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Leverage of Six-hour Bovine Blood Meal on Blood Profiles of Broiler Chickens

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

We investigated the effect of bovine blood meal (BBM) processed 6 hours post-storing on hematological and serum biochemical indices. A total of one hundred unsexed Abor Acre broiler chicks were randomly allocated into four dietary treatments of 25 birds and three replicates each in a Completely Randomized Design (CRD). Treatment 1 (T_1) contained no bovine blood meal and served as the control while T_2, T_3, and T_4 contained 1, 2, and 3% respectively to replace fish meal in the diet formulation. Adequate warmth, feeds, and water were provided *ad libitum* while routine vaccination and necessary medication were administered during the study period (8 weeks). By the end of the experiment, three birds per replicate were selected for sample collection serving for the determination of hematological (packed cell volume; PCV, hemoglobin; Hb, rell cell counts; RBC, while cell counts; WBCs, mean corpuscular hemoglobin concentration; MCHC, mean corpuscular hemoglobin; MCH, and mean corpuscular volume; MCV) and biochemical indices (Urea, Alkaline Phosphatase, Cholesterol, Albumin, and Total protein). Results showed significant ($P<0.05$) differences in PCV, Hb, MCV, WBC, neutrophils, and lymphocytes. T4 (3%) recorded the highest values in PCV (41.75%), Hb (11.00g/dl), and MCV (163.7f/l), but low values in WBC (9.94 x 10^3/ul), neutrophils (42.75%), and lymphocytes (46.00%). BBM 3% produced significant impacts on birds.

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Bovine blood meal at 3-5% is recommended for poultry dietary formulation as it is well-tolerated and devoid of any serious challenges (diseases). Further trials with keeping duration beyond 6 hours and another processing methods are suggested.

Keywords: Broiler chickens; bovine blood meal; haematology; serum biochemical metabolites.

1. INTRODUCTION

Broiler production has been the most enticing and productive sector of poultry business in Nigeria, given its numerous advantages over other types of animal husbandry in terms of small space requirement, low investible funds, quick income generation, short production cycle, fast growth rate, high yield, and easy management [1,2]. Apart from income generation and employment creation, broiler production holds a high potential of providing the populace with quality animal protein containing low cholesterol, in addition to being widely acceptable, with no known biases and trade-religious taboos against its consumption [3].

Today, the high cost of feed and feed ingredients has been the bane on the growth and expansion of once a vibrant industry, prompting reduced animal protein intake and may lure feed millers into cutting corners through feed adulteration and the use of sub-standard feedstuffs [1].

Nutrition remains one of the most challenging factors to broiler production in particular and other monogastrates, thus rising costs and scarcity of feed have continued unabated in Nigeria, hence feed cost is reported within the range of 70-80% of the total production cost [4]. As posited by Anoh and Akpet [5] increased total cost of production may be attributed to various circumstances including ethno-religious crisis, competition for staples with humans, natural disasters, political instability, poor government policies, and recent insurgency in Northern Nigeria whose supplies of grains to feed industry has been seriously hampered.

Serious and devastating feed costs have become, and the onus of finding lies with the feed millers and animal nutritionists who have not relented in exploiting cheaper, locally available alternatives to replace the expensive animal protein feedstuffs such as fish meal [6].

In the face of abundant by-products from abattoir operations, blood seems to be a promising option to fill the gap of costly animal protein sources. Blood is processed into blood meal, a product with an unpalatable taste, dark chocolate color, pungent smell, and hygroscopic properties [7].

Other feed products derived from blood are hemoglobin and plasma [7], but according to Caires *et al.,* [8], the most common animal products used in broiler diets are meat and blood meal because of their high protein levels which attributed to the economic and nutritional impacts [9].

Ofori and Hsieh [10] reported that blood meant for human and animal food is mostly obtained from bovine and porcine species as compared to others, perhaps due to the large volume of drained blood and the reasonable number of cattle slaughtered. During preparation, blood meal needs to be free from contaminants such as adulterants, insects, fungal infestation, offensive odor, and spores of *Bacillus anthracis* and *Clostridium* spp.

Blood meal (BM) has been reported to contain a minimum crude protein content of 80-85% [5] or 90-95% crude protein, fat (less than 1%), and ash (less than 5%) [7]. Of the various amino acids present in blood meal, lysine, methionine, histidine, arginine, leucine, and tryptophan quantities are reported to be reasonable except isoleucine and glycine are low [11,5,12, but Seifdavati *et al.,* [13] and Hardy [14] reported low arginine and methionine content in BM. BM is valued as a rich source of zinc and iron [7] but deficient in calcium [14].

From the nutritional perspective, BM is reported to have a poor amino acid balance as compared with other animal protein feedstuffs. Rations intended for non-ruminants must be formulated in a manner that ensures sufficient isoleucine content to achieve better productive performance (Ekwe et al., 2022). However, studies have attributed the variations in lysine and other amino acids to the processing methods employed during BM preparation. Mulik [14] reported the availability of lysine 84-89% for ring-dried BM in comparison with 62-77% obtained from a batch-dried BM. Regarding the inclusion level of BM in poultry diets, studies reported that 3-5% had been recommended due to poor digestibility

efficiency associated with it, unlike the fish meal [5] and for broilers, blood can replace 50-100% fish meal and 50% soybean meal for improved performance [14] Being rich in tryptophan with appreciable digestibility coefficiency has been a good consideration for the use of BM in a broiler's diet. The outcome of the blood meal inclusions varies with the amount added to the feed [9]. BM has been an excellent supplementary protein source for plant-based ingredients with low lysine content such as cereal grains and forages, as well as being a satisfactory replacement for other expensive protein sources [7]. Regardless of the enormous nutritional and economic benefits of BM as a quality protein source for poultry, there are still palpable fears and criticisms concerning its use as safe for animal feed ingredients, owing to the belief that blood drained from animals especially cattle is a haven for pathogens, allergens, toxin (dioxin), metabolites, transmissible spongiform encephalopathies (TES), and zoonotic Hepatitis E virus (HEV) [10]. However, from the standpoints of efficient hygienic abattoir practices, sterile collection utensils, and adequate processing methods, these drawbacks against blood meal can be addressed.

BM has been reported to enhance growth performance in poultry (broilers) [9] and previous studies have demonstrated positive effects of BM on the growth performance, hematology, and serum biochemical indices of birds [15]. In avian species, hematology and serum biochemistry are becoming important diagnostic tools for determining the health and breeding status of an individual or flock [16]. Blood examination remains a readily available and fast means of assessing animal health, nutritional status, and performance In feeding trials Lamidi *et al.,* [15]. While reference hematological values of healthy domestic chickens at specific ages and physiological status are necessary for accurate interpretation of blood tests [17], hematological and biochemical indices in animals are based on treatment effects [15]. Maidala et al. [18] reported different protein sources to have different amino acid compositions and may exert effects on the hematological and serum biochemical parameters of broiler chickens. Owen and Amakeri [19] reported on hematological parameters most commonly examined in nutritional studies including PCV, RBC, and WBC (Lymphocy, MCHC, MCV, and Clotting time).

While so many studies on various aspects of BM have been published, information on keeping duration before processing is non-existent, and since the price of fish meal has remained ever high, it is quite pertinent to replace it with cheaper feed ingredients such as BM. Therefore, blood meal processed 6 hours post-storage was added to broiler chickens' diet to evaluate its effects on the hematology and biochemical indices of the birds.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, University of Uyo, Akwa Ibom State, Nigeria. Uyo lies within Latitude 5^{o} 7^{l} and 5^{o} 27^{l} North of the Equator and Longitude 7^{o} 27^{l} and 7o58^{l} East of Greenwich, Uyo is also characterized by temperature ranges between 26– 28^{o}C, average and annual rainfall ranges of 2000mm – 3000mm while relative humidity ranges are reported at 78% - 93% [2]

2.2 Experimental Animals and Management

One hundred unsexed one-day-old Arbor Acre broiler chickens obtained from a credible distributor were randomly allocated to four treatment groups of 25 birds and three replicates each in a Completely Randomized Design (CRD) (after one week of acclimatization). These birds were housed in a deep litter management system in which adequate heat for warmth was supplied. Ample feed and portable water were provided *ad libitum*, while routine vaccinations and medications were effected as when due. Daily feed intake and weekly gain were monitored and recorded. The experiment lasted for only 8 weeks duration.

2.3 Experimental Diet and Design

Bovine blood meal (BBM) processed after 6 hours keeping duration using a cooking oven-drying method was used in formulating diets for four (4) treatment groups designated as T_1, T_2 T_3, and T_4. T_1 was the control without BBM while T_2 (1.0%), T_3 (2.0%), and T_4 (3%) had different percentages of BBM.

2.4 Data Collection and Haemato-Serum Analysis

At 8 weeks, two birds per replicate were randomly selected and fasted overnight, bled for

blood by **vene** puncture method. 2 mls of the blood samples were collected using a disposal syringe and needle into two (2) separate sterile blood sample bottles; with anticoagulant (EDTA) for hematology and without EDTA for serum metabolites. Blood samples were analyzed for Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), and White Blood Cell (WBC) according to Baker et al. [20]. Mean Corpuscular Volume (MCV), mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin concentration (MCHC) were calculated according to Jacquin [21] while the biochemical metabolites were determined by the Brurer reaction method after Bush [22].

2.5 Data Analysis

Data generated were subjected to analysis of variance (ANOVA) according to Steel and Torrie [23] Significant means were separated using the Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

The composition and calculated analysis of the diet containing BBM processed 6 hours post-keeping are shown in Table 1. Both metabolizable energy and crude protein content values were within the ranges reported.

For hematological indices, the results revealed significant (P<0.05) differences in PCV, Hb, MCV, WBC, lymphocytes, and Neutrophils of the broiler chickens fed BBM processed 6 hours post-keeping while RBC, MCHC, Eosinophil, Basophil, and Monocytes showed no significant differences. Amongst the treatments, T_4 (3%) recorded significant (P<0.05) differences in PCV, Hb, MCV, WBC, Lymphocytes, and Neutrophils. Significant values and trends were also observed across the treatments as the inclusion levels increased. All the hematological indices were within the normal ranges reported by Mitruka and Rawnsley (1977) except MCV and Neutrophils which indicated significantly (P<0.05) higher values than normal ranges in contrast to the values of Mitruka and Rawnsley (1977). It also agrees with the findings of Maidala *et al.,* [18] and Ozung et al., [24].

PCV, Hb, and MCV in this study may be attributed to the nutritional content of the blood and the processing method used. BBM is a rich source of iron as reported by Lamidi *et al.,* [15] and Mulik [7] and iron is an essential component of the heme present in hemoglobin an agent responsible for oxygen transport to tissues. The result has confinement the report

Table 1. Composition of experimental diet containing blood meal processed for 6 hours

Ingredients	T1 (control) 0%	T_2 1%	T_3 2%	T_4 3%
Maize	46.30	46.30	46.30	46.30
Soya bean	28.00	28.00	28.00	28.00
Fishmeal	5.00	4.00	3.00	2.00
Blood meal	0.00	1.00	2.00	3.00
Rice meal	17.00	17.00	17.00	17.00
Bone meal	3.00	3.00	3.00	3.00
Starter premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Total	**100**	**100**	**100**	**100**
Calculated analysis				
Crude protein (%)	22.00	21.71	21.44	21.17
ME (kcal/kg)	2985.75	2985.65	2985.55	2985.45
Ca (%)	2.081	2.023	1.965	1.898
EE (%)	5.182	5.182	5.116	5.083
CF (%)	4.921	4.921	4.95	4.97
P (%)	1.279	1.250	1.224	1.196
Lysine (%)	1.126	1.126	1.138	1.144
Methionine (%)	0.305	0.283	0.321	0.329

Vitamin/Mineral Premix = Vitamin A, D3, E, K, B1, B2, B6, B12, Niacin, Pantothenic Acid, Folic Acid, Biotin, Choline Chloride, Manganese, Zinc, Iron, Copper, Iodine, Selenium, Cobalt and Antioxidants

of Maidala et al., [18] who reported that hematological parameters' significant diagnostic values are those of PCV, Hb, and RBC, as well as Fasina *et al*., [16] who posited that the corpuscular elements of the blood have been a reliable indicator of the metabolic state of the experimental animals. About Hb, the value for T_4 recorded as the highest in this study also fell within the normal ranges reported by Mitruka and Rawnsley [25], aligned with values of Owen and Amakiri [19] and Lamidi *et al.,* [15] both of which reported that high Hb values are associated with high feed conversion efficiency, which might be responsible for the appreciable growth performance observed in broiler chickens. The significantly higher values in T_4 (3%) were in agreement with Anoh and Akpet [5] who posited that 3.5% BM recommended for poultry diets is the best for improved performance, thus inclusion level beyond 5% may be deleterious to growth and haemato-serum indicators.

The WBC values obtained showed a diminishing trend with increased inclusion levels. The respective values for WBC and lymphocytes are within the normal ranges reported by Mitruka and Rawnsley [25] except that of neutrophils with higher values than normal. Regardless of these high values, the blood level and immunity status of the broiler chickens were not negatively affected by treatment diets and the disease condition. The result was also at variance with Owen and Amakiri [19] that high lymphocyte values are an index associated with the ability of the broiler chicken to perform under stressful conditions, however, agrees with Yusuf et al., (2012) who asserted that dietary components have measurable effects or blood constituents such that significant changes in their values can be used to inference on the nutritive values of feed offered to the experimental animals.

Table 2. Haematological indices of broiler chickens supplemented with bovine blood meal (bbm) processed 6 hours post-keeping

Parameters	T1 (control) 0%	T_2 1%	T_3 2%	T_4 3%	SEM
Packed Cell Volume (%)	39.06^{ab}	39.60^{ab}	41.75^{ab}	43.38^{a}	1.84
Haemoglobin (g/dl)	9.90^{c}	8.85^{d}	10.14^{b}	11.00^{a}	0.41
Red Blood Cell ($x10^6$/l)	2.53	2.84	3.70	2.65	0.26
Mean Corpuscular Haemoglobin Concentrmchcation (%)	27.58	27.75	29.58	29.49	1.38
Mean Corpuscular Volume (MCV) (f/l)	146.8^{c}	143.0^{d}	153.8^{ab}	163.7^{a}	4.72
Mean Corpuscular Haemoglobin (MCH) (pg)	39.65	40.37	41.98	43.00	1.66
White Blood Cell (WBC) ($x10^3$/l)	17.71^{b}	17.91^{ab}	11.62	9.94^{a}	1.08
Neutrophils (%)	59.00^{a}	53.00^{b}	49.25^{c}	42.75^{d}	1.61
Basophils (%)	1.00	1.50	1.00	1.50	0.72
Eosinophib (%)	2.00	2.25	3.50	3.00	0.58
Lymphocytes (%)	49.00^{a}	48.75^{b}	47.00^{c}	46.00^{d}	0.79
Monocytes (%)	4.50	4.00	4.75	5.00	

Means in row with different superscripts are significantly (P<0.05) different a, b, c, d,
SEM = Standard Error of Mean, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin & MCHC = Mean Corpuscular Hemoglobin Concentration

Table 3. Biochemical profile of broiler chickens supplemented with bovine blood meal (bbm) processed 6 hours post-keeping

Parameters	T1 (control) 0%	T_2 1%	T_3 2%	T_4 3%	SEM
Serum urea (mg/dl)	7.52^{d}	8.50^{c}	8.92^{b}	9.26^{a}	0.14
Alkaline phosphatase (μ/l)	65.07	64.11	65.10	68.30	1.54
Cholesterol (g/dl)	70.98^{d}	77.16^{c}	80.10^{b}	94.84^{a}	0.78
Albumin (g/dl)	2.14	2.31	2.34	2.52	0.29
Total protein (g/dl)	3.25	3.47	3.56	3.97	0.32

a, b, c, d = Means in row with different superscripts are significantly (P<0.05) different

There were no significant differences among Alkaline phosphatase, Albumin, and total protein except for urea and cholesterol (P<0.05). ALP, Alb, and TP indicated trends in values across the treatments.

For urea, the result showed that birds on T_4 (3%) gave the highest value, followed by T_3 (2%), while T_2 (1%) gave the lowest urea value. Urea values of the present study are observed to be within the normal ranges for broiler chickens after Mitruka and Rawnsley [25].

The result is slightly higher than the values reported by Lamidi *et al.,* (2014) whose highest value for urea was 7.00mg/dl while entirely contrasting with lower values of 2.62mg/dl reported by Ilo *et al.,* [26] and numerically higher values than 7.67mg/dl reported by Ozung et al., [24].

Serum urea, often the end-product of protein metabolism, is regarded as an indicator of renal function, with normal values of 20 to 40 mg/dl although increases with age. Higher values of serum urea are reported to portend the incidence of renal diseases ([27,28]. Therefore, the serum values of the present study are below the recommended serum normal values, thus implying that the BBM had no negative effect on renal activities in broiler chickens.

Cholesterol values also indicated significant (P<0.05) differences. Unlike the urea values, cholesterol showed a progressive decrease in value as the inclusion level increased, but these values are within normal ranges reported by Mitruka and Rawnsley [25]. Results disagreed with the values reported by Ozung *et al.,* [24] who showed significantly lower values ranging from 2.52 – 5.74mg/dl, and Moji *et al*., [29] who reported low ranges of 4.13 – 5.05 mg/dl of cholesterol. Being among the desirable plasma lipids, cholesterol is synthesized in the liver and it is a fundamental element of all membranes and a precursor of various critical substances like adrenocortical and gonadal steroid hormones and bile acids [30]. However, serum cholesterol values have a positive correlation with nutritional impediments such as cardiovascular disease (atherosclerosis and myocardial infarction) [30]. Hypercholesterolemia is reported to connote unhealthy intake of fatty acids that may result in serious health setbacks to animals when the reduction of fat in poultry birds has become one of the prime focuses of nutritional research. Conversely, hypocholesterolaemia promotes decreased liver activity which may lead to liver dysfunction. Nevertheless, slightly high values observed in the present study are within the reported normal serum cholesterol ranges (125-200mg/dl) for broiler chickens, as age, sex, diet, species, genetics, and lipid composition are known to affect cholesterol quality in broiler chicken's blood [30-32].The ranges in the present study did not negatively affect the broiler chickens, perhaps High-density lipoprotein (HDL) known for its anti-atherogenic effects may constitute a reasonable portion of the cholesterol in BM.

4. CONCLUSION

The T4 with 3% inclusion of bovine blood meal (BBM) processed 6 hours post-keeping was the best of all the treatments. The bovine blood meal did not pose any threat or deleterious effect to the broiler chickens but rather promoted appreciable performance vis-à-vis hematology and biochemical metabolites have been adjudged to be promising to adopt. Bovine blood meal enhances the potential to alleviate the scarcity and high cost of animal protein feedstuffs in broiler formulation, but the recommended, 3-5% inclusion particularly for poultry species must not be exceeded to avoid undesirable effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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