Remedies Pvt. Ltd., Bengaluru, India, containing *Acacia nilotica* and *Curcuma longa* plant parts.

Ethical approval

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

Study design

A total of 700 one-day-old Ross AP95 broiler chicks weighing between 30 and 60 g were selected for the study. The experiment was performed at Agrivet Research & Advisory Pvt. Ltd., Kolkata, India for 42 days. Chicks were randomly assigned to seven groups with ten replicates having 10 birds each, namely positive control (PC; Treatment-T1) fed on basal diet plus CCL 60% (1000g), negative control (NC; T2) fed with diet containing high energy (5% increment), low protein (24% reduction of crude protein), high Cholesterol (2% increment), T3 (T2 plus 1000g of PHF for full cycle), T4 (T2 plus 2000g of PHF for full cycle), T5 (T2 plus 1000g of CCL 60% for full cycle), T6 (T5 plus 1000g of PHF for grower and finisher stage), T7 (T5 plus 2000g of PHF for finisher stage). The birds were fed *ad libitum* with a starter (1-10 d), a grower (11-24 d) and a finisher (25-42 d) diet; the composition and nutritive values of the diet were presented in Table 1.

Productive performance

The body weight (BW) of the birds was recorded pen-wise at weekly intervals. A measured quantity of the feed was offered to each pen and the cumulative feed intake (FI) was calculated during the periods of 1-14 d, 15-28 d and 29-42 d by subtracting the quantity of feed left in each pen from the total quantity offered during the respective period. Average daily gain (ADG) and average daily feed intake (ADFI) were calculated by the total weight gain or total weight of feed consumed (g) / total number of days in each respective period. The feed conversion ratio (FCR) was calculated as total feed consumption divided by total body weight gain. Mortality was recorded as and when it happened, and the weight of the dead birds was recorded to adjust the FCR data accordingly. Overall liveability was calculated for the cumulative period of 1-42 d.

Gross observation

At 42 d of age, the whole liver was removed carefully and kept on wooden sheet for gross anatomical observation. The severity of fatty liver was assessed by liver color and scored, as modified based on a study conducted by Zhu et al. (2020). Briefly, liver color was scored as follows: Score-1, dark red and no haemorrhage; Score-2, mild yellow and haemorrhages (mild case); Score-3, light yellowish red and haemorrhages (moderate case); Score-4, large and massive haemorrhages with putty-coloured livers (extreme case).

Serum biochemical parameters

At 42 d of age, after the final BW was recorded, 10 birds (1 bird / pen) were selected randomly in each dietary group, and whole blood was collected from the right brachial vein in vacutainer tubes without any anticoagulant and allowed to clot at room temperature for 2 hrs. Then the separated serum was harvested and analysed photometrically for the estimation of triacylglycerol using commercially available biochemical kits (Delta Lab®, Mumbai, India).

Histopathological examination

At 42 d of age, the whole liver was cut into small pieces of approximately 4-5 mm width and was preserved in 10% buffered formal saline solution. Paraffin embedded tissue was stained with standard Haematoxylin and Eosin method, mounted using DPX mounting medium (S.D. fine-chem Ltd., Bengaluru, India) and was observed under a microscope

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(Olympus Corporation, Tokyo, Japan) connected with the camera (DP20) for histopathological examination to evaluate the hepatic changes due to dietary treatments.

Statistical analysis

Data were analysed using the pen averages as the experimental units for the performance traits (ADG, feed intake, ADFI, FCR and liveability). For histological observations and serum biochemical assay, individual observations were considered as the experimental units. The data were analysed in the general linear model of SPSS (V. 26.0) using multivariate ANOVA and expressed in terms of mean and pooled standard error of the mean. Probability values of $p<0.05$ were described as statistically significant; whenever found significant, the means were separated by Tukey's B test.

Table 1 - Composition and calculated nutritive values (g/kg as fed basis unless stated otherwise) of the positive control diets

Ingredients (g/kg diet)	Starter (1-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Maize	578.13	616.24	644.1
Soybean meal Hi Pro	322.43	279.7	234.1
Palm oil	24.1	28.23	38.84
Cholesterol-91%	0	0	0
Maize gluten meal	34.7	40	49.2
Dicalcium phosphate	15.61	13.23	11.24
Limestone powder	9.5	8.86	8.8
Salt	2.72	2.22	2.2
Sodium bi carbonate	2	2	2
DL-methionine	2.54	2	1.82
L-lysine HCl	2.64	2.3	2.57
L-threonine	0.93	0.52	0.43
Vitamin premix+	1	1	1
Trace mineral premix++	0.5	0.5	0.5
NSPase enzyme	0.1	0.1	0.1
E. coli phytase 5000	0.1	0.1	0.1
Choline chloride	1	1	1
Filler (rice husk)+++	2	2	2
Nutrients			
AME kcal/kg	3000	3100	3200
Crude protein	23	21.5	20
SID amino acids %			
Lysine	1.28	1.15	1.06
Methionine	0.6	0.54	0.52
Met + Cys	0.95	0.87	0.66
Threonine	0.86	0.77	0.71
Tryptophan	0.25	0.22	0.2
Arginine	1.37	1.23	1.13
Isoleucine	0.91	0.85	0.76
Valine	0.98	0.93	0.85
Crude fibre	2.68	2.6	2.56
Crude fat	5.38	5.73	7.11
Calcium	0.96	0.87	0.81
Available P	0.48	0.44	0.41
Sodium	0.22	0.2	0.2
Potassium	0.94	0.84	0.79
Chloride	0.22	0.18	0.18
Choline ppm	1700	1600	1500

+: each kg contained vitamin A 13.5 MIU, vitamin D3 4.5 MIU, vitamin E 60 g, vitamin K3 3.5 g, vitamin B1 3.5 g, vitamin B2 8.0 g, vitamin B6 3.5 g, vitamin B 12 0.02 g, biotin 0.145 g, pantothenic acid 14.5 g, folic acid 2.25 g, niacin 60 g); ++: protein chelates of manganese 60 mg, iron 30 mg, zinc 50 mg, copper 10 mg, selenium 0.5 mg, chromium 0.4 mg, iodine 4.0 mg (as potassium iodide). +++: The test material was added by replacing an equivalent amount of the filler substance.

RESULTS

Effect of PHF on the performance trait

High energy and low protein along with high cholesterol diet suppressed the production performance of birds in the T2 when compared to the T1. However, all the groups showed an improvement in the ADG, ADFI, feed intake and FCR as compared to T2 ($P\leq0.01$). Supplementation of PHF (2000g/ton) as a top-up (along with CCL-60%) in during the finisher stage showed better performance as compared to other supplemented groups (Table 2).

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Effect of PHF on triglycerides and liver color score

Table 3 showed the effect of PHF on serum triglycerides and liver color score. The birds in T2 showed low serum triglycerides and a higher color score of liver (gross observation) as compared to T1 (Figure 1). On the other hand, product supplemented groups showed a significant increase the serum triglycerides level ($P \leq 0.01$) and numerical increment in the liver color score ($P > 0.05$) as compared to T2. This indicates that more fat is mobilized from liver to non-hepatic tissues in broilers supplemented with PHF and CCL. Supplementation of PHF (2000g/ton) as a top-up (along with CCL-60%) in T7 during the finisher stage showed higher serum triglycerides level and better color score as compared to other supplemented groups.

Effect of PHF on liveability (%)

Table 2 showed the effect of PHF on liveability %. When compared with T2 group, liveability % was improved in all the supplemented groups.

Effect of PHF on histopathological alteration

Table 4 showed the effect of PHF on histopathological alteration. Hepatic vacuolization along with necrosis was predominantly observed in T2 which was fed with high energy and low protein diet devoid of choline and the presence of cholesterol (Figure 2). This hepatic vacuolization along with hepatocellular necrosis is subject to fat deposition in hepatic tissue. Moreover, changes have become mild to moderate in different groups with the least pathologic changes observed in T7 (PHF 2000 g along with CCL 60% 1000 g- during finisher stage) followed by T5 (CCL 60% 1000 g) and T4 (PHF 2000 g).

Table 2 - Effect of PHF on the performance traits

Day	(T1) PC	(T2) NC	(T3) T2+PHF 1000g FC	(T4) T2+PHF 2000g FC	(T5) T2+CCL60% 1000g FC	(T6) T5+PHF 1000g GF	(T7) T5+PHF 2000g F	SEM	P-value
Average Daily Gain (ADG, g)									
1-14d	37.14[b]	31.14[a]	30.43[a]	30.49[a]	31.74[a]	30.78[a]	31.25[a]	0.29	0.0001
15-28d	74.61[b]	52.01[a]	54.01[a]	51.59[a]	54.71[a]	52.20[a]	55.90[a]	0.98	0.0001
29-42d	85.83[b]	71.62[a]	74.23[a]	76.23[a]	74.95[a]	75.40[a]	76.62[a]	0.73	0.0001
1-42d	65.86[c]	51.59[a]	52.89[ab]	52.77[ab]	53.80[ab]	52.79[ab]	54.59[b]	0.59	0.0001
Average Daily Feed Intake (ADFI, g)									
1-14d	43.52[c]	41.88[abc]	41.55[ab]	41.11[a]	42.96[bc]	41.82[ab]	41.90[abc]	0.17	0.001
15-28d	111.99[b]	95.01[a]	95.56[a]	94.76[a]	99.21[a]	96.43[a]	100.67[a]	0.94	0.0001
29-42d	156.93	146.88	151.73	152.09	152.25	151.52	152.03	1.14	0.488
1-42d	104.15[b]	94.59[a]	96.28[a]	95.99[a]	98.14[a]	96.59[a]	98.20[a]	0.54	0.0001
Feed Conversion Ratio (FCR)									
1-14d	1.172[a]	1.346[b]	1.366[b]	1.349[b]	1.354[b]	1.359[b]	1.341[b]	0.008	0.0001
15-28d	1.501[a]	1.828[b]	1.772[b]	1.839[b]	1.815[b]	1.849[b]	1.802[b]	0.016	0.0001
29-42d	1.829[a]	2.056[b]	2.042[b]	1.998[b]	2.033[b]	2.010[b]	1.985[b]	0.013	0.0001
1-42d	1.582[a]	1.834[b]	1.820[b]	1.821[b]	1.825[b]	1.830[b]	1.799[b]	0.011	0.0001
Liveability (%)									
1-42d	94	92	95	94	97	95	100	0.73	0.09

Means with dissimilar superscripts in a row varied significantly; PHF – Polyherbal formulation; CCL – Choline chloride; FC – Full cycle; GF – Grower & finisher; F – Finisher

Table 3 - Effect of PHF on triglycerides and liver color score

Parameters	(T1) PC	(T2) NC	(T3) T2+PHF 1000g FC	(T4) T2+PHF 2000g FC	(T5) T2+CCL60% 1000g FC	(T6) T5+PHF 1000g GF	(T7) T5+PHF 2000g F	SEM	P-value
TG (mmol/L)	1.336[a]	2.595[b]	3.061[b]	2.671[b]	2.614[b]	2.875[b]	2.812[b]	0.084	0.0001
Color	0.3	0.8	0.7	0.6	0.6	0.45	0.45	0.067	0.484

Means with dissimilar superscripts in a row varied significantly; PHF – Polyherbal formulation; TG – Triglycerides; CCL – Choline chloride; FC – Full cycle; GF – Grower & finisher; F – Finisher; Lower the color score indicates less fat accumulation; Liver color score: 1 – dark red, without any haemorrhagic spots and firm in consistency, 2 – firm in consistency but slightly yellowish in colour with few haemorrhagic spots, 3 – softer in consistency and moderate area of haemorrhagic patches were observed, 4 – pale yellow in colour with large haemorrhagic patches

Table 4 - Effect of PHF on histopathological alteration

Parameters	(T1) PC	(T2) NC	(T3) T2+PHF 1000g FC	(T4) T2+PHF 2000g FC	(T5) T2+CCL60% 1000g FC	(T6) T5+PHF 1000g GF	(T7) T5+PHF 2000g F
Hepatic vacuolization	0.3 (10)	2.3 (80)	2 (60)	1.5 (50)	1.5 (50)	1.7 (70)	1.2 (30)
Congestion	0	1.1	0.5	1	0.3	0.1	0.1
Haemorrhages	0	1.4	0.4	1	0.2	0	0.2
Inflammation	0.1	0.6	0.4	1	0	0	0.1
Hepatocellular necrosis	0.2 (0)	1.7 (40)	1.6 (50)	1.3 (40)	1.2 (40)	1 (20)	1.1 (20)

Values are expressed as Mean and Percentage in parentheses; n=10; PHF – Polyherbal formulation; CCL – Choline chloride; FC – Full cycle; GF – Grower & finisher; F – Finisher

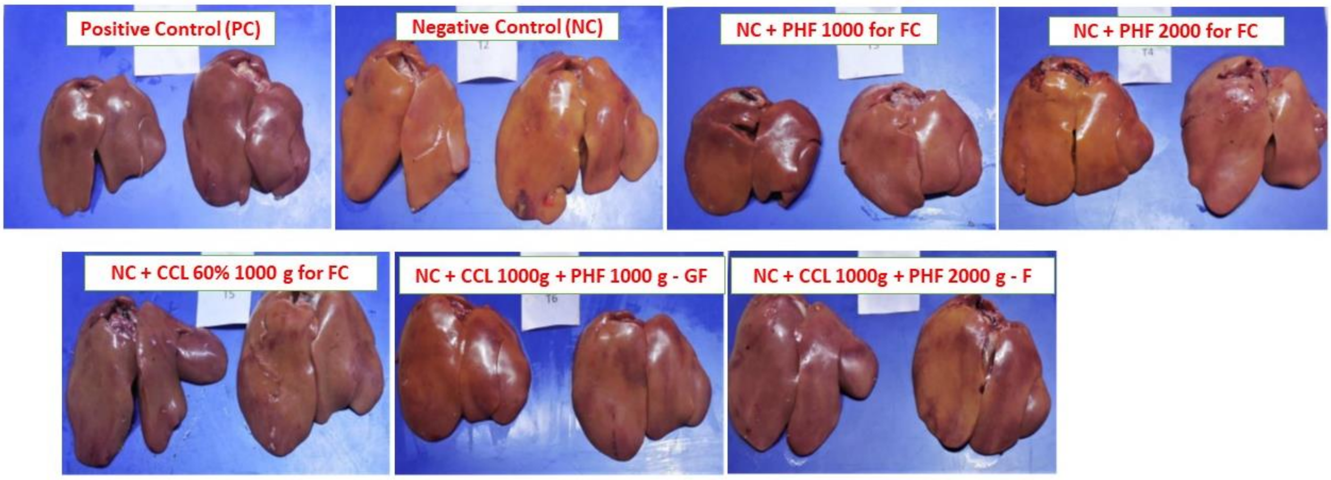

Figure 1 - Effect of PHF on liver color score. Liver color was scored as follows: 1: dark red, without any haemorrhagic spots and firm in consistency; 2: firm in consistency but slightly yellowish in color with few haemorrhagic spots; 3: softer in consistency and moderate area of haemorrhagic patches were observed; 4: pale yellow in colour with large haemorrhagic patches. Means with dissimilar superscripts in a row varied significantly; PHF: Polyherbal formulation; CCL: Choline chloride; FC: Full cycle; GF: Grower and finisher; F: Finisher

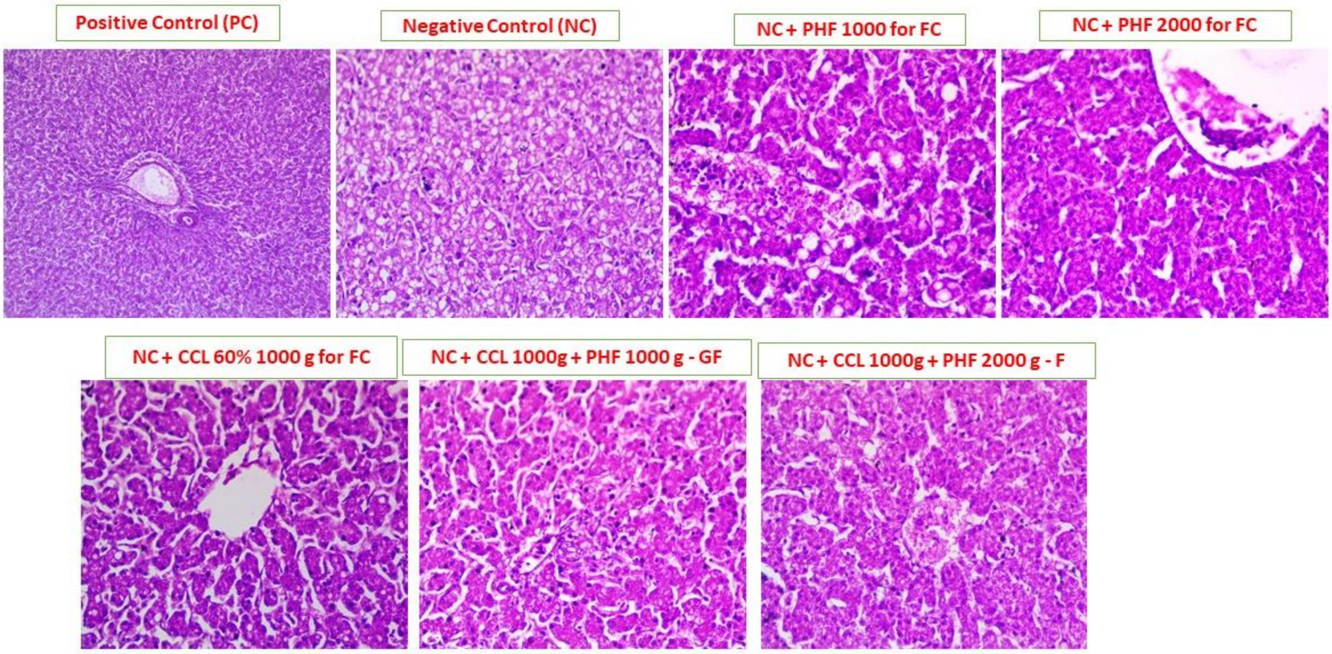

Figure 2 - Effect of PHF on histopathological alteration. A: normal hepatic parenchyma (Score 0); B: Severe hepatic vacuolization (Score 3); C: Moderate hepatic vacuolation (Score 2); D: No to rare hepatic vacuolization (Score 0); E: Mild hepatic vacuolization (Score 1); F: Mild hepatic vacuolization (Score 1); G: Mild hepatic vacuolization (Score 1); PHF: Polyherbal formulation; CCL: Choline chloride; FC: Full cycle; GF: Grower & finisher; F: Finisher

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DISCUSSION

Fatty liver disease is a serious problem to poultry sectors in several parts of the world even in well-managed farms (Whitehead, 1979). Poultry liver injury, a clinically common disease, can lead to low performance and even death, and has created a huge economic loss in the poultry industry (Lin et al., 2021). At present, due to the lack of effective hepatoprotective agent in the market, herbal based supplement has gained a significant importance to prevent the fatty liver disease in broiler chickens. Hence in the current study, we have employed a high energy and low protein with high cholesterol diet to induce fatty liver associated problems in broiler chickens. Furthermore, we have evaluated the effect of PHF alone or in combination with CCL on the prevention of fatty liver associated problems in broiler chickens.

High cholesterol with low protein and high energy diet suppressed the broiler's production performance, which was reversed by PHF alone or PHF plus CCL 60% in dose dependent manner. In addition, the current data revealed that supplementation of PHF (2000g/ton) as a top-up (along with CCL-60%) during the finisher stage showed better body weight gain (ADG), feed intake (ADFI) and FCR as compared to NC group. This agreed with the earlier reports who showed that PHF improved the production performance in broilers fed with choline deficient diet (Selvam et al., 2018). Similarly, this herbal supplement comprised of *A. nilotica* and *C. longa* was reported that it efficiently regulates the specific genes involved in liver protection and growth in broilers fed a choline-deficient diet (D'Souza et al., 2019; Marimuthu et al., 2022). Ncube et al., (2012) also reported that body weight gain was noticed in birds supplemented with *Acacia* supplemented diet. Hence, it indicates that the negative impact was reversed through the lipotropic action of herbal ingredients present in the PHF.

Lower serum triglycerides level and higher gross liver color score with histopathological alterations (hepatic vacuolization along with necrosis) reflect the fatty liver associated problems in broilers fed with fed high energy, low protein (HELP) diet devoid of choline and the presence of high cholesterol. The liver is the largest substantive vital metabolic organ, which has several complex physiological roles including metabolism, excretion, and detoxification (Alamri, 2018). It oxidizes the dietary fatty acids to produce the energy required for its own metabolism. Nevertheless, the major function of liver is to convert the surplus dietary fatty acids to triglycerides (TG) and mobilize it to the extrahepatic tissues. On the other hand, when the TG production is more than 5% of liver weight, it leads to the accumulation of fat in the hepatocyte that weakens the organ function (Heeren et al., 2021). Fat accumulation exceeds 5% to 10% of the liver's weight directly correlates with the presence of fatty vacuoles in the cytoplasm of hepatocytes (Nassir et al., 2015). Despite being useful energy sources, an excess of fat evokes excessive β-oxidation and eventually produces high ROS levels. These ROS mediated oxidative stress could result in the damage of hepatocellular membrane due to inactivation of antioxidant enzymes and lipid peroxidation in the liver (Bjørklund et al., 2017). Also, the current results were in accordance with Mei et al. (2020) report who demonstrated that the fatty liver condition induced hepatocytic vacuolation and hepatocyte necrosis in broilers. The above findings clearly demonstrated that high energy, low protein and high cholesterol diet without lipotropic-agents leads to the accumulation of excess TG in liver, which eventually diminish the production performance in broiler chickens. However, the negative impact such as high serum triglycerides and low liver color score with mild to moderate hepatic vacuolization was observed in all supplemented groups, especially the remarkable improvement which was detected in birds supplemented with CCL 60% plus PHF (during finisher stage) when compared with NC. Abdel-Razik et al. (2006) suggested that *Acacia nilotica* exerts hepatoprotective effects by providing maximum protection against CCl_4-induced liver injury. Furthermore, it was reported that *Acacia* species supplementation restored the normal architecture of hepatocytes in acetaminophen induced liver toxicity models (Kannan et al., 2013). Ikarashi et al. (2011) also suggested that polyphenol extracted from the bark of *Acacia* species lowered fat accumulation, indicating that *Acacia* polyphenols suppressed the fatty liver in mice fed with high-fat diet. Furthermore, Feng et al. (2012) reported that curcumin can effectively prevents the high fat diet induced liver steatosis in mice. This agreed with Xie et al. (2019), who reported that curcumin supplementation regulates the lipid metabolism and reduces the fatty liver in broiler chickens. The above points clearly showed that both *Acacia* and *Curcumin* were known to have hepatoprotective property in birds. Moreover, the current results demonstrated that supplementation of PHF with CCL 60% at finisher stage facilitates the fat mobilization in the broiler chickens fed with high energy, low protein and high cholesterol diet.

CONCLUSION

It is concluded that polyherbal formulation (PHF; 2000g/ton) with choline chloride (CCL 60 %) during the finisher stage can be used as a top-up to improve the performance parameters and to prevent the fatty liver condition in broiler chickens.

DECLARATIONS

Corresponding author
E-mail: saravana.k@naturalremedy.com

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Authors' contribution

All authors contributed equally to research work execution, analysing, interpreting the data and manuscript preparation.

Conflict of interests

The authors declare that they have no competing interests.

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COMPARATIVE EFFECT OF VITAMIN COMPLEX AND ORANGE EXTRACT ON PHYSIOLOGICAL AND BLOOD PARAMETERS OF TRANSPORTED PULLETS IN HUMID TROPICS

Mathew Oluwaseyi AYOOLA*, Foluke ADEREMI, Olufemi Mobolaji ALABI, Opeyemi OLADEJO and Micheal Abiola ABIODUN

Animal Science and Fisheries Management Unit, College of Agriculture, Engineering and Science, Bowen University, Iwo, Nigeria

Email: mathew.ayoola@bowen.edu.ng

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ABSTRACT: The comparative modulating effects of synthetic and natural source of ascorbic acid (AA) were investigated on transporting pullet birds in the hot-dry season of humid tropics. Ninety-six 16 weeks Isa-brown pullets were randomly allotted in a completely randomized design into four treatments of oral vitamin supplementation 5 days before transportation i.e; T_1 (ordinary water), T_2 (synthetic vitamin), T_3 (30% citrus-sweet orange), T_4 (50% citrus-sweet orange). Birds were crated and transported for 3 hrs covering 135km at 45km/hr. Meteorological values were monitored during the journey and no mortality was recorded. The results revealed that treatments had a significant effect ($p < 0.05$) on measured physiological parameters [body temperature (BTC), rectal temperature (RTC), respiratory rate (RR) and panting rate (PR)], hematological parameters and measured serum biochemical parameters as compared to the control group (T_1). The treatments group of orange at different inclusion ratios (T_3 and T_4) compared well with pullet birds on oral supplementation of synthetic vitamin (T_2) and were significantly different ($p < 0.05$) from birds on control water treatment (T_1). Birds on control (T_1) had the highest values for all measured physiological parameters which were significantly different from other groups ($p<0.05$). Birds in the control treatment (T_1) were more stressed as compared to other treatment groups, indicated by increased hematological and serum biochemical parameters except for a decrease in hemoglobin (Hb) as compared to other treatments. It can be deduced from this study that the oral supplementation of natural source of ascorbic acid (*Citrus sinensis)* and synthetic vitamin supplement helps to ameliorate the effect of transportation stress. *Citrus sinensis* extract can be a suitable alternative that is readily available for farmers and stakeholders.

Keywords: Ascorbic acid, Physiological measures, Pullets, Synthetic vitamin, Transportation stress.

INTRODUCTION

Livestock transportation is an important husbandry protocol during their lifecycle. Livestock are transported for different reasons such as reproduction, feeding, slaughtering, restocking, etc. (Shearer, 2021). The increase in demand for animal proteins to meet the growing global population and industrialization necessitated transportation across different ecological/climatic zones. Road transportation presents as the most popular means of transportation, which is capable of inducing stress directly affecting production in terms of both economy and welfare (Minka and Ayo, 2009, Bhatt et al., 2021).

The transportation of food animals on road can impair the optimum physiological condition through the changes in blood biochemical composition, muscles biochemical reactions, adverse effect on meat quality, loss in body weight, the spread of infectious diseases, increased morbidity, and subsequently mortality (Minka and Ayo, 2007; Minka and Ayo, 2009). Transportation-related stressors such as; handling, restraining, feed withdrawal, loading, unloading, vehicle vibration, ventilation, and transportation time, can also contribute to an adverse health condition which can increase the degree of pathogen infection/physiological disruption in subclinically infected animals (Ritter et al., 2009; Adenkola and Ayo, 2009a, Bhatt et al., 2021).

The prevailing climatic conditions of the immediate environment during transportation are an important factor capable of contributing to transportation stress (EFSA Panel on Animal Health and Welfare, 2022). Prolonged heat or cold stress is a stressor detrimental to the health of transported animals (Avero et al., 2008, Nielsen et al., 2011). Young and adult animals are likely to be more susceptible to extreme climatic conditions i.e. temperature (Lewis and Berry, 2006). The Onboard climatic control with appropriate densities during the transportation is a tool to achieve age-species specific transportation regime for effective animal welfare (Nielson et al., 2011, Ayoola et al., 2020).

The poultry industry is contributing immensely to the World economy, and a high in demand for major poultry products has led to the transportation of live birds (Minka and Ayo 2011, Lalonde et al., 2021). Although the awareness and establishment of standard regulation for transportation are in many countries (FAWC, 1992, EU, 2004), its strict

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compliance by farmers and relevant stakeholders is yet to be effective (Minka and Ayo, 2009, Jayaprakash et al., 2016). The bulk of previous studies on transportation stress are on broiler chickens, they are typically transported once in a production cycle for slaughter. The stress factor most often studied with transportation is thermal stress. Chickens are homeotherms, as a primary response to thermal stress, birds can modify their behavior to conserve or dissipate heat (Gou et al., 2021). If the bird continues to be subjected to heat stress, then physiological changes may probably be initiated.

Applications of amino acids, tranquilizers, and electrolytes have been reported to suppress stress in food animals (Ayo et al., 2005, Ahmadu, et al., 2016). However, the residual effects of a few of these substances contradict legislative regulations (Jacobs et al., 2017, Hussnain et al., 2019). Therefore, a need to source readily available, relatively cost-effective, reliable and non-toxic prophylactic material to ameliorate the adverse effect of road transportation stress is imminent.

Organic agriculture involves utilization of natural materials such as orange pulp, honey, *Telfairia occidentalis* leave extract etc. contribute to animal welfare as against the use of inorganic products in livestock production. *Citrus Sinensis* (sweet orange) is said to have a good content of Vitamin C (ascorbic acid) (Etebu and Nwauzoma, 2014). Vitamin C an active ingredient in orange extract is responsible for the biosynthesis of Corticosterone, a hormone that enhances energy supply during transportation stress. The ameliorating effect of Vitamin C is evident when the body's supply of ascorbic acid is depleted due to stress factors (Deters and Hansen, 2020).

The evaluation of sweet orange extract as oral supplements, a suitable alternative for synthetic vitamin C during road transportation of point of lay pullet birds belonging to Isa brown breed will be investigated. The research is aimed at evaluating the effect of transportation stress on physiological parameters, hematological, and serum indices with or without conventional vitamin and supplements, and orange juice at the varied concentration on pullet birds.

MATERIALS AND METHODS

Ethical approval

This study was carried out in accordance with the ethical regulation and approval of Bowen University research and ethical committee BUREC/2022/010. All animals used are in strict adherence to the guidelines.

Study area

The study was carried out at wonderful springboard farms, a commercial poultry farm, situated in Ibadan, Oyo State, southwest of Nigeria. The study area is on coordinate latitude 7°3047.7"N3°59'06.3"E.

Birds and management

One hundred and fifty pullets of Isa brown strain were obtained as day-old chicks from a reputable hatchery. They were raised following strict management practices and fed *ad libitum* with feed and water until they attained 12 weeks old. A total of 96 healthy pullet birds of body weight range (1.0 ± 0.1 kg) were selected and randomly allocated into oral supplementation treatment groups. Birds were allotted to four treatment groups; T_1 (ordinary water), T_2 (synthetic vitamin), T_3 (30% citrus-sweet orange), T_4 (50% citrus-sweet orange) for five days before loading and transportation. On the day of transportation, loading and crating were done carefully. Birds were crated according to treatment groups with 8 birds allotted to each crate at 0.25 m^2 / birds and replicated thrice for each treatment. Management practices such as vaccination, medication etc., were done according to (NVRI), Jos, Nigeria.

Experimental Unit

The experimental unit comprised of 16 weeks old Isa Brown birds. Birds were arranged into four oral treatments; water, conventional vitamin supplement, orange juice (30 ml/l of water), orange juice (50ml/l of water) with treatments administered for five days before transportation. The experimental birds were fed ad libitum and each treatment was replicated thrice.

Vehicle design and journey duration

A Toyota Hiace bus was used for the journey. The vehicle has an inner floor dimension of 4 × 1.2 m, with an iron roof top pilled with insulated foam and rug material. The vehicle floor was of metal, covered by a rug and foam underlay. The vehicle windows made of glass, located on both sides, with a sliding design for ventilation. The windows were opposite each other, placed at 800 cm measured from the inner floor, at (50×35 cm each) which allows adequate ventilation. Transportation crates containing the birds were carefully loaded to ensure similar conditions within the bus for all treatments and replicates (Buckham et al., 2008, Minka and Ayo 2011). The vehicle traveled on a mixture of un-tarred (rough) tarred roads for 3 hours, travelled through for 135 km with an average speed of 45 km/h.

Meteorological data

The ambient temperature (AT) and relative humidity (RH) were recorded at intervals for both inside and outside of the vehicle using a wet- and dry–bulb thermometer (Brannan, England). The average AT and RH outside the vehicle was 37.5^0 C and 54.7%, while inside the vehicle were 38.8^{0}C and 63.4%. The average wind speed during the journey was 37^{0}C.

The temperature humidity index (THI) was calculated using:

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THI = 0.8*T + RH*(T-14.4) + 46.4 (Scope et al., 2002)

Where T = ambient or dry-bulb temperature in °C

RH=relative humidity expressed as a proportion i.e. 75% humidity is expressed as 0.75

THI (Outside) the vehicle = 0.8*37.5 + 0.547* (37.5 – 14.4) + 46.4 = 89.04

THI (Inside) the vehicle = 0.8*38.8 + 0.634* (38.8 – 14.4) + 46.4= 92.91

Physiological data

The respiratory rate (RR), panting rate (PR), Body temperature (BR), and rectal temperature (RT) were measured for initial and final periods; before loading and transportation (pre-transportation) and immediately after the end of the journey (post-transportation). The physiological parameters were described as adapted from Oguntunji et al. (2019).

Hematological and serum biochemical data

Blood samples were collected before loading and transportation (pre-transportation) and immediately after the journey (post-transportation). 4 ml of the blood sample was collected via the wing vein. 2 ml of blood sample was collected in a heparinized bottle for hematology parameters while a plain container, left to clot was used for serum biochemical analysis. All blood samples were analyzed for hematology and serum biochemical according to Odere et al., (2004).

Statistical analysis

The physiological, hematological, and biochemical parameters were analyzed with the general linear model as follows: Yij =µ + tTi +Pj + eij

Yij = individual observation; µ = fixed effect of genotype; eij = experimental error

Statistical differences between means were determined using new Duncan multiple tests at a 5% probability level

RESULTS

The results of the effect of the treatments on physiological parameters as presented in Table 1. The treatment effect had ($p<0.05$) effect on the investigated physiological parameters. The treatments group of orange extract at different inclusion ratios (T_3 and T_4) compared well with pullet birds on oral supplementation of synthetic vitamin (T_2) and were significantly different ($p < 0.05$) from birds on control water treatment (T_1). Birds on control (T_1) had the highest values for all measured physiological parameters which are ($p<0.05$) different from other treatment groups. The period has an effect ($P< 0.05$) on measured physiological parameters. Initial measured parameters were ($p<0.05$) lower as compared to final measurements.

The results of treatment effect on hematological parameters are presented in table 2. The treatment has an effect ($p<0.05$) on measured blood parameters. The packed cell volume and red blood cells of birds on T_1 are significantly higher ($p<0.05$) as compared to other treatments. T_2 – T_4 is not significantly ($p>0.05$) different. T_1 had the lowest hemoglobin value (Hb) which is significantly ($p<0.05$) different from other treatments. Birds transported on water treatment alone (T_1) had the highest value for white blood cell (WBC) and its constituents, which is significantly ($p<0.05$) different from other treatments. The periods had a significant ($p<0.05$) effect on the treatments. Initial measured blood parameters are significantly ($p<0.05$) lower as compared to final values except for hemoglobin where the value is significantly higher ($p<0.05$) as compared to final measured blood parameters.

The results of treatment effects on serum biochemistry of transported birds as presented in table 3 revealed significant ($p<0.05$) effects of treatment on measured parameters. The glucose, aspartate amino transaminase, alanine amino transaminase, and creatinine level of birds on T_1 is higher ($p<0.05$) as across the treatments. The period has significant ($p<0.05$) effects on transported birds irrespective of the treatments. The initial period was significantly ($p<0.05$) lower for all measured serum blood biochemical parameters as compared to the final period.

DISCUSSION

Effect of treatments and period on physiological parameters of transported pullets

The fluctuations in measured physiological parameters have been attributed to variations in ambient temperature, duration of transportation, and vehicle vibration/mode of transportation (Piccione and Caola, 2002, Ayo and Minka, 2005). The measured panting rate (PR), rectal temperature (RT), body temperature (BT), and respiratory rate (RR) are described as the relevant on-spot diagnostic parameters to evaluate the welfare and adaptability of an animal subjected to stressors (Minka and Ayo, 2009, Hussnain et al., 2019, Gou et al., 2021). As reported in table 1, transported pullets with oral supplementation of fresh orange extract at different inclusion ratios (T_3 and T_4) compared well with pullet birds on oral supplementation of synthetic-vitamin (T_2) and were significantly different ($p<0.05$) from birds on control water treatment (T_1). The period also has a significant ($p<0.05$) effect on transported animals for measured parameters, as final measured parameters, are significantly ($p<0.05$) higher than initial.

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Table 1- Effect of treatments and periods on physiological parameter

Parameter	Treatment				Period	
	T_1 Water	T_2 Synthetic vitamin	T_3 30ml/L	T_4 50ml/L	Initial	Final
BTC ^{0}C	37.08±0.25^{a}	34.91±0.10^{b}	35.85±0.11^{b}	34.89±0.13^{b}	35.09±0.11^{b}	37.77±0.09^{a}
RTC ^{0}C	42.69±0.25 ^{a}	39.59±0.23^{b}	40.76±0.23^{b}	39.61±0.23^{b}	39.79±0.16^{b}	42.83±0.19^{a}
RR breath/min	26.00±13.09^{a}	20.63±14.16^{b}	19.75±12.95^{b}	19.38±12.68^{b}	0.00±0.00^{b}	40.38±10.45^{a}
PR breath/min	45.50±17.32^{a}	40.12±16.30^{b}	35.75±15.55^{bc}	33.88±14.98^{bc}	0.00±0.00^{b}	67.63±10.16^{a}

^{abc} values along the same row with different superscripts are significantly different ($p<0.05$). Where: BTC – Body temperature, RTC- rectal temperature, RR- Respiratory rate, PR- panting rate

Table 2- Effect of treatments and periods on hematological parameters

Parameters	Treatment				Period	
	T_1 Water	T_2 Synthetic-vitamin	T_3 30ml/L	T_4 50ml/L	Initial	Final
PCV (%)	29.25±4.21^{a}	25.20±2.13^{b}	20.75±1.65^{b}	24.20±2.22^{b}	23.10±1.99^{b}	28.38±1.28^{a}
HB (g/dl)	6.50±1.51^{b}	8.72±0.58^{a}	8.80±0.66^{a}	9.36±0.81^{a}	14.39±4.77^{a}	7.21±0.50^{b}
RBC (x10^{6}uL)	2.69±0.47^{a}	2.42±0.24^{b}	2.31±0.13^{b}	2.46±0.36^{b}	1.22±0.23^{a}	2.91±0.14^{b}
WBC X10^{3}UL	13925.00±314.58^{a}	13450.00±393.07^{a}	13325.00±178.54^{a}	13500.00±252.98^{a}	13355.00±177.865^{b}	13900.00±277.10^{a}
Platelet	12225.00±2015.56^{a}	134600.00±3187.48^{a}	132000.00±8755.95^{a}	127600.00±4643.27^{a}	125700.00±3907.40^{a}	13605.00±3190.04^{a}
LYM %	69.75±0.63^{a}	62.40±1.29^{b}	62.25±1.49^{b}	64.60±2.23^{b}	64.50±1.42^{b}	67.75±1.49^{a}
HET %	33.75±0.85^{a}	28.00±1.38^{b}	27.50±1.55^{b}	25.60±2.38^{a}	27.20±1.47^{b}	30.88±1.41^{a}
HET/LYM%	0.47±1.34^{a}	0.45±1.06^{b}	0.43±1.06^{b}	0.39±1.06^{bc}	0.42±1.03	0.45±0.94
MON %	3.50±0.75^{a}	2.80±0.37^{a}	3.20±0.65^{a}	3.30±0.40^{a}	2.90±1.47^{b}	3.50±0.44^{a}
EOS %	4.75±0.48^{a}	4.20±0.37^{a}	3.75±1.31^{a}	3.80±0.66^{a}	3.30±0.54^{b}	4.13±0.44^{a}
BAS %	0.55±0.25^{a}	0.60±0.241^{a}	0.00±0.00^{a}	0.40±0.24^{a}	0.40±0.15^{b}	0.41±0.18^{a}

^{ab} values along the same row with different superscript are significantly different ($p<0.05$)

Table 3. Effect of treatments and periods on serum biochemical indices

Parameters	Treatment				Period	
	T_1 Water	T_2 Synthetic-vitamin	T_3 30ml/L	T_4 50ml/L	Initial	Final
Glucose (mg/dl)	172.00±4.28^{a}	141.23±21.43^{b}	148.18±11.00^{b}	140.03±31.17^{b}	139.39±11.67^{b}	191.36±8.10^{a}
AST (i.u/L)	68.82±4.66^{a}	53.99±4.74^{b}	55.13±3.22^{b}	54.83±4.75^{b}	60.40±8.84^{b}	66.47±2.76^{a}
ALT (i.u/L)	2.50±1.13^{a}	1.80±0.55^{b}	1.32±0.40^{b}	1.25±1.04^{b}	1.35±2.17^{b}	4.38±0.34^{a}
Creatinine (mg/dl)	1.88±0.10^{a}	1.08±0.05^{b}	0.90±0.04^{b}	1.00±0.07^{b}	0.95±0.04^{b}	1.98±0.06^{a}

^{ab} values along the same row with different superscript are significantly different ($p<0.05$)

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This result agreed with other authors who reported that road transportation is generally considered a stressor for food animals. In addition, ambient temperature, length of travel, feed/water deprivation; density, and age of birds are factors to be considered (Ritter et al., 2004; Adenkola et al., 2008; Adenkola et al., 2009, Ayoola et al., 2020). Poultry lacks sweat glands, which other mammals use to mitigate thermal load during unfavorable conditions. Therefore, birds adopts panting and increased respiratory rate in an attempt to balance the thermal-induced stress as found in birds under investigation. The report of Oguntunji et al (2019) and Ayoola et al (2020) are per with this study, they reported that when the body temperature of birds rises above the thermal-optimum range due to metabolic activities or environmental conditions, birds' physiology is adjusted to ensure heat dissipation through increased respiratory rate, and panting rate.

The oral supplementation with synthetic-vitamin in this study was found to have an effect ($p<0.05$) on birds, as it suppress the effect of transportation stress on measured physiological parameters. This study was in line with the report of Adenkola et al. (2008) who found that administration of ascorbic acid which is the main component of synthetic-vitamin ameliorates the adverse effect of stress on transported pigs during 8 hours and 4 hours as evident in measured rectal temperature. This study also corroborates Jacobs et al (2017) who observed a reduction in rectal and body temperature of broilers and layers administered ascorbic acid after being subjected to heat stress. Citrus (sweet orange) used in this study compared well as an organic source of Vit C with synthetic- Vit C as observed for T_3 and T_4 with no significant difference ($p>0.05$) as compared to T_2 for all measured parameters. *Citrus sinensis* are rich sources of bioactive compounds, provide about 51% vitamin C and large quantities of carotenoids (Tiwari et al., 2009). To the best of our knowledge, previous reports on the comparative application of orange extract with synthetic vitamin C to reduce transportation stress in livestock are scarce, therefore, this study serves as one of the leading reports.

Effect of treatments and period on hematological parameters of transported pullets

In the report of Scope et al. (2002) on pigeons, it was reported that measured total leucocyte value had a progressive increase with length of travelling and peak at the end of transportation. Ayo et al (2006) and Minka and Ayo (2008), reported that transportation stress increases the H/L ratio, erythrocytes, hematocrit, and leucocytes as resulted from transportation stress, especially thermal stress induced by transportation in humid tropics.. In table 2, the measured value of packed cell volume (PCV), red blood cell (RBC), and white blood cell (WBC) were significantly increased ($p<0.05$) across the treatments post transportation. T_1 had the highest value which is significantly ($p<0.05$) different as compared to other treatments. Hemoglobin (Hb) value decreased significantly ($p<0.05$) across the treatments with T_1 having the least value post transportation.

The results of this study agreed with Minka and Ayo (2008) who found a significant ($p<0.05$) increase in hematocrit and total protein values of adult ostrich and attributed the changes to dehydration. Similarly, Adenkola and Ayo (2009) reported that the value of PCV can increase due to dehydration or splenic contraction due to transportation stress. However, our report differs from Tadich et al. (2005) and Deepanshu et al (2020) reported that PCV decreased after loading and subsequent transportation. Also, Cockram (2022) reported a low PCV for transported animals, they concluded that stressors can cause actual, than apparent reduction in hematocrit values. The observation on measured Hb in this study can be attributed to a reduction in the oxygen-carrying capacity of the blood due to transportation stress and dehydration during the journey (Ayoola et al., 2019).

Birds in the control treatment (T_1) were more stressed as compared to other treatment groups, indicated by increased WBC and its constituents. Lin et al. (2006) reported that H/L ratio is an indicator of chronic stress in food animals. Our study disagree with Lalonde et al. (2021), who found no impacts of either the temperature/relative humidity or duration of transportation on pullet H/L ratio, these differences can be attributed to the simulated transportation system used for their experiment, differences in atmospheric condition, and strain of pullet under investigation.

However, the results of this study were in agreement with the report of Borges et al. (2004) and Lalonde et al. (2021) that supplementation of vitamin C lowered the H/L ratio. Inclusion of natural source of vitamin C- citrus (sweet orange) at the two inclusion levels T_3 (30%) and T_4 (50%) compared well with the synthetic vitamin source (T_2). The present results for hematological parameters were in agreement with Guardia et al. (2009), Alam et al. (2018), and Cockram (2022).

Effect of treatments and period on serum biochemical parameters of transported pullets

Transportation stress, especially within the hot humid agro-ecological zone, causes physical stress which disrupted the thermal homeostasis, behavioral adaptation, and consequently physiological adaptation in livestock animals (Averos et al., 2008, Minka and Ayo, 2009, Bhatt et al., 2020). Notable physiological disruption includes an increase in the blood level of hormones and enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), plasma glucose, creatine phosphate kinase (CPK), cortisol, nitrogen urea, lactic acid, and uric (Parker et al., 2003, 2007; Ferguson and Warner, 2008, Minka and Ayo, 2009).

As reported in table 3; AST, ALT, creatine, and glucose were affected ($p<0.05$) by treatments and transportation stress. The control group (T_1) had the highest values for measured parameters which were significantly ($p<0.05$) different from other treatments. T_3 and T_4 compared well with T_2 with no significant ($p>0.05$) difference between the treatment groups. The rise in the value of measured liver function enzymes which include alanine amino transferase, aspartate

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amino transaminase, CPK occurs due to rough handling during pre-loading, loading and transportation (Averos et al., 2008, Deters and Hansen 2020, Deepanshu et al., 2020).

Transportation over long distance is a stressor for animals. Animals are require to ensure homeostasis, while contact between animals' during transportation can produce fatigue and bruising, touching the permeable membranes and led to the extrusion of enzymes into the blood (Lopez et al., 2006, Deepanshu et al., 2020, Gou et al., 2021). Plasma glucose level was considerably high (p concentration), primarily due to the breakdown of glycogen in the liver (Jacobs et al., 2017).

The significant difference between the control (T_1) and other treatment groups (T_2, T_3, and T_4) can be attributed to oral supplementation of ascorbic acid sources in the form of synthetic vitamins and citrus. The oral supplementation of these ascorbic sources ameliorates the effect of transportation on pullets as evident in measured serum blood parameters. The findings were in line with goats administered ascorbic acid (Minka and Ayo, 2007), in the broiler (Lin et al., 2006).

CONCLUSION

The oral supplementation of a natural source of ascorbic acid (Citrus-sweet orange) and synthetic Vitamin c supplement helps to reduce the effect of transportation stress during the hot humid season in the tropics. Their ameliorating effect improves the health status of the birds during transportation. In addition, the oral supplementation of citrus-sweet orange compared effectively with the synthetic vitamin, hence a suitable alternative that is readily available for farmers and stakeholders. Further studies are hereby recommended to evaluate other associated effects of *Citrus sinensis* on birds' welfare.

DECLARATION

Consent
Not Applicable

Competing Interest
The authors declare that there are no competing interest during the data collection or writing up of this article

Author's Contribution
M. O. contributed to data collection and article write up, F. A. contributed to writing of manuscript, O.M. Alabi contributed to research design and data collection, A. O. contributed to statistical analysis and result interpretation, O. A. Oladejo :Contributed to statistical analysis, M. A contributed to data collection and laboratory analysis.

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PHYSICO-CHEMICAL PROPERTIES AND DIGESTIBILITY OF AMMONIATED BAMBARA GROUNDNUT (*Vigna subterranea*) SHELL FOR RUMINANTS

Rina PUTRI[1], Sari Putri DEWI[2], Fariz Am KURNIAWAN[2], Muhammad RIDLA[3], and Yuli RETNANI[3]

[1]Graduate School of Animal Nutrition and Feed Science, IPB University, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia
[2]Livestock Management and Technology, College of Vocational Studies, IPB University, Bogor, West Java, Indonesia
[3]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, West Java 16680, Indonesia

Email: sariputri21@apps.ipb.ac.id

Supporting Information

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ABSTRACT: This experiment was conducted to evaluate the physical properties, chemical quality, and digestibility of the ammoniated Bambara groundnut (*Vigna subterranea*) shell as ruminant feed. Bambara groundnut shell (BGS) were collected, ground with a grinder machine, afterward added 0, 3 and 5 (% DM) urea levels into 500 g of sample. Samples were mixed until homogeneous, then put into plastic bottles, after that stored for 7 and 14 days. Opened, dried in the oven at 65ºC for 48 hours and ground. A completely randomized design (CRD) was used with 5 treatments of BGS ammoniation (T0= control, T1= BGS + 3% urea and 7 days storage, T2= BGS +5% urea and 7 days storage, T3= BGS + 3% urea and 14 days storage, and T4= BGS + 5% urea and 14 days storage time), 4 replications each. The result of this study showed that the increase of urea level and days storage time, can decrease crude fiber, neutral detergent fiber, acid detergent fiber, and hemicellulose contents of all samples ($P<0.05$) and increase the value of bulk density, tapped density, in vitro dry matter digestibility and in vitro organic matter digestibility in comparison to untreated samples ($P<0.05$). It was concluded that the T4 was the best treatment. The BGS ammoniated with 5% urea for a period 14 days of storage causes the lowest value of crude fiber, neutral detergent fiber, acid detergent fiber, and hemicellulose, and also causes the highest value of bulk density, tapped density, crude protein, in vitro dry matter digestibility and in vitro organic matter.

Keywords: Ammoniation, Fibrous feed, Bambara groundnut shell, Ruminant, Urea.

INTRODUCTION

Bambara groundnut (*Vigna Subterranea*) is more easily adaptable in the Bogor area and the eastern part of West Java, so it is better known as Bambara groundnut (Adhi and Wahyudi, 2018). Bambara groundnut shell is an agricultural by-product, that has high fiber and low protein content, so it can be used as a source of fiber feed but low quality so processing techniques are needed (Dewi et al., 2018). Treatment is needed to break down fiber so it can increase the quality of agricultural by-products (Sarnklong et al., 2010). Therefore, Bambara groundnut shells can be used as an alternative feed for ruminants. The treatment that reduces the fiber content and increases the quality of agricultural by-products is ammoniation (Lukman et al., 2020). The ammoniation process is an effort using urea which will be converted to ammonia in gastrointestinal tract livestock (Belanche et al., 2021; Datsomor et al., 2022). The ammonia process server increases crude protein levels (Tampoebolon et al., 2019), through the addition of non-protein nitrogen (NPN) components from ammonia in urea $(NH_2)_2CO$ (Amin et al., 2016).

The function of urea (ammonia) as an alkali can also stretch fiber bonds, and break lignin, and cellulose bonds as well as lignin and hemicellulose bonds (PPrastyawan et al., 2012). Using urea levels of 5% in empty palm oil marks using Fiber Cracking Technology (FCT) to improve the nutrient quality of empty palm marks and reduce the content of fiber fractions (Dewi et al., 2018). The urea can be converted to ammonia and induce cleavage in lignocellulose structures (Shi et al., 2015; Hildebrant et al., 2017). According to Lukman et al. *(*2020) two sources of ammonia that can be used for the ammoniation process, namely ammonium hydroxide (NH_4OH) in the form of a solution, ammonia (NH_3) in gas form and urea $(NH_2)_2CO$ in solid form. The advantages of urea from the use of the other two forms are ease of handling (because it is solid) and ease to obtain.

Urea contains 42-45% nitrogen (Cantarella et al., 2018; Lukman et al., 2020). According to Van Soest (2006) the level of urea that is safe to use is 1-6% of dry matter. Ammoniation has been used in several agricultural by-products such as rice straw (Bata et al., 2014), corn straw (Elkholy et al., 2009), and coconut leaves (Filho et al., 2013), and has been reported to increase crude protein content as much as 9.63%, 11.45%, 17.35%. Wanapat et al. (2013) stated of 2-3% urea on rice straw was able to increase dry matter intake, nutrient digestibility and rumen ecology. Dewi et al., (2018) reported the addition of 5% urea in corn plant waste (corn straw, corn husks and corn cobs) and sugarcane waste (sugarcane shoots and bagasse) had increased dry matter digestibility and organic matter digestibility in ruminants. The treatment with 14 days incubation time was the best method that could be used in ammoniated of banana stems so

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that, increasing crude protein and decreasing crude fiber in the ammoniated feed from banana stems (Irianto et al., 2022). Feeding with amniotic fiber has also been reported to increase livestock body weight gain by 147.1 g/head/day in sheep (Walandari et al., 2014) and by 120.0 g/head/day in cattle (Bata et al., 2014).

Evaluation of the degradation of feed components in the rumen can be done by several methods; one of them is the in vitro technique. In vitro technology is an indirect method of estimating digestibility in the laboratory by imitating the digestive process in the digestive tract of ruminants (Oluwo and Yaman, 2019; Mayulu et al., 2022). Compared to in vivo methods, in vitro techniques have the advantage of a short time and low cost (Getachew et al., 2004). Another advantage of in vitro is that feed samples are single-use and can measure the digestibility of one type of feed ingredient (Dijkstra et al., 2005).

This experiment was conducted to evaluate the physical properties, chemical quality, and digestibility of the ammoniated Bambara groundnut shell (*Vigna subterranea)* as ruminant feed, due to in vitro method.

MATERIALS AND METHODS

Location and time

This research was conducted in May–July 2022 at the Faculty of Animal Husbandry, IPB University, Indonesia. Proximate and Van Soest analyses were carried out at the Feed Science and Technology Laboratory. Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) analyses were carried out at the Dairy Animal Nutrition Laboratory.

Experimental design

This study used a completely randomized design (CRD), 5 treatments with 4 replications. The treatment consists of T0= Bambara groundnut shell + 0% urea (control), T1= T0 + urea 3% + 7 day storage, T2= T0 + urea 5% + 7 day storage, T3= T0 + urea 3% +14 day storage, T4= T0 +urea 5% + 14 day storage.

Research procedure

A 10 kg sample of Bambara groundnut shell have been used (randomly selected from Bogor City) and divided into 20 research samples (500 g per sample). To reduce the size of the Bambara groundnut shells, they were milled with a milling machine without a sieve. The ammoniation process is carried out by adding as much urea as 0, 3 and 5 from (%DM into 500 g of sample. Mixing is carried out until the homogenous first dissolving urea into distilled water in comparison (1:5). The treatment samples were put into 500 mL plastic bottles, then stored for 7 and 14 days. At the end of each time, the research sample storage is opened. Dried in the oven at 65^{0}C for 48 hours then ground. Milling result were used to the analysis of physical properties, proximate, Van Soest and in vitro digestibility.

Sample analysis procedure

Analysis of the characteristics of physical properties of bulk density (BS) and tapped density (TD) was carried out according to the method of Amidon et al. (2017). Dry matter analysis (DM), crude protein (CP), and crude fiber (CF) using method of AOAC (1990). Content Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed by the method of Van Soest (2006). In vitro dry matter digestibility (IVDMD) and in vitro organic matter digestibility (IVOMD) were analyzed by the method of Tilley and Terry (1963).

Statistical analysis

The data from this was an analysis of variance in Completely Randomized Design (CRD) using SPSS version 25 software. Data were analyzed of variance (ANOVA) if a difference is found ($P<0.05$) and further tested Duncan Multiple Range Test (DMRT) (Duncan, 1955). The significance value was determined based on a 5% significance level. Mathematical model of experimental design follows the mathematical model of Steel et al. (1997). The linear model used was: $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$

Where Y_{ij} is the observation values of each of the response variables arising as a result of μ= the overall mean; τ_i= observed effect of the ith dietary treatment; ε_{ij}= random or residual error due to the experimentation.

RESULTS

Composition physical properties and chemical structure

In present experiment, all samples are given the treatment value of physical properties bulk density (BS) and tapped density (TD; $P<0.05$) increasing along with an increase in urea level and storage time (Table 1). The addition of urea (ammonia) and storage time ($P<0.05$) increased the crude protein content of Bambara groundnut shell (Table 2). Untreated samples have higher values of crude fiber, NDF, ADF, and Hemicellulose than the samples with the addition of 3 and 5% urea (Table 3). All samples with the addition of 5% urea and storage time of 14 days have the lowest content of crude fiber, NDF, ADF, and hemicellulose.

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In vitro **characteristics**

All samples with the addition of urea and storage time can increase the digestibility of livestock. The addition of urea 0, 3 and 5% levels with storage time of 7 and 14 days on the samples increased IVDMD and IVOMD of Bambara groundnut shell (P<0.05; Table 4).

Table 1 - Physical properties of ammoniated Bambara groundnut shell bulk density (BS) and tapped density (TD)

Treatment	Bulk density (g L^{-1})	Tapped density (g L^{1})
T0	230±0.82^{e}	383±1.63^{e}
T1	237±0.58^{d}	395±0.82^{d}
T2	257±1.15^{b}	428±2.3b^{b}
T3	246±0.50^{c}	402±1.29^{c}
T4	266±0.96^{a}	444±1.41^{a}

T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% + 7 day storage, T3 = T0 + Urea 3% + 14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with superscripts are significantly different (P<0.05). P>0.05; ns, not significant; CP: crude protein; DM: Dry Matter.

Table 2 - The crude protein content of ammoniated Bambara groundnut shell

Treatment	DM (%)	CP (%)
T0	92.30±0.97^{b}	7.9±0.66^{c}
T1	87.28±1.54^{a}	10.85±0.64^{b}
T2	87.57±0.62^{a}	10.92±0.65^{b}
T3	87.63±1.29^{a}	12.15±0.68^{a}
T4	87.18±0.51^{a}	12.39±0.89^{a}

* T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% +7 day storage, T3 = T0 + Urea 3% + 14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with superscripts are significantly different (P<0.05). P>0.05; ns, not significant; CP: crude protein; DM: Dry Matter.

Table 3 - Content of ammoniation Bambara groundnut shell fiber components

Treatment	CF	NDF	ADF	Hemicellulose
T0	29.83±0.40^{a}	55.74±4.21^{a}	34.94±0.76^{a}	20.77±3.56^{a}
T1	26.51±0.45^{b}	49.25±2.11^{b}	33.12±0.52^{b}	16.13±1.64^{b}
T2	26.06±0.80^{bc}	47.99±2.06^{bc}	33.07±0.45^{bc}	14.91±2.11^{bc}
T3	25.34±1.19^{c}	44.81±02.03^{c}	32.01±0.68^{c}	12.80±1.57^{c}
T4	25.02±0.53^{c}	44.56±2.05^{c}	31.99±0.82^{c}	12.57±1.63^{ac}

* T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% + 7 day storage, T3 = T0 + Urea 3% + 14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with superscripts are significantly different (P<0.05). *, P>0.05; ns, not significant; CF: crude fiber (CF); NDF: Neutral detergent fiber; ADF: Acid detergent fiber; HS: Hemicellulose.

Table 4 - *In vitro* digestibility of ammoniated Bambara groundnut shell

Treatment	IVDMD	IVOMD
T0	59.75±0.57^{c}	59.21±0.55^{c}
T1	61.44±1.33^{b}	60.85±1.52^{b}
T2	62.14±0.69^{b}	61.57±0.56^{b}
T3	62.35±0.93^{b}	61.88±1.06^{b}
T4	64.25±0.71^{a}	63.78±0.57^{a}

* T0 = Bambara groundnut shell + 0 % urea (control), T1 = T0 + Urea 3% + 7 day storage, T2 = T0 + Urea 5% + 7 day storage, T3 = T0 + Urea 3% +14 day storage, T4 = T0 + Urea 5% + 14 day storage. * Means in the same column with different superscripts differ significantly (P<0.05). *, P<0.05; ns, non-significant; IVDMD: in vitro dry matter digestibility; IVOMD: in vitro organic matter digestibility.

DISCUSSION

Bulk density (BS) is a method used to assess the physical quality of a material. The value of density is determined with calculating the volume of the material to the weight that has been determined in a measuring cup (Amidon et al., 2017). Particle size affects space-filling, coarse particles occupy more space while fine particles tend to be denser (Toit et al., 2019). Stack density affects the space between particle size storage times (Jaelani et al., 2016). The tapped density obtained was measured by first inserting a known sample mass into a measuring cup and carefully leveling it and then shaking it for 10 minutes (Amidon et al., 2017).

The results of the study showed that increased levels of urea and increased time of storage can higher the content of BD and TD. This is caused by the content of values BD and TD are affected by moisture content and particle size. Ridla et al. (2023) reported that physical properties are highly correlated with nutrient values, they reported that crude protein positive correlation with the values of bulk density and tapped density. BS and TD values are increasing along with the increase in urea level and length of storage time. Compared to the control the value of physical BS and TD was found to be highest at the level of 5% urea addition with a storage time of 14 days of 266 – 444 g L^1 the next is the treatments of urea doses of 3% 14 days of storage 246 – 402 g L^1 the 5% urea treatment with a storage time 7 days and smallest treatment doses of 3% urea addition with storage time of 7 days of 237 -395 g L^1. This increase in BS and TD value is suspected because there has been a change in the nutrient content on Bambara groundnut shell treatment due to the addition of urea.

The addition of urea up to 5% and stored for 14 days can increase the crude protein content that is 12.39%, 7 days storage 10.92%, 0 days storage control 7.90%. This means that the higher the N content in a material the higher the value of crude protein. Bambara groundnut shells with 3 and 5% urea based on DM material, no changes were observed compared to the control treatment. The content of CP at 14 days of storage was found to be higher, presumably because the nitrogen fixation time ammonia formed in the ammoniated Bambara groundnut shell was higher than the fixation time of 7 days. The increase in crude protein content of feed ingredients (rice straw) due to ammoniating with urea is the result of the hydrolysis of urea which is fixed into the fiber network and the fixed nitrogen will be measured as crude protein (Amin et al., 2016). The increase in crude protein levels in ammoniated bagasse at 21 days of storage from 4.0% to 12.7% was reported by Lunsin et al. (2018).

The addition of urea level decreased the fiber component because of the changes in the structure of the cell wall due to ammonia treatment, the hydrolysis process of urea is able to break down lignocellulosic and lignohemicellulose bonds. Urea is alkali component, because it contains ammonia (NH_3) so in addition to having the ability to increase CP levels, it can also degrade fiber components (CF, NDF, ADF, and Hs) (Elihasridas et al., 2015). Urea ($(NH_2)_2CO$) causes fiber expansion so that it remodels complex carbohydrate components into simple carbohydrates (Lukman et al., 2020). In addition, high pressure can encourage the release of acetyl groups from the fiber structure which leads to an increase in substrate acidity and an increase in the solubility of the fiber (Thomsen et al., 2014; Jayanegara et al., 2018). Urea can be converted into ammonia and induce cleavage in lignocellulose structures (Shi et al., 2015; Hildebrandt et al., 2017). The combination of 1% urea and autoclave in rice straw increased digestibility but did not change the fiber content (Jayanegara et al., 2017). In another study, Jayanegara et al. (2018) reported that the combination treatment of 5% urea under pressure using an FCT (Fiber Cracking Technology) machine on empty oil palm fruit bunches could reduce fiber fraction and increase digestibility. According to Tampoebolon et al. (2018) different storage times (7,14,21 and 28 days) affect the crude protein, ash and crude fiber, 14 days of storage is the best treatment, because increasing the crude protein and decreases the crude fiber content.

In the present study, the storage time also does not affect the fiber component (Table 3); it is suspected that the added urea in the treatment sample has not worked optimally in hydrolyzing fiber bonds Bambara groundnut shell. Decrease in NDF content and weight it is also suspected that the increase in cell contents is supported by the increase in amniotic crude protein ammoniated Bambara groundnut shell.

Acid detergent fiber is a part of NDF, which is insoluble in acidic solutions composed of cellulose, lignin, and silica (Jannnah, et al., 2019). A higher concentration of ammonia can reduce the hemicellulose content (Yuan et al., 2015). Nuswantara et al. (2020) it was reported that ammonia reduced the content of NDF, ADF, Cellulose, Hemicellulose and lignin in the coconut belt. Decreased ADF levels are suspected because microorganism activity produces the enzyme cellulase in the ammonia process (Nuswantara et al. 2020). The hemicellulose content has decreased along with the increasing concentration of ammonia in the treatment (Table 3). Therefore, adding treatment 3 and 5% urea increased the fibrous feed quality of Bambara groundnut shell by lowering the fiber content substantially (Table 3). The decrease in the value of CF, NDF, ADF and Hemicellulose in Bambara groundnut shell after the addition of 5% urea in a row is 25.02%, 44.56%, 31.99% and 12.57% were observed.

The Increasing level of urea affects the higher value of IVDMD and IVOMD Bambara groundnut shells (Table 4), this is validated by the results of the NDF and ADF, which are decreasing simultaneously with increased urea levels (Table 3). Higher levels of urea addition of 5% with 14 days storage increased IVDMD and IVOMD of ammoniated Bambara groundnut shell by 64.25% and 63.78% (Table 4).

This is reinforced by the results of NDF and ADF a decrease with an increase in urea level with storage in Table 3. The highest increase in ammoniated Bambara groundnut shell IVDMD and IVOMD at the level 5% with a storage time of 14 days was due to the degradation of lignocellulose during the ammoniated process being able to break the bond between lignin and cellulose resulting in a simpler form of carbohydrates. According to Zulkiple et al., (2015), 5% urea treatment can loosen fiber components and open the lignocellulose structure so that cellulose becomes more accessible to fiber digestion microbes which break down polysaccharide polymers into sugar monomers. This makes microbial developments in the process of degradation of dry matter components and organic matter. According to Amin et al. (2016), IVDMD value increases as the storage of ammoniated increases. In addition, Dewi et al., (2018) reported that the 5% urea level causes a decrease in fiber in feed ingredients, significantly increasing the digestibility of dry matter and organic matter.

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CONCLUSION

Best treatment in present study was the treatment at 5% urea content and 14 days of storage. Bambara groundnut shell ammoniated with the additional level of urea 5% for 14 days of storage can reduce the lowest component of fiber such as crude fiber, Neutral Detergent Fiber, Acid Detergent Fiber, and hemicellulose, increase bulk density, tapped density, in vitro dry matter digestibility and in vitro organic matter digestibility highest. Therefore, the processed Bambara groundnut shell is promising for ruminants.

DECLARATIONS

Corresponding author
E-mail: sariputri21@apps.ipb.ac.id

Authors' contribution
Perform the experiment, data analysis by SPSS software and writing the original draft was done by R. Putri; conceptualization, investigation methodology, review and editing of the paper was done by S. P. Dewi; supervise the experiment and revise the paper was done by M. Ridla and Y. Retnani; supervise the experiment and review the paper was done by F. A. Kurniawan.

Conflict of interests
The authors declare that they have no conflicts of interest.

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IMPACT OF PHASE-FEEDING PROGRAMS ON PERFORMANCE OF BROILER CHICKENS IN NIGERIA

Victoria Nneoma EBEGBULEM, Emmanuel Ekpo ARCHIBONG, Thaddeus Nanevfa KPERUN, Esther Darlington IZUKI and Mary Amu UDAYI

Department of Animal Science, University of Calabar, Calabar, Nigeria

Email: vicnneb@gmail.com

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ABSTRACT: Phase-feeding is the feeding of several diets for a relatively short period of time to specifically meet an animal's nutrient requirements. The study evaluated the effect of different phase feeding methods on growth and carcass characteristics of broiler chickens. A total of 120-day-old chicks of the FIDAN strain were assigned to four dietary treatments of 30 birds each, 15 birds per replicate. Birds were fed at different phases: Phase 1 were fed broiler starter diet alone for 8 weeks; Phase 2 birds were fed starter diet from 0-4 weeks and 1st finisher diet from 5-8 weeks. Phase 3 birds were fed starter diet from 0-3 weeks, 1st finisher diet from 4-6 weeks and 2nd finisher diet from 6-8 weeks. Phase 4 birds were fed starter diet from 0-2 weeks, 1st finisher diet from 2-4 weeks, 2nd finisher diet from 4-6 weeks and 3rd finisher diet from 6-8 weeks of age. Result no significant differences ($p>0.05$) between the groups in body weight gain (2.91–2.47 kg/bird) and feed conversion ratio (2.03–2.34). Total feed intake was highest in phase 1 (6.70 kg/bird) followed by phase 2 birds (6.41 kg). Dressed weight in Phase 1 was significantly ($p<0.05$) higher than others, followed by Phase 2. Dressing percentage did not differ significantly ($p>0.05$) between the groups. Feed cost between treatments was however significantly ($p<0.05$) different, Phase 1 diet being costliest. Phase-feeding using phase 4 regime elicited reduced dietary cost without compromising optimal performance of the birds.

Keywords: Diet; Feed cost; Feed efficiency; Nutrition; Phase-feeding.

INTRODUCTION

Phase-feeding is a nutritional management strategy in which the ingredient and chemical composition of the diet is modified over time so that the nutrient composition of the diet more clearly meets the nutritional requirement of the animal (Warren and Emmert, 2000). It also describes the feeding of several diets for a relatively short period of time to more closely match an animal's nutrient requirements, minimizing over or under feeding of nutrients (Pope and Emmert, 2002; Moss et al., 2021). It is therefore an important part of establishing feed programs to meet animal performance and profitability goals.

The tremendous increase in grains costs over the years has increased focus on feed programs (Brown, 2019). Even small improvements in the feed programs used to produce meat or eggs can lead to substantial saving in feed costs and dramatically improve profitability. Pope and Emmert (2002) observed that weight gain and feed efficiency of broiler birds phase fed were greatly improved when compared with those fed NRC based diets. In another study (Tolimir et al., 2010) asserted that protein and amino acids requirements of broiler chickens change with age and feeding of one diet over a prolonged period of time give rise to shortfall or excess of nutrients in main part of the growth period. Restriction of feed in broiler chickens during the early stage of growth is reported to induce compensatory growth, improve feed efficiency and engenders reduced cost (Jalal and Zakaria, 2012; Bordin et al., 2021; Belaid-Gater et al., 2022).

Currently commercial feed companies produce different forms (mash, pellets or crumbles) of broilers feed in order to engender production performance (Saveewonlop et al., 2019). These forms affect directly the cost and efficiency of production as it impacts on the digestibility, conversion ratio and growth output. Mash is a feed form which is of fine texture and homogenous such that birds cannot easily separate out the composing ingredients while the pellets are compacted into hard dry pellets or grains (Saveewonlop et al., 2019). The authors observed that pellets offered many benefits including decreased feed wastage, reduced selective feeding among others. Crumbles are however prepared by firstly pelleting the mixed ingredients, then crushing the pellets into texture coarser than mash.

In order to derive maximum benefits from phase feeding, it is pertinent to establish diets and feed budget on the basis of actual animal performance and profitability or performance goals. Wenger Feeds (Wenger Feeds Co®. USA) assert that information from breeding companies can be useful in establishing expected outcome. Meremikwu and Obikaonu (2020) in their experiment where they fed a high – low – high nutrient phase diets to broilers reported a significantly

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higher feed intake by the nutrient restricted diet groups than the control group. The authors however observed a 25 % increase in cost of feed for the control diet above the nutrient restricted phase diets.

The study was conducted to evaluate the impact of feeding different planes of diets and at different periods of growth on performance and carcass characteristics of broiler chickens as well as to analyse the economics of production.

MATERIALS AND METHODS

Study which lasted for 8 weeks, between October and November, 2021, was carried out at the Poultry Unit of the Teaching and Research Farm, University of Calabar, Calabar, Nigeria. Feed ingredients were procured from local market in Calabar. African yam bean (*Sphenostylis stenocarpa*) seeds were processed by boiling in hot water at about 100˚C for one hour. It was then drained of water using aluminium basket, sundried and milled, as well as other feed ingredients. Four experimental diets were formulated as presented in Table 1.

One hundred and twenty day old chicks of the FIDAN strain were randomly assigned using Completely Randomized Design to four dietary treatments of 30 birds per treatment and 15 birds per replicate. Birds were fed at different phases: Phase 1 were fed broiler starter diet alone for 8 weeks; Phase 2 birds were fed starter diet from 0-4 weeks and first finisher diet from 5-8 weeks of age. Phase 3 birds were fed starter diet from 0-3 weeks, 1st finisher diet from 4-6 weeks and 2nd finisher diet from 6-8 weeks. Phase 4 birds were fed starter diet from 0-2 weeks, 1st finisher diet from 2-4 weeks, 2nd finisher diet from 4-6 weeks and lastly, 3rd finisher diet from 6-8 weeks of age. Birds were raised in deep litter system and fed *ad-libitum*. Data on feed intake, weight gain and feed conversion ratio were taken and recorded weekly.

Table 1 - Feed composition of experimental diets

Ingredient	Starter	1st finisher	2nd finisher	3rd finisher
Maize	50	53	54	55
African Yam Bean	5	6	6	6
Soya bean	30	27	25	23
Fish meal	4	2	2	1
Wheat offal	8	9	10	12
Bone meal	2	2	2	2
Salt	0.3	0.3	0.3	0.3
Vit/Min premix	0.3	0.4	0.4	0.4
Lysine	0.2	0.15	0.15	0.15
Methionine	0.2	0.15	0.15	0.15
Total	100	100	100	100
Cost per Kg diet ($)	0.900	0.202	0.178	0.175
Determined analysis				
CP (%)	29.31	27.12	25.37	23.62
CF (%)	5	6	7.75	8.25
ME (kcal/kg)	1705.6	1724	1772.9	1780.4

CP: crude protein, CF: Crude fibre, ME: Metabolizable energy

Carcass evaluation

At the end of the feeding trial, three birds per treatment were selected randomly and fasted for 24 hours in preparation for slaughtering and carcass evaluation. Each bird was weighed and slaughtered by severing the jugular vein. The feathers were plucked and primal cuts removed thereafter. All data collected was subjected to Analysis of Variance. Significant means were separated using Duncan Multiple Range Test.

Animal welfare and ethical approval

The ethical approval of university of Calabar Committee on Animal Welfare and Rights was obtained based on the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition of National Health and Medical Research Council - Canberra in 2013.

RESULTS AND DISCUSSION

The gross composition of experimental diets is presented in Table 1. Cost of each diet was also calculated. Table 2 shows the proximate composition of the experimental diets. Diet T_1 had the highest (29.31%) crude protein content as compared to 27.12 %, 25.37% and 23.625 for T_2, T_3 and T_4 respectively. Emmert and Baker (1997) remarked that levels of amino acid in diets could be gradually decreased in accordance with a bird’s lysine, sulphur amino acid and threonine

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requirements without compromising its growth and carcass yield. The growth performance of the birds is presented in Table 3. Weight gain and feed conversion ratio were not significantly different (p>0.05) among the dietary groups. Feed intake was however significantly different (p<0.05), Phase1 and 2 birds being superior in this regard. The result of the present study is in consonance with the reports of Warren and Emmert (2000) as well as Pope and Emmert (2002) who established that multi- Phase-feeding had no significant effect on body mass of broiler chickens. The mean weekly weight of the experimental birds was not significantly different (p>0.05). However, Tolimir et al (2010) differed with this research finding. Previous authors (Farhart et al., 2002) reported improvement in body weight of birds fed starter diets from 0-14 days and those fed finisher diets from 16–35 days. Contrary to the present research finding, Gajana et al. (2011) observed that birds fed single diet performed better in terms of feed conversion ratio than those fed two diets. Zubair and Leeson (1994) remarked that under nutrition is more detrimental to animals during the early stages of life than later. When birds are subjected to early feed-restriction they exhibit slow growth followed by a period of rapid growth and weight gain as they approach market weight to compensate for the delayed growth during the early restriction period. Feed restriction in this study was in terms of quality at the different phases of feeding. Feed intake is an important factor and birds on Phase 3 diet consumed averagely lower quantity than other groups, it therefore portends to reduced body weight gain with a resultant effect of reduced maintenance requirements.

Table 2 - Performance of broiler chickens subjected to phase-feeding

Parameters	Phase 1	Phase 2	Phase 3	Phase 4	SEM	P-value
Initial Weight (kg)	0.04	0.04	0.04	0.05	0	NS
Final weight (kg)	2.95	2.78	2.67	2.51	0.17	NS
Total weight gain (kg)	2.91	2.74	2.81	2.47	0.17	NS
mean weekly wt. gain	0.37	0.36	0.36	0.33	0.07	NS
Mean weekly body wt.	1.12	1.11	1.06	1	0.04	NS
Total feed intake (g)	6695.4	6405.7	5711.6	5738.1	10.32	NS
Mean weekly feed intake	836.93^{a}	800.71^{a}	713.95^{b}	717.26^{b}	3.65	*
Feed conversion ratio	2.27	2.37	2.17	2.33	0.04	NS
Total feed cost/kg ($)	1.884^{a}	1.736^{ab}	1.536^{b}	1.507^{b}	0.16	*

^{a,b} Means on the same row with different superscripts are significantly different; SEM= standard error of mean; *p<0.05; NS: not significant

Table 3 - Carcass characteristics of broiler chickens subjected to phase-feeding

Parameters	Phase 1	Phase 2	Phase 3	Phase 4	SEM	P-value
Live weight	2950.03^{a}	2780^{b}	2670.62^{c}	2510.1^{c}	11.2	*
Carcass weight	2067.39^{a}	1935.45^{b}	1834.79^{c}	1824.25^{c}	10.12	*
Dressing %	70.08	69.62	68.7	72.67	3.3	NS
Head	55.78	50.14	60.74	60.12	1.03	NS
Neck	91.97^{a}	73.80^{ab}	69.12^{b}	71.92^{ab}	1.5	*
Wings	216.6^{ab}	211.2^{ab}	210.08^{ab}	220.3^{a}	1.02	*
Thighs	274.76^{ab}	282.33^{a}	229.04^{c}	261.82^{b}	2.26	*
Drumstick	214.51^{a}	194.13^{ab}	200.91^{a}	185.34^{b}	1.63	*
Back	265.63^{a}	212.29^{ab}	192.52^{b}	200.46^{ab}	2.67	*
Breast	516.67^{a}	516.67^{a}	466.67^{b}	416.67^{c}	3.22	*
Shank	73.07	71.64	83.47	80.6	1.11	NS
Gizzard	38.03	36.03	35.21	33.68	0.31	NS
Abdominal fat	37.97	33.05	20.86	27.84	1.26	NS

^{a,b} Means on the same row with different superscripts are significantly different; SEM= standard error of mean; *p<0.05; NS: not significant

The cost per kg of the diets differed significantly (p<0.05). Feed cost per bird was higher (p<0.05) in the birds on single diet (phase 1) $0.900 than in others. Lowest feed cost per kilogram diet ($0.175) was recorded on the four-phase diet. This finding is at variance with report of Henry and Ammerman (1995) that single diet feeding programme may save merit in broiler production by saving on feeding cost. In line with result of this research, Meremikwu and Obikaonu (2020) affirms that the cost per kilogram ($0.42) of feed of broiler birds on the control (regular feed) was significantly (p<0.05) higher than the cost ($0.21 – 0.23) of nutrient restricted diets. It goes to confirm that it is more cost effective to feed broiler birds using different Phase- feeding regimes than the conventional diets.

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Result of carcass evaluation is presented in Table 4. Live and dressed carcass weights of birds on single diet were significantly (p<0.05) higher than those on 2, 3 and 4 phase diets. Similarly, birds on phase 2 phase diet were significantly (p<0.05) superior than those on 3 and 4 phase diets live and dressed carcass weights. Dressing percentage however, did not differ (p>0.05) significantly among the diet phases, values recorded ranged between 68.70 and 72.67%. The dressing percentage values obtained in this study fall within the range (68.66 – 70.30) reported by Jalal and Zakaria (2012) who fed broiler chickens at 100, 80, 65 and 50% feed intake respectively. Significance varied among traits and dietary phases. This result is at variance with the report of Farhart et al. (2002) that carcass weight was lower in the single diet group than those fed three phases. Abdelraheem et al. (2019) asserted that "carcass weight of broiler chickens can be controlled using different options of feed restriction programs according to the need of the market and the producer situation with special consideration to the economic return". Previous researches have lent credence to the fact that nutrition plays important role in broiler performance as feeding factors impact considerably on carcass composition of the birds (Abdelraheem et al., 2019; Banaszak et al., 2021). Abdelraheem et al. (2019) reported dressing percentage values of 72 – 75% for broiler birds fed 120g of feed per bird per day slaughtered at 32 days of age. Milczarek et al. (2022) similarly reported dressing percentage ranging between 75 and 78.5% for broiler chickens fed graded levels of guar meal diets. Differences between the findings of the present research and reports of previous authors could be attributed to genetic, nutritional and management variations among the studies.

CONCLUSION AND RECOMMENDATIONS

The study established that none of the Phase-feeding regimes engendered a significant performance among the bird, since total weight gain, feed conversion ratio and dressing percentage did not differ significantly among the feeding groups. It is recommended therefore that phase-feeding of broiler chickens using phase 4 feeding regime, (that is starter, first finisher, second finisher and third finisher diets) be embraced by poultry farmers as it would elicit reduced dietary cost without compromising optimum performance of the birds.

DECLARATIONS

Corresponding author

Dr. Victoria Nneoma Ebegbulem;
Email: vicnneb@gmail.com;
Phone: 2348064473566

Consent to publish

Not applicable

Authors' contributions

V.N. Ebegbulem: conceptualization, research methodology and writing of the first draft of the article;
E.E. Archibong: methodology, statistical analyses;
T.N. Kperun and M. Udayi: feeding trial, data collection;
E.D. Izuki: literature review, editing the manuscript and references.

Competing interest

There is no competing interest to this research and publication.

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EVALUATION OF FERMENTATION PROGRESS DURING STORAGE OF MILLET STOVERS SILAGE BASED ON pH-INDICATORS

Hamza Seydou KOROMBÉ[1], Vincent Boubié BADO[2], Nourou ABDOU[3], Clarisse UMUTONI[2], Abdoussalam IBRAHIMA[2], Abdoulaye Soumana GOURO[1]

[1]*Abdou Moumouni University, Faculty of Agronomy, BP: 10960, Niamey, Niger*
[2]*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), BP: 12404, Niamey, Niger*
[3]*National Institute for Agricultural Research, Department of Animal Production, BP: 429 Niamey, Niger*

Email: koseha2018@gmail.com

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ABSTRACT: This study aimed at evaluating the fermentation levels of pearl millet [*Pennisetum Glaucum* (L.) R. Br] stovers silage during storage based on pH evolution. A completely randomized experimental design in a 6×2×2 factorial scheme with three replications for each treatment was used to evaluate three factors (6 cultivars, 2 different cutting stages, and with or without salt addition to the cultivars). The silages were prepared in plastic bags and stored for 60 days at room temperature. The results revealed that the pH values of the treatments were significantly ($P<0.05$) higher on the first day than in the other periods and a rapid drop in pH, with significant differences ($P<0.05$), to levels below 4 was obtained on the third day of storage for the majority of local Sadoré and Siaka Millet silages (Niger). Four types of pH evolution were recorded and the variation was statistical significant among cultivars. Also, analysis of the relationships between pH, chemical composition parameters and In Vitro Digestibiliy of Organic Matter (IVDOM) showed that increasing pH values were associated with increasing Dry Matter content of stovers before silage (DM_BE), Dry Matter content of silages (DM_S), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) values and decreasing Crude Protein (CP), Metabolizable Energy (ME), IVOMD, and Ash values. However, the pH values obtained for all silages showed that all the millet stovers used were suitable for silage. At the maturity stage, it is thus possible to use the grain for human consumption and to ensile the stovers for animal feed. This study also shows that monitoring the pH in the silo makes it possible to evaluate the quality of the fermentations to avoid losses on the farms.

Keywords: Dual-purpose varieties; Harvesting stage; Monitoring of pH; Silage; Stovers conservation.

INTRODUCTION

Rain-fed agriculture and agro-pastoralism systems are the source of employment for about 80-90% of the population in the West Africa Sahel (Bado et al., 2021). However, crop production alone cannot meet basic human needs for nutrition or income generation. The demand for livestock products is increasing due to an increasing human population and the dietary changes that are driven by demographic changes of an urbanizing society (Thornton, 2010; Bado et al., 2021). Livestock plays a crucial role, in generating income, nutrition and creating the means to purchase more diverse diets, and paying for health and other household needs. However, the increasing demand for feed and seasonal shortage in feed and water, particularly during the long dry season remain the major constraints limiting livestock production (Lamega et al., 2021; Amole et al., 2022).

In Niger, nearly 66 % of the national livestock, mainly small ruminants, are raised in agricultural areas, in a sedentary mode. The immediate consequence is chronic underfeeding of the animals, resulting in a decline in their performance (Sourabie et al., 1995). Increasing feed availability for livestock and the demand for food for the population is possible through the improvement of both grain and biomass production of the dominant crops, such as millet (Ouendeba and Siaka, 2004; Malam et al., 2019).

But the main constraint of millet stovers in small farming systems is the gradual loss of their nutritional value, ingestibility, and digestibility during storage (Cai et al., 2020). These losses can be controlled by managing storage conditions. Silage is one of the technologies that have been found to preserve the quality of crop residues (AFSSA, 2004). However, during the silage process, a number of parameters fluctuate and include: pH, temperature, types and numbers of microorganisms, fermentation products and chemical composition (Cherney and Cherney, 2003). Thus, when the pH value drops rapidly, the wet fodder is preserved from spoilage microorganisms (Rooke and Hatfield, 2003).

Therefore, monitoring acidity fluctuation over time is the simplest and quickest way to assess the fermentation and final product quality of silage (Sprague and Taylor 1977; Decruyenaere et al., 2008; Ishiaku et al., 2020). But how does pH relate to the quality of the final silage product? According to Cherney and Cherney (2003), the chemical composition of the silage influences its nutrient quality. Furthermore, assessing the progression of fermentation during the silage process

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provides information on its relative success with different types of treatments (Sprague and Taylor, 1977; Cherney and Cherney, 2003; Ahmed et al., 2022).

Therefore, this study was carried out to assess the progress of fermentation during the ensiling process by analyzing the evolution of pH as function of cultivars, cutting stage and adding salt. Specifically, the aim is to: i) Characterise the different types of pH evolution in millet stover silages; ii) Compare the different types of pH evolution in millet stover silages according to cultivars, cutting stage and salt addition; and iii) determine the relationship between pH and chemical composition parameters of millet stover silages.

MATERIALS AND METHODS

Study area and material

The experiment was conducted in Niger at the International Crops Research Institute for the Semi-Arid Tropic (ICRISAT) research station at Sadoré, which is located between 13° 14' N and 2° 16' E. During the experiment, the average minimum and maximum temperatures were 22.85±4.05°C and 36.42±3.91°C respectively, with an overall average of 29.64±3.51°C. In this period, the total annual rainfall was 715.29 mm. The pearl millet stover was obtained from pearl millet grown at the Sadoré experimental station from 29 June to 14 November 2019. The number of days between the flowering and maturity stage varied between 31 to 55 days depending on the variety (Table 1).

Table 1 - Number of days between sowing and harvest (NDAS) according to cultivar and cutting stage.

Cultivars	Stage	NDAS	NDFM
Chakti	Flowering	45	31
	Maturity	76	
Millet of Siaka	Flowering	73	42
	Maturity	115	
ICRI-Tabi	Flowering	72	40
	Maturity	112	
Alambana	Flowering	71	55
	Maturity	126	
Maywa	Flowering	88	50
	Maturity	138	
Local Sadoré	Flowering	75	41
	Maturity	116	

NDAS: Number of Days After Sowing; NDFM: Number of Days between Flowering and Maturity.

Experiment design

A completely randomized experimental design was used in a 6x2x2 factorial scheme with three replications. Three factors were evaluated: cultivar, stage of cutting stover, and inclusion or not of salt (i) the cultivar, with six modalities consisting of four improved dual-purpose varieties Chakti, ICMV167005 (Millet of Siaka), ICMV167006 (ICRI-Tabi), ICMH177111 (Alambana) and two local varieties (Maywa and Local Sadoré). The four improved dual-purpose varieties were selected because they have been approved and evaluated in the field, while the local varieties were selected because they are widely used in the study area and generally in all Niger. (ii) Two stages of cutting were considered: flowering and maturity. Silages obtained from stover cut at the flowering stage were used as a basis for comparison, as this stage is probably the best time to ensile millet stover (Morales et al., 2015). (iii) The addition of salt was also studied in two ways (without salt and with salt). Salt was used at a rate of 10 kg per ton of fresh silage (Tamboura et al., 2005).

The treatments tested consisted of a combination of different modalities of the three factors, i.e. 6x2x2 giving 24 treatments. For each treatment, three repetitions were considered (Kim and Adesogan, 2006), giving 72 repetitions for the whole trial.

Silage- making process and data measurements

The stovers were harvested, using a machete, at different periods depending on the cultivar and desired stage of cutting for ensiling (Table 1). The stovers were chopped using a chopping machine, to obtain particles of approximately 2 to 3 centimeters (Trevisoli et al., 2017; Kang et al., 2018; Ishiaku et al., 2020). A 20 kg of fresh material was used for each repetition (Morales et al., 2011). Thus, 20 kg of stover was put and compacted, using a manual compactor, in 100 kg capacity plastic bags. The filled plastic bag was hermetically sealed with string and tape. Then introduced in a second bag to improve the anaerobic conditions. The plastic bags were kept in a covered area, at room temperature for 60 days (Costa et al., 2012). Before ensiling and at 60 days, when the plastic bags were opened, fresh silage samples were taken for dry matter determination in an oven at 65°C for 72 hours (Trevisoli et al., 2017) and stored in a freezer for laboratory pH measurements. The dried samples were then ground with a 1 mm grid for the determination of the chemical composition and the In Vitro Digestibility of Organic Matter (IVDOM).

The Direct pH and temperature of the silage were recorded on the 1st, 3rd, 45th, and 60th day of storage, using a Hanna instrument HI99161 portable pH meter, equipped with a penetration electrode for food products. The pH electrode FC2023 is equipped with an integrated temperature sensor for direct reading of both parameters. In this regard, Sprague and Taylor (1977), Cherney and Cherney (2003) and Shan et al. (2021) reported that pH and temperature during the fermentation process in experimental silos or on-farm silos can be monitored effectively using pH probe electrodes, for acceptable moisture levels.

For each repetition, the bag was perforated to place the pH meter electrode inside the silage. The flashing light disappears to indicate the stabilization of the device for pH and temperature readings. Thus, for each repetition, this operation was repeated three times, changing the position of the probe to take into account the heterogeneity inside the silage. Then averages were calculated for each repetition to find the pH and temperature corresponding to a given

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measurement period. At the end of each measurement, the pH meter probe was properly cleaned with distilled water and the hole formed at the introduction of the latter inside the bag was automatically and hermetically sealed with adhesive tape. For the laboratory pH measurement, 10 g of fresh silage was mixed with 100 ml of distilled water using a blender. After filtration, the laboratory pH was measured with a "WTW Multi 9620 IDS pH meter". The chemical composition and IVDOM were determined by Near Infra- Red Spectrometry (NIRS) at the International Livestock Research Institute (ILRI) Laboratory in Burkina Faso.

Statistical analysis of data

The analysis of the pH evolution pattern was performed using SPSS chi-deux tests according to the different factors.

A principal component analysis (PCA) was carried out using XLSTAT 2014. Relationships between Factors (cultivars, cutting stage and adding salt), direct pH, Laboratory pH, IVDOM and some parameters of the chemical composition of silages were established. The DM content was determined according to the ratio between the weight of the dried matter (Pf) and the weight of the initial sample (Pi) (Cherney and Cherney, 2003). According to the factors and their modalities, the frequencies of the different types of pH evolution have been calculated using the following formula:

$$FRtm = \frac{NOTEm}{TNSf} \times 100$$

FRtm: Frequency of the type of evolution in the modality under consideration;
NOTEm: Number of Occurrence of the Type of evolution in the modality;
TNSf: Total Number of Samples for the considered factor.

RESULTS

Characteristics of pH evolution in millet stover silages

The stovers were ensiled at DM levels ranging from 19.06 to 41.69% (Table 2). In general, as a function of the storage period, the pH decreased from day 1 to day 60 in all treatments (Figures 1 and 2). The pH values of the treatments were significantly higher on the first day than in the other periods. A rapid drop in pH, with significant differences, to levels below 4 was obtained on the third day of storage for the majority of the Local Sadoré and Millet of Siaka silages (Table 2). Overall, from this period onwards, the pH evolved until the 60th day of storage with a tendency to stabilize for the majority of silages (Table 2).

The pH values on the first day of storage did not vary significantly between treatments, except Maywa, which was significantly lower than the others for all the different interactions between factors, and Local Sadoré for all the silages at the maturity stage with and without salt. On the forty-fifth day of storage, for all silages at the flowering stage with and without salt, there were no significant differences between all cultivars for the pH means. For maturity stage silages with and without salt, the pH of Chakti and Maywa varieties was significantly lower than the others. At day 60 of storage, significant differences were recorded between treatments. For silages at the flowering stage with salt, ICRI-Tabi, Alambana, Chakti, and Millet of Siaka recorded the lowest pH values, while for silages at the flowering stage without salt, Millet of Siaka, ICRI-Tabi, Local Sadoré, and Alambana gave the most acidic silages. For maturity silages with salt, the varieties Chakti, Maywa, and Millet of Siaka recorded significantly lower pH values than the other varieties, while for maturity silages without salt, the same result was obtained with Chakti and Local Sadoré (Table 2).

Comparison between the different types of pH evolution in millet stover silages according to cultivars, cutting stage, and salt addition

Four types of pH evolution over time were identified, one normal (type 1) and three atypical (types 2-4). The type 1 showed a continuous fall in pH until stabilization, from the 1st to the 60th day of storage. The second type recorded pH drop from the 1st to the 3rd day, followed by a slight increase until the 45th day, then a slight decrease until the 60th day. Type 3 had a pH drop from the 1st to the 45th day, followed by a slight increase until the 60th day while the type 4 registered a pH falls from the 1st to the 3rd day, followed by an increase until the 60th day (Figures 1 and 2). Overall, type 1 evolution of pH was dominated in silages, followed by type 3 and then type 2, whereas the type 4 is weakly recorded (Table 3). However, this global analysis of the type of pH evolution hides several disparities depending on the stage of cutting, the addition of salt, and the cultivar. Regarding the cutting stage, although there was no significant difference ($P > 0.05$), type 2 has was dominated in silages from stover cut at flowering stage, while in silages from stover cut at maturity stage, type 1 dominated. Type 4 was only record by silages from stover cut at maturity (Table 3). There was no significant difference among silage from pearl millet stover with inclusion or not of salt ($P > 0.05$), but in these silages the type 1 was dominated. Specifically, in silages without salt type 3, was dominated compared to type 2, while the opposite was observed with silages with inclusion of salt (Table 3). The type of pH evolution was statistically different between cultivars ($P < 0.05$). Chakti silages have all recorded a type 1 pH evolution (Table 3). 50 % of silages from millet of Siaka showed type 2 pH evolution, while the 50% remaining were equally distributed in, types 1 and 3 pH evolution. In silages of ICRI-Tabi pH evolution was in order to types 1, 2 and 3. In the Alambana silages, the pH evolved in order according to type 1 and type 3. As for Maywa silages, the pH evolution was type 1 and type 3. The type 4 pH evolution was only recorded in the Local Sadoré silages (Table 3).

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Table 2 - Comparison of Dry Matter and pH values according to treatment and conservation period

Factors interaction		DM				pH					
		Conservation period (day)									
Cutting Stage × Adding Salt	Cultivar	1	60	*P*-value	Sign. level	1	3	45	60	*P*-value	Sign. level
Flowering × With Salt	Alambana	20.93^{cB}	23.57^{abA}	0.015	*	6.39^{aA}	4.42^{abcB}	4.33^{aB}	3.67^{bcB}	0.002	**
	Chakti	22.91^{bcA}	21.77^{bB}	0.038	*	6.62^{aA}	5.71^{aB}	4.31^{aC}	3.76^{bcC}	0.000	***
	ICRI-Tabi	27.75^{aA}	23.02^{bB}	0.022	*	6.67^{aA}	4.18^{bcB}	4.31^{aB}	3.64^{cB}	0.000	***
	Local Sadoré	26.03^{abA}	21.21^{bB}	0.049	*	6.49^{aA}	3.45^{cC}	4.38^{aB}	4.09^{abB}	0.000	***
	Maywa	26.05^{abA}	26.11^{aA}	0.969	NS	5.89^{bA}	5.08^{abB}	4.15^{aC}	4.30^{aC}	0.000	***
	Millet of Siaka	19.63^{cB}	22.19^{bA}	0.006	**	6.58^{aA}	3.56^{cC}	4.03^{aB}	3.80^{bcBC}	0.000	***
	P-value	0.000	0.000			0.001	0.002	0.517	0.002		
	Significance level	***	***			**	**	NS	**		
Flowering × Without Salt	Alambana	19.51^{aA}	17.36^{bA}	0.128	NS	6.71^{aA}	5.78^{aB}	4.11^{aC}	4.14^{bcC}	0.000	***
	Chakti	22.17^{aA}	17.77^{bA}	0.138	NS	6.86^{aA}	5.67^{aB}	4.65^{aC}	4.54^{aC}	0.000	***
	ICRI-Tabi	22.96^{aA}	22.15^{aA}	0.561	NS	6.59^{aA}	4.55^{bcB}	4.27^{aB}	3.97^{bcB}	0.000	***
	Local Sadoré	24.61^{aA}	19.24^{abA}	0.051	NS	6.71^{aA}	3.61^{cC}	4.22^{aB}	3.97^{bcBC}	0.000	***
	Maywa	22.47^{aA}	23.59^{aA}	0.316	NS	5.87^{bA}	5.39^{abB}	4.30^{aC}	4.31^{abC}	0.000	***
	Millet of Siaka	19.06^{aA}	20.74^{abA}	0.055	NS	6.74^{aA}	3.76^{cB}	4.43^{aB}	3.87^{cB}	0.000	***
	P-value	0.057	0.003			0.000	0.000	0.109	0.001		
	Significance level	NS	**			***	***	NS	**		
Maturity × With Salt	Alambana	38.90^{abA}	37.75^{abA}	0.684	NS	6.39^{abA}	5.56^{aB}	5.26^{aB}	5.09^{aB}	0.003	**
	Chakti	31.65^{dA}	26.46^{dB}	0.004	**	6.62^{aA}	5.39^{aB}	4.34^{cC}	3.98^{cC}	0.000	***
	ICRI-Tabi	41.69^{aA}	41.06^{aA}	0.782	NS	6.64^{aA}	5.56^{aB}	4.83^{bC}	4.78^{abC}	0.000	***
	Local Sadoré	33.33^{bcdA}	29.71^{cdB}	0.035	*	5.93^{bA}	5.13^{abB}	4.56^{bcC}	4.72^{abC}	0.000	***
	Maywa	31.90^{cdA}	32.97^{bcA}	0.187	NS	5.12^{cA}	4.79^{bB}	4.37^{cC}	4.29^{bcC}	0.000	***
	Mil of Siaka	37.47^{abcA}	39.41^{aA}	0.314	NS	6.11^{abA}	4.77^{bB}	4.46^{bcB}	4.40^{bcB}	0.000	***
	P-value	0.000	0.000			0.000	0.000	0.000	0.000		
	Significance level	***	***			***	***	***	***		
Maturity × Without Salt	Alambana	30.26^{bcA}	29.83^{bcA}	0.747	NS	5.92^{cA}	5.37^{abB}	4.70^{abC}	4.65^{bC}	0.000	***
	Chakti	30.96^{bcA}	24.87^{cB}	0.010	*	6.75^{aA}	4.92^{bB}	4.30^{bBC}	4.18^{cC}	0.000	***
	ICRI-Tabi	39.61^{aA}	36.44^{aA}	0.356	NS	6.60^{abA}	5.81^{aB}	4.96^{aC}	5.46^{aBC}	0.000	***
	Local Sadoré	29.44^{cA}	27.22^{cB}	0.005	**	6.08^{bcA}	3.58^{cC}	4.31^{bBC}	4.47^{bcB}	0.000	***
	Maywa	28.56^{cA}	30.91^{abcA}	0.287	NS	5.37^{dA}	5.07^{abAB}	4.83^{aBC}	4.60^{bC}	0.003	**
	Millet of Siaka	35.12^{abA}	35.98^{abA}	0.562	NS	6.24^{abcA}	4.81^{bB}	4.30^{bC}	4.57^{bBC}	0.000	***
	P-value	0.000	0.000			0.000	0.000	0.001	0.000		
	Significance level	***	***			***	***	**	***		

In each column and according to parameter and interaction between the modalities, means with at least one similar lower case letter in superscript are not statistically different at 5% level. In each row, according to parameter, means with at least one similar capital letter in superscript are not statistically different at the 5% level. Flowering x With Salt: Interaction between Flowering and With Salt ; Flowering x Without Salt : Interaction between Flowering and Without Salt ; Maturity x With Salt : Interaction between Maturity and With Salt; Maturity x Without Salt : Interaction between Maturity and Without Salt; Sign.: Significant; NS: Not Significant; *: *P-value < 0.05;* **: *P-value < 0.01;* ***: *P-value < 0.001.*

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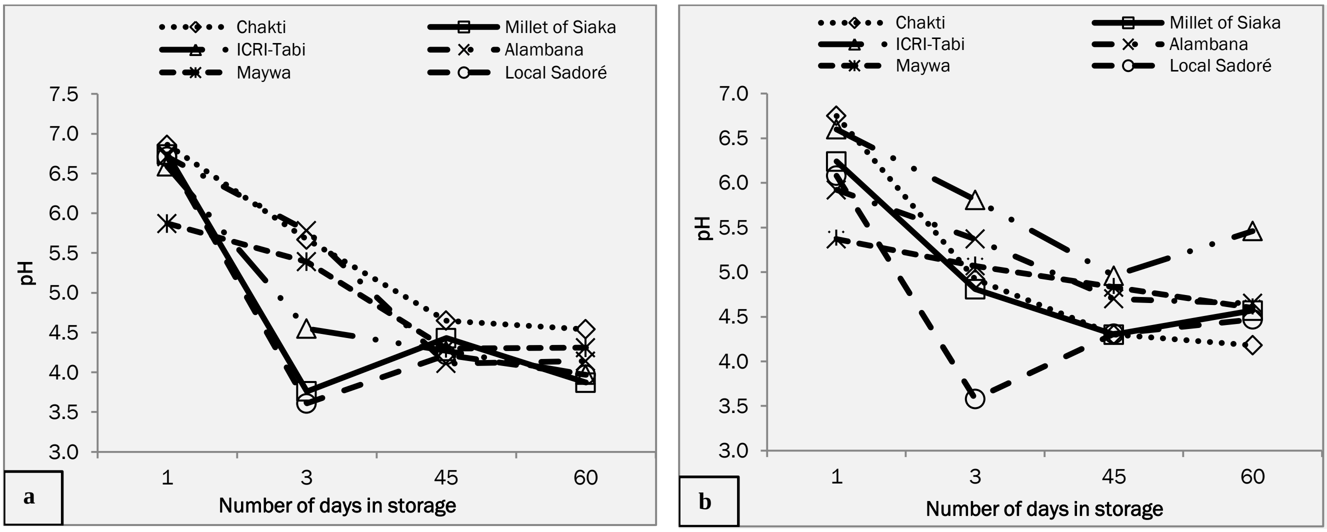

Figure 1 - pH of silages over time, (a) silages from stover cut at flowering stage, without addition of salt, and (b) silages from stover cut at maturity, without addition of salt.

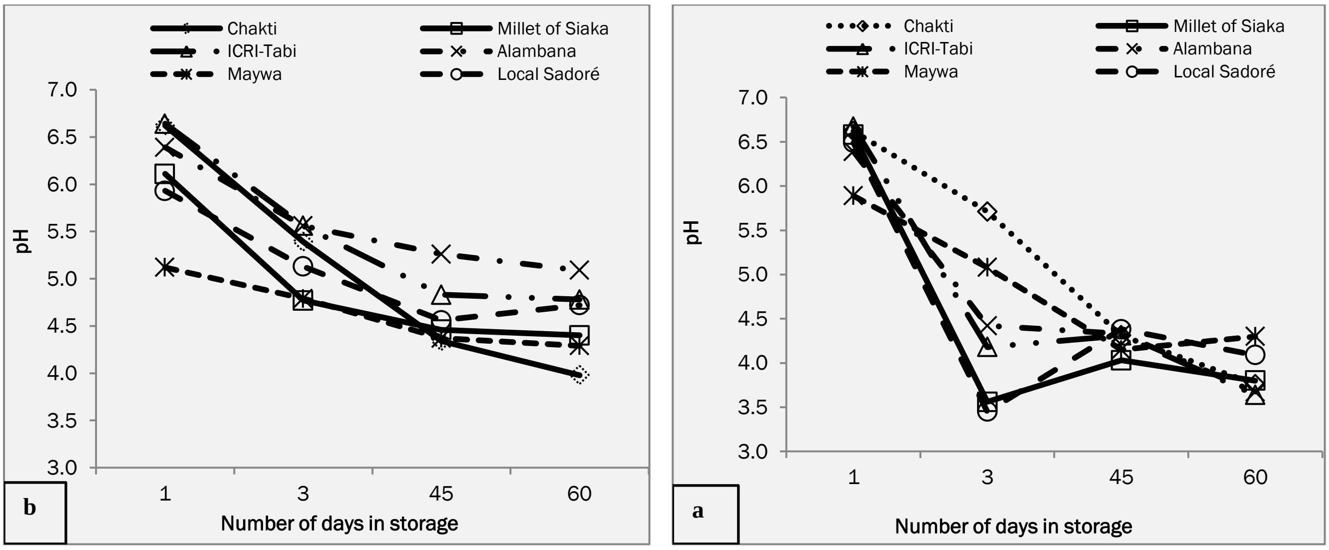

Figure 2 - pH of silages over time, (a) silages from stover cut at flowering, with added of salt (a) and (b) silages from stover cut at maturity, with added salt (b).

Table 3 - Mean of Dry Matter content at first day, mean of pH at day 60 and frequencies of different types of pH evolution according to variety, cutting stage and salt addition

Factors	Modalities	DM_BE_M (%)	pH_f	Types of pH evolution				
				Type 1 (%)	Type 2 (%)	Type 3 (%)	Type 4 (%)	Total (%)
Cultivars	Chakti	26.92^a	4.12^a	100	0	0	0	100
	Millet of Siaka	27.82^a	4.16^{ab}	25	50	25	0	100
	ICRI-Tabi	33.00^b	4.46^c	50	25	25	0	100
	Alambana	27.40^a	4.39^c	75	0	25	0	100
	Maywa	27.25^a	4.37^c	50	0	50	0	100
	Local Sadoré	28.35^a	4.31^{bc}	0	50	25	25	100
	P-value	0.000	0.000			0.000		
	Significance level	***	***			***		
Cutting stages	Flowering	22.84^a	4.00^a	33.3	41.7	25	0	100
	Maturity	34.08^b	4.60^b	66.7	0	25	8.3	100
	P-value	0.000	0.000			0.062		
	Significance level	***	***			NS		
Adding salt	Without salt	27.06^a	4.39^b	41.7	16.7	33.3	8.3	100
	With salt	29.85^b	4.21^a	58.3	25	16.7	0	100
	P-value	0.000	0.000			0.53		
	Significance level	***	***			NS		
Global Frequencies				50	20.8	25	4.2	100

DM_BE_M : Mean dry matter content at first day ; pH_f : Mean pH at day 60; NS: Not Significant; ***: *P-value < 0.001.*

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Relationships between pH and pararameters of chemical composition and In Vitro Digestibily of Organic Matter of millet stover silages

The results of the PCA between the factors (Cultivar, Salt addition, and Cutting stage), the pH was measured directly on the 60th day of storage (Direct pH), the pH was measured in the laboratory on the 60th day of storage (Laboratory pH), Chemical composition parameters (DM_BE, DM_S, CP, Ash, NDF, ADF, ADL, ME) and In Vitro Digestibility of Organic Matter (IVDOM) was indicated that there were strong significant correlations between some parameters (Table 4 and Figure 3). Figure 3 shows that all parameters are well represented on the first two axes of the plan, which together account for 81.02% of the information. Each parameter was well correlated positively or negatively with one of the two axes. Thus, all the studied parameters are well correlated with axis 1 (70.21% of the information), except for Salt addition and cultivar which are best represented on axis 2 (10.81% of the information). The analysis of Figure 3 and Table 4 shows a strong positive correlation between direct and laboratory pH. Two groups of correlation can be distinguished according to the analysis of the evolution of pH values on axis 1. The analysis of these relationships showed that the increase in pH values was associated with the increase in DM_BE, DM_S, NDF, ADF, and ADL. All these parameters move in opposite directions with CP, EM, MDMVI, and ash. The increase in these parameters is caused by the decrease in pH values. The analysis of Table 4 and Figure 3, also allows us to deduce that all the parameters of the chemical composition and the IVDOM studied can contribute to characterise the cutting stage, while only Ash contributes to separate the groups according to the addition of salt and no parameter allows identifying the cultivar groups. The results also show that there are no significant interactions between the three factors compared in pairs (Table 4).

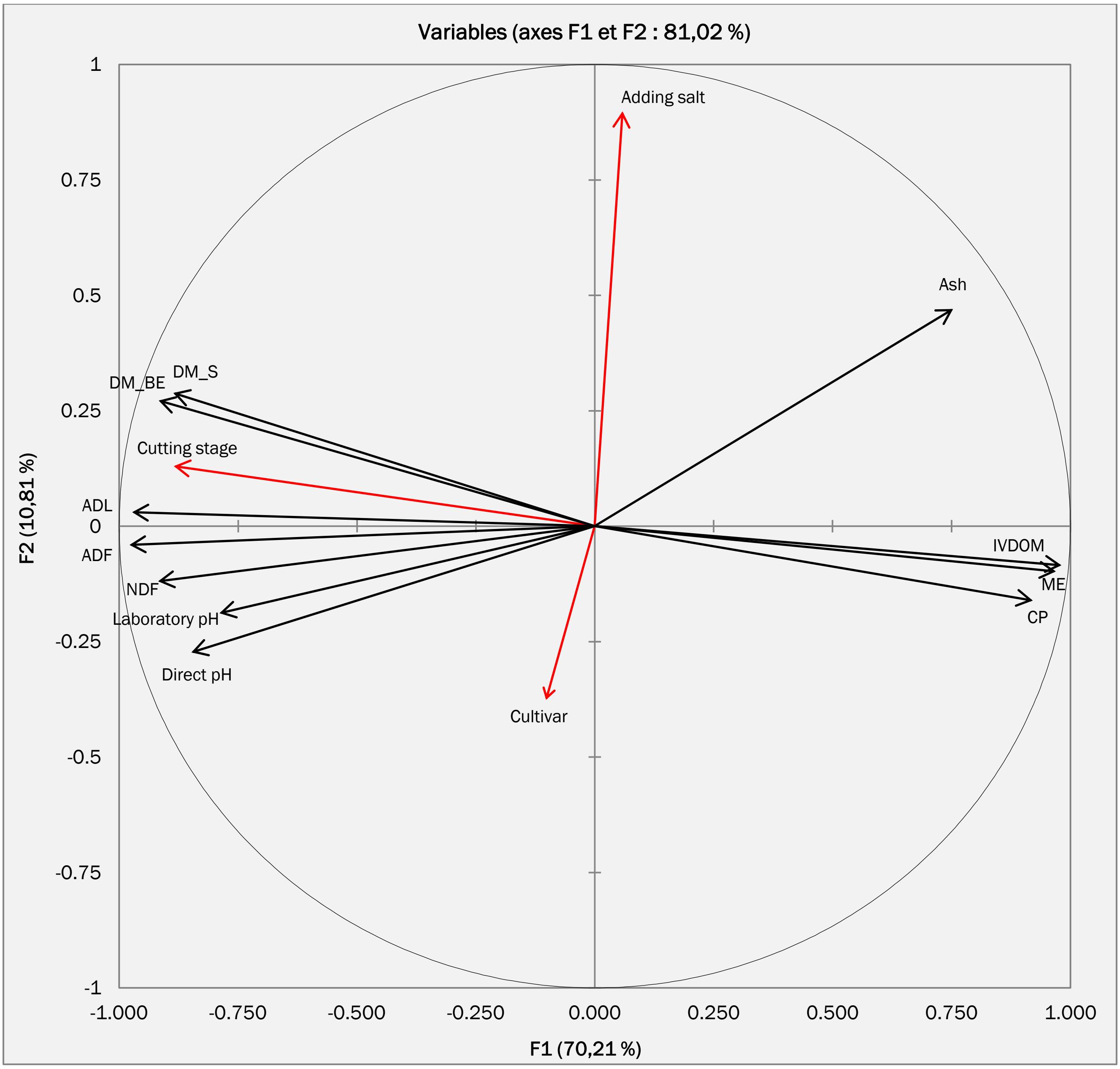

Figure 3 - Spatial representation of the relationships between some silage parameters. Direct pH: Hydrogen potential measured directly in silages at 60 days of storage; Laboratory pH: Hydrogen potential of silages measured in the laboratory after 60 days of storage; DM_BE: Dry Matter content of stovers before silage; DM_S: Dry Matter content of silages; CP: Crude Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin; ME: Metabolizable Energy; IVDOM: In Vitro Digestibility of Organic Matter.

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Table 4 - Matrix of correlation between factors, pH, different parameters of chemical composition and In Vitro Digestibility of Organic Matter

	Cultivar	AS	CS	Ash	CP	NDF	ADF	ADL	ME	IVDOM	Direct pH	DM_S	DM_BE	Laboratory pH
Cultivar	1													
AS	-0.024	1												
CS	0.024	0.000	1											
Ash	-0.210	0.447*	-0.582**	1										
CP	0.003	-0.028	-0.803**	0.600**	1									
NDF	0.081	-0.221	0.751**	-0.730**	-0.924**	1								
ADF	0.062	-0.117	0.830**	-0.716**	-0.908**	0.943**	1							
ADL	0.026	-0.088	0.920**	-0.709**	-0.915**	0.917**	0.967**	1						
ME	-0.115	-0.045	-0.837**	0.662**	0.922**	-0.913**	-0.967**	-0.964**	1					
IVDOM	-0.076	-0.013	-0.874**	0.689**	0.925**	-0.917**	-0.977**	-0.982**	0.994**	1				
Direct pH	0.204	-0.208	0.670**	-0.715**	-0.651**	0.707**	0.823**	0.750**	-0.766**	-0.772**	1			
DM_S	0.020	0.237	0.812**	-0.626**	-0.844**	0.727**	0.804**	0.825**	-0.844**	-0.855**	0.696**	1		
DM_BE	0.026	0.210	0.847**	-0.596**	-0.852**	0.758**	0.861**	0.871**	-0.888**	-0.896**	0.735**	0.918**	1	
Laboratory pH	0.206	-0.109	0.640**	-0.574**	-0.590**	0.610**	0.769**	0.680**	-0.705**	-0.716**	0.947**	0.660**	0.708**	1

*: The correlation is significant at the 0.05 level; **: The correlation is significant at the 0.01 level. AS: Adding Salt; CS: Cutting Stage; CP: Crude Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Detergent Lignin; ME: Metabolizable Energy; IVDOM: In Vitro Digestibility of Organic Matter; Direct pH: Hydrogen potential measured directly in silages at 60 days of storage; DM_S: Dry Matter content of silages; DM_BE: Dry Matter content of stovers before silage; Laboratory pH: Hydrogen potential of silages measured in the laboratory after 60 days of storage.

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DISCUSSION

Analysis of the different types of pH evolution in silages

The variation of pH during the storage period revealed four types of evolution reflecting the silage preparation conditions. Similar types of pH evolution in silage was reported by AFSSA (2004). The type 1 evolution suggests good silage preparation conditions and involve high concentration of soluble sugars in stover, which would allow sufficient production of lactic acid throughout the silage process. This probably led to a continuous decrease in pH from the first to the sixtieth day of storage. Indeed, Kang et al. (2018) reported that Plant carbohydrates are the substrates of the fermentation process, so their concentration in the original forage has a strong influence on the extent and nature of silage fermentation. Thus a high concentration of soluble sugars in fresh forage would result in good- quality silage as this would lead to high production of lactic acid which would be the basis for the gradual fall to stabilization of the pH of the silage. For type 2 pH evolution, after a drop in pH to values below 4 between days 1 and 3 corresponding to stability pH, the product becomes stable according to Adesogan and Newman (2021) because the development of undesirable microorganisms is inhibited. But, the slight increase in pH observed between the 3rd and 45th day suggests that during this period, the undesirable microorganisms have resumed their activities following air entry into the silos, because according to Adesogan and Newman (2021) the quality of the silage and consequently the stability pH can be maintained during storage as long as the silo remains sealed and air does not penetrate it. However, the rapid depletion of silo air and the acceptable level of soluble sugar in the product resulted in a slight decrease in pH between days 45 and 60. For pH evolutions of types 3 and 4, soluble sugars were probably insufficient in the stover to allow continuous production of lactic acid, to maintain a decrease of silage pH until the end of the process. Soluble carbohydrates play an important role in the fermentation process as they accelerate the acidification process. Their action, lead to a complete inhibition of any microbial activity (even that of the lactic flora) and of any proteolytic activity (Rooke and Hatfield, 2003; Baumont et al., 2011).

The rapid decrease of pH was observed in most of our silages between day 1 and 3 followed by pH stability during the storage period indicates a good fermentation process as was reported by Hassanat et al. (2007), Alhaag et al. (2019) and Hanif et al. (2020). Generally, pH is one of the quickest and simplest ways of evaluating silage quality (Ishiaku et al., 2020). The majority of pH values obtained in our silages at day 60 show that the making process would have gone well. This result shows good fermentation levels in these silages as was reported by Hassanat et al. (2007). Normally, good - quality silage is defined by a pH < 4 (Demarquilly et al., 1998).

Analysis of the different types of pH evolution in millet stover silages according to cultivars, cutting stage, and salt addition

For the cultivar, relatively low average pH (<4.5) and high frequencies of normal evolution (type 1) of pH recorded in the silages of Chakti (100%) and Alambana (75%) show that these silages were made under very good conservation conditions and that these cultivars produced stovers sufficiently rich in soluble sugars to make good silages. Moreover, the average pH of less than 4.5 obtained in the silages of the cultivars Millet of Siaka, Local Sadoré, Maywa and ICRI-Tabi, suggest that these cultivars produced stovers that are easily ensiled. However, the process of storage did not go well for some of the samples, which would have resulted in types 2, 3, and 4 of pH evolution in silages of these cultivars.

Regarding the cutting stage, normal (type 1) pH evolution dominated in silages at the maturity stage (66.7%), while atypical evolution of types 2 and 3 (66.7%) was predominantly recorded in silages at the flowering stage. However, the average pH (Table 3) obtained in silages at the flowering - stage (4.00) was statistically lower ($p<0.001$) than that recorded at the maturity stage (4.60). The frequency of atypical pH evolution in flowering stage silages would indicate inadequate conditions in the preparation and conservation process of these silages. On the other hand, the frequency of normal pH evolution obtained in silages from the maturity stage allows us to conclude that they were prepared under better conditions and that, according to the quality grid based on pH and DM proposed by Decruyenaere et al. (2008), the average pH of 4.6 and the average DM content of 34.08% (Table 3) would indicate that the stovers from the maturity stage are suitable for silage. The variations of the average pH according to the cutting stage can be explained, on the one hand, by the DM rate which is significantly higher in the silages of the maturity stage (Table 3), which would lead to a weak decrease of the pH in these silages and, on the other hand, by the fact that the stovers of the flowering stage contain more soluble sugars to allow a good decrease of the pH. These results are similar to those of Morales et al. (2014), who reported a variation in silage quality depending on the cutting stage. Costa et al. (2012), Morales et al. (2014 and 2015) noted an increase in silage pH and DM with plant age.

As for adding salt, silages with salt had a significantly lower pH ($p<0.001$) than silages without salt and a predominantly normal pH evolution (58.3%) compared to 41.7% in control silages. This shows that salt improved the fermentation conditions of the silages. These results corroborate those of Cai et al. (1997) who observed, by adding salt (NaCl), a decrease in pH and an improvement in silage quality. However, Shockey and Borger (1991) reported a negative effect of salt on silage storage. This can be explained by the differences in the amount of salt used in the two trials and the types of lactic acid bacteria present in the silage. Indeed, Amar and Manaa (2016) obtained three types of lactic acid bacteria growth depending on the salt dose.

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Analysis of Relationships between pH and parameters of chemical composition and In Vitro Digestibility of organic matter of millet silages

Our results showed that increasing pH values were associated with increasing DM_BE, DM_S, NDF, ADF, ADL values and decreasing CP, ME, IVOMD, Ash values. Indeed, Smith (1954), Seglar (2003) and DuPonte et al. (2016) reported that silage quality is closely related to the degree of acidity obtained during fermentation. According to Wilkinson et al. (2003) and Cai et al. (2020), low pH values were associated with stable, good-quality silages. Furthermore, hay and silage quality can be defined in several ways. It is associated with nutrients; protein, NDF, ADF and ADL, minerals, vitamins, lipids, carbohydrates, energy, and digestibility, and also sometimes animal production (Charmley 2001; Newman et al. 2009). Ephrem et al. (2015) reported that the CP content of the forage is the most important nutrient parameter to consider. Thus, the results of our study suggest that good quality silage is characterized by low pH, high CP, ME, IVOMD, Ash contents and low DM_BE, DM_S, NDF, ADF, ADL contents.

Also, CP, ME, NDF, ADF and ADL contents of silages were the most correlated with IVOMD. These results corroborate those of Yang et al. (2018) and Aïssa et al. (2021), who showed that nutrient digestibility and energy parameters are positively correlated with forage CP content, but negatively correlated with NDF and ADF content. These authors also showed that the CP, NDF and ADF contents of the forage most determine the digestibility of the nutrients.

CONCLUSION

This research revealed that the stover of six pearl millet varieties has the potential to provide good- quality silages based on their pH analysis as a function of DM before ensiling. Stovers cut at flowering gave the best silages. However, silages at the maturity stage are also suitable. This means that the grain can be used for human consumption and the stover silages for animal feed. The addition of salt at 1% has the benefit to improve the quality of silage. The dual-purpose pearl millet varieties Chakti and Siaka produced silage of better quality. However, the varieties of Local Sadoré and Siaka produced the most suitable stover for silage. On the other hand, this study has shown that the pH evolution in the silo can be monitored directly with an adapted pH meter in order to prevent silage fermentation problems. However, this study needs to be completed with fermentation profile of the silages.

DECLARATIONS

Corresponding author

Hamza Seydou KOROMBÉ; Email: koseha2018@gmail.com; ORCID: https://orcid.org/0000-0003-4027-173X

Authors' contributions

HS KOROMBÉ contributed to protocol development, trial conduct, data analysis, manuscript writing, review, and validation. **VB BADO, N ABDOU, C UMUTONI** and **AS GOURO** participated in protocol development, data analysis, manuscript review, and validation of the manuscript. **A IBRAHIMA** participated in protocol development, data collection, review and validation of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

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EFFECT OF THREE DIFFERENT PROCESSING TECHNIQUES OF SOYBEAN ON NUTRITIONAL AND GROWTH PERFORMANCE OF JAPANESE QUAIL (*Coturnix japonica*)

Anthony EKEOCHA[1*], Adeolu AGANGA[1], Pius OKIKI[2], Peter OLUBIYO[1], Joshua OLUWADELE[1]

[1]*Department of Animal Production and Health, Faculty of Agriculture, Federal University, Oye-Ekiti, Nigeria*
[2]*Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*

Email: anthony.ekeocha@fuoye.edu.ng

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ABSTRACT: The experiment investigated the effects of various soya bean groups (boiled, fermented, and roasted) on Japanese quail at 3 weeks old. 160 Japanese quail were randomly assigned to four treatments (control, boiling soya beans, fermented soya beans, roasted soya beans) with four duplicates each. The 12-week trial lasted. Data on weekly body weights and feed conversion ratio were analyzed using analysis of variance (ANOVA) and Tukey's honestly significant at 5% probability test. The result shows there are significant differences in weekly weights of Japanese quail at weeks 1 (828.12-1083.24g), 2 (1026.47-1362.02g), and 3 (1325.69-1528.20g) with the highest observed in birds in treatment 2 (boiled soya beans). The maximum FCR was in week 1 for all treatments, while the lowest was in treatment 3 for weeks 5 and 9 (0.83; P<0.05). Week 1 to week 12 feed conversion ratio decreases. The birds' feed conversion ratios varied significantly (P<0.05). The quails in treatment 4 (roasted soybeans) had the greatest weekly weight after the trial (1742.34g). Thus, quails in treatment 3 (roasted soybean) had the best development performance than the control, boiled and fermented. So it is advised that roasted soybean can be an efficient diet for Japanese quails for maximum performance.

Keywords: Growth performance, Feed conversion ratio, Nutrient; Processing techniques, Roasted soybean.

INTRODUCTION

Quail is a pheasant-family bird and Japanese quails are sexually dimorphic than the wild common quail (*Coturnix coturnix*). Japanese quail breeds vary in commercial outputs (Chang et al., 2005; Rehman et al., 2021). Popularity of Japanese quail is in related to meat and eggs; it's used for egg and meat production throughout Asia, Europe, America, and the Middle East. Small size, fast generation turnover, and high egg yield make it a popular bird (Vali, 2008). Quail meat and eggs are low in cholesterol, fat, but high in protein, making them suitable for diabetics and hypertensive individuals (Onyenweaku et al., 2018; Jeke et al., 2018). Quails provide economical animal protein to rural and urban inhabitants. This will improve meat and egg supply while reducing health risks (Akinola, 2018).

Soybean meal is the main plant protein source in commercial poultry diets (Fowler, 2018). Monogastric animals can't use raw Soya bean meal because of Anti-Nutritional Factors (ANFs) (Ebrahimi-Mahmoudabad and Taghinejad-Roudbaneh, 2011). Anti-nutritional factors (ANFs) affect metabolism and nutrition (Coulibaly et al., 2011). Raw unprocessed Soya bean meal (SBM) reduces broiler development, feed efficiency, pancreatic gland hypertrophy, and amino acid digestibility and availability (Gilani et al., 2005). Heat-processed Soya bean meal (SBM) enhanced broiler chicks' body weight, growth, feed intake, and conversion ratio. Extrusion, boiling, toasting, and roasting may diminish Transient Ischemic Attack (TIA) and Panick attack (PA) in soybeans to inactivate or eradicate ANFs in chicken diets (Ari et al., 2012). Cooking, autoclaving, and microwaving remove anti-nutritional factors from soybeans. Information of comparing soybean heat processing on chicken performance is limited (Akande and Fabiyi, 2010; Heger and Wiltafsky, 2016).

Encouraging Japanese quail production in Nigeria will enhance animal protein intake and improve farmers' and labs' understanding of these popular birds (Vali, 2008). Feeding data gaps have hampered commercial quail production; too much protein causes nitrogen excretion and poor egg production in quails (Dos Santos et al., 2016). Compared to hen eggs, quail eggs have more fat and more protein (Genchev, 2012) since their 1992 debut in the Nigerian poultry market, they've acquired popularity because of their short generation interval, quick development rate, and disease tolerance (Arora et al, 2011).

The aim of this experimental trial study is to evaluate the effect of three different processed diets of soya bean (boiled, fermented and roasted) on growth performance of Japanese quail. The Specific objective is to assess soybean diet with the best feed conversion ratio (FCR).

Citation: Ekeocha A, Aganga A, Okiki P, Olubiyo P, Oluwadele J (2023). Effect of three different processing techniques of soybean on nutritional and growth performance of Japanese quail (*Coturnix japonica*). *Online J. Anim. Feed Res.*, 13(1): 127-131. DOI: https://dx.doi.org/10.51227/ojafr.2023.19

MATERIALS AND METHODS

Experimental location

The study was carried out at a location that was 570 meters above mean sea level and had a longitude of 5.5145^0 East and latitude of 7.7983^0 North. According to the climate classification of the region under investigation, relative humidity levels range from 57 to 92 percent and mean daily temperatures range from 68 to 90 degrees Fahrenheit. The drier season runs from November to March, while the wetter season runs from April to October. The yearly rainfall ranges from 500 to 3000 millimeters.

Ethical approval

This study was carried out after being approved ethically Under the Animal Research Act of 1985, approved by an Animal Research Ethics Committee (AREC) of the university which is required for the use of any experimental animal for research and teaching.

Experimental birds

A total of 160 Japanese quail 3 weeks old, consisting of both males and females were purchased at a private farm in Nigeria. The birds were grouped into 4 treatments including the control, making 10 birds per replicate. On the first week of arrival, the birds were fed with the layer's marsh (control), which is an adjustment period before being grouped according to replicate. Sexing was done starting from the 5th week to the 7th week using the difference in breast coloration which was creamy coloration for females while reddish brown with a speckled pattern in males. Also, sexing was done by venting the cloaca (excretion of white foams which is only peculiar to the male only). The experimental trial study duration was for 12 weeks.

Experimental management

The poultry house was cleaned, washed (cement floor), and fumigated a week before the arrival of the birds with proper disinfectants. The surroundings were cleared of bushes and sprayed with contact herbicides to prevent insects and rodents that may endanger the birds. Cages were cleaned, and wood shavings were used as bedding, which is subject to change once a week. One week adjustment phase was carried out in which they were fed with layer marsh before feeding them as per treatment.

Experimental design

160 Japanese quails were used and assigned into four experimental treatments with 4 replicates of 10 Japanese quails each in a Completely Randomized Design (CRD). The experimental groups are: Treatment 1 (T_1); the control; which the purchase feed is; Treatment 2 (T_2); Boiled and dried soya bean with cassava flour; Treatment 3 (T_3); Fermented and Dry soyabean with cassava flour; Treatment 4 (T_4); Roasted soya bean with cassava flour.

Table 1 - The composition of three treatments diets.

Ingredients	T_1	T_2	T_3	T_4
Maize	58.4	58.4	58.4	58.4
Soybean	35.4	-	-	-
Fermented + dried soya bean	-	21.15	-	-
Roasted soya bean	-	-	21.15	-
Boiled + dried soya bean	-	-	-	21.15
Cassava flour	-	14.25	14.25	14.25
Limestone	1.5	1.5	1.5	1.5
Dicalcium phosphate	2	2	2	2
Fish meal	2	2	2	2
Salt	0.25	0.25	0.25	0.25
Layer premix	0.25	0.25	0.25	0.25
Lysine	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1
Total	100	100	100	100

Table 2 - Calculated composition diet for Japanese quails

Analytical values	T_1	T_2	T_3	T_4
Metabolizable Energy (kcal/kg)	2500	2985.12	2998.32	2885.37
Crude Protein (%)	16.5	18.3	18.1	17.8
Crude Fibre	6	5.2	5.29	8.53
Calcium	3.6	0.45	0.95	0.77
Phosphorus	0.45	0.16	0.15	0.16
Lysine	0.8	2.06	0.88	2.09
Methionine	0.34	0.53	0.43	0.57

Citation: Ekeocha A, Aganga A, Okiki P, Olubiyo P, Oluwadele J (2023). Effect of three different processing techniques of soybean on nutritional and growth performance of Japanese quail (*Coturnix japonica*). *Online J. Anim. Feed Res.*, 13(1): 127-131. DOI: https://dx.doi.org/10.51227/ojafr.2023.19

Experimental procedures

Birds randomly assigned to replicates by Completely Randomized Design (CRD). Each replicate received the appropriate treatment feed and water ad libitum. Leftovers were weighed weekly in plastic bags. Bird weight was measured weekly. Daily egg counts were taken. Weekly cleaning of trash picked up. When needed, birds were given antibiotics, anti-stress drugs, and vitamins.

Economic gain

Economic gain was calculated as a ratio between the return of weight gain and the cost of feed intake. The price of ingredients and selling one kg of quail ($1.00/kg) was calculated based on the price in the local market at the time of the experiment.

Statistical analysis

All data collected has been subjected to analysis of variance (ANOVA) using the general linear model (GLM) of SAS (2008) employed, means has been separated using Tukey's honesty significance at 5% probability test.

RESULTS AND DISCUSSION

Result of effect of the soybean diet on growth performance of Japanese quail is presented in table 3. The table shows that there are significant differences (P<0.05) in the weekly weights of Japanese quail at weeks 1(828.12 - 1083.24g), 2 (1026.47 - 1362.02g), and 3(1325.69 - 1528.20g). Thus, birds fed heat-processed soybean were considered to be more efficient users of feed in terms of growth performance, probably due to higher nutrient availability, than those fed raw soybean meal. This is similar to findings from several authors who reported that soybean meal heat procedures improve the nutritive value and remove anti-nutritive factors in poultry diets, causing better growth performance of broiler chickens: dry heating, extrusion, cooking, roasting (Prachayawarakorn et al., 2006). In addition, Tousi-Mojarrad et al. (2014) reported that broiler chickens fed full-fat roasted soybean showed inferior growth performances than those fed steamed or extruded products.

It was attributed to the fact that steaming was superior to roasting or extrusion in the destruction of TIA (Trypsin inhibitor activity). Ari et al. (2012) corroborated it because all the thermal processing soybean methods examined by them (extrusion, cooking, toasting and roasting) reduced TIA (trypsin inhibitor activities) and PA (phytic acid) compared to unprocessed soybean. The reduction in TIA observed in the experiment of Ari et al. (2012) was consistent with the report of Aviles-Gaxiola et al. (2017), who indicated that steaming was more effective than roasting in trypsin inhibitor activities (TIA) inactivation while PA reduction is best achieved through roasting. The observed variations From week 4 to week 12, were not significant which is similar to what Janocha et al. (2022), reported that heat-processed full-fat soybeans in broiler diets at a level of 15% and reported that Body weights of animals at 6 weeks of age was not adversely affected.

The result of Table 4 showed significant differences (P<0.05) in the feed conversion ratio of the birds. The highest FCR was observed in week 1 for all treatments but was not significant (P>0.05). There were no significant differences (P>0.05) at weeks 1, 3, 4, 6, 8, 10, 11, and 12, however, there are significant differences (P<0.05) at weeks 2(1.22–1.61), 5(0.83–1.11), 7(0.99–1.13) and 9(0.83–1.05) with birds on control diet having the highest feed conversion ratio. The lowest Feed Conversion Ratio was observed in treatment 3; Roasted soya beans for weeks 5 and 9 (0.83). The Feed Conversion Ratio was observed to be decreasing with increasing age from week 1 to week 12.

Table 3 - Effects of soya diets on growth performance of Japanese quails

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
T_1	833.12^{b}	1026.47^{a}	1364.29^{ab}	1438.72	1541.52	1510.26	1525.64	1502.4	1570.2	1599.82	1615.58	1632.14
T_2	1083.24^{a}	1362.02^{b}	1528.20^{a}	1493	1492.21	1551.54	1539.17	1521	1593.43	1606.46	1626.33	1655.98
T_3	828.12^{b}	1062.14^{a}	1330.76^{b}	1423.71	1581.84	1593.16	1569.84	1534	1562.34	1577.64	1599.41	1623.87
T_4	873.05^{ab}	1064.43^{b}	1325.69^{b}	1475.35	1560.32	1656.4	1667.04	1639	1678.09	1702.04	1717.65	1742.34
P-Value	*	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

^{a,b} means on the same row with different superscripts are significantly difference;. T_1: The control, T_2: Boiled soya bean, T_3: Fermented soya bean, T_4: Roasted soya bean. *p<0.05; NS; not significant

Table 4 - Effect of soya bean diets on feed conversion ratio of Japanese quails

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
T_1	1.56	1.22^{b}	1.08	1.15	1.11^{a}	1.08	1.09^{ab}	1.07	1.03^{a}	1.04	1.01	0.98
T_2	1.97	1.56^{a}	1.18	1.15	0.99^{ab}	1.03	1.04^{ab}	1.03	1.00^{a}	1.03	1.01	0.94
T_3	1.76	1.46^{ab}	0.91	1.04	0.83^{b}	0.94	0.99^{b}	0.98	0.83^{b}	0.96	0.95	0.9
T_4	1.97	1.61^{a}	1.15	1.13	1.00^{ab}	1.1	1.13^{a}	1.18	1.05^{a}	1.03	1.06	1.03
P-Value	NS	*	NS	NS	*	NS	*	NS	*	NS	NS	NS

^{a,b} means on the same row with different superscripts are significantly difference;. T_1: The control, T_2: Boiled soya bean, T_3: Fermented soya bean, T_4: Roasted soya bean. *p<0.05; NS; not significant

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CONCLUSION

The quails that were given the treatment of roasted soy beans had the greatest average weekly weight at the conclusion of the trial, which was 1742.34 grams. This weight was numerically larger than the average weekly weight of the quails that were given the other treatments. Therefore, the quails in treatment 3 (roasted soy beans) have the best development performance, which indicates that this therapy is the most successful compared to the others (control, boiled and fermented). The feed conversion ratio was seen to decrease with increasing age from week 1 to week 12, with the greatest feed conversion ratio happening in the first two weeks of life. This trend was observed from week 1 to week 12.

Ethical consideration

Ethical issues including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors which command respect in Nigeria by the ethical committed monitory team.

DECLARATION

Corresponding author

Email: anthony.ekeocha@fuoye.edu.ng

Authors' contribution

Ekeocha collated the whole manuscript; Oluwadele and Okiki analyzed the data, Aganga interpreted the data and Olubiyo involved in data collection

Conflict of interest

Author has not declared any conflict of interest

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THE EFFECTS OF DIFFERENT FEEDING CONDITIONS ON PERFORMANCE AND CARCASS CHARACTERISTICS OF PEKIN, LOCAL, AND CROSSBRED DUCKS

Aam GUNAWAN*[1], Abdul MALIK[1], Siti DHARMAWATI[1], Dwi KARTIKA[1], Nisa WULANDARI[1], and SAPRANI[2]

[1]*Department of Animal Science, Faculty of Agriculture. Islamic University of Kalimantan, Muhammad Arsyad Al Banjari, Banjarmasin, South Kalimantan, Indonesia*
[2]*Balai Pembibitan Ternak Unggul dan Hijauan Pakan Ternak Pelaihari, South Kalimantan, Indonesia*

Email: aamgunawan@yahoo.com

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ABSTRACT: The objective of the study was to investigate performance and carcass quality of Pekin, local, and cross-breed ducks raised under different feeding (varied in protein and fiber levels). A total of 180 male ducks aged 14 days, consisting of 60 Pekin ducks, 60 local ducks (Mojosari), and 60 cross-breed (Mojosari + Alabio) ducks were used in the study. Each type of duck was randomly divided into 36 units of cages, each of which was filled with 5 ducks. The data obtained from the study were analyzed according to a completely randomized design with 2×3 factorial pattern. Initial body weight of local ducks at 14 days was significantly (P<0.01) lower than that of Pekin and cross-breed ducks, while, Pekin ducks were higher than the other two types of ducks. On the other hand, feed consumption was significantly (P<0.01) influenced by the type of diet and breed. While carcass percentage was significantly (P<0.01) influenced by breed and diet types. The percentage of Pekin duck carcasses that received diet A (low protein and fiber) was significantly different (P<0.05) from cross-breed ducks, in comparison to local ducks. In conclusion the feed conversion rate and final body weight for diet A showed better results than diet B (high protein and high fiber) on the Pekin duck. Meanwhile, the carcass characteristics like abdominal fat of diet B (for Pekin, local duck, and cross breed) were better than diet A (for Pekin and local duck). It's suggested to use Pekin ducks with low protein and low fiber diet to get the better performance, in compared with local ducks which needs high protein and high fiber content in diet.

Keywords: Carcass characteristics, Cross-bred birds, Nutrients, Pekin Duck, Performance.

INTRODUCTION

Duck meat is very liked by almost all levels of society because it has a distinctive taste, and a higher fat content than chicken meat (Ali et al., 2007; Qiao et al., 2017). Pekin ducks are good type of duck and have several advantages including large size, fast growth, weight, and good carcass quality (Bugiwati et al., 2021). One of the local duck origins in Indonesia is Mojosari Alabio ducks mostly originate from cross-breeding of male Mojosari (Anas javanica) and female Alabio (*Anas platyrhynchos Borneo*). Mojosari Alabio male duck is a local duck that has a good daily weight gain (Subhan et al., 2022).

In general, broiler ducks are kept intensively fed a complete commercial feed in the form of granules or pellets (Blair, 2008). This maintenance pattern is relatively expensive, so it can increase production costs and reduce profits. Therefore, producers constantly seek cheaper ways to feed the duck. On the other hand, the demand for world duck meat production tends to increase, therefore knowledge of nutrition is needed for feed formulation to enable better meat production (Fouad et al., 2018).

Parameters of duck meat and quality of carcass depend on performance factors, including sex of birds and age, genotype, a system of management, and type of feed (Rahman et al., 2014; Smith et al., 2015). Furthermore, Ali et al. (2008), Zdanowska-Sąsiadek et al. (2013), and Naveen et al. (2016) reported that the qualities of meat duck also depend on the handled before and rearing period, during, and post-slaughter including the condition of meat storage. Meanwhile, the nature of the meat depends on the feed during the rearing period, handling before and during slaughter, and the storage conditions of the meat (Nurkhoeriyati et al., 2012; Zdanowska-Sąsiadek et al., 2013; Naveen et al., 2016). Furthermore, feeding a balanced-diet is a key factor for the growth and development of local ducks, especially protein requirements (Kuzniacka et al., 2014; Udayana et al., 2020).

Determining the nutritional needs of different types of ducks is very important for the efficient use of feeds (Fouad et al., 2018; Liu et al., 2019). In addition, comparing the performance and characteristics of carcasses with various nutritional profiles can provide important information for the progress of duck farmers. However, this data is restricted to the duck (Fouad et al., 2018). The objective of the research was to evaluate of performance and carcass characteristics of Pekin Ducks, local ducks, and the cross between Pekin with local ducks raised under different feeding formulates.

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MATERIALS AND METHODS

Experimental design

A total of 180 male ducks aged 14 days, consisting of 60 Pekin ducks, 60 local ducks called Mojosari, and 60 cross-breed ducks (Mojosari ducks with Alabio duck) were used in the study. Each group of breed were devided 12 cage. every cage consisted 5 ducks. Cages measuring 100 x 50 x 60 cm are equipped with a place to feed and drink. The cage floor was made from iron and designed using a slat system. Feed and water were offered ad-libitum throughout the experiment period of 28 days (14 to 42 days of age). The basal diet was formulated to the nutrient requirements of feed based on local plants as an energy source broiler according to NRC (1994). The design used was a completely randomized design with 6 replications. The treatments given were Diet A and Diet B which were given to three species of ducks, namely Pekin ducks, local ducks, and crossbred ducks. The composition and contain nutrients from experimental diets are shown in table 1.

Table 1 - Composition of feed for duck

Type of diet	Crude Protein (%)	Crude Fiber (%)	Crude fat (%)	Ca (%)	P (%)	ME (Kcal/kg)
Diet A	22.00	5.00	5.00	0.95	0.60	3,000
Diet B	23.05	13.67	5.01	3.41	0.79	2,327

Diet A: Feed composition consist of yellow corn, soybean meal, meat bone meal, corn gluten meal, palm oil, amino acid essential, mineral essential, premix and vitamin. Diet B: Feed composition consist of corn, rice bran, bran pollard, dried distillers grains with soluble (DDGS), soybean meal, palm kernel meal, flour binder, shrimp meal, sago, crude palm oil, limestone, amino acid essential, premix vitamin, premix mineral, mono-calcium phosphate (MCP), and enzyme.

Parameters of study

Ducks and feed were weighted based on parameters including initial and final body weight (BW), the gain of weight, feed intake (FI), and feed conversion ratio per kg of body weight gain, carcass qualities were measured. Recording of growth performance from the ducks was assessed by recording BW, weight gain, feed consumption, and feed conversion ratio (FCR) during the study. Body weight was measured weekly, and feed consumed on a per pen basis was recorded daily. After 28 days of rearing, 12 birds (2 from each group), with body weight close to the mean for the whole flock, were slaughtered for carcass and meat quality analysis. Then the carcass was removed manually and divided into pieces such as chest, legs, wings, neck, and belly fat for analysis. All processes parameters adopted from Wang et al. (2017) and Kokoszynski et al. (2020).

Statistical analysis

The data obtained from the study were analyzed for variance using the GLM procedure from SPSS version 24 according to a completely randomized design with 2x3 factorial pattern. The standard error of measurement (SEM) was also calculated. If the results of the F test show a significant or very significant difference, then the analysis is continued with the Tukey test at $P<0.01$ (Steel and Torrie, 1982).

Ethical approval

This study was approved by the Institutional Animal Care and Use Committee in Faculty of Agriculture, Islamic University of Kalimantan with number 003/U.CC/FP/IV/21.

RESULTS AND DISCUSSION

Performances

The growth performance of the Pekin ducks, local ducks, and cross-breed ducks fed diets A and B were presented in Table 2. The initial body weight of local ducks at 14 days was significantly different ($P<0.01$) lower than that of Pekin and cross-breed ducks, while, Pekin ducks were higher than the other two types of ducks. On the other hand, feed consumption was significantly ($P<0.01$) influenced by the type of diet and breed. Then, diet type and breed type were very significant ($P<0.01$) in influencing weight of gain and final body weight. The interaction was very significant ($P<0.01$) between diet type and breed type on body weight gain and final body weight. The Pekin ducks fed diet A significantly ($P<0.05$) had higher body weight gain than Pekin ducks fed diet B. Likewise, local and cross-breed ducks that received diet A significantly ($P<0.05$) had higher body weight gain than local and cross-breed ducks that received diet B. The final body weight of Pekin ducks fed diet A was significantly ($P<0.01$) higher than Pekin ducks fed diet B. While, local and cross-breed ducks fed diet A were significantly ($P<0.05$) higher in body weight than ducks local and cross-breeds on diet B. There was an interaction between breed and diet on weight gain and final body weight. Pekin duck was who received diet A produced the highest weight gain and final body weight. The pekins duck were broiler breeds, while local breeds and cross breeds are laying types.

Carcass characteristic

The carcass characteristics of Pekin ducks, local ducks, and cross-breed ducks were fed diets A and B and their interactions were presented in Table 3. Carcass percentage was significantly ($P<0.01$) influenced by breed type and diet

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type. The percentage of Pekin duck carcasses that received diet A was significantly different (P<0.05) from cross-breed ducks, but not significantly different from local ducks. Whereas, diet A produced a higher carcass percentage than diet B.

The percentage of meat was very significantly (P<0.01) influenced by the type of diet and significantly (P<0.05) was influenced by the type of breed. The percentage of thigh meat was significantly (P<0.01) influenced by the type of diet. The percentage of breast meat was very significant (P<0.01) influenced by diet type and significantly (P<0.05) influenced by breed type. Pekin ducks that received diet A produced a higher percentage of breast meat than Pekin ducks that received diet B.

The Pekin ducks were known as broiler ducks which have been genetically enhanced to obtain higher meat yields and lower levels of carcass fat deposition (Fouad et al., 2018). In our study, final body weight diet feeds a ranged from1.014 g to 1.500 g/bird. Similar results from the final body weight of duck by Kokoszynski et al. (2019a) and Starcvic et al. (2021). The variation in duck body weight can be influenced by several factors, including feed, genetic value, and the environment. On the other hand, the difference in final weight gain that occurs between types of diets is mostly due to the different nutrient content, especially protein and energy. This was supported by Wen et al. (2017) and Liu et al. (2019) stated that the level of metabolic energy and crude protein in the ration affected the growth performance of Pekin ducks. On the other hand, other researchers like Murawska (2012); Kokoszynski et al. (2015), and Kokoszynski et al. (2019a) revealed that final body weight of ducks was influenced by many factors such as duck strain, sex, age, chemical composition of feed, and feeding including a total of feed intake.

The consumption of feed A was 2.30 - 3.01 g/bird while the consumption of feed B was higher at 3.11 - 3.16 gr/bird. Ducks can regulate the total consumption of feed according to the amount of energy needed by the body. Ducks also will consume more feed when receiving a low-energy diet compared to a high-energy diet. While diet A contains higher energy (Table 1) than diet B, so the ratio consumption is lowest. The results of this study were almost the same as those of Wen et al. (2017) who reported that increasing ration energy can reduce feed intake and feed/gain. Liu et al. (2019) stated that feed consumption and feed conversion of Pekin ducks decreased linearly with increasing metabolizable energy (ME) and crude protein. The average consumption of the Pekin duck ration in this study was 3.14 g or 112.18 g/day, slightly lower than the results of the study by Kokoszynski et al. (2019a) the consumption of ducks aged 1-49 days was 123.2 g/day.

The results of our study, carcass percentages ranged from 50-57% Diet A, and 49-52% Diet B. meanwhile other authors have found higher carcass percentages in commercial Pekin ducks (Kwon et al., 2014; Baltic et al., 2017; Kokoszynski et al., 2019a; Kokoszynski et al., 2019b; Kokoszynski et al., 2020). The results of our study showed that the abdominal fat content of feed A was 1.41% on average while in feed B was 0.39%, the difference was due to variations in different nutritional content, like protein dang fiber. Meanwhile, Wang et al. (2017) reported that the percentage of duck belly fat will decrease as the protein content in the feed increases. This illustrates that the level of protein content in the feed has an impact on the duck's abdominal fat. In addition, the increase in crude fiber content in the feed also resulted in a decrease in the length of the small intestine, along with an increase in the relative weight of the proventriculus and gizzard (Freitas et al., 2014; Yokhana et al., 2016).

Table 2- Performances Pekin duck, Local duck, and Crossbred duck were given diets feed differences

Variable	Diet A			Diet B			SEM	P-value		
	Pekin	Local duck	Cross breed	Pekin	Local duck	Cross breed		D	B	D×B
Initial BW 14 days (g/bird)	363^{d}	210^{a}	299^{bcd}	338^{cd}	249^{ab}	277^{abc}	13.80	ns	**	ns
Feed Consumption/FC (g/bird)	3,077	2,399	2,918	3,164	3,118	3,161	78.90	**	*	ns
Weight Gain/WB (g/4 weeks)	1,136^{c}	803^{ba}	853^{b}	581^{a}	582^{a}	581^{a}	51.59	**	**	**
Feed conversation ratio (FCR)	2.75^{a}	3.02^{a}	3.41^{A}	5.44^{b}	5.36^{b}	5.45^{b}	0.29	**	ns	ns
Final body weight (g/bird)	1,500^{c}	1,014^{ab}	1,196^{b}	920^{a}	831^{a}	858^{a}	58.84	**	**	**

D = Diet factor; B = Duck breed factor; R×B = interaction between diet and duck breed factors. Within a row, means with a different superscript letter significantly differ a, b: P < 0.05; A, B: P<0.01); ns = no significance (P > 0.05); *: P < 0.05); **: P < 0.01.

Table 3- Carcass characteristics Pekin duck, Local duck, and Crossbred duck were given diets feed differences

Variable	Diet A			Diet B			SEM	P-value		
	Pekin	Local duck	Cross breed	Pekin	Local duck	Cross breed		D	B	D×B
Carcass (%)	57.41^{c}	54.96^{abc}	50.86^{ab}	52.76^{abc}	49.85^{a}	49.02^{a}	0.81	**	**	ns
Abdominal fat (%)	1.48	1.57	1.20	0.53	0.30	0.36	0.15	**	ns	ns
Meat:Slaughter weight (%)	30.88^{b}	28.66^{ab}	26.14^{ab}	25.83^{ab}	23.45^{a}	24.74^{a}	0.67	**	*	ns
Thigh meat in Carcass (%)	20.91^{a}	22.89^{ab}	22.14^{ab}	24.13^{ab}	24.30^{ab}	26.41^{b}	0.52	**	ns	ns
Breast meat in carcass (%)	19.17^{b}	14.70^{ab}	16.49^{ab}	12.04^{a}	10.55^{a}	12.63^{a}	0.79	**	*	ns

D = Diet factor; B = Duck breed factor; D×B = interaction between diet and duck breed factors. Within a row, means with a different superscript letter significantly differ (a, b: P < 0.05; A, B: P<0.01); ns = no significance (P > 0.05); *: P < 0.05); **: P < 0.01.

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CONCLUSION

Based on the description of the results and discussion, In conclusion it was showed the feed conversion rate and final body weight for Diet A were better than Diet B (high protein and high fiber) on the Pekin duck. Meanwhile, the carcass characteristics like abdominal fat of Diet B (for Pekin, local duck, and cross breed) were better than Diet A (for Pekin and local duck). It's suggested to use Pekin ducks with low protein and low fiber diet to get the better performance, in compared with local ducks which needs high protein and high fiber content in diet.

DECLARATIONS

Corresponding author

Aam Gunawan; E-mail: aamgunawan@yahoo.com; ORCID: https://orcid.org/0000-0001-5208-1437

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Authors' contributions

A. Gunawan designed of the research; A. Malik drafted the manuscript; Dharmawati, Kartika, Wulandari and Saprani collected of the data and done the statistical analysis.

Competing interest

All authors have no competing interest in the research.

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ANTIOXIDANT ACTIVITY OF RAW AND COOKED ONIONS IN RABBIT DOE NUTRITION

Ammar Abdulrazzaq TAWFEEQ[1*], Entisar Nadhim SHALLAL[1], Ali M ABDULWAHID[2] and Bilal J M ALDAHHAM[1]

[1]*College of Applied Sciences, University of Anbar, Heet, Iraq*
[2]*Iraqi Ministry of Health, Anbar Health Department, Heet General Hospital, Iraq*

Email: ammarabta@uoanbar.edu.iq

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ABSTRACT: The aim of this research is to examine how raw and cooked onions affect some antioxidant enzymes and some tissues in female rabbits. Twenty-four female albino rabbits weighing (1-1.5 Kg), (5-6 months age), non-pregnant, were used for the experiment, and they were divided into three groups for a duration of 28 days. A 20 g/kg raw onion and same amount of cooked onion were added to the second and third groups' diets respectively for comparison of results with control groups without any addition of onion. Results showed that diets supplemented with raw and cooked onion significantly increased the superoxide dismutase (SOD) activity. The cooked onion group showed normal and no pathological changes in liver, kidney, and heart tissues, while liver tissues of both control and raw onion groups suffered extreme congestion in the central veins of the liver lobules and in kidney tissues of only control rabbits, developed hemorrhagic foci was observed. In the control and raw onion groups, the heart tissue showed the development of hemorrhagic foci and necrosis in the heart muscle fibers. In conclusion, both raw and cooked onions boosted the activity of SOD enzyme, but the cooked onions showed to be more effective than raw at protecting liver, kidney, and heart tissues against cell necrosis caused by oxidative processes.

Keywords: Cooked onion, Oxidative stress, Rabbits, Superoxide dismutase, Tissue.

INTRODUCTION

Many biologically active compounds are found in onions, including organosulfur compounds, thiosulfinates, and polyphenols, which include flavonoids and fructooligosaccharides. Anthocyanins, quercetin, and quercetin derivatives are among the most biologically active flavonoids (Bystrická et al, 2013; Sagar et al, 2022). The biological activity of these compounds is important for preventing cell damage caused by free radicals and oxidative stress, which can lead to serious diseases such as cancer (Alpsoy et al, 2013). These compounds also have other important biological activities, such as antimicrobial, anti-diabetic, and other physiological activities (Kumar et al, 2022). Cooking onions causes fundamental changes in their structure, which increases the extractability of phenolic compounds due to the decomposition of cell walls and the release of phenolic compounds bound to fibers (Cattivelli et al, 2021).

The highest concentration of phenolic compounds was found in barbecue onions, followed by baked, boiled, and fried onions, and it was discovered (Cattivelli et al, 2022) that all heat treatments of onions increased the total phenolic compounds when compared with raw onions. Barbecue was also found to be the heat treatment that was able to preserve anthocyanins after digestion in the laboratory. Another study found that the concentration of Quercetin increased when onions were baked or fried, but decreased when they were cooked by boiling (Lombard et al 2005). According to Sans et al (2019), roast onion increased the concentration of most nutrients except for fiber and the total content of phenols, flavonoids, calcium, sodium, and iron, which decreased after grilling, but increased the antioxidant activity of onions. They compared roasted onions to raw onions after *in vitro* gastric digestion and attributed the increase in antioxidant activity to the release of antioxidant compounds from insoluble parts or the formation of new compounds with antioxidant properties during the cooking process.

Numerous earlier studies focused on the beneficial effects of raw onions and the active ingredients they contain in the field of veterinary medicine, particularly the antioxidant properties. According to Sabir et al (2019), adding onion juice or powder to the diet of male rabbits caused a substantial increase in the levels of superoxide dismutase and total antioxidant capacity as well as a significant decrease in malondialdehyde in serum when compared to control rabbits. Additionally, treatment of onion aqueous extract to diabetic rabbits enhanced catalase, superoxide dismutase, and glutathione peroxidase levels while decreasing glutathione and malondialdehyde levels in the liver (Ogunmodede et al, 2012). Previous studies lack a comparison between the effect of raw and cooked onions on the tissues of the liver, kidneys, stomach and heart, and because the protection of the mentioned tissues is linked to the mechanisms of anti-

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oxidation, so it is necessary to follow up the essential anti-oxidant enzymes while adding both types of onions to the ration.

The aim of this study is to evaluate the efficacy of cooking onions on the level of antioxidant enzymes in the blood serum, as well as the protective role it plays by protecting cells in the rabbit liver, kidneys, heart, and stomach from oxidative stress-induced damage.

MATERIALS AND METHODS

Twenty-four female rabbits (Albino), with a weight (1-1.5 Kg), (5-6 months age), non-pregnant, have been chosen to reveal the effect of cooked onions on their tissues. The rabbits were divided into three groups, with eight rabbits in each group. The first group was used as a control group. The diet of the second group was supplemented with 20g/ Kg of raw onions, while the third group's diet was supplemented with 20g/ Kg of cooked onions.

The onions were obtained from a local market. They were washed and the external shells were removed. The internal shell was cut into small pieces and was divided into two parts. The first one was added directly to the diet while the second one was boiled for 15 minutes before being added to the diet of the third group.

In order to measure the characteristics of whole blood using the Auto Analyzer of Blood provided by (IRMA company, Japan), 5 ml of blood from each rabbit's heart was taken directly after the experiment. This blood was then divided into two tubes, the first of which contained an anticoagulant (Ethylenediaminetetraacetic acid - EDTA). For the aim of evaluating the levels of lipid index components and the activity of antioxidant enzymes, including Total Superoxide Dismutase, Glutathione Peroxidase and Xanthine Oxidase (Paglia andand Valentine, 1967; Stripe et al, 1969), the second tube is free of any anticoagulant substance and is used to centrifuge the serum from the cellular portion of the blood (Paoletti andand Mocali, 1990).

Statistical analysis

Data were analyzed by one-way analysis of variance, using the general linear model (GLM) procedures of SAS. The significant differences among the means were tested by Duncan's multiple range test at probability value ($p \leq 0.05$; Duncan, 1955).

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.

RESULTS

According to the findings in Table 1, there were no significant differences among all tested blood parameters for the groups under study. The same appears to be the case for the results presented in Table 2, which show that there are no notable variations in the characteristics of the fat index among the groups under study.

The activity of the superoxide dismutase enzyme in the blood serum is much higher in the rabbits from the two groups of raw and cooked onions than it is in the rabbits from the control group, as shown in Figure 1. The levels of the enzymes xanthine oxidase and glutathione peroxidase in the blood serum did not significantly differ across research groups. In the control group of rabbits, histological sections of the liver demonstrated severe congestion in the sinusoids with severe hydronephrosis and enlargement of hepatocytes in the hepatic lobule area, as well as the central veins of the liver lobules and the portal area. Additionally, it was discovered that the liver tissue of the rabbits in the raw onion group exhibited swollen hepatocytes, watery overall degeneration, and congestion in the central and portal veins of the liver lobules. On the other hand, the cooked onion group's liver tissue showed some slight congestion in the central veins of the liver lobules with regularity of the shape of hepatic lobule and the hepatocyte ropes. At the same time, the shape of most of the cells appeared normal with the appearance of simple water degeneration in a few of them as in Figure 2, the cooked onion group's liver tissue in rabbits showed no such congestion.

The histological sections of the kidneys in the control rabbits showed the formation of multiple hemorrhagic foci in the inter-renal cortex tissue. Along with a general clouded degeneration of the renal tubules, it also appeared as though the renal tubules were obviously enlarged. On the other hand, in the cortex and pulp of the kidneys of the raw onion group rabbits, the renal tubules and glomeruli had a normal look, with a small congestion of the arteries between the renal tubules. While the kidney cortex and pulp histological sections in the cooked onion group of rabbits did not exhibit any pathological alterations and appeared normal (Figure 3).

Figure 4 illustrates the histological section of heart in different groups. Pictures of cooked onion group appeared normal and did not show any pathological changes compared to other study groups as suffering from coagulative necrosis and clots within cardiac cavities in control groups and group of crude onion suffered from large, severe hemorrhage with edema, atrophy, and foci of necrotic and degenerative myocardium. The rabbits in the three groups' stomach mucosa histological sections displayed a normal appearance and bore no signs of pathological alterations (Figure 5).

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Table 1 - Effect of raw and cooked onion on the hematological blood tests in rabbits

Item	Control	Raw onion	Cooked onion	*P*-value
WBC (thousand cell/ml^3)	4.62 ± 0.852	4.80 ± 1.11	15.7 ± 9.20	N.S.
Lymphocytes%	57.3 ± 5.42	57.4 ± 8.51	62.6 ± 9.01	N.S.
Monocytes%	9.41 ± 1.23	10.8 ± 1.10	6.38 ± 1.28	N.S.
Granulocytes%	33.2 ± 4.85	31.7 ± 7.61	31.0 ± 8.32	N.S.
Lymphocytes	2.48 ± 0.456	2.60 ± 0.633	12.0 ± 9.93	N.S.
Monocytes	0.387 ± 0.093	0.500 ± 0.147	0.380 ± 0.198	N.S.
Granulocytes	1.75 ± 0.386	1.70 ± 0.484	2.06 ± 1.06	N.S.
RBC (million cell/ml^3)	6.68 ± 0.713	5.75 ± 0.447	5.76 ± 1.31	N.S.
HGB (gm/100 ml)	10.6 ± 1.28	9.82 ± 0.539	10.8 ± 1.25	N.S.
HCT %	43.5 ± 4.76	36.9 ± 2.64	44.5 ± 6.10	N.S.
MCV	65.1 ± 0.742	64.2 ± 1.03	66.8 ± 1.47	N.S.
MCH	15.4 ± 0.729	17.1 ± 0.545	16.5 ± 0.797	N.S.
MCHC	23.7 ± 1.20	26.6 ± 0.512	24.8 ± 1.35	N.S.
RDW	13.3 ± 0.286	13.5 ± 0.476	13.5 ± 0.294	N.S.
PLT (thousand cell/ml^3)	401 ± 106	401 ± 118	285 ± 99.8	N.S.
MPV	6.90 ± 0.403	6.62 ± 0.460	6.48 ± 0.284	N.S.
PCT %	0.263 ± 0.067	0.250 ± 0.070	0.184 ± 0.063	N.S.
PDW	7.67 ± 0.994	8.07 ± 0.828	8.01 ± 0.695	N.S.

Mean ± Standard Error. N.S.: Non-Significant at probability value (P≤0.05).

Table 2 - Effect of raw and cooked onion on the lipid profile in serum of rabbits

Item	Control	Raw onion	Cooked onion	*P*-value
Cholesterol (mg/dl)	51.7 ± 6.32	54.7 ± 8.33	43.5 ± 15.3	N.S.
Triglyceride (mg/dl)	147 ± 33.9	139 ± 49.7	81.0 ± 15.2	N.S.
High Density Lipoprotein (mg/dl)	9.12 ± 1.18	6.87 ± 1.73	6.03 ± 1.62	N.S.
Low Density Lipoprotein (mg/dl)	17.6 ± 6.03	20.0 ± 3.87	25.8 ± 15.8	N.S.
Very Low Density Lipoprotein (mg/dl)	29.5 ± 6.74	27.7 ± 10.1	16.1 ± 3.08	N.S.

Mean ± Standard Error. N.S.: Non-Significant at probability value (P≤0.05).

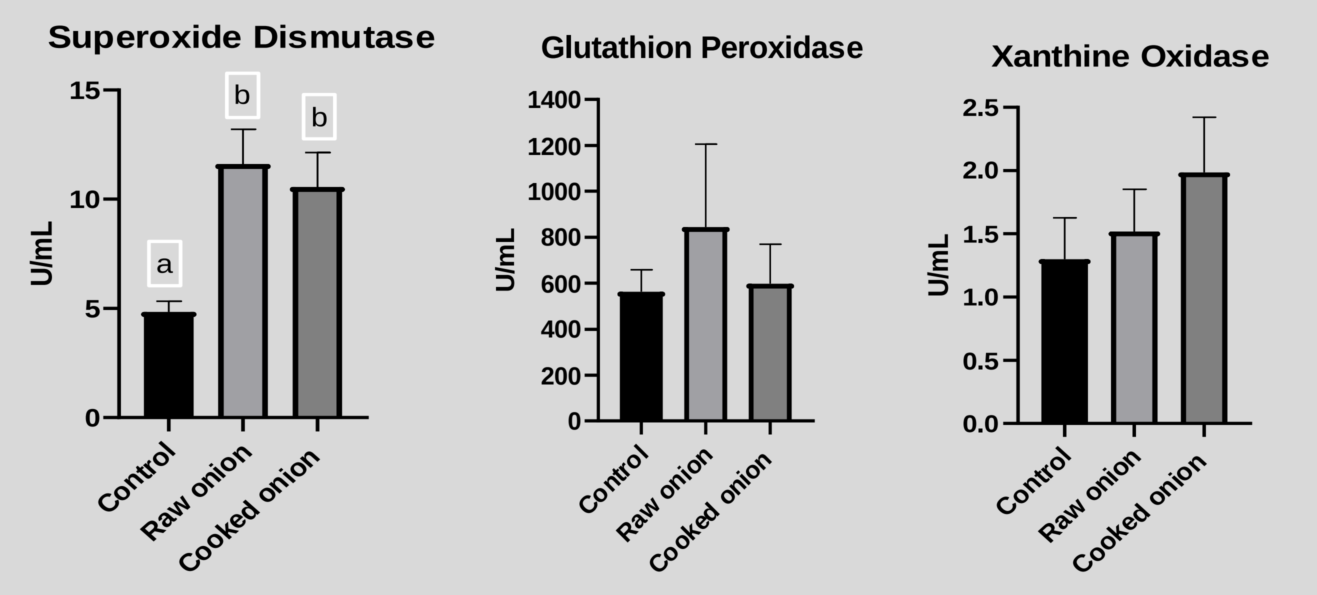

Figure 1 - Effect of raw and cooked onion on the antioxidant enzymes activity in serum of rabbits (mean ± standard error), P<0.05

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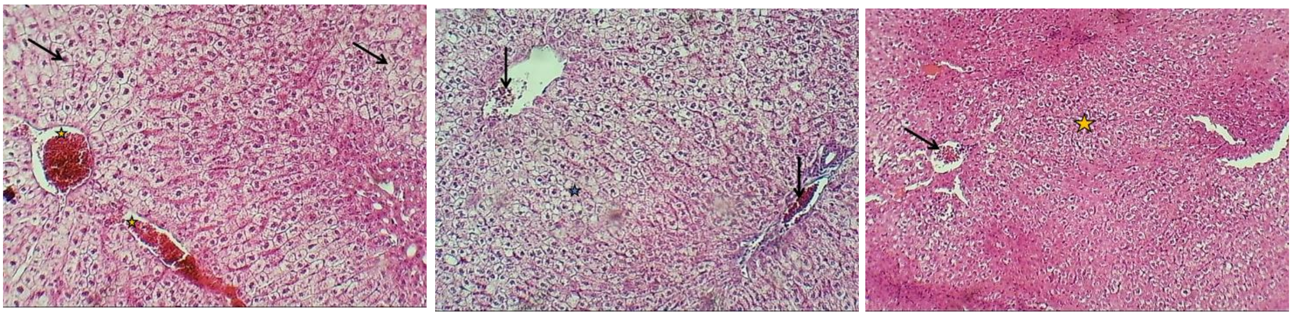
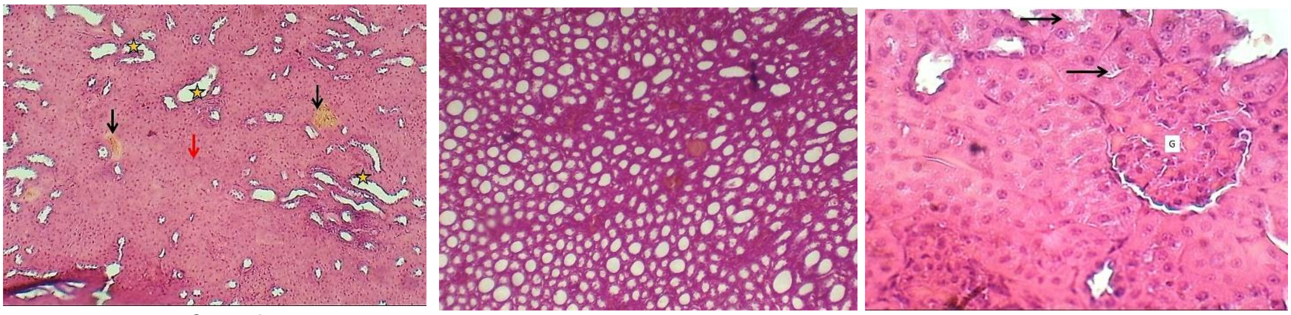


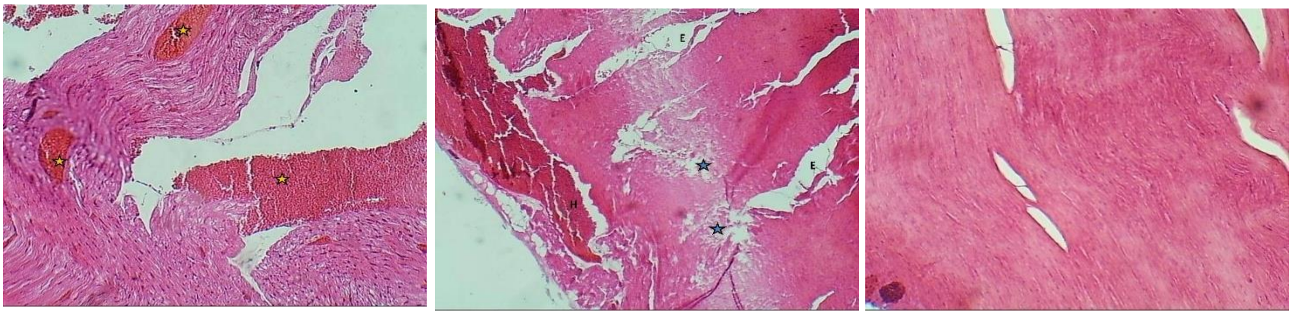
Control | Raw onion | Cooked onion

Figure 2 - Photomicrograph of liver from rabbits stained with HandE (x100) (arrows and star)



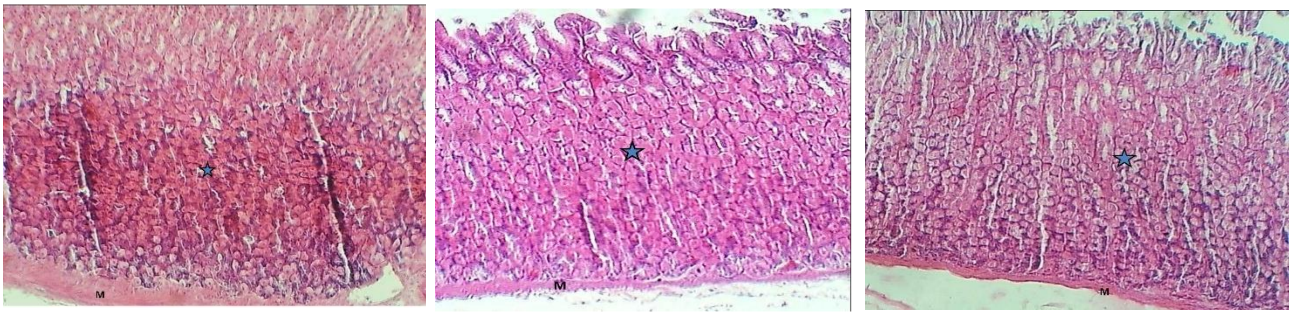
Control | Raw onion | Cooked onion

Figure 3 - Photomicrograph of kidney from rabbits stained with HandE (x100) (arrows)



Control | Raw onion | Cooked onion

Figure 4 - Photomicrograph of heart from rabbits stained with HandE (x100) (stars)



Control | Raw onion | Cooked onion

Figure 5 - Photomicrograph of gastric mucosa from rabbits stained with HandE (x100) (stars)

DISCUSSION

In this study, the addition of raw or cooked onions had a limited impact on whole blood characteristics, as decrease in the rate of corpuscular hemoglobin (MCH) and erythrocytes (RBC). This comes in agreement with (Ajao and Ola, 2022) findings. Additionally, these findings supported those of Zeweil et al (2016), who discovered no impact of adding raw onions to the diet of rabbits on whole blood characteristics. While they found that adding raw onions to rabbit ration at (400 and 800 mg/kg) significantly reduced the level of total fats, triglycerides, total cholesterol and low density

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lipoproteins. These findings conflicted with those of the current investigation, in which we discovered that adding onions, both cooked and raw, had no influence on the lipid index.

The liver, kidneys, and heart tissues were affected, particularly in the rabbits in the control group. This damage may be the result of the feed's lipids being held at high temperatures, which can lead to oxidation and the formation of free radicals (Lin et al., 1998). Additionally, unsanitary feed storage conditions promote the growth of mold, which results in mycotoxins being produced in the fodder (Alam et al., 2014). Free radicals in the body start protein and lipid breakdown, which changes cell permeability and affects the movement of enzymes between cells and serum (Nakamura et al, 1985).

Flavonoids are the main phenols in onions, which can be classified into their sub-families (flavones, flavanones, flavonols, isoflavones, flavanonols, flavanols, chalcones, and anthocyanins), and the most abundant in onions are flavonols (Liguori et al., 2017). Razavi and Kenari (2016) believe that there is an association between the antioxidant activity and free radical elimination of onions' phenolic content. By increasing the concentration of total phenols, the ability of the total phenols extracted from onions to remove free radicals (DPPH, FRAP, and OH) *in vitro* was further enhanced.

Protection of tissues depends on cellular protein synthesis and then entire proper cell performance, which necessarily depends on contribution of antioxidant system. Therefore, several factors may have contributed to maintaining the normal tissue of the kidney and liver without any focal lesions in the rabbits exposed to the two groups of onions, especially the cooked onion group and to a lesser extent in the raw onion group (Ahmed et al., 2017). These factors mainly arose from; role of complete phenols by inhibiting Xanthine oxidase activity (Ouyang et al., 2018), free radical chain reaction stopping by the phenolic OH groups that behave as hydrogen or electron donors (Ahmed et al, 2017), and phenols effective function in reducing malondialdehyde oxidation role.

This study is unique in that it focuses on releasing phenolic compounds, which lose a lot of their active components when plants are cooked, particularly onions, where many studies have shown that cooking has a significant impact on adjusting the content of phenolic compounds in onions (Cattivelli et al, 2021). Some substances, including anthocyanins, may disintegrate at high temperatures or be released into the medium as flavonols after boiling (Palermo et al, 2014). According to results reported by Pellegrini et al. (2009), the release of antioxidant compounds from onion briquettes after they were softened by high heat, the creation of new antioxidant compounds, or polyphenol oxidation to intermediate oxidation states showing higher efficiency in free radical removal were all possible explanations for the increase in total antioxidant capacity of cooked onions. Price et al. (1997) stated that onions that have been fried for five minutes have more quercetin than those that have not. Another study of Lombard et al. (2005) found that baking or frying onions boosts their flavonol concentration and that the cooking duration should not be less than five minutes validated this.

According to the results of the current study, both raw and cooked onions boosted the activity of some antioxidant enzymes, and cooked onions were more effective than raw onions at protecting liver, kidney, and heart tissues against cell necrosis caused by oxidative processes.

Onions are abundant in phenolic compounds, which act as antioxidants. It can be concluded that onions require heat treatment through cooking in order to achieve the greatest benefits from phenolic chemicals. Boiling onions have a protective effect on the liver, kidneys, and cardiac tissues because they protect them from free radicals and other oxidative agents. Increasing the activity of superoxide dismutase is one of those mechanisms.

DECLARATIONS

Corresponding author
Ammar Abdulrazzaq Tawfeeq; E-mail: ammarabta@uoanbar.edu.iq

Authors' contribution
A.A. Tawfeeq was carrying out the experiment of rabbits rearing, and all the other practical parts of the study as well as discussion of results. E.N. Shallal did histological analysis and interpretation. A.M. Abdulwahid examined blood parameters. B.J.M. Aldahham was the owner of the research idea.

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Competing interests
The authors declared that there is no any competing interest.

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THE QUALITY OF FERMENTED RICE STRAW WITH *Trichoderma viride* INOCULUM

Badat MUWAKHID[1], Umi KALSUM[1], Hilarius Yosef SIKONE[2], and RIFA'I[3]

[1]Faculty of Animal Science, University of Islam Malang, Jl. Mayjen Haryono 193 Malang, East Java, Indonesia
[2]Study Program Animal Husbandry, Faculty of Agriculture and Animal Husbandry, University of Katolik Indonesia Santu Paulus Ruteng, East Nusa Tenggara, Indonesia
[3]Faculty of Animal Science, University of Kahuripan Kediri, Kediri, East Java, Indonesia

Email: badatmuwakhid@unisma.ac.id

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ABSTRACT: Rice straw has several nutritional weaknesses, namely its high silica and lignin content, and its low level of protein, minerals and vitamins, so the impact on digestibility is also low. Aim of present study was to evaluating nutritional efficacy of rice straw after fermentation with *Trichoderma viride*. The study was conducted by using complete random design. There were three different treatments with four replicates for each treatment. Fermented rice straws were treated with varying concentrations of *Trichoderma viride* inoculum as follows; 0.5% (T1), 1% (T2), and 1.5% (T3). Fermented rice straw's nutrients, including dry ingredients, organic material, crude fiber, crude protein, dry matter digestibility coefficients, and organic matter digestibility coefficients were measured in this study. T3 (1.5% of *T. viride*) treatment performed a proper nutrient, with 80.02% dry ingredients, 80.03% organic materials, 31.68% crude fiber, 5.72% protein, 38.46% dry matter digestibility coefficient, and 61.05% organic matter digestibility coefficient. In conclusion, using 1.5% *Trichoderma viride* to improve the quality of rice straw, as stimulator of fermentation process can be efficient in ruminant or non-ruminant nutrition.

Keywords: Agricultural by-product; Crude fiber; Digestibility; Ruminants; *Trichoderma viride*.

INTRODUCTION

Mainly, forage is major feedstuff for ruminants (Minson, 2012). However, the limited stock of forage is the major obstacle for farms. So, to increase the nutritional efficacy of ruminants, high quality and quantity of forage are required (Guyader et al., 2016; Mahanta et al., 2020). The high sustainability of forage is directly proportional to the high productivity of ruminants (Guyader et al., 2016). Supplying ruminants with high energy food sources; such as grass, leguminosae, and agricultural by-products will increase the quality of the animal production (Roy et al., 2019; Yusriani et al., 2021).

An agricultural by-product that is commonly processed for animal feed is rice straw (Aquino et al., 2020). It is supposed to be an energy source for ruminants (Aquino et al., 2020). Even so, rice straw contains high concentrations of silica and lignin and also fewer vitamins and minerals (Malik et al., 2015; Aquino et al., 2020). It has a low digestibility level, which is not applicable enough to be a food stock (Sarnklong et al., 2010). The low digestibility level is caused by the old supporting tissue that undergoes lignification (Huang and Lo, 2019). The lignocellulose and hemicellulose of rice straws are the main causes of low digestibility levels (Tama et al., 2020).

Improving crude protein (CP) is an essential nutrient for improving rice straw quality (Sufyan et al., 2022); Yanuartono et al. (2017) reported that rice straw contained 3-5% crude protein, lower than leguminosae and grass. Setiarto (2013) explained that the fermentation and ammoniation methods can elevate rice straw nutrition. Fermentation is one of the food/feed processing technologies involving microorganisms to degrade coarse fiber and reduce lignin concentration to increase the quality of food/feed (Xia et al., 2018; Shen et al., 2018). One of fermenter agent was *Trichoderma viride* it has exhibited with high protein, and fat, also with potential of crude fiber reduction (Kasmira et al., 2023). Stand out among the cellulose-producing bacteria. The most studied species of filamentous fungi are *Trichoderma viride* (Gautam et al., 2011). However the optimum concentration of *Trichoderma viride* for improving rice straw nutritional quality is remaining unclear. Therefore, we investigated the optimum concentration of *Trichoderma viride* to improve rice straw nutrients.

MATERIALS AND METHODS

This study was conducted in July-September 2022 at Teaching Farm and Feed Chemical Analysis Laboratory of Islamic University Malang.

Production of fermented rice straws

The experimental design of this study was complete randomized design (CRD) with three treatments and four repetitions. Three various concentrations of *Trichoderma viride*, included T1: rice straw + 0.5% *T. viride* inoculum; T2: rice

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straw + 1% *T. viride* inoculum; and T3: rice straw + 1.5% *T. viride* inoculum, were used for rice straw fermentation. The fermented rice straws were incubated for 21 days. Proximate analysis (dry ingredients, organic materials, crude fiber, and crude protein) was measured on the fermented rice straw to analyzed the fermented rice straw quality (AOAC International, 2015). The dry matter digestibility and organic digestibility coefficients were analyzed *in vitro,* referred to Tilley and Terry (2006).

Data analysis

The nutrients of fermented rice straws were statistically analyzed by one-way ANOVA, followed by Duncan test with significant P<0.05. Statistical analysis was conducted by SPSS software version 20.

RESULTS AND DISCUSSION

The nutrition composition of fermented rice straws

Various *Trichoderma viride* concentrations in 21 days rice straw fermentation impacted the quality of rice straw (Table 1). T1 (0.5% of *T. viride)* increased the organic materials and crude fiber significantly (P<0.05*)*. Besides that, T1 did not show significantly dry ingredients higher than T2 and T3. Muck et al. (2018) reported that the dry ingredients of rice straw fermented by local microorganisms ranged from 34.04% to 38.18%, lower than this study. Various effects of dry ingredients in fermented sources were caused by microorganism population. A higher population of microbes was effectively breakdown the substrate for energy production. Increased hydrolysis reaction of microorganisms decreased the dry ingredients of food stock. Furthermore, fermentation elevated the digestibility of raw fermented food (Jasin, 2014). According to Chen et al. (2019) statement, the decrease in silage dry matter is influenced by respiration and fermentation. Respiration will cause a lot of nutrient content to decompose so that it will reduce dry matter, while fermentation will produce lactic acid and water. Furthermore Surono et al. (2006) stated that the increase in the level of additive (*Trichoderma viride* inoculum) is thought to stimulate fermentation activity, causing H_2O production to also increase. The increase in water content in ensiling causes the dry matter content of the silage to decrease, causing an increase in dry matter loss.

The organic materials of fermented rice straws in Table 1 ranged from 80.03% to 82.50%. Similar to dry ingredients, T3 performed lowest organic materials, significantly (P<0.05*)*. Organic materials of fermented food were affected by lactic acid bacteria. Growth microorganisms metabolized carbohydrates and protein to organic materials (Zahra et al., 2020). It could also affect the nutrition value of fermented feed nutrition, especially the reduction of organic materials. Muck et al. (2018) described that chemical change of carbohydrate occurred during fermentation processes to generated energy. Surono et al. (2006) that in general it is known that lactic acid in ensilages is produced from organic components, especially carbides, thereby increasing the formation of lactic acid. Loss of organic matter in silage which mainly comes from carbohydrates, namely BETN with the main components of starch and sugar is used by bacteria to produce lactic acid. Carbohydrates were converted into alcohol, organic acid, water, and carbon dioxide. Daning and Karunia (2018), found that Trichoderma produced cellulase enzymes to hydrolyze cellulose and crystal, which caused the percentage of coarse fiber in the substrate.

The crude fiber showed the significantly high on the T1, followed by T2 and T3. The 1.5% dose of *T. viride* inoculum (T3) might have ideal performation to decompose rice straw and produced crude fiber. Fermented rice straw using *Trichoderma viride* for 21 days produced crude protein, ranged from 5.55% to 5.72%. Suningsih et al. (2019) identified the activity of proteolitic activity in the fermented raw food, which fermentation increased proteolytic activity and exhibited different value of crude protein. The amount of water-soluble N and water-soluble solid will be increased during fermentation. The increase of water-soluble N is caused by protease enzymes that decompose protein into water-soluble fragments. Then, microbes used the water-soluble fragments for their growth and contributed to the single cell protein, increasing microbes mass and substrate's coarse protein, respectively (Suningsih et al., 2019). The presence of *T. viride* inoculum is suspected as an energy source for lactic acid bacteria so that it can work optimally in fermentation where bacterial acid is a microbe that plays a role in the crude protein content of silage to ferment sugar into lactic acid where the bacteria are protein contributors of microbial origin. Furthermore, Santoso et al. (2013) stated that lactic acid bacteria have an important role in forage fermentation and affect the quality of the resulting silage. According to Afrianti (2022) results, fermentation improved the nutritional value and degraded raw food sources, indicating easier to digest.

Digestibility of fermented rice straws

Digestibility is the percentage of nutrients that were absorbed in the digestive tract. It was obtained from the difference of intake nutrients with the nutrients in feces. The dry matter digestibility coefficient of fermented rice straws ranged from 35.93% to 38.46% (Table 2).

The variance analysis showed that fermentation with *Trichoderma viride* to rice straws was significantly different (P<0.05). The Duncan test showed that T1 was not different with T2, but showed significantly different results with T3. The 1.5% of *T. viride* inoculum increased the activity of decomposing microbes and produced higher energy. With so many decomposing microorganisms, it functions to digest crude fiber, namely as a digester of cellulose as well as hemicellulose and starch.

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Energy source stimulates microbes to degrade crude protein (Owens and Basalan, 2016). Jasin (2014) explained that Volatile fatty acids (VFAs) are important for microorganisms' growth that digest the crude fiber in the rumen and be the source of carbon chain for protein synthesis. Ouyang et al. (2019) reported that high digestibility in ruminants showed high digested-nutrition by rumen's microbes. The increase of rumen's metabolites will affect the increase of growth followed by livestock weight gain as reported by Cantalapiedra-Hijar et al. (2018) and Mwangi et al. (2022).

Digestibility of organic matter in ruminants' intestines includes carbohydrates, protein, fat, and vitamin. This indicator also relates to the dry matter digestibility coefficient because most of the dry matter in feed consisted of organic and inorganic material. According to the study, fermented rice straws' organic matter digestibility coefficient ranged from 56.88% to 61.05%. However, in the variance analysis, the fermentation with *T. viride* inoculum showed insignificant difference ($P<0.05$). Similarly, dry matter digestibility coefficient, the high level of *T. viride* inoculum resulted in a high organic matter digestibility coefficient. From this result, it could be assumed that the high microorganism level was directly proportional to the digestibility coefficient. The high percentage of organic matter digestibility coefficients was caused by cellulose's complete digest (Nahak et al., 2019; Xia et al., 2017). Daning and Karunia (2018) explained that *Trichoderma* could convert the organic materials in the feed, causing high organic matter digestibility coefficient.

Table 1 - Nutrition contents of fermented rice straws

Nutrition (%) / Treatment	T1	T2	T3
Dry ingredients	80.98 ± 1.16^{a}	80.41 ± 2.29^{a}	80.02 ± 2.21^{a}
Organic materials	82.50 ± 0.34^{a}	81.19 ± 0.19^{b}	80.03 ± 0.06^{c}
Crude fiber	32.88 ± 0.45^{a}	32.35 ± 0.04^{ab}	31.68 ± 0.35^{b}
Crude protein	5.55 ± 0.35^{a}	5.70 ± 0.36^{a}	5.72 ± 0.49^{a}

T1=rice straw + 0.5% *Trichoderma viride* inoculum; T2=rice straw + 1,% *T. viride* inoculum; T3= rice straw + 1.5% *T. viride* inoculum; Mean ± standard error; Different superscripts in the same row represented significantly different ($P<0.05$).

Table 2 - Percentage of digestibility of fermented rice straws

Digestibility coefficient (%) / Treatment	T1	T2	T3
Dry matter digestibility coefficient	35.93 ± 0.78^{a}	36.69 ± 1.00^{ab}	38.46 ± 1.37^{b}
Organic matter digestibility coefficient	56.88 ± 1.79^{a}	59.08 ± 1.82^{a}	61.05 ± 2.56^{a}

T1=rice straw + 0.5% *Trichoderma viride* inoculum; T2=rice straw + 1,% *T. viride* inoculum; T3= rice straw + 1.5% *T. viride* inoculum; Mean ± standard error; Different superscripts in the same row showed significantly different effects ($P<0.05$).

CONCLUSION

Fermentation of rice straws using 1.5% *Trichoderma viride* inoculum showed the better nutritional contents , with the score of dry ingredients of 80.02%, organic materials 80.03%, coarse fiber 31.68%, crude protein 5.72%, dry matter digestibility coefficient 38.46%, and organic matter digestibility coefficient 61.05%. *Trichoderma viride* inoculum can be used in fermentation of fibrous feedstuffs in alternative ways.

DECLARATION

Author's contribution

B. MUWAKHID and U. KALSUM designed the study, and manuscript writing; RIFA'I collected samples and data, H.Y. SIKONE performed data analysis and manuscript writing. All authors drafted, revised and approved the final manuscript.

Conflict of interests

The author has no possible conflicts of interest in this paper's research, authorship, or publishing.

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EFFECTS OF SUBSTITUTION OF FERMENTED CHICKEN LITTER WITH CONCENTRATE ON NUTRIENT DIGESTIBILITY AND PERFORMANCE OF SHEEP

Marry CHRISTIYANTO, Eko PANGESTU, Limbang Kustiawan NUSWANTARA, SURONO, and Cahya Setya UTAMA

Department of Animal Sciences Faculty of Animal and Agricultural Sciences, University Diponegoro, Jl. Prof. H. Soedarto, S.H, Semarang City, Central Java 50275, Indonesia

Email: marrychristiyanto@gmail.com

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ABSTRACT: The study aimed to investigate the effects of supplementing fermented chicken litter on feed consumption, nutrient digestibility (dry matter/DM, organic matter/OM, crude fiber/CF, extract ether/EE, crude protein/CP), total digestible nutrients (TDN), and average daily gain (ADG) in sheep. A completely randomized design with 4 treatments and 3 replications, namely T0 = concentrate without the addition of fermented litter, T1 = 90% concentrate + 10% fermented litter, T2 = 80% concentrate + 20% fermented litter, T3 = 70% concentrate + 30% fermented litter and T4 = 60% concentrate + 40% fermented litter was used. The parameters studied were dry matter digestibility (DMD), organic matter digestibility (OMD), extract ether digestibility (EED), crude fiber digestibility (CFD), crude protein digestibility (CPD), TDN, feed consumption and average daily gain. The results revealed that sheep fed different levels of fermented litter did not affect OMD, DMD, EED, CPD, CFD, TDN, dry matter consumption, and average daily gain (ADG). It was concluded that fermented chicken litter can be incorporated in sheep diet, without considerable negative effects.

Keywords: Digestibility, Feed, Fermentation, Litter, Sheep.

INTRODUCTION

Poultry farming in Indonesia is the largest livestock production sector with the fastest population growth. Statistics Indonesia (BPS) recorded that the broiler population in 2018 - 2020 in Indonesia reached 3 trillion heads (Statistics Indonesia, 2020). Poultry litter is a material used as a base for cages and has several functions such as absorbing excreta, ammonia, and heat insulation (Munir et al., 2019; Pepper and Dunlop, 2021). The development of broiler cages that are getting wider has increased the amount of litter/manure waste that has the potential to pollute the environment and disrupt human health (Wang et al., 2019). Statistics Indonesia (2020) noted that the increase in broiler chicken production in Indonesia caused waste in the form of litter and manure by 15.72%, so handling and processing efforts were needed.

Litter has a crude protein content of 25 – 50% and TDN of 55 – 60% (Rahimi et al., 2018). Litter contains nitrogen proteins such as uric acid, purines, and allantoin which serve as the basic ingredients for the synthesis of rumen microbes (Van Ryssen, 2001), with acid detergent fiber (ADF) content (26.17 ± 0.40%), neutral detergent fiber (NDF) (40.11 ± 0.54%), lignin (6.91 ± 0.37%), CuO (1.15%), MgO (42.53%) and Al_2O_3 (10.19%) which can be degraded by microorganisms during the fermentation process (Utama and Christiyanto, 2021).The litter must go through a processing process so that it can be used optimally and not harmful to livestock (Utama and Christiyanto, 2021).

Fermentation is a process of microorganism activity in obtaining the energy needed for metabolic processes through the breakdown of organic compounds both aerobically and anaerobically and resulting in changes in the substrate (Owens and Basalan, 2016). The activity of these microorganisms is expected to reduce crude fiber levels and improve the quality of feed ingredients (Supriyati et al., 2014).

This study aimed to examine the feeding of fermented litter as a substitute for sheep concentrates on dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), crude fiber digestibility (CFD), total digestible nutrients (TDN), dry matter consumption and average daily gain.

MATERIALS AND METHODS

The material used in the study was 15 female local sheep with a weight of ±11 kg. The research design used was a completely randomized design (CRD) with 4 treatments and 3 replications, namely TO = concentrate without the addition

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of fermented litter, T1 = 90% concentrate + 10% fermented litter, T2 = 80% concentrate + 20% fermented litter, T3 = 70% concentrate + 30% fermented litter and T4 = 60% concentrate + 40% fermented litter.

Litter was fermented using starter *exceed* for 6 weeks then the fermented product was ground until smooth. Sheep were adapted to treatment for 14 days and then followed by data collection for 10 days. Maintenance was carried out for 4 weeks with feeding 2 times in one day. The feed is provided in the form of forage and concentrate. Stool collection was carried out 1 × 24 hours for 10 days, then continued with proximate analysis and calculated digestibility. The concentrate consists of rice bran, corn, Corn Gluten Feed (CGF), palm cake, soybean groats, molasses, minerals, salt, and fermented litter. The composition of the treatment ration can be seen in Table 1.

Table 1 - Composition feed of the treatment.

Feed ingredients / Treatments	T0	T1	T2	T3	T4
Bran	40	28	19	17	18
Corn	8	14	14	14	10
Palm kernel meal	20	17	17	17	11
Soybean groats	8	7	6	6	7
Corn gluten meal	20	20	20	12	10
Salt	1	1	1	1	1
Molasses	2	2	2	2	2
Mineral	1	1	1	1	
Litter fermentation	0	10	20	30	40

T0 = concentrate without the addition of fermented litter, T1 = 90% concentrate + 10% fermented litter, T2 = 80% concentrate + 20% fermented litter, T3 = 70% concentrate + 30% fermented litter and T4 = 60% concentrate + 40% fermented litter

Parameter estimate

The parameters observed in this study were dry matter consumption, dry matter digestibility (DMD), organic matter digestibility (OMD), extract ether digestibility (EED), crude fiber digestibility (CFD), crude protein digestibility (CPD), Total Digestible Nutrients (TDN), and average daily gain (ADG). The measurement of DMD, OMD, EED, CFD, CPD, and TDN is calculated using the formula of Alsersy et al. (2014):

$$\text{DMD} = \frac{\text{DM Consumption - DM Excreted}}{\text{DM Consumption}} \times 100\%$$

$$\text{OMD} = \frac{\text{OM Consumption - OM Excreted}}{\text{OM Consumption}} \times 100\%$$

$$\text{CFD} = \frac{\text{CF Consumption - CF Excreted}}{\text{CF Consumption}} \times 100\%$$

$$\text{EED} = \frac{\text{EE Consumption - EE Excreted}}{\text{EE Consumption}} \times 100\%$$

$$\text{CPD} = \frac{\text{CP Consumption - CP Excreted}}{\text{CP Consumption}} \times 100\%$$

TDN = % digestible crude fiber + % digestible NFE + % digestible crude protein + 2,25 % digestible extract ether

Measurement of average daily gain and feed consumption was calculated using the formula of Abebe and Tamir (2016):

$$\text{Average daily gain (ADG)} = \frac{\text{Final Weight (kg)-Initial Weight (kg)}}{\text{"Maintenance Length (days)"}}$$

$$\text{Dry matter consumption (Kg DM/head/day)} = \frac{\text{"Total Feed given (kg DM) - Total Remaining Feed (kg DM)"}}{\text{"Maintenance Length (days)"}}$$

Data analysis

Research data were analyzed using analysis of variance (ANOVA). When the results of the analysis showed a real effect, it was continued with Duncan's difference test at the 5% level.

Animal ethical regulation

The treatment of experimental animals was carried out in accordance with the "Guidelines for the Care and Utilization of Laboratory Animals" from Diponegoro University. All procedures carried out in this study involving animals have been following ethical standards and approved by the Feed Technology Laboratory of the Faculty of Animal Husbandry and Agriculture, University Diponegoro.

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RESULTS AND DISCUSSIONS

Nutrient digestibility data, DM consumption, daily body weight gain sheep

Based on the research results in Table 2 showed that there was no significant effect (P>0.05) of different feed treatments on nutrient digestibility, DM consumption and daily body weight gain of sheep.

Table 2 - Nutrient digestibility, and daily body weight gain in Sheep.

Parameters \ Treatments	T0	T1	T2	T3	T4	P-values (P<0.05)
Dry matter consumption (kg/head/day)	0.55	0.55	0.55	0.54	0.54	NS
DMD (%)	71.36 ± 6.37	67.64 ± 5.91	67.42 ± 7.05	69.56 ± 3.34	67.41 ± 3.47	NS
OMD (%)	73.73 ± 5.44	70.00 ± 5.72	70.00 ± 6.45	71.54 ± 3.36	69.58 ± 3.26	NS
EED (%)	57.25 ± 4.95	65.77 ± 8.88	68.24 ± 10.02	55.97 ± 5.67	70.91 ± 3.68	NS
CFD (%)	63.40 ± 6.97	49.11 ± 10.28	52.51 ± 10.15	56.26 ± 6.11	53.72 ± 9.07	NS
CPD (%)	76.99 ± 5.19	76.94 ± 5.20	75.30 ± 5.19	75.61 ± 2.85	73.21 ± 1.58	NS
TDN (%)	65.55 ± 4.85	66.23 ± 6.98	62.86 ± 5.89	60.80 ± 2.90	59.00 ± 2.76	NS
Average daily gain (kg/head/day)	0.19 ± 0.04	0.21 ± 0.07	0.20 ± 0.04	0.22 ± 0.02	0.20 ± 0.03	NS

NS: non-significant (P>0.05); Dry matter digestibility (DMD), organic matter digestibility (OMD), extract ether digestibility (EED), crude fiber digestibility (CFD), crude protein digestibility (CPD), and Total Digestible Nutrients (TDN). T0 = concentrate without the addition of fermented litter, T1 = 90% concentrate + 10% fermented litter, T2 = 80% concentrate + 20% fermented litter, T3 = 70% concentrate + 30% fermented litter and T4 = 60% concentrate + 40% fermented litter

Dry matter consumption

Based on Table 2, it can be seen that the average DM consumption of local sheep feed is 0.54 – 0.55 kg/head/day. This value is by the standard by Gerlach et al. (2015) that the consumption of DM feed that has high quality can reach 3.5% of body weight. The nutritional quality of the feed given will affect livestock productivity. McGrath et al. (2018) stated that feed consumption in ruminants was influenced by several factors such as palatability, energy requirements, feed form, physiological status, and production. Scherer et al. (2015) stated that the ability to consume DM shows an effort to fulfil the body's nutritional needs for development.

Dry matter digestibility (DMD)

Results showed that the administration of fermented litter in treatments T0, T1, T2, T3, and T4 did not affect the DMD value of sheep. The highest DMD value of 80.97% with T0 treatment could occur because the nutrient content in the ration was easily digested by rumen microbes. This value is higher than the results of research by Al-Galbi (2013) which states that the provision of broiler excreta in feed provides a DMD value of 61.39 – 65.56%. The high DMD value is thought to be caused by the ability of microbes to break complex bonds such as the lignin content in the ration to be simpler. Langda et al. (2020) stated that high levels of lignin in feed caused microbes in the rumen unable to degrade nutrients in cells so that the digestibility produced was low. The high DMD value in the T3 treatment indicated that the dry matter ration was able to be digested by microbes.

Organic matter digestibility (OMD)

The results showed that the increase in fermented litter substitution in concentrate did not affect the OMD value of sheep. The absence of this difference is presumably because the DMD values are not different. Gao et al. (2015) stated that ration OMD can be an indicator that OM ration is easy to be degraded by rumen microbes and digested by post-rumen digestive enzymes. The highest OMD value was 82.13% in the T0 treatment, while the lowest OMD value was 79.01% in the T2 treatment. This value is higher than the results of the study by Shahowna et al. (2013) that the value of OMD litter added to the ratio ranged from 67.35 – 79.79%. Gao et al (2015) stated that the high and low of OMD are related to DMD because organic matter is part of dry matter.

Extract ether digestibility (EED)

Statistical test results showed that the concentrate substitution treatment with fermented litter gave no significant results. Extract Ether digestibility increased no-significantly with increasing fermentative litter composition. This is caused by the binding of triglyceride complexes to the feed with the addition of fermented litter. Lam et al. (2010) stated that high-fat triglyceride bonds do not break down into simple bonds such as fatty acids and alcohols so the evaporation process due to alcohol does not occur. Irungu et al. (2018) added that the main effect of the high digestibility value of EE is influenced by the chemical structure of fat which is highly digestible by livestock compared to protein. The main effect of increasing fat absorption is on the amount of triglyceride content rather than free fatty acids. Patra (2014) stated that

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the ability to digest fat increases when it is dominated by unsaturated fatty acid bonds, there are short - chain fatty bonds, and contains more triglyceride molecules compared to free fatty acids.

Crude fiber digestibility (CFD)

The digestibility of CF sheep fed different fermented litter feeds in vivo showed that the results had no effect. Litter fermentation on extract ether had no significant effect, presumably due to the influence of cellulose degradation. The crude fiber in sheep has a role in balancing the buffer by helping the process of rumen saliva production. The highest CFD value was 76.88% with T0 treatment, while the lowest CFD value was 68.79% with T1 treatment. Lignin can be a major factor in the high content of crude fiber. Islam et al. (2017) stated that CF has a relationship with digestibility, the lower the CF, the higher the digestibility of the ration. Lignin as a component of CF is a complex substance that is difficult to digest. Behan et al. (2019) stated that the digestibility of CF is influenced by high and low cell wall fractions. Hemicellulose and cellulose are cell wall components that can be digested by rumen microbes.

Crude protein digestibility (CPD)

Crude protein digestibility of sheep fed different fermented litter feeds in vivo showed that there was no significant effect. Crude protein digestibility value is a percentage of CP contained in the consumed ration and not found in livestock feces, CPD is influenced by CP value. The CPD value was influenced by the protein content in the ration. Tilman et al. (2005) stated that CPD depends on protein content and the amount of protein that enters the digestive tract, the higher the protein content, the higher the digestibility. The highest CPD value was 84.22% with the T1 treatment, while the lowest CPD value was 69.67% with the T0 treatment. The high CP content in the ration will cause the rate of reproduction and the number of microbes in the rumen to increase. Soltan et al (2018) stated that an increase in the number of microbes in the rumen will cause more enzymes that digest CP and an increase in CPD. The DMD value of a ratio is closely related to the CPD value. Pazla et al. (2021) stated that the CPD value is directly proportional to the DMD.

Total digestible nutrients (TDN)

The results showed that the treatment with fermented litter did not affect the TDN value of the sheep. TDN shows the amount of energy consumed by livestock. Omer et al. (2019) stated that the TDN value is an illustration of the total energy consumed by livestock from feed or rations. The TDN value is influenced by the nutritional content in the feed ration. Van Soest (1994) stated that the TDN value was obtained from the digestibility value of the fiber, protein, fat, and carbohydrate components present in the feed. Alshelmani et al. (2016) added that the TDN consumed by livestock will be high because the NFE consumed is high, a high TDN will support an increase in ration efficiency.

Average daily gain (ADG)

The substitution of concentrate with fermented litter did not statistically give any difference in the value of average daily gain. The results showed that the average value of daily gain for sheep ranged from 0.187 to 0.223 kg. This value is higher than the research of Abad et al. (2015) who reported that the average daily gain of local goats ranging from 3 to 6 months of age was 40 g. Body weight gain is thought to be influenced by the nutrient content in the ration. Madeira et al. (2017) stated that the factors affecting body weight gain were influenced by the palatability of the ration and the nutrient content in the ration such as adequate protein and energy.

CONCLUSION

Based on the results of this research, it can be concluded that giving fermented litter can be used as a substitute for sheep feed concentrate because it shows the same performance as giving without fermented litter. It was concluded that fermented chicken litter can be incorporated in sheep diet, without considerable negative effects.

DECLARATIONS

Corresponding author

Email: marrychristiyanto@gmail.com

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Authors' contribution

MCH and CSU provide recommendations and suggestions on research topics, article preparation and finalization of scientific articles; EPA and LKN conduct research and analysis of productivity and performance parameters; SRN conducts article preparation and research data processing

Conflict of interests

The authors declare that they have no competing interests.

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