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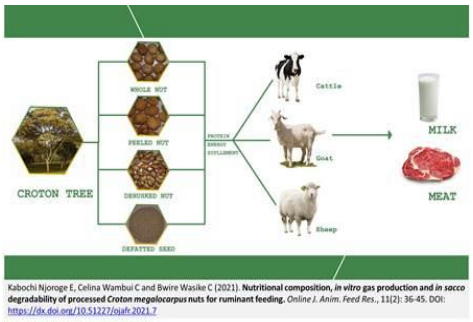
Volume 11 (2); March 25, 2021

Research Paper

Nutritional composition, *in vitro* gas production and *in sacco* degradability of processed *Croton megalocarpus* nuts for ruminant feeding.



Kabochi Njoroge E, Celina Wambui C✉ and Bwire Wasike C.

Online J. Anim. Feed Res., 11(2): 36-45, 2021; pii: S222877012100007-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.7

Abstract
This study was conducted to evaluate the effects of processed croton nut on chemical composition, *in vitro* gas production and *in sacco* degradability. Four forms of croton nut namely: whole nut (WN), peeled nut (PN), De-husked nut (DhN) and De-fatted seed (DfS) were subjected to proximate analysis, Van Soest fibre fractionation, mineral composition analysis, phytochemical and aflatoxin tests. Degradability analyses were conducted using *in vitro* gas production and *in sacco* degradability techniques. Defatted seeds recorded significantly high level of CP and NFE (198 g/kg and 174 g/kg), whereas, ash content and ether extract (EE) were significantly high in WN (59 g/kg) and DhN (362 g/kg) respectively. Low fibre fractions of NDF (556 g/kg) and ADF (490 g/kg) were observed in DhN, while the mineral content was high in DfS which had calcium at 2.13 g/kg and phosphorus at 5.04 g/kg. High level of flavonoid was recorded in WN (124 g/kg), whereas low level of alkaloids was found in DfS (60 g/kg) and tannins in PN (7.1 g/kg). The potential *in vitro* gas production (*a*+*b*) was highest in DfS (22.2 ml/0.2 gDM) while potential *in sacco* degradability (*a*+*b*) was highest in DhN (58.4 %). High level of organic matter digestibility (OMD) (41 %) was observed in DfS. At *kp*=0.025 rumen outflow rate, DhN had the highest effective degradability of dry matter (56.6%), while the rate effective crude protein degradability was 80.0 %. Processing through peeling and dehusking improved the protein, energy and mineral content of DhN and DfS while crude fibre content reduced. Nutritional composition and degradability characteristics of all forms of croton nuts imply that they could be used in a total mixed ration (TMR) to supply requisite nutrients for maintenance of ruminant animals, while DhN and DfS could be used to supplement energy and protein for increased productivity.
Keywords: Chemical composition, Croton nut, degradability, Gas production technique, Processing.

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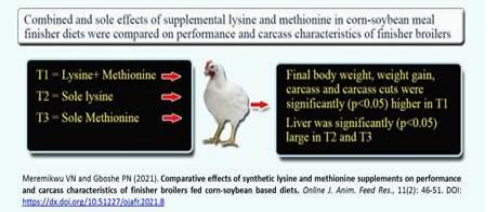
Research Paper

Comparative effects of synthetic lysine and methionine supplements on performance and carcass characteristics of finisher broilers fed corn-soybean based diets.



Meremikwu VN✉ and Gboshe PN.

Online J. Anim. Feed Res., 11(2): 46-51, 2021; pii: S222877012100008-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.8

Abstract
The aim of this research was to investigate the effects of lysine and methionine supplements in corn-soybean meal diets for finisher broilers, by comparing their combined and sole effects on performance and carcass characteristics of the birds. Parameters measured were performance (body weight, weight gain, feed intake, feed conversion ratio and mortality), dressed weight, dressing percentage, carcass cuts and internal organs. The experimental diets were: T1 (control) = lysine + Methionine, T2 (sole lysine) and T3 (sole methionine) supplements. Final body weight, weight gain, carcass and carcass cuts were significantly higher in the control (lysine + methionine) than in the sole supplemented diets, while sole supplementation with methionine (T3) produced significant higher values than sole lysine (T2) in the above mentioned parameters The liver was significantly enlarged in the birds that received the sole supplemented diets. Due to the enlarged liver of the birds fed the sole supplemented diets, it was concluded that supplementation with both lysine and methionine is indispensable in corn- soybean meal based diets for finisher broilers.

Keywords: Amino acid, Broiler, Lysine, Methionine, Supplement.

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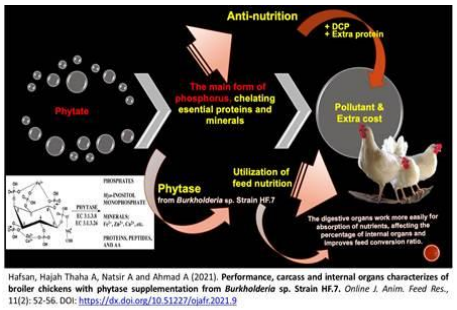
Research Paper

Performance, carcass and internal organs characterizes of broiler chickens with phytase supplementation from *Burkholderia* sp. Strain HF.7.

Hafsan✉, Hajah Thaha A, Natsir A and Ahmad A.

DOI: https://dx.doi.org/10.51227/ojafr.2021.9



Hafsan, Hajah Thaha A, Natsir A and Ahmad A (2021). **Performance, carcass and internal organs characterizes of broiler chickens with phytase supplementation from *Burkholderia* sp. Strain HF.7.** *Online J. Anim. Feed Res.*, 11(2): 52-56. DOI: https://dx.doi.org/10.51227/ojafr.2021.9

Abstract
Feed formulation with phytase supplementation is an innovation in the feed industry to improve monogastric feed quality without increasing production costs. This study aims to determine the carcass weight of broilers and the percentage of internal organs by providing various feeds, including those supplemented with phytase in phytase units (FTU) from *Burkholderia* sp. strain HF.7. A completely randomized experimental design was used in this study, using 108 broilers for five weeks of maintenance in three treatments with six replicas, each replica consisting of six broilers. The experimental feed given to broilers was basal feed without phytase supplementation (P1), basal feed + 750 FTU phytase (P2) and commercial feed (P3), each with the category of starter phase and finisher phase. Carcass weight and percentage of organs in broilers (liver, heart, gizzard, and lymph) were measured in each treatment unit. The results showed that broilers that consumed phytase supplemented feed had a higher carcass weight with a lower feed conversion value than broilers fed basal feed without phytase. These findings also indicate that the addition of phytase from *Burkholderia* sp. HF.7 strain at 750 FTU/kg feeds does not interfere with the organs of broilers' physiological function because of no increase in the percentage of the liver, heart, gizzard, and lymph.

Keywords: Broiler, Burkholderia, Internal organs, Performance, Phytase.

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Research Paper

Effects of pre-determined level of folic acid supplement on performance and carcass characteristics of broiler chickens.

Meremikwu VN✉ and Izuki ED.

Online J. Anim. Feed Res., 11(2): 57-62, 2021; pii: S222877012100010-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.10

Meremikwu VN and Izuki ED (2021). **Effects of pre-determined level of folic acid supplement on performance and carcass characteristics of broiler chickens.** *Online J. Anim. Feed Res.*, 11(2): 57-62. DOI: https://dx.doi.org/10.51227/ojafr.2021.10

Abstract
A pre-determined level of folic acid supplement (30 mg per litre of drinking water) was fed for varying durations (7, 10 and 14 days) from day-one of age to determine the effect on performance and carcass characteristics of broilers. The objective was to confirm the high levels of abdominal fat pads in previous trials with graded levels of folic acid, to clarify the mechanism underlying adipose tissue growth in broilers. Parameters measured were body weight, weight gain, feed intake, feed conversion ratio, folic acid intake, mortality and dressed weight, dressing percentage, carcass cuts and internal organs. Data obtained were analyzed using statistical package for social sciences. The outstanding result of this research was on the conformation of the dressed carcasses of the folic acid treated birds, characterized by expanded abdominal regions filled with large mass of abdominal fat pads. There was no difference between the control and the folic acid birds in other parameters measured, except the group on the longest duration of folic acid supplementation, which had higher feed intake. Folic acid intake increased significantly with increase in the duration of administration. It was concluded that, the large mass of abdominal fat pads of the folic acid birds were as a result of cell multiplication (hyperplasia) due to the fact that folate-mediated one-carbon units transfer reactions support rapid proliferation of cells and are important during periods of active cell division.
Keywords: Abdominal fat, Broiler, Folic acid, Pre-determined level, Supplement.

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Short Communication

The protein digestibility of the broiler chickens fed jamu formula, a local herbal solution.

Rusny R, Hidayat MN, Kalsum U and Masri M✉.

Online J. Anim. Feed Res., 11(2): 63-67, 2021; pii: S222877012100011-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.11

Rusny R, Hidayat MN, Kalsum U and Masri M (2021). **The protein digestibility of the broiler chickens fed jamu formula, a local herbal solution.** *Online J. Anim. Feed Res.*, 11(2): 63-67. DOI: https://dx.doi.org/10.51227/ojafr.2021.11

Abstract
Jamu (local herbal drinking) have been known for a long time by inhabitants in Indonesia as conventional home grown pharmaceutical and to progress digestion system within the body. Jamu, not as it were for people but also for creatures.

Local farmers have moreover utilized jamu for chicken for a long time, and it's utilize is expanding. This Research points to decide the impact of jamu to extend protein in vivo digestibility in broilers and for knowing the ideal level of jamu for optimum protein digestibility in broilers. The strategy utilized in this investigate is Completely Randomized Design (CRD) with 4 treatment and 5 replications, each redundancy comprises of 1 broiler chickens, so there are 20 chickens. The treatment comprises of P0 (control), T1 (jamu 1.5 mL/500 mL), T2 (jamu 2.5 mL/500 mL) and T3 (jamu 3.5 mL/500 mL). The parameters watched were digestibility protein in broilers. Based on the examination of fluctuation, it appears The treatment had no critical impact on chicken protein broilers' digestibility given jamu. However, seeing each treatment's average value, T1, T2 and T3 tend to increase to 99.62%, 99.68% and 99.71%, respectively. In conclusion, supplemented with jamu formula does not significantly affect broiler chicken protein's digestibility, but the digestibility increases with increasing formula, up to the formula 3.5 mL/500 mL (T3) as the ideal level.
Keywords: Broiler, Digestibility, Herbal treatment, Jamu, Protein.

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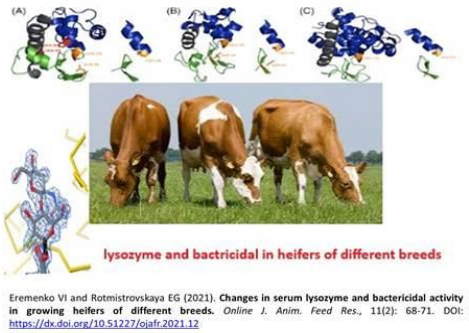
Short Communication

Changes in serum lysozyme and bactericidal activity in growing heifers of different breeds.

Eremenko VI ✉ and Rotmistrovskaya EG.

Online J. Anim. Feed Res., 11(2): 68-71, 2021; pii: S222877012100012-11

DOI: https://dx.doi.org/10.51227/ojafr.2021.12



Abstract
The study presents the results of a study of the bactericidal and lysozyme activity of blood serum of heifers of different breeds. The experiment involved 4 groups of heifers, 10 heads in each group: 1) Black-and-white Holstein; 2) Simmental; 3) Aberdeen-Angus; and 4) crosses of Simmental and Aberdeen-Angus breeds. Animals of all groups were kept in the same feeding and housing conditions. During the experiments, the animals were fed according to generally accepted standards. Blood was taken from animals from the tail vein in the morning before the first feeding in compliance with the aseptic rules. It was found that with an increase in gestation, the activity of serum bactericidal activity (SBA) and serum lysozyme activity (SLA) in the blood of heifers gradually increases.in conclusion, during pregnancy, the level of SBA and SLA in the blood of heifers depended on the month of pregnancy and the breed of animals. During pregnancy, hybrid heifers have higher levels of SBA and SLA, and relatively low levels of SBA and SLA are observed in Black-and-White, Simmental and Aberdeen Angus heifers.

Keywords: Aberdeen-Angus, Bactericidal, Heifer, Lysozyme activity, Simmental.

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796-704 Montrose St, Winnipeg, Manitoba R3T 2N2, CANADA
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NUTRITIONAL COMPOSITION, *IN VITRO* GAS PRODUCTION AND *IN SACCO* DEGRADABILITY OF PROCESSED *Croton megalocarpus* NUTS FOR RUMINANT FEEDING

Elias KABOCHI NJOROGE, Caroline CELINA WAMBUI and Chrilukovian BWIRE WASIKE

Livestock Efficiency Enhancement Group (LEEG), Department of Animal Science, School of Agriculture & Food Security, Maseno University, P.O. Private Bag, 40105, Maseno, Kenya

Email: wambuicc@gmail.com; ORCID: 0000-0002-0007-3096

Supporting Information

RESEARCH ARTICLE

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ABSTRACT: This study was conducted to evaluate the effects of processed croton nut on chemical composition, *in vitro* gas production and *in sacco* degradability. Four forms of croton nut namely: whole nut (WN), peeled nut (PN), De-husked nut (DhN) and De-fatted seed (DfS) were subjected to proximate analysis, Van Soest fibre fractionation, mineral composition analysis, phytochemical and aflatoxin tests. Degradability analyses were conducted using *in vitro* gas production and *in sacco* degradability techniques. Defatted seeds recorded significantly high level of CP and NFE (198 g/kg and 174 g/kg), whereas, ash content and ether extract (EE) were significantly high in WN (59 g/kg) and DhN (362 g/kg) respectively. Low fibre fractions of NDF (556 g/kg) and ADF (490 g/kg) were observed in DhN, while the mineral content was high in DfS which had calcium at 2.13 g/kg and phosphorus at 5.04 g/kg. High level of flavonoid was recorded in WN (124 g/kg), whereas low level of alkaloids was found in DfS (60 g/kg) and tannins in PN (7.1 g/kg). The potential *in vitro* gas production (*a+b*) was highest in DfS (22.2 ml/0.2 gDM) while potential *in sacco* degradability (*a+b*) was highest in DhN (58.4 %). High level of organic matter digestibility (OMD) (41 %) was observed in DfS. At *kp*=0.025 rumen outflow rate, DhN had the highest effective degradability of dry matter (56.6%), while the rate effective crude protein degradability was 80.0 %. Processing through peeling and dehusking improved the protein, energy and mineral content of DhN and DfS while crude fibre content reduced. Nutritional composition and degradability characteristics of all forms of croton nuts imply that they could be used in a total mixed ration (TMR) to supply requisite nutrients for maintenance of ruminant animals, while DhN and DfS could be used to supplement energy and protein for increased productivity.

Keywords: Chemical composition, Croton nut, degradability, Gas production technique, Processing.

Abbreviations: WN: whole nut; PN: peeled nut; DhN: dehusked nut; DfS: defatted seeds

INTRODUCTION

The livestock sector accounts for 40% of agriculture's Gross Domestic Product (GDP) in developing countries and is not only a source of food and livelihood but enhances resilience against climate change extremities such as drought (Herrero et al., 2013; Nabarro and Wannous, 2014). The continuously growing human population as well as increased per capita income has led to increased demand for livestock-based products (Otte et al., 2019). Thus, livestock production ought to increase so as to meet the rising demand. Ruminant animals in tropical arid and semi-arid areas (ASAL) continue to play a key role of the rural households in developing countries where they are a major source of nourishment from products such as meat and milk as well as play social economic roles by providing income and acting as an economic safety net (Herrero et al., 2013).

Livestock production in the tropics is constrained by various factors which include inadequate nutrition, breeding and reproduction, disease and parasites among others (Kahi and Wasike, 2019). In confined systems, feeds account for up to 70% of the total cost of production (Makkar, 2014). Hence, variation in quantity and quality of feeds becomes a major constraint to livestock production. The problem of feed scarcity is further exacerbated by increased food – feed competition between human and livestock and decline in available land for feed production. Majority of ruminant animals (cattle, sheep and goats) in Kenya are reared in arid and semi-arid Counties (KNBS, 2019). In these areas, effects of climate change such as drought greatly reduces available feed resources consequently leading to low productivity and at time causing mortality of the animals (Makkar, 2014). Feeding strategies that optimise utilisation of available feed resources are thus critical to maintain ruminant productivity and preventing mortality.

Identification and introduction of alternative feed resources is a major avenue that could be used to mitigate feed scarcity. Locally available, low-cost feed resources could enhance resilience and adaptability of small holder farmers and pastoralists by allowing them to transit through adverse effects of climate change (Makkar, 2014). Evaluation of non-conventional feed resources for potential inclusion in mainstream livestock offers a preliminary step in determining the suitability of the identified feed resource before it can be included in livestock diets (Quansah and Makkar, 2012). One

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such feed with potential is croton nuts from *Croton megalocarpus* tree. Croton tree is adapted to different agro-ecological zones in the tropics and has multipurpose use such as provision of wood fuel, acting as a live fence and a source of bio fuel (Ndegwa et al., 2011). Croton tree produces up to 25 kg of nuts per year (Jacobson et al., 2018) which are reported to contain high CP content of up to 18%, crude fat (30%) and hence could be exploited for feeding livestock (Thijssen et al., 1996; Ndegwa et al., 2011). Farmers have been observed to collect and use croton nuts for feeding cows and goats during extreme dry seasons. However, there is limited information on the chemical composition, ant - nutritive factors and degradability of croton nuts. Moreover, there is also limited information on effects of processing various forms of croton such as peeling, dehusking and oil extraction on the nutritive value for effective utilization of this underutilized feed resource. This study was therefore conducted to evaluate nutritional and phytochemical composition and ruminal degradation of the various processed forms of croton nut to facilitate its use in ruminant feeding.

MATERIALS AND METHODS

Site description

Samples of Croton nut were collected from Laikipia West and East sub counties of Laikipia County, which is located North - West of Mount Kenya at an altitude of between 1600 m and 2300 m above sea level with a total area of 9,700 km^2. The area experiences a bimodal rainfall pattern with long rains between March and June and short rains between October and December separated by dry seasons (MoALF, 2017). The annual precipitation varies between 400 mm to 900 mm and average temperature is between 16 ^{o}C and 26 ^{o}C. The area lies in semi - humid, semi - arid, arid to very arid agro ecological zones IV - VII, and is considered arid and semi-arid (ASAL) (MoALF, 2017).

Collection and processing of croton nuts

Mature croton nuts were collected from the ground, air dried under shade and processed into four forms which included whole nuts (WN), peeled nut (PN), dehusked nut (DhN) and defatted seeds (DfS). The whole nuts (WN) form comprised of unprocessed whole croton nuts with the outer peel (exocarp) and the hard woody husk (endocarp) intact. Peeled nuts (PN) consisted of nuts whose outer seed coat (peel/exocarp) was removed leaving the hard woody endocarp intact. De-husked nuts (DhN) consisted of the inner seeds after the removal of both the outer peel (exocarp) and the hard woody husk (endocarp). Defatted seeds (DfS) also referred to as Croton cake was the by-product of the seeds after oil extraction using a cold press. The DfS form was obtained from a commercial plant that extracts bio-diesel from croton in Laikipia County. After processing into various forms, the samples were then ground using a hummer mill to pass through a 2 mm screen and stored in air tight glass containers pending analyses.

Chemical analyses

Ground samples of the various processed forms of croton were subjected to proximate analysis to determine dry matter (DM), ash, crude fibre (CF), ether extract (EE) and crude protein (CP) which were expressed on dry matter basis according to AOAC (1990). Nitrogen free extract (NFE) was calculated as the difference of the sum (%) of crude protein, crude fibre, ether extract and total ash from 100%. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), were sequentially determined using the method of Van Soest et al. (1991). Hemicellulose content was calculated as the difference between NDF and ADF, whereas cellulose was the difference between ADF and ADL. Gross energy (MJ/kg) was determined from 0.5 g of sample using a digital bomb calorimeter (CAL2K of Digital Data Systems (pty) ltd South Africa). Neutral detergent insoluble nitrogen and acid detergent insoluble nitrogen were determined from the residues of NDF and ADF using Kjeldahl method (AOAC, 1990). Nitrogen obtained was multiplied with a conversion factor (6.25) to obtain neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP).

Sodium (Na) and potassium (K) concentration was determined using atomic emission in a flame photometer while total available phosphorus (P) concentration was determined using Ultra Violet (UV) colorimeter (AOAC, 1990). Calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) concentrations were determined using an atomic absorption spectrophotometer (AAS) (AOAC, 1990).

Phytochemicals and aflatoxin analyses

Flavonoids were extracted from the samples using organic solvents and expressed gravimetrically as outlined by Harbone (1984). Alkaloid determination was done by extraction from the samples using acetic acid dissolved in ethanol (Harbone, 1984). Tannin content was determined using Folin-Coicalteu reagent and determination of absorbance was done at 725 nm using a UV-visible spectrophotometer (AOAC, 1990). Aflatoxins were extracted using methanol and levels determined by ELISA testing kit. The amount of aflatoxin was expressed in parts per billion (ppb) calculated from the standard aflatoxin curve (Leszczyñska et al., 2001).

In vitro gas production

In vitro gas procedure was conducted following the procedure of Menke and Steingass (1988). Rumen liquor was drawn in the morning from two mature fistulated Friesian steers. The steers had 450±25 kg live weight and were fed on Rhodes grass (*Chloris gayana*) hay and wheat bran at 90% and 10% respectively of the total ration at 3% of their body

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weight at 08:00 hours and 17:00 hours for maintenance purposes. Mineral licks and water were provided adlibitum. This was done so as to maintain a stable rumen environment before the rumen liqour was collected. Collected rumen liqour was strained through four layers of cheese cloth into a pre-warmed, vacuum flask and kept at 39°C under CO_2 atmosphere. About 0.2g of 1mm ground samples (WN, PN, DhN and DfS) were weighed into the glass syringes. A mixture of 30ml of rumen liquor and buffer in the ratio of 1:2 was added into each of the 100ml calibrated glass syringes that were pre-warmed to 39°C. Oil was applied to the pistons to facilitate ease of movement and prevent gas escape. Two blank syringes with rumen liquor without a feed sample were included as controls. All syringes were incubated in a water bath maintained at 39°C and shook periodically. Gas production readings were recorded at 0 and after 3, 9, 12, 24, 48, 72 and 96 hours of incubation.

The gas production characteristics were computed by fitting the mean gas volumes to the exponential equation of Ørskov and Mcdonald (1979) using Neway Excel Computer program (Chen X. B., Rowett Research Institute, Aberdeen UK).

$$Y = a + b(1 - e^{-ct}) \quad \text{(Ørskov and Mcdonald, 1979)} \quad (1)$$

Where: *Y* is gas production (ml/0.2g) at time *t*, *a* is gas production (ml) from immediately soluble fraction, *b* is gas production (ml) from insoluble fraction, *a+b* is gas production from potential degradable fraction, *c* is the rate constant of gas production per hour (h), *t* is the incubation time in hours and *e* is the exponential constant (2.718).

In vitro gas production parameters were used to estimate organic matter digestibility (OMD), metabolisable energy (ME), Dry Matter intake (DMI) and short chain fatty acids (SCFA) using the models presented in Equations 2 to 5.

$$OMD(\%) = 14.88 + 0.889\ GV + 0.45\ CP + 0.0651\ XA \quad \text{(Menke and Steingass, 1988)} \quad (2)$$

$$ME(\text{MJ/Kg}) = 2.20 + 0.136\ GV + 0.057\ CP \quad \text{(Makkar and Becker, 1996)} \quad (3)$$

$$DMI(\text{kg/day}) = 1.66 + 0.49a + 0.0297b - 4c \quad \text{(Blümmel and Ørskov, 1993)} \quad (4)$$

$$SCFA(mmol/L) = 0.0222\ GV - 0.00425 \quad \text{(Makkar, 2005)} \quad (5)$$

Where: *GV* is gas production after 24 hours, *CP* is crude protein and *XA* ash content of the processed form of croton, *a, b* and *c* are constants as described in Equation 1.

In sacco degradation (nylon bag technique)

In sacco degradation of the various forms of croton was carried out using Nylon bag technique as described by Ørskov (2000). Two mature fistulated Friesian steers weighing 450±25 kg live weight were used. The steers were fed on Rhodes grass (*Chloris gayana*) hay and wheat bran at 90% and 10% respectively of the total ration at 3% of their body weight at 08:00 hours and 17:00 hours for maintanance purposes. Mineral licks and water were provided adlibitum. This was done so as to maintain a stable rumen environment. Five grams of each processed sample of croton was weighed into duplicate nylon bags (12cm by 6cm, 50µm pore size). The bags were incubated for 0, 9, 12, 16, 24, 48 and 72 hours in the rumen. Zero-hour washing was measured by soaking nylon bags containing the sample in water maintained at 39 °C for 1 hour. Bags from zero hour washing and those retrieved from the rumen were washed thoroughly under running cold water for 15 minutes until the washing water was clear. The bags with the residue were then dried at 60°C for 48 hours in a forced air oven and dry matter loss determined as the difference from the original weight. Crude protein and neutral detergent fibre (NDF) from the residue were then analysed. The DM, CP and NDF degradability characteristics were determined by fitting the degradability data to the exponential Equation 6 of Ørskov and Mcdonald (1979) using Neway Excel Computer program (Chen X. B., Rowett Research Institute, Aberdeen UK).

$$P = a + b(1 - e^{-ct}) \quad \text{(Ørskov and Mcdonald, 1979)} \quad (6)$$

Where: *P* is the degradability of (DM, CP and NDF) incubated in the rumen at time *t* in hours, *a* is the percentage of rapidly soluble fraction, *b* is the percentage of insoluble but fermentable fraction, *a+b* is potential percentage of degradability, *c* is the rate of constant degradation per hour (h^{-1}) and *e* is the exponential constant (2.718).

Effective degradability (ED) of DM, CP and NDF was calculated using Equation (7).

$$ED = a + b\left(\frac{c}{c+kp}\right) \quad \text{(McDonald, 1981)} \quad (7)$$

Where: *a+b* is the potential degradability, *c* is the rate constant degradability per hour (h), *kp* is the ruminal outflow rate. The following outflow rates (*kp*) per hour were considered (0.025, 0.05 and 0.08). Rumen undegradable protein (RUP) was calculated by subtracting effective degradable CP% from 100%. The DM index value (IV) which denotes the fraction of the feed that would provide nutrients to the animal for its maintenance needs was calculated using Equation 8. A feed with an index value above 33 would provide sufficient nutrients to the animal for its maintenance needs.

$$IV = a + 0.4b + 200c \quad \text{Ørskov and Shand (1997)} \quad (8)$$

Where: *a*, *b* and *c* are as described in Equation 6.

Statistical analysis

Analysis of variance (ANOVA) was carried out on proximate composition, fibre fractions, minerals composition, gross energy (GE) and phytochemicals as well as *in vitro* gas production and *in sacco* degradability parameters. The analysis was based on completely randomized design using STATA (2017). Significant differences between the means were tested using Tukey's honest significance difference (THSD). The following statistical model was used

$$y_{ij} = \mu + f_i + e_{ij} \quad (9)$$

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Where: y_{ij} = chemical composition, *in vitro* gas production and *in sacco* degradability parameters, μ = mean of the different forms of *Croton megalocarpus,* f_i = forms of croton nuts (*i*= WN, PN, DhN and DfS), e_{ij} = error term.

Ethical approval

All process of in vivo study was in according to animal welfare rules and approved by university ethical committee.

RESULTS

Proximate composition

Proximate composition of the various forms of croton nut is presented in Table 1. Peeled nut had significantly high DM content while WN did not differ significantly from DhN and DfS (P<0.05). Defatted seeds had significantly high CP content compared to other forms while the lowest level of CP was recorded in WN and PN which were not significantly different (P<0.05). The CF content was significantly low in DhN compared to the other forms while the ash content was significantly high in WN followed by DfS, but no significant difference was observed between PN and DhN. The EE content did not differ significantly between WN and PN but was significantly high in DhN at 363g/kg and significantly low in DfS (113g/kg; P<0.05). The NFE in all forms did not differ significantly. Gross energy was highest in the DhN (21.1MJ/kg) and lowest in PN (17.3MJ/Kg) although the differences were not significant.

Fibre composition

Fibre composition of the various forms of croton nut is presented in Table 2. Processing by dehusking and defatting resulted to lower NDF content in DhN and DfS to 576 g/kg and 556g/kg respectively, compared to WN and PN forms (P<0.05). Hemicellulose content was highest in PN (205 g/kg) (P<0.05). The cellulose level ranged between 94g/kg in DhN to 181g/kg in WN. The NDICP ranged between 16 in PN to 24 in DfS while ADICP ranged between 16 to 21 in both WN and DfS (P>0.05). There were no significant differences in ADF, ADL, cellulose, NDICP and ADICP among the croton forms (P>0.05).

Table 1 - Proximate composition of the various forms of croton nut (g/kg)

Nutritional parameter	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
Dry matter	893[a]	963[b]	917[c]	919[c]	0.763	P<0.001
Crude protein	89[a]	80[a]	158[b]	198[c]	1.487	P<0.001
Crude fibre	522[a]	579[b]	336[c]	476[a]	2.747	P<0.001
Ether extract	185[a]	175[a]	363[b]	113[c]	2.824	P<0.001
Ash	59[a]	23[b]	24[b]	38[c]	0.449	P<0.001
Nitrogen free extract	143[a]	143 [a]	120 [a]	174 [a]	0.847	P<0.001
Gross energy (MJ/kg)	18.1	17.3	21.1	19.3	0.826	NS

[a,b,c;] Means in the same row without common letter are different at P<0.05; SEM = standard error of the mean; Prob.= probability; NS = not significant.

Table 2 - Fibre composition of the various forms of croton nut (g/kg)

Fibre components	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
NDF	686[a]	741[a]	576[b]	556[b]	2.36	P<0.001
ADF	506	536	482	490	1.10	0.357
ADL	341	377	392	367	1.62	0.781
Hemicellulose	180[ab]	205[b]	94[ac]	66[c]	2.02	P<0.010
Cellulose	181	168	94	132	1.39	0.089
NDICP	22	16	23	24	0.159	0.155
ADICP	21	16	17	21	0.115	0.063

[a,b,c;] Means in the same row without common letter are different at P<0.05; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein; SEM = standard error of the mean; Prob. = probability.

Minerals composition

Mineral content of the various forms of croton nut is presented in Table 3. Processing by defatting enhanced the macro minerals (Ca, P, Mg and Na) in DfS (P<0.05). Whole nut recorded the highest level of potassium (14.27 g/Kg)

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(P<0.05). Amongst the micro minerals, Fe was highest in WN (0.113 g/kg), Mn in DhN (0.047g/kg) and Zn in DfS (0.049 g/kg) at P<0.05 compared to the other forms.

Table 3 - Mineral composition of the various forms of croton nut (g/kg)

Mineral composition	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
Macro minerals						
Calcium	1.51^{a}	1.69^{ab}	1.82^{ab}	2.13^{b}	0.084	0.0324
Phosphorus	3.21^{ab}	2.78^{a}	4.21^{bc}	5.04^{c}	0.284	<0.001
Magnesium	0.46^{ab}	0.35^{a}	0.57^{bc}	0.71^{c}	0.042	<0.001
Sodium	0.79^{a}	0.14^{b}	0.34^{c}	2.27^{d}	0.251	<0.001
Potassium	14.27^{a}	4.36^{b}	3.66^{b}	5.41^{c}	1.294	<0.001
Micro minerals						
Iron	0.113^{a}	0.051^{b}	0.063^{bc}	0.075^{c}	0.0071	<0.001
Manganese	0.024^{a}	0.029^{a}	0.047^{b}	0.046^{b}	0.0031	<0.001
Zinc	0.022^{ab}	0.017^{a}	0.034^{bc}	0.049^{c}	0.004	0.001
Copper	0.007	0.015	0.019	0.008	0.0027	0.401

$^{a,b,c,d:}$ Means in the same row without common letter are different at P<0.05; SEM = standard error of the mean; Prob. = probability.

Phytochemicals and aflatoxin content

Phytochemical composition and aflatoxin levels of the various forms of croton nut is presented in Table 4. Flavonoid content in WN was significantly higher (124 g/kg) (P<0.05) from other forms. Alkaloids ranged from 60g/kg in DfS to 69g/kg in WN (P>0.05). Both WN and DfS had the highest tannin level (9.6 g/kg) (P<0.05). Aflatoxin level was highest in DhN (21.1 ppb) and least in PN (6.4 ppb).

In vitro gas production

In vitro gas production fermentation characteristics of the various forms of croton nut are presented in Table 5. There was no difference in gas production from the readily soluble fraction (*a*) among the forms (P>0.05). However, highest gas production of (*b*) and (*a*+*b*) were observed in DfS at (18.6 ml) and (22.2 ml) respectively (P<0.05). Defatted seeds recorded the highest OMD (41.0%), ME (5.9 MJ/kg), and SCFA (0.419 mmol/L) while PN had the least OMD (29.8%), ME (4.3 MJ/kg) and SCFA (0.271 mmol/L).

In sacco DM degradability

In sacco DM degradability characteristics of the various forms of croton nut are presented in Table 6. Dehusked nut had highest rapidly soluble DM fraction (*a* ~ 42.8%) and potentially degradable DM fraction (*a*+*b* ~ 58.4%) (P<0.05), with the rate constant of degradation (*c*) ranging between 0.02 in WN to 0.2 in DhN. Effective dry matter degradability (EDDM) among the various forms was observed to reduce as the rumen outflow rate increased. Dehusked nut (DhN) had consistently higher percentages of EDDM and at all rumen outflow rates and a converse trend was true for PN. Dehusked nut also recorded the highest IV 90.1 (P<0.05).

In sacco CP degradability

In sacco CP degradability characteristics of the various forms of croton nut are presented in Table 7. Rapidly degradable fraction of protein (*a*) was highest in WN (4.1%) (P<0.05). At p<0.05, slowly degradable fraction (*b*) and potential degradable fraction (*a*+*b*) were highest in DhN (87.8%) and (87.9%) and lowest in WN (59.4%) and (63.5%) respectively. The rate constant of degradability per hour (*c*) was highest in PN (0.26) and lowest in DhN (0.02) whereas highest rumen undegradable protein was recorded in WN (36.4%) and the lowest recorded in DhN (12.0%). At *kp*=0.025, effective degradable crude protein for DhN (80.0%) and DfS (65.1%) were different (P<0.05) from that of WN and PN. The rumen undegradable protein (RUP) among all forms of croton nuts at 0.025 *kp* was low compared to RUP at 0.05*kp* and 0.08*kp* (P<0.05).

In sacco NDF degradability

The NDF degradability characteristics of the various forms of croton nut are presented in Table 8. Significant difference in NDF degradability was observed in rapidly degradable fraction (*a*) which was highest in DhN (17.2%) compared to the other forms of croton nut (P<0.05). There was no significant difference among the various forms of croton nut for *b*, *a*+*b*, and ***c***. At 0.025*kp* and 0.08*kp,* effective degradability NDF was significantly high in DhN compared to the other forms. However, at 0.05*kp* there was no significance difference among all forms of croton nut in EDNDF.

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Table 4 - Phytochemical and aflatoxin content of the various forms of croton nut (g/kg).

Anti-nutritive factors	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
Flavonoids	124[a]	57[b]	43[b]	64[b]	1.01	P<0.0014
Alkaloids	69	67	62	60	1.85	0.307
Tannins	9.6[a]	7.1[b]	8.9[ab]	9.6[a]	0.04	P<0.021
Aflatoxin (ppb)	14	6.4	21.1	9.9	3.13	ND

[a,b,c;] Means in the same row without common letter are different at (P<0.05); SEM = standard error of the mean; Prob. = probability; ND = not determined.

Table 5 - *In vitro* gas production of the various forms of croton nut (ml gas/0.2g dry matter).

Gas production parameters	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
a	1.4	4.2	2.4	3.7	0.465	P<0.083
b	18.6[a]	10.6[b]	14.3[ab]	18.4[a]	1.28	P<0.004
a+b	20.1[ab]	14.9[a]	16.8[ab]	22.2[b]	1.13	P<0.019
c	0.08	0.06	0.10	0.08	0.007	P<0.096
OMD	34.1[ab]	29.8[a]	35.3[b]	41.0[c]	1.54	P<0.004
ME (MJ/Kg)	5.0[a]	4.3[a]	5.1[a]	5.9[b]	0.218	P<0.005
DMI (kg/day)	2.6	3.8	2.8	3.6	0.226	P<0.103
SCFA (mmol/L)	0.37[a]	0.27[ab]	0.32[ab]	0.41[b]	0.022	P<0.035

[a,b,c;] Means in the same row without common letter are different at P<0.05; *a* = gas production (ml) from immediately soluble fraction; *b* = gas production (ml) from insoluble fraction, *a+b* = potential gas production (ml); c = the rate constant of gas production per hour; OMD = organic matter digestibility; ME = metabolisable energy; DMI = dry matter intake; SCFA = short chain fatty acids; SEM = standard error of the mean ; Prob. = probability.

Table 6 - *In sacco* DM degradability characteristics for various forms of croton nut (%).

DM degradability parameters	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob
a	29.5[a]	26.4[b]	42.8[c]	33.4[d]	2.33	P<0.001
b	18.8[ab]	22.5[b]	15.6[ab]	12.5[a]	1.49	P<0.028
a+b	48.3[a]	49.0[a]	58.4[b]	46.0[a]	1.87	P<0.011
c	0.02	0.01	0.20	0.05	0.032	P<0.051
EDDM (*kp*=0.025)	37.8[a]	30.7[b]	56.6[c]	41.8[d]	3.56	P<0.001
EDDM (*kp*=0.05)	34.9[a]	28.8[b]	55.1[c]	39.7[d]	3.68	P<0.001
EDDM (*kp*=0.08)	33.2[a]	28.0[b]	53.8[c]	38.3[d]	3.64	P<0.001
IV	41.0[a]	36.6[a]	90.1[b]	48.5[a]	8.44	P<0.017

[a,b,c,d;] Means in the same row without common letter are different at P<0.05; a = is the rapidly soluble fraction; b = is the insoluble but fermentable fraction; *a+b* = is the potentially degradable fraction; c = is the rate constant of degradation; IV = index value; EDDM = effective degradability of dry matter; kp = rumen outflow rate; SEM = standard error of the mean; Prob. = probability.

Table 7 - *In sacco* CP degradability characteristics of the various forms of croton nut (%).

CP degradability	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
a	4.1[a]	0.02[b]	0.08[b]	0.4[b]	0.660	P<0.002
b	59.4[a]	75.0[b]	87.8[c]	74.7[b]	3.81	P<0.001
a+b	63.5[a]	75.0[b]	87.9[c]	75.1[b]	3.26	P<0.001
c	0.06[a]	0.26[b]	0.02[b]	0.16[c]	0.0312	P<0.001
EDCP (*kp*=0.025)	46.2[a]	68.5[a]	80.0[b]	65.1[b]	4.59	P<0.001
EDCP (*kp*=0.05)	36.8[a]	63.0[b]	73.5[c]	57.5[d]	5.06	P<0.001
EDCP (*kp*=0.08)	29.8[a]	57.4[b]	66.9[c]	50.4[d]	5.16	P<0.001
RUP (*kp*=0.025)	53.7[a]	31.4[a]	19.9[b]	34.8[b]	4.59	P<0.001
RUP (*kp*=0.05)	63.1[a]	36.9[b]	26.4[c]	42.4[d]	5.06	P<0.001
RUP (*kp*=0.08)	70.1[a]	42.5[b]	33.0[c]	49.5[d]	5.16	P<0.001

[a,b,c,d;] Means in the same row without common letter are different at P<0.05; a = is the rapidly soluble fraction; b = is the insoluble but fermentable fraction; a+b = is the potentially degradable fraction; c = is the rate constant of degradation; EDCP = effective degradability of crude protein; kp = rumen outflow rate; RUP = rumen undegradable protein; SEM = standard error of the mean; Prob. = probability.

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Table 8 - *In sacco* NDF degradability characteristics of the various forms of croton nuts (%)

NDF degradability	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
A	6.5[a]	2.0[a]	17.2[b]	1.6[a]	2.41	0.002
B	15.1	9.4	10.1	15.8	1.28	0.144
a+b	21.6	11.4	27.3	17.4	2.44	0.065
C	0.03	0.01	0.33	0.35	0.0052	0.714
EDNDF (*kp*=0.025)	15.0[a]	5.9[b]	22.9[c]	9.1[b]	2.47	P<0.001
EDNDF (*kp*=0.05)	11.2	7.5	21.2	6.8	2.45	0.053
EDNDF (*kp*=0.08)	10.2[a]	3.7[b]	20.1[c]	5.5[b]	2.43	P<0.001

[a.b,c,d]: Means in the same row without common letter are different at P<0.05; a = is the rapidly soluble fraction; b = is the insoluble but fermentable fraction; *a+b* = is the potentially degradable fraction; c = is the rate constant of degradation; EDNDF = effective degradability of neutral detergent fibre; *kp* = rumen outflow rate; SEM = standard error of the mean; Prob. = probability.

DISCUSSION

Nutritional composition

The DM content in all forms was above 86%, which is the recommended level for storage of feeds. Conversely, this implied low moisture content that is critical in preventing growth of fungi and reducing aflatoxin contamination (Mahato et al., 2019). The high moisture content in WN suggests that the peel acts as a barrier against loss and itself contains moisture. Whole nuts and DfS had high ash contents indicating that they could be good sources of minerals for grazing animals during the dry seasons hence averting the effects of mineral deficiencies such as impaired growth, poor health and reduced reproductive performance in ruminants (Lengarite et al., 2012). This is corroborated by mineral results whereby, Ca and P levels of all forms of croton nut in this study were within the recommended critical maintenance level (1.2 - 2.6g/kg Ca) and (1.4g/kg P) respectively for ruminant animals (ARC, 1980). The K level in WN was above 8g/kg even though, the Mg level in all forms was below (2g/kg) recommended level for grazing animals, (Mirzaei, 2012). The level of Fe was above the recommended level (0.05g/kg) for grazing animals (ARC, 1980). Both DhN and DfS contained the recommended critical level of Zn (0.03g/kg) which is sufficient for cattle, sheep and goats (ARC, 1980).

Removal of the husks and defatting effectively elevated CP content as reflected in DhN and DfS forms. The CP in all croton forms was above the recommended (80g/kg) required for maintenance in grazing ruminant animals (NRC, 2001). Moreover, DhN and DfS CP levels were within 140g/kg to 165g/kg recommended for growth and increased milk production in lactating animals (NRC, 2001). Defatting reduced the EE content considerably in DfS making it suitable for storage by reducing the amount of oils which when oxidised cause rancidity hence feed spoilage.

Removal of the outer peel and husks (hard woody endocarp encasing the seeds) lowered the fibre levels considerably in DhN and DfS. Neutral detergent fibre level in these forms was between 450 g/kg to 650 g/kg. These forms may be classified as medium quality feed, a predominant characteristic of tropical feed stuffs (Singh and Oosting, 1992). Feeds in this category can achieve the required gut health of ruminant animals by enhancing optimum feed intake, stimulating rumen function and increasing chewing of cud (Singh and Oosting, 1992). Moderate crude protein levels (80 – 90 g/kg) in WN and PN could play a fundamental role in mitigating lowered fibre digestibility that may be occasioned by the high NDF through availing of rumen ammonia nitrogen necessary for optimal functioning of the rumen ecosystem (Van Soest, 1994). There was no difference in NDICP among all forms of croton nut an indication that the degradability of insoluble-protein fraction was similar in all forms. NDICP represent the insoluble fraction of protein that remains after extraction with neutral detergent solution and is usually assumed to be insoluble (Mustafa et al., 2001). This fraction is a measure of nitrogen availability and constitutes a major portion of ruminal undegradable protein content (Mustafa et al., 2001).

High flavonoid content in all forms of croton nuts is an indication that croton nut could be included in ruminant feed rations to confer improved growth performance, health and improved rumen fermentation (Panche et al., 2016). A study by Kong et al. (2019) showed that flavonoid supplementation improved the average daily gain by alleviating stress during weaning of Holstein calves. Low level of tannins (<50 g/kg) similar to those recorded in this study could confer beneficial effects to ruminant animals such as reduction in ruminal protein degradation thus availing essential amino acids for absorption in the small intestines (Frutos et al., 2004). The level of aflatoxin observed in this study was within the minimum recommended level of 20ppb for complete and complementary feed materials used for feeding cattle, sheep and goats except for DhN (Kotinagu et al., 2015). The high level of aflatoxin in DhN could be attributed to high level of oil which provides conducive environment for growth of fungi resulting in production of aflatoxins (Filazi and Sireh, 2013). Therefore, proper handling and storage of DhN is crucial to prevent conditions that could encourage growth of fungi.

In vitro gas production

Amount of gas produced in *in vitro* gas digestibility method is an indicator of the rate and extent of feed digestion (Makkar, 2005). Gas production is affected by the composition, bioavailability of nutrients and presence of anti-nutritive factors in a feed. The higher levels of gas production observed in DfS compared to other forms of croton could be

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attributed to high levels of fermentable carbohydrates and protein which produce more gas when acted upon by rumen microbes (Makkar, 2005). Quality of roughage in a feed determines the nutritive value that the feed would confer when fed to an animal. The presence of high amount of fibre in a feed increases the rumen pool of indigestible fibre lignin which impedes the action of fibrolytic microbes that act on cellulose and hemicellulose (Venkateswarlu et al., 2013). This consequently reduces fermentable fibre as observed in PN.

Observed reduced fermentation characteristics in DhN could be attributed to high levels of EE in this form. Although the type of fat was not differentiated in present study, presence of poly unsaturated fatty acids (PUFA) has been shown to reduce activity of fibre degrading microbes resulting to lower degradation and low gas production as observed in this study (Maia et al., 2010). It has been shown that excess oil of the long fatty acids in a feed (more than 3 - 5%) of the dry matter has a toxic effect on ruminal microorganisms especially bacteria which form the major fibrolytic colonies (Castillo-Gonzáleza et al., 2014). High predicted OMD and DMI in DfS implied better nutritive value in this form indicating that ruminant animals could consume higher amounts compared to the other forms (Negesse et al., 2016). The markedly high level of SCFA produced by DfS indicated that this form was better placed to supply the ruminant animals with the requisite energy to support production.

***In sacco* degradability**

High dry matter degradability of rapidly degradable fraction (*a*) in DhN is an indication of high soluble nutrients which could be combined with low quality roughages to provide protein and energy needed by microbes. Slowly degradable fraction (*b*) of DM in all forms of croton nut was low compared to rapidly degradable fraction. Low fibre quality limit the ability of microbes in effectively degrading the feed by making it difficult for rumen microorganism to attach on the feed particles (Venkateswarlu et al., 2013). The dry matter rate constant of degradation (*c*) was comparable to various conventional feed resources such as coconut meal, peanut meal and whole cotton seeds (0.2-0.05 per hour) (Chumpawadee et al., 2005). This rate is important as it determines rumen fill and exerts direct effect on intake (Chumpawadee et al., 2005). At rumen outflow rate of *kp*=0.05, effective degradability (DM) of various forms of croton in this study were within the range (24.3 – 60.9%) observed for conventional protein sources which include soy bean meal, whole cotton seed, coconut meal and fish meal (Chumpawadee et al., 2005). This fraction represents the total amount of nutrients which can be captured by rumen microbes for their growth, production of VFAs and synthesis of microbial protein (Lanyasunya et al., 2006). The IV of all croton forms in this study were within the acceptable level of >33 as recommended by (Ørskov and Shand 1997). This level indicates sufficient nutrients that an animal needs to consume to meet its daily maintenance needs.

The low level of rapidly soluble fraction of CP (*a*) observed in this study is within the recommended <40% for effective degraded protein (Lanyasunya et al., 2006). At this level, the (*a*) fraction does not overwhelm rumen microbes through production of excess nitrogen in form of ammonia, thus, maintaining an optimal protein-energy balance. Feeds with high slowly degradable fraction (*b*) avail required nitrogen in small amounts which are effectively utilized by rumen microbes. Effective degradability of crude protein provides an estimate of the total amount of protein captured by the rumen microbes for growth and synthesis of microbial protein (Lanyasunya et al., 2006). This fraction was high in DhN an indication that a considerable amount of protein in this form was degraded in the rumen. The remaining amount of protein regarded as rumen undegradable protein (RUP) represents the fraction of protein that is not degraded in the rumen and is termed as rumen by pass protein (Gao et al., 2015). Rumen by pass protein is available at the lower gut (small intestines) where combined with microbial protein contribute to protein requirements of the animal for maintenance and production. In this study WN was a good source of RUP and could be used to provide this form of protein in ruminant diets.

CONCLUSION

Processing through dehusking and defatting had the most significant impact on the nutritional composition of croton nuts. The two methods improved the nutritional profiles of protein, energy and mineral contents while reducing the fibre fractions compared to where peeling or no-processing was done. Degradability of dehusked and defatted forms of croton nuts was also high compared to the peeled and unprocessed whole nut forms. However, nutritional value of all forms of croton nuts was adequate and could be used in a total mixed ration (TMR) for maintenance purposes. In particular, dehusked and defatted forms have potential utilisation as protein supplements which could additionally supply energy and minerals for increased ruminant productivity on low quality basal diets. Microbial, enzymatic or chemical pre - treatment of the WN and PN forms prior to feeding could be explored to improve any observed lowered feed digestibility. Further studies to assess the effect of feeding croton on palatability, level of intake and production performance of ruminants are required.

DECLARATIONS

Corresponding author
E-mail: wambuicc@gmail.com

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Authors' contribution

All authors contributed equally to this work.

Conflict of interests

The authors declare that there are no competing interests.

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COMPARATIVE EFFECTS OF SYNTHETIC LYSINE AND METHIONINE SUPPLEMENTS ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FINISHER BROILERS FED CORN-SOYBEAN BASED DIETS

Victoria Nnenna MEREMIKWU and Peter Noah GBOSHE

Department of Animal Science, Faculty of Agriculture and Forestry, Obubra Campus, Cross River University of Technology, PMB 112, Cross River State Nigeria

Email: victoriameremikwu@yahoo.com; ORCID: 0000-0002-3904-8234

Supporting Information

ABSTRACT: The aim of this research was to investigate the effects of lysine and methionine supplements in corn-soybean meal diets for finisher broilers, by comparing their combined and sole effects on performance and carcass characteristics of the birds. Parameters measured were performance (body weight, weight gain, feed intake, feed conversion ratio and mortality), dressed weight, dressing percentage, carcass cuts and internal organs. The experimental diets were: T1 (control) = lysine + Methionine, T2 (sole lysine) and T3 (sole methionine) supplements. Final body weight, weight gain, carcass and carcass cuts were significantly higher in the control (lysine + methionine) than in the sole supplemented diets, while sole supplementation with methionine (T3) produced significant higher values than sole lysine (T2) in the above mentioned parameters The liver was significantly enlarged in the birds that received the sole supplemented diets. Due to the enlarged liver of the birds fed the sole supplemented diets, it was concluded that supplementation with both lysine and methionine is indispensable in corn- soybean meal based diets for finisher broilers.

Keywords: Amino acid, Broiler, Lysine, Methionine, Supplement.

RESEARCH ARTICLE

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INTRODUCTION

Broilers are domestic chickens (*Gallus domesticus)* of either sex, specially bred for rapid growth and meat production, commercially. They reach an average live weight of 2.2 to 2.8kg at 5 to 8 weeks of age on consumption of 3.3 to 5.0kg of feed depending on the nutrient content of the diet (Smith, 2001). Broiler production involves two phases in a production cycle namely; a "starter phase" from day one to week four of age, on a starter diet of 22-24% crude protein (CP) and a "finisher phase" from week four to week eight of age on a finisher diet of 19% CP (Aduku, 2004). Meremikwu and Gboshe (2007) reported an average feed intake of 130.8g/bird/day during the finisher phase against the 45.0g /bird/day at the starter phase. This resulted to high cost of feed/kg weight gain at the finisher phase (Meremikwu and Gboshe, 2007; Tandoğan and Çiçek, 2016). Although feed intake is very high at the finisher phase growth rate is also very high. Smith (2001) reported that the peak growth rate for the broiler is achieved between five to eight weeks of age. This is about 64.0g/day against 31.0g/day at the starter phase (Meremikwu and Gboshe, 2007).

Protein is a vital nutrient in poultry nutrition because of its biological role in enhancing growth, egg production, immunity and adaptation to environment (Esmnil, 2016). The biological function of protein is attributed to specific amino acids (Lee et al., 2020). Lysine and methionine have universally been recognized as the limiting amino acids in most of the practical diets for broilers especially in diets base on corn and soybean meals which are the basal ingredients in most poultry diets (Farkhoy et al., 2012; Lee et al., 2020). Dietary deficiencies of lysine and methionine have been shown to impair chicken growth. Wen et al. (2014) reported that broilers fed methionine deficient diet exhibited low concentration of insulin-like growth factor 1 (IGF-1) within two days of the feeding, which resulted to low performance and low breast muscle growth. Cacew et al. (2005) reported that Lysine deficient diet increased fat synthesis at the expense of body protein accretion and energetic efficiency in broilers.

The supplementation of poultry feeds with Lysine and methionine in crystalline form is very common in the poultry industry. Fishmeal is also described as an excellent source of high quality protein in cereal – based diet for poultry because of the natural balance of essential nutrients including high content of lysine and methionine. However, the use of fish meal in most developing countries is limited by cost. Fishmeal is also reported to be a source of food-borne pathogen especially salmonella specie (Novoslavskij et al., 2016). Research has shown that industrial amino acids are competitively available and can replace protein sources in poultry diets to match amino acid requirements (Farrell, 2005). According to Farrell (2005), little or no-dietary protein sources can be used in poultry diets because protein sources are both scarce and expensive and nitrogen excretion is high.

This research was designed to investigate the basic traits (essentialities) of lysine and methionine supplements in corn – soybean meal based diets for finisher broilers by comparing their combined and sole effects on performance and carcass characteristics of finisher broilers. Parameters measured were performance (body weight, body weight gains, feed intake, feed conversion ratio and mortality), dressed weight, dressing percentage, carcass cuts and internal organs.

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MATERIALS AND METHODS

Experimental site

The study was carried out at the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture and Forestry, Obubra Campus, Cross River University of Technology (CRUTEH), Cross River State Nigeria. The location of the study lies along Latitude 6" 4.6032' N and Longitude 8" 19.9446' East (Date and Time Information, 2020).

Experimental treatments and design

The experiment comprised three treatments each of which was replicated four times in a Complete Randomized Design (CRD). The treatments are as follows: T_1 (control): Methionine and Lysine; T_2: Lysine only; T_3: Methionine only.

Experimental diets

The feed ingredients used for the diets were based on availability and cost. The cheapest and most available ingredients were used in formulation of the diets. They include; maize, Soybean meal, wheat offal, bone meal, mineral/vitamin premix, synthetic lysine and methionine and common salt. The diets were formulated according to NRC 1994 specification. The experimental diets and their calculated chemical composition are presented in table 1. The chemical composition of the rations was obtained by calculation. The calculation was carried out using the spreadsheet method as described by Smith (2001).

Table 1 - Experimental diets and their calculated nutrient composition

Ingredients (%)	T_1 (control) Methionine + Lysine	T_2 Lysine	T_3 Methionine
Maize	48.94	49.26	49.26
Soybean meal	36.56	36.49	36.49
Wheat offal	10.00	10.00	10.00
Bone meal	3.25	3.25	3.25
Common salt	0.25	0.25	0.25
Vitamin premix*	0.50	0.50	0.50
DL-methionine	0.25	0	0.25
Lysine – Hcl	0.25	0.25	0
Total	100.00	100.00	100.00
Calculated composition of experimental diets.			
Crude protein (%)	20.00	20.00	20.00
ME (Kcal/kg)	3073.10	3081.77	3081.77
Crude fibre (%)	3.99	4.00	4.00
Methionine (%)	0.98	0.32	0.57
Lysine (%)	1.58	1.33	1.08

* Each 2.5 kg of premix contained: Vitamin A, 8,000,000 IU; Vitamin D_1,,600,000 IU; Vitamin E, 20,000 IU; Vitamin K, 2,000mg; Vitamin B_1, 1,5000mg; Vitamin B_2, 4,000mg; Vitamin B_6, 2,000mg; Vit. B_{12}, 10mg; Niacin, 15,000mg; Panthotenic Acid, 5,000mg; Folic Acid, 500mg; Biotin, 20mg; Choline Chloride, 200,000mg; Manganese, 80,000mg; Zinc, 50,000mg; Iron, 20,000mg; Copper, 5,000mg; Iodine, 1,000mg; Selenium, 200mg; Cobalt, 500mg; Antioxidant, 120,000mg.

Management of experimental animals

One hundred and twenty finisher broilers were used for the feeding trial which lasted for twenty-eight days. The birds were selected after brooding and randomly allotted to the twelve experimental units (10 birds per unit). They were housed in deep litter house partitioned into experimental units of 8ft × 12ft (width × length). Feed and water were given *ad libitum*. The birds were managed using standard husbandry practices for rearing broilers.

Data collection

The birds were weighed at the beginning of the experiment to get their initial body weight. They were weighed thereafter on weekly basis. Weight gain and feed conversion ratio were deduced from the weekly body weights. Feed offered daily was weighed and the left over weighed the following morning. Feed intakes were obtained by subtracting the leftover from the quantity supplied the previous day. At the end of the experiment at eight weeks of age, four birds were randomly selected from each treatment for carcass analysis. The birds were starved of feed but not water for twelve hours before slaughtering. The slaughtering and dressing of the birds were carried out using standard practices for processing broilers.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using Minitab Statistical Package. Significant means were separated using the Fisher Least Significant Difference (FLSD) that is containing in the statistical software.

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Ethical approval

Birds were handled and managed in accordance with rules and recommendations in the "Guide for the Care and use of Animals", presented in the Faculty of Agriculture and Forestry Obubra Campus, Cross River University of Technology, Cross River State, Nigeria (ethical committee).

RESULTS AND DISCUSSION

The calculated chemical composition of the experimental diets are presented in table 1, while the results of the performance and carcass characteristics of the experimental birds are presented in tables 2 and 3, respectively.

Experimental diets

The limitations of Lysine and methionine in corn-soybean meal diets for this experiment are revealed in the calculated chemical composition of the diet in table 1. The calculated lysine levels of all the diets including sole methionine diet (T3) were up to minimum requirement for finisher broilers, while the methionine levels in the sloe lysine diet (T2) was below minimum requirement (NRC, 1994). This result has revealed that methionine is the first limiting amino acid in corn-soybean meal based diets for broilers. This is supported by the report of Byrne (2018) that methionine is the first limiting essential amino acid in corn-soybean meal based diets for broilers.

Performance

The final body weights and body weight gains of the experimental birds differed significantly ($P<0.05$) between the treatments and were highest ($P<0.05$) in the control (T1, Lys + Meth) and lowest ($P<0.05$) in the sole lysine (T2). The sole methionine group (T3) were in-between the control and the sole lysine groups in the said parameters i.e. lower ($P<0.05$) than the control and higher ($P<0.05$) than the sole lysine. Feed intake did not differ ($P<0.05$) between the treatments. Feed conversion ratio followed the same trend with body weight and body weight gain, being highest ($P<0.05$) in the control and lowest ($P<0.05$) in the sole lysine group. Mortality was zero percent for all the treatments. The significant ($P<0.05$) higher performance of the control birds (T1, Lysine + methionine) over the sole lysine (T2) and sole methionine (T3) could be due to complementary effect of the amino acids to each other. This is supported by the report of Si et al. (2001 and 2014) that there were no interaction between Lysine and Methionine when they were fed equal to or in excess of NRC recommendations in broiler diets. Rather, each of the amino acids supplied a complimentary effect to meet specific deficiencies. The significant ($P<0.05$) low performance of the sole lysine birds (T2) in comparison to the sole methionine birds (T3) could be due to absence of complimentary effect of methionine and it is an indication that methionine is the first limiting essential amino acid in corn-soybean meal diet for broilers. This is also supported by the report of Neutkens (2005) that DL-methionine or Methionine hydroxyl is the first-limiting amino acid for birds, while Lysine is the first-limiting amino acid in corn-soybean meal based diet for pigs. According to Neutkens (2005), to use crystalline amino acids in low- protein diets effectively, and to minimize nitrogen excretion, you must first understand their limitation i.e. the order in which they are limiting in various feedstuffs, and second the magnitude of difference between them. The absence of supplemental Methionine in the sole Lysine diet (T2) reduced the methionine content of the diet below minimum requirement as revealed in the calculated chemical composition of the diets in table 1, resulting to poor performance of the birds.

Table 2 - Performance of finisher broilers fed supplemented diets (lysine and methionine).

Parameters (g) \ Treatments	T_1 (control) Meth + Lys	T_2 (Lys)	T_3 Meth	SEM
Initial body weigh t (kg)	0.62	0.62	0.62	-
Final body weight (kg)	2.80^a	2.15^c	2.50^b	0.13
Weight gain (g/day)	77.86^a	54.64^c	67.14^b	4.59
Feed-intake (g/day)	127.00	130.75	128.50	5.424ns
FCR (g of feed/g of gain)	1.63^c	2.40^a	1.92^b	0.12
Mortality (%)	0.00	0.00	0.00	

Mean with different superscript are significantly ($p<0.05$) different. FCR= Feed conversion ration; SEM=Standard Error of Mean; Ns=Not significant.

Carcass and carcass cuts

The results of carcass and carcass cuts followed the same trend with that of performance parameters. The control birds (T1, Lys + Meth) had significant ($P<0.05$) higher values for carcass parameters (including dressed weight, dressing percentage, breast and thigh) than the birds in treatments T2 and T3 (sole lysine and sole methionine, respectively) table 3. The sole lysine birds (T2) had the lowest ($P<0.05$) values in carcass parameters mentioned above, while the sole methionine birds (T3) were in-between the control (Lys + meth) and the sole Lysine birds (T2) in the said parameters. The significant ($P<0.05$) higher carcass values of the control birds (Lys + Meth) over the sole lysine and sole methionine birds confirm the complimentary effect of the two amino acids to each other to meet specific deficiencies and enhance the

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productivity of birds as reported by Zhai et al. (2016). The significant (P<0.05) higher performance of the sole methionine (T3) over the sole lysine groups (T2) in body weight and body weight gains reflected in significant (P<0.05) higher breast and thigh values. This is supported by the report of Wen et al. (2014) that methionine increased the concentration of insulin-like growth factor in broilers with subsequent improvement in performance and breast muscle growth. The significant (P<0.05) lower carcass values of the sole Lysine birds (T2) in comparison to the sole Methionine birds (T3) could be due to the low dietary content of methionine in the sole lysine diet as revealed in the calculated chemical composition of the diets in table 1.

Table 3 - Carcass and Internal organ weights of finisher broilers fed supplemental lysine and methionine

Parameters (g) \ Treatments	T_1 (control) Meth + Lys	T_2 (Lys)	T_3 Meth	SEM
Pre-slaughter weight (kg)	2.80^a	2.15^c	2.50^b	0.102
Dressed weight (kg)	2.00^a	1.30^c	1.55^b	0.104
Dressing percentage (%)	71.43^a	60.47^c	62.00^b	1.86
Carcass cuts (% of pre-slaughter weight)				
Drumstick	10.73	9.26	9.96	1.96^{ns}
Breast/wing	37.67^a	29.13^c	34.91^b	1.43
Thigh	12.24^a	11.04^c	11.96^b	0.192
Back	10.89^a	10.04^c	10.99^a	0.27
Internal organ (% of pre-slaughter weight)				
Gizzard	2.50	2.53	2.51	0.07^{ns}
Heart	1.09	1.10	1.10	0.026^{ns}
Liver	1.96^b	2.21^a	2.20^a	0.053
Abdominal fat	1.27^b	1.28^a	1.27^b	0.168^{ns}

Ns=Not significant; SEM=Standard error of mean.

Table 4 - Economics of supplementation with lysine and methionine in broiler nutrition.

Ingredients	Unit cost (₦)*	Quantity/100kg of feed			Amount (₦ /100kg of feed)		
		T_1 (Control)	T_2 (Lys)	T_3 (Met)	T_1	T_2	T_3
Lysine	1,500	0.25	0.25	0.00	375	375	0
Methionine	1,500	0.25	0.00	0.25	375	0	375
Total cost (₦)					750	375	375
Cost of supplementation /kg of feed (Total cost divide by 100) (₦)					7.50	3.75	3.76
Cost/kg of feed (₦)					159.5	155.75	155.75
Feed conversion ratio (FCR)					1.63	2.40	1.92
Cost of supplementation/kg weight gain (₦)					12.25	9.00	9.00
Cost of feed/kg weight gain(₦)					260.0	373.8	299.04
Relative cost of feed/kg weight gain (%)					27;87	40.07	32.05

*(₦) = Nigerian Naira (official money of Nigeria)

Internal organs

There was no significant difference (P<0.05) between the treatments in the sizes of the internal organs, apart from the liver that was significantly (P<0.05) larger in the sole lysine and the sole methionine birds in comparison with the control (lys + met). The significant (P<0.05) larger sizes of liver in the sole lysine (T2) and sole methionine (T3) birds could be due to increased metabolic activities to cope with imbalance of the amino acids. This is supported by the report of Park (2006) that one of the biochemical responses of animals fed amino acid imbalance diets is an increase in the activities of

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the enzymes involved in the catabolism of the limiting amino acid leading to increase in liver size. This is supported by the reports of Zaefarian et al. (2019) that increased liver size in avian is considered a positive indicator associated with higher metabolic activity and higher energy expenditure.

Economics of supplementation with lysine and methionine

The result of economics of supplementation with lysine and methionine is presented in Table 4. The birds fed the sole supplemented diets had about 4.18 – 12.2% higher cost of feed/kg weight gain than the control even though the cost of supplementation was higher in the control than the sole supplemented. The higher cost of feed/kg weight gain exhibited by birds in the sole supplemented diets (T1 and T2) could be due to poor utilization of feed by these birds. This is supported by the fact that the enlarged liver of the birds in the sole supplemented diets is associated with increased metabolic activities and higher energy expenditure (Zaefarian et al., 2019).

CONCLUSION

From the results of this study, it was observed that supplementation with both lysine and methionine produced significant enhanced effect than sole supplementation. Sole lysine supplementation produced significant ($P<0.05$) lower values in all parameters measured including performance, carcass and carcass cuts compared to sole methionine supplementation. Sole supplementations with either lysine or methionine caused increase in liver size. Although sole methionine supplementation gave significant ($P<0.05$) enhanced effects than sole lysine, it was concluded from this research that supplementation with both lysine and methionine is essential in corn-soybean meal based diet for finisher broilers to avoid increased catabolic activities that result to enlarged liver in the birds.

DECLARATION

Corresponding author

Victoria N. Meremikwu, Ph.D., Department of Animal Science, Faculty of Agriculture and Forestry, Obubra Campus, Cross River University of Technology, Cross River State, Nigeria. Email: victoriameremikwu@yahoo.com.

Availability of data

Data can be availed to the journal upon request.

Consent to publish

Not applicable.

Conflict of interest

The authors declare that they have no competing interest.

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PERFORMANCE, CARCASS AND INTERNAL ORGANS CHARACTERIZES OF BROILER CHICKENS WITH PHYTASE SUPPLEMENTATION FROM *Burkholderia* sp. Strain HF.7

HAFSAN[1], Aminah HAJAH THAHA[2], Asmuddin NATSIR[3] and Ahyar AHMAD[4]

[1]*Biology Department, Faculty of Science and Technology, Universitas Islam Negeri Alauddin, Sultan Alauddin Street, Gowa 92118, Indonesia*
[2]*Animal Science Department, Faculty of Science and Technology, Universitas Islam Negeri Alauddin, Sultan Alauddin Street, Gowa 92118, Indonesia*
[3]*Department of Nutrition and Animal Feed, Faculty of Animal Sciences, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar 90245, Indonesia*
[4]*Chemistry Department, Mathematics and Natural Science Faculty, Hasanuddin University, Perintis Kemerdekaan Street Km. 10 Tamalanrea, Makassar 90245, Indonesia*

Email: hafsan.bio@uin-alauddin.ac.id; ORCID: 0000-0001-5821-0164

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RESEARCH ARTICLE

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ABSTRACT: Feed formulation with phytase supplementation is an innovation in the feed industry to improve monogastric feed quality without increasing production costs. This study aims to determine the carcass weight of broilers and the percentage of internal organs by providing various feeds, including those supplemented with phytase in phytase units (FTU) from *Burkholderia* sp. strain HF.7. A completely randomized experimental design was used in this study, using 108 broilers for five weeks of maintenance in three treatments with six replicas, each replica consisting of six broilers. The experimental feed given to broilers was basal feed without phytase supplementation (P1), basal feed + 750 FTU phytase (P2) and commercial feed (P3), each with the category of starter phase and finisher phase. Carcass weight and percentage of organs in broilers (liver, heart, gizzard, and lymph) were measured in each treatment unit. The results showed that broilers that consumed phytase supplemented feed had a higher carcass weight with a lower feed conversion value than broilers fed basal feed without phytase. These findings also indicate that the addition of phytase from *Burkholderia* sp. HF.7 strain at 750 FTU/kg feeds does not interfere with the organs of broilers' physiological function because of no increase in the percentage of the liver, heart, gizzard, and lymph.

Keywords: Broiler, Burkholderia, Internal organs, Performance, Phytase.

INTRODUCTION

Broiler farming is a prospective productive farming and the increase of nutrient conscious and public consumption of food animal-based (Walker et al., 2005; Manning et al., 2006; Bonham et al., 2006). This opportunity encourages (Benton and Bailey, 2019). One of the essential and economic aspects of broiler maintenance is feed (Tallentire et al., 2016). The content and availability of nutrients determine feed quality to meet broilers' needs during the maintenance period (Wenk, 2000; Abdollahi et al., 2013). Various feed formulations have been arranged in such a way to achieve maximum productivity (Daghir, 2009; Santoso, 2012; Krishnasamy et al., 2015). This effort, feed cost is one of the main obstacles of broiler production. Recently, various efforts have made in order to find ways to reduce feed costs.

One effort that can accomplish in order to improve the feed quality while reducing the production cost is adding feed additive which is a material or combination of ingredients (Tallentire et al., 2016). The supplementation of phytase is efforts that can be an option in innovative feed formulation to improve the nutritional value of broiler feed through improving nutrient utilization; increase utilization of phosphorus and calcium in the feed (Augspurger et al., 2003; Aureli et al., 2011; Hafsan et al., 2017), amino acid absorption (Cowieson et al., 2004) and the ability to digest feed ingredients (Rutherfurd et al., 2012). Increase utilization of nutrients by phytase in performing the phosphate group release function of the Mio-inositol ring on phytate compounds which the main form of phosphorus storage in broiler is feed ingredients. The phosphate group's release implies the release of other essential proteins and minerals bound to the phytate complex, hence its availability in the feed has a unique effect (Hirvonen et al., 2019).

The usage of phytase with high stability to temperature and pH at specific dosage has reported significantly improved broiler performance as it improved nutrient digestibility in the feed (Shirley and Edwards, 2003; Hafsan et al., 2018; Cowieson et al., 2006). Extra-phosphorus effects as well as the release of amino acids, and cations bound such as calcium, magnesium and iron lead to absorbed nutrients increased in metabolic and biosynthetic processes, affected of higher energy retention leading to increased broiler performance (Cowieson et al., 2006; Selle et al., 2000). Indicators of broiler metabolic processes reflected in a good performance as well as lead to excellent carcass properties and a balanced percentage of internal organs (Angel et al., 2006; Çimrin and Demirel, 2008) as a result of increased availability of phosphate, higher nitrogen retention and increased solubility of phytate complexes in the digestive tract of broilers

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(Shirley and Edwards, 2003). The maximum utilization of nutrients such as protein, phosphorus, and calcium by broilers is the increased performance indicated by weight gain at harvest. Besides, feed conversion value will decrease due to the maximum absorption of nutrients (Humer et al., 2015; Savita et al., 2017; Maas et al., 2021). Anti-nutritional substances, including phytic acid, will cause the digestive organs to work longer to cause physiological disorders, including the digestive organs' weight. Therefore, this study reveals the effect of phytase giving by *Burkholderia* sp. Strain HF.7 (Hafsan et al., 2018) on the profile of broilers' internal organs.

MATERIALS AND METHODS

This study was an experimental study using complete randomized design, namely three treatments with six replications. The variables observed in this study were the appearance of broiler production, which included carcass weight and percentage of internal organs (liver, heart, gizzard, and lymph). Day Old Chick (DOC) broiler Cobb strain used without separating males and females (unsexed). Maintenance performed for five weeks. The cage used roofed with a litter system with dimensions is 250 × 250 × 80 cm, which is equipped with lighting and functions to warm the cage. Ten broilers occupied each plot. The cage equipment used was two feedings vessel of 500 g and drinking water containers of 500 mL capacity. Measurement of the temperature and humidity of the cage environment using a thermometer and thermo hygrometer. The average temperature of the cage was 27.69°C, and the average humidity was 75.88%. Feed and drinking water were given in ad libitum every morning, afternoon and evening based on treatment. Phytase powder from *Burkholderia* sp. strain HF.7 was added to every 5 kg of feed a homogenized before fed the broiler. Treatment feed was given based on the maintenance period, namely starter and finisher.

Feed experiments using three types of feed. The basal feed used in this study was obtained from conventional feed mills. The ingredients of the basal feed composition are yellow corn, rice bran, soybean meal, Meat and Bone Meal, coconut oil, CaCO3, dicalcium phosphate, DL-methionine, L-Lysine, premix. A basal feed with the composition is used as P1 feed and basal feed supplemented with 750 FTU of *Burkholderia* strain HF.7 as P2. A comparison feed (P3) shows that commercial feed is obtained from one Poultry shop in Makassar, without knowing its ingredients. The chemical composition of the fodder with its nutritional content is presented in Table 1.

Table 1 – Composition and nutrient of broiler feed ingredients.

Feed Composition	Starter (%)			Finisher (%)		
	P1	P2	P3	P1	P2	P3
Corn	53	53	-	60	60	-
Rice bran	6	6	-	5	7	-
Soybean meal	28	28	-	21.2	19.2	-
Meat and bone meal	8	8	-	8.3	8.3	-
Coco oil	3	3	-	3.3	3.3	-
CaCO3	0.8	0.8	-	1	1	-
Dicalcium phosphate	0.1	0.1	-	0	0	-
DL-methionine	0	0	-	0.2	0.2	-
L-lysine	0.3	0.3	-	0.5	0.5	-
Premix	0.5	0.5	-	0.3	0.3	-
Total (%)	100	100	100	100	100	100
Chemical composition						
Phytase (FTU/kg)	0	750	0	0	750	0
Crude Protein	22.75	22.75	22.80	20.11	0.80	22.40
Raw fat	3.60	3.60	3.85	3.30	0.30	3.45
Phosphorus	0.79	0.79	0.37	0.71	0.45	0.60
Calcium	1.43	1.43	1.03	1.43	1.03	1.20
Phytate	0.33	0.33	0.20	0.29	0..29	0.26
Metabolic energy (Kl/kg)	3.03	3.03	3.05	3.14	3.14	3.16

P1 = basal feed without phytase; P2 = basal feed + 750 FTU phytase; P3 = commercial feed; FTU=phytase units.

Ethical approval

This research was conducted in accordance with the recommendations of research ethics approval using animal subject by Health Research Ethics Committee of Universitas Islam Negeri Alauddin Makassar, referred to Legislation of the Republic of Indonesia No. 18, 2009.

Statistical analysis

The gathered data is analyzed with analyzed with ANOVA of Complete Randomized Design. Estimated conversion of feed consumed by broilers is determined by calculating the ratio between the amount of feed consumed and the resulting weight gain. Bodyweight gain was calculated from the chicken's weight last week minus the initial bodyweight of the chicken. Percentage determination of internal organ was obtained from dividing internal organ weight by broiler's live weight in 100% after fat dismissing.

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RESULTS AND DISCUSSION

As a parameter to determine feed quality, the feed conversion value of each type of feed treated was determined beforehand by compared between the amount of feed consumed with broiler body weight gain during maintenance has ended. Feed conversion value indicates the level of feed efficiency, meaning the lower the value of feed conversion, the higher the feed efficiency and more economical. Figure 1 shows that the average value of feed conversion produced in this study was 1.922 - 2.591.

Variance analysis has shown very significant differences between treatments (P<0.01). The basal feed which was not added phytase (P1) was indicated to produce a significantly high feed conversion compared to other treatments. P2 feed given to broiler shows the lowest feed conversion value and relatively similar (not significantly different) to P3. This disparity indicates that the best feed conversion obtained in P2 with the addition of 750 FTU of *Burkholderia* sp. strain HF.7. However, statistically, there is no significant difference from the commercial feed, which has been used extensively by farmers. The lowest feed conversion rate among these treatments (1.922) has provided an optimal description of this broiler's digestive system in converting 1.922 kg of feed into 1 kg of the carcass. According to (Dos Santos et al., 2013; Liu et al., 2014), the lower feed conversion rates indicate that broilers are better at converting feed into meat and feed can be said to be of good quality. This study has proven to indicate that the lowest feed conversion rate by giving 750 FTU/kg phytase from *Burkholderia* sp. strain HF.7 has improved broiler metabolism. Feed nutrition is increased in availability can metabolism. The feed can optimally be converting to meat.

The percentage determination of carcass and organ in broilers with a basal diet without phytase additives, feed with phytase supplementation from *Burkholderia* sp. strain HF.7 as a feed additive, and commercial feed for five weeks of maintenance is present in Table 2.

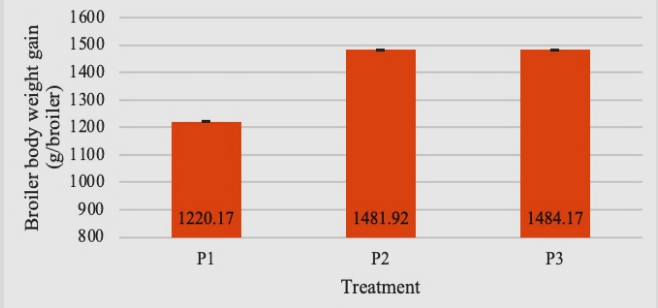

Figure 1 - Broiler body weight gain (g/broiler) with broiler maintenance for 35 days: P1 (basal feed without phytase); P2 (basal feed + 750 FTU phytase); and P3 (commercial feed).

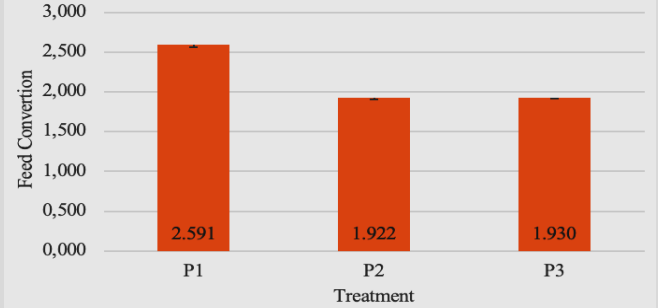

Figure 2 - Value of feed conversion with broiler maintenance for 35 days: P1 (basal feed without phytase); P2 (basal feed + 750 FTU phytase); and P3 (commercial feed).

Table 2 - Average and standard percentage of broiler's carcass and weight of internal organs.

Percentage	P1	P2	P3	Standard Percentage	P-Values
Carcass weight	61.2 ± 0.934 [a]	69.6 ± 0.842 [b]	69.2 ± 1.011 [b]	67–72	P<0.05
Liver (%)	2.116 ± 0.926	2.021 ± 0.450	1.995 ± 0.264	1.7–2.8	P>0.05
Heart (%)	0.803 ± 0.086	0.792 ± 0.065	0.798 ± 0.035	0.5–1.4	P>0.05
Gizzard (%)	2.016 ± 0.752	1.902 ± 0.784	1.899 ± 0.881	1.6–2.3	P>0.05
Lymph (%)	1.503 ± 0.284	1.533 ± 0.857	1.592 ± 0.721	1.4–1.9	P>0.05

Different superscript in the same line shows a significant effect (P<0.05); P1 = basal feed without phytase; P2 = basal feed + 750 FTU phytase; P3 = commercial feed.

The difference in broiler growth between treatments P1 and P2 is caused by the occurrence of protein and mineral metabolic disorders which one of the causes is the presence of phytate as in table 1. Addition of 750 FTU / kg phytase *Burkholderia* sp. strain HF.7 in feed, significantly increased the growth of broiler experiments (P<0.01) and this fact strengthens the results of some study, that increased growth of broilers that received feed with the addition of phytase showed significant weight gain (Augspurger et al., 2003; Cowieson et al., 2006; Rutherfurd et al., 2012; Fernandes et al., 2019). A different trend occurred between P2 and P3 treatments, in which different broiler body weight gain is not accurate. Bodyweight gain is almost the same as those treatments as in Figure 1 thought to be caused by broilers' ability to be about equal in metabolizing feed the body. Feed nutrition is converted into available nutrients. Digestion and absorption can occur, and the rest that is not absorbed is excreted in the faeces. If digestible energy is converted into metabolic energy that can produce heat and energy for basic life and production activities, the expected growth of broilers will always be average and as expected (Moss et al., 2019).

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The carcass is part of broiler's body after slaughtered and separated from feathers, abdominal fat, internal organs except for lungs, kidneys, legs, head, neck, and blood (Çimrin and Demirel, 2008). Carcass weight percentage was obtained by dividing carcass weight with broiler life weight. The statistical analysis result in table 2 showed that phytase supplementation from *Burkholderia* sp. strain HF.7 gave a significant effect ($P<0.05$) on carcass weight percentage. Overall, 750 FTU phytase from *Burkholderia* sp. strain HF.7 in each kilogram of feed, increased the carcass weight compared with a basal diet without phytase supplementation. These findings are in agreement with Nourmohammadi et al. (2010) that there is an increase in carcass weight by the addition of Natuphos phytase to roosters and hens fed with low phosphorus levels. some researchers also that phytase in *Aspergillus oryzae* has a positive effect on carcass weight for poultry fed with low phosphorus levels reported (Angel et al., 2006; Ghosh et al., 2016; Akter et al., 2016; Barzegar et al., 2020). Similarly, other studies with feed containing low levels of phytate phosphate and various commercial phytase levels have a positive effect on carcass weight (Cufadar et al., 2010).

The positive effect on carcass weight by phytase supplementation is due to the absorption of the maximum nutrients in the diet due to the phytase ability to release essential minerals, amino acids, and energy bound from the phytate complex of feed (Dersjant-Li et al., 2015). Nutrients in the feed are released and absorbed, so they can be used for metabolism and help broilers' growth (te Pas et al., 2020). Hence the role of protein has a very substantial in the growth of chicken tissue. Protein absorption ultimately provides faster growth and improves broiler carcass (Kamran et al., 2008; Rezaei et al., 2018). The trend of increasing carcass weights shows in the treatment of feeding using the commercial feed as in table 2. The improvement shows that the quality of basal feed with phytase supplements equals the quality of commercial feed, even without the addition of dicalcium phosphate (DCP), increasing the cost of feed production. The study focus also showed that the percentage of carcasses with P2 and P3 feeds meet the usual broiler carcass percentage standard of about 65-75% of the weight of live broiler (Aletor et al., 2000).

Analyses of variance indicate that phytase supplementation from *Burkholderia* sp. strain HF.7 as feed additive does not affect ($P>0.05$) the weight percentage of organs in the broiler on liver, heart, gizzard or lymph. The average weight percentage of organs in the broiler on each treatment P1, P2, and P3 are list in Table 2. Percentage of liver weight in each treatment ranging from 1.9 to 2.2% was in the standard range of the liver's healthy weight percentage (1.7-2.8). The percentage of heart weight in each treatment by 0.8% was in the standard range of healthy heart weight percentage of 0.5 to 1.4. Likewise, the percentage of gizzard and lymph were in the normal range that means the three feed treatments did not disturb the equilibrium percentage of liver, heart, stomach, and lymph of broiler. This data also shows that the addition of phytase from *Burkholderia* sp. strain HF.7 will not interfere with the broiler organs' physiological function. Thus, it is relatively safe to utilize as a feed additive in the future (Sari and Ginting, 2012; Kokoszyński et al., 2017).

Overall, after the three types of feed in broiler chickens reared for 35 days, feed supplemented with 750 FTU phytase/kg to produce the best feed conversion value and directly proportional to the weight of carcass produced. Supplementation of phytase has proven that the lower feed conversion rate means better feed quality. The high value of feed conversion indicates the need for the amount of feed needed to increase body weight higher and lower feed efficiency. The value of feed conversion is closely related to production costs, primarily feed costs, because the higher the conversion of feed, the cost of feed will increase because the amount of feed consumed to produce body weight in a certain period is higher (McNitt, 1983; Kokoszyński et al., 2017).

CONCLUSION

The supplementation of *Burkholderia* sp. strain HF.7 to 750 FTU/kg of feed may stimulate maximum absorption of nutrients, so that carcass weight is greater and decreases feed conversion ratio. Increased carcass weight and do not affect the percentage of liver, heart, gizzard, and lymph in the broiler.

DECLARATIONS

Corresponding Author
E-mail: hafsan.bio@uin-alauddin.ac.id; ORCİD: 0000-0001-5821-0164

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

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

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EFFECTS OF PRE-DETERMINED LEVEL OF FOLIC ACID SUPPLEMENT ON PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILER CHICKENS

Victoria Nnenna MEREMIKWU[1] and Esther Darlington IZUKI[2]

[1]*Department of Animal Science, Faculty of Agriculture and Forestry, Obubra Campus, Cross River University of Technology, PMB 112, Cross River State, Nigeria*
[2]*Department of Animal Science, Faculty of Agriculture, University of Calabar, Cross River State, Nigeria*

Email: victoriameremikwu@yahoo.com; ORCID: 0000-0002-3904-8234

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ABSTRACT: A pre-determined level of folic acid supplement (30 mg per litre of drinking water) was fed for varying durations (7, 10 and 14 days) from day-one of age to determine the effect on performance and carcass characteristics of broilers. The objective was to confirm the high levels of abdominal fat pads in previous trials with graded levels of folic acid, to clarify the mechanism underlying adipose tissue growth in broilers. Parameters measured were body weight, weight gain, feed intake, feed conversion ratio, folic acid intake, mortality and dressed weight, dressing percentage, carcass cuts and internal organs. Data obtained were analyzed using statistical package for social sciences. The outstanding result of this research was on the conformation of the dressed carcasses of the folic acid treated birds, characterized by expanded abdominal regions filled with large mass of abdominal fat pads. There was no difference between the control and the folic acid birds in other parameters measured, except the group on the longest duration of folic acid supplementation, which had higher feed intake. Folic acid intake increased significantly with increase in the duration of administration. It was concluded that, the large mass of abdominal fat pads of the folic acid birds were as a result of cell multiplication (hyperplasia) due to the fact that folate-mediated one-carbon units transfer reactions support rapid proliferation of cells and are important during periods of active cell division.

Keywords: Abdominal fat, Broiler, Folic acid, Pre-determined level, Supplement.

INTRODUCTION

Folic acid (Pteroyl-L-glutamic acid) is the synthetic form of a water soluble vitamin (vitamin B9) which occurs naturally as folate. Folate is richly available in dark green vegetables while folic acid is often recommended as a nutritional supplement and for fortification of foods (Chan et al., 2015). Folic acid is referred to as a growth promoting factor because of its role in the production and maintenance of new cells (Wagner 2001; Armando, 2018). It is reported to be particularly important during periods of active cell division such as pregnancy and infancy (Armando, 2018; Tjong and Mohiuddin, 2019). It is essential in cell metabolism because of its use in the biosynthesis of components of nucleic acid needed for cell division (Barry, 2001; Tjong and Mohiuddin, 2019).

Folic acid itself is not biologically active but it is converted to its biological active form tetrahydrofolic acid (THF) after its conversion to dihydrofolic acid by dihydrofolic reductase in the liver (Fernández-Villa et al., 2019). Tetrahydrofolic acid is a cofactor in one-carbon metabolism which is a universal metabolic process that serves to activate and transfer one-carbon units for biosynthetic processes in the body (Barry, 2001). Tetrahydrofolic acid can also be converted to other one-carbon transport forms by serving as a carrier molecule for one –carbon groups e. g. methyl – THF, methylene – THF, methenyl–THF (Fernández-Villa et al., 2019). These folic acid derivatives especially tetrahydrofolic acid serve as coenzymes in one-carbon units transfer reactions during cellular activities. Folate-mediated single-carbon transfer reactions are important in biosynthetic pathways leading to DNA and RNA synthesis, amino acid metabolism, methylation and remethylation reactions (Barry, 2001).

Tetrahydrofolic acid plays key role in DNA synthesis by serving as a direct donor of one-carbon units in the synthesis of components of nucleic acid (the critical base pairs) needed by DNA for replication i.e. the synthesis of DNA from its precursors (thymidine and purine) is dependent on folate-mediated one-carbon unit transfer reactions (Field, 2018). According to Field (2018), folate-mediated one-carbon units transfer reactions support high proliferation rate of normal cells and cancer cells because of many activated precursors of nucleic acid (thymidine and purine). This body of knowledge had led to addition of folic acid to virtually all cell culture media because, it enhances the growth of cells in serum-free culture (Media Experts, 2020). Deficiency of folic acid leads to impaired cell division due to impairment in thymidine and purine synthesis, resulting from impairment in one-carbon metabolism (Greenberg et al., 2011; Field, 2018). In avian species, folic acid deficiency is characterized by poor growth, very poor feathering and anaemic appearance in chicks (Poultry DVM, 2020). Supplemental folic acid nutrition has not been a major concern in the poultry industry. However, some researchers had investigated the growth promoting potentials of folic acid on the growth performance of the broiler chicken. Meremikwu et al. (2008) fed graded levels of folic acid per litre of drinking water for the first five days of age of the broiler. The result of the trial showed enhanced performance in the folic acid treated birds

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over the control, with higher levels of abdominal fat in the folic acid birds than the control. There was however no difference between the folic acid treated birds. A second trial was conducted with the same levels of folic acid supplement as in the previous trial but for a longer period of seven days from day-one of age of the broiler (Meremikwu et al., 2015). The result of the later trial showed a clearer picture of the effect of the different levels of folic acid supplementation on broiler performance. There were conspicuous and significant higher levels of abdominal fat pads in the folic acid treated birds than the control, with 30 mg level of supplementation eliciting a better effect than the other two levels (15 and 45mg) in all the performance parameters measured.

The high levels of abdominal fat pads in the folic acid treated birds recorded in the two previous trials calls for a further research to clarify the mechanism underlying adipose tissue development in broilers. This may enhance efforts to develop measures to reduce the accumulation of excess body fat in broilers. This is necessary since excessive accumulation of fat in adipose tissue of broilers have been a major problem in the broiler industry. This is because high body fat in broilers is associated with obesity and several metabolic disorders especially those affecting the cardiovascular system which are responsible for a majority of flock mortality in broilers (Whitehead, 2001). The use of abdominal fat content has been reported to be very useful in reducing fat deposition in broilers in the short- term than the use of selection procedures in the long-term. This is because, abdominal fat grows faster than other fat tissues and it is a reliable parameter for judging total body fat content because it is directly linked to total body fat content in avian species (Tumova and Teimouri, 2010).

The present research was designed to feed the pre-determined 30mg of folic acid per liter of drinking water for varying durations from day-one of age of the broiler and determine the effect on performance and carcass characteristics with emphasis on abdominal fat. The objective was to confirm the high levels of abdominal fat pads recorded in the folic acid treated birds in the previous trials so as to clarify the mechanism underlying adipose tissue growth in broilers. Parameters measured were: performance (body weight, body weight gains, feed intake, folic acid intake, feed conversion ratio, feed efficiency and mortality), carcass (dressed weight, dressing percentage, breast with wings, thigh with drumstick and back), internal organs (Heart, gizzard, abdominal fat and liver).

MATERIALS AND METHODS

Ethical approval

Chickens were handled and managed in accordance with the recommendations in the Guide for the Care and use of Animals, at the Faculty of Agriculture and Forestry Obubra Campus, Cross River University of Technology, Cross River State Nigeria.

Experimental site

The research was carried out at the Teaching and Research Farm of the Department of Animal Science, Faculty of Agriculture and Forestry, Obubra Campus. Cross River University of Technology, Cross River State Nigeria. The location of the study lies along Latitude 6" 4.6032' N and Longitude 8" 19.9446' East (Date and Time Information, 2020).

Experimental treatments and design

The treatments comprised: a control and three different durations of folic acid administration from day-one of age. These includes: T1 (control)= no folic acid supplementation; T2= 30mg of folic acid /litre of drinking water for 7 days (0-7 days of age); T3= 30mg of folic acid / litre of drinking water for 10 days (0-10 days of age); T4= 30mg of folic acid / litre of drinking water for 14 days (0-14 days of age); Each treatment was replicated three times in a complete randomized design i.e. twelve experimental units (pens). The folic acid supplements were purchased from one of the patent medicine stores within the vicinity of the experiment. The folic acid pills were put into the drinking trough of water and allowed to dissolve before stirring with a spatula to avoid loss of particles.

Management of experimental birds

Sixty day-old broiler chicks were purchased from a commercial distributor. The birds were divided into twelve groups and each group was randomly assigned to an experimental unit. Each group was brooded separately in deep litter pens measuring 1m × 1.5m (width × length). The birds were raised in these pens by dismantling the brooding compartments after brooding. Feed and water were given *ad libitum* throughout the duration of the experiment which lasted for eight weeks (56 days). Management during the brooding and rearing periods was based on standard husbandry practices for broiler production. Commercial diets were used for the experiment. Chemical compositions of the experimental diets are presented in table 1.

Table 1 - Chemical composition of experimental diets for broilers.

Composition	Starter	Finisher
Crude protein (g/kg)	21.00	19.00
Fat (g/kg)	8.50	8.50
Crude fibre (g/kg)	5.00	5.00
Phosphorus (g/kg)	0.45	0.41
Calcium (g/kg)	1.20	1.20
Metabolizable energy (Kcal/kg)	2,800	2,900

*Source: Grand Cereal and Oil Mills Limited (Jos, Plateau State Nigeria).

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Data collection and analysis

The birds were weighed at the beginning of the experiment and thereafter at weekly intervals. Feed offered daily were weighed and the leftover weighed the following morning. Feed intakes were obtained by subtracting the leftover from the quantity supplied the previous day. Weight gain and feed conversion ratio (FCR) were deduced from the live weight records. Mean values of folic acid consumed per bird per day were calculated by simple proportion using mean water intake per bird per day in relation to 30mg of folic acid per litre of drinking water. At the end of the experiment at eight weeks of age, three birds were randomly selected per treatment (one from each replicate). The birds were weighed, slaughtered and dressed for carcass analysis. The slaughtering and dressing were carried out using standard procedures for processing broilers. The internal organs were removed, separated, weighed and recorded. Data generated were analyzed using statistical package for social sciences SPSS Version 16.0 (Student's version). Significant means were separated using Duncan's Multiple Range Test of the same software.

RESULTS

The results of the performance of the experimental birds are presented in table 2, while the results of the carcasses, carcass cuts and internal organ weights are presented in table 3.

Performance

There was no difference (P>0.05) in the final body weight and weight gains of the experimental birds including the control. There was also no difference (P>0.05) in feed intake apart from the group on the longest duration of folic acid supplementation (14 days, T4). This group (T4) exhibited higher feed intake (P<0.05) than all the other groups including the control which resulted to low feed utilization efficiency for this group i.e. higher (P<0.05) feed conversion ratio. Folic acid intake increased significantly (P<0.05) with increase in the duration of administration due to increase in water intake as the birds ages. Mild mortality was spread across the treatments.

Table 2 - Performance of the experimental birds fed pre-determined level of folic acid for varying periods.

Treatments / Parameters	No folic acid	30mg of folic acid/litre of water			SEM
	T1 (control)	T2 (7days)	T3 (10 days)	T4 (14 days)	
Initial body weight (g)	40.00	40.00	40.00	40.00	0
Final body weight (kg)	2.48	2.45	2.47	2.48	0.13^{ns}
Feed intake (g/day)	116.37	116.18	116.31	127.27	8.97^{ns}
Body weight gain (g/day)	44.28	43.75	44.11	44.28	0.92^{ns}
FCR (g of feed/g of gain)	2.63	2.66	2.64	2.87	0.18^{ns}
FE (g of gain/g of feed)	0.38^{a}	0.376^{a}	0.38^{a}	0.347^{b}	0.02*
Folic acid intake (mg/bird/day)	0	0.71	0.98	1.33	0.12^{ns}
Mortality (%)	6.67	6.67	0	6.67	0

Means on the same row with different super scripts are significantly (P<0.05) different. Amount of folic acid used = 30mg per litre of drinking water; SEM=Standard Error of Mean; *= P<0.05; ns=Not significant; FCR=Feed conversion Ratio; FE=Feed Efficiency.

Carcass and carcass cuts (visual appraisal)

The outstanding effect of the 30 mg of folic acid supplementation on the broiler chickens was the physical manifestation on the dressed carcasses of the experimental birds (body conformation). The folic acid treated birds had expanded abdominal region that were filled with large mass of abdominal fat pads irrespective of the duration of supplementation, while the control birds had normal carcass conformation that is characteristic of normal growth. The dressed carcasses of the experimental birds are shown in the figure 1. The result of the carcass and carcass cuts (Figure 1) followed the same trend with that of performance parameters. There was no significant difference (P>0.05) between the treatments including the control in all he parameters measured, except the group on the highest duration of folic acid supplement (14 days, T4). The significant (P<0.05) low feed efficiency of the T4 group (Table 2) manifested in significant (P<0.05) low dressed weight and dressing percentage (Table 3). The low (P<0.05) dressed weight of the T4 group reflected in the thigh and drumstick.

Internal organs

Apart from the abdominal fat which was significantly (P<0.05) higher in all the folic acid treated birds than the control, there was no difference (P<0.05) between the control and the folic acid treated birds in all the internal organs measured. The folic acid treated birds did not differ (P>0.05) in their abdominal fat irrespective of the duration of administration.

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Figure 1. Dressed carcasses of the experimental birds.

Table 3 - Carcass, carcass cuts and internal organ of the experimental birds fed pre-determined level of folic acid for varying durations.

Parameters \ Treatments	No folic acid	30mg of folic acid/litre of water			SEM
	T1 (control)	T2 (7days)	T3 (10 days)	T4 (14 days)	
Pre-slaughter weight (kg)	2.40	2.40	2.40	2.40	0.08^{ns}
Dressed weight (kg)	1.744^{a}	1.743^{a}	1.70^{a}	1.650^{b}	0.04
Dressing percentage (%)	72.64^{a}	72.62^{a}	72.50^{a}	68.75^{b}	0.90
Carcass cuts (% of pre-slaughter weight)					
Thigh + drumstick	24.03^{a}	24.02^{a}	24.00^{a}	21.28^{b}	0.89
Back	15.28	15.27	15.20	14.28	1.27^{ns}
Breast + wing	33.33	33.33	33.30	33.10	0.68^{ns}
Internal organs (% of pre-slaughter weight)					
Heart	0.43	0.43	0.42	0.42	0.025^{ns}
Liver	1.94	1.94	1.93	1.93	0.031^{ns}
Abdominal fat	1.17^{b}	3.50^{a}	3.48^{a}	3.50^{a}	0.43
Gizzard	1.65	1.65	1.66	1.66	0.03^{ns}

Means on the same row with different super scripts are significantly (P<0.05) different. Amount of folic acid used = 30mg per litre of drinking water; SEM=Standard Error of Mean; ns=Not significant.

DISCUSSION

The folic acid supplemented birds had expanded abdominal regions that were filled with large mass of abdominal fat pads irrespective of the duration of supplementation. This result agrees with the report of Meremikwu et al. (2015) that, the folic acid treated birds in their research were dramatically different from the control birds in carcass appearance and conformation.

The enlarged abdominal regions of the folic acid treated birds that were filled with large mass of abdominal fat pads could be due to cell multiplication resulting from Folate-mediated one-carbon units transfer reactions. This is supported by the reports of Wang et al. (2007) and Guo et al. (2011) that fat growth in chicken within the first fourteen (14 days) of age is by hyperplasia (cell multiplication) after which hypertrophy (cell enlargement) of existing adipose cells becomes responsible for increases in the mass of these fat depots. This is also supported by the reports of Field (2018) and Tjong and Mohiuddin (2019) that Folate-mediated one-carbon units transfer reactions are important during periods of active cell division and support rapid proliferation of cells due to ample supply of nucleotides with many activated precursors of nucleic acid (thymidine and purine). The non-significant (P>0.05) difference in carcass cuts (especially the breast) between the control and the folic acid treated birds implied that folic acid supplement given for seven to fourteen days of age of the broilers had no effect on factors that influence muscle growth in broiler. This is supported by the fact that the hyperplasic period of muscle development is said to be nearly complete at hatch (Rutz, 2015) and folic acid is reported to be effective during periods of active cell division (Armando, 2018; Tjong and Mohiuddin, 2019). The high feed intake of the group on the longest duration of folic acid could be due to the fact that folic acid is said to be an appetite stimulant when taken in high doses (Marshal, 2016). According to Marshal (2016), folic acid is a nutrient with two-edged sword i.e. it is an appetite stimulant and a hunger deregulator, in which case , if an individual is deficient in folic acid, the first thing that goes is the appetite.

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CONCLUSION

Thirty mg of folic acid supplement per litre of drinking water administered for 7 - 14 days from day-one of age, has confirmed the results of previous trials with graded levels of folic acid supplement by producing visual morphological effect on the dressed carcasses of the folic acid treated birds, characterized by expanded abdominal regions that were filled with large mass of abdominal fat pads. It was concluded from this research that, the excessive growth of abdominal fat depots in the folic acid treated broiler chickens were by cell multiplication (hyperplasia), due to the fact that Folate-mediated one-carbon transfer reactions are important during periods of active cell division and supports high rate of cell proliferation because of many activated precursors of nucleic acid (thymidine and purine). This research has confirmed the fact that adipose tissue growth in chicken within the first fourteen days of age is by hyperplasia. It is therefore recommended that, folic acid or any other supplement that supports cell multiplication should not be used within the first 7 to 14 days of age of the broiler since this will induce adiposity. Further research should be conducted to determine the appropriate age to administer folic acid supplement to exploit the growth promoting (cell proliferation) effect to maximize muscle tissue growth in broilers.

DECLARATIONS

Corresponding author

Victoria Nnenna. Meremikwu, Ph.D., Department of Animal Science, Faculty of Agriculture and Forestry, Obubra Campus, Cross River University of Technology, Cross River State, Nigeria. Email: victoriameremikwu@yahoo.com

Ethics and consent to participate

This paper has been submitted with full responsibility of both authors following due ethical standards and there is no duplicate publication or plagiarism.

Consent to publish

Not applicable.

Conflict of interest

The authors declare that they have no competing interest.

Authors' contribution

Both authors contributed equally to the work.

Availability of data

Data can be availed to the journal upon request.

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THE PROTEIN DIGESTIBILITY OF THE BROILER CHICKENS FED JAMU FORMULA, a LOCAL HERBAL SOLUTION

Rusny RUSNY[1], Muhammad Nur HIDAYAT[1], Ummi KALSUM[1] and Mashuri MASRI[2*]

[1]*Department of Animal Husbandy, Faculty of Science and Technology, State Islamic University Alauddin Makassar, South Sulawesi, Indonesia*
[2]*Department of Biology, Faculty of Science and Technology, State Islamic University Alauddin Makassar, South Sulawesi, Indonesia*

Email: mashuri.masri@uin-alauddin.ac.id; ORCID: 0000-0003-1148-7208

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ABSTRACT: Jamu (local herbal drinking) have been known for a long time by inhabitants in Indonesia as conventional home grown pharmaceutical and to progress digestion system within the body. Jamu, not as it were for people but also for creatures. Local farmers have moreover utilized jamu for chicken for a long time, and it's utilize is expanding. This Research points to decide the impact of jamu to extend protein *in vivo* digestibility in broilers and for knowing the ideal level of jamu for optimum protein digestibility in broilers. The strategy utilized in this investigate is Completely Randomized Design (CRD) with 4 treatment and 5 replications, each redundancy comprises of 1 broiler chickens, so there are 20 chickens. The treatment comprises of P0 (control), T1 (jamu 1.5 mL/500 mL), T2 (jamu 2.5 mL/500 mL) and T3 (jamu 3.5 mL/500 mL). The parameters watched were digestibility protein in broilers. Based on the examination of fluctuation, it appears The treatment had no critical impact on chicken protein broilers' digestibility given jamu. However, seeing each treatment's average value, T1, T2 and T3 tend to increase to 99.62%, 99.68% and 99.71%, respectively. In conclusion, supplemented with jamu formula does not significantly affect broiler chicken protein's digestibility, but the digestibility increases with increasing formula, up to the formula 3.5 mL/500 mL (T3) as the ideal level.

Keywords: Broiler, Digestibility, Herbal treatment, Jamu, Protein.

INTRODUCTION

The demand for chicken meat increases along with increasing incomes and awareness of the importance of animal protein (Wilkie, 2005). Developing broiler production, and provide commercial feeds has fulfilled legal needs for farmers (Variani et al., 2017). Despite the price relatively expensive because some of the ingredients are still imported, some commercial feed ingredients are widely available and easy to obtain. Besides, it contains additional feed ingredients (feed additives) needed by livestock (Alqaisi et al., 2017).

Protein is a necessity nutrient for humans and livestock to be affect the growth period, age, physiology, production, and body condition. Protein digestibility is the ability of the protein to be hydrolyzed into amino acids by digestive enzymes (Hou et al., 2017). If protein digestibility is high, the protein can be well hydrolyzed into amino acids, so the number of amino acids that can be absorbed and used by the body sufficiently (Ketnawa and Ogawa, 2019). If the protein digestibility is a combined process to be hydrolyzed into amino acids then the amount Amino acids that can be absorbed and used by the body are in low rate due in part large will be disposed of by the body with feces (Deb-Choudhury et al., 2018). Its well-known, protein is very important in tissue repair energy metabolism and for obtaining vital substances in body functions such as enzymes (Shah et al., 2020).

Herbal formulation (Jamu) have been known for a long time by residents in Indonesia as traditional medicine and to improve metabolism in the body (Elfahmi et al., 2014). Jamu has been used for special targets not only for humans (Mosihuzzaman, 2012; Zhu, 2020) but also for animals (Alagawany et al., 2019; Zhu, 2020). Local farmers have also used jamu for chicken for a long time, and its use is increasing (Gaucher et al., 2015; Galli et al., 2020). Based on information in the field, some breeders who use jamu can increase their livestock productivity, for example Galli's research fed jamu in breeders which increase quality of meat in fatty acid profile (Galli et al., 2020).

Agustina et al. (2017) showed that jamu in liquid or powder form can inhibit Gram-positive and Gram-negative bacteria, because the ingredients contain bioactive substances. It was necessary to reduce the types of materials suspected of having the same bioactive substances. The use of jamu in liquid form as much as 2.5 mL/L of drinking water, is the best result of performance and histopathological abnormalities of internal organs. The use of 0.15% herbal concoction powder in feed effectively improves performance, reduces the number of deaths, abdominal fat, blood cholesterol, and gives the highest OD (Optic Density) value, which indicates that herbal concoction powder can prevent viruses (using a lubricant kit to test IFNγ (Interferon-gamma). Based on this description, it is necessary to conduct a

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research on the use of herbal medicine in drinking water to determine the effect protein digestibility in broilers. Aim of present study was to determine the effect of jamu to expand protein *in vivo* digestibility in broilers and for knowing the perfect level of jamu for ideal protein digestibility in broilers

MATERIALS AND METHODS

The materials used in this study were 40 broilers, husk, and herbal solution with 250 g of a mixture of ingredients, namely garlic (*Allium sativum* L.), leaves betel (*Piper betle* L.), cinnamon (*Cinnamomum verum* L.), EM-4 (Effective Microorganisms-4) and molasses. The feed used comes from a commercial feed, namely B11A with the composition of corn, rice bran, soybean meal, fish meal, meat bone meal, corn gluten meal, pollard, stone flour, crude palm oil, sodium bicarbonate premix, vitamins and trace minerals. While the material used to calculate digestibility protein, namely sample (feces), selenium ± 1 gram, 25 mL concentrated H_2SO_4, distilled water 100 mL, 10 mL 2% H_3BO_3, 4 drops indicator solution and 10 mL 30% NaOH.

Research design

This study used a completely randomized design (CRD) consisting of 4 treatments and 5 replications, each replication consisted of 2 broilers so that there are 40 experimental units with treatment (T), namely: T0: control; T1: Jamu 1.5 mL/500 mL/drinking water; T2: Jamu 2.5 mL/500 mL/drinking water; T3: Jamu 3.5 mL/500 mL/drinking water.

Broiler preparation and maintenance

The cage must be prepared before day old chick (DOC) entered, cage preparation is done carefully and carried out to install curtains and cleaning and sterilization around the cage with how to spray detergent and the tools to be used and wait until dry. After that, it is covered with husks with a thickness of seven cm feed, and the area of the cage unit used is 60 × 100 cm. Preparations are maintained from DOC until the age of 30 days with a cage covered with husks. The treatment is given to chickens since the chicken entered the cage unit experiment until harvest. The number of treatment chickens was 40 chickens selected randomly and put into the cages of each experimental unit 2 tails. Each experimental unit enclosure is equipped with a 25 watt incandescent lamp as many as 20 pieces.

Production of Jamu

Materials used to manufacture herbal such as garlic, betel leaf, cinnamon first cleaned, then weighing 250 g each, then crushed use a blender for garlic and betel leaves, except for cinnamon ground using a mortar until smooth. Next third, the ingredients are mixed in one container. Addition of molasses and EM-4 (effective microorganisms-4) was also carried out each as much 1 L then add 10 L of water. Stir until all ingredients to be homogeneous (Jamili et al., 2014).

Table 1 - Ingredients of Jamu used in present study.

Ingredients	Composition
Garlic	250 g
Betel leaf	250 g
Cinnamon	250 g
EM-4	1 L
Molasses	1 L
Well water	10 L

Source: Primer Data.

Table 2 - Energy content of B11A feed used in present study.

Content	Composition (%)
Water	13.0
Protein	22.0-23.5
Fat	5.0
Fiber	5.0
Ash	7.0
Calcium	0.9
Phosphorus	0.6

Source: PT. New Hope Indonesia, 2019

Feed and drinking water

Feeding is done a few hours after drinking DOC (3-4 hours after the DOC is drinking). The provision of drinking water is carried out *ad libitum* (continuously), and in giving it must be clean and fresh, and the drinking water has been mixed with the herbal herbs that are given each day until the age of 30 days, and the giving is done according to treatment that has been determined in this study. The nutritional content of commercial feed B11A produced by PT. New Hope Indonesia is used in this study is presented in Table 2.

Protein digestibility calculation process

After going through the maintenance process, at the end of the study, fecal samples were taken from each treatment in the form of fresh ones that had been weighed previously to determine their fresh weight for further observation in the laboratory by the method of calculating protein digestibility, namely by weighing carefully weighing ± 0, 5 g of the sample, then put it in the Kjeldahl flask. A mixture of selenium (±1 g) and 25 mL of concentrated H_2SO_4 was added. The Khjedhal flask and its contents were shaken until all samples were wetted with H_2SO_4 then digested in a fume

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hood until it was clear. Let it cool, then pour into a 100 mL volumetric flask and rinse with distilled water. Let it cool again, squeeze it to the mark with distilled water and then shook it until it was homogeneous. After that, a pan consisting of 10 mL H_3BO_3 2% + 4 drops of mixed indicator solution prepared into Erlenmeyer, then Pipette 5 mL of sample solution into a distillation flask, add 10 mL of 30% NaOH and 100 mL of distilled water. Then it was distilled until the reservoir volume became ± 50 mL. Rinsed the distiller's end with distilled water, then the container and its contents were titrated with a 0.0171 N H_2SO_4 solution (Adedokun et al., 2008).

$$\%\,CrudeProtein = \frac{VxNx14x6.25xP}{sampleweight(gr)} \times 100\%$$

Description: V: volume of sample titration; N: normality of H_2SO_4 solution; P: dilution factor.

Protein digestibility test by taking 1 sample from each test

Observation of protein digestibility by knowing the data on feed consumption that has been added with herbal herbs to drinking water and weighing the feces in the ileum. The collection method of ileal digesta is by fasting for 14 hours. It is given commercial feed as much as 100 g/head and drinking water for 10 hours before slaughtering after being fast. Then the chicken is slaughtered. Digesta was taken from the small intestine part of the ileum, after 1 cm from Meckel's diverticulum to a limit of 1 cm before the ileum-cecal junction. After that, the digesta is removed, and then the initial weight is weighed in fresh form from each treatment. After that, the digesta was collected and then analyzed *in vivo* (Adedokun et al., 2008). According to Li et al. (2017), regarding the digestibility calculation method protein, namely the following formula:

$$\%ProteinDigestibility = \frac{(\Sigma Ax\%B) - (\Sigma Cx\%D)}{(\Sigma Ax\%B)} \times 100\%$$

Description: A: consumption of ration (g); B: food substances in the ration (protein, %); C: number of feces (g); D: food substance in feces (protein; %).

Statistical analysis

The data obtained will be analyzed through variance using a completely randomized design (CRD) with 4 treatments and 5 replications. If the treatment has a significant effect, then the Duncan multiple area test is continued to see the differences in each treatment sample. According to Ervina et al. (2019) the mathematical model of the CRD is as follows:

Y ij = μ + αi + € ij

Description: Yij: The observed value of the i^{th} treatment of jamu; μ: Real average value; αi: effect of treatment at level I; € ij: error; i: T0, T1, T2, T3 (treatment); j: 1, 2, 3 (repeat).

Ethical approval

The *in vivo* study was supervised by The Animal Ethics Committee of the Universitas Islam Negeri Alauddin and conducted in accordance with the basic animal husbandry and health protocols referred to in Legislation of the Republic of Indonesia No. 18, 2009.

RESULTS AND DISCUSSION

The results of the 23 days feeding jamu-treatment against protein digestibility in the cobb-500 broiler chicken presented in Table 3. The results of this analysis of variance indicated that the treatment has not significant effect (P>0.05) on protein digestibility. The treatments were T0 (99.56%), T1 (99.62%), T2 (99.68%) and T3 (99.71%).

Protein digestibility is the amount of protein that is absorbed from food into particles absorbed by the digestive tract (Jonker and Yu, 2017; Cholis et al., 2018). In Table 3, the average value of T3 (99.71%), which is given herbal herbs in chicken drinking water as much as 3.5 mL, showed the value of protein digestibility as the highest among other treatments. In comparison, the lowest average protein digestibility value was P0 (99.56%) of all treatments. The treatment statistically has no significant effect on protein digestibility, but seen from the trend of research data, the feed of jamu with a dose of 3.5 mL can increase protein digestibility, this treatment has the highest value of all treatments with a value of 99.71%. Alagawany et al. (2019) stated that cattle that consume high protein could affect their body cells' metabolism to run correctly.

Table 3 - Average digestibility and standard deviation of protein in broiler chickens fed jamu for 23 days.

Variable	Treatment				P-value
	T0	T1	T2	T3	
Protein digestibility	99.56±0.95	99.62±0.15	99.68±0.13	99.71±0,07	0.24

T0 = Control. T1 = Jamu 1.5 mL/500 mL/drinking water. T2 = Jamu 2.5 mL/500 mL/drinking water. T3 = Jamu 3.5 mL/500 mL/drinking water. ±(standard deviation).

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In the present study, the treatment given was in the form of jamu from several ingredients such as garlic, betel leaf, and cinnamon which had almost the same content as alicin, essential oils, flavonoids, tannins (Castillo-López et al., 2017; Alagbe et al., 2020), it's were able to increase protein digestibility in broilers and could be antibacterial (Alagawany et al., 2019; Alagbe et al., 2020). The working system of feeding jamu in livestock, which can improve metabolism, the digestive system and reduce pathogenic bacteria that can affect feed consumption absorption (Alagawany et al., 2019). Reduced pathogenic bacteria in the digestive system of livestock so that the protein also produced increases (Galli et al., 2020).

All ingredients' content works following their respective mechanisms that interfere with and even damage pathogenic bacteria so that their growth is blocked or dies (Alagawany et al., 2019; Galli et al., 2020). According to Castillo-López et al. (2017), alicin is one of the most active biological components in garlic (Castillo-López et al., 2017). Previously, Cardoso-Ugarte et al. (2016) argued that cinnamon's content has many compounds, namely essential oils (Cardoso-Ugarte et al., 2016). According to Jamili et al. (2014) when the betel leaf, garlic, and betel leaf are all mixed, it will have a robust inhibitory compound against *Staphylococcus aureus* and *Salmonella thypi* bacteria, namely tannins, essential oils, alisin, flavonoids, etc. which have their way to inhibit bacteria.

The contents of the materials used which have antibacterial properties work according to their respective mechanisms, for example, flavonoids, tannin alkaloids, and essential oil, which work to form more complex compounds then disrupt and even damage the test bacterial cell membranes so that the bacterial life activity is inhibited or dies (Alagbe, et al., 2020; Galli et al., 2020). Previously, Cheng et al. (2014) and Rabinowitch (2002) stated that allicin could inhibit the growth of negative and positive gram bacteria, and prevent abnormalities in the small intestine to better the intestine's protein absorption process (Cheng et al., 2014; Rabinowitch, 2002). The effect of this study was not significant ($P>0.05$) because it could be caused by several factors such as provision of feed, bulkhead conditions, environmental conditions, provision of drinking water added with jamu in each treatment. According to Dersjant et al. (2015) and Olijhoek et al. (2018), the high and low digestibility of feed ingredients is influenced by several factors, including types of livestock, feed, types of feed ingredients in rations, crude protein content, and the way of providing rations, however this also shows that one of the factors that makes it insignificant is the amount of broiler consumption influenced by the form of feed and the protein content of the feed (Dersjant-Li et al., 2015; Olijhoek et al., 2018).

In present research, the form of feed used is commercial feed produced in pelleted form. According to Milanovic (2018), good feed for broilers such as pellets and crumble is because poultry has high palatability to add to its digestibility, poultry feed dramatically determines the level of protein digestibility so that the amount of feed and protein content that enters the digestive tract (Milanovic, 2018). The protein content in the feed used in each treatment was an average of 22.75% from the starter-finisher period. Kaewtapee et al. (2017) and Olijhoek et al. (2018) stated that rations with low protein content generally have low digestibility and vice versa. The level of protein digestibility depends on the protein content of the feed ingredients, the amount of protein that enters the digestive tract, and the influence of the use of doses of antibiotics and probiotics given (Liao and Nyachoti, 2017; Clavijo and Flórez, 2018; Galli et al., 2020; Zaghari et al., 2020). The addition of doses from each treatment also dramatically determines the effect on the digestibility of the protein itself, the doses used in this study started from T1, T2, and T3 treatments, respectively, namely 1.5 mL/500 mL/drinking water, 2.5 mL/500 mL/drinking water and 3.5 mL/500 mL/drinking water, following the research of Kusbiyantari et al. (2017) which uses a betel leaf solution with a dose of 5% per liter of drinking water to increase protein digestibility.

CONCLUSION

The feeding of jamu had no significant effect on digestion of protein in broilers. T1, T2 and T3 tend to increase; 99.62%, 99.68% and 99.71%, respectively. In summary, supplementation with jamu does not essentially influence broiler chicken protein's digestibility, but the digestibility increments with expanding equation, up to 3.5 mL/500 mL (T3) as the ideal level. Further studies with other local herbs and herbal solutions are suggested.

DECLARATIONS

Corresponding Author
E-mail: mashuri.masri@uin-alauddin.ac.id; ORCID: 0000-0003-1148-7208

Authors' Contribution
All authors contributed in research and writing, equally.

Conflict of Interests
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CHANGES IN SERUM LYSOZYME AND BACTERICIDAL ACTIVITY IN GROWING HEIFERS OF DIFFERENT BREEDS

Viktor I. EREMENKO[1] and Elena G. ROTMISTROVSKAYA[2]

[1]*Head of the Department, Ph.D., Biology, Professor, I.I. Ivanov Kursk State Agricultural Academy, Kursk, Russia*
[2]*Senior Lecturer, Ph.D. in Biology, I.I. Ivanov Kursk State Agricultural Academy, Kursk, Russia*

Email: vic.eriomenko@yandex.ru; ORCID: 0000-0003-3780-7724

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ABSTRACT: The study presents the results of a study of the bactericidal and lysozyme activity of blood serum of heifers of different breeds. The experiment involved 4 groups of heifers, 10 heads in each group: 1) Black-and-white Holstein; 2) Simmental; 3) Aberdeen-Angus; and 4) crosses of Simmental and Aberdeen-Angus breeds. Animals of all groups were kept in the same feeding and housing conditions. During the experiments, the animals were fed according to generally accepted standards. Blood was taken from animals from the tail vein in the morning before the first feeding in compliance with the aseptic rules. It was found that with an increase in gestation, the activity of serum bactericidal activity (SBA) and serum lysozyme activity (SLA) in the blood of heifers gradually increases.in conclusion, during pregnancy, the level of SBA and SLA in the blood of heifers depended on the month of pregnancy and the breed of animals. During pregnancy, hybrid heifers have higher levels of SBA and SLA, and relatively low levels of SBA and SLA are observed in Black-and-White, Simmental and Aberdeen Angus heifers.

Keywords: Aberdeen-Angus, Bactericidal, Heifer, Lysozyme activity, Simmental.

INTRODUCTION

Increasing the productivity of farm animals is the key challenge of modern animal husbandry (Eremenko and Kretova, 2007). To achieve this goal, in addition to improving the quality of feeding and improving the management of the industry, it is necessary to carry out systematic selection work on the most important inherited traits of animals (Rauw and Gomez-Raya, 2015; Balzani and Hanlon, 2020). To improve the productive qualities of cattle, it is necessary to study in more detail the mechanism of the formation of natural resistance, and its relationship with the future productive qualities of animals, and in the future to recommend the most resistant breeds of cattle for their use in breeding work (Eremenko and Polianskii, 2013; Marshal et al., 2019).

The main indicator of natural resistance of livestock is the indicators of bactericidal and lysozyme activity of blood serum (Zhou et al., 2019). This indicator is widely used in fish health detection and aquaculture (Das and Sahoo, 2014; Panase et al., 2017). Especially in dairy cows it's documented that lyzozyme level in milk is an important indicator for immune status and it's differed with breeds of cows (Król et al., 2010).

Thus, serum bactericidal activity (SBA) and serum lysozyme activity (SLA) are a combined manifestation of the body's natural defenses (Carroll and Jasper, 1977). SLA can be different in depends on breed of farm animal (Sotirov et al., 2007), and it's documented in local studies (Sotirov et al., 2006; 2007). The study of these mechanisms will make it possible to purposefully use these indicators in the selection of cattle (Król et al., 2010). Aim of present study is to determination of these indicator activities in different breeds of cows.

MATERIALS AND METHODS

The studies were carried out on Black-and-White Holstein, Simmental, Aberdeen-Angus and crossbred heifers of Black-and-White breeds. Heifers were analogous in age and gestational age, 10 heads in each group. They were grown in the same conditions, which ensured their normal growth, development and, subsequently, milk and meat productivity, characteristic of each breed. During the experiments, the animals were fed according to generally accepted standards. Blood was taken from animals from the tail vein in the morning before the first feeding in compliance with the aseptic rules. SBA and SLA indices were determined according to generally accepted methods. The obtained research results were subjected to biometric processing by variation statistics using Microsoft Office Excel.

Statistical analysis

The One-way AOVA method used for statistical comparison of treatments. For comparison of means, SAS software, version 10 ($P<0.05$) was used, and Duncan multiple range test for comparison of means. Experimental groups consisted: 1-Black-and-White Holstein, 2-Simmental, 3-Aberdeen-Angus and 4crossbred heifers of Black-and-White (n: 10).

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Ethical approval

The *in vivo* and *in vitro* studies were approved by ethical committee of department of Biology, I.I. Ivanov Kursk State Agricultural Academy of Russia.

RESULTS

Blood serum bactericidal activity (SBA)

SBA indices in the first month of pregnancy in all groups were within normal limits and did not differ significantly across groups. The data shown in Figure 1 show that in the first month of lactation, the bactericidal activity of blood serum in heifers of different breeds was between 82.7±3.8% and 89.4±4.2%.

By the second month of pregnancy, this indicator in all compared breeds of heifers increased slightly and was between 82.4±2.9% and 90.1±4.6%. In Black-and-White Holstein heifers, SBA was 82.4±2.9%, in Simmental was 83.3±3.3%, in Aberdeen-Angus was 90.1±4.6% and in crossbred animals was 89.0±3.7%. By the third month of pregnancy, SBA slightly increased in heifers of the first group to 82.7±2.8%, in the second group to 83.7±3.1%, in the fourth group to 89.3±4.5%. In the group of Simmental heifers, SBA slightly decreased to 88.9±4.7%. Later, at the 4th and 5th months of pregnancy, the value of this indicator in all groups ranged between 82.3±3.1% and 92.4±4.0%. The research found an increase in the bactericidal activity of blood serum by the 6th month of pregnancy in experimental animals. In Black-and-White Holstein, SBA was 80.0±2.9%, in Simmental was 84.2±3.7%, in Aberdeen-Angus was 88.8±4.2% and in crossbred heifers was 93.7±4.2%. By the 9th month of pregnancy, the bactericidal activity of blood serum continued to increase and amounted to 82.0±4.1% in Black-and-White Holstein, 87.4±3.7% in Simmental, 91.3±4.2% in Aberdeen-Angus, and 97.7±5.0% in crossbred heifers. During month 7, 8, and 9 of gestation, crossbred animals showed statistically significant differences in relation to the data of the Black-and-White Holstein heifers ($P<0.05$). Comparing the indicators of bactericidal activity of blood serum between the experimental groups of heifers, it should be noted that before the 9th month of pregnancy, this indicator was slightly higher in crossbred animals. Thus, the bactericidal activity of blood serum depends on the breed of heifers and the duration of pregnancy. Relatively low SBA was noted in Black-and-White Holstein in relation to the compared breeds of heifers, and higher SBA was noted in crossbred animals.

Blood serum lysozyme activity (SLA)

The lysozyme activity of blood serum in the first month of pregnancy in experimental heifers was approximately at the same level and amounted to 22.6±1.5% in Black-and-White Holstein, 22.3±1.5% in Simmental, 26.5±1.3% in Aberdeen-Angus, and 29.5±1.6% in crossbred heifers (Figure 2).

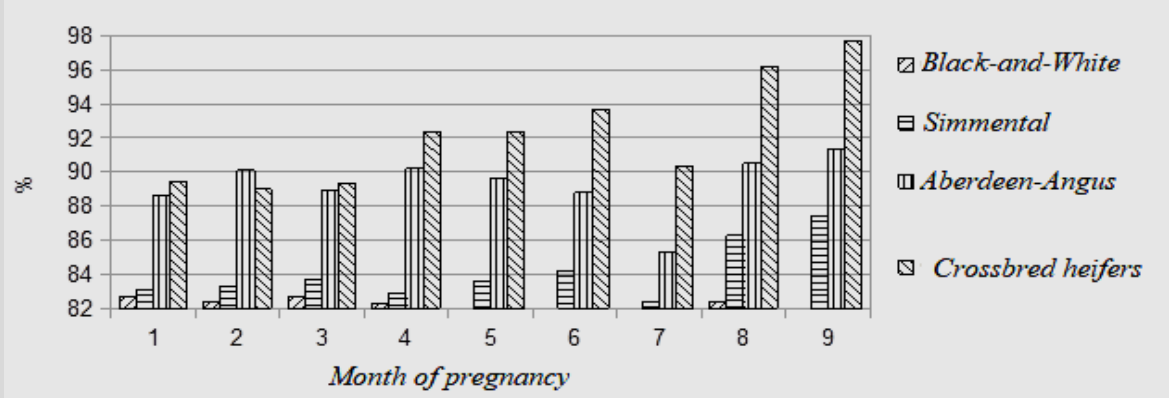

Figure 1 - Changes in bactericidal activity of blood serum in heifers of different breeds.

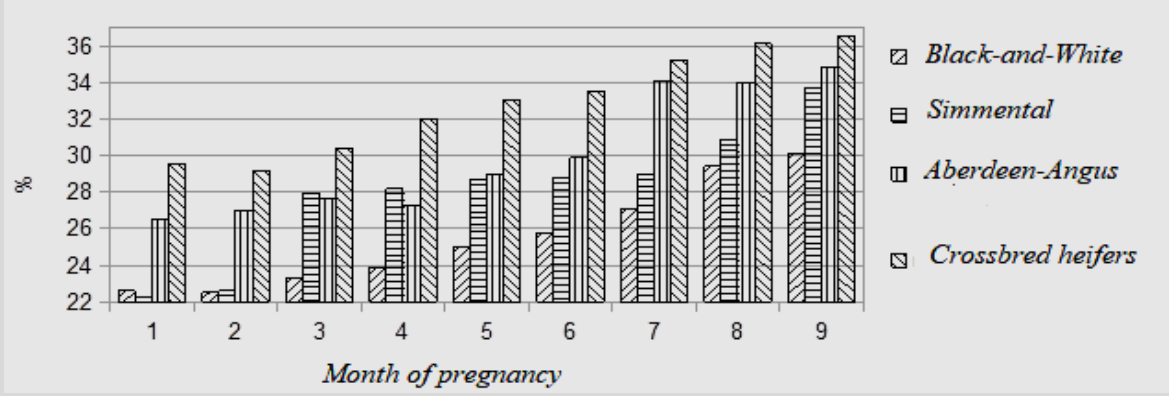

Figure 2 - Changes in lysozyme activity of blood serum in heifers of different breeds.

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Statistically significant differences during the 1st month of gestation were noted between hybrid animals, Black-and-White and Simmental heifers (P<0.05). By the second month of pregnancy, the lysozyme activity of the blood serum of animals was also lower in Black-and-White Holstein. Crossbred animals showed the highest SLA level - 29.2±1.3%. Statistically significant differences at 2 months of gestation were noted between Black-and-White, Simmental breed of heifers and crossbred animals (P<0.05). Mentioned differences in breeds (for SLA activity) has fully recognized in goat breeds by Marmaryan (2013).

Analyzing the data in Figure 2, it can be noted that by the third month of pregnancy, SLA was slightly lower in heifers of Black-and-White Holstein breed - 23.3±1.1%. The highest level of SLA was also in crossbred animals - 30.4±1.4%. In Black-and-White heifers, lysozyme activity during months 3, 4, and 5 of gestation was significantly lower in relation to crossbred animals (P<0.05). In the second half of pregnancy, all breeds of animals showed an increase in lysozyme activity, especially pronounced these changes were noted in the Aberdeen Angus breed and crossbred animals. During the 6th months of pregnancy, SLA was 25.8±1.5% in Black-and-White Holstein, 28.8±1.4% in Simmental, 29.9±1.6% in Aberdeen-Angus, and 33.5±1.5% crossbred heifers.

Crossbred heifers had significantly higher lysozyme activity during the 6th months of gestation in relation to Black-and-White Holstein and Simmental heifers (P<0.05). The activity of lysozyme during the 9th month of pregnancy was 30.1±1.8% in Black-and-White Holstein; 33.7±1.8% in Simmental, 34.8±2.0% in Aberdeen-Angus, and 36.5±2.0% in crossbred animals. Black-and-white heifers at the end of pregnancy had a significantly lower activity than crossbred heifers (P<0.05).

Thus, the lysozyme activity of blood serum in crossbred animals during all months of pregnancy exceeded those of the Simmental and especially the Black-and-White breed. This finding is in according to Puppel et al. (2019) who review and noted changes lysozyme activity in different breeds and in colostrum composition. An increase in SLA during pregnancy indicates an increase in serum lysozyme during pregnancy. This is obviously due to the evolutionary reaction of the nonspecific protection on the part of the mother to the birth of a viable calf.

CONCLUSION

With respect to the above it can conclude that with an increase in gestation, serum bactericidal activity (SBA) and serum lysozyme activity (SLA) of heifers gradually increases. During pregnancy, the level of SBA and SLA in the blood of heifers depended on the month of pregnancy and the breed of animals. During pregnancy, hybrid heifers have higher concentrations of SBA and SLA. During pregnancy, Black-and-White, Simmental, and Aberdeen Angus heifers have lower concentrations of SBA and SLA.

DECLARATIONS

Corresponding Author

E-mail: vic.eriomenko@yandex.ru; ORCID: 0000-0003-3780-7724

Authors' Contribution

Both authors contributed in research, experiments and writing, equally.

Conflict of Interests

Authors declare no competing interests.

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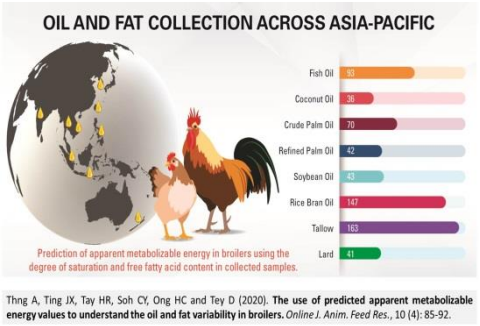

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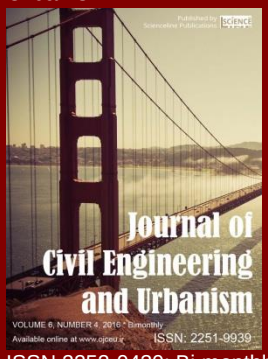

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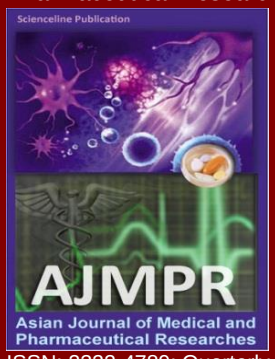

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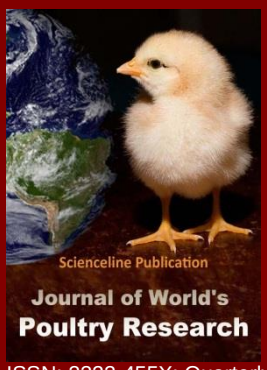

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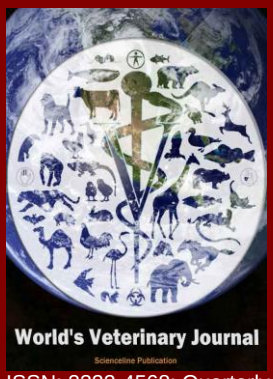

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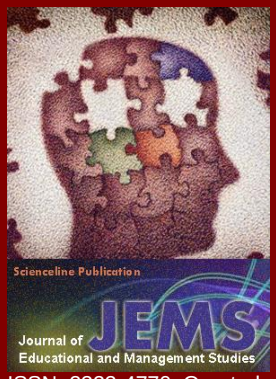

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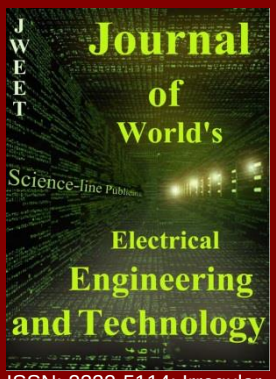

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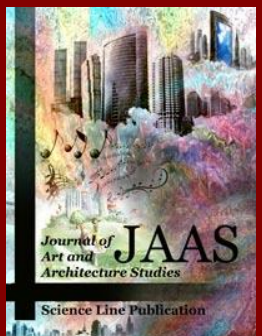

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