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# **ARTICLE Effect of Metabolic Excesses from Dietary Proteins on Blood Profile of Heat-stressed Broilers**

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## ABSTRACT

Although, dietary proteins play a crucial role in poultry profit maximization, through the sustenance of birds' welfare, growth and development, yet metabolic excesses from crude protein (CP) degradation is detrimental to broiler chickens (BC) affected by heat stress. This study evaluated the effect of dietary protein levels on blood profile of heatstressed BC at starter phase (SP) and finisher phase (FP). Arbor Acre BC (n=288) were randomly allotted to four dietary treatments (T1- 23% CP; T2- 21% CP; T3- 19% CP; and T4- 17% CP) with six replicate groups in a completely randomized design. Data were subjected to descriptive analysis, analysis of variance (p=0.05) and correlation statistics. Protein intake (PI) was not significantly affected by varying CP in diets at SP, but not at FP, where PI significantly increased with increasing dietary CP. PER had a negative correlation with PCV (r= -0.89, p<0.01), Hb (r= -0.88, p<0.01), RBC (r= -0.93, p<0.01) and PI (r= -0.78, p<0.01). Metabolic excesses including heat dissipation from dietary proteins influenced PCV, Hb, platelets and glucose of heat-stressed broilers.

## 1. Introduction

As an established scientific fact, blood parameters showcase the health status of animals, and they are crucial in the evaluation of the physiology of farm animals. Dietary proteins are important, as they play crucial roles in coordinated feed intake, regulation of homeostasis and metabolism of nutrients, among others. When ingested, proteins undergo hydrolysis in the gastro intestinal tract (GIT) and release smaller units known as amino acids (AA) that functions in structural development, blood components, and hormonal activities in the body <sup>[1]</sup>. According to Beski *et al.* <sup>[2]</sup>, proteins are vital in livestock feeds and they sustain life alongside other nutrients such as carbo-

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hydrates, fats, crude fibre, water, vitamins and minerals. These proteins are structural polymers, consisting of linkages of AA to form compounds possessing carbon structure, amino and carboxyl groups<sup>[3]</sup>.

Dietary proteins from plant based products are complex and differ as some are heterogeneous and include those obtained from cereals and may exhibit cytoplasmic and storage functions. Storage proteins are rich in large volume of amino acids, while cytoplasmic proteins contain less of some amino acids, but more of arginine and lysine, and are therefore considered more nutritionally advantageous than storage proteins <sup>[4]</sup>. The metabolic fate of ingested dietary proteins involves proteinase action of degradation into a pool of biologically active peptides that are further hydrolyzed by peptidases into small-sized peptides that are either absorbed or form free amino acids. However, these sets of degraded proteins exhibit a variety of functions in the gastrointestinal tract <sup>[5]</sup>. Yvon *et al.* <sup>[6]</sup> noted that various degraded protein segments function in the GIT to modulate nutrient absorption, digestive enzymes and metabolism.

Pulses contain considerable amounts of proteins, although unprocessed pulse seeds contain anti-nutritional factors that decrease protein digestibility if not properly processed <sup>[7]</sup>. Soy proteins from soyabeans contain high protein content of 35 to 40% on dry weight<sup>[8]</sup>. According to <sup>[5]</sup>, about 90% of these sova proteins exist as storage proteins, and are primarily glycoprotein composed of both acidic and basic polypeptides. Biolo et al. [9] noted that the splanchnic tissues retain 20 to 50% of essential AA from ingestion of foods by humans. However, branched chain AA are exceptions, with approximately 80% of dietary proteins being found in blood circulation <sup>[10]</sup>, and may influence physiological functions. Popoola et al. [11] opined that poultry meat quality reduction and loss of customers' preference to meat of chickens under heat stress may persist in the 21<sup>st</sup> century poultry farming systems and necessitates prompt solutions <sup>[12]</sup>. Therefore, this study was implemented to establish the effect of metabolic excesses from dietary proteins on blood profile of heat-stressed BC.

#### 2. Materials and Methods

This study began shortly after the decision of the Institution's Animal Care and Use Committee was relayed through the Department of Animal Science. The experimental location was the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Nigeria. A day-old Arbor Acre BC (n=288) with initial body weight of 41±3g were randomly allotted to four dietary treatments (CP, %: 23, 21, 19, 17) at pre-starter phase and 21, 19, 17 and 15% CP at finisher phase with six replicate groups, in a completely randomized design in an attempt to establish the influence of metabolic excesses from dietary proteins on blood profile of BC at starter and finisher phases. Aggregate DEB were computed as defined by Popoola and Iyayi <sup>[13]</sup>, and the assumptions that guarded the derived equations were put forward by Popoola *et al.* <sup>[11]</sup> in a concept for an ideal DEB.

Feed intake (FI, g/bird) was calculated by subtracting the amount of feed left over in a specific time from the amount of feed initially supplied. This difference was thereafter divided by the number of birds in each replicated group to obtain estimated mean feed intake values per bird. Body weight gain was calculated by subtracting the initial weight from the final weights on a weekly basis. Birds were served drinkable water as described by Popoola *et al.*<sup>[14]</sup>. Mean environmental temperature and relative humidity were recorded daily. At the end of each phase, venous blood samples (5 mL) were collected using sterilized needles to puncture the brachial vein in two birds per replicate pen whose weights were closest to the mean class weight. Samples were fractionated, with a portion (2 mL) put into anticoagulant (EDTA) bottles for determination of haematology and others (3 mL) saved for blood glucose determination. Parameters measured for haematology includes the volume of packed cells (PCV), count of white blood cells (WBC) and differentials [15],[16], haemoglobin (Hb) (determined by Sahli's haemoglobinometer), and Red blood cells (RBC). The differential leukocyte counts were made on monolayer blood films and fixed in absolute methanol for five minutes and were subsequently stained and examined under a light microscope as described by Dacie and Lewis<sup>[17]</sup>. Blood glucose (GLU) was also assessed. Proximate analysis of the feeds was determined according to AOAC [18] procedure. Sodium and potassium concentrations in feeds were determined using Flame spectrophotometer; and chloride using titration<sup>[19]</sup>. Data obtained were descriptively analyzed, and through the analysis of variance statistics, differences among treatment means were determined using SAS package <sup>[20]</sup> and means were compared using Duncan Multiple Range Test at a 95% level of probability. Also, correlation statistics were done to evaluate the relationship between different dependent variables.

### 3. Results

#### **3.1 Chemical Composition of Dietary Treatments**

Table 1 shows the chemical composition of dietary treatments offered to heat-stressed broiler chickens at both starter and finisher phases. It was observed that dietary crude protein levels were the only differentiating factor contributing to variations among raised broiler chickens, with a 2% sequential reduction from the standard CP levels at SP and FP.

#### 3.2 Performance of Heat-stressed Broilers

Table 2 shows the performance of heat-stressed broilers on different dietary crude protein at starter and finisher phases. Feed intake (FI) was not substantially (P=0.58) affected by varying CP levels at SP, but at finisher phase. Body weight gain (BWG) of birds on T1 and T2 were similar and were higher (P=0.00) compared to T3 and T4 at finisher phase. Although, at starter phase, no vivid differences were observed in BWG values of birds on T1, T2 and T3. At starter phase, feed conversion ratio (FCR) value in T1 (1.64) was lower compared to T4 (2.03), but was similar to T2 and T3. However, at finisher phase, T2 (1.87) was lower in FCR value compared to T1 (2.11) and T4 (2.35), but was similar to T3 (1.98).

Feed efficiency (gain: feed) in T1 (0.63) was higher than T4 (0.50), but did not differ from T2 (0.55) and T3 (0.57) at starter phase. However, at finisher phase, T2 had the highest feed efficiency value. Protein intake (PI) in heat-stressed birds was not affected (P=0.58) by varying CP diets at starter phase, but not at finisher phase, where PI increased (P=0.00) with increasing dietary CP. Protein efficiency ratio (PER) was substantially affected by varying CP levels at starter and finisher phases with non-transverse effects observed with increasing levels of dietary CP. Higher CP levels in diets reduced (P=0.01) PER value at finisher phase, while at starter phase, an increase in PER was observed with increasing dietary CP.

#### **3.3 Haematology and Blood Glucose of Heat**stressed Broilers

Table 3 shows the haematology and blood glucose of heat-stressed broiler chickens on varying dietary protein at starter phase. Haemoglobin value, RBC, lymphocytes, heterophils, monocytes, eosinophils, basophils, platelets, heterophils: lymphocytes and blood glucose values of heat-stressed birds were not affected by varying dietary CP and values ranged from 9.18 to 10.25g/dL, 3.16 to 3.44 ( $10^6/\mu$ L), 9.84 to 11.50 ( $10^3/\mu$ L), 5.27 to 6.20 ( $10^3/\mu$ L), 0.51 to 0.64 ( $10^3/\mu$ L), 0.59 to 0.76 ( $10^3/\mu$ L), 0.03 to 0.06 ( $10^3/\mu$ L), 15.58 to 16.98 (x10<sup>4</sup>), 0.49 to 0.61, and 267.10 to 293.16mg/dL, respectively. However, PCV in T1 (31.00) and T4 (31.17) were higher compared to T3 (28.33), but did not differ from T2 (29.33). The lowest WBC value was observed in T1 (16.96), while T3 (19.07) had the highest WBC value.

Table 4 shows the haematology and blood glucose of heat-stressed broiler chickens on varying dietary protein

at finisher phase. Higher value of PCV was observed in T1 (29.33) compared to other dietary treatments. However, the T2 (26.83) and T3 (27.00) had similar PCV values. The Hb value observed in T1 (9.82) was higher compared to T2 (8.98), but was similar to T3 (9.03) and T4 (9.23). Higher basophils value was observed in birds on T4 (0.12)compared to T2 (0.00), but was similar to T1 (0.06) and T3 (0.03). Platelets count in birds on T2 (27.28) was higher compared to T1 (18.67) and T4 (16.36), but was similar to T3 (23.78). Birds on T2 (284.58) had higher GLU value compared to other dietary treatments. However, the RBC, WBC, lymphocytes, heterophils, monocytes, eosinophils, and H: L values were not substantially (P=0.94) affected by varying CP and values ranged from  $3.14 \text{ to} 3.49(10^6/$  $\mu$ L), 17.19 to 19.22 (10<sup>3</sup>/ $\mu$ L), 11.49 to 12.84 (10<sup>3</sup>/ $\mu$ L), 4.27 to 5.16 ( $10^{3}/\mu$ L), 0.42 to 0.56 ( $10^{3}/\mu$ L), 0.71 to 0.86 ( $10^{3}/\mu$ L) μL), and 0.38 to 0.43, respectively.

PCV-Packed cell volume, Hb- Haemoglobin concentration, RBC- Red blood cell, WBC- white blood cell, Lymp- Lymphocyte, Hetero-Heterophil, Mono-Monocyte, Eos- Eosinophils, Baso- Basophils, GLU- Glucose, SEM-Standard error of mean, P Value- probability, Dietary electrolyte balance: 240mEq/Kg.

#### **3.4 Correlations between Protein Intake Efficiency** and Blood Parameters of Heat-stressed Broilers

Table 5 shows the correlation between protein intake efficiency and blood parameters of heat-stressed birds at finisher phase. It was observed that the PCV was strongly and positively correlated with Hb (r=0.92, p<0.01), RBC (r= 0.76, p<0.01), and basophil (r= 0.48, p<0.01), but had a strong and negative correlation with WBC (r= -0.55, p<0.05), lymphocytes (r= -0.65, p<0.01), eosinophils (r= -0.34, p<0.01), platelets (r= -0.67, p<0.05), GLU (r= -0.41, p<0.01) and PER (r= -0.89, p<0.01). Hb was also positively correlated with PI (r= 0.69, p<0.01), PCV (r=0.92, p<0.01), RBC (r=0.86, p<0.01), monocytes (r= 0.69, p<0.01), and basophil (r=0.37, p<0.01). Lymphocytes had a strong and positive correlation with PI (r=0.09, p<0.01) and PER (r=0.32, p<0.05). Monocytes was strongly and positively correlated with PI (r=0.59, p<0.05), but was negatively correlated with PER (r= -0.47, p<0.01). However, monocyte was also strongly correlated with other blood parameters such as PCV, Hb, RBC, lymphocytes, eosinophils, basophils and blood glucose. The PI was positively correlated with PCV (r=0.55, p<0.05), Hb (r=0.69, p<0.01), RBC (r=0.89, p<0.01), heterophils (r= 0.58, p<0.05), monocytes (r=0.59, p<0.05), and H:L (r=0.71, p<0.05), but was strongly and negatively correlated with eosinophils (r = -0.73, p < 0.01), basophils (r = -0.38, p < 0.01) and PER (r = -0.78, p < 0.01).

		Starte		Finisher	phase			
Nutrients (%)	T1	T2	Т3	Τ4	T1	T2	Т3	T4
Crude protein	22.87	21.10	18.80	17.20	20.89	18.83	17.20	15.33
ME, kcal/kg	3062.08	3047.30	3046.63	3081.40	3038.90	3046.63	3083.08	3087.65
Ether extract	3.88	3.83	3.80	3.82	3.84	3.80	3.81	3.78
Crude fibre	3.45	3.44	3.28	3.08	3.49	3.28	3.08	2.93
Calcium	1.00	1.03	1.00	1.04	1.03	0.99	1.02	1.02
Total phosphorus	0.80	0.74	0.70	0.69	0.74	0.69	0.67	0.65
NPP	0.43	0.43	0.42	0.41	0.43	0.42	0.41	0.40
Ca:NPP	2.33	2.39	2.38	2.54	2.39	2.36	2.49	2.55

#### Table 1. Chemical composition (g/100g) of dietary treatments fed to heat-stressed broiler chickens

ME- Metabolizable energy, NPP-Non-phytate phosphorus, Ca- Calcium, CP- Crude protein

Phases	Parameters	T1	T2	Т3	T4	SEM	P Value
	FI (g/bird)	421.50	413.33	392.50	379.67	23.34	0.58
	BWG (g/bird)	261.83ª	228.11 <sup>ab</sup>	222.44 <sup>ab</sup>	187.16 <sup>b</sup>	18.32	0.07
Starter	FCR (g/g)	1.64 <sup>b</sup>	1.85 <sup>ab</sup>	1.83 <sup>ab</sup>	2.03 <sup>a</sup>	0.12	0.21
	Gain: Feed (g/g)	0.63 <sup>a</sup>	0.55 <sup>ab</sup>	0.57 <sup>ab</sup>	0.50 <sup>b</sup>	0.04	0.17
	PI (g/bird)	96.95	95.07	90.28	87.32	5.37	0.58
	PER (g/g)	2.73 <sup>a</sup>	2.39 <sup>ab</sup>	2.47 <sup>ab</sup>	2.17 <sup>b</sup>	0.17	0.17
	FI (g/bird)	1777.33 <sup>a</sup>	1617.83 <sup>b</sup>	1485.58°	1462.08 <sup>c</sup>	24.63	0.00
	BWG (g/bird)	866.08ª	869.08 <sup>a</sup>	757.50 <sup>b</sup>	623.08 <sup>c</sup>	40.57	0.00
Finisher	FCR (g/g)	2.11 <sup>b</sup>	1.87 <sup>c</sup>	1.98 <sup>bc</sup>	2.35 <sup>a</sup>	0.10	0.02
	Gain : Feed (g/g)	0.49 <sup>b</sup>	0.54 <sup>a</sup>	0.51 <sup>ab</sup>	0.43 <sup>c</sup>	0.02	0.02
	PI (g/bird)	373.24 <sup>ª</sup>	307.39 <sup>b</sup>	252.55 <sup>c</sup>	219.31 <sup>d</sup>	4.25	0.00
	PER (g/g)	2.32 <sup>b</sup>	2.83 <sup>a</sup>	2.99 <sup>a</sup>	2.85 <sup>a</sup>	0.12	0.01

Table 2. Performance of heat-stressed broilers on different dietary crude protein.

<sup>abed</sup> Means along a column with different superscripts differed significantly (P<0.05) using DMRT. Starter treatments (T1-23% CP; T2-21% CP; T3-19% CP; T3-17% CP), FI-Feed intake, BWG-Body weight gain, FCR-Feed conversion ratio, PI-Protein intake, PER-Protein efficiency ratio.

Crude protein (%)	PCV (%)	Hb (g/ dL)	RBC (106/µL)	WBC (103/µL)	Lymp (103/µL)	Hetero (103/µL)	Mono (103/ μL)	Eos (103/μL)	Baso (103/ μL)	Platelets (x 104)	GLU (mg/ dL)	Hetero: Lymp
T1 (23%)	31.00 <sup>a</sup>	10.00	3.44	16.96 <sup>b</sup>	9.84	5.99	0.51	0.59	0.06	16.49	267.10	0.61
T2 (21%)	29.33 <sup>ab</sup>	9.53	3.24	17.55 <sup>ab</sup>	10.91	5.27	0.64	0.67	0.06	15.58	282.91	0.51
T3 (19%)	28.33 <sup>b</sup>	9.18	3.31	19.07 <sup>a</sup>	11.50	6.20	0.57	0.76	0.03	16.98	293.16	0.54
T4 (17%)	31.17 <sup>a</sup>	10.25	3.16	17.97 <sup>ab</sup>	11.32	5.42	0.59	0.59	0.03	15.86	271.37	0.49
SEM	1.29	0.45	0.22	1.00	1.80	0.57	0.21	0.32	0.02	1.84	19.96	0.07
P Value	0.37	0.36	0.82	0.52	0.64	0.56	0.81	0.93	0.86	0.95	0.79	0.69

Table 3. Haematology and blood glucose	of heat-stressed broiler chickens on	varving dietary protein at starter phase
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PCV-Packed cell volume, Hb- Haemoglobin concentration, RBC- Red blood cell, WBC- white blood cell, Lymp- Lymphocyte, Hetero-Heterophil, Mono-Monocyte, Eos- Eosinophils, Baso- Basophils, GLU- Glucose, SEM- Standard error of mean, P Value-probability, Dietary electrolyte balance: 270mEq/Kg.

Crude protein (%)	PCV (%)	Hb (g/ dL)	RBC (106/µL)	WBC (103/µL)	Lymp (103/µL)	Hetero (103/µL)	Mono (103/μL)	Eos (103/ μL)	Baso (103/µL)	Platelets (x 104)	GLU (mg/dL)	Hetero:Lymp
T1 (21%)	29.33ª	9.82 <sup>a</sup>	3.49	17.63	11.49	4.82	0.56	0.71	0.06 <sup>ab</sup>	18.67 <sup>b</sup>	230.70 <sup>b</sup>	0.43
T2 (19%)	26.83 <sup>b</sup>	8.98 <sup>b</sup>	3.29	19.22	12.84	5.16	0.42	0.80	0.00 <sup>b</sup>	27.28 <sup>ª</sup>	284.58 <sup>ª</sup>	0.43
T3 (17%)	27.00 <sup>b</sup>	9.03 <sup>ab</sup>	3.14	17.99	11.87	4.83	0.51	0.75	0.03 <sup>ab</sup>	23.78 <sup>ab</sup>	225.70 <sup>b</sup>	0.41
T4 (15%)	27.83 <sup>ab</sup>	9.23 <sup>ab</sup>	3.22	17.19	11.52	4.27	0.43	0.86	0.12 <sup>ª</sup>	16.36 <sup>b</sup>	239.17 <sup>b</sup>	0.38
SEM	1.14	0.40	0.21	1.23	1.18	0.71	0.24	0.21	0.03	2.45	15.28	0.07
P Value	0.41	0.46	0.68	0.69	0.97	0.94	0.75	0.68	0.07	0.02	0.04	0.94

Table 4. Haematology and blood glucose of heat-stressed broiler chickens on varying dietary protein at finisher phase

<sup>ab</sup>Means for treatments within a column with no common superscript showed significant (P < 0.05) differences using DMRT.

Also, PER had a strong and negative correlation with PCV (r= -0.89, p<0.01), Hb (r= -0.88, p<0.01), RBC (r= -0.93, p<0.01), monocytes (r= -0.47, p<0.01), basophil (r= -0.18, p<0.01) and PI (r= -0.78, p<0.01) and positively correlated with eosinophils (r= 0.47, p<0.01).

### 4. Discussion

The extent of adaptation to heat stress conditions by poultry birds is dependent on the enhancing strategies adopted during thermal conditioning in chicks<sup>[21]</sup>. Popoola et al. <sup>[22]</sup> affirmed that tropical regions face more difficulty raising fast-growing chickens because birds suffer from physiological disorders that limit efficient productivity. Ideal DEB is prerequisite to blood acid-base balance and reduced incidences of hemodilution in heat-stressed broilers, and if supplied in adequate amount, there would be a substantial positive feedback <sup>[23]</sup>. Similarly, dietary proteins and their respective degraded segments (peptides and amino acids) may also affect physiological functions through the alteration of endogenous peptides stability. According to McGuinness et al. [24], components obtained from protein degradation in the digesta enhanced the release of cholecystokinin by inhibiting duodenal cholecystokinin-releasing factor break down. Trypsin inhibitor compounds increase cholecystokinin release and the interaction between protein-digested products and gut peptides also influence gastrointestinal tract functions and dictates the metabolic responses.

The results of current study corroborates the reports of Si *et al.* <sup>[25]</sup> who also noted reduced appetite in birds fed low-CP diets. Current findings are consistent with the reports of Yakout et al. [26] and Moustafa et al. [27] who noted improved feed conversion ratio in hens fed diets with high crude protein. Contrarily, Meluzzi et al. [28] and Bunchasak et al. [29] noted that feed intake and feed conversion ratio were not significantly affected by varying crude protein diets in laying hens at first stage of production cycle. In an attempt to optimize the use of dietary crude protein in poultry nutrition, as well as reducing the cost of feed and overall production process, indiscriminate use of dietary crude protein has resulted in diversity in choice of nutritional manipulations and detrimental outcomes. Current study affirms the reports of Zeng et al. [30], who opined that the role of nutritionists is to reduce the cost of feeds compounded while ensuring the efficiency of either low or high protein diets to meet amino acid standards, as proper nutrition will optimize production performance and reduce the adverse effects of the environment. Popoola et al. [31] noted that altered blood acid-base balance elevates body temperature and consequently reduce European production index in broilers. Therefore, avoiding excess supply of proteins and amino acids in feeds fed to broilers could help reduce nitrogen release in litters <sup>[32]</sup> and the amount of faecal nitrogen available for denitrification, while ensuring

	PCV	Hb	RBC	WBC	Lymp	Hetero	Mono	Eos	Baso	Plat	GLU	H:L	PI	PER
PCV		0.92**	0.76**	-0.55*	-0.65**	-0.31 <sup>ns</sup>	0.54 <sup>ns</sup>	-0.34**	0.48**	-0.67*	-0.41**	0.22 <sup>ns</sup>	0.55 <sup>ns</sup>	-0.89**
Hb	0.92**		0.86**	-0.47 <sup>ns</sup>	-0.61*	-0.17 <sup>ns</sup>	0.69**	-0.53 <sup>ns</sup>	0.37**	-0.59 <sup>ns</sup>	-0.40*	$0.32^{\text{ns}}$	0.69**	-0.88 <sup>ns</sup>
RBC	0.76**	0.86**		-0.04 <sup>ns</sup>	-0.18 <sup>ns</sup>	0.20 <sup>ns</sup>	0.50**	-0.55**	0.03 <sup>ns</sup>	-0.24 <sup>ns</sup>	0.04 <sup>ns</sup>	0.44 <sup>ns</sup>	0.89**	-0.93**
WBC	-0.55*	-0.47 <sup>ns</sup>	-0.04		0.97**	0.90**	-0.31*	-0.03 <sup>ns</sup>	-0.91 <sup>ns</sup>	0.95 <sup>ns</sup>	0.82**	0.51**	$0.28^{*}$	0.21 <sup>ns</sup>
Lymp	-0.65**	-0.61*	-0.18	0.97**		$0.78^{**}$	-0.52**	0.18*	-0.81 <sup>ns</sup>	0.91**	0.89 <sup>ns</sup>	0.34**	0.09**	0.32*
Hetero	-0.31 <sup>ns</sup>	-0.17 <sup>ns</sup>	0.20	0.90**	$0.78^{**}$		0.11 <sup>ns</sup>	-0.43 <sup>ns</sup>	-0.96 <sup>ns</sup>	0.89**	0.56**	0.69 <sup>ns</sup>	0.58 <sup>ns</sup>	-0.02 <sup>ns</sup>
Mono	$0.54^{*}$	0.69**	$0.50^{*}$	-0.31 <sup>ns</sup>	-0.52*	0.11 <sup>ns</sup>		-0.87**	-0.02*	-0.22 <sup>ns</sup>	-0.66*	0.34 <sup>ns</sup>	0.59*	-0.47**
Eos	-0.34 <sup>ns</sup>	-0.53*	-0.55*	-0.03 <sup>ns</sup>	0.18 <sup>ns</sup>	-0.43 <sup>ns</sup>	-0.87 <sup>ns</sup>		0.35*	-0.11 <sup>ns</sup>	0.37 <sup>ns</sup>	-0.49 <sup>ns</sup>	-0.73 <sup>ns</sup>	$0.47^{*}$
Baso	0.48 <sup>ns</sup>	0.37 <sup>ns</sup>	0.03	-0.91**	-0.81**	-0.96**	-0.02 <sup>ns</sup>	0.35 <sup>ns</sup>		-0.96**	-0.53**	-0.61**	-0.38 <sup>ns</sup>	-0.18 <sup>ns</sup>
Plat	-0.67**	-0.59*	-0.24	0.95**	0.91**	0.89**	-0.22**	-0.11*	-0.96 <sup>ns</sup>		0.63**	0.47**	0.15**	0.38*
GLU	-0.41 <sup>ns</sup>	-0.40 <sup>ns</sup>	0.04	0.82**	0.89**	0.56*	-0.66 <sup>ns</sup>	0.37 <sup>ns</sup>	-0.53 <sup>ns</sup>	0.63**		$0.26^{*}$	0.13 <sup>ns</sup>	0.08 <sup>ns</sup>
H:L	0.22 <sup>ns</sup>	0.32 <sup>ns</sup>	0.44	0.51*	0.34 <sup>ns</sup>	0.69**	0.34 <sup>ns</sup>	-0.49 <sup>ns</sup>	-0.61 <sup>ns</sup>	$0.47^{*}$	0.26 <sup>ns</sup>		0.71 <sup>ns</sup>	-0.43 <sup>ns</sup>
PI	$0.55^{*}$	0.69**	0.89**	0.28 <sup>ns</sup>	0.09 <sup>ns</sup>	$0.58^{*}$	0.59*	-0.73**	-0.38**	0.15 <sup>ns</sup>	0.13 <sup>ns</sup>	0.71*		-0.78**
PER	-0.89**	-0.88**	-0.93**	0.21 <sup>ns</sup>	0.32 <sup>ns</sup>	-0.02 <sup>ns</sup>	-0.47**	0.47**	-0.18**	0.38 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.43 <sup>ns</sup>	-0.78**	

\*\*P<0.01; \*P<0.05; ns- not significant.

broiler growth and efficient productivity <sup>[33]</sup>. The report of current study corroborates the assertions of Akhavan *et al.* <sup>[34]</sup> who reported that the interactive effect of ingested dietary proteins and glucose depends on some factors such as AA composition, digestion kinetics and AA utilization in the GIT. Krebs<sup>[35]</sup> noted that AA released from dietary proteins have direct and indirect effects on the metabolism of glucose. The direct effects are substrate-mediated, while the indirect effects are hormone-mediated. The observed differences in blood glucose levels in heat-stressed broiler chickens in present study could be well explained by Roden et al. <sup>[36]</sup> who noticed that an elevation of amino acids in the plasma led to an increase in the secretion and ratio of insulin and glucagon that affects hepatic glucose metabolism. Lynch et al. [37] noted that amino acids such as arginine and leucine are most potent in stimulating pancreatic insulin release and therefore decreasing plasma glucose concentration.

Corzo et al. [38] reported that minimizing excess dietary AA resulted in improved performance of broiler chickens when low crude protein diet was fed. Hindrichsen et al. <sup>[39]</sup> reported that altering feeding practices to achieve improved efficiency of nutrients in animals could result in significant reduction in the amounts of ammonia released during production and nitrous oxide released from animal manure during storage. Present findings agree with the observations of <sup>[40]</sup> who opined that diet is one of the factors influencing RBC and WBC in livestock. From current study, the protein intake had a strong and positive correlation with PCV, Hb, RBC, heterophils, monocytes and H: L, while PER had a strong and negative correlation with PCV, Hb, RBC, monocytes, basophil and PI. Present findings were consistent with the reports of <sup>[41]</sup> who noted that the reduction in the concentration of PCV in blood usually suggests the presence of a toxic factor, and it is worthy to note that relatively lower PCV value in heat-stressed broilers on low CP diets depicted an inefficient dietary protein utilization, owing to the strong negative correlation existing between PER and PCV and these suggest birds' physiological disposition to nutrition.

### 5. Conclusions

Unabsorbed amino acids reflect low dietary protein efficiency and dissociate into ions that metabolically alter blood ionic balance and physiological status of heatstressed broiler chickens. These metabolic excesses including heat dissipation from dietary protein degradation collectively influenced PCV, Hb, platelets and blood glucose of heat-stressed broilers.

#### References

- [1] Abbasi, M. A., Mahdavi A. H., Samie, A.H., and Jahanian, R. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. Braz. J. Poult. Sci., 2014, 16:35-44.
- [2] Beski, S. S. M., Swick, R. A., and Iji, P. A. Specialized protein products in broiler chicken nutrition: A review. Anim. Nutri., 2015, 1:47-53.
- [3] Cheeke, P. R. Applied Animal Nutrition: Feeds and Feeding. 3<sup>rd</sup> Edn., Pearson Prentice Hall, Upper Saddle River, USA., 2005. ISBN-13:9780131133310, pages: 604.
- [4] Lásztity, R. The Chemistry of Cereal Proteins, 1996, 2nd ed.; CRC Press: Boca Raton, FL, USA.
- [5] Fukushima, D. Soy Proteins. In Proteins in Food Processing; Yada, R.Y., Ed.; Woodhead Publishing: Cambridge, UK, 2004; pp. 100-122.
- [6] Yvon, M., Beucher, S., Guilloteau, P., Le Huerou-Luron, I., Corring, T. Effects of caseinomacropeptide (CMP) on digestion regulation. Reprod. Nutr. Dev. 1994, 34, 527-537.
- [7] Boye, J., Zare, F., and Pletch, A. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. Food Res. Int. 2010, 43, 414-431.
- [8] Torres, N., Torre-Villalvazo, I., and Tovar, A. R. Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. J. Nutr. Biochem. 2006, 17, 365-373.
- [9] Biolo, G., Tessari, P., Inchiostro, S., Bruttomesso, D., Fongher, C., Sabadin, L., Fratton, M.G., Valerio, A., Tiengo, A. Leucine and phenylalanine kinetics during mixed meal ingestion: A multiple tracer approach. Am. J. Physiol., 1992, 262, E455-E463.
- [10] Layman, D. K. and Baum, J. I. Dietary protein impact on glycemic control during weight loss. J. Nutr. 2004, 134, 968S-973S.
- [11] Popoola, O.R., Popoola, I.O., and Olusola, O. Preservative Effect of Newbouldia laevis (Boundary Tree) Leaf Extract on Shelf-Life of Fresh Chicken Meat under Tropical Conditions. Journal of Zoological Research, 2020a; Volume 2 (1):8-13.

DOI: https://doi.org/10.30564/jzr.v2i1.2015.

- [12] Popoola, I. O. Combating Heat Stress for Optimum Profitability in the 21st Century Poultry Farming Systems. Book Publisher International, First Edition, 2020, ISBN 978-93-90431-14-4 (Print); 978-93-90431-15-1 (eBook).
- [13] Popoola, I. O., and Iyayi, E. A. Response of Heat-

Stressed Broiler Chicks to Varying Dietary Electrolyte Balance at Pre-Starter and Starter Phases. Proceeding of 43rd Annual Conference of the Nigerian Society for Animal Production, Owerri, 18-22 March 2018, 189-191.

- [14] Popoola, I. O., Oshibanjo, D. O., Popoola, O. R., Okuneye, T. A., Ilaboya, I. I., Iyayi, E. A. Effect of Dietary Electrolyte Balance on Water Intake, Litter Moisture and Production of Broiler Chicks at Pre-Starter and Starter Phases. Open Journal of Animal Sciences, 2019; 9, 472-480. DOI: https://doi.org/10.4236/ojas.2019.94036.
- [15] Dein, F.J. Laboratory Manual of Avian Haematology. Association of Avian Veterinarian, East North Port, 1984.
- [16] Natt, P. M. and Herrik, C. A. A New Blood Diluent for Counting the Erythrocytes and Leucocytes of Chicken. Poultry Science, 1952, 31, 735-738. DOI: https://doi.org/10.3382/ps.0310735.
- [17] Dacie, J. U. and Lewis, S. M. Practical Haematology. Churchill Livingstone, London, 1975.
- [18] AOAC. Official methods of analysis, 2005, 18th edition, AOAC Inc. Arlington, VA.
- [19] Lacroix, R. L., Keeney, D. R., and Welsh, L. M. Potentiometric Titration of Chloride in Plant Tissue Extracts Using the Chloride Ion Electrode. Communications in Soil Science and Plant Analysis, 1970, 1, 1-6. DOI: https://doi.org/10.1080/00103627009366233.
- [20] Statistical Analysis System, 2012. SAS Users Guide: Statistics. SAS Institute Inc., Cary.
- [21] Popoola, I. O., Popoola, O. R., Olaleru, I. F., Busari, I. O., Oluwadele, F. J., Olajide, O. O. Early Thermal Acclimatization in Pre-Starter and Starter Chicks Fed Varying Crude Protein Diets Fortified with Optimum Electrolyte Balance. Central European Journal of Zoology, 2020b, Vol. 6 (1): 3-17. DOI: https://doi.org/10.13187/ceiz.2020.1.3.
- [22] Popoola, I.O., Popoola, O.R., Adeyemi, A.A., Ojeniyi, O.M., Olaleru, I.F., Oluwadele, F.J., Akinwumi, E.O. Overall Performance, Carcass Yield, Meat Safety Potentials and Economic Value of Heat-Stressed Broilers Fed Diets with Balanced Electrolytes. Food and Nutrition Sciences, 2020c, 11, 615-628. DOI: https://doi.org/10.4236/fns.2020.117044.
- [23] Popoola, I. O., Popoola, O. R., Ojeniyi, M. O., Olajide, O. O., Iyayi, E. A. The Roles of Key Electrolytes in Balancing Blood Acid-Base and Nutrient in Broiler Chickens Reared under Tropical Conditions. Natural Science, 2020d, 12, 4-11.

DOI: https://doi.org/10.4236/ns.2020.121002.

[24] McGuinness, E.E., Morgan, R.G., Levison, D. A.,

Frape, D. L., Hopwood, D., Wormsley, K.G. The effects of long-term feeding of soya flour on the rat pancreas. Scand. J. Gastroenterol. 1980, 15, 497-502.

- [25] Si, J., Fritts, C. A., Burnham, D. J., Waldroup, P. W. Extent to which crude protein may be reduced in corn-soybean meal broiler diets through amino acid supplementation. Int. J. Poult. Sci., 2004, 3:46-50.
- [26] Yakout, H. M., Omare, M. E., Marie, Y., and Hasan, R. A. Effect of incorporating growth promoters and different dietary protein levels into Mandarah hens Layers's diets. Egypt Poult. Sci. J., 2004, 24:977-994.
- [27] Moustafa, M., El-Kloulb, E.K., Hussein, A. A., and Gad El-Hak, M. K. A study on the energy and protein requirement of Mamoura local strain chickens during laying period. Egypt Poult. Sci., 2005, 25:637-651.
- [28] Meluzzi A., Sirri, F., Tallarico, N., and Franchini, A. Nitrogen retention and performance of Brown laying hens on diets with different protein content and constant concentration of amino acids and energy. Br. Poult. Sci., 2001, 42: 213-217.
- [29] Bunchasak, C., Poosuwan, K., Nukraew, R., Markvichitr, K., and Choothesa, A. Effect of dietary protein on egg production and immunity responses of laying hens during peak production period. Int. J. Poult. Sci., 2005, 4:701-708.
- [30] Zeng, Q. F., Cherry, P., Doster, A., Murdoch, R., Adeola, O., and Applegate, T. J. Effect of dietary energy and protein content on growth and carcass trait of Pekin ducks. Poult. Sci., 2015, 94:384-394.
- [31] Popoola, I. O., Popoola, O.R., Olajide, O.O., Adeyemi, A.A. and Alegbejo, Q.T. Reducing Sharp Fluctuations in Body Temperature and Optimizing Production Index of Broilers Using Dietary Electrolytes. Open Journal of Animal Sciences, 2020e, 10, 266-277.

DOI: https://doi.org/10.4236/ojas.2020.102015.

- [32] Soliva, C. R., Takahashi, J., and Kreuzer, M. Greenhouse Gases and Animal Agriculture: An Update. International Congress Series, 2006, The Netherlands: Elsevier.
- [33] Monteny, G. J., Bannink, A., and Chadwick, D. Greenhouse gas abatement strategies for animal husbandry. Agriculture, Ecosystems and Environment, 2006, 112, 163-170.

DOI: http://dx.doi.org/10.1016/j.agee.2005.08.015.

[34] Akhavan, T., Luhovyy, B. L., Brown, P. H., Cho, C. E., Anderson, G. H. Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults. Am. J. Clin. Nutr., 2010, 91, 966-975.

- [35] Krebs, M. Amino acid-dependent modulation of glucose metabolism in humans. Eur. J. Clin. Invest. 2005, 35, 351-354.
- [36] Roden, M., Perseghin, G., Petersen, K.F., Hwang, J.H., Cline, G.W., Gerow, K., Rothman, D.L., and Shulman, G.I. The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. J. Clin. Invest. 1996, 97, 642-648.
- [37] Lynch, C.J., Hutson, S.M., Patson, B.J., Vaval, A., and Vary, T.C. Tissue-specific effects of chronic dietary leucine and norleucine supplementation on protein synthesis in rats. Am. J. Physiol. Endocrinol. Metab. 2002, 283, E824-E835.
- [38] Corzo, A., McDaniel, C. D., Kidd, M. T., Miller, E. R., Boren, B. B., and Fancher, B. I. Impact of dietary amino acids concentration on growth, carcass yield and uniformity of broilers. Australian Journal of Agricultural Research, 2004, 55, 1133-1138. DOI: http://dx.doi.org/10.1071/AR04122.
- [39] Hindrichsen, I. K., Wettstein, H. R., Machmüller, A., and Kreuzer, M. Methane emission, nutrient degradation and nitrogen turnover in dairy cows and their slurry at different production scenarios with and without concentrate supplementation. Agriculture, Ecosystems and Environment, 2006, 113, 150-161. DOI: http://dx.doi.org/10.1016/j.agee.2005.09.004.
- [40] Talebi, A., Asri-Rezaer, S., Rozeh-Chai, R. and Sahraei, R. Comparative studies on haematology values of broiler strains (Ross, Cobb, Arbor-Acre and Arian). Int. Journal of Poultry Science, 2005, 4(8), 573-579.

DOI: http://dx.doi.org/10.3923/ijps.2005.573.579.

[41] Oyawoye, E.O. and Ogunkunle, M. Physiological and biochemical effects of raw jack beans on broilers. Proceedings of Annual Conference of Nigerian Society of Animal Production, 2008, 23:141-142.

#### Abbreviations

AA-Amino acids **Baso-Basophils BC-Broiler** chickens BWG-Body weight gain **CP-**Crude protein DEB-Dietary electrolyte balance EDTA- Ethylene diamine tetra-acetic acid **Eos-** Eosinophils FCR-Feed conversion ratio FI- Feed intake **FP-Finisher Phase** GIT-Gastro intestinal tract **GLU-** Glucose Hb-Haemoglobin Hetero-Heterophils, Lymp-Lymphocyte Mono-Monocytes PCV-Packed cell volume, PER-Protein efficiency ratio PI-Protein intake RBC- Red blood cells SEM- Standard error of mean, SP-Starter phase WBC-White blood cells