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ORIGINAL RESEARCH ARTICLE

Effects of Supplementation of Protease to Low Crude Protein Diets on Performance of and Nutrient Digestibility in Heat Stressed Broiler Chickens

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ABSTRACT

The effect of supplementation of protease to low crude protein diet on the performance and nutrient digestibility of heat-stressed broiler chickens was investigated in a 35-day experiment. One hundred and sixty-eight 7-day-old broiler chickens were randomly allotted to four treatments with 6 replicates of 7 birds each in a completely randomised design. Diets 1 and 2 contained 23% CP without or with enzyme, while diets 3 and 4 had low 17% CP without or with enzyme at the starter phase. Similarly, normal 21% CP diet without or with enzyme and low 15% CP without or with enzyme supplementation at the finisher phase respectively. At starter phase, final weight, weight gain and feed intake of birds fed 23% CP diets (without or with protease supplementation) were similar to those fed 17% CP without protease. Higher (P<0.05) protein intakes (PI) were recorded in birds fed 23% CP without or with enzyme supplementation when compared to birds on 17% CP. At finisher phase, PI of birds on 21% CP without enzyme was significantly higher (P<0.05) than those on other diets. Highest PER (10.12) was observed in birds on 15% CP with enzyme supplementation while least PER (3.57) was recorded for birds on 21% CP with enzyme. Glucose concentration of birds sampled on day 14 did not differ (P>0.05) significantly within treatments. Lymphocyte values of birds on 17% CP without or with enzyme were similar to those of birds on 23% CP without or with enzyme supplementation. Similar trend was observed in heterophil values and heterophil to lymphocyte ratio of birds on dietary treatments. Lymphocytes of birds on 21% CP without or with enzyme were similar to lymphocyte of birds on 15% CP without or with protease. Diets had no effect on apparent crude protein and gross energy digestibility of birds on dietary treatments. Least dry matter digestibility (28.27%) was recorded in birds fed 21% CP with enzyme supplementation compared to other diets. In conclusion, protease supplementation did not significantly improve performance of heat-stressed birds in both phases. Blood parameters measured were not altered in response to high temperature except lymphocyte counts.

Keywords: *Heat inducement, Protease supplementation, Crude protein, Broilers*

INTRODUCTION

Adequate nutrition for broilers under high temperatures is extremely important for broiler chicken industry (Annison, 1991). There is considerable disagreement as to what is the ideal temperature range for different classes and age groups of poultry (Charles, 2002). This is probably due to the fact that many factors influence the reaction of poultry to temperature changes. The most important are humidity, wind velocity and previous acclimatization of the birds (Arjona *et al.,* 1990). Birds perform well within a comfort zone relatively wide range of temperatures, whether they are broilers, layers or turkeys, this range extends between 10 and 27°C. Kampen (1984) found that the highest growth rate of broilers occurs in the range of 10-22°C while maximum feed efficiency is at about 27°C. Charles (2002) postulated that optimum temperature for birds' performance is 18-22°C. It is known, however, that what is ideal for growth might not be ideal for feed efficiency.

The most important factor affecting performance in broilers subjected to high temperature is reduced feed intake. It is well know that birds reduce feed intake under heat stress conditions. According to Baziz *et al.* (1996), broiler chickens reduce feed intake around 3.6% for every increase (1%) in environmental temperature from 22 to 32°C. This reduction aims to avoid increasing of endogenous heat production since heat production is high when feed intake increase (Koh and Macleod, 1999; Longo, 2000).

Attempts have been made at reducing heat-stress mortality in broilers by acclimatization (Charles, 2002).

Raising house temperature prior to the onset of a heat wave has been shown to reduce mortality. Arjona *et al.* (1990) observed that exposure to 35-38° C for 24 hours at 5 days of age reduced mortality when these birds were heat stressed for 8 hours at 44 days of age. It has been suggested that a temperature range of 36-37.5°C at three days of age is optimum for early conditioning of broilers (Yahav and McMurty, 2001). Incubating eggs at high temperature has also shown to improve the tolerance of fast-growing broilers to heat stress (Yalcin *et al.*, 2008). Although this practice of acclimatization is still in the experimental stage, it has strong potential for the broiler industry. Similarly, earlier intervention strategies including environmental management and dietary supplementation have been inconsistent in poultry for mitigating heat stress. However, some innovative approaches, such as genetic marker-assisted selection of poultry breeds for increased heat tolerance and application of molecular techniques in poultry breeding to improve poultry productivity in a sustainable manner had been reviewed (Pawar *et al.*, 2016).

The performance of poultry is greatly dependent on the quality of feed and the bioavailability of the nutrients in the feed (Kocher *et al.*, 2002). However, poultry birds do not benefit from the presence of microorganisms producing endogenous enzymes as ruminants (Ramesh *et al.,* 2013). Exogenous enzymes have been shown to improve digestibility of starch, protein, fat and apparent metabolisable energy in broilers fed diets containing wheat (Annison and Choct, 1991; Bedford, 1995). More so, studies with intestinal digesta confirmed that protein was linked to the viscous gels and combined action of proteolytic and pentosanase enzymes which lead to a higher reduction of intestinal viscosity than that from pentosanase alone (Finnfeed, 1991). Exogenous enzymes help to make more nutrients available especially when feed ingredients are of inferior quality and or with low bioavailability (Kocher *et al.,* 2002; Cowieson and Adeola, 2008). However, reports have shown that low-protein diets have negative effects on broiler performance when environmental temperature is high, because during heat stress, low feed intake associated to a low diet protein induces amino acid deficiencies (Kamran *et al.*, 2010; Fru-Nji *et al.*, 2011).

Other studies have also shown that broilers fed low-protein diets increase their energy requirement for maintenance with higher heat production (Kerr and Kid, 1999; Kamran *et al.*, 2010). It is imperative to manage both the ambient temperature and nutritional conditions to avoid negative effects on poultry production. It was therefore the aim of the study to determine the effect of protease supplementation on performance and nutrient digestibility in heat stressed broiler chickens fed low crude protein diets.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Oyo State in the South West geographical zone of Nigeria and within the Tropical rain forest region.

Management of birds and experimental diets

One hundred and sixty eight one-day-old Abor acre broiler chicks were used for this study. The birds were reared in a well-ventilated and illuminated standard poultry house. The birds were tagged and weighed at one -day-old and brooded for seven days after which they were randomly allotted to four dietary treatments of six replicates with seven birds each and transferred to cage. Starter and finisher diets formulated were offered to the birds from day 8 to 21 and 22 to 35 respectively. At starter phase, treatments 1 and 2 had 23% crude protein without or with enzyme supplementation, while treatments 3 and 4 had 17% crude protein without or with enzyme inclusion. Similarly, at finisher phase, treatments 1 and 2 contained 21% crude protein with or without enzyme, while treatments 3 and 4 had 15% crude protein without or with enzyme supplementation (Tables 1 and 2). Experimental diets were given *ad libitum* and birds had access to water through nipple drinkers of the cage. Routine management programmes were strictly adhered to. The birds were subjected to heat stress for a period of 6 to 8 hours and at temperature between 38 to 40°C (monitored with the use of digital thermometer) from day 7 to day 27. However, from day 28 till day 35, birds were not subjected to heat stress.

Experimental design

The experimental design was a Completely Randomized design (CRD).

Source of heat: The 100-watt electric bulbs were complemented with kerosene stoves and charcoal pots.

Table 1: Gross composition (g/ kg DM) of experimental broiler starter diets

Ingredients	23% CP without enzyme	23% CP with enzyme	17% CP without enzyme	17% CP with enzyme
Maize	500.00	495.00	670.00	665.00
Soyabean meal	431.00	431.00	266.00	266.00
Soya oil	35.00	35.00	30.00	30.00
Dicalcium phosphate	15.00	15.00	15.00	15.00
*Broiler premix	2.50	2.50	2.50	2.50
Limestone	12.00	12.00	12.00	12.00
DL-Methionine	1.00	1.00	1.00	1.00
L-Lysine	1.00	1.00	1.00	1.00
Table salt	2.50	2.50	2.50	2.50
Protease	0.00	5.00	0.00	5.00
TOTAL	**1000**	**1000**	**1000**	**1000**
Calculated nutrients (g/kg)				
Crude protein	232.14	232.00	173.99	173.57
Energy ME (kcal/kg)	2930.13	2930.10	3089.68	3072.93
Crude fat	35.09	35.09	36.11	35.91
Crude fibre	41.17	41.17	33.36	33.25
Calcium	9.09	9.09	8.65	8.65
Total phosphorus	6.71	6.71	6.04	6.03

*Composition of premix per kg of diet: vitamin A,12,000,000 I.U; vitamin D_3,3,000,000 I.U; vitamin E, 30,000mg; vitamin K_3,, 2,500mg; vitamin B_1, 2,000mg; vitamin B_2, 5,000mg; vitamin B_6, 3,500 niacin, 40,000mg; ; vitamin B_{12}, 20mg; choline chloride, 200,000mg; folic acid, 1,000mg; biotin, 80mg; manganese, 70,000mg; iron,40,000mg; zinc, 60,000mg; copper, 8,000mg; iodine, 1,200mg; cobalt, 250mg; selenium, 250mg; Anti-oxidant, 126,000mg.

DATA COLLECTION

Performance indices

The body weight gain and feed consumption were taken weekly for the duration of the experiment. Feed conversion ratio, protein intake and protein efficiency ratio were calculated.

Haematology and serum biochemical indices

At day 14, blood samples were taken from the jugular vein of one bird per replicate after the birds had been subjected to heat stress for a period of 6 to 8 hours and at temperature between 38 to 40°C to assay for the blood glucose. At day 21, blood samples were again taken as described above. The blood glucose, total protein, albumin, lymphocytes, heterophils, monocytes, eosinophils, basophils, were analysed and heterophil/lymphocyte ratio was calculated. However, at day 28, the same procedures as described above were carried out, but without subjecting the birds to heat stress.

Nutrient digestibility

On day 33, two birds were taken from each replicate for nutrients digestibility assay. Fresh excreta were collected in the morning daily (across the replicates) for a period of 3 days, weighed and oven dried. The oven dried samples were analysed for dry matter, crude protein and gross energy (using a bomb calorimeter) and values subsequently used for digestibility calculations. Apparent nutrient digestibility was calculated as follows:

Table 2: Gross composition (g/kg DM) of experimental broiler finisher's diets

Ingredients	21% CP without enzyme	21% CP with enzyme	15% CP without Enzyme	15% CP with enzyme
Maize	541.00	536.00	721.00	716.00
Soyabean meal	375.00	375.00	210.00	210.00
Soya oil	50.00	50.00	35.00	35.00
Dicalcium phosphate	15.00	15.00	15.00	15.00
*Broiler premix	2.50	2.50	2.50	2.50

Limestone	12.00	12.00	12.00	12.00
DL-Methionine	1.00	1.00	1.00	1.00
L-Lysine	1.00	1.00	1.00	1.00
Table salt	2.50	2.50	2.50	2.50
Protease	0.00	5.00	0.00	5.00
TOTAL	**1000**	**1000**	**1000**	**1000**
Calculated nutrients (g/kg)				
Crude protein	210.99	210.59	153.69	153.26
Energy ME (kcal/kg)	3068.60	3067.00	3177.65	3160.90
Crude fat	34.77	34.77	36.19	35.99
Crude fibre	38.15	38.15	30.56	30.45
Calcium	8.94	8.94	8.50	8.49
Total phosphorus	6.44	6.44	5.80	5.79

*Composition of premix per kg of diet: vitamin A,12,000,000 I.U; vitamin D_3,3,000,000 I.U; vitamin E, 30,000mg; vitamin K_3,, 2,500mg; vitamin B_1, 2,000mg; vitamin B_2, 5,000mg; vitamin B_6, 3,500 niacin, 40,000mg; ; vitamin B_{12}, 20mg; choline chloride, 200,000mg; folic acid, 1,000mg; biotin, 80mg; manganese, 70,000mg; iron,40,000mg; zinc, 60,000mg; copper, 8,000mg; iodine, 1,200mg; cobalt, 250mg; selenium, 250mg; Anti-oxidant, 126,000mg.

Apparent Nutrient Digestibility (%) =

$$\frac{\text{Nutrient Intake} - \text{Nutrient Output}}{\text{Nutrient Intake}} \times 100$$

PROXIMATE ANALYSIS

The proximate composition of the test diets and excreta samples was determined according to the methods of AOAC (2000). Gross energy was determined using bomb calorimeter.

STATISTICAL ANALYSIS

Data obtained were analyzed using descriptive statistics and ANOVA ($P \leq 0.05$) SAS (2008). Mean differences were separated using Duncan Multiple Range Test (DMRT) of the same software.

RESULTS

The results on the performance characteristics of heat-stressed birds fed protease with low crude protein at the starter and finisher phases are shown in Tables 3 and 4 respectively. Significant differences were observed in the initial weight (IW) and the feed conversion ratio (FCR) across the treatments at the starter phase. There were significant ($P<0.05$) differences observed in the final weight (FW), weight gain (WG), feed intake (FI), protein intake (PI) and protein efficiency ratio (PER) of birds on experimental diets at starter phase. The FW, WG and FI of birds fed 23% CP diets (without or with protease supplementation) were similar ($P>0.05$) to those fed diet containing 17% CP without protease supplementation.

Significantly higher ($P<0.05$) protein intakes were recorded in birds fed 23% CP without or with enzyme supplementation compared to birds on 17% CP without or with protease. However, PER of birds on 23% CP diets were similar and significantly ($P<0.05$) lower than what was recorded in birds on 17% CP diets.

Table 3: Effect of protease to low crude protein on the performance of heat-stressed broiler chickens in the starter phase

Parameters	23% CP without enzyme	23% CP with enzyme	17% CP without enzyme	17% CP with enzyme	SEM	P value
IW (g/b)	102.17	104.67	103.50	103.00	2.38	0.900
FW (g/b)	283.00[a]	277.83[a]	256.83[ab]	236.33[b]	12.43	0.058
WG (g/b)	180.83[a]	173.17[a]	153.33[ab]	133.33[b]	12.39	0.057
FI (g/b)	186.17[a]	175.67[ab]	161.33[b]	154.33[b]	7.90	0.043
FCR	1.04	1.03	1.09	1.18	0.06	0.346
PI (g/b)	42.82[a]	40.40[a]	27.43[b]	26.24[b]	1.56	0.0001

PER	4.23^{b}	4.29^{b}	5.58^{a}	5.05^{a}	0.30	0.013

Mean on the same row with different superscripts are significantly (P<0.05) different. *IW – Initial weight; FW – Final weight; WG – Weight gain; FI – Feed intake; FCR – feed conversion ratio; PI – Protein intake; PER – Protein efficiency ratio.

There were significant differences (P<0.05) observed in the weight gain (WG), feed intake (FI), protein intake (PI) and protein efficiency ratio (PER) of birds on experimental diets at the finisher phase. Significantly lower weight gain was recorded for birds on diet 21% CP with protease supplementation, however, WG of birds fed 21% CP without enzyme supplementation was similar to 15% CP with enzyme inclusion. Feed intakes of birds fed diet containing 21% CP without enzyme supplementation were identical with birds fed 15% CP without or with enzyme supplementation. Protein intake of birds on 21% CP without enzyme was significantly higher (P<0.05) than PI of birds on other diets. Similar PER was recorded in birds fed 21% CP and 15% CP without enzyme. Highest PER (10.12) was observed in birds on 15% CP with enzyme supplementation while least PER (3.57) was recorded for birds on 21% CP with enzyme Diet had no effect on the final weight and feed conversion ratio of birds on the experimental diets.

Table 4: Effect of protease to low crude protein on the performance of heat-stressed broilers chickens at the finisher phase

Parameters	21% CP without enzyme	21% CP with enzyme	15% CP without enzyme	15% CP with enzyme	SEM	P value
IW (g/b)	283.00^{a}	277.83^{a}	256.83^{ab}	236.33^{b}	12.43	0.058
FW (g/b)	600.80	444.50	491.17	591.17	42.37	0.047
WG (g/b)	330.80^{a}	148.50^{c}	234.33^{b}	334.33^{a}	38.96	0.010
FI (g/b)	256.00^{a}	198.50^{b}	221.50^{ab}	222.67^{ab}	11.92	0.026
FCR	0.78	1.34	1.02	0.87	0.13	0.250
PI (g/b)	53.76^{a}	41.69^{b}	33.23^{b}	33.40^{b}	2.28	0.0003
PER	6.18^{b}	3.57^{c}	7.17^{b}	10.12^{a}	1.19	0.142

Mean on the same row with different superscripts are significantly (P<0.05) different. * IW – Initial weight; FW – Final weight; WG – Weight gain; FI – Feed intake; FCR – feed conversion ratio; PI – Protein intake; PER – Protein efficiency ratio; BM – Breast meat; AF – Abdominal fat.

The results of the haematology and serum biochemical indices at 21 day of blood sampling, of heat stressed broiler chickens fed with low crude protein supplemented with protease are shown in Table 5. Diets had no influence on the monocyte, eosinophil, basophils, glucose, total protein, albumin, and globulin of birds on the experimental diets. Glucose concentration of birds sampled on day 14 did not differ (P>0.05) significantly. However, there were significant differences (P< 0.05) observed in the lymphocyte, heterophil and heterophil to lymphocyte ratio across the treatments. Lymphocyte values of birds on 17% CP without or with enzyme were similar to those of birds on 23% CP without or with enzyme supplementation. Similar trend was observed in heterophil values and heterophil to lymphocyte ratio of birds on dietary treatments.

Table 5: Effect of protease to low crude protein on the haematology and serum biochemical indices of heat-stressed broiler chickens at the starter phase

Parameters	23% CP without enzym	23% CP with enzyme	17% CP without enzyme	17% CP with enzyme	SEM	P value
Lymphocytes (%)	71.83^{a}	61.50^{b}	65.67^{ab}	66.83^{ab}	2.53	0.045
Heterophils (%)	21.83^{b}	32.50^{a}	27.17^{ab}	27.17^{ab}	2.54	0.059
Monocytes (%)	2.00	2.17	2.67	3.33	0.46	0.202
Eosinophil (%)	4.17	3.17	3.83	2.50	0.59	0.230

Basophil (%)	0.17	0.17	0.33	0.17	0.18	0.883
Heterophil:Lymphocyt ratio	0.33^{b}	0.54^{a}	0.42^{ab}	0.42^{ab}	0.06	0.001
Glucose day 7 (mg/dL)	236.13	234.52	233.33	223.61	9.66	0.976
Total protein (g/dL)	2.71	2.82	2.56	2.72	0.16	0.733
Albumin (g/dL)	1.43	1.39	1.17	1.24	0.33	0.290
Globulin (g/dL)	1.28	1.43	1.39	1.48	0.17	0.846
Glucose day 14 (mg/dL	243.46	231.46	224.34	243.53	12.88	0.656

Mean on the same row with different superscripts are significantly (P<0.05) different.

The results of the haematology and serum biochemical indices of broiler chickens without heat inducement are shown in Table 6. There were significant (P< 0.05) differences observed in the lymphocyte, heterophil, heterophil : lymphocyte ratio, glucose and albumin of birds on experimental diets. Lymphocytes of birds on 21% CP with enzyme and 15% CP without enzyme supplementation were similar to lymphocyte values of birds on 21% CP without enzyme and 15% CP with enzyme inclusion. The same trend was recorded in heterophils and heterophil : lymphocyte ratio of birds on the experimental diets. Glucose concentration was significantly (P<0.05) higher in birds fed 21% CP without enzyme and 15% CP with enzyme compared with other diets.

Table 6: Effect of protease to low crude protein on the haematology and serum biochemical indices of broiler chickens without heat inducement at the finisher phase (28th day blood sampling)

Parameters	21% CP without enzyn	21% CP with enzyme	15% CP without enzyme	15% CP with enzyme	SEM	P value
Lymphocytes (%)	49.17^{b}	55.50^{ab}	53.67^{ab}	62.00^{a}	3.39	0.023
Heterophils (%)	43.83^{a}	36.75^{ab}	38.33^{ab}	30.40^{b}	3.45	0.011
Monocytes (%)	2.83	3.75	3.67	2.60	0.69	0.622
Eosinophils (%)	4.00	3.25	4.17	4.80	0.61	0.510
Basophils (%)	0.17	0.15	0.17	0.20	0.18	0.171
Heterophil:Lymphocyte ratio	0.93^{a}	0.74^{ab}	0.75^{ab}	0.50^{b}	0.11	0.036
Glucose (mg/dL)	262.37^{a}	206.77^{b}	209.96^{b}	251.21^{a}	20.07	0.193
Total protein (g/dL)	3.25	2.79	2.65	3.11	0.25	0.354
Albumin (g/dL)	2.44^{a}	2.05^{ab}	1.98^{b}	2.23^{ab}	0.13	0.045
Globulin (g/dL)	0.81	0.74	0.67	0.88	0.23	0.934

Mean on the same row with different superscripts are significantly (P<0.05) different.

The result on apparent nutrient digestibility of birds fed low protein diet with or without enzyme supplementation at the finisher phase is shown in Table 7. There was no significant (P> 0.05) difference in the apparent crude protein and gross energy digestibilities of birds fed diets without or with protease supplementation. However, apparent dry matter digestibility showed significant (P< 0.05) differences, least apparent dry matter digestibility (28.27%) was recorded in birds fed 21% CP with enzyme supplementation compared to other diets.

Table 7: Effect of apparent nutrient digestibility of broiler chickens on experimental diets at the finisher phase

Parameter	21% CP without enzyme	21% CP with enzyme	15% CP without enzyme	15% CP with enzyme	SEM	P value
App CPD (%)	26.15	21.41	15.88	20.77	4.950	0.055
App DMD (%)	39.41^{a}	28.27^{b}	37.28^{a}	44.24^{a}	3.75	0.053
App GED (%)	81.26	78.46	80.69	82.83	1.16	0.377

Mean on the same row with different superscripts are significantly (P<0.05) different.
APP CPD – Apparent Crude Protein Digestibility; APP DMD – Apparent dry matter Digestibility; APP GED – Apparent gross energy Digestibility

DISCUSSION

Elevated environmental temperature is a stressor and has been characterized with reduced body weight gain, feed intake, gain : feed ratio or feed efficiency and increased mortality of chickens (Geraert *et al.*, 1996; Cooper and Washburn, 1998; Mujahid *et al.*, 2009). The positive effect of protease supplementation to low crude protein on performance has been reported in broilers (Angel *et al.*, 2011). In this study, there were considerable differences observed in birds on the standard diets (23% CP) without or with enzyme supplementation and those on low crude protein diet (17% CP) without or with protease for both starter and finisher phases. Birds fed low protein diets (17% and 15% CP) without or with protease compared favourably with those on 23% and 21% CP without or with inclusion of enzyme in the starter and finisher phases. Reduced feed intake was observed in birds fed low protein diets without or with enzymes in the starter phase. According to Geraert *et al.* (1996), during periods of heat stress, feed intake is reduced in broilers, limiting the intake of nutrients and therefore limiting growth performance. In this study, it was also observed that enzyme supplementation did not significantly improve performance. This is contrary to findings of Ajayi (2014) who reported that performance of birds on low CP diets was enhanced by supplementation with protease. The author recorded a significant increase in the body weight gain of birds fed 17.5% CP and 15.5% CP diets when protease was added in addition to a significant decrease in feed intake (FI) without protease supplementation for some birds. Freitas *et al.* (2001) observed a general decrease in FI of birds fed protease supplemented diets on the contrary. There was no significant difference in the feed conversion ratio (FCR) of birds on dietary treatments. This could be due to the fact that the birds were subjected to similar environmental stress leading to low feed intake.

However, Fru-Nji *et al.* (2011) reported no significant difference in FCR but higher weight gain in low CP diets supplemented with exogenous protease compared with normal 23% CP level diets. In this study, birds were subjected to heat stress, and as such no remarkable difference was observed in the FCR across the diets probably due to low FI. Daghir (2008) reported that broilers are able to adapt themselves to a heat stress challenge through various mechanisms that include increasing peripheral blood flow to dissipate excess heat, decrease FI to reduce heat increment as well as panting to facilitate evaporative cooling. Cowieson and Adeola (2008) noted that body weight gain (BWG) of birds fed low protein diet was not significantly increased with enzyme supplementation when compared with a control diet. The authors reported that this response was not because the enzymes were not effective but that it was due to reduction in the energy content of the diets. It was further explained that birds on the control diet (23% CP with enzyme supplementation) did not benefit from protease addition, probably, because with adequate CP in their diet they require no increase in amino acid pool to maintain nitrogen balance.

Several blood parameters may be altered in response to high temperature, especially during the onset of heat stress or acute heat stress. Changes take place in the blood during periods of heat stress that reflect changes in respiration, electrolyte balance, serum metabolites and pH (Borges *et al.,* 2003; Sahin *et al.,* 2003; Toyomizu *et al.,* 2005). Several studies have investigated the effect of haematology and serum biochemical indices on heat stressed broiler chickens. Khan *et al.* (2002) reported that birds exposed to heat between 35 - 40°C showed increased level of glucose as compared to the birds kept on 28-32°C. However, these reports are contrary to the result of this study. No significant difference was observed in the blood glucose concentration of heat stressed birds at days 14 and 21 of blood sampling. However, there was higher blood glucose levels in birds fed 21% CP without enzyme supplementation and 15% CP with addition of enzyme when the birds were not subjected to heat stress compared to when they were heat stressed. This could be due to unclear stressor in the blood as a result of the initial heat inducement.

Khan *et al.* (2002) reported that the serum concentration of total protein affected birds when they are exposed to high temperature. In this study, there were no remarkable changes in the amount of total protein when birds were induced with or without heat. This could be due to the fact that the heat inducement was introduced at the initial stage which was later withdrawn; birds could have been acclimatized to the ambient temperature. The effects of heat stress on the lymphocyte, heterophil, eosinophil, basophil, monocytes and heterophil/lymphocyte ratio in broiler chickens had been investigated (Altan *et al.*, 2000). The authors recorded a significantly reduced lymphocyte when birds were induced with heat between 35 – 40°C and raised heterophil value which resulted in increased heterophil/lymphocyte ratio. This is not in agreement with the findings of this study because the value of lymphocytes was significantly higher than lymphocyte value of birds without heat inducement which resulted in reduced heterophil/lymphocyte ratio (Table 5).

Mitchell *et al*. (1992) and Maxwell *et al*. (1992) reported an increase in basophil counts after heat stress. On contrary to the result obtained in this study, there were no marked differences in the basophil count in birds on experimental diets or without heat inducement (Tables 5 and 6). Maxwell *et al*. (1992) and Maxwell (1993) suggested that an increase in the H/L ratio may be a response to mild or moderate stress but a basophilia condition may result from extreme stress. It has been reported that there is remarkable reduction in eosinophil and monocyte when birds are subjected to heat stress (Maxwell *et al.*, 1992). Inducement of heat did not affect monocyte and eosinophil counts of birds in this study. Maxwell and Robertson (1998) averred that eosinophils disappear from circulation while basophils increase in circulation during stress, particularly acute stress.

External influences such as physiological, disease and environmental stress can exert an effect on the digestibility of individual feed component as well as feed dry matter as a whole (Keshavarz and Fuller, 1980). Akinola *et al*. (2015) reported that apparent crude protein and dry matter digestibility of broilers showed no obvious differences when low CP diet of 18% were fed to broiler chickens under heat stress. In this study, apparent dry matter digestibility of birds fed 21% CP without enzyme was similar to those on other diets except for birds fed 21% CP with enzyme. Low apparent crude protein and dry matter digestibility in heat-stressed birds was observed in this study. This was confirmed in an experiment conducted by Wallis and Balnave (1984) and Zuprizal *et al*. (1993). It was asserted that crude protein digestibility in broilers was significantly affected when birds were subjected to increasing ambient temperature. Similarly, Bonnet *et al*. (1997) found that 4-week-old broilers held under a constant 32°C for 2 weeks showed significantly lower digestibility (69.1%) of a corn-soy diet. Hai *et al*. (2000) affirmed that market weight broilers exposed to temperature of 32°C for a 6-day period showed decrease activities of trypsin and chymotrypsin, suggesting reduced capacity for protein digestibility. Various studies have shown slightly increased or non-significant changes in metabolizable enegy (ME) during high environmental temperature in broiler chickens (El Husseiny and Creger, 1980; Keshavarz and Fuller, 1980). Other studies have shown that increased environmental temperature decreased ME content of the diets fed to broilers (Yamazaki and Zi-Yi, 1982). In an experiment conducted by De Faria Filho *et al*. (2007) on the effect of nutrient digestibility on low crude protein diet in broiler chickens, it was discovered that there were no obvious differences in the dry matter, crude protein and gross energy digestibility. This corroborates the results obtained in this study with exception of dry matter digestibility.

CONFLICT OF INTEREST

The authors declare no conflict of interest with respect to this study.

CONCLUSION

Protease supplementation did not significantly improve performance of and, apparent crude protein and dry matter digestibilities in heat-stressed birds in both starter and finisher phases. Blood parameters measured were not altered in response to high temperature except lymphocyte counts.

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