In the Name of God

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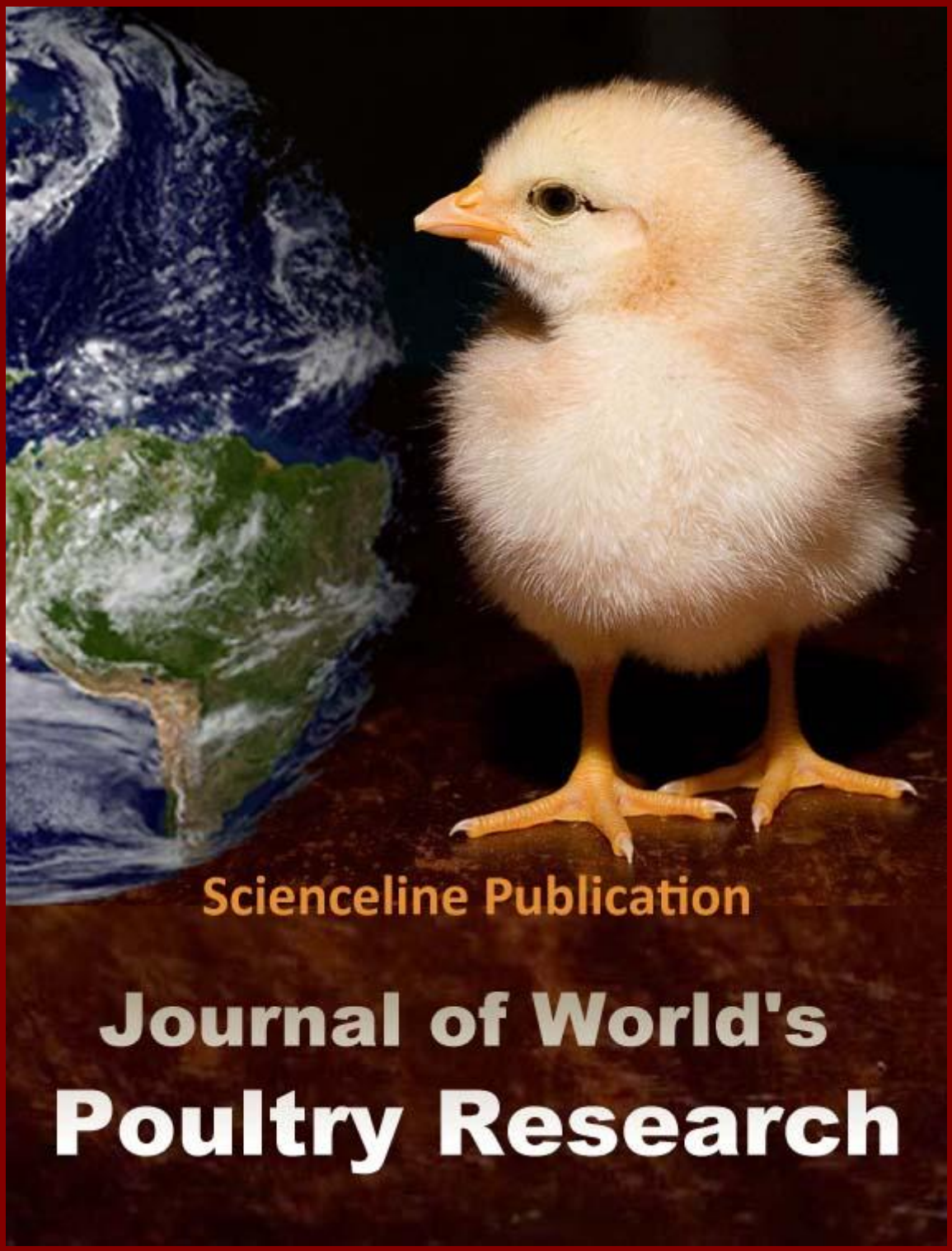

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and incubated until hatched. Forty five females and 45 males offspring quails were collected from each treatment. The second stage consisted of 3 groups; K0: offspring of quail whose parents were not supplemented by turmeric powder (P0); K1: offspring of quail whose parents were supplemented by turmeric powder 54 mg/quail/day (P1); K2: offspring of quail whose parents were supplemented by turmeric powder 108 mg/quail/day (P2). This study implemented completely randomized design experimental method. It is proven that turmeric powder supplementation increased the levels of vitellogenin, HDL, vitamin B12, vitamin A, white egg protein, linoleic acid, arachidonic acid in eggs. In contrast, the cholesterol levels, LDL and total fat of eggs decreased. However, no significant changes of the oleic acid level were observed. On the second stage for both K1 and K2 in the case of the first female offspring of Japanese quail, the weight of carcass, SGPT, cholesterol serum, triglycerides serum and liver cholesterol increased, but LDL and SGOT serum decreased. Beside the weight of carcass, there were no significant changes for other parameters of the first male offspring of Japanese quail.

Key words: Japanese quail, Quality of egg, Physiological condition, Turmeric powder

[Full text-PDF] [RICeST] [DOAJ] [AGRIS]

Research Paper

Quality Characteristics of Whole Guinea Fowl Egg as Binder in Beef and Chevon Burgers.

Adzitey F, Birteeb P and Kwasi Holdbrook B.

J. World Poult. Res. 6(2): 66-72; pii: S2322455X1600010-6

ABSTRACT:

This study was conducted to determine the cohesiveness of whole guinea fowl egg as a binder in chevon and beef burgers. The study also investigated the sensory characteristics, nutritional content, cooking loss, lateral shrinkage, welling and doming of the beef and chevon burgers prepared using whole guinea fowl eggs. A total of 3 kg beef and 3 kg chevon were used. The meats were assigned using complete randomized design into 3 levels. The 3 levels of inclusion of the whole guinea fowl egg per kilogram of meat were 0 g, 50 g and 100 g which corresponds to each treatment that is B1 (control), B2 (5 %) and B3 (9 %) for beef, and C1 (control), C2 (5 %) and C3 (9 %) for chevon, respectively. Thus each treatment contained 1 kg meat, 0.5 g red pepper, 1.0 g black pepper, 1.0 g white pepper, 2.0 g mixed spice (adobo®), 5 g salt and whole guinea fowl egg (0 g, 50 g or 100 g). The meat and spices were minced and moulded manually into burgers using a cylindrical tube to obtain uniform shapes and sizes. They were vacuum-packed in transparent packaging bags and stored overnight at 4 °C prior to processing. The processed samples were evaluated for their sensory, nutritional and binding properties. Sensory characteristics of beef and chevon burgers (cohesiveness, colour, juiciness, texture, taste, flavor and overall liking) showed no significant differences ($P > 0.05$). In absolute terms beef and chevon burgers with the highest inclusion level (9 %) of whole guinea fowl egg were most preferred. There were also no significant differences ($P > 0.05$) in moisture content, crude protein content, pH, cooking loss, lateral shrinkage and doming of the beef and chevon burgers. Significant difference ($P < 0.05$) occurred in the crude fat content of chevon burger but not beef burger. Welling was not observed in the beef and chevon burgers.

Key words: Binder, Burgers, Guinea fowl eggs, Nutritional, Sensory

[Full text-PDF] [RICeST] [DOAJ] [AGRIS]

Research Paper

Supplementation of Different Level of Deep Stacked Broiler Litter as a Source of Total Mixed Ration on Digestibility in Sheep and Their Effects on Growth Performance.

Tahir Khan M, Bhutto ZA , Abbas Raza SH, Saeed M, Arain MA, Arif M, Fazlani SA, Ishfaq M, Siyal FA, Jalili M and Moshaveri A.

J. World Poult. Res. 6(2): 73-83; pii: S2322455X1600011-6

ABSTRACT:

Poultry litter from rigorous poultry production plants has impact on environmental pollution. Feedstuffs for animal are getting with time expensive, to reduce the feed cost which could be achieved through the assimilation of relatively inexpensive and non-conventional feed ingredients, like poultry litter. The objective of this study was to explore the nutritive value of deep stacked broiler litter in ruminant's total mixed ration. Four non castrated male sheep were used into 4×4 Latin Square Design (LSD) to 1 of the 4 dietary treatment groups that different in deep stacked broiler litter (DBL) as percentage of concentrate diet to investigate the nutritive value of DBL as a ruminant feed. The effect of dry matter intake and digestibility of DBL in sheep studied. Nitrogen retention was determined in total mixed ration at each level in the diets fed to sheep. Microsoft excels was used to balance experimental rations A, B, C, and D. Ration A was containing 0% DBL and served as control. Ration B contains 15% DBL, C was containing 30% DBL while Ration D containing 45% DBL. All the diets were prepared according to requirement of critical nutrients. All the diets were prepared isocaloric, isonitrogenous with or without DBL. Dry matter intake gradually decreased ($P < 0.05$) with the levels of broiler litter increased in the four diets. Means values of DMI (g/day) in rations A, B, C and D was 1040.7, 945.3, 840.9 and 786.8. Nitrogen retention (% of the total N consumed) were decreased ($P < 0.05$) as the broiler litter level increased in the diet. Up to 30% poultry litter in the supplement diets of sheep contributes as non-conventional source of nitrogen, and could be used for replacing traditional nitrogen sources like cotton seed cake. The findings of the present study suggested that inclusion of broiler litter up to 30% has no adverse effect on the health and apparent weight.

Keywords: Deep stacked, Mixed ration, Litter, Digestibility, Sheep

[Full text-PDF] [RICeST] [DOAJ] [AGRIS]

Research Paper

Awareness of Farmers on Newcastle Disease, its Vaccination and Antibody Titre in Commercial Chickens.

Modupe Lola O, Philip O, Yakubu D, Israel B, Lawal S, Paul A and Sunday Blessing O.

J. World Poult. Res. 6(2): 84-91; pii: S2322455X1600012-6

ABSTRACT:
Newcastle disease is a highly contagious viral disease which affects existing or developing poultry industries. This study was performed to assess the level of awareness of farmers on Newcastle disease and its control through vaccination and also to determine the level of Newcastle disease virus antibody (Ab) titer in commercial layer chicken sera using haemagglutination inhibition test in Jos South Local Government Area, Plateau State, Nigeria. A structured questionnaire was shared to farmers to fill. Thirty four farms were visited and nine districts were randomly selected. A total of 354 sera were collected from commercial chickens; ten from each flock. There was a high level of awareness of farmers (100%) on ND and its vaccination (100%) and all the farmers (100%) had vaccinated their chickens against ND. The HI test revealed that, out of the 354 sera tested, 9 (2.5 %) chickens were negative for NDV Abs, which means had NDV antibody titer below the minimum protective titer of log2 3 and 345 chickens (97.5%) were positive for NDV Abs; had NDV antibody titer above log2 3. It was concluded that the level of awareness of farmers on ND and its control through vaccination was incredibly high, also, the level of protection to ND in vaccinated chickens was also very high, in that a higher percentage of the chickens had NDV Antibodies between log2 6 and log2 8, however, inspite of these, ND is still a continual threat to the poultry industry in Nigeria. It is therefore recommended that, other biosecurity measures, such as good management practice, proper hygiene and surveillance be emphasized and ensured, in order to prevent ND infection among flocks.

Key words: Newcastle disease, Antibody titer, Haemagglutination inhibition, Commercial chickens

[Full text-PDF] [RICeST] [DOAJ] [AGRIS]

Research Paper

Growth Performance and Gastrointestinal Tract Morphometry in Growing Japanese Quails Fed with Moringa oleifera Leaf Meal as Partial Replacement of Dietary Soya Beans Meal.

Mahmud Muhammd A, Peter S, James G, Ruth N, Wosilat A, Musa M and Alhaji Abubakar M.

J. World Poult. Res. 6(2): 92-98; pii: S2322455X1600013-6

ABSTRACT:
One hundred and twenty (120) day old Japanese quails were bought and allocated to four dietary treatments of thirty (30) birds per treatment with the aim of studying the growth performance and gastrointestinal tract morphometry of growing Japanese quails fed with graded levels of Moringa oleifera meal as partial replacement of dietary soybean meal. Each treatment had three replicates of 10 birds each in a completely randomized design. The experiment lasted for six weeks. Four diets containing 24% crude protein for the growing phase (0-6 weeks) were formulated in which Moringa oleifera leaf meal replaced soya bean meal at 0, 5, 10 and 15% as T0, T1, T2, T3 respectively. The mean initial body weights, the mean final body weight and the mean total weight gains of the four treatments were significantly different from one another. However, feed conversion ratio, protein efficiency, performance efficiency factor and production number significantly varied across the four treatments; with T1 having the best result than the others. The mean spleen weights, mean breast weights, mean thigh weights, mean drumstick weights, mean wing weights and mean liver weights of the growing Japanese quails in the four treatments were not significantly different from one another except the heart weight of T1. The mean weights, lengths, width and thickness of proventriculus, ventriculus, duodenum, jejunoileum, caeca and colon of the growing Japanese quails in the four treatments were not significantly different from one another, except the lengths of jejunoileum. It is therefore concluded that in growing Japanese quails, Moringa oleifera leaf meal may replace dietary soya beans meal up to 15%, with optimum level of 5% and no apparent adverse effects on gastrointestinal tract morphometry.

Key words: Growth performance, Gastrointestinal tract, Soya beans, Moringa oleifera, Japanese quail

[Full text-PDF] [RICeST] [DOAJ] [AGRIS]

Research Paper

Crude protein and energy requirements of Japanese quail (*Coturnix coturnix Japonica*) during rearing period.

Omidiwura BRO, Odu O, Agboola AF, Akinbola DD and Iyayi EA.

J. World Poult. Res. 6(2): 99-104; pii: S2322455X1600014-6

ABSTRACT:
Present experiment was conducted to evaluate the effect of diets containing different levels of metabolizable energy (3000, 3100 and

3200 kcal metabolizable energy/kg) and crude protein (20, 22, 24 and 26% crude protein) on performance of growing Japanese quail. 288 two-week old quail chicks were assigned into 12 treatments and 3 replicates with 8 birds in each. Birds were randomly allocated to each dietary treatment. For 3000, 3100 and 3200 kcal metabolizable energy/kg levels of energy, crude protein levels of 26, 24, 22 and 20% were assigned. Data on performance and nutrient digestibility were recorded and analyzed using a completely randomized design with a 4×3 factorial arrangement during 6 weeks of age. Metabolizable energy significantly affected ($P < 0.05$) total and daily feed intake. Level of crude protein also had a significant effect on the crude protein intake and protein efficiency ratio of growing Japanese quails. Level of crude protein and metabolizable energy had no significant effect on the body weight gain. The metabolizable energy significantly affected ($P < 0.05$) the ether extract digestibility while crude protein significantly affected ash digestibility. The results indicated that a diet of 26% crude protein and 3200 kcal metabolizable energy/kg is suitable for optimum performance of Japanese quail in terms of weight gain.
Keywords: Japanese quail, Crude protein, Metabolizable energy, Digestibility, Performance
[Full text-PDF] [RICeST] [DOAJ] [AGRIS]

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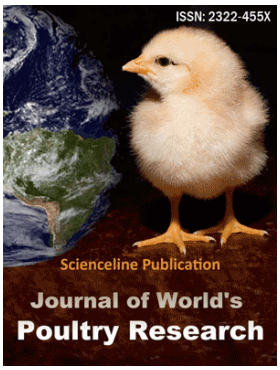
The Journal of World's Poultry Research



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Research Paper, PII: S2322455X1600007-6

Quality Improvement of Broiler Chicken Breasts by Nisin and Lactic Acid

Fathy Ahmed Khalafalla, Fatma Hassan Mohammed Ali and Amal Hamdy Abd-Elfattah Hassan*

Food Hygiene and Control Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, 65211, Egypt
*Corresponding author`s Email: emyhamdy41@gmail.com

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ABSTRACT

The present study was conducted to evaluate the effect of nisin, lactic acid and their combination on quality parameters and shelf life of broiler chicken breasts during chilling storage at 3 ± 1 °C. For achieving this goal, broiler chicken breasts were collected and divided into control and treated groups by dipping in each of 1% lactic acid, 2%lactic acid, nisin 50µg, nisin 100µg, nisin 50µg followed by 1% lactic acid, and nisin 100µg followed by 1% lactic acid. The samples were packaged and stored at 3 ± 1 °C. Control and treated groups were examined periodically at day zero and every three days until spoilage with chemical and microbiological methods. The results revealed that nisin, lactic acid and their combination improved the quality and extended the shelf life of broiler chicken breasts for 3 to 12 days during chilling storage. The use of nisin followed by lactic acid would be promising to provide a new hurdle technology for poultry meat decontamination.

Keywords: Broiler chicken breasts, Quality improvement, Lactic acid, Nisin, Chemical examination, Microbiological examination

INTRODUCTION

Poultry meat is easy to prepare at home and widely used in restaurants and fast food establishments. There is no primary religious restriction on the consumption of poultry meat (Mulder, 1999). Poultry meat is an excellent substrate for the growth of a wide variety of microorganisms including pathogens and spoilage microorganisms. Other properties influence the growth of microorganisms such as water activity and pH value, as well as the skin, which harbors many microorganisms while serving as a physical barrier to microorganisms (ICMSF, 1998). However, poultry meat is highly perishable with a relatively short shelf life even when it is kept under refrigeration (Mantilla et al., 2012). Thus, the application of decontamination treatments during processing could be highly useful. Several decontaminants have been reported to be effective in destroying disease-causing pathogens, decontaminating, preserving and extending shelf-life of poultry carcasses and cuts. These treatments can be classified as physical, chemical, and biological which include chlorine, organic acids, tri-sodium phosphate, bacteriocin, hydrogen peroxide, ozone, water, ultrahigh pressure, irradiation, pulsed-field electricity, ultrasonic energy and UV (Bolder, 1997 and Capita et al., 1999 a, 1999 b).

Lactic acid is an organic acid with proven effectiveness as a decontaminant with different kinds of food. It can delay the proliferation of spoilage microorganisms, prevent the generation of undesirable chemicals, improve the levels of sensory attributes, and extend the shelf life of chicken during refrigerated storage (Smaoui et al., 2012). Lactic acid is used as a decontaminant in different concentrations and treatment of chicken breast samples with 3% lactic acid gave the highest initial reduction in aerobic mesophilic and psycrophilic bacteria (Cosansu et al., 2011).

Nisin is a bacteriocin successfully used as an antibacterial agent in various food products. It is generally recognized as safe for use as a biopreservative in food systems (Jay, 2000; FDA, 2008**).** It is a 3,500-Da polypeptide produced by *Lactococcus lactis subsp lactis* that inhibits growth of Gram positive organisms. Nisin's spectrum of inhibitory activity could be extended to include gram negative bacteria when combined with agents such as disodium ethylenediamine tetraacetate (EDTA), lactate, citrate, irradiation and vacuum packaging (Cutler and Siusa, 1995**;** Cosby et al. 1999**;** Long and Phillips, 2003 and Zahran, 2015). Therefore, the aim of this work was to evaluate the effect of lactic acid and nisin applied

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singly or in combination to improve the quality and extend the shelf life of broiler chicken breasts during refrigerated storage.

MATERIALS AND METHODS

Preparation of broiler chicken breasts

Fresh broiler chicken breasts (each breast was about 600 g) were purchased from a retail poultry shop at Beni-Suef City, Egypt. Each broiler chicken breast was divided into four parts of average weight 150 g, then wrapped in sterile polyethylene bags and directly transferred in sterile ice box as soon as possible to the laboratory of food hygiene department, faculty of veterinary medicine, Beni-Suef university, Egypt.

Preparation of treatment solutions:

Lactic acid: lactic acid 1, 2% (v/v) solutions were prepared using pure lactic acid liquid (2010/1, ADWIC, Egypt) and distilled water.

Nisin: nisin 50, 100µg/ml (w/v) solutions were prepared using nisin (Aplin and Barrelt Ltd. Trobridge, U K) and distilled water.

Samples treatment

Broiler chicken breasts were divided into seven groups as follow: Group one was used as a control (untreated); Group two was dipped in lactic acid 1% (v/v) solution for 10 minutes; Group three was dipped in lactic acid 2 % (v/v) solution for 10 minutes; Group four was dipped into nisin 50µg/ml (w/v) solution for 30 minutes; Group five was dipped into nisin 100µg/ml (w/v) solution for 30 minutes; Group six was dipped into nisin 50µg/ml (w/v) solution for 30 minutes followed by lactic acid 1% (v/v) solution for 10 minutes and Group seven was dipped into nisin 100µg/ml (w/v) solution for 30 minutes followed by lactic acid 1% (v/v) solution for 10 minutes.

Packaging and storage

Samples of control and treated groups were aerobically packed as triplicates inside fiber dishes and stored at 3± 1°C. Samples were examined chemically and microbiologically at day zero and periodically every three days until spoilage (0, 3rd, 6th, 9th, 12th, and 18th).

Examination techniques

Deterioration criteria

Determination of Thiobarbituric Acid-Reactive Substances Value (TBA-RS): The technique of Taraldgis et al. (1960) with additional modification of Pikul et al. (1983) was applied by blending ten grams of broiler chicken breast sample with 50 mL of distilled water in food blender for 2 min. The mixture was transferred to Kjeldahl flask by washing with additional 47.5 mL of distilled water. Then, the pH was adjusted to 1.5 by addition of 2.5 mL of 4 N HCl solution. Antioxidant (butylated hydroxytoluene (BHT) and anti pumping stones were added. Apparatus and heat flasks were assembled at the highest heat obtainable on the Kjeldahl distillation apparatus. Five mL of the mixed distillate were pipetted into 50 mL glass stoppered tube and 5 mL of TBA reagent were added. The tubes were stoppered and their contents were mixed, and immersed in a boiling water bath for 35 minutes. A blank solution was prepared by distilled water and TBA reagent and treated the same as the samples. After heating, the tubes were cooled under tap water for 10 min. A portion was transferred to a cuvette and the optical density of sample (D) was determined against the blank at a wavelength of 538 nm of the spectrophotometer.

Calculation:

TBA value =D×7.8 (mg malondialdehyde (MDA) / kg of flesh).

Determination of Total Volatile Basic Nitrogen Value (TVB-N): The technique recommended by Food and Agriculture Organization (1986) was applied. Briefly, 10 gm of minced broiler chicken breast sample were homogenized with 100 mL of distilled water in a food blender for two minutes. Sample was washed into distillation flask with a further 200 mL of water, then two grams magnesium oxide and two drops of antifoaming agent were added. The mixture was boiled for 10 minutes and distilled for exactly 25 minutes, using the same rate of heating, into 25 ml of 2% boric acid solution with few drops of screened methyl red indicator in a 500 ml flask. The heating was stopped and the condenser washed down with distilled water. Then, the contents of the flask and the blank solution (25 mL of 2% boric acid) were titrated with 0.1 N H2SO4 (titer).

Calculation: Total volatile base (mg N/100g flesh) = 14 (titer–blank).

Bacteriological examination

Preparation of samples: Preparation of broiler chicken breasts samples was carried out according to International Commission on Microbiological Specifications for Food, ICMSF (1986). Ten grams of broiler chicken breast (skin and muscle) were homogenized with 90 ml of 0.1% sterile peptone water at 2000 rpm for 2.5 minutes using a sterile homogenizer (MPW 302, Universal Laboratory Aid, made in Poland). Ten fold serial dilutions up to 10^{-6} were done**.**

Determination of aerobic mesophilic and psychrophilic counts: The applied technique

recommended by Association of Official Analytic Chemists, AOAC (1990) was used by standard plate count agar (6G2307, Biolife, Italy) tempered at 45 °C, then inoculated plates were incubated in inverted position at 35 °C for 48 hours and at 7 ºC for 10 days for mesophiles and psychrophiles respectively.

Coliforms, fecal coliforms and E.coli (Most Probable Number): The three tubes method (MPN) recommended by AOAC (1990) for coliform, fecal coliform, and *E.coli* was applied.

Staphylococcus aureus count

The applied technique recommended by American public Health Association, APHA (1992) was used by Baired-Parker medium (BP Biolife, Italy) then the inoculated plates were incubated at 35 ºC for 48 hours for *S. aureus* count.

Statistical analysis

Data were subjected to analysis of variances (one way-ANOVA) according to Knapp and Miller (1992) using (SPSS Statistics 20.0) software program. Differences among the mean values of the various treatments were determined by the Least Significant Difference (LSD) test, and the significance was defined at $P < 0.05$.

RESULTS AND DISCUSSION

Deterioration criteria

Thiobarbituric Acid-Reactive Substances (TBA-RS): TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content. MDA formed through hydro peroxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez et al., 1997).

It was cleared that there is no significant differences between all groups ($P < 0.05$) at day zero of storage except nisin (50µg/ml) treated group which significantly higher than lactic acid 2% treated group Figure 1.

A significant increase with storage time ($P < 0.05$) in TBA-RS values was observed in all groups**.** This was in agreement with Alasnier et al. (2000); Ali et al. (2007) and Rahman et al. (2012). Significant differences ($P < 0.05$) were appeared between control and groups treated with nisin 50µg/ml + lactic acid 1%, and nisin 100µg/ml + lactic acid 1% at 3rd day of storage.

TBA-RS values of control group were the highest allover other groups. On the other hand, nisin + lactic acid treated one had the lowest TBA-RS values, this difference was significant ($P < 0.05$). Lipid oxidation is an important factor of oxidative deterioration of poultry meat. TBA is a measure of malondialdehyde (MA), one of the degradation products of lipid hydro peroxides formed through oxidation of unsaturated fatty acids. This is in accordance with Nawar (1996); Botsoglou et al. (2002) and Gatellier et al. (2007).

Concerning the permissible limit of TBA value in poultry meat (0.9 mg MDA/kg) recommended by Egyptian organization for standardization, EOS (2005), only control and treated groups with each of nisin 50µg/ml+lactic acid 1% and lactic acid exceeded such limit in the last occasion of examination.

Total volatile basic nitrogen

The TVB-N content in chicken, as an important reference index, has been being used to evaluate chicken's freshness (Fatih and Yeşim, 2000 and Castro et al., 2006). TVB-N compounds in chicken contain mainly ammonia, trimethylamine (TMA) and dimethylamine (DMA) and the levels of TVB-N compounds increase with spoilage by either bacterial or enzymatic degradation**.**

TVB-N values of control and treated groups were illustrated in Figure 2. There were no significant differences between all groups ($P < 0.05$) at day zero. Nearly similar results were detected by Rukchon et al. (2011) and Smaoui et al. (2011 and 2012). Higher values were reported by Balamatsia et al. (2007).

Control group was significantly higher than all treated groups at 3rd and 6th days of storage. Broiler chicken breasts treated with nisin + lactic acid were lower than all groups during all days of storage, this indicates that treatment of broiler chicken breasts with nisin + lactic acid has strong effect on microbial and enzymatic activities.

There was a significant increase ($P<0.05$) in TVB-N values with storage time in all groups**.** A clear relationship was found between the microbiological quality of broiler chicken breasts and level of TVB-N formation**,** this is in agreement with Rukchon et al. (2011). The TVBN is related to protein breakdown (Egan et al., 1981) and the observed increases may be attributed to the formation of ammonia or other basic compounds due to microbial activity (Banwart, 1981).

TVB-N values of all treated groups exceeded the limit recommended by EOS (2005) at last days of storage**.** Regarding the permissible limits of TVB-N (40mg/100g) suggested by Balamatsia et al. (2007), it was cleared that none of the samples exceeded such limit even at the last day of storage life time of each group. In this respect**,** Patsias et al. (2008) reported that the initial TVB-N value of 12 mg/100g sharply increased in the chilled chicken fillets stored in air resulting in high TVB-N values (49 g/100g) after 9 day of storage. Further more**,** Rukchon et al. (2011) stated that TVB-N was markedly detected in fresh chicken and its level increased with storage time.

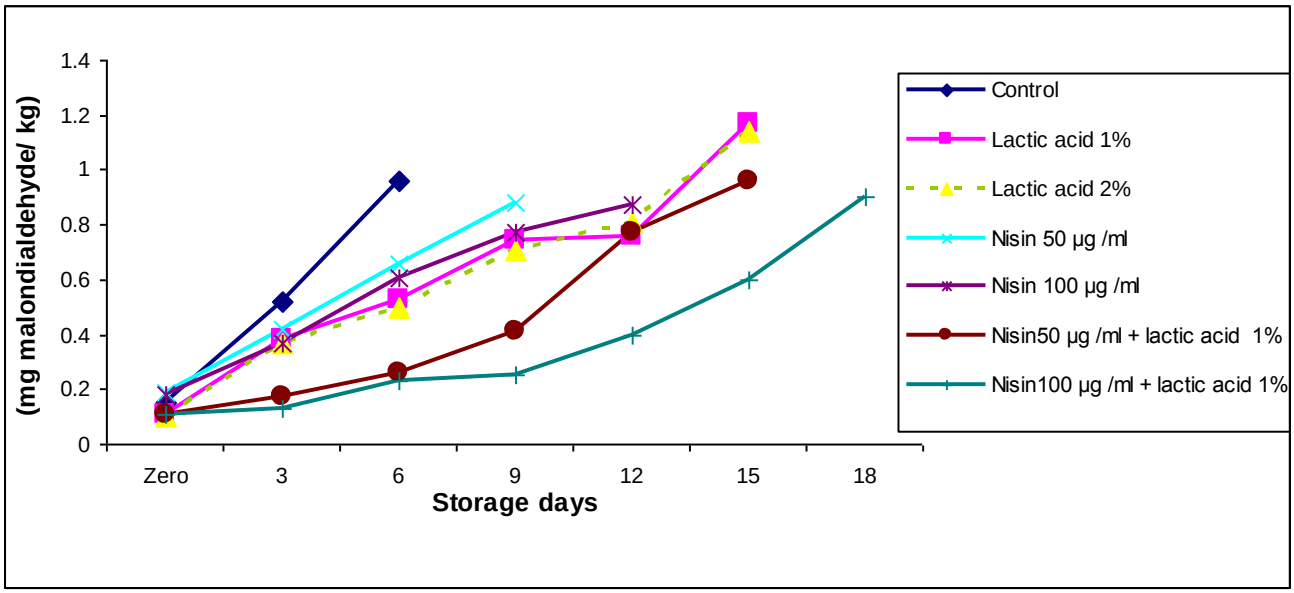

Figure 1. Effect of treatments on Thiobarbituric Acid-Reactive substance values of packed broiler chicken breast samples during chilled storage at 3±1°C (mg malondialdehyde/ kg).

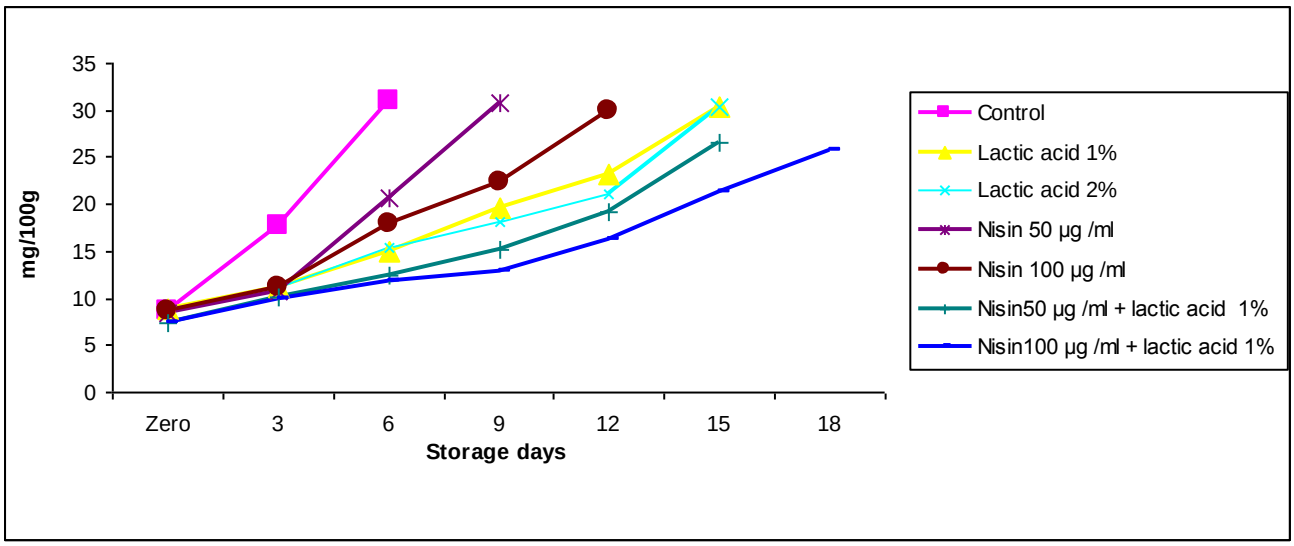

Figure 2. Effect of treatments on total volatile basic nitrogen values of packed broiler chicken breast samples during chilled storage at 3±1°C (mg /100g flesh).

Bacteriological examination

Aerobic mesophilic count: From Figure 3, it was cleared that the highest reduction in log CFU/g mesophilic bacteria were recorded in nisin (100µg/ml) + lactic acid (1%) treated group while the lowest reduction was determined in nisin 50µg/ml treated one. Nearly similar results were obtained by Tosun and Tamer's (2000); Sinhamahapatra et al. (2004) and Anang et al. (2010), low results were observed by Morshedy and Sallam (2009). High results were recorded by Ismail et al. (2001). Initially 2% lactic acid was not found significantly more effective in reducing colony counts than 1% lactic acid, this in accordance with Marcel et al. (1988).

The microbial counts in the control and treated groups gradually increased during storage time, but those for the treated groups were significantly lower than the control; this is in accordance with that reported by Ismail et al. (2001) and Gu et al. (2011).

Improper cleaning and disinfection of machines in poultry abattoirs may lead to contamination of poultry meat during processing. Bean and Griffin (1990) reported that quantifying the total mesophiles is an excellent indicator of contamination which has taken place during processing and is a useful tool to asses microbiological safety and sanitation conditions during processing.

The results indicated that nisin alone was less effective on aerobic mesophilic count. However it was cleared that dipping of broiler chicken breast in nisin solution followed by lactic acid had a synergistic effect on the aerobic mesophilic count. Thus, it can be clearly

expressed that nisin should be applied as combined with lactic acid application, rather than it's alone application. This greater inhibition of nisin + lactic acid 1% may be due to the lactic acid's increasing effect of nisin's penetration into Gram-negative bacteria, by decomposing the cell wall prior to nisin application (Helander, 2000). Regarding to the acceptability limit recommended by ICMSF (1986) for total viable count in processed chickens (7 $\log_{10}$ CFU/g flesh), it could be observed that all groups exceeded such limit at last day of the chilling storage of each group.

Aerobic psychrophilic count

The illustrated results as shown in Figure 4 revealed that treated groups with nisin (100µg/ml) + lactic acid (1%) had the highest reduction in log CFU/g at day zero of storage. Nearly similar results were obtained by Ellerbroek et al. (1996), while lower results were observed by Morshedy and Sallam (2009) and Hecer and Guldas (2011). The psychrophilic counts in control group was significantly ($p < 0.05$) higher than other treated groups at day zero of chilled storage. A gradual increase in aerobic psychrophilic count was observed during chilled storage which was significantly lower than that of control group. This is in agreement with Vatansever et al. (2008) and Hecer and Guldas (2011).

Nisin (50µg/ml) + lactic acid (1%) and nisin (100µg/ml) + lactic acid (1%) treated groups were lower than other groups at each occasion of examination. This suggested a synergistic effect occurred between nisin and lactic acid. The antimicrobial effect of lactic acid is the result of a decrease in pH and a specific antimicrobial effect of non-dissociated molecule (Debevere, 1987 and Smulders, 1987).

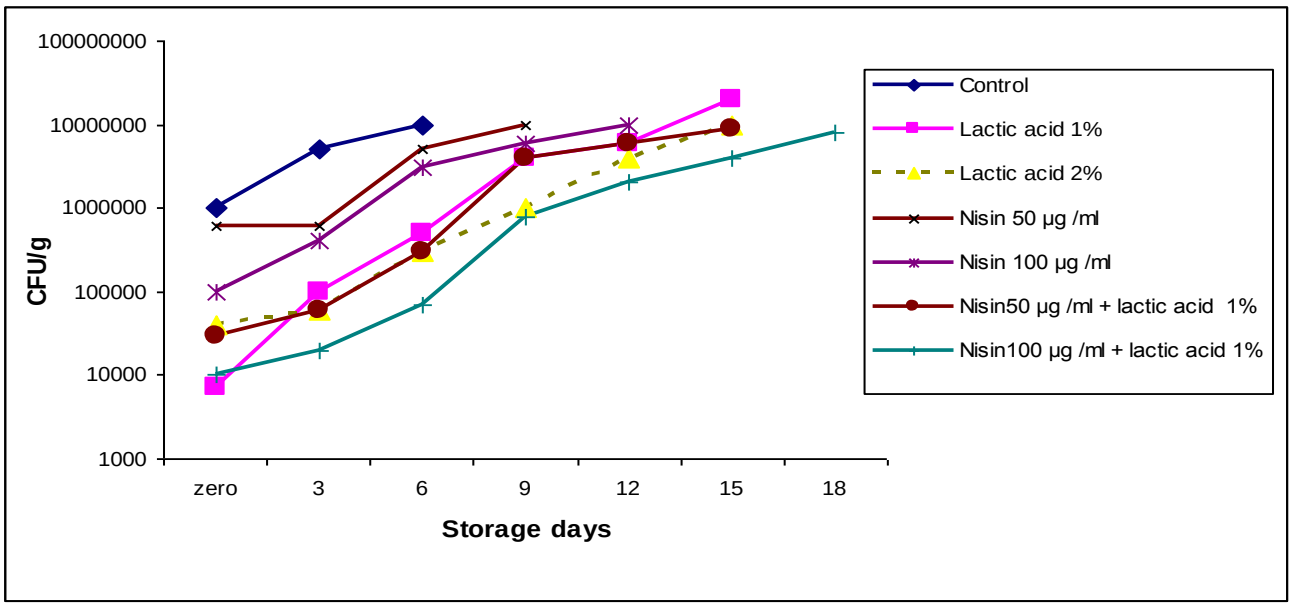

Figure 3. Effect of treatments on mesophilic counts of packed broiler chicken breast samples during chilled storage at 3±1°C.

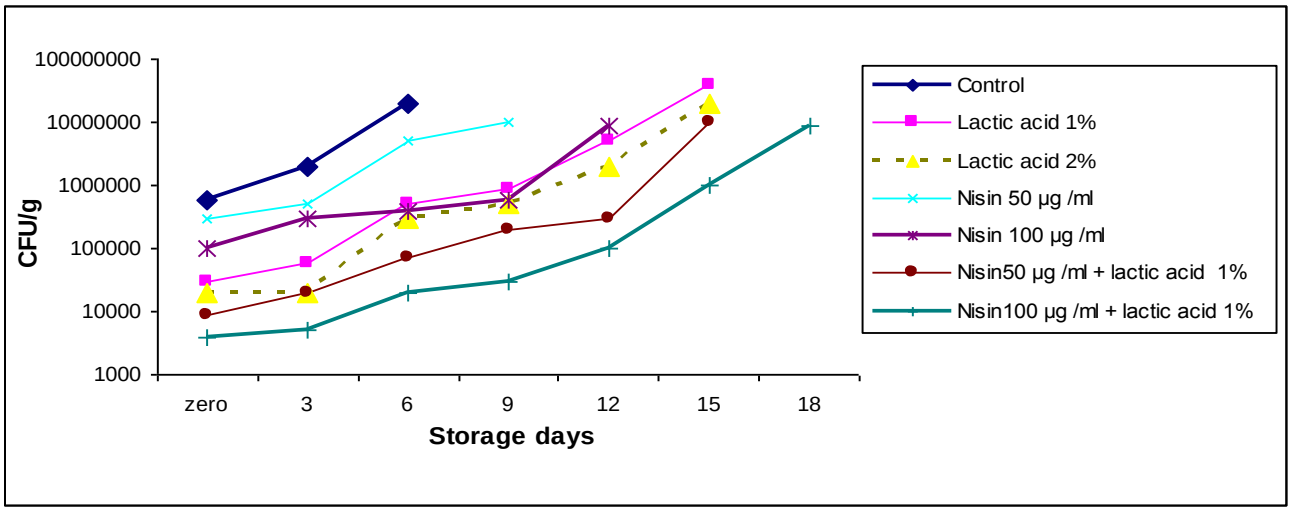

Figure 4.Effect of treatments on psychrophilic counts of packed broiler chicken breast samples during chilled storage at 3±1°C.

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Coliforms, fecal coliforms and *E. coli* (MPN)

There was a significant difference ($p < 0.05$) in coliforms (Most probable number) between control and treated groups except nisin (50µg/ml), and nisin (100µg/ml) treated one at day zero of chilled storage. Lactic acid (1 and 2%) treated groups had a higher significant reduction in coliforms (MPN) than nisin (100µg /ml) treated group Figure 5.

Nearly similar results were obtained by Tosun and Tamer (2000); Sinhamahapatra et al. (2004) and Gulmez et al. (2006) however higher values were recorded by Vatansever et al. (2008) While low values were recorded by Sinhamahapatra et al. (2004) and Killinger et al. (2010).

There was a gradual increase in coliforms (MPN) with chilled storage duration; this is in accordance with Vatansever et al. (2008). On contrary Gulmez et al. (2006) recorded that coliforms and fecal coliforms counts of lactic acid treated wings decreased during storage days. On the other hand the results indicated that nisin alone was less effective on coliforms (MPN) as their cell wall were less permeable to nisin. However it was cleared that dipping of broiler breast in nisin solution followed by lactic acid had a synergistic effect on the total coliforms.

As shown in Figure 6, nisin + lactic acid treated groups had the highest reduction in fecal coliforms counts at day zero of storage. Nearly similar results were obtained by Gulmez et al. (2006), however higher results were recorded by Vatansever et al. (2008). It was noticed that control group was significantly higher than all treated groups at 3rd day of storage. Fecal coliforms (MPN) decreased then gradually increased from 6th day of storage and through storage days. This is in agreement with Sinhamahapatra et al. (2004). On contrary Gulmez et al. (2006) recorded that coliforms and fecal coliforms counts of lactic acid treated wings decreased during storage days.

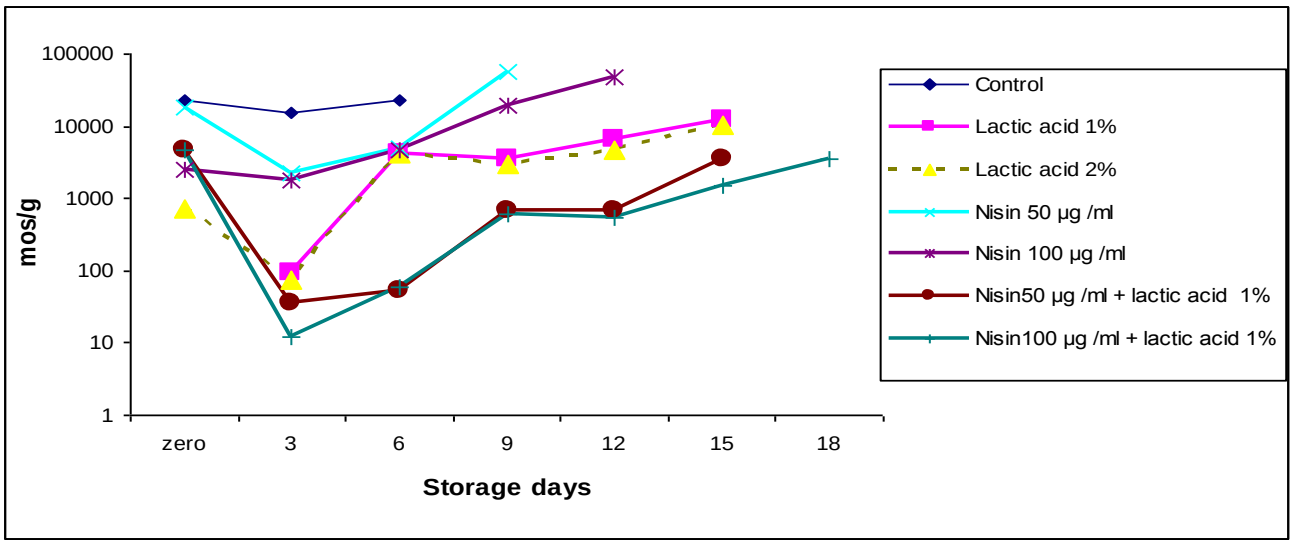

Figure 5. Effect of treatments on coliforms (MPN) of packed broiler chicken breast samples during chilled storage at 3±1°C.

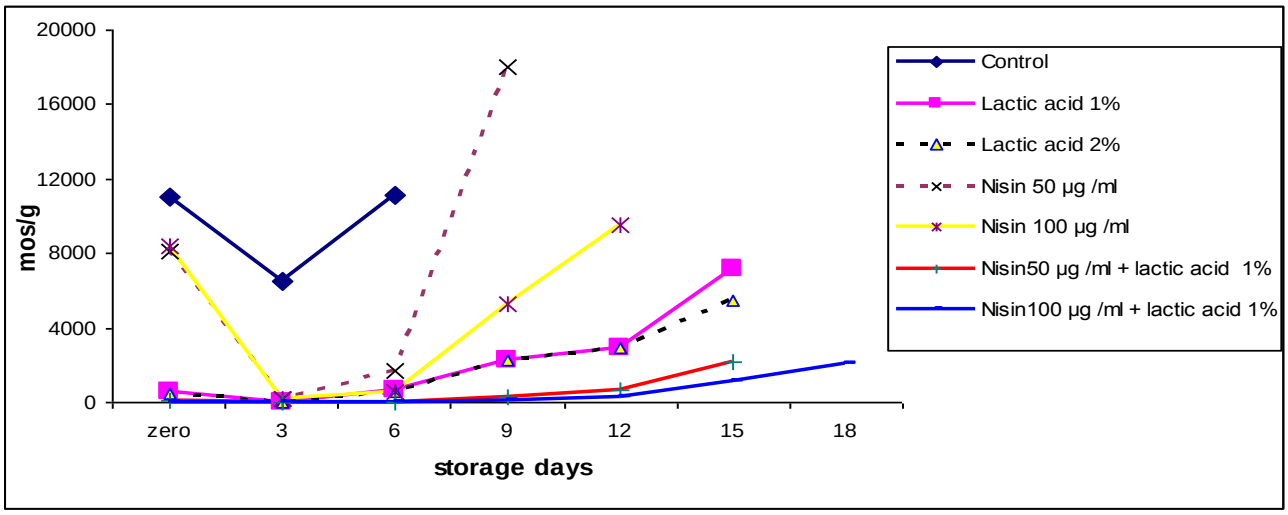

Figure 6. Effect of treatments on fecal coliforms (Most Probable Number) of packed broiler chicken breast samples during chilled storage at 3±1°C.

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E.coli (MPN) in control group was significantly higher than all treated groups at day zero of chilled storage, while the lowest values were recorded in nisin + lactic acid treated groups Figure 7. Nearly similar results were recorded by Hecer and Guldas (2011), higher results were recorded by Tosun and Tamer (2000). At 3rd day and 6th of storage control group was significantly higher than all treated groups, while at 9 th day of storage nisin (50µg/ml) treated group was significantly higher than other treated groups. Nisin 50µg/ml + lactic acid 1%, and nisin 100µg/ml + lactic acid 1% treated groups were more effective in reducing *E.coli* than other treated groups, thus suggesting that lactic acid increased the effect of nisin against *E.coli*. Presence of coliforms, fecal coliforms and *E. coli* in chicken carcasses indicates fecal contamination which may be attributed to the system of manual evisceration and unsatisfactory hygienic measures of handling and processing, this is in agreement with Whyte et al. (2004).

Gram negative bacteria are resistant to nisin because their cell walls are far less permeable than those of Gram positive bacteria. However, any treatment of Gram negative bacteria to make their cell walls permeable to nisin makes them susceptible to nisin. Such treatments include exposure to chelating agents, sub-lethal heat, osmotic shock and freezing (Delves-Broughton, 2005).

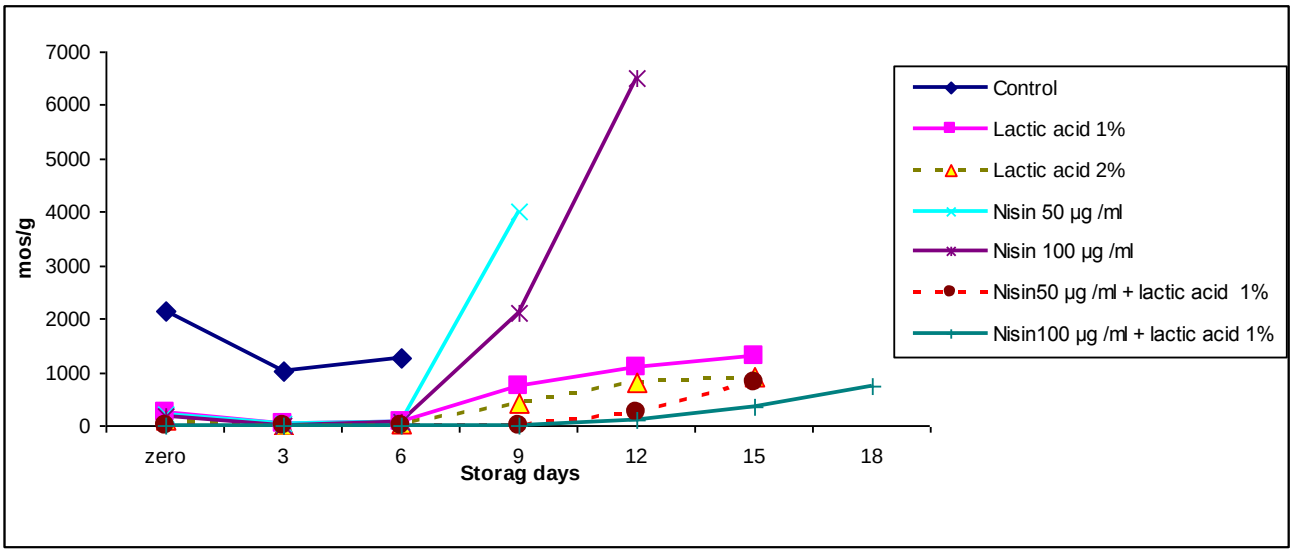

Figure 7. Effect of treatments on *E.coli* (MPN) of packed broiler chicken breast samples during chilled storage at 3±1°C.

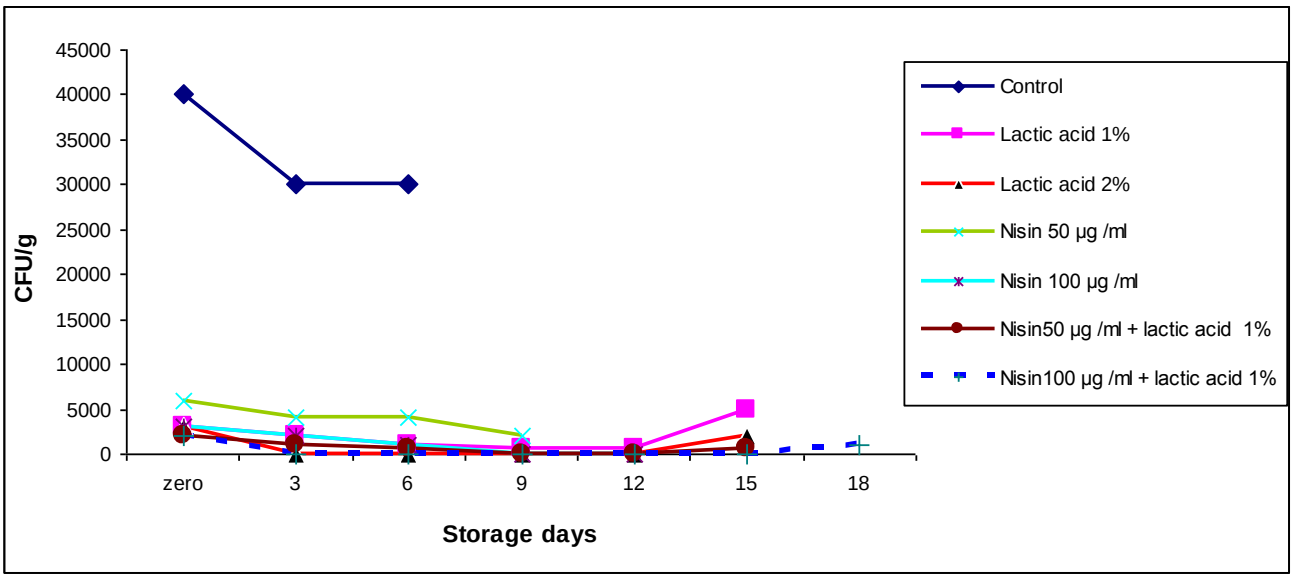

Figure 8. Effect of treatments on *S. aureus* count of packed broiler chicken breast samples during chilled storage at 3±1°C.

***Staphylococcus aureus* count**

The initial *S. aureus* count Figure 8 of control group was significantly higher than all treated groups at day zero, 3rd and 6th day of chilled storage ($P < 0.05$). Lactic acid (1%) treated group was significantly higher than lactic acid (2%), nisin (100µg/ml), nisin (50µg

/ml) + lactic acid (1%), and nisin (100µg/ml) + lactic acid (1%) treated groups at 12th day of storage. Nearly similar reduction effect was recorded by Marcel et al (1988); Antown (2002) and Smaoui et al (2011 and 2012)**.**

This decrease in count during storage days was in accordance with Hwang and Beuchat (1995) and Antown (2002). On contrarily Smaoui et al. (2011 and 2012) found a gradual increase in *S. aureus* counts during chilled storage. In this respect, Grisi and Gorlach-Lira (2005) found that in pure cultures, the growth of *S. aureus* was strongly inhibited by nisin for eight hours. *S. aureus* has often been tested in poultry products to assess microbiological safety, sanitation conditions, and product quality during processing and storage. The presence of *S. aureus* could occur due to inappropriate techniques applied, with regard to personal hygiene, abdomen opening, hand deboning or hand washing (Tompkin, 1983).

Nisin affects several Gram-positive bacteria such as *Staphylococcus spp*. but does not inhibit the majority of Gram-negative bacteria. Nisin initially forms pores in cell membrane and allows the efflux of essential cellular components resulting in inhibition or death of the bacteria (Abee et al. 1994 and Delves-Broughton, 2005).

CONCLUSION

From the previously mentioned data, it could be concluded that the dipping of broiler chicken breasts into nisin, lactic acid and their combination before refrigeration can retain the quality attributes and extend the shelf life for about 3-12 days more than the control during refrigerated storage. It was cleared that dipping of broiler chicken breasts in nisin solution followed by lactic acid had a synergistic effect on the bacterial load. Thus, it can be clearly expressed that nisin should be applied as combined with lactic acid application, rather than it's alone application.

Competing interests

The authors have no competing interests to declare.

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Influence of Feed Withdrawal Length on Carcass Traits and Technological Quality of Indigenous Chicken Meat Reared Under Traditional System in Benin

Ulbad Polycarpe Tougan[1,2,3]*, Gabriel Assouan Bonou[3], Thibaut Gbaguidi[3], Benoît Gbetondjingninougbo Koutinhouin[3], Serge Ahounou[3], Chakirath Salifou[3], Serge Mingnissè Zannou[1], Appolinaire Guy Mensah[4,5], Yves Beckers[2], Nadia Everaert[2], André Théwis[2], and Issaka Abdou Karim Youssao[3]

[1]*High National School of Agro-Pastoralism (ENSAP-Gogounou), University of Parakou, BP 2760, Abomey-Calavi, Republic of Benin*
[2]*Animal Sciences Unit, Gembloux Agro-Bio Tech, University of Liege, Passage des Déportés, 2, 5030 Gembloux, Belgium*
[3]*Laboratory of Animal Biotechnology and Meat Technology, Department of Animal Production and Health, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 BP 2009, Cotonou, Republic of Benin*
[4]*University of Abomey-Calavi, Faculty of Agronomic Science, Republic of Benin*
[5]*Agricultural Research Center of Agonkanmey, National Institute of Agricultural Research of Benin, 01 BP 884, Cotonou 01, Republic of Benin*
*Corresponding author's E-mail: ulcaless71@yahoo.fr

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ABSTRACT

The aim of the current study was to evaluate the effects of different feed withdrawal durations (0, 12 and 24 hours) on carcass traits and meat technological quality in local chicken of Benin. 30 South ecotype chickens of Benin were divided into 3 groups and slaughtered for the study after 12 hours of feed withdrawal. These chickens were all reared in free range according to the same traditional breeding system. The pH, weight of each carcass and the color of meat (breast and thigh) were determined. It appears that longer feed withdrawal periods significantly increased weight loss in chicken. The highest carcass weight, breast weight and carcass yields were recorded after 12 hours of feed withdrawal ($P < 0.05$). Technologically, the lowest pH values in the breast muscle at 1 hour, 8 hours, 16 hours and 20 hours post mortem were found in chickens slaughtered without any feed withdrawal ($P < 0.05$). At 12 and 24 hours post mortem, the highest pH values were noted in chickens slaughtered after 12 hours of feed withdrawal ($P < 0.01$). The live weight of control chickens and those slaughtered after 12 hours of feed withdrawal was highly and positively correlated with carcass weights ($P < 0.001$) but weakly and positively associated to breast weight and thigh-drumstick weight ($P < 0.05$); while after 24 hours of feed withdrawal, the live weight was moderately and positively correlated with the thigh-drumstick weight ($P < 0.01$, $r = 0.9$) but weakly associated to hot carcass weight and cold carcass weight ($P < 0.05$). After 24 hours of feed withdrawal, carcass yield was negatively correlated to breast drip loss ($P < 0.05$). Overall, longer feed withdrawal increased weight loss, pH, luminance and yellowness of meat but reduced its redness, water holding capacity and shear force.

Keywords: Indigenous chicken, Feed withdrawal, Carcass traits, Meat quality, Benin.

INTRODUCTION

Chickens in developing countries have more diverse use and benefits to household (Padhi, 2016). In countries of sub-Saharan Africa where food products are relatively in deficit, traditional chicken represents approximately 80% of the total poultry population and contributes to a significant proportion of meat production (25-70%) and eggs (12 to 36%). In Benin, the poultry provides a part of the nutritional needs of the family and more than 50% of farmers produce for subsistence and sometimes generation of some cash income by the commercialization of livestock products in the local market (Youssao et al., 2013). The indigenous chickens represent 81.3% of the national poultry flock (CountryStat, 2013) and are an important source of animal protein supply for the population and an income for producers and poultry sellers. Most of the national poultry production comes from the family poultry breeding which is composed mainly of local population of the species *Gallus gallus* (Tougan et al., 2013b). This population is composed of a variety of ecotypes: North, South, Sahoue, Fulani and Holli ecotypes (Tougan et al., 2013b). Among these ecotypes, the North ecotype in the north region of Benin and South ecotype in the South of Benin are the predominant breeds. These indigenous chicken populations have a remarkable heterogeneity in phenotypical and polymorphism traits. Several works were done on these local genetic types (Youssao et al., 2010 and Tougan et al., 2013b). Recent works carried out on carcass composition (Tougan et al., 2013a) and technological meat quality of these five ecotypes according to the breeding mode and slaughter age

(Tougan et al., 2013c) showed that important differences exist in meat quality. Moreover, the chemical composition of these local chicken meats was also affected by these factors (Tougan et al., 2013d). However, no data is available on the impact of pre-slaughter feed withdrawal periods on the carcass traits and technological quality of the local chicken populations of Benin. This work assesses the carcass traits and meat technological quality attributes of local chicken meat of South ecotype of Benin according to the pre-slaughter feed withdrawal periods and establishes the relationships between carcass traits and technological meat quality.

MATERIALS AND METHODS

Area of study

The study was conducted in Laboratory of Animal Biotechnology and Meat Technology of the Department of Animal Production and Health of of "Ecole Polytechnique d'Abomey-Calavi (EPAC)" in Benin. Chickens used in the current study were reared under traditional breeding system in the Commune of Abomey-Calavi (Figure 1) situated at a latitude of 6 ° 27 'North and at a longitude of 2 ° 21' East. The Commune of Abomey-Calavi covers an area of 650 km^2 with a population of 307,745 inhabitants (INSAE, 2010). This area exhibits climatic conditions of sub-equatorial type, characterized by two rainy seasons with an uneven spatial and temporal distribution of rainfall: major (from April to July) and minor (from September to November). These two seasons are separated by a dry season. Average rainfall is close to 1200 mm per year. The monthly average temperatures vary between 27 and 31°C and the relative air humidity fluctuates between 65%, from January to March, and 97%, from June to July. The study on the carcass composition was carried out.

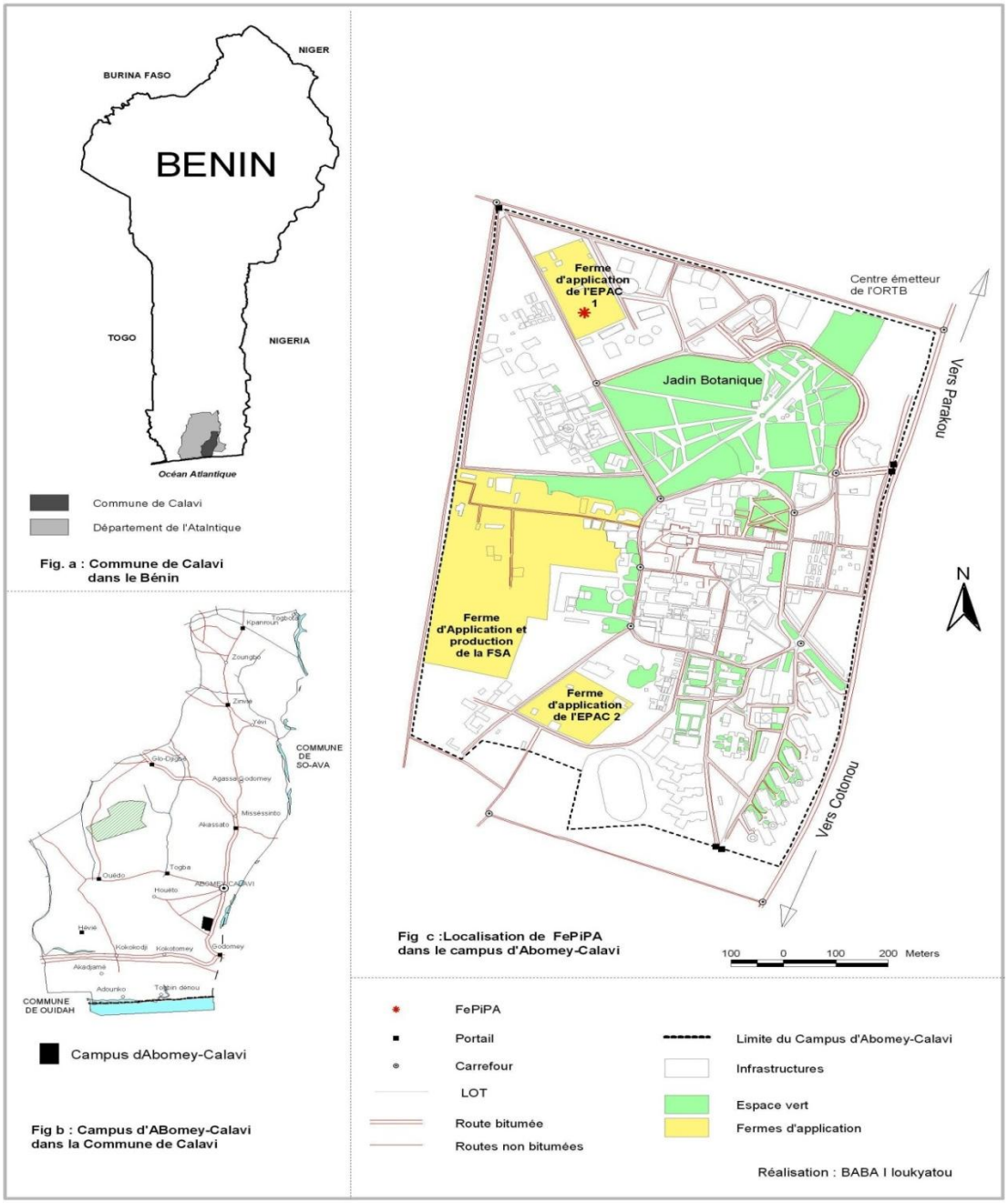

Figure 1. Area of study (Abomey-Calavi, Benin)

Birds rearing and sampling

Birds slaughtered in the current study are local chickens of South ecotypes of Benin. A total of 30 cockerels of 6 months old and 845 grams as live weight were divided into 3 groups of 10 chickens. These chickens were all reared in free range according to the same traditional breeding system. The scavenging of birds was the rule in this breeding system. The birds were in free range during the day but housed at night in rudimentary shelters (traditional henhouse made of mud, straw or wicker) or kept outside on any support that could serve as a perch. There are neither quantitative nor qualitative standards in their feeding. The birds fed themselves around concessions, by gleaning here and there, and occasionally receiving some grain supplement from the traditional breeder. Their diet was composed of energetic elements (kitchen waste, bran and etc), vitamins (green fodder, sprouted grains), minerals (salt, pounded shells) and protein (termites, legumes) (Youssao et al., 2013). Drinkable water was distributed in rudimentary watering tank. Various discarded containers were often used for drinking. In this type of farming, no health follow-up and no prophylactic standard were observed (Tougan et al., 2013b).

Feed withdrawal, slaughtering process and carcass cutting

The effect of feed withdrawal duration was evaluated from 3 different lots of 10 South ecotype chicken of Benin. Birds of lot 1 had undergone no feed withdrawal; and then slaughtered after being fed. In contrast, birds of lot 2 had undergone 12 hours of feed withdrawal; while birds of Lot 3 had undergone 24 hours of feed withdrawal.

The chickens were bled by section of the jugular vein and then scalded in hot water (70-80 °C) and plucked manually. Then, they were eviscerated and the heart, the kidney, the crop and the intestines were taken off. The legs were sectioned at the tibiotarsus-metatarsal articulation and the head separated from the neck at the cranium-atlas junction. The abdominal and thoracic cavity organs were then removed as well. The bird carcasses were refrigerated at 4°C for 24 hours and weighed. A cut of each carcass was used to determine the weights of breast, thigh-drumstick, wings and the rest of the carcass (Tougan et al., 2013b and Pripwai et al., 2014).

Data collecting

The live weight at slaughter, hot carcass weight at 1 hour post mortem, cold carcass weight at 24 hour post mortem, carcass cuts weight (breast, thigh-drumstick, wings, neck, tarsi and the rest of carcass) and the weight of the abdominal viscera (gizzard, liver and heart) were recorded at 24 hours post mortem. The abdominal fat was measured. The percentages of each carcass cut and abdominal offal component were calculated from the carcass weight. By the same way, carcass yield at 1 hour and 24 hours post-mortem were calculated.

The pH was measured in the breast muscle (Pectoralis major) and the thigh muscle (Ilio tibialis) at 2 cm depth. The pH was measured using a pH-meter (Hanna Instruments Inc., model HI99161) provided with a specialized probe and temperature control system. This apparatus was calibrated with two buffers pH-meter: pH = 4.0 and pH = 7.0 following a procedure provided by manufacturer. Between the measurements the muscle was stored at 4°C in an individual plastic container.

The colour of skin and meat (breast and thigh) were determined at 24 h post mortem using a Minolta chromameter CR-400 (Japan) in the trichromatic system (CIE L * a * b*). The surface was exposed to air for 20 min at room temperature before determining the color of the muscle. The readings were taken on equivalent positions. The tip of the chromameter measuring head was placed flat against the surface of the skin or of the meat for breast and thigh. For each reading, 6 measurements were performed and the average of these readings was considered as the final value. Meat color were expressed in the CIE L*a*b* dimensions of lightness (L*), redness (a*) and yellowness (b*). The colorimeter was calibrated using the specific white board (Minolta CR 400), before measurement began.

Drip loss was measured on intact fillets of the right side of the pectoralis major and thigh muscle (5 cm×3 cm×2 cm) and packaged in plastic bags, and then hung on wooden supports at 4 °C for 24 h, and calculations were made as a percentage of weight loss during storage.

The Water Holding Capacity was calculated by the sum of drip loss and cooking loss. Then, the breast and thigh-drumstick cuts used for drip loss were separately weighed and wrapped by placing inside vacuum bags (COPVAC 17025, Vigoclima S.L., Vigo, Spain), sealed without vacuum, and cooked placing vacuum-package bags in bain-marie (Memmert GmbH + Co, GK, Germany) until the core temperature reached 70°C (Franco et al., 2012). The core temperature was controlled by inserting the electrode of a digital thermometer (TestoAG, Lenzkirch, Germany) into the center of the meat sample for the duration of the boiling process. After boiling the samples were removed, cooled to room temperature, and reweighed. The cooking loss was calculated as the loss of weight during the boiling process and was expressed as a percentage as follows:

$$\text{Cooking loss (\%)} = \frac{\text{Weight loss}}{\text{Initial fresh meat weight}} \times 100$$

The samples prepared for the determination of the cooking loss were subsequently used for the Warner-Bratzler shear force analysis according to Bratcher et al. (2005). Cores with a diameter of 1.27cm were removed from the sample at different positions parallel to fiber orientation (longitudinal axis of the myofibres) and sheared as described by Honikel (1998). Shear force determinations were conducted on a texture analyzer LF plus (LLOYD Instruments) equipped with a Warner-Bratzler shear force head vertical to the fiber direction. The Warner-Bratzler single blade was used. The shear velocity was 200 mm/min. Each value was an average of at least 5 measurements.

Statistical analysis

The data collected on the carcass traits and meat technological quality were analyzed using the software SAS (Statistical Analysis System, 2006). The general linear model procedure of SAS was used for the analysis of variance. Means were compared pairwise by the Student t test. Comparisons between the parameters of the technological meat quality were also made between the 3 lots by type of muscle (thigh and breast). The threshold of significance used herein is 5%. The F test was used to determine the significance of effect of feed withdrawal duration on the carcass traits and meat technological quality.

RESULTS

Variation of carcass traits according to the feed withdrawal periods

The carcass traits of chicken of South ecotype of Benin are given by feed withdrawal periods in table 1. With the exception of breast weight, heart weight, spleen weight and the carcass yields measured at 1 hour and 24 hours post mortem, the other carcass traits did not vary significantly according to the length of the feed withdrawal ($P > 0.05$). Indeed, the breast weight, the heart weight, the spleen weight, the breast percentage and the carcass yields at 1 and 24 hours post-mortem was the highest ($P < 0.05$) in chickens that had undergone 12 hours of feed withdrawal.

Table 1. Variation of carcass traits according to the feed withdrawal periods

Variables	Feed withdrawal periods (hours)			ANOVA
	0	12	24	
	Mean ±S E	Mean ± SE	Mean ± SE	
Live weight (g)	870±33.26[a]	846.87±33.26[a]	816.5±33.26[a]	NS
Hot carcass weight (g)	624.82±22.78[a]	661.02±22.78[a]	625.99±22.78[a]	NS
Cold carcass weight (g)	614.44±21.59[a]	648.68±21.59[a]	618.56±21.59[a]	NS
Breast weight (g)	116.12±4.77[ac]	126.93±4.77[c]	109.25±4.77b[c]	*
Thigh-drumstick weight (g)	171.25±7.09[a]	177.42±7.09[a]	171.62±7.09[a]	NS
Neck weight (g)	38.12±1.62[a]	39.75±1.62[a]	40.25±1.62a	NS
Head weight (g)	30.75±1.23[a]	32±1.23[a]	31.75±1.23[a]	NS
Wing weight (g)	84.75±3.02[a]	90.62±3.02[a]	90.87±3.02[a]	NS
Tarsi weight (g)	29.87±1.9[a]	25.25±1.9[a]	27.5±1.9[a]	NS
Back weight (g)	96.75±4.86[a]	105.32±4.86[a]	105.25±4.86[a]	NS
Gizzard weight (g)	24.12±2.29[a]	24.87±2.29[a]	23.62±2.29[a]	NS
Liver weight (g)	17.5±1.06[a]	18.05±1.06[a]	14.62±1.06[a]	NS
Heart weight (g)	3.5±0.37[ac]	5.39±0.37[b]	2.75±0.37[c]	***
Spleen weight (g)	1.69±0.37[ac]	3.06±0.37[b]	1.21±0.4[c]	**
Carcass yield at 1 hour PM (%)	71.96±1.25[a]	78.11±1.25[b]	76.91±1.25[b]	**
Carcass yield at 24 hours PM (%)	70.79±1.1[a]	76.67±1.1[b]	75.97±1.1[b]	**
Breast percentage (%)	18.86±0.49[ab]	19.610.49±[a]	17.67±0.49[b]	*
Thigh-drumstick percentage (%)	27.89±0.48[a]	27.32±0.48[a]	27.71±0.48[a]	NS
Neck percentage (%)	6.21±0.2[a]	6.12±0.2[a]	6.51±0.2[a]	NS
Head percentage (%)	5.02±0.19[a]	4.95±0.19[a]	5.15±0.19[a]	NS
Wing percentage (%)	13.84±0.32[a]	13.99±0.32[a]	14.7±0.32[a]	NS
Tarsi percentage (%)	4.87±0.29[a]	3.9±0.29[a]	4.5±0.29[a]	NS
Back percentage (%)	15.7±0.37[a]	16.2±0.37[a]	16.97±0.37[a]	NS
Gizzard percentage (%)	3.89±0.28[a]	3.82±0.28[a]	3.81±0.28[a]	NS

*: p<0.05 ; **: p<0.01 ; ***: p<0.001; NS: Non Significant; ANOVA: Analysis of Variance; SE: Standard Error; The means between the classes of the same line followed by different letters differ significantly at the threshold of 5%.

Variation of technological quality

The technological meat quality properties varied depending on the length of feed withdrawal (Tables 2, and 3). In breast muscle, the pH recorded at 1 hour (pH1), 8 hours (pH 8), 16 hours (pH16) and 20 hours post-mortem (pH20) in chickens that hadn't undergone any feed withdrawal were higher than those measured in chickens submitted to 12 hours and 24 hours of feed withdrawal ($P < 0.05$). In thigh muscle, only the pH recorded at 24 hours post-mortem (PH24) varied significantly according to the time of feed withdrawal with the lowest value recorded in chickens that had not undergone any feed withdrawal while the highest value was recorded for feed withdrawal of 24 hours ($P < 0.05$). Similarly, the drip losses, the cooking losses and the water holding capacity did not vary according to the length of the feed withdrawal ($P > 0.05$).

In the breast meat, the different values of the luminance (L*) and redness (a*) measured at 1 hour, 8 hours, and 24 hours post mortem did not vary significantly according to the length of the feed withdrawal ($P > 0.05$). Nevertheless, the luminance of the breast meat recorded at 16 hours post mortem increased with the duration of FW ($P < 0.05$) while the redness (a*) decreased significantly with the length of feed withdrawal ($P < 0.05$). Furthermore, the yellowness (b*) recorded at 1 hour, and 8 hours post mortem decreased with the duration of FW with the highest value recorded in chickens that had not undergone any feed withdrawal ($P < 0.05$).

In thigh meat, the different values of the luminance measured at 1 hour, 8 hours, 16 hours and 24 hours post mortem were not affected by the feed withdrawal ($P > 0.05$). Similarly, except for the redness (a*) measured at 16 hours post mortem which decreased significantly with the duration of the feed withdrawal, the different a* values measured 1 hour, 8 hours, and 24 hours post mortem didn't vary according to the length of feed withdrawal ($P > 0.05$). Moreover, the yellowness values found at 8 hours, 16 hours and 24 hours post mortem remained constant regardless of the length of the FW ($P > 0.05$).

Correlations between carcass traits and offal components

The relationships between carcass traits and offal components of south chicken are given by feed withdrawal length in tables 4 and 5. Table 4 presents the correlations between carcass traits and offal components of south chicken that hadn't undergone any feed withdrawal. Table 5 presents under diagonal the correlations between carcass traits and offal components of south chicken that had undergone 12 hours of feed withdrawal and on top of the diagonal those of south chicken that had undergone 24 hours of feed withdrawal.

The live weight of chickens slaughtered without any feed withdrawal was highly and positively correlated with the hot carcass weight and the cold carcass weight ($0.91 \leq r \leq 0.93$; $P < 0.001$) but weakly and positively associated to the breast weight and thigh-drumstick weight ($0.75 \leq r \leq 0.76$; $P < 0.05$). No significant correlations were found between the carcass yields and the carcass traits and technological quality parameters (pH, L*, a*, b*, drip loss, cooking loss and water holding capacity. However, the wing weight of chickens slaughtered without any feed withdrawal was negatively and moderately correlated with pH 24 ($P < 0.01$, $r = -0.91$). Moreover, cooking loss of thigh were strongly and positively correlated ($P < 0.001$, $r = 0.98$) with the water holding capacity (WHC) of the thigh. Similarly, cooking loss in the breast was moderately and positively correlated ($P < 0.01$, $r = 0.92$) with the WHC of the breast.

In broilers slaughtered after 12 hours of feed withdrawal, the analysis of the correlation matrix also shows associations between different parameters of the carcass composition and technological meat quality. The live weight of chickens that had undergone 12 hours of feed withdrawal was strongly and positively correlated with the hot carcass weight, the cold carcass weight and thigh-drumstick weight ($P < 0.001$, $r \leq 0.94 \leq 0.96$) but moderately and positively associated with the breast weight, wing weight and thigh-drumstick weight. As found for broiler slaughtered without any feed withdrawal, no significant correlations were found between the carcass traits and technological quality parameters (pH, L*, a*, b*, drip loss, cooking loss and water holding capacity) except the positive correlation between the carcass yield recorded at 24 hours post-mortem and the pH 24 ($P < 0.05$, $r = 0.78$). However, cooking loss of thigh were strongly and positively correlated ($P < 0.001$, $r = 0.99$) with the water holding capacity of the thigh. Similarly, cooking loss in the breast was highly and positively correlated ($P < 0.001$, $r = 0.99$) with the water holding capacity of the breast.

In broilers slaughtered after 24 hours of feed withdrawal, the degree of linkage between the carcass traits and technological quality parameters (pH, L*, a*, b*, drip loss, cooking loss and water holding capacity) decreased compared to the chickens slaughtered without feed withdrawal and those slaughtered after 12 hours of FW. Indeed, the live weight of broilers that had undergone 24 hours of feed withdrawal was moderately and positively correlated with the thigh-drumstick weight ($P < 0.01$, $r = 0.9$) but weakly associated to the hot carcass weight and the cold carcass weight ($P < 0.05$, $0.82 \leq r \leq 0.85$). No significant correlations were found between the carcass traits and technological quality parameters (pH, L*, a*, b*, drip loss, cooking loss and water holding capacity) except the correlation between the wing weight and the thigh drip loss ($P < 0.05$, $r = -0.79$) and breast cooking loss ($P < 0.05$, $r = 0.78$). However, cooking loss of thigh were strongly and positively correlated ($P < 0.001$, $r = 0.96$) with the water holding capacity of the thigh. Similarly, cooking loss in the breast was highly and positively correlated ($P < 0.001$, $r = 0.99$) with the water holding capacity of the breast.

Table 2. Variation of meat pH, drip loss, cooking loss, water holding capacity and shear force according to the feed withdrawal periods

Variables	Muscle	Feed withdrawal periods			ANOVA
		0	12	24	
		Mean ± SE	Mean ± SE	Mean ± SE	
pH at 1 hour post mortem	Breast	5.74±0.08^{a}	5.71±0.08^{a}	5.43±0.08^{b}	*
	Thigh	5.93±0.07^{a}	5.96±0.07^{a}	5.77±0.07^{a}	NS
pH at 4 hours post mortem	Breast	5.35±0.05^{a}	5.52±0.05^{b}	5.29±0.05^{c}	**
	Thigh	5.55±0.08^{a}	5.76±0.08^{a}	5.68±0.08^{a}	NS
pH at 8 hours post mortem	Breast	5.41±0.04^{a}	5.37±0.04^{a}	5.25±0.04^{b}	*
	Thigh	5.62±0.04^{a}	5.59±0.04^{a}	5.58±0.04^{a}	NS
pH at 12 hours post mortem	Breast	5.33±0.03^{ab}	5.37±0.03^{a}	5.26±0.03^{b}	*
	Thigh	5.61±0.03^{a}	5.62±0.03^{a}	5.64±0.03^{a}	NS
pH at 16 hours post mortem	Breast	5.4±0.03^{a}	5.37±0.03^{ab}	5.28±0.03^{b}	*
	Thigh	5.75±0.05^{a}	5.63±0.05^{a}	5.67±0.05^{a}	NS
pH at 20 hours post mortem	Breast	5.39±0.03^{a}	5.38±0.03^{a}	5.3±0.03^{b}	*
	Thigh	5.67±0.04^{a}	5.58±0.04^{a}	5.64±0.04^{a}	NS
pH at 24 hours post mortem	Breast	5.42±0.03^{a}	5.34±0.03^{a}	5.38±0.03^{a}	NS
	Thigh	5.64±0.04^{a}	5.63±0.04^{a}	5.81±0.04^{b}	*
Drip loss (%)	Breast	3.06±0.26^{a}	4.29±0.26^{b}	4.84±0.26^{b}	***
	Thigh	2.65±0.19^{a}	2.82±0.19a	3.27±0.19^{a}	NS
Cooking loss (%)	Breast	30.21±1.47^{a}	31.31±1.47^{a}	26.19±1.47^{b}	*
	Thigh	31.7±1.13^{a}	32.06±1.13^{a}	30.59±1.13^{a}	NS
Water Holding Capacity (%)	Breast	33.27±1.47^{a}	35.6±1.47^{a}	31.01±1.47^{a}	NS
	Thigh	34.35±1.16^{a}	34.92±1.16^{a}	33.86±1.16^{a}	NS
Shear Force (N)	Breast	39.35±2.21^{a}	38.65±2.21^{a}	38.45±2.21^{a}	NS
	Thigh	50.21±1.18^{a}	49.25±1.18^{a}	49.48±1.18^{a}	NS

*: p<0.05; **: p<0.01; ***: p<0.001; NS: Non Significant; ANOVA: Analysis of Variance; SE : Standard Error; The means between the classes of the same line followed by different letters differ significantly with the threshold of 5%.

Table 3. Variation of meat color (L*, a*, b*; CIE Lab) according to the feed withdrawal periods

Variables		Feed withdrawal periods (hours)			ANOVA
		0	12	24	
		Mean ±S E	Mean ± SE	Mean ± SE	
Thigh Color at 1 hour PM	L*	40.79±1.21^{a}	39.31±1.21^{a}	43.1±1.21^{a}	NS
	a*	12.77±0.88^{a}	12.86±0.88^{a}	11.26±0.88^{a}	NS
	b*	5.66±0.63^{a}	3.04±0.63^{b}	6.79±0.63^{a}	**
Breast Color at 1 hour PM	L*	58.25±2.55^{a}	54.87±2.55^{a}	60.33±2.55^{a}	NS
	a*	1.51±0.62^{a}	1.68±0.62^{a}	2.31±0.62^{a}	NS
	b*	4±0.95^{a}	3.16±0.95^{c}	2.31±0.95^{bc}	*
Thigh Color at 8 hours PM	L*	38.68±1.27^{a}	40.75±1.27^{a}	37.81±1.27^{a}	NS
	a*	11.72±0.79^{a}	11.51±0.79^{a}	13.18±0.79^{a}	NS
	b*	4.86±0.85^{a}	4.45±0.85^{a}	4.01±0.85^{a}	NS
Breast Color at 8 hours PM	L*	54.59±2.06^{a}	53.93±2.06^{a}	52.28±2.06^{a}	NS
	a*	3.65±0.6^{a}	2.51±0.6^{a}	3.06±0.6^{a}	NS
	b*	8.6±0.71^{a}	4.15±0.71^{b}	8.23±0.71^{a}	***
Thigh Color at 8 hours PM	L*	38.19±1.69^{a}	40.04±1.69^{a}	41.37±1.69^{a}	NS
	a*	12.38±0.79^{a}	10.99±0.79^{a}	13.81±0.79^{a}	NS
	b*	5.81±0.62^{a}	4.57±0.62^{a}	6.15±0.62^{a}	NS
Breast Color at 8 hours PM	L*	51.22±2.77^{a}	53.65±2.77^{a}	53.96±2.77^{a}	NS
	a*	3.06±0.64^{a}	2.23±0.64^{a}	3.35±0.64^{a}	NS
	b*	7.36±0.81^{ab}	4.98±0.81^{a}	8.19±0.81^{b}	*
Thigh Color at 16 hours PM	L*	38.01±1.81^{a}	40.87±1.81^{a}	40.59±1.81^{a}	NS
	a*	12.47±0.79^{ac}	9.54±0.79^{b}	11.87±0.79^{c}	*
	b*	6.18±0.68^{a}	5.86±0.68^{a}	7.65±0.68^{a}	NS
Breast Color at 16 hours PM	L*	51.14±2.32^{ac}	58.09±2.32^{bc}	53.38±2.32^{c}	*
	a*	3.8±0.64^{a}	2.39±0.64^{b}	3.52±0.64^{a}	*
	b*	8.01±0.75^{c}	6.16±0.75^{a}	9.77±0.75^{b}	*
Thigh Color at 24 hours PM	L*	41.42±2.16^{a}	43.49±2.16^{a}	41.7±2.16^{a}	NS
	a*	11.86±0.99^{a}	10.72±0.99^{a}	10.06±0.99^{a}	NS
	b*	4.54±0.79^{a}	5.49±0.79^{a}	7.17±0.79^{a}	NS
Breast Color at 24 hours PM	L*	50.34±3.79^{a}	50.29±3.79^{a}	45.7±3.79^{a}	NS
	a*	3.8±0.7^{a}	2.8±0.7^{a}	3.82±0.7^{a}	NS
	b*	6.83±0.89^{a}	5.4±0.89^{a}	7.7±0.89^{a}	NS

*: p<0.05; **: p<0.01; ***: p<0.001; NS: Non Significant; ANOVA: Analysis of Variance; SE: Standard Error; L*: Luminance, a*: red index, b*: Yellow index; CIE Lab: Laboratory of the International Centre of illumination. The means between the classes of the same line followed by different letters differ significantly with the threshold of 5%.

Table 4. Correlations between carcass traits and technological quality of meat of indigenous broilers slaughtered without feed withdrawal

Variables	LW	HCW	CCW	BW	TDW	WW	CY24	L*TD24	a*TD24	b*TD24	L*BR24	a*BR24	b*BR24	pH24BR	pH24TD	DLTD	DLBR	CLTD	CLBR	WHCTD	WHCBR
LW	1																				
HCW	0.91**	1																			
CCW	0.93***	0.99***	1																		
BW	0.76*	0.87**	0.86**	1																	
TDW	0.75*	0.82*	0.83*	0.48^{NS}	1																
WW	0.51^{NS}	0.56^{NS}	0.57^{NS}	0.3^{NS}	0.74*	1															
CY24	-0.54^{NS}	-0.15^{NS}	-0.19^{NS}	-0.55^{NS}	-0.11^{NS}	-0.04^{NS}	1														
L*TD24	0.48^{NS}	0.59^{NS}	0.59^{NS}	0.61^{NS}	0.35^{NS}	0.55^{NS}	0.09^{NS}	1													
a*TD24	-0.36^{NS}	-0.44^{NS}	-0.44^{NS}	-0.61^{NS}	-0.15^{NS}	-0.38^{NS}	-0.02^{NS}	-0.74*	1												
b*TD24	-0.43^{NS}	-0.17^{NS}	-0.19^{NS}	0.005^{NS}	-0.62^{NS}	0.04^{NS}	0.68^{NS}	0.06^{NS}	-0.15^{NS}	1											
L*BR24	-0.33^{NS}	-0.61^{NS}	-0.58^{NS}	-0.68^{NS}	-0.42^{NS}	-0.51^{NS}	-0.45^{NS}	-0.53^{NS}	0.17^{NS}	0.47^{NS}	1										
a*BR24	-0.21^{NS}	-0.17^{NS}	-0.16^{NS}	-0.32^{NS}	0.27^{NS}	0.60^{NS}	0.19^{NS}	0.001^{NS}	0.22^{NS}	0.55^{NS}	-0.13^{NS}	1									
b*BR24	-0.66^{NS}	-0.52^{NS}	-0.53^{NS}	-0.46^{NS}	-0.46^{NS}	-0.28^{NS}	0.54^{NS}	0.19^{NS}	-0.26^{NS}	0.31^{NS}	0.25^{NS}	0.06^{NS}	1								
pH24BR	-0.7^{NS}	-0.63^{NS}	-0.66^{NS}	-0.45^{NS}	-0.72*	-0.91**	0.35^{NS}	-0.51^{NS}	0.17^{NS}	0.08^{NS}	0.39^{NS}	-0.49^{NS}	0.51^{NS}	1							
pH24TD	-0.02^{NS}	-0.09^{NS}	-0.08^{NS}	-0.26^{NS}	0.005^{NS}	-0.32^{NS}	-0.14^{NS}	0.003^{NS}	-0.34^{NS}	-0.38^{NS}	0.67^{NS}	-0.32^{NS}	0.48^{NS}	0.31^{NS}	1						
DLTD	-0.46^{NS}	-0.28^{NS}	-0.31^{NS}	-0.15^{NS}	-0.39^{NS}	-0.47^{NS}	0.54^{NS}	-0.39^{NS}	0.28^{NS}	-0.05^{NS}	0.05^{NS}	-0.38^{NS}	0.20^{NS}	0.60^{NS}	0.06^{NS}	1					
DLBR	-0.004^{NS}	0.11^{NS}	0.09^{NS}	0.43^{NS}	-0.21^{NS}	-0.49^{NS}	0.18^{NS}	0.13^{NS}	-0.55^{NS}	0.47^{NS}	-0.25^{NS}	-0.37^{NS}	-0.55^{NS}	0.17^{NS}	0.37^{NS}	-0.07^{NS}	1				
CLTD	-0.14^{NS}	-0.19^{NS}	-0.17^{NS}	-0.19^{NS}	-0.05^{NS}	0.25^{NS}	-0.01^{NS}	0.17^{NS}	-0.23^{NS}	-0.01^{NS}	0.32^{NS}	0.40^{NS}	0.31^{NS}	-0.30^{NS}	0.27^{NS}	0.07^{NS}	-0.05^{NS}	1			
CLBR	-0.13^{NS}	-0.32^{NS}	-0.28^{NS}	-0.50^{NS}	0.11^{NS}	-0.14^{NS}	-0.34^{NS}	-0.50^{NS}	0.12^{NS}	0.04^{NS}	0.68^{NS}	0.37^{NS}	0.01^{NS}	0.02^{NS}	0.46^{NS}	-0.32^{NS}	-0.11^{NS}	0.24^{NS}	1		
WHCTD	-0.24^{NS}	-0.24^{NS}	-0.23^{NS}	-0.21^{NS}	-0.14^{NS}	0.14^{NS}	0.13^{NS}	0.09^{NS}	-0.18^{NS}	-0.02^{NS}	0.32^{NS}	0.30^{NS}	0.34^{NS}	-0.16^{NS}	0.27^{NS}	0.27^{NS}	-0.45^{NS}	0.98***	0.15^{NS}	1	
WHCBR	-0.13^{NS}	-0.27^{NS}	-0.24^{NS}	-0.31^{NS}	0.01^{NS}	-0.34^{NS}	-0.25^{NS}	-0.45^{NS}	-0.08^{NS}	0.23^{NS}	0.55^{NS}	0.21^{NS}	0.03^{NS}	0.17^{NS}	0.41^{NS}	-0.33^{NS}	0.29^{NS}	0.02^{NS}	0.92^{**}	-0.05^{NS}	1

***** : p<0.05; **** :** p<0.01; ***** :** p<0.001; NS: Non Significant ; LW: Live Weight; HCW: Hot Carcass Weight; CCW: Cold Carcass Weight; BW: Breast Weight; TDW: Thigh-drumstick Weight; WW: Wing Weight; CY24: Carcass Yield at 24 hours PM; L*TD24: Luminance of Thigh-drumstick at 24 hours PM; a*TD24: Redness of Thigh-drumstick at 24 hours PM; b*TD24: Yellowness of Thigh-drumstick at 24 hours PM; L*BR24: Luminance of breast at 24 hours PM; a*BR24: Redness of breast at 24 hours PM; b*BR24: Yellowness of breast at 24 hours PM; pH24BR: pH of breast at 24 hours PM; pH24TD: pH of Thigh-drumstick at 24 hours PM; DLTD: Drip loss of Thigh-drumstick; DLBR: Drip loss of breast; CLTD: Cooking loss of Thigh-drumstick; CLBR: Cooking loss of Breast; WHCTD: Water Holding Capacity of Thigh-drumstick; WHCBR: Water Holding Capacity of Breast.

Table 5. Correlations between carcass traits and technological quality of meat of indigenous broilers slaughtered after 12 hours (below diagonal) and 24 hours (above diagonal) of feed withdrawal

Variables	LW	HCW	CCW	BW	TDW	WW	CY24	L*TD24	a*TD24	b*TD24	L*BR24	a*BR24	b*BR24	pH24BR	pH24TD	DLTD	DLBR	CLTD	CLBR	WHCTD	WHCBR
LW	1	0.82*	0.85*	0.7^{NS}	0.9**	0.47^{NS}	-0.33^{NS}	0.67^{NS}	0.42^{NS}	0.50^{NS}	-0.06^{NS}	0.15^{NS}	-0.66^{NS}	-0.61^{NS}	-0.11^{NS}	-0.53^{NS}	0.51^{NS}	0.51^{NS}	0.52^{NS}	0.33^{NS}	0.57^{NS}
HCW	0.94***	1	0.99***	0.4^{NS}	0.98***	0.8*	0.26^{NS}	0.69^{NS}	0.33^{NS}	0.53^{NS}	-0.42^{NS}	0.41^{NS}	-0.47^{NS}	-0.61^{NS}	-0.22^{NS}	-0.60^{NS}	-0.00003^{NS}	0.43^{NS}	0.56^{NS}	0.24^{NS}	0.56^{NS}
CCW	0.96***	0.99***	1	0.44^{NS}	0.99***	0.8*	0.21^{NS}	0.72^{NS}	0.29^{NS}	0.50^{NS}	-0.37^{NS}	0.43^{NS}	-0.53^{NS}	-0.62^{NS}	-0.20^{NS}	-0.64^{NS}	0.04^{NS}	0.44^{NS}	0.62^{NS}	0.25^{NS}	0.62^{NS}
BW	0.85**	0.78*	0.81*	1	0.45^{NS}	0.11^{NS}	-0.49^{NS}	0.77*	0.03^{NS}	0.06^{NS}	0.01^{NS}	-0.18^{NS}	-0.91^{NS}	0.07^{NS}	0.55^{NS}	-0.51^{NS}	0.62^{NS}	-0.09^{NS}	0.40^{NS}	-0.24^{NS}	0.46^{NS}
TDW	0.85**	0.94***	0.93***	0.61^{NS}	1	0.76*	0.11^{NS}	0.69^{NS}	0.34^{NS}	0.53^{NS}	-0.28^{NS}	0.42^{NS}	-0.53^{NS}	-0.67^{NS}	-0.24^{NS}	-0.61^{NS}	0.14^{NS}	0.51^{NS}	0.61^{NS}	0.31^{NS}	0.62^{NS}
WW	0.92**	0.91**	0.93***	0.93***	0.79*	1	0.59^{NS}	0.55^{NS}	-0.03^{NS}	0.01^{NS}	-0.35^{NS}	0.72^{NS}	-0.37^{NS}	-0.49^{NS}	-0.02^{NS}	-0.79*	-0.36^{NS}	0.12^{NS}	0.78*	-0.09^{NS}	0.74^{NS}
CY24	-0.31^{NS}	0.02^{NS}	-0.04^{NS}	-0.28^{NS}	0.13^{NS}	-0.15^{NS}	1	0.07^{NS}	-0.30^{NS}	-0.06^{NS}	-0.53^{NS}	0.49^{NS}	0.24^{NS}	0.06^{NS}	-0.10^{NS}	-0.18^{NS}	-0.87*	-0.20^{NS}	0.16^{NS}	-0.22^{NS}	0.07^{NS}
L*TD24	0.55^{NS}	0.67^{NS}	0.66^{NS}	0.24^{NS}	0.75*	0.49^{NS}	0.22^{NS}	1	-0.23^{NS}	0.13^{NS}	-0.33^{NS}	0.15^{NS}	-0.87**	-0.02^{NS}	0.33^{NS}	-0.75^{NS}	0.11^{NS}	0.06^{NS}	0.67^{NS}	-0.14^{NS}	0.67^{NS}
a*TD24	-0.39^{NS}	-0.22^{NS}	-0.25^{NS}	-0.22^{NS}	-0.35^{NS}	-0.29^{NS}	0.50^{NS}	0.01^{NS}	1	0.57^{NS}	-0.22^{NS}	-0.28^{NS}	0.27^{NS}	-0.67^{NS}	-0.46^{NS}	0.21^{NS}	0.24^{NS}	0.43^{NS}	-0.36^{NS}	0.45^{NS}	-0.33^{NS}
b*TD24	0.29^{NS}	0.24^{NS}	0.24^{NS}	0.18^{NS}	0.39^{NS}	0.16^{NS}	-0.16^{NS}	0.06^{NS}	-0.70^{NS}	1	-0.22^{NS}	-0.08^{NS}	0.07^{NS}	-0.43^{NS}	-0.67^{NS}	0.32^{NS}	0.22^{NS}	0.65^{NS}	-0.26^{NS}	0.70^{NS}	-0.24^{NS}
L*BR24	-0.22^{NS}	-0.30^{NS}	-0.30^{NS}	-0.43^{NS}	-0.21^{NS}	-0.29^{NS}	-0.20^{NS}	-0.33^{NS}	-0.42^{NS}	-0.14^{NS}	1	0.28^{NS}	-0.09^{NS}	0.03^{NS}	0.12^{NS}	0.25^{NS}	0.65^{NS}	-0.07^{NS}	0.09^{NS}	-0.01^{NS}	0.16^{NS}
a*BR24	-0.26^{NS}	-0.11^{NS}	-0.12^{NS}	-0.07^{NS}	-0.001^{NS}	-0.14^{NS}	0.51^{NS}	0.08^{NS}	0.16^{NS}	0.45^{NS}	-0.67^{NS}	1	-0.18^{NS}	-0.36^{NS}	-0.07^{NS}	-0.40^{NS}	-0.14^{NS}	0.01^{NS}	0.68^{NS}	-0.09^{NS}	0.66^{NS}
b*BR24	0.22^{NS}	0.28^{NS}	0.27^{NS}	0.36^{NS}	0.28^{NS}	0.34^{NS}	0.16^{NS}	-0.26^{NS}	-0.41^{NS}	0.6^{NS}	-0.04^{NS}	0.35^{NS}	1	-0.09^{NS}	-0.58^{NS}	0.70^{NS}	-0.47^{NS}	0.13^{NS}	-0.69^{NS}	0.33^{NS}	-0.73^{NS}
pH24BR	-0.37^{NS}	-0.11^{NS}	-0.15^{NS}	-0.40^{NS}	0.03^{NS}	-0.35^{NS}	0.78*	0.35^{NS}	0.58^{NS}	-0.03^{NS}	-0.51^{NS}	0.67^{NS}	-0.21^{NS}	1	0.63^{NS}	0.24^{NS}	-0.11^{NS}	-0.72^{NS}	-0.30^{NS}	-0.61^{NS}	-0.31^{NS}
pH24TD	0.11^{NS}	-0.04^{NS}	-0.01^{NS}	0.18^{NS}	-0.11^{NS}	-0.06^{NS}	-0.40^{NS}	-0.44^{NS}	-0.06^{NS}	0.002^{NS}	0.11^{NS}	-0.38^{NS}	-0.13^{NS}	-0.26^{NS}	1	-0.37^{NS}	0.17^{NS}	-0.85^{NS}	0.24^{NS}	-0.92**	0.26^{NS}
DLTD	-0.25^{NS}	-0.02^{NS}	-0.06^{NS}	-0.42^{NS}	0.13^{NS}	-0.29^{NS}	0.62^{NS}	0.59^{NS}	0.44^{NS}	0.02^{NS}	-0.45^{NS}	0.58^{NS}	-0.32^{NS}	0.91**	-0.48^{NS}	1	0.11^{NS}	0.02^{NS}	-0.90**	0.28^{NS}	-0.88**
DLBR	-0.05^{NS}	-0.14^{NS}	-0.11^{NS}	0.04^{NS}	-0.23^{NS}	-0.08^{NS}	-0.27^{NS}	0.27^{NS}	0.40^{NS}	-0.19^{NS}	-0.64^{NS}	0.24^{NS}	-0.60^{NS}	0.25^{NS}	-0.12^{NS}	0.38^{NS}	1	0.12^{NS}	0.03^{NS}	0.12^{NS}	0.13^{NS}
CLTD	-0.42^{NS}	-0.55^{NS}	-0.54^{NS}	-0.55^{NS}	-0.54^{NS}	-0.64^{NS}	-0.35^{NS}	-0.20^{NS}	0.24^{NS}	-0.39^{NS}	0.29^{NS}	-0.43^{NS}	-0.82*	0.03^{NS}	0.47^{NS}	0.06^{NS}	0.35^{NS}	1	0.08^{NS}	0.96***	0.09^{NS}
CLBR	0.31^{NS}	0.33^{NS}	0.34^{NS}	0.13^{NS}	0.53^{NS}	0.27^{NS}	0.04^{NS}	0.35^{NS}	-0.52^{NS}	0.26^{NS}	0.25^{NS}	-0.11^{NS}	-0.06^{NS}	0.01^{NS}	0.23^{NS}	-0.001^{NS}	-0.34^{NS}	-0.02^{NS}	1	-0.16^{NS}	0.99***
WHCTD	-0.45^{NS}	-0.55^{NS}	-0.54^{NS}	-0.60^{NS}	-0.52^{NS}	-0.66^{NS}	-0.27^{NS}	-0.14^{NS}	0.28^{NS}	-0.38^{NS}	0.24^{NS}	-0.36^{NS}	-0.84**	0.13^{NS}	0.41^{NS}	0.17^{NS}	0.38^{NS}	0.99***	-0.02^{NS}	1	-0.15^{NS}
WHCBR	0.32^{NS}	0.32^{NS}	0.33^{NS}	0.14^{NS}	0.22^{NS}	0.27^{NS}	0.01^{NS}	0.40^{NS}	-0.49^{NS}	0.25^{NS}	0.18^{NS}	-0.08^{NS}	-0.14^{NS}	0.05^{NS}	0.22^{NS}	0.05^{NS}	-0.22^{NS}	0.02^{NS}	0.99***	0.02^{NS}	1

***** : p<0.05; ****:** p<0.01 ; *****:** p<0.001; NS: Non Significant; LW: Live Weight; HCW: Hot Carcass Weight; CCW : Cold Carcass Weight; BW: Breast Weight; TDW : Thigh-drumstick Weight; WW: Wing Weight; CY24: Carcass Yield at 24 hours PM; L*TD24: Luminance of Thigh-drumstick at 24 hours PM; a*TD24: Redness of Thigh-drumstick at 24 hours PM ; b*TD24: Yellowness of Thigh-drumstick at 24 hours PM; L*BR24: Luminance of breast at 24 hours PM; a*BR24: Redness of breast at 24 hours PM; b*BR24: Yellowness of breast at 24 hours PM; pH24BR: pH of breast at 24 hours PM; pH24TD: pH of Thigh-drumstick at 24 hours PM; DLTD: Drip loss of Thigh-drumstick; DLBR: Drip loss of breast; CLTD: Cooking loss of Thigh-drumstick; CLBR: Cooking loss of Breast; WHCTD: Water Holding Capacity of Thigh-drumstick; WHCBR: Water Holding Capacity of Breast.

DISCUSSION

Variability of the carcass traits according to the feed withdrawal periods

The weight of the different carcass cuts did not vary significantly according to the length of feed withdrawal. These observations are comparable to the findings of Contreras-Castillo et al. (2007). The similarity observed between the pre-slaughter live weights of the birds at the beginning of the experiment shows that the three experimental groups are statistically homogeneous. The average slaughter live weight of the south local chicken recorded herein at 6 months old (845 ± 26.8 grams) is consistent with the values reported by Tougan et al. (2013a) for the same ecotype of chicken.

In the current study, as carcass yields, the breast weight, the breast percentage, the heart weight, the spleen weight were affected by the duration of the water diet. These results are consistent with those reported by Contreras-Castillo et al. (2007) and Eman (2014). According Rosenvold et al. (2001), carcasses yields in chicken increased with the extension of the feed withdrawal duration. This important change in carcass yield of broilers depending on the feed withdrawal length could be due to the fact that the changes in digestive tract content is most important in birds that had undergone a short feed withdrawal time (Lyon et al.,2004). As found by Petracci et al. (2010), length of fasting is important because it affects carcass yield (live weight losses), carcass contamination and product safety (pathogenic and spoilage bacteria) and quality (ultimate muscle pH). The recommended length of time without feed for birds before slaughtering is between 8 and 12 hours, as this allows the majority of the flock to evacuate remaining fecal matter, and minimizes any negative effects on body weight and carcass (Bilgili, 2002 and Northcutt et al., 2003). Weight loss of the birds during the period between FW and processing is called as live shrink or shrinkage (Bilgili, 2002). After broilers have been without feed for more than 6 hours, they begin to draw moisture and nutrients from their own body tissues and this weight loss may then affect edible yield (Northcutt, 2010). Birds lose 0.18% of body weight per hour during the withdrawal period, to a maximum of 0.42% (Northcutt, 2001; Nijdam et al., 2005 and Petracci et al., 2010). In the first 4-6 hours, weight loss in birds is mainly due to gastric emptying, so carcass yield is not negatively influenced (Petracci et al.,2010). After 6 hours, there are losses in moisture and nutrients from body tissues, which can affect carcass yield (Petracci et al., 2010). Pripwai et al. (2014) had recorded a carcass yield of 70% in Thai indigenous chickens at 14 weeks of age, after fasting for 12 hours before slaughter.

Overall, the results of carcass traits parameters found in this study at 28 weeks old are below those reported by Choo et al. (2014), Cassandro et al. (2015), Liu et al. (2015), Raphulu et al. (2015) and Padhi et al. (2016).

This variability among results can be due to several factors such as genotype, age, sex, production system, feeding, feed and water withdrawal, transport, slaughter process, post mortem aging time (Tougan et al., 2013a). These factors can promote a significant difference in carcass traits and parameters of technological quality in chicken meat (Thobela et al., 2015 and Padhi et al., 2016).

Variation of technological quality of meat according to the feed withdrawal periods

The variation observed between pH values according to the feed withdrawal period herein confirms the findings of Contreras-Castillo et al. (2007) and Eman (2014). The different pH values recorded herein varying between 5 and 6 are comparable to those reported in the literature (Hasan, 2012; Tougan et al., 2013b and 2013c). The highest pH values found in meat after 24 hours of feed withdrawal may be due to the starvation and dehydration that can lead to depletion of muscle glycogen and reduction in weight (Adzitey, 2011).

The thigh and breast meat color (CIELAB; L * a * b *) were also affected by the length of the liquid diet. This variation of the color parameters of the meat according to the feed withdrawal periods is in agreement with the results reported by Rosenvold et al. (2001) and Eman (2014). Lyon et al. (2002) have also shown that feed withdrawal increased the luminance of meat in chicken (from 46.1 to 48.9) and the yellowness (from 2.8 to 3.7) but reduced the redness (from 4.1 to 3.1).

Correlations between carcass traits and technological quality of meat

Overall, the live weight of chickens slaughtered without any feed withdrawal was highly and positively correlated with the hot carcass weight and the cold carcass weight but weakly and positively associated to the breast weight and thigh-drumstick weight, while in broilers slaughtered after 24 hours of feed withdrawal, the live weight was moderately and positively correlated with the thigh-drumstick weight but weakly associated to the hot carcass weight and the cold carcass weight. This difference in the relationships between the carcass traits and quality according to the feed withdrawal period could be due to the variation of digestive tract contents at slaughtering time. According to Tougan et al. (2013a) and Tougan et al. (2013b), the slaughter live weight in South chicken slaughtered after

12 hours of feed withdrawal is highly and positively correlated with the hot carcass weight, breast weight, thigh-drumstick weight, rest of carcass weight, tarsi weight, neck weight and head weight ($P<0.001$; $0.62 \leq r \leq 0.99$), moderately associated to the cold carcass yield and wing weight, weakly and positively associated to the gizzard weight ($P < 0.05$; $r = 0.44$), but negatively correlated with the carcass drip loss ($P<0.01$, $r= -0.51$). Moreover, according to these authors, the dry matter content of the meat is positively correlated with the index of redness (a *), the chromaticity (C *), the protein content and fat content but negatively associated the luminance among five types studied genetic. Furthermore, similar relationships to the current findings between carcass traits and meat technological quality properties are reported by Musa et al. (2006b) in chickens Anka and Rugao strains and Olawumi (2013) in chickens and Arbor Acre in Nigeria. Moreover, Ojedapo et al. (2008) showed strong phenotypic positive correlations between live weight and carcass weight (0.95), thigh weight (0.93) and breast weight (0.97) in broilers. Similar correlations were also obtained by Musa et al. (2006) and Chabault et al. (2012).

CONCLUSION

The present study carried out on the variability of carcass characteristics and technological meat quality of broilers of South ecotype of Benin according to the pre-slaughter feed withdrawal periods reveals that the weight loss decreases when feed withdrawal period increases confirming that increasing feed withdrawal times will result in higher carcass yield. The highest carcass yields can be obtained with a feed withdrawal of 12 hours. Technologically, luminance and the yellow index of the meat increases with longer feed withdrawal period while the index of red decreases. Furthermore, it is important to note that the breast was most affected by the variation of feed withdrawal periods compared to the thigh meat.

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Competing interests

The authors have no competing interests to declare.

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Physiological Condition of First Female and Male Offspring of Japanese Quail (*Coturnix japonica*) whose Parents were Supplemented by Turmeric Powder

Tyas Rini Saraswati, Silvana Tana
Department of Biology, Faculty of Science and Mathematic, Diponegoro University, Semarang, Central Java, Indonesia.
*Corresponding author's Email: tyasrinis63@gmail.com

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ABSTRACT

The study was carried out to determine the physiological condition of the first female and male offspring of Japanese quail (*Coturnix japonica*) whose parents were supplemented by turmeric powder. This study consisted of two stages. In the first stage, 45 female quails aged 1 week were divided into 3 groups; P0: control; P1: supplemented by 54 mg turmeric powder/quail/day, P2: supplemented by 108 mg turmeric powder/quail/day. Each group consisted of 15 quails. Fertile eggs were collected from each treatment and incubated until hatched. Forty five females and 45 males offspring quails were collected from each treatment. The second stage consisted of 3 groups; K0: offspring of quail whose parents were not supplemented by turmeric powder (P0); K1: offspring of quail whose parents were supplemented by turmeric powder 54 mg/quail/day (P1); K2: offspring of quail whose parents were supplemented by turmeric powder 108 mg/quail/day (P2). This study implemented completely randomized design experimental method. It is proven that turmeric powder supplementation increased the levels of vitellogenin, HDL, vitamin B12, vitamin A, white egg protein, linoleic acid, arachidonic acid in eggs. In contrast, the cholesterol levels, LDL and total fat of eggs decreased. However, no significant changes of the oleic acid level were observed. On the second stage for both K1 and K2 in the case of the first female offspring of Japanese quail, the weight of carcass, SGPT, cholesterol serum, triglycerides serum and liver cholesterol increased, but LDL and SGOT serum decreased. Beside the weight of carcass, there were no significant changes for other parameters of the first male offspring of Japanese quail.

Key words: Japanese quail, Quality of egg, Physiological condition, Turmeric powder

INTRODUCTION

Growth and development of quail offspring after hatching depends on the development of embryo during incubation. Normal embryonic growth and development depend on a complete supply of all required nutrients within the egg (Wilson, 1997). The quality of chemical substances in the yolk can be improved by giving turmeric powder supplement in the feed of the quail. This idea is rooted from the fact that turmeric powder contains curcumin of 7.97 % (Saraswati et al., 2013), antioxidant, anti-inflammatory, antibacterial and antivirus substances (Vashan et al., 2012; Kilany et al., 2014; Khosravifar et al., 2014; Alagawany et al., 2015) that have a role in improving liver function (Saraswati et al., 2013 and Nabavi et al., 2014). Liver is a place for vitellogenin biosintesis as a material to form yolk (Saraswati et al., 2013 and Moussavi et al., 2009). The triggering factor of vitellogenin synthesis is estrogen hormone that is synthesized under the axis hipotalamus-hipofisis-gonad regulation (Levi et al., 2009 and Hachfi et al., 2012). Estrogen receptor is located in cytosol or nucleus of targeted cells (Wollf et al., 2011; Khoshnoud et al., 2011 and Kocanova et al., 2010). Turmeric powder contains 6.79% of phytoestrogen (Saraswati et al., 2013), which has an activity to bind to the estrogen receptor (Turner et al., 2007 and Harris et al., 2005). Phytoestrogen with estrogen receptor binding causes an increase in vitellogenin synthesis (Levi et al., 2009).

Supplementation of turmeric powder increases levels of DNA in liver (Saraswati et al., 2013) with regard to the role of curcumin to induce several transcription factors, cytokinase, growth factors, and other enzymes. Turmeric powder supplementation causes estriol hormone fluctuation resulting in shortening one ovulation cycle for 2 hours 35 min (Saraswati et al., 2014).

Turmeric powder supplementation by 54 mg/ quail/ day could improve the physiological condition of quail,

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increase vitellogenin level, and also increased the quality of yolk (Saraswati et al., 2013). Turmeric powder supplementation up to 108 mg/quail/day decreased the triglyceride level in the blood (Putra et al., 2015). Vitellogenin in the form of VLDL is transported from the liver through the blood stream system and brought to the ovary (Ito et al., 2003 and Vezina et al., 2003).

Egg yolk is the main source of protein, minerals, vitamins and lipids for the development of the embryo (Duce et al., 2011). Supplementation of turmeric powder accelerates the incubation period. Turmeric powder supplementation up to 108 mg/quail/day shortened the incubation period of quail embryo (Saraswati et al., 2015), because turmeric powder supplementation improved the physical and chemical quality of the first eggs of Japanese quail. Based on the above information, we studied the physiological condition of the first offspring Japanese quail females and males whose parents were given turmeric powder supplements.

MATERIAL AND METHODS

Material

One hundred *Coturnix japonica* at age of one day was obtained from Colomadu, Solo, Central Java, Indonesia. Turmeric powder was made from 8 kg turmeric rhizome at age of 9 months. Cages and equipments were fumigated with disinfectants before used. The DOQ was acclimated for 1 week. Each cage consisted of three quails.

Acclimation on battery cage

Acclimation was followed in a battery cages for one week. During the experiment feed and drinking water were provided *ad libitum*. Vita chick (PT. Medion, Indonesia) at dosage of 5 g/12 L water were given to the quail on the third day through drinking water. Anti-stress vitamins were given to increase endurance and prevent stress at the time before and after vaccination, after cutting the beak, transfer to other cage, bad weather, and a falling feather. Vaccination with Newcastle disease vaccine was given at the age of 3 days old and also at the age of 36 days old. These vaccines were delivered via eye drops.

Methods

This experiment consisted of two stages. In the first stage, a total of 45 female quails aged 1 week were divided into three experimental groups, the control group that received basal diet without turmeric powder (P0), second group treated by turmeric powder 54 mg/quail/day (P1), third group treated by turmeric powder 108 mg/ quail/ day (P2). Each group consisted of 15 quails. The treatment was given every day, starting at age of 15 days for 1 month. Each cage was given 1 male quail age three months so that the produced eggs were fertile eggs. The observed parameters were the content of vitellogenin, cholesterol, HDL, LDL, vitamin B12, vitamin A, protein of white egg, fat, oleic acid, linoleic acid and arachidonic acid of eggs that were produced in third month.

In the second stage, the physiological conditions of post-hatching quail offspring were observed. The fertile eggs were collected from eggs that were laid by quails at aged of 3 months from the controlled treatment and the one that was produced by quails treated with turmeric powder. The eggs were incubated in an incubator at temperature 37-38^{o}C with humidity 60% until hatched. Forty five female and 45 male offspring of quail that hatched from each treatment were collected. The experiment was divided into 3 groups; K0: offspring of quail whose parents were not supplemented by turmeric powder (P0); K1: offspring of quail whose parents were supplemented by turmeric powder 54 mg/quail/day (P1); K2: offspring of quail whose parents were supplemented by turmeric powder 108 mg/quail/day (P2). So, there were 135 female offsprings and 135 male offsprings of Japanese quail. They were maintained by giving the standard feed intake (crude protein 18.8%, fat content 3.5%, ash 10%, total carbohydrate 50.78% and crude fiber 6%) and drinking water *ad libitum.* Physiological condition of the offspring quails were observed by measuring the carcass weight and analyzing the chemical components of the blood taken at the end of the treatment. Blood was collected via vena jugularis and the serum was taken. Chemical analysis of blood consisted of SGPT and SGOT levels by Reitman and Frankle method (Bigoniya et al., 2009), glucose and cholesterol with CHO-PAP method (Elwakkad et al., 2012), triglycerides with GPO-PAP method (Bekal et al., 2011), serum HDL and LDL, and liver cholesterol by the Liebermann Burchard method (Puwastien et al., 2011).

The obtained data were analyzed using analysis of variance (ANOVA), Analyses were done by software SAS 9.1 for windows. Variant means were separated using Duncan's Multiple range Post hoc Test. P values <0.05 were considered significant.

RESULTS

The result of the first stage included the chemical quality of eggs produced in the third month from quails in control group and quails in supplemented by turmeric powder

group at dose of 54 mg/quail/day and 108 mg/quail/day presented in table 1. Results of the analysis of turmeric powder supplementation showed significant results in level of vitellogenin, cholesterol, HDL, LDL, vitamin B12, vitamin A, white protein, total fat, linoleic acid, arachidonic acid of eggs ($P<0.05$). There was no significance at levels of oleic fatty acid ($P> 0.05$). The levels of vitellogenin, HDL, vitamin B12, vitamin A, protein of white egg, linoleic acid and arachidonic acid increased, but the levels of cholesterol and fat decreased.

Physiological condition of the first offspring of female quails whose parents were supplemented by turmeric powder for a month before being sexually mature are described in table 2. The results showed that turmeric powder supplementation had a significant effect on the physiological condition of the first offspring of female quails including the levels of SGPT, SGOT, triglycerides serum, serum LDL, liver cholesterol and carcass weight ($P <0.05$), but not significant on levels of blood glucose and serum HDL ($P> 0.05$). Levels of SGPT, serum cholesterol and triglycerides, liver cholesterol, and carcass weight increased, however, levels of serum SGOT and LDL decreased.

Physiological condition of the first male offspring of quails whose parents were supplemented by turmeric powder for a month before being sexually mature presented in table 3. The analysis results of the effect of turmeric powder supplementation to the physiological condition of the first male offspring of quails indicated that they were not significant in level of SGPT, SGOT, glucose, cholesterol, triglycerides, LDL, HDL serum, liver cholesterol, and carcass weight ($P > 0.05$).

Table 1. Chemical quality of eggs produced by Japanese quail at age of three month after turmeric powder supplementation

Parameter	P0 (Control)	P1 54 mg/quail/day	P2 108 mg/quail/day
Vitellogenin (mg/100g)	0.77±0.31^{b}	0.95±0.11^{b}	1.13±0.57^{a}
Cholesterol (mg/100g)	747.63±23.82^{a}	689.51±19.45^{b}	653.27±12.69^{c}
HDL (mg/100g)	108.33±4.07^{b}	112.91±4.98^{b}	121.06±5.79^{a}
LDL (mg/100g)	139.56±6.74^{a}	128.71±2.99^{b}	121.04±4.86^{c}
Vitamin B12(mg/100g)	1.29±0.05^{c}	1.48±0.11^{b}	1.66±0.07^{a}
Vitamin A (SI)	543.72±12.44^{c}	555.39±17.13^{b}	575.47±11.23^{a}
Protein of white egg (%)	16.12±0.87^{b}	17.56±0.67^{a}	17.55±0.62^{a}
Fat(%)	31.15±1.64^{a}	30.03±0.21^{b}	28.92±0.77^{b}
Oleic acid (%)	1.99±0.12	2.06±0.06	2.09±0.08
Linoleic acid (%)	0.49±0.06^{b}	0.53±0.04^{b}	0.64±0.03^{a}
Arachidonic acid (%)	0.034±0.01^{b}	0.04±0.01^{b}	0.06±0.01^{a}

Different small letters in superscript at the same row indicate a significant result ($P < 0.05$); P0: Control group; P1: Supplemented by 54 mg turmeric powder/quail/day; P2: Supplemented by 108 mg turmeric powder/day

Table 2. Biochemical parameters and carcass weight of the first female offspring of Japanese quail aged two months whose parents were supplemented by turmeric powder

Parameter	K0	K1	K2
Carcass weight (g)	65.56±0.82^{b}	71.40±3.29^{a}	69.03±0.88^{a}
SGPT (U/L)	33.47±0.95^{b}	33.44±0.19^{b}	34.07±0.05^{a}
SGOT (U/L)	31.92±0.36^{b}	31.32±0.33^{ab}	31.25±0.05^{a}
Blood glucose (mg/dl)	109.59±0.24^{a}	107.99±1.12^{b}	109.8±0.5^{a}
Cholesterol serum (mg/dl)	99.82±0.5^{b}	100.09±0.01^{b}	101.08±0.62^{a}
Triglycerides serum(mg/dl)	53.25±0.16^{b}	52.18±0.11^{b}	54.08±0.96^{a}
LDL serum (mg/dl)	42.73±0.16^{a}	42.4±0.9^{b}	42.15±0.01^{c}
HDL serum (mg/dl)	44.81±1.56^{a}	44.59±0.61^{a}	44.08±0.52^{a}
Liver Cholesterol (mg/100g)	199.1±0.92^{b}	200.19±0.19^{b}	202.99±1.69^{a}

Different small letters in superscript at the same row indicate a significant result ($P < 0.05$); K0: offspring of quail whose parents were not supplemented by turmeric powder (P0); K1: offspring of quail whose parents were supplemented by turmeric powder 54 mg/quail/day (P1); K2: offspring of quail whose parents were supplemented by turmeric powder 108 mg/quail/day (P2).

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Table 3. Biochemical parameters and carcass weight of the first male offspring of Japanese quail aged two months whose parents were supplemented by turmeric powder

Parameter	K0	K1	K2
SGPT (U/L)	34.05±0.2[a]	34.13±0.02[a]	34.46±0.64[a]
SGOT (U/L)	31.7±0.75[a]	31.78±0.8[a]	32.2±0.15[a]
Blood glucose (mg/dl)	109.33±0.73[a]	110.83±0.82[a]	108.24±4.89[a]
Cholesterol serum (mg/dl)	98.83±1.19[a]	101.89±0.9[a]	101.52±3.15[a]
Triglycerids serum(mg/dl)	54.37±0.45[a]	54.96±0.4[a]	54.89±1.25[a]
LDL serum (mg/dl)	42.26±0.19[a]	43.07±0.2[a]	43.04±0.76[a]
HDL serum (mg/dl)	45.97±0.14[a]	44.79±0.7[a]	44.81±1.28[a]
Liver cholesterol (mg/100g)	198.03±6.72[a]	199.47±1.47[a]	203.67±7.09[a]

Note: Different small letters in superscript at the same row showed a significant result ($P < 0.05$); K0: offspring of quail whose parents were not supplemented by turmeric powder (P0); K1: offspring of quail whose parents were supplemented by turmeric powder 54 mg/quail/day (P1); K2: offspring of quail whose parents were supplemented by turmeric powder 108 mg/quail/day (P2).

DISCUSSION

The increase in dose of turmeric powder supplementation up to 108 mg/quail/day increased the levels of vitellogenin in yolk. The results of previous studies showed that turmeric powder increased levels of vitellogenin in plasma (Saraswati et al., 2013). The more vitellogenin were synthesized, there were more follicles developed (Saraswati et al., 2013). As more follicle hierarchies were developed, vitellogenin were distributed to many follicles, which lead to the reduction in cholesterol levels as a constituent of vitellogenin. Finally, egg cholesterol levels decreased. According to Qinna et al. (2012), curcumin induces changes in the expression of genes involved in cholesterol homeostasis. The homeostasis of cholesterol in body mainly depends on its synthesis, absorption from intestine and secretion of the bile, of which the metabolic process is under precise regulation (Li et al., 2015). Curcumin acts on the stimulation of the enzyme activity of hepatic cholesterol-7α-hydroxylase, which catalyzes the conversion of cholesterol into bile salts. Due to stimulation of this enzyme by curcumin, the changes in hepatic cholesterol into bile salts were increased, resulting in decreased levels of cholesterol. Since curcumin has the ability to activate genes in liver cells to increase production of LDL receptors, turmeric powder supplementation lowered LDL levels quail eggs (Peschel et al., 2007). With the increase in the available LDL receptors, liver cells could eliminate a large amount of LDL cholesterol in the blood so that LDL in eggs also decreases. Reduction of LDL in the egg was followed by an increase of HDL levels in eggs.

The results indicated that turmeric powder can increase the level and absorption of vitamin B12 and vitamin A in eggs. Vitamin B 12 is soluble in water, while vitamin A is fat soluble (Park et al., 2015). Turmeric helps in the digestion of food and absorption of fats and fat-soluble vitamins. Turmeric is also known to assist and improve the intestinal flora. Supplementation by 2 g/kg of curcumin increases mucous colonies 1.8 mol/g (Irving et al., 2011). Curcumin could alter the motility of *Salmonella*, a bacterium pathogen in the intestine (Marathe et al., 2016), so that the absorption in the intestine can be advanced.

The protein in eggs was found in the white part of the egg. The white part of the egg is secreted when the egg passes through the magnum part of the reproductive tract. The more hierarchies' follicles developed; there would be more follicles that ovulated. It will lead the cells in the magnum to synthesize and secrete the white egg, so that proteins in egg will be increased. From the analysis of essential fatty acids in eggs, it was indicated that turmeric powder could improve metabolic processes of essential fatty acids which was shown with the increase of the content of essential fatty acids include linoleic acid, arachidonic acid, but had not led to an increase in oleic acid. Results of the first stage of the study showed an increase in chemical quality of quail eggs which were supplemented by turmeric powder.

Supplementation of turmeric powder to quail for a month before becoming sexually mature affects the physiological condition of the first female offspring quails. Improvement of physiological conditions of the first offspring quails female were indicated by an increase in carcass weight. This is in line with the research conducted by (Yudha et al., 2013), turmeric powder supplementation increases quail's muscle diameter. Carcass weight is a product of metabolism produced by the organism.

Physiological conditions of the first offspring of quails were determined by the development of embryo during the incubation period. Embryo of quail whose parents were given turmeric powder supplements before becoming sexually mature will be born 2 days faster than usual (Saraswati and Tana, 2016). The development during embryonic period was influenced by the nutrients stored in the yolk.

Along with the increased activity of liver in the vitellogenin biosynthesis, there was also an increase in metabolic processes, as shown by an increase of cholesterol in liver and also cholesterol and triglycerides in serum. Vitellogenin biosynthesis occurs in the liver, so that the liver cholesterol levels increased. Vitellogenin subsequently transported through the bloodstream to the ovaries and will accumulate as the yolk, so that the levels of cholesterol and triglycerides in the blood experienced a slight increase, but still in the normal range. Increased activity of liver cells can cause damage in some cells. This damage was indicated by the increased levels of SGPT level, but the SGPT was still at normal level (<35 U/L). Increased SGPT is an indicator of liver damage (Parmar et al., 2016). There was no significant difference in SGOT and it was at normal levels (< 45) which suggests that the internal organs were also in good condition.

Increased physiological conditions of the first female offspring of quail were also shown by the decrease of LDL levels in serum. Turmeric powder supplementation was able to induce the formation of LDL receptors, resulting in decreased levels of LDL serum in the first female offspring of quail. Curcumin induces genes activation in the liver cells to increase production of mRNA, thereby increases the production of LDL receptors in the liver. With the increased amount of available LDL-receptors, liver cells can eliminate a large amount of LDL cholesterol from the body. Curcumin can enhance the liver cells to synthesize LDL receptor mRNA seven times more compared to cells that did not receive curcumin (Peschel et al., 2007). The decrease in LDL levels has not been followed by an increase in serum HDL levels. There was significance for the level of blood glucose between K0 and K1 and also between K1 and K2, but between K0 and K2 there was no significant differences. However, blood glucose level for all K0, K1, and K2 were still in normal condition. This suggests that the metabolic processes that occur in the body are running well.

Physiological conditions of the first male offspring of quails did not show any significant in all parameters and at normal conditions. The insignificance was due to the physiological activity of male quails was simpler than the physiological activity of female quail. The first female offspring of quails whose parents were given turmeric powder supplements started to produce eggs at the age of 42 days, so that the metabolism activity became more complex and happened at early age.

CONCLUSION

From the results of this study, it can be concluded that supplementation of turmeric powder was capable to improve the chemical quality of eggs. Eggs are a good source of nutrients for the development of the embryo, so that better physiological conditions of male and female offspring of quail can be obtained after consuming the turmeric powder.

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Competing interests

The authors of present study have no competing interest to declare.

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Quality Characteristics of Whole Guinea Fowl Egg as Binder in Beef and Chevon Burgers

Frederick Adzitey*, Peter Birteeb and Benjamin Kwasi Holdbrook
Department of Animal Science, Faculty of Agriculture, University for Development studies, P.O. Box TL 1882, Tamale, Ghana
*Corresponding author's Email: adzitey@yahoo.co.uk

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ABSTRACT

This study was conducted to determine the cohesiveness of whole guinea fowl egg as a binder in chevon and beef burgers. The study also investigated the sensory characteristics, nutritional content, cooking loss, lateral shrinkage, welling and doming of the beef and chevon burgers prepared using whole guinea fowl eggs. A total of 3 kg beef and 3 kg chevon were used. The meats were assigned using complete randomized design into 3 levels. The 3 levels of inclusion of the whole guinea fowl egg per kilogram of meat were 0 g, 50 g and 100 g which corresponds to each treatment that is B1 (control), B2 (5 %) and B3 (9 %) for beef, and C1 (control), C2 (5 %) and C3 (9 %) for chevon, respectively. Thus each treatment contained 1 kg meat, 0.5 g red pepper, 1.0 g black pepper, 1.0 g white pepper, 2.0 g mixed spice (adobo®), 5 g salt and whole guinea fowl egg (0 g, 50 g or 100 g). The meat and spices were minced and moulded manually into burgers using a cylindrical tube to obtain uniform shapes and sizes. They were vacuum-packed in transparent packaging bags and stored overnight at 4 °C prior to processing. The processed samples were evaluated for their sensory, nutritional and binding properties. Sensory characteristics of beef and chevon burgers (cohesiveness, colour, juiciness, texture, taste, flavor and overall liking) showed no significant differences ($P > 0.05$). In absolute terms beef and chevon burgers with the highest inclusion level (9 %) of whole guinea fowl egg were most preferred. There were also no significant differences ($P > 0.05$) in moisture content, crude protein content, pH, cooking loss, lateral shrinkage and doming of the beef and chevon burgers. Significant difference ($P < 0.05$) occurred in the crude fat content of chevon burger but not beef burger. Welling was not observed in the beef and chevon burgers.

Key words: Binder, Burgers, Guinea fowl eggs, Nutritional, Sensory

INTRODUCTION

Meat is the edible muscle, fat and other tissues obtained from an animal after it has been slaughtered (Lawrie and Ledward, 2006). Meat obtained from cattle (beef) and goats (chevon) are red meat (Warriss 2000 and Adzitey, 2013). Red meat is important source of various nutrients. Red meat contains high amount of essential amino acids that play a major role in the growth and development of our bodies (Warriss, 2000). It is a source of long-chain omega-3 polyunsaturated fats, riboflavin, pantothenic acid, selenium and vitamin D (Williams, 2007). Red meat is an excellent source of Vitamin B6 (pyridoxine), Vitamin B12 (cobalamin), Vitamin B3 (niacin), iron, zinc and phosphorus (Williams, 2007; McAfee et al., 2010 and FOA, 2014). It is also sources of a range of endogenous antioxidants and other bioactive substances including taurine, carnitine, carnosine, ubiquinone, glutathione and creatine (Williams, 2007). The nutrient composition and importance of red meat calls for the need for Africans particularly people in rural communities to consume enough red meat. To reduce wastage, preserve and add value to red meats there is the need to process them into various meat products.

Meat processing refers to the procedures such as addition of ingredients and/or mechanical action that convert meat into specific products (Teye, 2007). Meat can be processed into different meat products such as sausages, frankfurter, bacon, meat loaf, burgers, meatballs and many more (FAO, 1991; Adzitey et al., 2014; Adu-Adjei et al., 2014; Teye et al., 2014; Adzitey et al., 2015a; Haslia et al., 2015a; Haslia et al., 2015a; Teye et al., 2015a; Teye et al., 2015b and Ossom et al., 2016). Burgers are among the meat products of importance and are prepared from minced meats with the addition of spices, additives and other ingredients, normally shaped into a circular form. There are different types of burgers on the market made from the meat of different animals. For

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instance beef burger, chicken burger and lamb burger are consumed by many people (FAO, 1991; Adu-Adjei et al., 2014; Anonymous, 2014). The characteristics and quality of burgers influence consumers' acceptability. The characteristics of burgers are influenced by the ingredients that are used to prepare the burger. When meat extenders are added they can help to improve yield, improve meat emulsification stability, improve water binding stability, enhance texture and flavour, reduce shrinkage during cooking, improve slicing characteristics and reduce formulation cost (FAO, 1991; Warriss, 2000; Adu-Adjei et al., 2014). Non-meat ingredients such as water, salt, sugar, fillers, binders and spices are used to impact flavour, slow bacterial growth and increase the yield of meat products (Tronsky et al., 2004; FAO, 2010). It has been reported that eggs are suitable as binders in burgers (Chen, 1999).

Guinea fowl meat is a favourite meat for many Ghanaians because of it nutritional value and low fat content (Gyebi, 2012). The demand for guinea fowl meat in Ghana far exceeds the supply and the implication is that guinea fowl production will continue to increase (Adzitey et al., 2015b). With the increasing production and demand for guinea fowl meats, its exploitation for use as meat products is important. Nevertheless, the effect of whole guinea fowl egg as a binder in burgers is unknown. This work investigates the effects of adding whole guinea fowl eggs to beef and chevon burgers. This was to evaluate the extent to which the use of whole guinea fowl egg to beef and chevon burgers would influence the sensory, nutritional and binding properties of the burgers.

MATERIALS AND METHODS

Study area

The study was conducted at the Meat Processing Unit of the University for Development Studies (UDS), Nyankpala, Ghana. Chemical analysis of the meat products were conducted at the Spanish Laboratory of UDS, Nyankpala, Ghana.

Preparation of guinea fowl egg, beef and chevon burgers

Table eggs from guinea fowls were cracked and whisked to ensure that the yolk and albumen were well mixed. Three kilogram each of lean beef and chevon were obtained from the UDS Meat Processing Unit and used for the experiment. The meat was thawed overnight at 4^{o}C and minced using table top mincer (Teller Ramon, Spain) through a 5 millimeter sieve. The minced beef and chevon were divided into three treatments per kilogram each, mixed with spices of 0.5 gram (g) red pepper, 1.0 g black pepper, 1.0 g white pepper, 2.0 g mixed spice (adobo®) and 5 g salt. Each treatment was mixed until a desired consistency was obtained. The three experimental treatments of both beef and chevon were formulated with 0 g, 50 g and 100 g inclusion level of whole guinea fowl egg per kilogram of beef and chevon which corresponded to products B1 (0g, control), B2 (50 g, 5 %), B3 (100 g, 9 %), C1 (0 g, control), C2 (50 g, 5 %) and C3 (100 g, 9 %), respectively. The mixed meat with spices was then moulded into circular shapes. The products were stored in a deep freezer for further processing and analyses.

Welling, doming, lateral shrinkage and cooking loss of beef and chevon burgers

These were done as previously described by earlier workers (Adzitey et al., 2014). Welling is the accumulation of fluid in vacuole of a burger and it is determined by observation. Doming (thickness) is the rise in height of a burger and was determined by measuring the height of burger before and after cooking. Lateral shrinkage (diameter) is the shrinkage of burger towards a central direction, that is, a circular shaped burger looking oval after cooking and was determined by measuring the diameter of the burger at different directions before and after cooking. Cooking loss was determined by weighing the burger before and after cooking.

Selection of taste panel and preparation of beef and chevon burgers for sensory analysis

Fifteen panelists were randomly selected and trained according to the British method of sensory evaluation to evaluate the product (BSI, 1993). The frozen burgers were grilled to a core temperature of 70^{o}C for 15 minutes by the use of a griddle oven (Turbofan, Blue seal, UK). The products were then sliced into pieces of equal sizes of 1.8cm^2 x 2.5cm^2 each and wrapped in a coded aluminium foil to keep it warm. Each panelist was served with the test burger in addition to a piece of bread and water to act as a neutralizer between tests. Panelists were asked to indicate the eating qualities of the various samples with the aid of the 5-point hedonic scale shown in table 1.

Nutritional/physical analyses of beef and chevon burgers

Beef and chevon burgers were analyzed for moisture, crude protein (Kjeldhal method) and fat contents (Soxtec apparatus) (AOAC, 1999). For the determination of pH, 10 g beef burger of each treatment was ground with a laboratory mortar and pestle, homogenized with 50 ml distilled water, and pH values were measured with a digital pH-meter (CRISON, Basic 20, Spain).

Statistical analysis

Data obtained was analyzed using Analysis of Variance (ANOVA) of the Genstat Edition 4. Means were separated at 5% significant level. Data obtained from beef and chevon burgers were analyzed separately. Similar data from beef and chevon have been combined in a table for convenience and to reduce the number of tables.

RESULTS

Sensory characteristics of beef and chevon burgers

Table 2 shows the sensory characteristics of beef burgers prepared using whole guinea fowl egg as a binder. From table 2 there were no significant differences ($P > 0.05$) in colour, juiciness, texture, taste, flavour, cohesiveness and overall liking of beef and chevon burgers. Though there were no differences, there was a trend with the cohesiveness of beef burgers prepared using 100 grams whole guinea fowl eggs being most preferred by the panelists. Table 3 shows the sensory characteristics of chevon burgers prepared using whole guinea fowl egg as a binder. From table 3 there were statistically insignificant differences ($P > 0.05$) in colour, juiciness, texture, taste, flavour, cohesiveness and overall liking of chevon burgers. Similarly to beef burgers, chevon burgers with the highest inclusion level of whole guinea fowl eggs were most preferred.

Nutritional/physical qualities of beef and chevon burgers

The moisture, crude fat, crude protein and pH contents of the beef burgers are shown in table 4. Table 5 shows the moisture, crude fat, crude protein and pH contents of the chevon burgers. From table 4 there were insignificant differences ($P > 0.05$) in moisture, crude protein and pH of beef burgers but significant difference ($P < 0.001$) occurred in the crude fat content. B1 (3.67) was significantly higher than B2 (2.15) and B3 (2.00). Table 5 also shows that there were insignificant differences ($P > 0.05$) in moisture, crude fat, crude protein and pH contents of chevon burgers prepared using whole guinea fowl eggs.

Cooking loss, doming, lateral shrinkage and welling of beef and chevon burgers

Table 6 shows the cooking loss, doming, lateral shrinkage, and welling of beef burgers, while. Table 7 shows the cooking loss, doming, lateral shrinkage, and welling of chevon burgers. From table 6 and table 7, there were no significant differences ($P > 0.05$) in cooking loss, doming, and lateral shrinkage of beef and chevon burgers. The trend of cooking loss, doming and lateral shrinkage was least in B3 (19.90 g), B1/B3 (0.10 cm) and B1 (0.70 cm), respectively for beef burger. In chevon burgers, cooking loss, doming and lateral shrinkage was least in C3 (27.88 g), C3 (0.87 cm) and C/C2 (0.10 cm). Welling was not observed in the both beef and chevon burgers. Figure 1 shows beef burgers before and after cooking prepared without whole guinea fowl eggs. Figures 2 and 3 show beef burgers before and after cooking prepared with whole guinea fowl eggs as a binder at various inclusion levels.

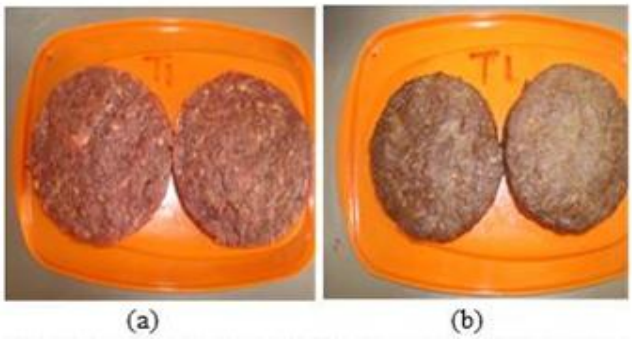

Figure 1. Beef burgers before (a) and after (b) cooking prepared without using whole guinea fowl eggs.

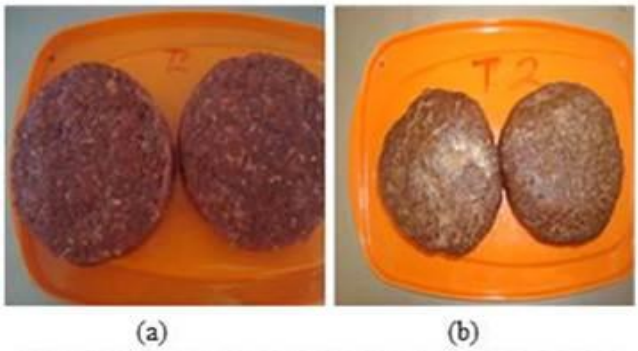

Figure 2. Beef burgers before (a) and after (b) cooking prepared using whole guinea fowl eggs at 5%.

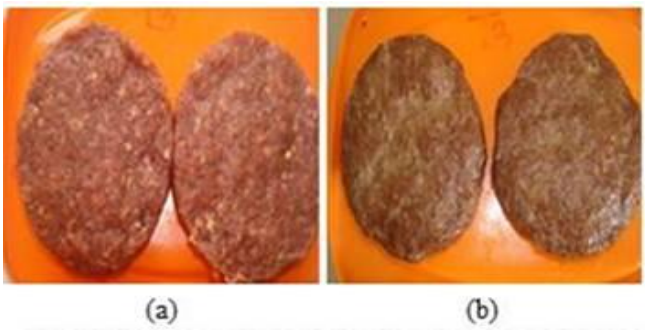

Figure 3. Beef burgers before (a) and after (b) cooking prepared using whole guinea fowl eggs at 9%.

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DISCUSSION

Sensory characteristics of the beef and chevon burgers

The incorporation of whole guinea fowl egg at 5 % and 9 % inclusion level in the beef and chevon burgers did not have any effect on the sensory quality of the burgers. Cohesiveness is the ability to hold solids and liquid together or the state of materials in a product holding together. Even though there was no significant difference in cohesiveness, beef and chevon burgers with the (9%) inclusion of whole guinea fowl eggs tended to be firmer and most liked/preferred by the panelists (Tables 2 and 3). These results agree with that of Adzitey et al. (2014) who reported that whole egg used as binder in beef burger showed no significant difference in texture, taste, juiciness, flavor, colour, cohesiveness and overall liking. The cohesive effect of the whole guinea fowl egg in both products also showed that B3 and C3 which had a 9 % inclusion level was scored lower in terms of likeness (2.40 and 2.13, respectively) as compared to the other treatments (B1=2.47, B2=2.53, C1=2.33, C2=2.53) which explains that most consumers liked the cohesive or binding effect of the whole guinea fowl egg in B3 and C3. Protein coagulates during thermal processing, resulting in the formation of gel-like structures which bind together the batter structural units (Barbut, 1995). The protein in guinea fowl eggs could have contributed to the cohesiveness of B3 and C3.

Nutritional Qualities of Beef Burger and Chevon Burgers

The beef burger, B1 had significantly higher crude fat content ($P < 0.001$) than B2 and B3. The whole guinea fowl egg could have reduced the crude fat content of the test beef burgers. Chevon burgers did not differ significantly ($P > 0.05$) in their crude fat content. USDA (1985) indicated that guinea fowl has 8.9% fats while beef contains 28.0% fat. Significant differences in moisture, crude protein and crude fat content of beef burgers prepared using whole chicken eggs at inclusion level of 5 %, 10 % and 15 % have been reported elsewhere (Adzitey et al., 2014). Moisture, crude protein, crude fat and pH contents in burgers contribute to it taste, shelf life and acceptability. Burgers with very high moisture content will have lots of drip during grilling which will have negative impact on it acceptability. Protein and fat are important nutrients needed by humans for growth, repair of worn out tissue and/or energy. Fat improve flavour and taste of meat and meat products (Warriss, 2000). pH measured the acidity and alkalinity of the burgers. The lower the pH, the less it supports the reproduction, growth and proliferation of pathogenic and spoilage microorganisms (Warriss, 2000 and MAFRA, 2011).

Table 1. Five-point hedonic scale used for the sensory evaluation

ATTRIBUTE	SCALE				
COLOUR:	1- Very light	2- Light	3- Intermediate	4- Dark	5- Very dark
FLAVOUR:	1- Like very much	2- Like	3- Intermediate	4- Dislike	5- Dislike very much.
JUICINESS:	1- Very juicy	2- Juicy	3- Intermediate	4- Dry	5- Very dry.
TEXTURE:	1- Very rough	2- Rough	3- Intermediate	4- Smooth	5- Very smooth.
TASTE:	1- Like very much	2- Like	3- Intermediate	4- Dislike	5- Dislike very much.
COHESSIVENESS:	1- Like very much	2- Like	3- Intermediate	4- Dislike	5- Dislike very much.
OVERALLIKING:	1- Like very much	2- Like	3- Intermediate	4- Dislike	5- Dislike very much.

Table 2. Sensory characteristics of beef burgers prepared using whole guinea fowl egg as a binder

Beef burger	B1 (control)	B2 (5 %)	B3 (9 %)	S.e.d	P value
Colour	2.93	2.80	3.07	0.254	0.580
Juiciness	2.60	2.20	2.13	0.317	0.293
Texture	2.93	2.80	3.07	0.339	0.736
Taste	2.53	2.40	2.53	0.352	0.909
Flavour	2.27	2.47	2.20	0.314	0.679
Cohesiveness	2.47	2.53	2.40	0.304	0.908
Overall liking	2.40	2.13	1.93	0.330	0.374

S.e.d: Standard error of difference; P value = Probability value; B1 = Beef burger containing 0 g whole guinea fowl egg; B2 = Beef burger containing 50 g whole guinea fowl egg; Beef burger containing 100 g whole guinea fowl egg.

Table 3. Sensory characteristics of chevon burgers prepared using whole guinea fowl egg as a binder

Chevon burger	C1 (control)	C2 (5 %)	C3 (9 %)	S.e.d	P value
Colour	2.80	2.67	2.87	0.345	0.841
Juiciness	2.73	2.80	2.60	0.410	0.884
Texture	2.53	2.80	3.07	0.304	0.226
Taste	2.67	2.27	1.93	0.334	0.102
Flavour	2.27	2.07	1.87	0.262	0.321
Cohesiveness	2.33	2.53	2.13	0.323	0.470
Overall liking	2.13	1.87	1.67	0.269	0.232

SED: Standard error of difference; P value = Probability value; C1= Chevon burger containing 0 g whole guinea fowl egg; C2= Chevon burger containing 50 g whole guinea fowl egg; C3= Chevon burger containing 100 g whole guinea fowl egg.

Table 4. Nutritional/physical properties of beef burgers prepared using whole guinea fowl egg as a binder

Beef burger	B1 (control)	B2 (5 %)	B3 (9 %)	S.e.d	P value
Moisture	60.40	54.70	51.30	4.030	0.221
Crude fat (g)	3.67^{a}	2.15^{b}	2.00^{b}	0.123	0.001
Crude protein (g)	14.28	14.49	13.16	0.404	0.085
pH	5.86	5.89	5.93	0.025	0.125

SED: Standard error of difference; P value= Probability value; Means in the same row with different superscript are significantly different ($P < 0.05$).

Table 5. Nutritional/physical properties of chevon burgers prepared using whole guinea fowl

Chevon burger	C1 (control)	C2 (5 %)	C3 (9 %)	S.e.d	P value
Moisture	64.00	63.20	60.00	2.91	0.451
Crude fat (g)	3.33	5.00	4.00	0.86	0.292
Crude protein (g)	14.40	13.50	17.60	2.60	0.370
pH	6.09	6.09	6.22	0.05	0.142

SED: Standard error of difference; P value= Probability value; Means in the same row with different superscript are significantly different ($P < 0.05$).

Table 6. Lateral shrinkage, doming and cooking loss of beef burgers prepared using whole guinea fowl egg as a binder

Beef burger	B1 (control)	B2 (5 %)	B3 (9 %)	S.e.d	P value
Cooking loss (g)	20.20	21.10	19.90	2.620	0.887
Lateral shrinkage (cm)	0.70	0.87	0.90	0.228	0.662
Doming (cm)	0.10	0.20	0.10	0.125	0.670

SED: Standard error of difference; P value= Probability value.

Table 7. Lateral shrinkage, doming and cooking loss of chevon burgers prepared using whole guinea fowl egg as a binder

Chevon burger	C1 (control)	C2 (5 %)	C3 (9 %)	S.e.d	P value
Cooking loss (g)	20.20	21.10	19.90	2.620	0.887
Lateral shrinkage (cm)	0.70	0.87	0.90	0.228	0.662
Doming (cm)	0.10	0.20	0.10	0.125	0.670

SED: Standard error of difference; P value= Probability value.

Cooking loss, doming, lateral shrinkage and welling of beef and chevon burgers

Welling which is the accumulation of fluid in vacuole of a burger and determined by observation was not found in the control and the burgers prepared using whole guinea fowl egg as a binder. Accumulation of fluid in a burger after any form of cooking will make it unattractive and can be rejected by consumers. The burgers were weighed or measured before and after cooking to determine the cooking loss, the rise in height (doming) and lateral shrinkage (shrinkage towards a direction). The results obtained for the cooking loss, doming and lateral shrinkage is contrarily to earlier reports (Adzitey et al., 2014). Significant differences were observed ($P < 0.05$) in cooking loss, doming and lateral shrinkage of beef burgers prepared using whole chicken egg at 5 %, 10 % and 15 % inclusion level (Adzitey et al., 2014).

CONCLUSION

This study showed that the addition of whole guinea fowl eggs to both beef and chevon burgers at inclusion levels of 50 g and 100 g had no influence on the sensory characteristics, cooking losses, lateral shrinkages and doming of the products. Furthermore, significant effects were not observed in the nutritional qualities of the burgers except for the crude fat content of the beef burger. Though the inclusion of whole guinea fowl egg in both beef and chevon burgers had no effect on the flavour, taste, cohesiveness and overall likening of the products, consumers seem to prefer the flavour, taste and cohesiveness of both beef and chevon burgers with the 9 % inclusion level. Welling was not observed in both beef and chevon burgers.

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Competing Interests

The authors declare that they have no competing interests.

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Supplementation of Different Level of Deep Stacked Broiler Litter as a Source of Total Mixed Ration on Digestibility in Sheep and Their Effects on Growth Performance

Muhammad Tahir Khan[1]*, Zohaib Ahmed Bhutto[2], Sayed Haider Abbas Raza[3], Muhammad Saeed[3], Muhammad Asif Arain[2], Muhammad Arif[4], Sarfraz Ahmed Fazlani[2], Muhammad Ishfaq[1], Farman Ali Siyal[5], Mehdi Jalili[6] and Arman Moshaveri[6]

[1]*Faculty of Animal Husbandry and Veterinary Sciences, the University of Agricultural Peshawar 25000, Pakistan*
[2]*Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water & Marine Sciences, Uthal Baluchistan 38000, Pakistan*
[3]*College of Animal Sciences, Northwest A and F University, Yangling 712100, China*
[4]*Department of Animal Sciences College of Agriculture University of Sargodha, Pakistan*
[5]*Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Pakistan*
[6]*Department of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Iran*

*Corresponding author`s Email: tahirjhagra@gmail.com

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ABSTRACT

Poultry litter from rigorous poultry production plants has impact on environmental pollution. Feedstuffs for animal are getting with time expensive, to reduce the feed cost which could be achieved through the assimilation of relatively inexpensive and non-conventional feed ingredients, like poultry litter. The objective of this study was to explore the nutritive value of deep stacked broiler litter in ruminant's total mixed ration. Four non castrated male sheep were used into 4×4 Latin Square Design (LSD) to 1 of the 4 dietary treatment groups that different in deep stacked broiler litter (DBL) as percentage of concentrate diet to investigate the nutritive value of DBL as a ruminant feed. The effect of dry matter intake and digestibility of DBL in sheep studied. Nitrogen retention was determined in total mixed ration at each level in the diets fed to sheep. Microsoft excels was used to balance experimental rations A, B, C, and D. Ration A was containing 0% DBL and served as control. Ration B contains 15% DBL, C was containing 30% DBL while Ration D containing 45% DBL. All the diets were prepared according to requirement of critical nutrients. All the diets were prepared isocaloric, isonitrogenous with or without DBL. Dry matter intake gradually decreased ($P < 0.05$) with the levels of broiler litter increased in the four diets. Means values of DMI (g/day) in rations A, B, C and D was 1040.7, 945.3, 840.9 and 786.8. Nitrogen retention (% of the total N consumed) were decreased ($P < 0.05$) as the broiler litter level increased in the diet. Up to 30% poultry litter in the supplement diets of sheep contributes as non-conventional source of nitrogen, and could be used for replacing traditional nitrogen sources like cotton seed cake. The findings of the present study suggested that inclusion of broiler litter up to 30% has no adverse effect on the health and apparent weight.

Keywords: Deep stacked, Mixed ration, Litter, Digestibility, Sheep

INTRODUCTION

Feed resources for livestock in Pakistan is 121 million heads of animals need about 10.9 and 90.36 million tons of Crude Protein (CP) and Total Digestible Nutrients (TDN) correspondingly but to them the obtainable CP and TDN are 67 and 69 million tons. In Pakistan feed resources are green fodder, crop residues, grazing rangelands, post-harvest grazing, cereal by products, and oilseed cakes. In these the chief one which provided about 51% of the feed resource is green fodder and crop residues (Sarwar et al., 2002). The broiler litter can be used as a supplement for conventional protein sources, but at higher inclusion levels, it needs to be augmented by increased fermentable energy (Lawrence et al., 2015). Reducing the cost of gain, the inclusion of layer litter in lambs' finishing diets is potentially valuable and can be considered as cheap alternative in livestock feeding strategies reported by Obeidat et al. (2016). Using heat- processed litter (HBL) up to 210 g/kg dry matter in diet was possible without any effect on feed intake, growth performance and animal health, but reduced loin fat, internal fat and cost per unit production reported by Bello and Tsado (2014) and Ayoub et al. (2015). Recommended that, dried poultry dropping can satisfactorily supplement sorghum stover up to 80% inclusion level for good performance and without any deleterious effects (Ayoub et al., 2015). Fodder supplies the broad fraction of nutrient to

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ruminants, but lack of these nutrients occurred throughout the dry season as a consequence a quick decline in the quality of forages take placed, leading to low forage intake and digestibility reflects poor animal performance. Low quality roughages fed to ruminants without supplementation throughout the dry season caused severe weight losses and at last animal lead to death (Adegbola, 2002). The market prices of the conventional feed sources of protein in livestock ration have risen unreasonably high and this has force the search for inexpensive protein sources (Akinmutimi, 2004). Deprived nutrient supplied, both in quantity and quality and low reproductive capabilities are identified one of the main reason limiting animal production. In this aspect, poultry litter has been recognized as one of the non-conventional feeds substances for ruminant production. Broiler litter is a secondary product of poultry industry, which is high in crude protein, quickly degraded in the rumen and low to moderate in available energy concentration (Saleh et al., 2003).

Poultry litter is usually used as fertilizer, but in addition it has a probable to use as a ruminant feed and it is more valuable as feed constituents than as a fertilizer. The use of poultry litter as a dietary supplement in ruminant ration could have a sensible result on reducing costs, insufficiency of protein in diet. The germ which there in the broiler litter is efficiently eliminated during deep stacking (Elemam et al., 2010). The disposals of poultry litter from rigorous poultry production plants have its impact on environmental pollution. Furthermore, feedstuffs for animal are getting with time expensive. Therefore to reduce the feed cost which could be achieved through the assimilation of relatively inexpensive and non-conventional feed ingredients, like poultry litter. Therefore, the present study was planned to explore the nutritive value of deep stacked broiler litter in ruminants.

MATERIALS AND METHODS

Processing of poultry litter

Before mixing with other feed ingredients, litter was processed to restrict the population of pathogenic microorganisms present in raw poultry litter. For this deep stacking technique was used for poultry litter processing. The collected poultry litter was dried for five hours in sun light. The phenomenon of sunlight drying was to bring the level of the moisture content in poultry litter up to 30%. When the litter was analyzed in lab the level of moisture content was less than 30%. To bring the level of moisture content of litter up to 30%, water was sprinkled on the litter. Later on, in the lab to confirm the level of moisture content in litter and the result showed that the litter contains moisture level up to 30 %. After that deep stacked at three cubic feet for 21 days. Stacked poultry litter was pressed tightly and then covered by plastic sheath. The air was totally excluded during deep stacking of poultry litter to develop an aerobic condition to kill the microorganisms. The fermentation process occurred which heated up the stacked poultry litter up to 140^{o} to 160^{o} F for four hours which killed all the potential microorganisms e.g. *E.coli* or *Salmonella*. After 21 days the processed litter was used for mixing with other feed ingredients (Ruffin and McCaskey, 1990).

Experimental design and animals

Experiment was design involving four male sheep and four diets. Each period was consisted of 10 days adaptation and 7 days data collection period. The diets were switched over to the 4 sheep in each period according to the following scheme (Table 1). Animals used in the experiment were four male sheep each was nearly the same age and weight. Animals were treated against ecto and endo parasites a week before the starting of trail and were kept in a well ventilated shed and each individual metabolic cage was used for separate feeding, watering and collection of feces and urine. Before starting the trial all the metabolic cages including feeders and water troughs was cleaned thoroughly.

Table 1. Experimental lay out for offering different feeding rations

Periods (Day)	Sheep			
	I	II	III	IV
1	A	B	D	C
2	D	C	A	B
3	C	A	B	D
4	B	D	C	A

Ration A = 0% broiler litter+100%Total mixed ration; Ration B = 15% broiler litter+85% Total mixed ration; Ration C = 30% broiler litter+70% Total mixed ration; Ration D = 45% broiler litter+55% Total mixed ration

Feeding

In diet A, B, C and D the deep stacked broiler litter was added at a level of 0, 15, 30 and 45% along with other feed ingredients. The diet A was control and contained (0%) deep stacked broiler litter. During the adaptation period of 10 days animals were adopted to their respective diets by gradual replacement of the previous diet. The physicochemical compositions of experimental diets are given in (Table 3 and 4). The diets were fed twice daily at 9:00 am and 5:00 pm. The basal diet and each of the supplements were weighed according to the ingredient composition of the experimental ration. Feed refusal of the previous day was recorded daily before offering the fresh feed. Clean water was available to the experimental animals in the drinker fixed with each cage.

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Samples collection from feed, feces and urine

After the adaptation period data collection period was started, and remained continued for seven days. Before offering fresh feed in the morning, the refusal of the previous day was weighed and representative samples of the feed offered and feed refused was collected in a labeled polythene bags and was shifted immediately to the freezer for storage with minimum loss of moisture. Similarly, the quantities of feces excreted by each animal during 24 hour were weighed. A representative sample equivalent to 20% of the total weight was collected in a labeled polythene bag to store in a freezer. Urine excreted during the last 24 hour was collected from individual animal in labeled bottles containing 100 ml of 2.5 mol/liter sulfuric acid. Urine volume was measured and representative sample equivalent to 20% of the total volume was collected.

Chemical analysis

About 50 gram, in duplicate of the pooled feces and feed samples after thawing and mixing were taken for dry matter (DM) analysis and the remaining was air dried at 60°C for 72 hour. The air-dried samples were ground in Thomas-Willey laboratory mill to a particle size of 1 mm and stored at room temperature in labeled bottles. The sample was analyzed for dry matter, organic matter, moisture, crude protein, crude fiber, ether extract and nitrogen free extract according to (AOAC, 1997).

Ether Extract (EE)

Dried sample (2-4 gram) in a clean previously dried extraction thimble (Whatman) was taken and plugged it with absorbent cotton wool. This was kept in an extractor and fixed under the condenser of the Soxhlet extraction apparatus. 150 ml of the solvent was added to the receiving flask and connected it to the apparatus. Then the water and heater was on. Extraction was continued for 10 hours at a rate of condensation at 3-4 drops/second and then the thimble was removed from the extractor. Just before the solvent drying in the flask, the extraction was stopped and the flask was be removed. The extract were transferred into clean evaporating basin with ether were hinges, dryness was evaporated on water bath, after that basin was placed in oven at 105°C for 2 hours. Further cooled in desiccator for 30 minutes and reweighed. The percentage of EE was calculated as under:

$$\% \text{ EE (DM)} = \frac{\text{Weight of ether extract}}{\text{(Sample weight)}} \times 100$$

Converting to as fed basis:

$$\% \text{ EE as fed} = \frac{\text{Weight of ether extract}}{\text{(Sample weight)}} \times (100 - \% \text{ moisture})$$

Crude Fiber (CF)

It is the organic residues that remain when a moisture free sample is digested first with weak acid solution (H_2SO_4) and then with a weak alkaline solution (NaoH). The residues collected after digestion is ignited and the loss in weight on burning is registered as crude fiber. Moisture free sample (1-2 gram) was taken in a tall from beaker. Two hundred ml boiling dilute H_2SO_4 was added and was digested for 30 minutes on crude fiber extraction apparatus. Then was filtered through glass buchner funnel with an aid of suction air pump. Then was wash with hot water until it became acid free (15 ml filtrate is collected and 1 drop N/10 NaoH and 1 drop Phenolphthalein indicator was added. Pink color is an indicator of being acid free). Transferred again to tall beaker and 200 ml boiling dilute NaoH was added. Then was filtered through glass buchner funnel with an aid of suction air pump. Then was washed with 10 ml hot dilute H_2SO_4 and then with hot water until it became acid free. It was transferred to a prepared gooch crucible, and then with 10ml ethanol crucible was held. Then sample was dried in an oven at 135°C for 2 hours. Then was cooled in desiccator for 30 minutes and weighted. Samples were further ignited in muffle furnace at 600°C for 30 minutes. Ignition residues were cooled in desiccator for 1 hour and reweighed. The percentage of CF was calculated as under:

$$\% \text{ CF (sample)} = \frac{\text{(crucible weight + dried residue) - (crucible weight + ash residue)}}{\text{(Crucible weight + sample) - empty crucible weight)}} \times 100$$

$$\% \text{ EE (DM)} = \frac{\text{CF \% in sample}}{\text{DM \% in sample}} \times 100$$

Nitrogen Free Extracts (NFE)

It was found by the difference after the analyses of all other items mentioned in proximate analysis. It is as under: (%Moisture + %Crude protein + %Ether extract + %Crude fiber + %ash + %NFE) = 100

Therefore, %NFE = 100 - (Moisture + Crude protein + Ether extract + Crude fiber + Ash)

Dry matter and ash

For the estimation Dry Matter (DM) and ash, about 2 gram samples were taken in clean and pre-weighted crucibles in duplicate. The crucibles were then placed in laboratory oven for 18h at 100°C. After drying in an oven the samples was cooled in a desiccator for 30 minutes and reweighed. The DM% was determined by using the following formula.

$$\text{DM (\%)} = \frac{\text{C-A}}{\text{B-A}} \times 100$$

A = weight of empty crucible

B = weight of crucible + sample (pre drying)

C = weight of crucible + sample (post drying).

The samples were incinerated in a muffle furnace at 550°C for 6 hour to estimate its ash content. After incineration the samples were cooled again in a desiccator and were re-weighed. Ash was calculated as under:

$$\text{Ash (\%)} = \frac{\text{D-A}\times 100}{\text{C-A}}$$

A = weight of empty crucible
C = weight of crucible + sample (post drying)
D = weight of crucible + ash

Organic Matter (OM) was calculated after subtracting ash from DM

OM = (100-Ash % in DM)

Crude protein

Crude Protein in the representative sample of feed was determined with kjeldhal method (AOAC, 1997). In this method samples was digested with concentrated Sulphuric acid and was followed by distillation and titration. Samples (about 0.5 gram) in duplicate were taken in the Tecator digestion tubes and were added with 3 gram of catalyst (Potassium sulphate 93%, Copper sulphate 7%) and 5 ml concentrated sulfuric acid. Acetanilide (0.1 gm) was processed as standard for recovery of nitrogen. The digestion tubes were heated in Tecator digestion block. The tubes were then allowed to cool at room temperature. About 15 ml distill water was added with the tubes containing digested samples. After dispensing required amount of sodium hydroxide (NaOH) solution (40%) in the tubes to alkaline the sample and the contents were distilled for about seven minutes. The resulting ammonia was collected in conical flask containing 10 ml boric acid and 3-4 drops of methylene red. The titration of distillate was carried out with sulfuric acid solution of known normality. To determine the blank values duplicate tubes containing 15 ml distilled water and 5 ml sodium hydroxide was processed for distillation and titration. The percentage of nitrogen was calculated as under:

$$\text{N (\%)} = \frac{\text{(V1-V2)} \times 14.0) \times \text{mol/liter (N) of titrate}}{\text{(Sample in milligram)}} \times 100$$

V1= Titration reading of sample
V2= Titration reading of blank
14.01= Atomic weight of Nitrogen (N)

Crude protein was determined for feed sample, by multiplying the nitrogen content of the sample by 6.25. The results were corrected for dry matter as given under:

$$\text{N (\%) in DM} = \frac{\text{N\% in sample}}{\text{DM \% in sample}} \times 100$$

Feed intake

Feed intake was measured by subtracted the feed refused from total offered feed. The feed intake during experimental trials was calculated by the following manner.

Feed intake = Total feed offered - feed refused by the animal

Nutrients digestibility

Digestibility of DM, OM, and nutrients was calculated by the difference between the nutrients consumed and voided in feaces by the sheep using following equation.

$$\text{Digestibility \%} = \frac{\text{A-B}}{\text{A}} \times 100$$

Where:

A = Quantity of nutrients i.e. DM, OM, consumed by the Animal (gram/day)

B = Quantity of the above nutrients excreted by the animal in feaces (gram/day)

Nitrogen retention

Nitrogen retention (gram/day) was calculated by subtracting the total nitrogen excreted in feaces and urine (gram/day) from the total dietary nitrogen (gram/day) consumed.

In vitro Dry matter digestibility (IVDMD)

In vitro dry matter digestibility (IVDMD) was measured by the procedure as decribed by Tilly and Terry, (1963). Samples about 0.5 gram in triplicate were incubated in 60 ml centrifuge tubes fitted with Bunsen valve. Rumen liquor was collected from a rumen fistulated cow. Rumen liquor was filtered through double layers of muslin cloth and mixed with buffer solution 1:3 ratio. An aliquot of 10 ml was dispensed in each tube with simultaneous flushing of CO2 to establish anaerobic conditions in the tubes. The tubes were closed and incubated at 37 ºC for 48 hours. Tubes containing rumen fluid without sample and tubes with standard samples were also included in each run for the determination of blank values. All possible measures were adopted to maintain microflora of rumen liquor during collection, filtration and dispensing. The contents of tubes were mixed two times by gentle swirling at twelve hours interval. On termination of the incubation (48 hours), the tubes were centrifuged at 3000 rotation per minute for 5 minutes. The supernatant was discarded. The tubes with the precipitate were dried in an oven at 70-72 ºC. Finally after cooling in a desiccator the tubes were weighed.

The IVDMD was calculated as under:

$$\text{IVDMD\%} = \frac{\text{A} - (\text{B} - \text{C})}{\text{A}} \times 100$$

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A = weight of sample (DM)

B = weight of undigested dried residues in a tube.

C = weight of undigested dried residues of rumen fluid in the blank tube.

Statistical analysis

The data obtained from digestibility study was subjected to analysis of variance (ANOVA) is according to statistical analysis system (SAS version 6.04, 2000). The mean values were compared for estimating LSD and its significance level set as $P<0.05$.

Economics

The cost of the control and experimental rations was calculated according to the prevailing market rates. Similarly increases or a decrease in digestibility was also being considered for the respective ration.

Table 2. Analysis of variance of the experimental design

Source of Variation	Sum of square	Degrees of freedom	Mean square	F= 0.05
Between diets, α_i	SSα	4 – 1 = 3 (a - 1)	$SS_\alpha/3=MS_\alpha$	$MS_\alpha/MS\varepsilon$
Between animals, β_i	SSβ	4 – 1 = 3 (b - 1)	$SS_\beta/=MS_\beta$	$MS_\beta/MS\varepsilon$
Between Periods, γ_κ	SSγ	4 – 1 = 3 (c - 1)	$SS_\gamma/3=MS_\gamma$	$MS_\gamma/MS\varepsilon$
Experimental error, $Є_{ij\kappa}$	SSε	3×3×3 = 27 (a -1) (b - 1) (c-1)	SSε/27=MSε	
Total	SST	64-1=63 abc-1		

Yijk = μ + αi + βj +γk + Єij k; where: Yijk = Combine effect of periods, animals and diets; μ = Population mean; αi= Effect of diets i.e. A, B, C and D; βj = Effect of animals i.e. i, ii, iii and vi; γk = Effect of periods i.e. 1, 2, 3 and 4; Єijk = Random error N (o, б2).

Table 3. Physical composition of experimental sheep rations during 8-10 months old

Ingredient	Ration (%)			
	A	B	C	D
DBL	0.00	15.0	30.0	45.0
CSC	20.0	13.0	6.0	0.00
Wheat Bran	24.0	20.0	16.0	7.00
Molasses	10.0	10.0	10.0	10.0
DCP	1.00	1.00	1.00	1.00
Wheat Straw	24.0	20.0	19.0	21.0
Maize fodder	20.0	20.0	17.0	15.0
Salt	1.00	1.00	1.00	1.00
Total	100	100	100	100

Ration A= 0% broiler litter+100%Total mixed ration; Ration B= 15% broiler litter+85% Total mixed ration; Ration C= 30% broiler litter + 70% Total mixed ration; Ration D= 45% broiler litter+55% Total mixed ration; DBL= Deep stacked broiler litter, CSC= Cotton seed cake, DCP= Dicalcium phosphate.

Table 4. Chemical composition of experimental sheep rations during 8-10 months old

Ingredient	DM	TDN	DE	ME	CP	Ca	P
	%		M cal/Kg		%		
DBL	87	50	0.00	0.00	20.0	2.30	1.60
CSC	91.40	69	3.04	2.49	26.50	1.00	1.40
Wheat Bran	89	70	1.40	0.73	17.00	0.12	1.38
Molasses	76	75	3.30	2.70	5.00	1.19	0.11
DCP	99	0	0.00	0.00	0.00	3.75	2.00
Maize fodder	70	67	2.82	2.31	9.00	0.50	2.50
Salt	96	0	0.00	0.00	0.00	0.00	0.00
Wheat Straw	91	42	1.94	1.58	3.00	0.16	0.05

*Feed composition of the ingredients used for formulating experimental rations (NRC, 1989). DM= Dry matter, TDN= Total digestable nutrients, DE= Digestable ether extract, ME= Metabolizable energy, CP= Crude protein, Ca= Calcium, P= Phosphorus, DBL= Deep stacked broiler litter, CSC= Cotton seed cake, DCP= Dicalcium phosphate.

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RESULTS

The present experiment was conducted to incorporate broiler litter in total mixed ration for sheep. Four non castrated male sheep were used into 4×4 latin square design to 1of the 4 dietary treatment groups that different in deep stacked broiler litter as percentage of concentrate diet to investigate the nutritive value of deep stacked broiler litter as a ruminant feed. The effect of dry matter intake and digestibility of deep stacked broiler litter in sheep studied. Nitrogen retention was determined in total mixed ration at each level in the diets fed to sheep.

A computer program Microsoft Excels was used to balance Experimental Rations A, B, C, and D. Ration A was containing 0% dry stacked broiler litter and served as control. Ration B contains 15% DBL, C was containing 30% DBL while Ration D containing 45% DBL. All the diets were prepared according to requirement of critical nutrients. All the diets were prepared isocaloric, isonitrogenous with or without DBL. The data of analysis of variance subjected to experimental design were shown in table 2.

Feed intake

In table 5 mean values of dry matter intake (DMI) for sheep are presented. These measures were found not significantly different ($P<0.05$) among the four rations. The mean value of dry matter intake g/d for ration A, B, C and D were 1011.7, 995.3, 940.9 and 886.8 respectively. Dry matter intake as gram/kilogram metabolic body weight were not found to be different from the 15 and 30% inclusion rate of deep stack broiler litter except for higher inclusion rate which increased ($P<0.05$). However dry matter intakes were lower for the 45% DBL than ration A, B, C during the study. Similarly intake of organic matter (OM), ether extract (EE), ash (A) and nitrogen free extract (NFE) and nitrogen (N) significantly different ($P<0.05$) among the four diets. These were high in ration A than that in ration D. whereas intake of the crude fiber (CF) and ash (A) content significantly different ($P<0.05$) among the all diets these were larger in case of high inclusions of DBL.

In vivo digestibility of nutrients

The mean value of *in vivo* digestibility of (DMD), organic matter (OMD), nitrogen (ND), crude fiber (DCF), ether extract (DEE) and nitrogen free extract (DNFE) for sheep are presented in table 6. These measured were found significantly different ($P<0.05$) among the four rations. Sheep receiving ration A was the highest DM digestibility (75.37%) and low for ration D that (67.19%). Similarly Organic Matter (OM), Ether Extract (EE) and Nitrogen Free Extract (NFE) digestibility were also decreased for those feed which have larger amount of deep stacked broiler litter as compared to the ration which have zero DBL. The mean digestibility of crude fiber and nitrogen significantly different ($P<0.05$) among the four ration. The crude fiber digestibility was high in ration C 69.54% and lowest for ration A 32.14%, in case of nitrogen the digestibility increased as the inclusion of the DBL increased among the ration

Nitrogen retention

Nitrogen consumed and retained by the sheep on the four diets is shown in the table 7. Diet composition significantly influenced ($P<0.05$) the quantity of nitrogen consumed and excreted in the feaces and urine by the experimental animals. The mean results were compared it was found that sheep receiving high level of broiler litter supplements in total mixed ration excreted greater quantity of nitrogen in feaces and urine ($P<0.01$) as compared to control diet. These differences reflected in Nitrogen Retention (NR, % of the total nitrogen) in the body of sheep. The nitrogen retention(NR) were expressed as percent of total N consumed of the rations A, B, C, and D. were 67.49, 53.36, 47.57 and 41.94 % respectively. The NR was appreciably greater in the rations containing low level of deep stacked broiler litter as compared to that rations containing highest inclusion of broiler litter.

In vitro Dry Matter Digestibility (IVDMD)

The *in vitro* dry matter digestibility (IVDMD %) of four experimental diets A, B, C, and D are shown in the (Table 8). The percent digestibility decreased as the inclusion of deep stacked broiler litter increased in the diets. The higher digestibility were recorded in control ration A (0%DBL) while the lowest digestibility in the ration contained high proportion of deep stacked broiler. The *in vitro* digestibility for diet A, B, C and D were 67.22, 60.91, 57.58 and 54.72%.Which were gradually decreased among the ration

Economics

The cost of the control and experimental rations were calculated according to the prevailing market rates. Similarly up and down in digestibility were also being considered for the respective ration. The result of present study given in the Table 9 shows that as the broiler litter inclusion increased in the diet cost of the diet reduced. Best result obtained for ration C on which the nitrogen and crude fiber digestibility is high compared to other three diets.

Table 5. Means dry matter, organic matter, crude fiber, ash, ether extract, nitrogen and nitrogen free extract intake of the experimental rations fed to sheep aged 8-10 months

Intake [b] (g/d)	Rations [a]			
	A	B	C	D
DMI	1011.7[a]	995.3 [a]	940.9 [a]	886.8[b]
OMI	842.46[a]	627.57[b]	590.07[c]	585.23[bc]
CFI	198.72[c]	212.13b[c]	226.41[b]	294.79[a]
AI	81.57b[c]	86.61[c]	94.59[b]	104.43[a]
EEI	67.11[a]	51.18[b]	46.25[bc]	38.97[c]
NI	22.92[a]	20.98[ab]	18.58[bc]	16.52[c]
NFE	523.24[a]	455.18[b]	404.87[bc]	378.87[c]

Mean in the same row with different superscripts are significantly different (P<0.05); Ration A = control (0 % broiler litter); Ration B = 15% broiler litter; Ration C = 30% broiler litter; Ration D = 40% broiler litter; bDMI= Dry Matter Intake; OMI= Organic Matter Intake; NDI = Nitrogen Intake; EEI= Ether Extract Intake; AD= Ash Intake; CFI=Crude Fiber Intake and NFEI= Nitrogen Free Extract Intake.

Table 6. Means dry matter, organic matter, crude fiber, ash, ether extract, nitrogen and nitrogen free extract digestibility (%) of the experimental rations fed to sheep aged 8-10 months

Digestibility (%)	Ration			
	A	B	C	D
DMD	75.37[a]	71.46[ab]	68.07[bc]	67.19[c]
OMD	73.11[a]	70.25[a]	60.62[b]	53.30[c]
CFD	32.14[c]	51.12[b]	69.54[a]	51.42[b]
AD	62.54[a]	57.03[b]	39.10[c]	37.07[c]
EED	68.80[a]	58.73[b]	48.71[bc]	43.27[b]
ND	64.29[a]	66.50[a]	70.44[a]	53.49[b]
NFED	69.88[a]	41.49[b]	41.38[b]	34.97[c]

Mean in the same row with different superscripts are significantly different (P<0.05); Ration A = control (0% broiler litter); Ration B = 15% broiler litter; Ration C = 30% broiler litter; Ration D = 40% broiler litter; bDMD= Dry Matter Digestibility; OMD= Organic Matter Digestibility; ND = Nitrogen Digestibility; EED= Ether Extract Digestibility; AD= Ash Digestibility; CFD=Crude Fiber Digestibility and NFED= Nitrogen Free Extract Digestibility.

Table 7. Mean nitrogen retention (%) in experimental diet fed to sheep aged 8-10 months

Ration	Broiler litter/Total mix ration (%)	Nitrogen Retention (%)
A	0/100	67.69[a]
B	15/85	53.36[b]
C	30/70	47.57[bc]
D	45/55	41.94[c]

Mean in the same column with different superscripts are significantly different (P<0.05); Ration A = 0% broiler litter+100%Total mixed ration; RationB = 15% broiler litter+85% Total mixed ration; Ration C = 30% broiler litter+70% Total mixed ration; Ration D = 45% broiler litter+55% Total mixed ration.

Table 8. In vitro dry matter digestibility of the experimental rations used in sheep aged 8-10 months old

Ration	Broiler litter/Total mix ration (%)	In vitro dry matter digestibility (%)
A	0/100	67.22[a]
B	15/85	60.91[b]
C	30/70	57.58[c]
D	45/55	54.72[d]

Mean in the same column with different superscripts are significantly different (P<0.05); Ration A = 0% broiler litter+100%Total mixed ration; Ration B = 15% broiler litter+85% Total mixed ration; Ration C = 30% broiler litter+70% Total mixed ration; Ration D = 45% broiler litter+55% Total mixed ration.

Table 9. Market price of experimental ration fed to sheep aged 8-10 months in Pakistan

Ration	Broiler litter/ Total mix ration (%)	Cost/Kg
A	0/100	Rs=17.95
B	15/85	Rs=15.87
C	30/70	Rs=13.80
D	45/55	Rs= 11.44

Ration A = 0% broiler litter+100%Total mixed ration; Ration B = 15% broiler litter+85% Total mixed ration; Ration C = 30% broiler litter+70% Total mixed ration; Ration D = 45% broiler litter+55% Total mixed ration.

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DISCUSSION

The experiment was conducted to add deep stacked broiler litter for sheep in the total mixed ration to ascertain its effect on the intake and digestibility of dry matter, organic matter, crude fiber, nitrogen and nitrogen free extract. Nitrogen retention was determined for DBL at each level it was increased in the diets which have 0 level of broiler litter. The results obtained from the present study that presented in the preceding chapter were opened here for further discussion.

Dry matter intake

The results of the present study showed Dry matter intake as g/kg metabolic body weight were not found non-significant among the treatments except for higher inclusion rate which increased ($P<0.05$). When inclusion rate of deep stack broiler litter is increased the lambs tend to consume more water therefore, gradually reduction ($P<0.05$) in dry matter intake (DMI) in diet by rising broiler litter level. Ensminger and Olentine, (1978) reported almost same results as our study; he demonstrated that, low intake of dry matter having broiler litter may be due to low acceptability of broiler litter and its high mineral contents decreased the desire of food. Whereas, NRC (1984) described that, the ash content of broiler litter is 6% more as compared to other diets which decreased the appetite of animal and ultimately feed intake decreased. Nadeem et al. (1993) reported that, reduction was observed in dry matter intake when barbari goat kids were fed diet contain high level of broiler litter compared to those fed broiler litter free diet. He explained the variation in the feed intake is due to the component of basal diet and of the metabolized energy level of the diet. Obeidet et al. (2012) reported that, dry matter intake decreased in the diet which containing BL compared to those which have 0BL due to the digesta passage rate for basal dietary component with broiler litter has been slower with relative to diet which has no broiler litter so this limited the feed intake. Goatsch and Aiken (2000) discussed that, the reason for low intake might be due the presence of rocks, muds, and other debris; Beside this increased level of ash could be due to soil contamination which caused low dry matter intake. Rossi et al. (1998) reported that, the most important factor which reduced dry matter intake and digestibility might be the particle sized and source of broiler litter. They further explained that broiler litter into fraction have greater intake compared to as a whole.

In vivo digestibility of nutrients

The results of our study on In vivo digestibility of nutrients showed that there was gradual decreased ($P<0.05$) dry matter (DM), organic matter (OM), ether extract (EE) and nitrogen free extract (NFE) digestibility as the level of the deep stacked broiler litter increased in the diets. Whereas, crude fiber and nitrogen digestibility increased among the ration A to D; The low digestibility of dry matter having broiler litter may be due to low acceptability of broiler litter and its high mineral content. Bello and Tsado, (2014) reported that, rams fed diets supplemented with dried poultry dropping had significantly better feed intake; body weight gain and feed conversion ratio. The nutrient digestibility was significantly improved with DPD supplementation. It is recommended that dried poultry dropping can provide sorghum Stover up to 80% inclusion level for good performance and without having any deleterious effects in growing Yankasa rams. Lawrence et al. (2015) reported DBL as potential protein supplement in growing/finishing ruminants. However, it has been noted that optimum levels of inclusion can lead to production efficiencies that are comparable with standard feeds. There is potential to further increase the inclusion levels of DBL in fattening diets if the availability of fermentable energy can be matched to ammonia produced from uric acid degradation. Similarly, NRC (2001) agreed with these results and reported that the ash content of broiler litter was 6% more as compared to other diets which decreased digestibility. Obeidet et al. (2012) reported that dry matter digestibility decreased in the diet which containing DBL compared to those which have zero DBL because the digesta passage rate slow for those diets which contain DBL compared to diet not contains broiler litter. Animals supplemented with dried poultry droppings based diet had the best intake and apparent digestibility coefficient Bello and Tsado (2013). Nadeem et al. (1993) supported the present results that reduction occurred in the digestibility of diet which contained deep stacked broiler litter (DBL) compared to 0 DBL. the reason of low digestibility was due high minerals content present in deep stacked broiler litter which declined the digestibility of dry matter. Abebe et al. (2004) founded that organic matter digestibility decreased as broiler litter increased in the diet. The reason for it could be low level of water soluble carbohydrates which are not properly fermented in the rumen and the low pH decreased the organic matter digestibility. Another authors Nasr Sayed and Fathy (2010) reported that nitrogen digestibility increased in the diet contained broiler litter due to increased microbial protein synthesis in the rumen caused by more degradable protein in the form of ammonia nitrogen being available to rumen microbes.

Elemam et al. (2009) observed low DM digestibility in lambs during 9 to 12 month of age when fed a diet containing 300 gram/kilogram DBL

compared to those were fed control diet due to ash content. The nitrogen digestibility increased as the incorporation of poultry litter increased in the ration compared to the controlled diet. The improvement in crude protein digestibility in broiler litter ration due to increased microbial protein synthesis in the rumen caused by more degradable protein in the form of NH3–nitrogen being available to rumen microbes Nasr Sayed and Fathy (2010); Goatsch and Aiken (2000) also reported that the crude fiber of poultry litter is relatively high digestibility in ruminants. This may be due to the exposure of poultry litter fiber to the enzymes and organisms in the digestive tract of the poultry making it more available and efficiently utilized by the microorganisms in the rumen. Alrukyan et al. (1998) is in line with our results that nitrogen digestibility increased as the level of poultry waste increased in the diet. Due to more digestible nitrogen which quickly converted in to ammonia and efficiently utilized by rumen microbe for synthesis structural protein and finally microbes digested in the small intestine. Similarly Evans et al. (1993) reported that 33% increase in ash content in the diet containing poultry waste result 14% decreased in the dry matter digestibility.

Nitrogen retention

The result of present study shown that nitrogen retention was significantly ($P<0.05$) different among the four diet; A highest, nitrogen retention recorded for the diet A and lowest for diet D in the body of sheep as the inclusion level of broiler litter increased nitrogen retention was reduced Goatsch and Aiken (2000) are agreed with our results and reported that that nitrogen retention decreased as the level of inclusion of poultry litter increased. Reason for low nitrogen retention for increased level broiler litter could be due to low available energy in poultry litter and when energy amount is less than the required rumen microbe, unable to convert ammonia into structural protein and the ammonia entered into urea cycle excreted in urine. Feeding layer litter at 150 g/kg improves feed intake, N retention, and growth performance of finishing Awassi lambs thus reducing the cost of gain. Alternatively, the inclusion of layer litter at 300 g/kg of the diet resulted in similar intake and growth performance to the control group while low- erring the cost of production a little further. Thus, due to reducing the cost of gain, the inclusion of layer litter in Awassi lambs' finishing diets is potentially valuable and can be considered as cheap alternative in livestock feeding strategies (Obeidat et al., 2016). Whereas, Awawdeh et al. (2011) reported that some alternative feedstuffs (AF) can be safely included in diets for Awassi sheep at different production stages to lower the feed and production cost without deteriorating the animal performance. With appropriate inclusion, AF can be safely included in diets for Awassi sheep without negatively affecting the quantity or quality of products such as meat or milk. McDonald et al. (2002) observed that high excretion of Nitrogen (N) in urine supplemented with diet containing poultry litter may have contributed to the high rumen degradability of N whereas high excretion of N in urine is associated with high rumen N degradability.

In vitro dry matter digestibility

The results of present study shown that, in vitro dry matter digestibility of diet (A, B, C and D) containing (0, 15, 30 and 45%) broiler litter linearly decreased ($P<0.05$) as the inclusion of broiler litter increased in vitro digestibility of a diet decrease as the broiler litter increased in the diet. The reason might be due to high mineral content. Usually 8% lime stone was added to broiler ration as source of calcium most of them excreted in the faeces. In vitro dry matter digestibility decreased as the inclusion of broiler increased in the diets. The reason could be due to decrease the water soluble carbohydrate and pH by increasing broiler litter in feed reported by Hadjipanayiotou (1994) and Park et al. (1995).

Economics

The results in our study showed that, the cost of feed linearly decreased ($P<0.05$) as the level of broiler litter in total mixed ration increased that among the four diet the ration C has best result in term of the digestibility of nitrogen and crude fiber. Anakalo et al. (2009) reported that low crude protein content diets based on cane forage require a large quantity of nitrogen. Therefore economics of feeding sugar cane could be improved by using chicken manure as an alternative and inexpensive source of nitrogen. Ayoub et al. (2015) reported that, using the heat processed broiler litter in the diet of Moghani fattening lambs up to 210 g/kg of dietary DM had no effect on growth performance, feed intake and animal health however, back-fat thickness, internal fat, dissected loin fat and cost per unit production reduced. HBL can be a cheap and safe feedstuff for use as a nitrogen source in sheep diet. Moreover, the use of HBL as a feedstuff can reduce environmental pollution. Using poultry litter as feed is significance about $100 per ton as compared to conventional feeds prices. Usually, the price of poultry litter is $10 per ton. Even after transporting the litter 200 miles, the total price of the litter, including transportation, is about $30 per ton. Another advantage of feeding poultry litter is a good alternative of hay, especially during the lean periods due to drought (Fontenot and Joseph, 2000).

CONCLUSION

Based on the results of this study, it is concluded that poultry litter can be included up to 30% as a supplementation of sheep diet, to full fill the nitrogen requirement. It can be used as a substitute of traditional nitrogen sources like cotton seed cake without having any adverse effect on the health and performance of animal. Further study is needed to investigate the toxins and metal content before use as a feeding source.

Recommendations

Laboratory analyses should be carried out of biding material particularly total ash, crude fiber and crude protein these providing useful estimates of available energy, as metabolized energy. Further, suggested that before using as feed ingredient toxin level and silver content should be determined. The harvesting period of broiler litter should be determining before use, properly deep stacked before use it to ruminants.

Competing interests

The authors have declared that there are no competing interests.

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Awareness of Farmers on Newcastle Disease, its Vaccination and Antibody Titer in Commercial Chickens in Jos South, Nigeria

Oluwadare, Modupe Lola*[1], Okewole, Philip[2], Dashe, Yakubu[3], Barde Israel[3], Sa'idu, Lawal[4], Abdu, Paul[4] and Oladele, Sunday Blessing[5]

[1]*National Veterinary Research Institute, Akure Zonal Office, Ondo State Veterinary Hospital Complex, Hospital road, Ondo State, Nigeria*
[2]*National Veterinary Research Institute, Diagnostics and Extension, Vom, Plateau State, Nigeria*
[3]*National Veterinary Research Institute, Central Diagnostic Division, Vom, Plateau State, Nigeria*
[4]*Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria*
[5]*Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria*
*Corresponding author`s Email: modupelola.o@gmail.com

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ABSTRACT

Newcastle disease is a highly contagious viral disease which affects existing or developing poultry industries. This study was performed to assess the level of awareness of farmers on Newcastle disease and its control through vaccination and also to determine the level of Newcastle disease virus antibody (Ab) titer in commercial layer chicken sera using haemagglutination inhibition test in Jos South Local Government Area, Plateau State, Nigeria. A structured questionnaire was shared to farmers to fill. Thirty four farms were visited and nine districts were randomly selected. A total of 354 sera were collected from commercial chickens; ten from each flock. There was a high level of awareness of farmers (100%) on ND and its vaccination (100%) and all the farmers (100%) had vaccinated their chickens against ND. The HI test revealed that, out of the 354 sera tested, 9 (2.5 %) chickens were negative for NDV Abs, which means had NDV antibody titer below the minimum protective titer of $\log_2$ 3 and 345 chickens (97.5%) were positive for NDV Abs; had NDV antibody titer above $\log_2$ 3. It was concluded that the level of awareness of farmers on ND and its control through vaccination was incredibly high, also, the level of protection to ND in vaccinated chickens was also very high, in that a higher percentage of the chickens had NDV Antibodies between $\log_2$ 6 and $\log_2$ 8, however, inspite of these, ND is still a continual threat to the poultry industry in Nigeria. It is therefore recommended that, other biosecurity measures, such as good management practice, proper hygiene and surveillance be emphasized and ensured, in order to prevent ND infection among flocks.

Key words: Newcastle disease, Antibody titer, Haemagglutination inhibition, Commercial chickens

INTRODUCTION

For the past 50 years, poultry production has recorded greater changes than in any other world's livestock sub sector of the agricultural production sector (David, 2000). Current trends in livestock production indicate that the global production of poultry meat and dairy will double by 2050 (David, 2000). Newcastle disease (ND) is one of the most important animal diseases in the world; both for the number of animals affected every year and the severe economic impact on the poultry industry (Thompson, 2015). ND is a highly contagious viral disease which is caused by Newcastle disease virus (NDV) which belongs to the Family, Paramyxoviridae and Genus, Rubulavirus (Alexander, 1997). The disease has been reported to be the most important poultry disease (Sonaiya et al., 2000).

ND is also the most important cause of mortality in chickens and many species of domesticated and wild birds have been found susceptible to this disease; mortality of 100% is common (Nguyen, 1992; Wernery et al., 1992; Alders and Spradbrow, 2001; Saidu and Abdu, 2008). ND usually affects the respiratory, gastrointestinal and nervous systems with common signs of listlessness, increased respiratory rate, yellowish to greenish diarrhea and weakness followed later by prostration and death (Chansiripornchai and Sasipreeyajan, 2006).

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The NDV has been reported endemic in many developing countries of Africa such as Kenya (Njue et al., 2001and Njagi, 2010); Cameroon (Ekue et al., 2002 and Mai et al., 2014); Tanzania (Salam et al., 2002); Ethiopia (Chaka et al., 2013); Egypt (Mohammed et al., 2011) and Nigeria (Sa'idu et al, 2004; El –Yukuga et al., 2009; Musa et al., 2009; Yakubu, 2010; Okwor and Eze, 2010 and Salihu et al., 2012).

The first recorded and confirmed outbreak of ND in Nigeria was between December, 1952 and February, 1953 in and around the city of Ibadan, the Oyo State capital (Hill et al., 1953). The disease has been a notable problem in the country since then (Oladele et al., 2002). ND is widespread in domestic and exotic chickens (Fatumbi and Adene, 1979). The most dreaded poultry disease in Nigeria as reported by Abdu et al. (1992) is ND. Fatumbi and Adene, (1979); Adu et al. (1986); Echeonwu et al. (1994); Sa'idu et al. (1994); Alders and Spradbrow, (2001), also reported that ND is enzootic in Nigeria. Olabode et al. (1992) reported that ND is a threat to poultry industry in Nigeria and it continues to cause havoc to different species of poultry.

Newcastle disease is characterized by signs, such as coughing, gasping, sneezing and rales, nervous signs, greenish-white diarrhea and cessation of egg production (Alexander, 1997 and 2001). ND has become endemic in Nigeria in both local and commercial poultry with annual epidemics recorded in highly susceptible flocks (Halle et al.,1999; Saidu and Abdu, 2008) with pockets of outbreaks occurring between the annual epidemic periods.

Commercial chickens in Nigeria are exclusively exotic chickens which are reared intensively or semi-intensively. In most parts of the country, ND is seen and diagnosed throughout the year in commercial flocks and the incidence varies with season (Chabauf, 1990; Alders and Spradbrow, 2001).

Newcastle disease is an economically important disease of poultry for which vaccination is carried out as a preventive measure in many countries. Orakaja et al. (1999) reported vaccination as the only safe option in control strategies of infection (Orakaja et al., 1999). Mariana et al. (2016) also reported that vaccination of chickens is able to prevent internal egg contamination, reducing egg shell surface contamination and reducing shedding from digestive and respiratory tracts in virulent NDV challenged hens (Mariana et al., 2016).

Nevertheless, outbreaks of ND have been reported in vaccinated poultry populations (Van Boven et al., 2008). Vaccination is practiced widely and different types of vaccines are available but the most successful and widely used ones are the mild live virus vaccines known as the Hitchner B1 and La Sota types (Rathore *et al*., 1987 and Aliyu et al., 2014). The typical vaccination schedule in layers in Plateau state, Nigeria is as follows; the birds are vaccinated against Newcastle disease (Hitchner B1) intraoccular at day one of age on the hatcheries before being sold; they are also given Lasota vaccine orally via the drinking water at the third week of age; at week six of age, they are given kumorov vaccine either subcutaneously or intramuscularly; between week eleven and fifteen, they are given Lasota vaccine orally via drinking water; at week sixteen of age, they are given kumorov vaccine either subcutaneously or intramuscularly and at every three months interval, they are also given kumorov vaccine either subcutaneously or intramuscularly; and the typical schedule of vaccination in broilers is as; birds are given first dose of Lasota vaccine orally at two weeks of age via the drinking water and at forth week of age, they are given the second dose of Lasota vaccine orally via the drinking water.

Newcastle disease alone accounts for more than 50% of total losses in Africa's poultry flocks (Ezeibe et al., 2006 and Musa et al., 2009). In response to the threat presented by ND, several attempts have been made to put in place vaccination programmes to prevent epidemics of disease. However, outbreaks have been reported in vaccinated populations (Okwor et al., 2010).

Serological tests are useful tools in the diagnosis of infection. Haemagglutination Inhibition (HI) test is the most commonly used test for detection of immune response in affected birds (Alexander and Senne, 2008). Value of serology in diagnosis of disease depends on the vaccination history of birds and on prevailing disease conditions (OIE, 2012). Because NDV can cause a wide variety of disease presentation, it is important to enhance the awareness of field personnel (Thompson, 2015). In view of this and the economic importance of ND, this study was carried out in order to determine the level of awareness of farmers on ND and its control through vaccination and also to determine ND virus antibody titre using haemagglutination inhibition test in commercial chicken sera in Jos South Local Government Area, Plateau State, Nigeria.

MATERIALS AND METHODS

The birds that were sampled in this study were layers (commercial birds). Layers of between age eighteen weeks and twenty four weeks were sampled. A Structured questionnaire was given to farmers. The farmers' awareness of ND and its vaccination, type of

ND vaccine given, route of administration of ND vaccine, whether or not cold chain was maintained and how it was maintained, service provider, any outbreak or infection with NDV and how the disease was treated; all these information were obtained from the farmers. Table one shows the typical Newcastle disease vaccination in layers and broilers in Plateau state, Nigeria.

Convenience sampling procedure was used to select nine districts under Jos South L.G.A. These districts were; Federal low cost, Rantya, Bukuru, Rayfield, Da zarmangada, Dung village, Dadinkowa, Gyel and State low cost. Furthermore, 1- 3 farms (depending on the number of farms in each district) were also randomly selected from each of the districts and 10 to 20 birds were selected at random from each flock, depending on the number of farms in each district and the flock size, respectively. Structured questionnaire was administered to farmers. Two milliliters of blood was collected aseptically via the wing vein of each bird, using a 21–G sterile hypodermic needle and 5 ml syringe in adult birds (layers) and 23-G sterile hypodermic needle and 2 ml syringe in young birds (pullets). The samples were labeled with the name and location of the farm, type of bird and date of collection. The blood samples were kept in a slanting position at room temperature to allow for clotting and sera formation. The sera were separated by transferring into a labeled sterile bottle for HI test which was stored frozen at -20°C and sent in a cold pack to regional laboratory for avian influenza and other transboundary animal diseases at the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. Newcastle disease antigen and ND positive and negative serum were obtained from the National Veterinary Research Institute, Vom, Nigeria. The HI test was carried out by the method described by Office Internationale des Epizooties (2005).

Preparation of chicken red blood cell suspension

A total of 4 ml of blood was collected aseptically from ND antibody- negative chicken in a disposable syringe containing 1 ml of Acid Citrate Dextrose (ACD) as anticoagulant. Cells were washed three times in Phosphate Buffered Saline (PBS) of pH 7.2 by centrifuging at 447.2 g for 5 minutes (Allan and Gough, 1984). One percent RBC (packed cell V/V) suspension was prepared by adding 99 ml of Phosphate Buffered Saline (PBS) to 1 ml of washed RBC.

Statistical analysis

Data obtained through questionnaire administration were noted. The number recorded for each question was then converted into percentages and tabulated. The Statistical Package for Social Sciences (SPSS) program, version 12 was used to analyze data. Values of $P \leq 0.05$ were considered significant.

Table 1. Typical Schedule of Newcastle Disease Vaccination in Layers and Broilers in Plateau State, Nigeria

Type of bird	Age of bird at administration	Name of vaccine	Type of vaccine	Route of administration
Layer	Day one	Hitchner B1	Live	Intraoccular
	Three weeks	Lasota	Live	Oral via drinking water
	Six weeks	Kumorov	Live	Subcutaneous or intramuscular
	Between eleventh and fifteenth weeks	Lasota	Live	Oral via drinking water
	Sixteenth weeks	Kumorov	Live	Subcutaneous or intramuscular
	At three months Interval	Kumorov	Live	Subcutaneous or intramuscular
Broiler	Two weeks	Lasota	Live	Oral via drinking water
	Four weeks	Lasota	Live	Oral via drinking water

RESULTS

The questionnaire administered to farmers revealed that all of the commercial poultry farmers (100%) were aware of ND and had vaccinated their birds against ND (Table 2).

Out of the 354 sera tested, 9 (2.6 %) chickens were negative for NDV Abs, that is, had NDV antibody titer below the minimum protective titer of $\log_2$ 3 (that 7 (2.0%) chickens had NDV Ab titer of $\log_2$ 1 and 2 (0.6%) chickens had NDV Ab titer of $\log_2$ 2). 345 (97.5%) chickens were positive for NDV Abs, that had NDV antibody titer $\log_2$ 3 and above $\log_2$ 3 (Table 3). Results in table 3 indicated that a higher percentage of the chickens (64.2%) had protective NDV antibodies between $\log_2$ 6 and $\log_2$ 8.

Table 2. Awareness of Newcastle disease and its vaccination in commercial chickens and Respondents (farmers) in Jos South Local Government Area, Plateau State, Nigeria.

Awareness of Newcastle disease and its vaccination in commercial chickens	Respondents (farmers) (%)
Yes	34 (100)
No	0 (0)
Total	34 (100)

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Table 3. Distribution of Newcastle disease virus antibody titers and the corresponding number (percentage) of chickens in February, 2015 in Jos South local Government Area, Plateau State, Nigeria.

Antibody Titre (log_2)	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	2^{11}	2^{12}
Number of chickens	7	2	2	11	12	30	37	160	38	28	16	11
(Percentage)	2.0%	0.6%	0.6%	3.1%	3.4%	8.5%	10.5%	45.2%	10.7%	7.9%	4.5%	3.1%

Sera with NDV antibody titre ≥ log_2 3 were considered positive.

DISCUSSION

The result of this study showed that all the farmers (100%) involved in commercial poultry production in Jos South Local Government Area, were aware of ND. This observation points to the fact that ND is indeed of great economic importance as it is said to be enzootic in Nigeria as reported by Sa'idu et al. in 1994 and also said to be a major disease problem of poultry in many other countries of the world, especially in Africa and Asia as reported by Spradbrow (1992); Awan et al. (1994) and Oladele et al. (2005).

This study also showed that commercial chickens in Jos South LGA of Plateau State, had NDV antibodies. This indicated that farmers in this area often vaccinate their birds against ND. This supports the report by Sa'idu et al. (2006) that commercial poultry are routinely vaccinated against ND.

The OIE (2000) recommended that haemagglutination Inhibition (HI) antibody titer between log_2 0 and log_2 3 is considered negative because they produce no antibody against the virus while HI antibody titer between log_2 3 and log_2 12 is protective for chickens because it produces antibodies against the virus (Alders and Spradbrow, 2000 and Aldous et al., 2003). An HI antibody titer of log_2 4 and the above ones is indicative of exposure to the virus at one time or the other and eventual production of neutralizing antibodies to protect the chicken up to the point of sale (Joseph et al., 2014). The high HI antibody titer may be due to an infection by a virulent strain of the virus such as mesogenic strains which are viruses causing clinical signs consisting of respiratory and neurological signs with low mortality and lentogenic strains which are viruses causing mild infection of the respiratory tract without visible morbidity and mortality (Seal et al., 2000).

A ND-HI titer of log_2 3 (i.e, 1:8) and above is generally accepted as an indicative of specific immunity (Allan and Gough, 1974; Spradbrow et al., 1980 and Jagne et al., 1991). Using this criterion in this present study, 2.6% of the total number of commercial chickens tested, showed no serological evidence of specific immunity to NDV while 97.5% of the total number of chickens tested showed serological evidence of the presence of NDV. It is noteworthy that the majority of the chickens tested had a protective level of antibodies to NDV. The HI test revealed that 9 of the chickens tested showed a NDV Ab titer of < log_2 3 (1:2 to 1:4). This result showed that the serum antibody titers were too low to protect the birds from NDV infection. Similar results had been described by Awan et al. (1994). There are several possible reasons for this low level of protection in these birds; these may include, vaccine failure, impaired immune competence due to immunosuppressive diseases. Low NDV antibody prevalence is suggestive of an inter-epidemic phase or early phase of ND infection (Awan et al., 1994). Problem of ND outbreak or infection is envisaged and expected in these particular chickens unless the vaccination practice is improved substancially. 345 (97.5%) out of the total number of chickens tested had NDV Ab titer that varied between log_2 3 and log_2 12. Differentiation between vaccine titer and field challenge is difficult (Awan et al., 1994). In practice, a high antibody titer is indicative of a recent infection (Luc et al., 1992). The wider range of NDV Ab titer in some chickens may be due to natural infection with pathogenic field strain which is known to produce higher antibody titer than vaccination (Luc et al., 1992).

In Nigeria, ND has been noted to be more common during the cold harmattan periods and this is in agreement with the observations of high prevalence from November to February (Abdu et al., 1992 and Saidu et al., 1994). The harmattan period of November to February in Nigeria is characterized by wind drop in ambient temperature, dryness and other harsh weather conditions and this is believed to lower the immune status of birds making it possible for ND to manifest in commercial birds that have ordinary or lowered herd immunity to ND. Some migratory birds and birds of prey are common during this period of harmattan and their role in the epidemiology of ND may be very important.

Newcastle disease was reported prevalent in most parts of the Northern Nigeria with outbreaks seen in Bauchi State (Nwankiti et al., 2010); Borno State (El-Yuguda et al., 2009); Jigawa State (Wakawa et al., 2009); Nassarawa State (Salihu et al., 2012);

Kaduna State (Nwanta et al., 2006) and Plateau State (Musa et al., 2009). A study carried out by Lawal et al. (2015) in Gombe State, Northeastern, Nigeria, revealed an overall prevalence rate of 55.5% of ND in the State. This concurred with previous studies by Nwankiti et al. (2010) who reported prevalence rate of 56.3% in Bauchi State, Northeastern Nigeria. However, it was relatively higher than the prevalence rates of 51.9% as reported by Musa et al. (2009) in Plateau State; 52.2% reported by Sadiq et al. (2011) in Borno State and lower than 73.3% reported by Nwanta et al. (2006) in Kaduna State, Northwestern Nigeria. The most commercial poultry farmers in the study area claimed to have been vaccinating their flocks. Mariana et al. (2016) reported in their study that vaccination of chickens is able to prevent internal egg contamination, reducing egg shell surface contamination and also reducing shedding from digestive and respiratory tracts in virulent NDV challenged hens (Mariana et al., 2016).

In the US, however, the virus has been eradicated due to stringent adherence to poultry management rules and any virulent strains are of foreign origin from places where strict compliance to management regulations and good sanitary practices is lacking (Qin et al., 2008).

The higher NDV antibody titer of between $\log_2$ 8 to $\log_2$ 12 in this present study may be suggestive of ND infection and this seems consistent with the findings of Sa'idu et al. (2006) and Nwanta et al. (2006), where both reported the disease to be common during the dry harmattan period (November – March) with cold stress also been reported to exacerbate the epidemiology of the ND. Alders and Spradbrow (2001) reported that the windy harmattan encourages the spread of the NDV. Although, Aliyu et al. (2015), has a contrary findings. The results of the study by Aliyu et al. (2015) showed that the difference in the prevalence of ND in the dry season and in the rainy season was significant. The findings in that study were not in agreement with reports made by Sa'idu et al. (1994) and Halle et al. (1999) on the seasonality of ND, which revealed that the highest prevalence of the disease occurred between October and March, possibly because of the cold weather with high wind velocity (Abdu et al., 1992).

Shafqat et al. (2015) presented a field data suggesting that, despite high levels of anti-NDV antibody titers of >$\log_2$ 3 HI in 99% of the tested birds in different farms and localities, there was a very high incidence of the disease (Shafqat et al., 2015).

One of the reasons attributed to this change in findings was that poultry farmers are more enlightened about the need for reporting disease outbreak to Veterinary clinic, thus, it was deduced under probability that the high prevalence in the layers may be due to arbitrary vaccination of birds within the egg production period.

Exclusive dependence on the erratic power supply for vaccine storage may lead to vaccine failure (Okwor et al., 2009). The availability of poor quality vaccines and presence of rampant unreliable vaccination schedules against ND could have contributed to the increased rate of the disease. However, the history of vaccination program is very important in the interpretation of results.

CONCLUSION

In conclusion, there is a high level of awareness of farmers in Jos South Local Government Area, Plateau State, of Newcastle disease and its control through vaccination. High percentage of chickens that were positive to NDV Ab in this study indicates that ND is a common and endemic disease of chickens; also, the level of protection of commercial chickens in this study was found satisfactory.

It is therefore recommended, that strict regulations must be adopted against outbreak of NDV infection, such as restriction of movements in and around the farms. Biosecurity measures and continuous surveillance must also be applied. Continual boosting of immunity of birds with NDV vaccine must also be included in order to reduce economic losses usually caused by Newcastle disease outbreak. Farmers must also source and vaccinate their flocks with the help of veterinarians and in accordance with the recommended vaccination program.

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Competing interests

Authors have declared that no competing interests exist as regards this manuscript.

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Growth Performance and Gastrointestinal Tract Morphometry in Growing Japanese Quails Fed with *Moringa oleifera* Leaf Meal as Partial Replacement of Dietary Soya Beans Meal

Muhammad Abdullahi Mahmud[1]*, Shaba Peter[1], Gana James[2], Ndagimba Ruth[1], Abdulsalam Wosilat[1], Ma'aji Musa[1] and Mohammed Alhaji Abubakar[1]

[1]*Department of Animal Health and Production Technology, Niger State College of Agriculture, Mokwa, Niger State, Nigeria*
[2]*Department of Agricultural Education, Federal College of Education, Kontagora, Niger State, Nigeria*
*Corresponding author's Email: drmahmud2@gmail.com

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ABSTRACT

One hundred and twenty (120) day old Japanese quails were bought and allocated to four dietary treatments of thirty (30) birds per treatment with the aim of studying the growth performance and gastrointestinal tract morphometry of growing Japanese quails fed with graded levels of *Moringa oleifera* meal as partial replacement of dietary soybean meal. Each treatment had three replicates of 10 birds each in a completely randomized design. The experiment lasted for six weeks. Four diets containing 24% crude protein for the growing phase (0-6 weeks) were formulated in which *Moringa oleifera* leaf meal replaced soya bean meal at 0, 5, 10 and 15% as T_0, T_1, T_2, T_3 respectively. The mean initial body weights, the mean final body weight and the mean total weight gains of the four treatments were significantly different from one another. However, feed conversion ratio, protein efficiency, performance efficiency factor and production number significantly varied across the four treatments; with T_1 having the best result than the others. The mean spleen weights, mean breast weights, mean thigh weights, mean drumstick weights, mean wing weights and mean liver weights of the growing Japanese quails in the four treatments were not significantly different from one another except the heart weight of T_1. The mean weights, lengths, width and thickness of proventriculus, ventriculus, duodenum, jejunoileum, caeca and colon of the growing Japanese quails in the four treatments were not significantly different from one another, except the lengths of jejunoileum. It is therefore concluded that in growing Japanese quails, *Moringa oleifera* leaf meal may replace dietary soya beans meal up to 15%, with optimum level of 5% and no apparent adverse effects on gastrointestinal tract morphometry.

Key words: Growth performance, Gastrointestinal tract, Soya beans, *Moringa oleifera*, Japanese quail

INTRODUCTION

The prices of soya bean and fish meals which are widely and successfully used as conventional protein sources for livestock have been escalating continuously in recent times, whilst their availability is often erratic. The problem has been worsened due to the increasing competition between humans and livestock for these protein ingredients as food. According to Odunsi (2003), the rapid growth of human and livestock population, which is creating increased needs for food and feed in the less developed countries, demands that alternative feed resources must be identified and evaluated. One possible source of cheap protein is the leaf meals of some tropical legume browse plants. Leaf meals do not only provide protein source but also some essential vitamins such as vitamins A and C, minerals and oxycarotenoids (Egwui et al., 2013). The constraints to enhanced utilization of leaf meals reside chiefly on factors such as fibre content, the presence of anti-nutritive compounds and deficiencies of certain amino acids (Olvera – Castillo et al., 2011). Several treatment such as soaking, fermenting, sun drying, auto claving, boiling and toasting have been investigated to reduce or remove the anti-nutritional factors (Adegoke et al., 2010).

Recently, there has been interest in the utilization of *Moringa oleifera* commonly called horseradish tree or drumstick tree, as a protein source for livestock (Makker and Becker, 1997 and Sarwatt et al., 2002). Moringa leaf has quality attributes which make it a potential replacement for soybean meal or fish meal in non-ruminant diets. Moringa can easily be established in the field, has good coppicing ability, as well as good potential for forage production. Furthermore, there is the possibility of obtaining large amounts of high quality forage from moringa without expensive inputs due to favorable soil and climatic conditions for its growth (Makker and Becker, 1997). Sarwattet al. (2004) reported that moringa foliages are a potential inexpensive protein source for livestock feeding. The advantages of using moringa for a protein resource are numerous, and include the fact that it is a perennial plant that can be harvested several times in one growing season and also has the potential to reduce feed cost.

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Moringa oleifera is in the group of high-yielding nutritious browse plants with every part having food value (Duke, 1998). Despite the high Crude Protein (CP) content of 25-27% in moringa leaf meal, and being extensively exploited in both broiler and layer chickens diets, there is little information available on the use of this unconventional feed resource, especially as an alternative protein source in growing Japanese quails. Therefore, this work highlights the growth performance and gastrointestinal tract morphometry as influenced by partial replacement of dietary Soya beans meal with *Moringa oleifera* leaf meal in growing Japanese quails.

MATERIALS AND METHODS

The study was carried out at the poultry unit of the Niger state College of Agriculture, College Livestock Farm, Mokwa, Nigeria. Mokwa is located at latitude 9°17'38" North and longitude 5°3'16 East (Google maps, 2015). 120 day-old Japanese quails were bought and allocated to four dietary treatments of 30 birds per treatment. Each treatment had three replicates of 10 birds per replicate in a completely randomized design. The experiment lasted for six weeks. Four diets containing 24% CP for the growing phase (0-6 weeks) were formulated in which *Moringa oleifera* leaf meal replaced Soya beans meal at 0, 5, 10 and 15% as T_0, T_1, T_2, T_3 respectively. The moringa leaves were air dried in shade until they were crispy to touch, while retaining their greenish coloration. The leaves were then milled using a hammer mill of sieve size 3 mm, to obtain a product herein referred to as moringa leaf meal (MOLM) which was stored in sacs until needed. Diet 1, which was designated as T_0 served as the control diet and contained soybean meal as the main protein source with no moringa leaf meal. Diet 2 designated as T_1, diet 3 as T_2 and diet 4 as T_3. All diets met the nutrient requirements of Japanese quail as set out by National Research Council (NRC) (1994). Table 1 represents the ingredients composition of the experimental diets.

Performance characteristics monitored included initial body weight, final body weight, final body weight gain, total feed intake, Feed Conversion Ratio (FCR), Protein Efficiency (PE), Performance Efficiency Factor (PEF) and Production Number (PN). The carcass parts weight analysis was done by weighing each of the parts using digital weighting scale with sensitivity of 0.01 gram. The gross morphometry of the Gastrointestinal Tract (GIT) was done by measuring weight (gram), length (cm), width (cm) and thickness (mm) of its segments in each treatment using digital weighting balance with sensitivity of 0.01 gram, thread stretched on a meter rule and digital Vernier caliper.

PE was calculated as the ratio of body weight gain to the crude protein fed as described by Sidduraju and Becker (2003). The PEF was calculated by dividing the live body weight of the flock by FCR and number of chicks purchased, multiplied by 100 as described by Ghosh and Samanta (2008).

The PN was calculated as described by Ghosh and Samanta (2008) using the following formular:

$$PN = \frac{\text{Daily growth x Survivability (\%)}}{\text{FCR X 10}}$$

$$\text{Daily growth} = \frac{\text{Average Final Weight/bird}}{\text{Average Fattening Period}}$$

Survivability = 100 - % mortality

One-Way Analysis of Variance (ANOVA) according to Steel and Torrie (1980) using Statistical Package for Social Sciences (SPSS) 17 at 95% confidence interval (CI) was used to determine level of significant difference in mean values of the data. Values of ($P \leq 0.05$) were considered significant. Where there were differences in means, Duncan's Multiple Range Test (DMRT) was used to separate the means (Duncan, 1955).

Table 1. Ingredient inclusion (%) of grower ration fed to Japanese quails

Ingredients (%)	Experimental diets			
	$T_{0(0\%)}$	$T_{1(5\%)}$	$T_{2(10\%)}$	$T_{3(15\%)}$
Maize	38.22	38.22	38.22	38.22
Soybean (Full Fat)	36.02	34.22	32.42	30.62
Maize offal	9.55	9.55	9.55	9.55
Fish meal	12.01	12.01	12.01	12.01
MOLM	0.00	1.80	3.60	5.40
Limestone	1.50	1.50	1.50	1.50
Bone meal	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Total	100	100	100	100
Chemical analysis (%)				
Crude Protein	25.64	25.28	24.92	24.56
Crude Fibre	3.47	3.53	3.59	3.63
ME (Kcal/Kg)	3083	3049	3016	2983

T_0 = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3. MOLM=*Moringa oleifera* Leaf Meal

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RESULTS

Growth performance

The results for growth performance of growing Japanese quails fed graded levels of *Moringa oleifera* leaf meal as partial replacements for Soybean meal are shown in table 2.

Mean initial body weight

The mean initial body weights of growing Japanese quails in T_0, T_1, T_2 and T_3 were 15.33 ± 0.88 gram, 16.00 ± 0.57 gram, 18.00 ± 0.57 gram and 18.00 ± 0.57 gram respectively. There was no statistical significant (P>0.05) difference in the mean initial body weights of the four treatments.

Mean final body weight

The mean final body weights of growing Japanese quails in T_0, T_1, T_2 and T_3 were 160.87 ± 1.01 gram, 170.08 ± 4.49 gram, 157.78 ± 11.84 gram and 150.78 ± 2.30 gram respectively. There was no statistical significant (P>0.05) difference in the mean final body weights of the four treatments.

Mean final body weight gain

The mean final body weight gains of growing Japanese quails in T_0, T_1, T_2 and T_3 were 145.54 ± 0.71 gram, 154.08 ± 4.69 gram, 139.26 ± 12.17 gram and 132.78 ± 2.63 gram respectively. There was no statistical significant (P>0.05) difference in the mean final body weight gain of the four treatments.

Total feed intake

The mean total feed intake of growing Japanese quails in T_0, T_1, T_2 and T_3 were 462.17 ± 0.65 gram, 500.55 ± 0.63 gram, 452.48 ± 0.79 gram and 462.16 ± 0.64 gram respectively. There was no statistical significant (P>0.05) difference in the mean total feed intake of the four treatments.

Feed conversion ratio

The mean FCRs of growing Japanese quails in T_0, T_1, T_2 and T_3 were 3.18 ± 0.04, 3.25 ± 0.01, 3.25 ± 0.01 and 3.48 ± 0.01 respectively. There was statistically significant (P≤0.05) difference in the mean FCRs of the four treatments. Though the FCR of T_0 was numerically lower, it was not significantly (P>0.05) different from those of T_1 and T_2. The FCRs of T_0, T_1 and T_3 however, differed significantly (P≤0.05) from the FCR of T_3.

Protein efficiency

The mean PEs of growing Japanese quails in T_0, T_1, T_2 and T_3 were 5.68 ± 0.01, 6.09 ± 0.01, 5.59 ± 0.01 and 5.41 ± 0.01 respectively. There was statistically significant (P≤0.05) difference in the mean PEs of the four treatments. The PE of T_1 was significantly (P≤0.05), the highest of the four treatments, while that of T_3 was significantly (P≤0.05) the lowest of the four treatments.

Performance efficiency factor

The mean PEFs of growing Japanese quails in T_0, T_1, T_2 and T_3 were 4290.05 ± 0.01 (%), 5233.23 ± 0.01 (%), 4094.34 ± 0.01 (%) and 3069.00 ± 0.58 (%) respectively. There was statistically significant (P≤0.05) difference in the mean PEFs of the four treatments. The PEF of T_1 was significantly (P≤0.05), the highest of the four treatments, while that of T_3 was significantly (P≤0.05) the least of the four treatments.

Production number

The mean PNs of growing Japanese quails in T_0, T_1, T_2 and T_3 were 9.75±0.01, 11.80±0.01, 9.30± 0.01 and 6.98±0.01 respectively. There was statistically significant (P≤0.05) difference in the mean PNs of the four treatments. The PN of T_1 was significantly (P≤0.05), the highest of the four treatments, while that of T_3 was significantly (P≤0.05) the least of the four treatments.

Carcass parts weight analysis

The results for carcass parts weight analyses of the growing Japanese quails fed graded levels of *Moringa oleifera* leaf meal as partial replacements for soya beans meal are shown in table 3.

The mean spleen weights, mean breast weights, mean thigh weights, mean drumstick weights, mean wing weights and mean liver weights of the growing Japanese quails in the four treatments were not significantly (P>0.05) different from one another. Whereas the weight of the heart in T_0 (1.33 ± 0.07 gram), T_2 (1.32 ± 0.05 gram) and T_3 (1.33 ± 0.95 gram) were significantly (P≤0.05) lower than the value of 1.58 ± 0.06 gram obtained in T_2.

Table 2. Performance of growing Japanese quails fed graded levels of *Moringa oleifera* for six weeks as partial replacement for soybean meal

Parameters	Treatments			
	T_0 (0%)	T_1 (5%)	T_2 (10%)	T_3 (15%)
Initial body weight (gram)	15.33±0.88[a]	16.00±0.57[a]	18.00±0.57[a]	18.00±0.57[a]
Final body weight (gram)	160.87±1.01[a]	170.08±4.49[a]	157.78±11.84[a]	150.78±2.30[a]
Body weight gain (gram)	145.54±0.71[a]	154.08±4.69[a]	139.26±12.17[a]	132.78±2.63[a]
Total feed intake (gram)	462.17±0.65[a]	500.55±0.63[a]	452.48±0.79[a]	462.16±0.64[a]
FCR (g/g)	3.18±0.04[a]	3.25±0.01[a]	3.25±0.01[a]	3.48±0.01[b]
PE	5.68±0.01[c]	6.09±0.01[d]	5.59±0.01[b]	5.41±0.01[a]
PEF (%)	4290.05±0.01[c]	5233.23±0.01[d]	4094.34±0.01[b]	3069.00±0.58[a]
PN	9.75±0.01[c]	11.80±0.01[d]	9.30±0.01[b]	6.98±0.01[a]

[a, b, c, d] Means ± SEM within the same row with different superscripts are significantly different (P≤0.05) from each other; T_0 = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3; FCR= Feed Conversion Ratio, PE= Protein Efficiency, PEF= Performance Efficiency Factor, PN= Production Number.

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Gastrointestinal tract gross morphometry

The results for weights and lengths of the segments of the GIT of the growing Japanese quails fed graded levels of *Moringa oleifera* leaf meal as partial replacements for soya beans meal are shown in diagrams 1 and 2 respectively, while widths and thickness of the proventriculus and ventriculus in growing Japanese quails fed with graded levels of *Moringa oleifera* for six weeks as partial replacement for soya bean meal are shown in tables 4 and 5 respectively.

The mean weights, lengths, width and thickness of proventriculus, ventriculus, duodenum, jejunoileum, caeca and colon of the growing Japanese quails in the four treatments were not significantly (P>0.05) different from one another, except the lengths of jejunoileum. The length of jejunoileum (1.80 ± 0.22 cm) of T_1 was significantly (P≤0.05) the highest of the four treatments while that of T_3 with the value of 1.00 ± 0.11 cm was significantly (P≤0.05) the least.

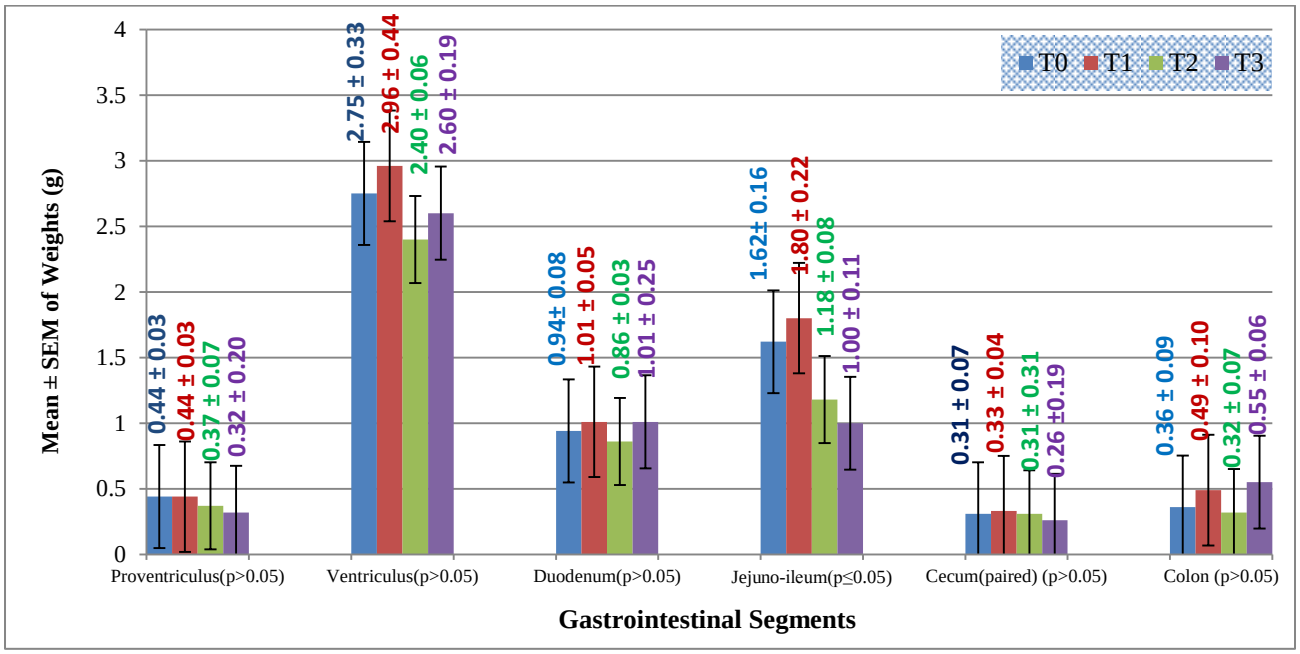

Diagram 1. Mean ± SEM weights of segments of the gastrointestinal tract in growing Japanese quails fed with graded levels of *Moringa oleifera* as partial replacement for soya bean meal. T_0 = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.

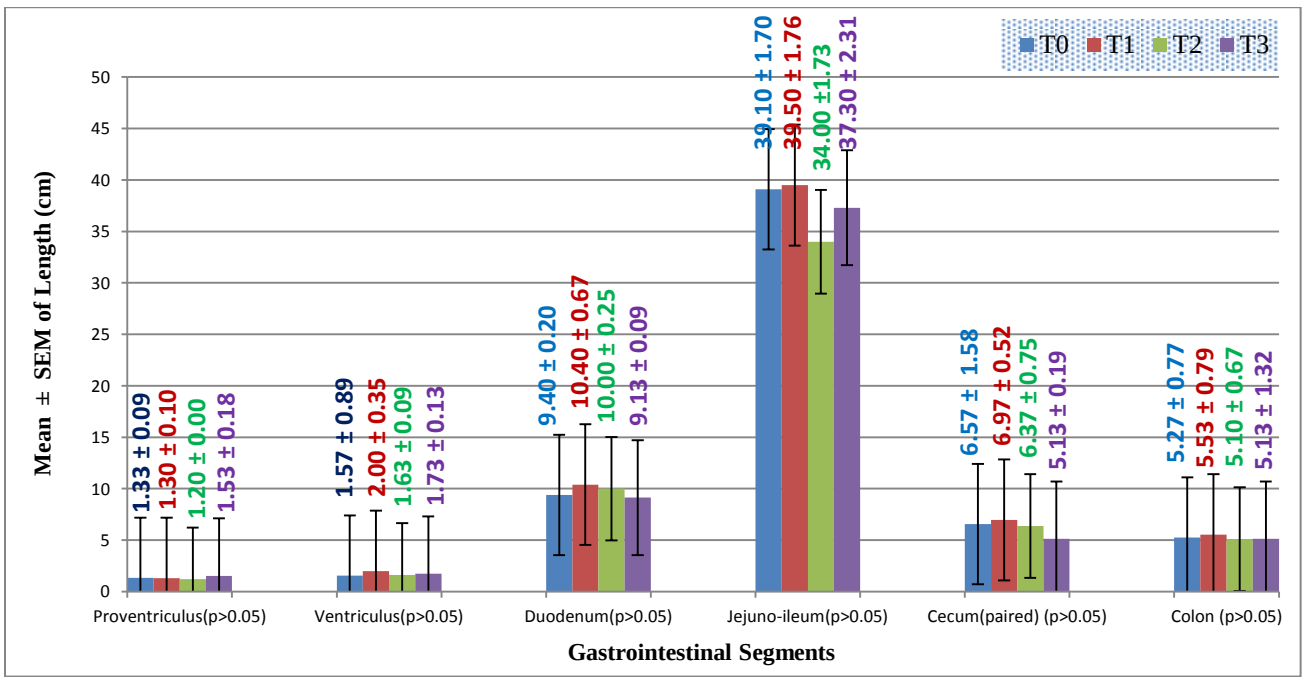

Diagram 2. Mean ± SEM length of segments of the gastrointestinal tract in growing Japanese quails fed with graded levels of *Moringa oleifera* as partial replacement for soya bean meal. T_0 = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.

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Table 3. Mean weight of carcass organs in growing Japanese quails fed with graded levels of *Moringa oleifera* for six weeks as partial replacement for soya bean meal

Parameter	Treatments			
	T_0 (0%)	T_1 (5%)	T_2 (10%)	T_3 (15%)
Spleen (gram)	0.04 ± 0.00	0.11 ± 0.05[a]	0.06 ± 0.01	0.55 ± 0.06
Breast (gram)	30.40 ±2.93	34.77 ± 0.93[a]	30.88 ± 3.43	28.25 ± 2.06
Thigh(gram)	10.93 ± 0.06	10.43 ± 0.03[a]	10.87 ± 0.39	10.37 ± 0.19
Drumstick (gram)	1.16 ± 0.09	1.43 ± 0.02[a]	1.28 ± 0.03	1.40 ± 0.09
Wing (gram)	4.74 ± 0.28	4.91 ± 0.23[a]	4.03 ± 0.32	4.40 ± 0.01
Heart (gram)	1.33 ± 0.07	1.58 ± 0.06[b]	1.32 ± 0.05	1.33 ± 0.05
Liver (gram)	2.06 ± 0.26	2.30 ± 0.26[a]	2.45 ± 0.16	1.89 ± 0.15

[a, b,] Means ± SEM within the same row with different superscripts are significantly different (P≤0.05) from each other; T_0 = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.

Table 4. Mean ± SEM width of proventriculus and ventriculus in growing Japanese quails fed with graded levels of *Moringa oleifera* for six weeks as partial replacement for soya bean meal

Parameters	Treatments			
	T_0 (0%)	T_1 (5%)	T_2 (10%)	T_3 (15%)
Proventriculus (cm)	0.50±0.06	0.57±0.03	0.53±0.07	0.53±0.33
Ventriculus (cm)	2.33±0.18	2.23±0.03	2.20±0.06	2.17±0.07

T_0 = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.

Table 5. Mean ± SEM thickness of proventriculus and ventriculus in growing Japanese quails fed graded levels of *Moringa oleifera* as partial replacement for soya beans meal.

Parameters	Treatments			
	T_0 (0%)	T_1 (5%)	T_2 (10%)	T_3 (15%)
Proventriculus (mm)	3.56±0.19	2.32±0.68	2.43±0.49	2.42±0.33
Ventriculus (mm)	8.23±1.39	10.03±0.12	8.64±1.64	8.11±1.45

T_0 = Control, T_1 = Treatment 1, T_2 = Treatment 2, T_3 = Treatment 3.

DISCUSSION

The finding of non-significant difference in the mean body weight gain and feed intake of the present study in the four treatments were similar to the earlier reports of Gadzirayi et al. (2012) in broiler chickens fed with different levels of moringa leaf meal. This study confirms previous findings that indicated Moringa Leaf Meal promoted good growth and productivity in poultry is attributed to its nutrients and phytochemicals (Kakengi et al., 2007).

The findings on FCR of the control group (T_0) to be numerically lower than the quails of T_1 and T_2 but the PE, PEF, and PN of T_1 were higher than the control group (T_0) are in line with the reports of Fuglie (1999) and Ebenebe et al. (2012) who reported high performance of livestocks fed on moringa based diet. This finding might be due to natural enzymes in moringa which facilitate digestion of fibrous food in animals and improve bioavailability of nutrients (Foild et al., 2011).

The significantly lower FCR of the control group (T_0) than those of T_2 and T_3 but with higher PE, PEF, and PN as moringa leaf meal inclusion increased, is similar to the reports of Ash and Petaia (1992) and Olugbemi et al. (2010) that increasing inclusion level of moringa leaf meal in broiler diets results in depressed growth performance. This observation could be generally traced to increasing fibre content of the diet which may have impaired nutrient digestibility and absorption (Ige et al., 2006). The negative effect of the anti-nutritional factors and phytochemical compounds present in *Moringa oleifera* leaf meal on the quails could be responsible for decreasing the performance.

The non-significant difference found in mean spleen weights, mean breast weights, mean thigh weights, mean drumstick weights, mean wing weights and mean liver weights of the growing Japanese quails in the four treatments are similar to the finding of Zanu et al. (2012) and Safa and Tazi (2012) who indicated that, none of the parameters measured for carcass characteristics in birds fed with diets containing *Moringa oleifera* leaf meal was significantly affected by inclusion of Moringa leaf meal. However, the significant difference observed in the weight of the heart between T_1 and other treatments are contrary to the non-significant difference reported by Safa and Tazi (2012) in broiler chicks.

The finding of non-significant difference on the many GIT parts gross morphometry could not be compared to the findings of other workers due to paucity of available relevant literature however, the findings indicated that inclusion of graded levels of *Moringa oleifera* leaf meal into diets of Japanese quails up to 15% as partial replacement for soybean meal has no apparent adverse morphometric effects on GIT.

The finding of significant difference observed in the present study in the length of jejunoileum of T_1 than that of the control group (T_0) could mean that more intestinal villi that are responsible for feed absorption might be more in T_1 than in T_0. This probably explains the better feed utilization observed in this study in the T_1 compared to T_0 and remaining treatments.

CONCLUSION

The mean initial body weights, mean final body weight, and mean total weight gains of the four treatments were significantly different from one another. However, FCR, PE, PEF and PN significantly varied across the four treatments; with T_1 having the best of the four treatments. The mean spleen weights, mean breast weights, mean thigh weights, mean drumstick weights, mean wing weights and mean liver weights of the growing Japanese quails in the four treatments were not significantly different from one another except the heart weight of T_1. The mean weights, lengths, width and thickness of proventriculus, ventriculus, duodenum, jejunoileum, caeca and colon of the growing Japanese quails in the four treatments were not significantly different from one another, except the lengths of jejunoileum. It is therefore concluded that in growing Japanese quails, *Moringa oleifera* leaf meal may replace dietary soya bean meal up to 15%, with optimum level of 5% and no apparent adverse effects on GIT morphometry.

Competing interests

The authors have no competing interests to declare.

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Crude Protein and Energy Requirements of Japanese Quail (*Coturnix coturnix japonica*) During Rearing Period

Babatunde Richard Olusegun Omidiwura*, Olatunbosun Odu, Adebisi Favour Agboola, Deborah Dara Akinbola and Eustace Ayemere Iyayi

Department of Animal Science, University of Ibadan, Nigeria
*Corresponding author's Email: richardwura@gmail.com

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ABSTRACT

Present experiment was conducted to evaluate the effect of diets containing different levels of metabolizable energy (3000, 3100 and 3200 kcal metabolizable energy/kg) and crude protein (20, 22, 24 and 26% crude protein) on performance of growing Japanese quail. 288 two-week old quail chicks were assigned into 12 treatments and 3 replicates with 8 birds in each. Birds were randomly allocated to each dietary treatment. For 3000, 3100 and 3200 kcal metabolizable energy/kg levels of energy, crude protein levels of 26, 24, 22 and 20% were assigned. Data on performance and nutrient digestibility were recorded and analyzed using a completely randomized design with a 4×3 factorial arrangement during 6 weeks of age. Metabolizable energy significantly affected ($P < 0.05$) total and daily feed intake. Level of crude protein also had a significant effect on the crude protein intake and protein efficiency ratio of growing Japanese quails. Level of crude protein and metabolizable energy had no significant effect on the body weight gain. The metabolizable energy significantly affected ($P < 0.05$) the ether extract digestibility while crude protein significantly affected ash digestibility. The results indicated that a diet of 26% crude protein and 3200 kcal metabolizable energy/kg is suitable for optimum performance of Japanese quail in terms of weight gain.

Keywords: Japanese quail, Crude protein, Metabolizable energy, Digestibility, Performance

INTRODUCTION

One way of increasing the protein supply is to diversify poultry production as well as increasing the production of other micro-livestock species with a short generation interval (Mandel et al., 2006). Japanese quail (*Coturnix coturnix japonica*) is among such micro-livestock animals which described as an excellent and cheap source of animal protein for Nigerians (Babangida and Ubosi, 2006).

The nutrient requirements of Japanese quail have been documented to a greater extent in some regions of the world than those of other game bird species (Ayasan and Okan, 2006), largely due to the bird's widespread functionality as a producer of meat and eggs renowned for high quality protein, high biological value, low caloric content (Haruna et al., 1997; Olubaniwa et al., 1999), nutritional and medicinal value (Dowarah and Sethi, 2014), their use as research animals and ease in handling, propagation, and reproduction for amateur bird fanciers and hobbyists. Adequate energy must be supplied by the diet to make efficient use of dietary protein. It has been discovered that production results are determined not by protein amount, but first of all by energy to protein ratio (Zofia et al., 2006). Alaganawy et al. (2014) reported that adequate amino acid balance is the most important nutrient for Japanese quails, while Reda et al. (2015) reported crude protein and energy levels of 22% and 2900 kcal ME/kg, respectively, as adequate during the first few weeks of growth. Jahanian and Edriss (2015) reported CP and energy levels of 26% and 3000 kcal ME/kg respectively, for the same period.

Japanese quail requirements for energy and protein in Nigeria (a tropical country) as well as the efficiency of feed utilization are still poorly documented. Thus, it was the aim of this study to investigate the energy and crude protein requirements of the Japanese quail in tropical environment during the rearing period by feeding different dietary levels of protein and energy to growing Japanese quail.

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MATERIALS AND METHODS

Three hundred and twenty 1-day-old Japanese quail chicks were purchased from a local hatchery. They were fed a basal diet for two weeks *ad libitum*. At the end of two weeks, they were weighed and 288 of them were randomly distributed into 12 treatments with 3 replicates in each treatment and 8 birds per replicate. Birds were reared in cages of dimension 60 cm × 60 cm. The study was conducted at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Nigeria.

The experimental diets were corn-soybean based with 4 levels of protein (20, 22, 24 and 26% CP) at either of 3 levels of energy (3000, 3100 and 3200 kcal ME/kg diet). The composition of the experimental diets is presented in table 1. The birds in all the treatments were subjected to similar management practices throughout the experimental period. Birds in each replicate were group weighed at weekly interval to know the gain in weight and weekly feed intake was also recorded on replicate basis. At the end of week six, total faeces voided by each replicate group was collected and weighed consecutively for three days, bulked and frozen until needed for further analysis. The faeces were dried at 55^0C in a hot air oven. Dried faeces were grounded and analyzed for proximate composition, gross energy, phosphorus and calcium.

Table 1. Summary of the composition of experimental diets fed to quails during 6 weeks of age

Treatment Number	Dietary metabolizable Energy (Kcal ME/ kg)	Metabolizable Energy level	Dietary crude Protein (%)
1	3000	Low	20
2	3100	Medium	20
3	3200	High	20
4	3000	Low	22
5	3100	Medium	22
6	3200	High	22
7	3000	Low	24
8	3100	Medium	24
9	3200	High	24
10	3000	Low	26
11	3100	Medium	26
12	3200	High	26

Data analysis

Data of feed offered and body weight were recorded weekly and used to calculate feed intake, weight gain, feed conversion ratio and protein efficiency ratio.

Nutrient digestibility

Proximate compositions of the feed and the faecal samples were determined using methods of AOAC (1996). The proximate compositions of the feed and excreta samples were used to calculate the percent digestibility of nutrients on dry matter basis using the following formula;

Digestibility (%) = [Nutrient intake - Nutrient output / Nutrient intake] × 100

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS statistical package (SAS, 2003) as a 4×3 factorial arrangement in a completely randomized design. Significant means were separated using Duncan multiple range test at $P<0.05$.

RESULTS AND DISCUSSION

The results of performance of growing Japanese quail fed varying levels of CP and energy are presented in table 2. The different dietary protein levels had a significant effect ($P<0.05$) on the total feed intake/bird. Birds on the 26% CP had the highest total feed intake when compared with birds fed with other protein levels. This supports the result of Dowarah et al. (2014) who reported that Japanese quail fed with 26% CP recorded the highest feed intake at 4-5 weeks of age. It could be concluded that the increase in feed intake at the finisher phase compensated for the reduced feed intake at the starter phase so that the overall effect of reduced intake becomes unnoticeable at the end of finishing phase. It was also observed from the study that total feed intake was reduced as the energy level increased. This supports the findings of Barque et al. (1994) and Attia et al. (2006) who reported that bird fed ration containing 3000 kcal ME/kg feed apparently consumed less feed compared to birds fed 2600 and 2800 Kcal ME/kg diet. Abbasali et al. (2011) further explained that a higher feed intake with decreased dietary energy concentration was mainly to compensate the energy intake, most importantly at finisher phase during which their energy requirements is relatively higher than starter and grower phases.

The results of the effect of protein and energy on body weight gain showed no significant differences ($P>0.05$) although 26% CP fed group recorded the highest (107.7 gram) weight gain, which comes from the fact that proteins build muscles. Abbasali et al. (2011) observed that the mean body weight was significantly higher and influenced by increased dietary protein level ($P<0.05$) in the growing phase thus emphasizing the importance of dietary protein and also amino acid concentrations in growing quail diet. The increase in weight gain at 6 weeks of age obtained in the present study though not significant can be attributed to the increased weight gain of chicks at

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starter phase although the different phases in the rearing periods were not considered separately in the present study.

Dietary energy level had no significant effect (P>0.05) on the body weight gain of birds. This is in agreement with the findings of Barque et al. (1994) who reported that various levels of energy did not affect weight gain of quail chicks. Elangovan et al. (2004) reported that body weight gain was significantly higher in the diet with 2900 and 2700 Kcal ME/kg than 2500 Kcal ME/kg diet. This is in agreement with the present study where it was observed that birds on the HE (3200kcal ME/Kg) and ME (3100Kcal ME/Kg) had the highest body weight gain.

Table 3 shows the effect of different levels of protein, energy and their interaction on the performance of growing Japanese quails. Inclusion of varying levels of protein in the diet of Japanese quails had a significant effect (P<0.0001) on crude protein intake, average daily crude protein intake and protein efficiency ratio of the birds. Also, energy had a significant effect (P<0.0001) on the total feed intake and average daily feed intake of birds. The interaction of protein and energy on all the performance parameters was not significant.

Table 2. Performance of growing Japanese quail birds fed with varying levels of protein, energy and different combinations of protein and energy during 6 weeks of age

Treatments	Initial body weight (gram)	Final body weight (gram)	Weight Gain (gram)	Daily weight gain (gram)	Feed Intake (gram)	Daily feed intake (gram)	CP intake (gram)	Daily CP intake (gram)	PER	FCR
VLP-LE	49.88	148.25	98.38	3.51	585.21^{a}	20.90^{a}	108.03^{i}	3.86^{i}	0.91^{bcd}	5.98
VLP-ME	49.71	152.92	103.21	3.69	572.04^{bc}	20.43^{bc}	106.40^{j}	3.80^{j}	0.97^{ab}	5.56
VLP-HE	50.00	160.29	110.21	3.94	565.54^{c}	20.20^{c}	104.80^{k}	3.74^{k}	1.05^{a}	5.16
LP-LE	50.08	156.13	106.04	3.79	584.29^{a}	20.87^{a}	118.60^{g}	4.24^{g}	0.89^{bcde}	5.51
LP-ME	50.54	160.04	109.50	3.91	569.13^{bc}	20.33^{bc}	115.93^{h}	4.14^{h}	0.94^{abc}	5.23
LP-HE	50.13	146.67	96.54	3.45	567.58^{c}	20.27^{c}	115.17^{h}	4.11^{h}	0.84^{cdef}	5.89
MP-LE	50.18	156.29	106.21	3.79	586.92^{a}	20.96^{a}	130.77^{d}	4.67^{d}	0.81^{def}	5.54
MP-ME	50.25	153.25	103.00	3.68	573.00^{bc}	20.46^{bc}	128.06^{e}	4.57^{e}	0.80^{def}	5.57
MP-HE	49.67	151.50	101.83	3.64	568.08^{c}	20.29^{c}	125.69^{f}	4.49^{f}	0.81^{def}	5.63
HP-LE	49.79	154.46	104.67	3.74	591.00^{a}	21.11^{a}	142.17^{a}	5.08^{a}	0.74^{f}	5.66
HP-ME	50.04	157.21	107.17	3.83	576.25^{b}	20.58^{b}	139.35^{b}	4.98^{b}	0.77^{ef}	5.39
HP-HE	50.00	160.58	110.58	3.95	568.42^{c}	20.30^{c}	137.02^{c}	4.89^{c}	0.81^{def}	5.18
SEM±	0.85	4.97	4.67	0.17	2.33	0.08	0.47	0.02	0.04	0.25

^{a,b,c,e,f,g,h,i,j} Means in the same row with different superscripts are significantly different (P<0.05). VLP: Very Low Protein (20%), LP: Low Protein (22%), MP: Medium Protein (24%), HP: High Protein (26%), LE: Low Energy (3000 kcal ME/kg), ME: Medium Energy (3100 kcal ME/kg), HE: High Energy (3200 kcal ME/kg)

Table 3. Effect of different levels of protein, energy and their interaction on the performance of growing Japanese quails at 6 weeks of age

Parameters	P value		
	Protein	Energy	Protein × Energy
Initial body weight (g/bird)	0.950	0.941	0.998
Final body weight (g/bird)	0.770	0.841	0.302
Weight gain (g/bird)	0.723	0.849	0.256
Daily weight gain (g/bird/day)	0.723	0.849	0.256
Total feed intake (g/bird)	0.071	< 0.0001	0.849
Daily feed intake (g/bird/day)	0.071	< 0.0001	0.849
Crude protein intake (g/bird)	< 0.0001	0.370	0.274
Daily crude protein intake (g/bird/day)	< 0.0001	0.370	0.274
Protein efficiency ratio	< 0.0001	0.324	0.180

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Feed conversion ratio	0.822	0.348	0.221

Table 4. Effect of different levels of protein, energy and their combinations on nutrient digestibility of growing Japanese quail at 6 weeks of age

Treatments	Dry matter	Crude protein	Ether extract	Crude fibre	Ash
Effect of protein					
VLP	66.59	35.07	73.59	42.15	29.92^{b}
LP	65.67	35.53	75.11	40.83	29.53^{b}
MP	69.69	45.89	78.08	48.51	50.51^{a}
HP	65.73	38.12	76.69	39.79	40.89^{ab}
SEM±	2.69	4.91	2.02	5.21	4.50
Effect of energy					
LE	64.91	34.43	72.76^{b}	40.58	33.48
ME	69.15	43.29	79.46^{a}	46.59	44.28
HE	66.70	38.23	75.68^{ab}	41.28	35.39
SEM±	2.33	4.25	1.95	4.51	3.89
Effect of Protein × Energy					
VLP-LE	64.58^{ab}	31.43^{ab}	71.10^{b}	41.59	19.82^{c}
VLP-ME	69.03^{ab}	40.55^{ab}	78.53^{ab}	47.20	32.43^{bc}
VLP-HE	66.16^{ab}	33.23^{ab}	72.32^{b}	37.66	37.49^{bc}
LP-LE	70.58^{ab}	45.77^{ab}	75.53^{ab}	46.52	38.07^{bc}
LP-ME	61.73^{ab}	28.94^{b}	74.24^{ab}	34.74	26.82^{bc}
LP-HE	64.71^{ab}	31.87^{ab}	75.56^{ab}	41.21	23.71^{bc}
MP-LE	59.24^{b}	26.66^{b}	68.93^{b}	34.06	33.98^{bc}
MP-ME	77.14^{a}	59.73^{a}	85.42^{a}	69.85	69.48^{a}
MP-HE	72.69^{ab}	51.27^{ab}	79.89^{ab}	51.63	48.08^{ab}
HP-LE	65.22^{ab}	33.88^{ab}	75.48^{ab}	40.17	42.03^{bc}
HP-ME	68.71^{ab}	43.95^{ab}	79.64^{ab}	44.57	48.39^{ab}
HP-HE	63.24^{ab}	36.54^{ab}	74.95^{ab}	34.51	32.26^{bc}
SEM±	4.65	8.50	3.50	9.02	7.79

^{a,b,} Means in the same row with different superscripts are significantly different (P<0.05) VLP: Very Low Protein (20%), LP: Low Protein (22%), MP: Medium Protein (24%), HP: High Protein (26%), LE: Low Energy (3000 kcal ME/kg), ME: Medium Energy (3100 kcal ME/kg), HE: High Energy (3200 kcal ME/kg)

Table 5. Effect of different levels of protein, energy and their interaction on the nutrient digestibility of Japanese quail at 6 weeks of age

	P value		
Parameters	**Protein**	**Energy**	**Protein× Energy**
Dry matter digestibility (%)	0.687	0.444	0.200
Crude protein digestibility (%)	0.393	0.351	0.190
Ash digestibility (%)	0.008	0.133	0.083
Ether extract digestibility (%)	0.512	0.040	0.288
Crude fibre digestibility (%)	0.643	0.595	0.529

Nutrient digestibility

All levels of protein had no effect on digestibility of dry matter, crude protein, ether extract and crude fibre. Ash digestibility for 24% CP fed group was highly significant (P<0.05) when compared with HP, LP and VLP. The LP and VLP ash digestibility was not significant. Ether extract digestibility was numerically highest at MP 24% CP fed diet. This is partly in support with Dowarah and Sethi (2014) who observed the highest significant differences in ether extract digestibility in white and colour plumage Japanese quail fed 25% CP diet. Crude fibre and ash digestibility were best in MP fed diet. This supports the findings of Dowarah and Sethi (2014) that the highest CF digestibility was achieved at 25% CP supplemented diet. Crude protein digestibility was the highest in the MP fed group. This implies that the HP fed group was unable to efficiently utilize the protein intake as the MP fed group. All the nutrient digestibility parameters

with the exception of crude fibre across diets were significantly different (P<0.05). No significant effect was observed in the dry matter and crude protein digestibility of VLP, LP and HP fed group regardless of their energy levels. The DM digestibility was the highest (77.14%) in the MP-ME fed group. Ash, ether extract and crude protein digestibility were also the highest in birds fed with MP-ME combination as compared with other combinations (P<0.05). It can then be summarized that the MP-ME fed group were able to digest and utilize their nutrients better than the birds fed with other energy-protein combinations. These findings are comparable to the reported CP level of 26% and ME of 3000 kcal ME/kg by Jahanian and Edriss (2015) which resulted in efficient nutrient utilization by growing Japanese quails. The crude fiber digestibility was not affected by the different dietary levels of energy and protein combinations.

Table 5 shows a significant effect (P<0.05) of protein on ash digestibility. The inclusion of different levels of energy resulted in a significant effect (P<0.05) on ether extract digestibility of the birds. Ether extract digestibility was the highest in the diet with high inclusion of soya oil, this is emphasizing its use in increasing fat content as supported by Dowarah and Sethi (2014). The interaction effect of protein and energy on Japanese quail birds had no significant effect (P>0.05) on the digestibility parameters.

CONCLUSION

It can be concluded from the study that the optimum level of dietary metabolizable energy and protein are 3200 kcal ME/kg and 26% CP respectively for weight gain during finisher period. Digestibility of nutrients also caused the best result at 24% CP and 3100 kcal ME/kg. This indicated that regardless of the total feed intake for each category of birds, the 24% CP and 3100 kcal ME/kg fed group efficiently utilized their intake mostly for performance.

Competing interests

Authors have declared that there is no competing interest.

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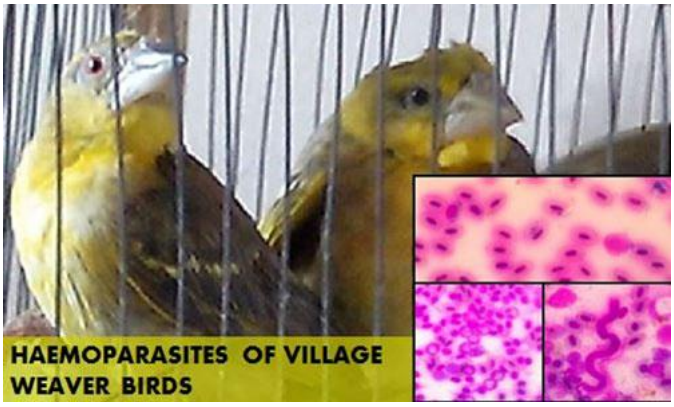

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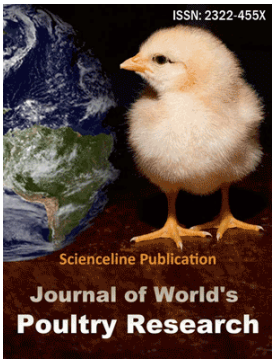

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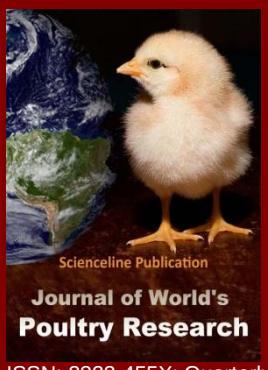

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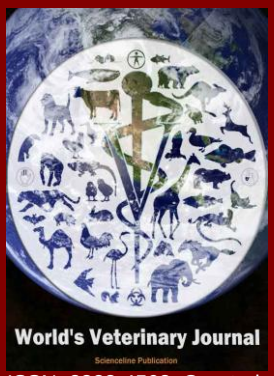

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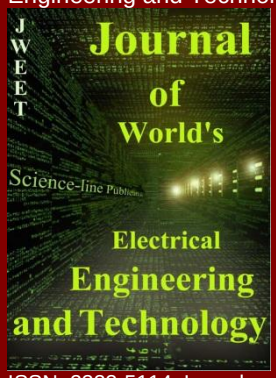

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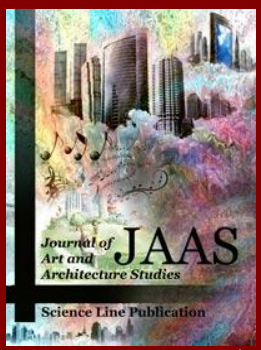

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