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Original Research Article

Blood profile as a health indicator in broiler chickens fed diets of different particle sizes supplemented with multienzyme

Damilola Uthman **Kareem**[1,4], Adedoyin Titi **Amos**[1], Olubukola Precious Adepeju **Idowu**[2,3], Lucas Pimentel **Bonagurio**[4], Olusegun Mark Obafemi **Idowu**[1]

[1]*Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, P.M.B 2240, Abeokuta, Nigeria*
[2]*Department of Animal Production and Health, College of Animal Science and Livestock Production, Federal University of Agriculture, P.M.B 2240, Abeokuta, Nigeria*
[3]*Agricultural Media Resources and Extension Centre, Federal University of Agriculture, P.M.B 2240, Abeokuta, Nigeria*
[4]*Department of Animal Science, São Paulo State University (UNESP), Faculty of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, 14884-900, Brazil*

Correspondence to:
D.U. Kareem, Department of Animal Nutrition, College of Animal Science and Livestock Production, Federal University of Agriculture, P.M.B 2240, Abeokuta, Nigeria; E-mail: kareemdu@funaab.edu.ng

Abstract

This study evaluated the health effects of feeding diets of different feed particle sizes supplemented with multienzyme to broiler chickens. Cobb500 (n = 450) broiler chicks (as hatched) were randomly distributed to nine treatments, with each treatment consisting of five replicates of ten birds. The experiment was arranged in a 3 × 3 factorial (three feed particle sizes [3, 4, and 5 mm] and three multienzyme supplementations [0, 1, and 2 g/kg]). The blood indices of the chickens were evaluated. The data were subjected to Analysis of Variance (ANOVA), and means were compared using the Tukey Test at a 5% probability level. The correlation between the growth performance and blood indices was also calculated. Birds fed the 3 mm particle sizes had decreased ($p < 0.05$) red blood cell (RBC) and lymphocyte counts with increased multienzyme at the starter phase, while the other haematological indicators increased as the multienzyme supplementation increased. Feeding the 4 mm particle size yielded similar ($p < 0.05$) total protein, triglyceride, and uric acid for all multienzyme inclusions. An increase in the multienzyme inclusion increased ($p < 0.05$) the RBC of birds fed the 5 mm particles with a decreased ($p < 0.05$) white blood cell (WBC) and eosinophil counts. At the finisher phase, serum albumin increased ($p < 0.05$) while alkaline phosphatase (ALP) decreased ($p < 0.05$), with increased multienzyme inclusion in birds fed the diet of 3 mm particles. Birds fed 4 mm particles had increased ($p < 0.05$) ALP and high-density lipoprotein (HDL) with increased multienzyme, while those fed the 5 mm particle size had increased ($p < 0.05$) albumin, aspartate transaminase (AST) as multienzyme supplementation increased. The correlation between growth performance and blood indices showed that feed intake (FI) has a significant ($p < 0.05$) impact on the blood profile of broiler chickens. The study concluded that other growth performance indicators, except the feed intake, have no impact on the blood profile of broilers. Feed particle sizes impact feed intake in broiler chickens which consequently impacts their blood composition. Therefore, the size of broiler feed particles has an impact on the health and well-being of the birds.

Keywords: Broiler health; correlation; feed intake; feed particle size; haematology; multienzyme; serum biochemistry

INTRODUCTION

Feeding has been reported to be a major determinant of the success of a poultry enterprise (Aguzey et al., 2018), as it represents 60 to 70% of the total cost of poultry production (Willems et al., 2003; Aliakbarpour et al., 2013). Therefore, it is important to put much emphasis on the types of feed given to birds to boost production (Attia et al., 2014ab; Al-Harthi et al., 2019). A study by Dozier et al. (2010) showed that maximum feed intake in poultry depends on several factors such as feed characteristics, environmental temperature, type of housing, and health status. Besides these factors, several researchers (Choct et al., 2004; Thorsten, 2011; Ball et al., 2015) also reported feed structure (particle size) and feed form (mash, pellets) to play important roles in the optimal nutrient utilisation in poultry.

According to Gentle (1979), birds distinguish the differences in feed particle size by mechanoreceptors located in the beak; it was thus suggested by Axe (1995) that both particle size and shape may influence birds' performance. Chickens are known to prefer larger feed particles (Schiffman, 1968), which is observed at all ages (Portella et al., 1988), and particle size preference is thought to increase with age (Nir et al., 1994a). However, while Nir et al. (1994a) observed a positive relationship between gizzard weight and particle sizes of feed, Jacobs et al. (2010) reported a decrease in weight gain and feed efficiency in broilers with an increase in the particle size of maize. Some previous studies (Douglas et al., 1990; Lott et al., 1992; Parsons et al., 2006; Xu et al., 2015; Kareem et al., 2022) have also reported that when young birds are fed large particles of maize as mash, there is a detrimental effect because the gizzard is not matured enough to grind coarse particles; however, this is believed to be remediable by the inclusion of enzyme cocktails (multienzyme) in the diet. According to Amerah et al. (2011), the effectiveness of enzymes in broiler chickens can be impacted by the sizes of the feed particles as well as their distribution. The bulk of the available studies on enzyme and feed particle size interaction were carried out on wheat (Wu et al., 2004; Peron et al., 2006) and literature on maize to this effect is relatively sparse (Amerah et al., 2011).

Several studies (Attia 2003; Meng et al., 2005; Attia et al., 2006; 2008; Costa et al., 2008) have been carried out on the individual effects of feed particle size or multienzyme supplementation on some parameters of broiler chickens. Kareem et al. (2022) observed that feed intake increased as the particle size of diets increased, which in turn negatively impacted the feed conversion ratio (FCR) of the birds when the particle size was perhaps too coarse at the starter phase; however, how this impacts their health is yet to be documented. Also, it has been shown that feed particle size affects feed intake (Dozier et al., 2010; Amerah et al., 2007; Kareem et al., 2022) at all ages (Portella et al., 1988), which in turn shows in nutrient deposition and consequently the health of the birds. Although these (health impacts) are postulations at the moment, there is a need to establish how feed particle sizes could impact the birds' health. The most common medium to determine the health status of the birds in nutritional studies is via blood profiles (Adeyemi et al., 2000). This study therefore aimed to establish that some growth performance parameters can explain how feed particle sizes impact broilers' health. It was hypothesised that the impact of feed particle sizes on broilers' health can be explained by the correlation between blood profile and some growth performance parameters.

MATERIALS AND METHODS

The trial was carried out at the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria (Google Earth, 2019). The procedures and methodologies implemented during this study were in agreement with the guidelines endorsed by the Project Review Committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State.

Experimental birds, management and diet

In this study, unsexed day-old Cobb500 broiler chicks (n = 450) were used. In the first 2 weeks, the birds were fed standard starter diets and were managed according to the guidelines recommended for broiler chickens by Cobb-Vantress (2018).

On day 14, the birds were randomly allotted in a 3 × 3 factorial arrangement to experimental groups of 50 birds per treatment, with each treatment having five replicates of ten birds each. The experimental units (replicates) contained wood shavings (litter), tubular drinkers, and feeders. The factorial design comprised three mash feed particle sizes (3, 4, and 5 mm; corresponding to 886.52, 1184.49, and 1329.13 µm GMD, respectively) and three multienzyme supplementations (0, 1, and 2 g/kg). Experimental diets and water were provided *ad libitum*. The diets were supplied for two weeks (weeks 3 and 4) for the starter and two weeks (weeks 5 and 6) for the finisher phases. The poultry house was open-sided, with the cover opened or closed depending on the ambient temperature. The diets fed to the birds in both phases are shown in Table 1. The procedures for the granulometric analyses of the feed particle sizes have been described in Kareem et al. (2022).

Table 1. Percentage composition of basal diets at starter and finisher phases

Ingredients (%)	Starter	Finisher
Maize	50.00	55.00
Soya Bean Meal	35.15	30.15
Fish meal (72%)	2.00	1.00
Wheat offal	7.00	7.50
Bone Meal	3.00	3.50
Limestone	2.00	2.00
Lysine	0.20	0.20
Methionine	0.20	0.20
Premix	0.25	0.25
Salt (NaCl)	0.20	0.20
Total	100	100
Calculated analysis		
M.E. (kcal/kg)	2772.75	2798.50
CP (%)	22.25	20.01
CF (%)	4.40	4.26
Fat (%)	3.99	3.92
Calcium (%)	1.88	2.03
Phosphorus (%)	0.85	0.89

Vitamin/Mineral premix: Vitamin A, 12,000,000 i.u; Vitamin D3, 2,500,000 i.u; Vitamin E, 30,000 i.u; Vitamin K, 2000 mg; Vitamin B1, 2,250 mg; Vitamin B2, 6,000 mg; Vitamin B6, 4,500 mg, Vitamin B12, 15 mcg; niacin, 40,000 mg; Pantothenic Acid, 15,000 mg; Folic Acid, 1,500 mg; Biotin, 50 mcg; Choline chloride, 300,000 mg; Manganese, 80,000 mg; Zinc, 50,000 mg; Iron, 20,000 mg; Copper, 5,000 mg; Iodine, 1,000 mg; Selenium, 200 mg; Cobalt, 500 mg; Antioxidant, 125,000 mg. M.E. – Metabolisable energy; CP – Crude protein; CF – Crude fibre

Multienzyme composition

The multienzyme used in this experiment was Multizyme Pro®; a product of XVet GmbH, Hamburg, Germany, containing xylanase, *β*-glucanase, *α*-amylase, and 6-phytase. It is a white, powdery substance packed in a 200 g sachet by the manufacturer. The composition of Multizyme Pro® as informed by the manufacturer is presented in Table 2.

Table 2. Composition of MultiZyme Pro®

S/N	Enzyme	Enzyme Commission (EC) Number	Composition
1	Xylanase	EC 3.2.1.8	7,000,000 U
2	*β*-Glucanase	EC 3.2.1.6	822,500 U
3	*β*-Glucanase	EC 3.2.1.4	1,400,000 U
4	*α*-Amylase	EC 3.2.1.1	140,000 U
5	6-Phytase	EC 3.1.3.26	35,000 FYT

Source: XVet GmbH, Hamburg, Germany

DATA COLLECTION

Haematology and serum biochemical indices

On 28 and 42 d post-hatch, two birds whose weights were close to the average weight of the group were randomly selected from each replicate. A blood sample (2.5 ml) was collected from the brachial vein of each bird into tubes containing ethylene diamine tetra-acetate (EDTA). The packed cell volume (PCV), red blood cell (RBC), and haemoglobin concentration (Hb) were determined according to the methods of Jain (1986). The white blood cell (WBC) and WBC differential counts were assessed following the method described by Coles (1989). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated from the haemoglobin concentration, RBC, and PCV values, as previously described by Duncan et al. (1994).

Following the same protocol as for haematology, another blood sample (2.5 ml) was collected from the brachial vein of each bird, into tubes that did not contain EDTA, for serum analyses, as detailed by Baker and Silverton (1985). The total serum protein, albumin, globulin, and creatinine of the blood samples were determined (Baker and Silverton, 1985), whereas the liver enzymes (alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)) were determined using the enzymatic colorimetric method of Schmidt and Schmidt (1975).

Statistical Analysis

Data were subjected to ANOVA in a 3 × 3 factorial arrangement using the SAS® ODA Software (SAS Institute Inc., USA). Mean comparisons were carried out using the contained in the software at a 5% significant level. Growth performance (Table 11) data from Kareem et al. (2022) were correlated with the blood variables (as a result of feed particle sizes), with permission, using Pearson Correlation of the SPSS software (SPSS ver. 20) to determine if and how the growth performance parameters affected the blood indices of the broiler chickens. Mean comparisons were carried out using the Tukey test contained in the software at a 5% significant level.

RESULTS

Main and interaction effects of feed particle sizes and multienzyme on haematological indices (starter phase)

The main effects of feed particle sizes and multienzyme on haematological indices of broiler chickens at the

Table 3. Main effects of feed particle sizes and multienzyme on haematological parameters of broiler chickens at starter phase

	Multienzyme (g/kg)					Particle sizes (mm)					Reference ranges
Variables	**0**	**1**	**2**	**SEM**	**p-value**	**3**	**4**	**5**	**SEM**	**p-value**	
PCV (%)	29.33	29.00	31.00	1.12	0.2233	30.33	29.67	29.33	1.14	0.6969	30–40*
Haemoglobin (g/L)	98.70	97.00	102.80	3.80	0.3368	100.7	99.5	9.84	3.80	0.8432	90–120*
RBC ($x10^{12}$/L)	2.45	2.40	2.60	0.10	0.1481	2.53	2.48	2.43	0.10	0.6200	2.5–3.2****
WBC ($x10^{9}$/L)	12.97	12.75	12.13	0.57	0.3413	14.75^{a}	11.08^{b}	12.02^{b}	0.58	0.0001	9–31****
Heterophil (%)	28.50	30.83	29.83	1.18	0.1847	29.67^{ab}	31.50^{a}	28.00^{b}	1.13	0.0328	25–30****
Lymphocyte (%)	68.17	66.67	67.67	0.93	0.4089	67.33	66.17	69.00	0.85	0.0615	55–60****
Het : Lymph	0.42	0.46	0.44	0.02	0.2018	0.44^{ab}	0.48^{a}	0.41^{b}	0.02	0.0299	
Eosinophil (%)	1.00	1.17	0.67	0.32	0.5407	1.00	0.33	1.50	0.31	0.0574	0–2**
Basophil (%)	0.33	0.83	0.83	0.15	0.1342	0.67	1.00	0.33	0.14	0.0751	0–5**
Monocyte (%)	2.00^{a}	0.50^{b}	1.00^{b}	0.27	0.0029	1.33	1.17	1.00	0.27	0.6818	0–3**
MCV (fl)	119.82	120.85	119.30	0.62	0.2102	119.72	119.52	120.73	0.69	0.3348	81.60–89.10***
MCH (pg)	40.35^{a}	40.42^{a}	39.55^{b}	0.26	0.0183	39.73	40.08	40.50	0.27	0.0651	27.20–28.90***
MCHC (%)	33.65^{a}	33.45^{b}	33.17^{c}	0.09	0.0001	33.18^{b}	33.55^{a}	33.53^{a}	0.10	0.0001	32.41–33.37***

$^{a,b,c;}$ *Means with different superscripts along the same row are significantly ddifferent ($p < 0.05$)*
SEM: Standard Error of the Mean
PCV: Packed Cell Volume, RBC: Red Blood Cell, WBC: White Blood Cell, Het : Lymph; Heterophil : Lymphocyte ratio, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration
* Nanbol et al. (2016); ** Miesle (2011); *** Wikivet (2013); **** Swenson (1970)

starter phase are presented in Table 3. Basophils, monocytes, MCH, and MCHC were significantly (*$p < 0.05$*) affected by the supplementation of multienzyme in the diets of broiler chickens at graded inclusions. MCHC decreased ($p < 0.05$) with an increase in multienzyme supplementation. The WBC, heterophil, heterophil: lymphocyte counts, and MCHC were all affected ($p < 0.05$) by the varying particle sizes of feeds. While WBC was higher ($p < 0.05$) for birds fed 3 mm feed particle size, heterophil and heterophil:lymphocyte had the highest ($p < 0.05$) values for birds fed 3 mm and 4 mm feed particle sizes. It was also observed that feeding 3 mm particle sizes to the birds yielded lower ($p < 0.05$) MCHC.

Table 4. Interaction effect of feed particle sizes and multienzyme on haematological parameters of broiler chickens at starter phase

Particle size (mm)	**3**			**4**			**5**			**SEM**	**p-value**	**Reference ranges**
Multienzyme (g/kg)	**0**	**1**	**2**	**0**	**1**	**2**	**0**	**1**	**2**			
Treatment	**3P0E**	**3P1E**	**3P2E**	**4P0E**	**4P1E**	**4P2E**	**5P0E**	**5P1E**	**5P2E**			
PCV (%)	31.45	30.00	29.50	30.50	29.00	29.50	26.00	28.00	34.00	1.03	0.0638	30–40*
Haemoglobin (g/L)	105.00	100.50	96.50	102.50	97.50	98.50	88.50	93.00	113.50	3.30	0.0780	90–120*
RBC ($\times 10^{12}$/L)	2.65^{ab}	2.50^{abc}	2.45^{abc}	2.55^{abc}	2.40^{bc}	2.50^{abc}	2.15^{c}	2.30^{bc}	2.85^{a}	0.10	0.0428	2.5–3.2****
WBC ($\times 10^{9}$/L)	14.90^{a}	14.40^{ab}	14.95^{a}	10.35^{c}	12.40^{bc}	10.50^{c}	13.65^{bc}	11.45^{c}	10.95^{c}	0.90	0.0002	9–31****
Heterophil (%)	27.50	29.50	32.00	31.50	33.50	29.50	26.50	29.50	28.00	1.22	0.0660	25–30****
Lymphocyte (%)	69.00	67.50	65.50	65.50	65.50	67.50	70.00	67.00	70.00	1.19	0.1402	55–60****
Het : Lymph	0.40	0.44	0.49	0.48	0.51	0.44	0.38	0.44	0.41	0.02	0.0596	
Eosinophil (%)	0.50	2.00	0.50	0.50	0.00	0.50	2.00	1.50	1.00	0.29	0.1597	0–2**
Basophil (%)	0.50	0.50	1.00	0.50	1.00	1.50	0.00	1.00	0.00	0.13	0.0730	0–5**
Monocyte (%)	2.50^{a}	0.50^{bc}	1.00^{abc}	2.00^{ab}	0.00^{c}	1.00^{abc}	1.50^{abc}	1.00^{abc}	1.00^{abc}	0.35	0.0415	0–3**
MCV (fl)	118.70	120.00	120.45	119.65	120.85	118.05	121.10	121.70	119.40	0.82	0.3262	81.60–89.10***
MCH (pg)	39.60^{b}	40.20^{ab}	39.40^{b}	40.20^{ab}	40.65^{ab}	39.40^{b}	41.25^{a}	40.40^{ab}	39.85^{b}	0.36	0.0364	27.20–28.90***
MCHC (%)	33.30^{de}	33.50^{bcd}	32.75^{e}	33.60^{bc}	33.65^{b}	33.40^{bcde}	34.05^{a}	33.20^{e}	33.50^{cde}	0.09	0.0001	32.41–33.37***

a,b,c,d; Means with different superscripts along the same row are significantly different ($p < 0.05$)
SEM: Standard Error of the Mean
PCV: Packed Cell Volume, RBC: Red Blood Cell, WBC: White Blood Cell, Het : Lymph; Heterophil:Lymphocyte ratio, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration
* Nanbol et al. (2016); ** Miesle (2011); *** Wikivet (2013); **** Swenson (1970)
3P0E: 3 mm feed particle with 0 g/kg multienzyme, 3P1E: 3 mm feed particle with 1 g/kg multienzyme, 3P2E: 3 mm feed particle with 2 g/kg multienzyme, 4P0E: 4 mm feed particle with 0 g/kg multienzyme, 4P1E: 4 mm feed particle with 1 g/kg multienzyme, 4P2E: 4 mm feed particle with 2 g/kg multienzyme, 5P0E: 5 mm feed particle with 0 g/kg multienzyme, 5P1E: 5 mm feed particle with 1 g/kg multienzyme, 5P2E: 5 mm feed particle with 2 g/kg multienzyme

Table 5. Main effects of feed particle sizes and multienzyme on haematological parameters of broiler chickens at finisher phase

Parameters	Multienzyme (g/kg)					Particle sizes (mm)					Reference ranges
	0	1	2	SEM	p-value	3	4	5	SEM	p-value	
PCV (%)	36.67	34.33	37.55	0.91	0.0821	38.17[a]	37.00[a]	33.33[b]	0.94	0.0063	32–45*
Haemoglobin (g/L)	123.20	115.00	124.80	3.00	0.0979	127.70[a]	124.20[a]	111.20[b]	3.10	0.0049	90–120*
RBC (×10¹²/L)	3.12	2.88	3.13	0.08	0.0889	3.22[a]	3.13[a]	2.78[b]	0.08	0.0042	2.5–3.2****
WBC (×10⁹/L)	10.88	11.50	11.70	0.71	0.5792	12.00	10.93	11.15	0.46	0.3917	9–31****
Heterophil (%)	27.33	29.33	28.33	0.99	0.3216	28.00	28.33	28.67	0.91	0.8752	25–30****
Lymphocyte (%)	70.00[a]	66.33[b]	68.50[ab]	0.88	0.0190	68.33	68.83	67.67	0.81	0.6134	55–60****
Het : Lymph	0.39	0.44	0.42	0.02	0.1366	0.41	0.41	0.43	0.02	0.7890	
Eosinophil (%)	0.83	1.00	1.33	0.34	0.3526	1.83[a]	0.83[b]	0.50[b]	0.30	0.0029	0–2**
Basophil (%)	0.67	0.33	0.50	0.21	0.1938	0.67[a]	0.67[a]	0.17[b]	0.21	0.0146	0–5**
Monocyte (%)	1.17	1.33	1.33	0.26	0.8679	1.17	1.33	1.33	0.34	0.8679	0–3**
MCV (fl)	117.75	119.12	119.77	0.60	0.0541	118.13	118.68	119.82	0.60	0.1195	81.60–89.10***
MCH (pg)	39.55	39.87	39.88	0.23	0.5053	39.70	39.65	39.95	0.25	0.6036	27.20–28.90***
MCHC (%)	33.58[a]	33.47[ab]	33.28[b]	0.10	0.0179	33.45	33.55	33.33	0.10	0.1011	32.41–33.37***

a,b; Means with different superscripts along the same row are significantly different (p < 0.05)
SEM: Standard Error of the Mean
PCV: Packed Cell Volume, RBC: Red Blood Cell, WBC: White Blood Cell, Het : Lymph; Heterophil:Lymphocyte ratio, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration
* Nanbol et al. (2016); ** Miesle (2011); *** Wikivet (2013); **** Swenson (1970)

Table 4 shows that there were significant ($p < 0.05$) effects of the interaction of feed particle sizes and multienzyme supplementation on all the variables measured, across the treatments, except the PCV, haemoglobin, heterophil, lymphocyte, het:lymph, eosinophil, and MCV. Birds fed diets of 3 mm particles had decreased ($p < 0.05$) RBC with an increase in multienzyme inclusion, while basophil and MCV increased as the multienzyme supplementation increased. An increase in multienzyme supplementation increased ($p < 0.05$) the values of RBC for birds fed diets of 5 mm particle size, and a decrease ($p < 0.05$) was recorded for WBC with this increase. The changes of other variables did not follow any trend.

Main and interaction effects of feed particle sizes and multienzyme on haematological indices (finisher phase)

Multienzyme supplementation affected ($p < 0.05$) the lymphocyte and MCHC of the birds (Table 5). The lymphocyte count of the birds fed with 2 g/kg multienzyme supplementation was similar ($p < 0.05$) to that of those fed the diets containing 0 and 1 g/kg multienzyme supplementation while MCHC were higher ($p < 0.05$) for birds fed diets supplemented with 0 and 1 g/kg multienzyme supplementation. PCV, haemoglobin, RBC, eosinophil, and basophil counts were affected ($p < 0.05$) by the varying particle sizes of feeds. The birds fed 5 mm particle size had consistently lower ($p < 0.05$) PCV, haemoglobin, RBC and basophil counts than those of 3 and 4 mm feed particle size. However, the 3 mm particle size group had a higher ($p < 0.05$) eosinophil count than the others.

The interaction of feed particle sizes and multienzyme affected ($p < 0.05$) all the haematological indices considered except for MCH and monocytes whose values across the varying treatments were not significantly ($p > 0.05$) affected (Table 6). The PCV, haemoglobin, and RBC of the birds were similar across almost all the treatments except for birds fed 5 mm particle size and supplemented with 1 g/kg multienzyme which was distinctly lower compared to the others. Basophils were ($p < 0.05$) highest for birds fed diets of 3 mm particle size with 0 g/kg multienzyme up to 5 mm particle size with 0 g/kg multienzyme. Values recorded for heterophil and lymphocyte counts were similar across almost all the treatments. The discrepancy observed among all the significantly ($p < 0.05$) affected haematological parameters was observed to be minimal.

Main and interaction effects of feed particle sizes and multienzyme on serum biochemical indices (starter phase)

Multienzyme supplementation in the diets of the broiler chickens influenced ($p < 0.05$) the globulin, glucose, ALT, total cholesterol, triglyceride, high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) as shown in Table 7. Globulin and LDL were higher ($p < 0.05$) for birds fed diets supplemented with 0 g/kg multienzyme, while those fed 1 and 2 g/kg multienzyme recorded similar values. Birds fed diets supplemented with 0

Table 6. Interaction effect of feed particle sizes and multienzyme on haematological parameters of broiler chickens at finisher phase

Particle size (mm)	3			4			5			SEM	p-value	Reference ranges
Multienzyme (g/kg)	0	1	2	0	1	2	0	1	2			
Treatment	3P0E	3P1E	3P2E	4P0E	4P1E	4P2E	5P0E	5P1E	5P2E			
PCV (%)	38.50^{ab}	35.50^{ab}	40.50^{a}	36.00^{ab}	37.50^{ab}	37.50^{ab}	35.50^{ab}	30.00^{c}	34.50^{b}	0.72	0.0221	30–40*
Haemoglobin (g/L)	129.50^{ab}	118.50^{ab}	135.00^{a}	122.00^{ab}	126.50^{ab}	124.00^{ab}	118.00^{ab}	100.00^{c}	115.50^{bc}	2.40	0.0200	90–120*
RBC (×10^{12}/L)	3.30^{ab}	3.00^{ab}	3.35^{a}	3.05^{ab}	3.15^{ab}	3.20^{ab}	3.00^{ab}	2.50^{c}	2.85^{bc}	0.06	0.0205	2.5–3.2****
WBC (×10^{9}/L)	10.75	11.95	13.30	10.85	12.00	9.95	11.05	10.55	11.85	0.33	0.4354	9–31****
Heterophil (%)	28.50^{abcd}	32.00^{ab}	23.50^{d}	27.50^{bcd}	29.00^{abc}	28.50^{abcd}	26.00^{cd}	27.00^{bcd}	33.00^{a}	0.69	0.0153	25–30****
Lymphocyte (%)	68.50^{ab}	65.50^{b}	71.00^{a}	69.50^{ab}	68.50^{ab}	69.0^{ab}	72.00^{a}	65.50^{b}	65.50^{b}	0.42	0.0312	55–60****
Het : Lymph	0.42^{abc}	0.49^{ab}	0.33^{c}	0.40^{bc}	0.43^{abc}	0.41^{abc}	0.36^{c}	0.41^{abc}	0.50^{a}	0.01	0.0115	
Eosinophil (%)	2.00^{ab}	1.00^{bcd}	2.50^{a}	0.00^{d}	1.00^{bcd}	1.50^{abc}	0.50^{cd}	1.00^{bcd}	0.00^{d}	0.19	0.0064	0–2**
Basophil (%)	0.50^{ab}	0.50^{ab}	1.00^{a}	1.00^{a}	0.50^{ab}	0.50^{ab}	0.50^{ab}	0.00^{b}	0.00^{b}	0.09	0.0382	0–5**
Monocyte (%)	0.50	1.00	2.00	2.00	1.50	0.50	1.00	1.50	1.50	0.16	0.1815	0–3**
MCV (fL)	116.70	118.40	120.95	118.15	118.95	117.30	118.40	120.00	121.05	0.39	0.0503	81.60–89.10***
MCH (pg)	39.25	39.50	40.35	40.05	40.10	38.80	39.35	40.00	40.50	0.15	0.0843	27.20–28.90***
MCHC (%)	33.65^{ab}	33.35^{bcd}	33.35^{bcd}	33.90^{a}	33.70^{ab}	33.05^{d}	33.20^{cd}	33.35^{bcd}	33.45^{bc}	0.06	0.0019	32.41–33.37***

a,b,c,d,e; Means with different superscripts along the same row are significantly different ($p < 0.05$)
SEM: Standard Error of Mean
PCV: Packed Cell Volume, RBC: Red Blood Cell, WBC: White Blood Cell, Het : Lymph; Heterophil:Lymphocyte ratio, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration
* Nanbol et al. (2016); ** Miesle (2011); *** Wikivet (2013); **** Swenson (1970)
3P0E: 3 mm feed particle with 0 g/kg multienzyme, 3P1E: 3 mm feed particle with 1 g/kg multienzyme, 3P2E: 3 mm feed particle with 2 g/kg multienzyme, 4P0E: 4 mm feed particle with 0 g/kg multienzyme, 4P1E: 4 mm feed particle with 1 g/kg multienzyme, 4P2E: 4 mm feed particle with 2 g/kg multienzyme, 5P0E: 5 mm feed particle with 0 g/kg multienzyme, 5P1E: 5 mm feed particle with 1 g/kg multienzyme, 5P2E: 5 mm feed particle with 2 g/kg multienzyme

Table 7. Main effects of feed particle sizes and multienzyme on serum biochemical indices of broiler chickens at starter phase

Parameters	Multienzyme (g/kg)					Particle sizes (mm)					Reference ranges
	0	1	2	SEM	p-value	3	4	5	SEM	p-value	
Total protein (g/L)	57.80	54.50	55.30	2.50	0.1435	54.30	55.80	57.50	2.30	0.1927	46.30–48.10*
Albumin (g/L)	29.20	27.80	29.70	0.60	0.2849	27.30	29.20	30.20	0.80	0.0698	32.80–34.80*
Globulin (g/L)	28.20^{a}	26.30^{b}	25.80^{b}	2.50	0.0189	26.30	26.50	27.50	2.20	0.2927	11.50–15.30*
Glucose (mg/L)	1591.50^{a}	1295.20^{b}	1500.30^{ab}	78.40	0.0305	1309.70^{b}	1623.70^{a}	1453.70^{ab}	56.00	0.0248	720–1800***
Creatinine (mg/L)	13.20	12.70	11.50	0.80	0.7781	11.80^{ab}	9.00^{b}	16.50^{a}	0.80	0.0447	8.80–9.50*
Uric Acid (mg/L)	59.80	56.30	51.80	2.73	0.8325	65.50^{a}	38.30^{b}	64.20^{a}	2.81	0.0447	44.60–45.40*
AST (IU/L)	106.00	106.33	102.67	6.81	0.4745	196.50	101.67	106.83	8.41	0.2331	≤330**
ALT (IU/L)	26.33^{b}	28.00^{a}	26.33^{b}	1.62	0.0435	27.00	26.33	27.33	1.62	0.3704	
ALP (IU/L)	26.83	25.83	25.83	1.78	0.8314	28.83^{a}	22.33^{b}	27.33^{a}	1.68	0.0076	20–50***
T. Chol (mg/L)	1323.70^{a}	1094.50^{ab}	956.80^{b}	42.40	0.0150	1176.70	1169.70	1028.70	53.10	0.3583	
Trig. (mg/L)	771.20^{ab}	662.80^{b}	843.70^{a}	29.80	0.0295	828.20	706.80	742.70	30.00	0.1611	
HDL (mg/L)	724.50^{a}	619.20^{ab}	495.50^{b}	25.90	0.0187	628.30	654.80	560.50	34.40	0.4315	
VLDL (mg/L)	154.20^{ab}	132.50^{b}	168.80^{a}	6.00	0.0289	165.70	141.30	148.50	6.00	0.1598	
LDL (mg/L)	445.00^{a}	342.80^{b}	292.50^{b}	17.10	0.0129	387.20	373.50	319.70	21.50	0.3302	

a,b; Means with different ssuperscripts along the same row are significantly different ($p < 0.05$)
SEM: Standard Error of the Mean
AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, T. Chol: Total cholesterol, Trig.: Triglyceride, HDL: High Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, LDL: Low-Density Lipoprotein
* Wikivet (2012); ** Miesle (2011); *** Nanbol et al. (2016)

and 2 g/kg multienzyme recorded higher ($p < 0.05$) and similar glucose, triglyceride and VLDL, while those fed diets supplemented with 1 g/kg multienzyme recorded the highest ($p < 0.05$) ALT. Furthermore, differences ($p < 0.05$) were observed among the values recorded across the varying feed particle sizes for glucose, creatinine, uric acid, and alkaline phosphatase. The increase and/or decrease ($p < 0.05$) of all the variables with an increase in feed particle sizes were observed not to follow a particular trend. Birds fed the diet of 4 mm particle size had the lowest values of creatinine and alkaline phosphatase (ALP), but

Table 8. Interaction effect of feed particle sizes and multienzyme on serum biochemical indices of broiler chickens at starter phase

Particle size (mm)	**3**			**4**			**5**			**SEM**	**p-value**	**Reference ranges**
Multienzyme (g/kg)	**0**	**1**	**2**	**0**	**1**	**2**	**0**	**1**	**2**			
Treatment	**3P0E**	**3P1E**	**3P2E**	**4P0E**	**4P1E**	**4P2E**	**5P0E**	**5P1E**	**5P2E**			
Total protein (g/L)	51.50^{b}	55.00^{b}	56.50^{b}	57.50^{b}	55.00^{b}	55.00^{b}	64.50^{a}	53.50^{b}	54.50^{b}	0.09	0.0194	46.30-48.10*
Albumin (g/L)	26.00^{c}	27.50^{bc}	28.50^{bc}	27.50^{bc}	28.00^{bc}	32.00^{ab}	34.00^{a}	28.00^{bc}	28.50^{bc}	0.6	0.0215	32.80-34.80*
Globulin (g/L)	24.50^{de}	26.50^{bcd}	28.00^{abc}	29.50^{ab}	27.00^{bcd}	23.00^{e}	30.50^{a}	25.50^{cde}	26.50^{bcd}	0.5	0.0007	11.50-15.30*
Glucose (mg/L)	1196.50^{de}	1466.50^{abcd}	1266.00^{cde}	1814.50^{a}	1382.00^{bcde}	1674.50^{abc}	1763.50^{ab}	1037.00^{e}	1560.50^{abcd}	60.50	0.0045	720–1800***
Creatinine (mg/L)	9.50^{bc}	26.00^{a}	14.00^{bc}	14.50^{bc}	3.00^{c}	9.50^{bc}	15.50^{b}	9.00^{bc}	11.00^{bc}	1.50	0.0152	8.80-9.50*
AST (U/L)	102.00	111.00	106.50	105.50	98.50	101.00	110.50	109.50	100.50	1.41	0.2659	≤330**
ALT (U/L)	26.00	28.00	27.00	26.00	26.50	26.50	27.00	29.50	25.50	0.33	0.1048	
T. Chol (mg/L)	1464.50	1067.00	998.50	1307.00	1149.50	1052.50	1199.50	1067.00	819.50	51.60	0.1447	
Trig. (mg/L)	853.00^{ab}	607.00^{b}	1024.50^{a}	649.00^{b}	745.50^{b}	726.00^{b}	811.50^{ab}	636.00^{b}	780.50^{b}	31.90	0.0284	
Uric Acid (mg/L)	44.00^{abc}	64.50^{abc}	88.00^{a}	47.50^{abc}	27.00^{c}	40.50^{bc}	88.00^{a}	77.50^{ab}	27.00^{c}	5.90	0.0216	44.60-45.40*
ALP (U/L)	27.50^{a}	27.50^{a}	31.50^{a}	25.00^{ab}	24.00^{ab}	18.00^{b}	28.00^{a}	26.00^{a}	28.00^{a}	0.94	0.0440	20–50***
HDL (mg/L)	757.00	637.50	477.00	754.00	629.00	581.50	662.50	591.00	428.00	32.00	0.2064	
VLDL (mg/L)	170.50^{ab}	121.50^{b}	205.00^{a}	130.00^{ab}	149.00^{b}	145.00^{b}	162.00^{ab}	127.00^{b}	156.50^{ab}	6.40	0.0285	
LDL (mg/L)	537.00	308.00	316.50	423.00	371.50	326.00	375.00	349.00	235.00	22.20	0.0780	

a,b,c,d,e; Means with different superscripts along the same row are significantly different ($p < 0.05$)
SEM: Standard Error of the Mean
AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, T. Chol: Total cholesterol, Trig.: Triglyceride, HDL: High Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, LDL: Low-Density Lipoprotein
* Wikivet (2012); ** Miesle (2011); *** Nanbol et al. (2016)
3P0E: 3 mm feed particle with 0 g/kg multienzyme, 3P1E: 3 mm feed particle with 1 g/kg multienzyme, 3P2E: 3 mm feed particle with 2 g/kg multienzyme, 4P0E:
4 mm feed particle with 0 g/kg multienzyme, 4P1E: 4 mm feed particle with 1 g/kg multienzyme, 4P2E: 4 mm feed particle with 2 g/kg multienzyme, 5P0E: 5 mm feed particle with 0 g/kg multienzyme, 5P1E: 5 mm feed particle with 1 g/kg multienzyme, 5P2E: 5 mm feed particle with 2 g/kg multienzyme

interestingly, these same birds had the highest blood glucose concentration.

The interaction of feed particle sizes and multienzyme supplementation impacted ($p < 0.05$) the total protein, albumin, globulin, glucose, creatinine, triglyceride, uric acid, ALP, VLDL, and LDL (Table 8). The highest ($p < 0.05$) total protein was recorded for birds fed the 5P0E diet and was above the recommended reference values for broiler chickens. The albumin of the birds, however, was highest ($p < 0.05$) for birds fed diets of 4 mm particle size supplemented with 2 g/kg multienzyme and 5 mm particle size supplemented with 0 g/kg multienzyme. While creatinine was highest ($p < 0.05$) for those fed 3 mm feed particle size supplemented with 1 g/kg multienzyme, other significantly affected variables were similar for almost all the other serum biochemical values considered in the study.

Main and interaction effects of feed particle sizes and multienzyme on serum biochemical indices (finisher phase)

The multienzyme supplementation in the diets had significant ($p < 0.05$) effects on albumin, creatinine, AST, ALT, total cholesterol, HDL and LDL of the birds (Table 9). The patterns of increase or decrease of the variables whose values were significantly ($p < 0.05$) affected by the multienzyme supplementation were observed to be inconsistent. Albumin and AST were higher ($p < 0.05$) for birds fed diets supplemented with 2 g/kg multienzyme, whereas the highest total cholesterol and LDL were recorded for birds fed diets supplemented with 1 and 2 g/kg multienzyme. There were significant ($p < 0.05$) differences among the values recorded across the varying feed particle sizes for total protein, globulin, glucose, AST, total cholesterol, ALP, and LDL. Total protein increased ($p < 0.05$) whereas the ALP decreased ($p < 0.05$) with an increase in the particle sizes of diets fed to the broiler chickens. Other significant ($p < 0.05$) variables were observed not to follow a particular pattern in increase and/or decrease.

The interaction had a significant ($p < 0.05$) effect on albumin, creatinine, AST, ALT, total cholesterol, triglyceride, uric acid, ALP, HDL, VLDL and low-density lipoprotein (LDL) of the birds (Table 10). Albumin was highest ($p < 0.05$) for birds fed 3 mm, 4 mm, and 5 mm feed particle sizes supplemented with 2 g/kg multienzyme. While AST was significantly ($p < 0.05$) highest for birds fed 3 mm particle size supplemented with 2 g/kg multienzyme, ALT on the other hand, was

Table 9. Main effects of feed particle sizes and multienzyme on serum biochemical indices of broiler chickens at finisher phase

Parameters	Multienzyme (g/kg)					Particle sizes (mm)					Reference ranges
	0	1	2	SEM	p-value	3	4	5	SEM	p-value	
Total protein (g/L)	53.70	52.70	58.70	1.30	0.1878	56.30^{ab}	59.30^{a}	49.30^{b}	1.40	0.0230	46.30-48.10*
Albumin (g/L)	32.00^{b}	32.60^{b}	35.70^{a}	0.90	0.0007	32.80	34.30	33.20	0.90	0.1947	32.80-34.80*
Globulin (g/L)	22.20	20.20	23.30	0.80	0.6586	23.50^{ab}	25.70^{a}	16.50^{b}	0.90	0.0413	11.50-15.30*
Glucose (mg/L)	1181.70	1164.30	1192.20	98.10	0.9527	1108.50^{b}	1066.50^{b}	1363.20^{a}	98.30	0.0083	720–1800***
Creatinine (mg/L)	117.00^{a}	88.00^{b}	103.00^{ab}	26.00	0.0139	103.00	97.00	108.00	24.00	0.4117	88.00-95.00*
AST (U/L)	121.33^{b}	119.67^{b}	162.17^{a}	2.34	0.0001	131.33^{b}	145.50^{a}	126.33^{b}	2.21	0.0047	≤330**
ALT (U/L)	23.00	20.67	24.33	0.51	0.1070	22.67	22.38	21.50	0.51	0.3866	
T. Chol (mg/L)	985.00^{b}	1231.50^{a}	1120.80^{a}	75.30	0.0041	1176.80	1035.70	1124.80	88.20	0.1076	
Trig. (mg/L)	1198.80	1157.80	1194.20	48.30	0.4519	1225.20	1151.30	1174.30	51.80	0.1237	
Uric Acid (mg/L)	133.80	144.50	175.00	10.50	0.2982	171.20	158.30	128.30	9.30	0.2107	44.60-45.40*
ALP (U/L)	27.17	24.83	28.33	1.66	0.2336	29.50	26.67	24.17	1.38	0.0506	20–50***
HDL (mg/L)	519.70^{b}	677.00^{a}	585.80^{b}	46.30	0.0019	625.20	561.00	596.30	54.80	0.2517	
VLDL (mg/L)	239.80	231.50	239.00	9.60	0.4388	245.20	230.20	235.00	10.40	0.1191	
LDL (mg/L)	225.50^{b}	323.00^{a}	296.00^{a}	33.00	0.0027	306.50	244.50	293.50	38.30	0.0508	

a,b; Means with different superscripts along the same row are significantly different ($p < 0.05$)
SEM: Standard Error of the Mean
AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, T. Chol: Total cholesterol, Trig.: Triglyceride, HDL: High Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, LDL: Low-Density Lipoprotein
* Wikivet (2012); ** Miesle (2011); *** Nanbol et al. (2016)

Table 10. Interaction effect of feed particle sizes and multienzyme on serum biochemical indices of broiler chickens at finisher phase

Particle size (mm)	3			4			5			SEM	p-value	Reference ranges
Multienzyme (g/kg)	0	1	2	0	1	2	0	1	2			
Treatment	3P0E	3P1E	3P2E	4P0E	4P1E	4P2E	5P0E	5P1E	5P2E			
Total protein (g/L)	55.50	51.00	62.50	58.00	56.50	63.50	47.50	50.50	50.00	1.60	0.1215	46.30-48.10*
Albumin (g/L)	31.50^{c}	32.00^{bc}	35.00^{ab}	33.40^{bc}	32.50^{bc}	37.00^{a}	31.00^{c}	33.50^{bc}	35.00^{ab}	0.50	0.0108	32.80-34.80*
Globulin (g/L)	24.00	19.00	27.50	25.50	24.00	27.50	17.00	17.50	15.00	1.50	0.3148	11.50-15.30*
Glucose (mg/L)	1098.50	957.50	1269.50	1109.00	1038.00	1052.50	1337.50	1497.50	1254.50	44.20	0.0566	720–1800***
Creatinine (mg/L)	11.00^{ab}	11.00^{ab}	9.00^{c}	10.00^{b}	9.50^{b}	9.50^{b}	14.00^{a}	6.00^{c}	12.50^{ab}	0.50	0.0032	8.80-9.50*
AST (U/L)	110.00^{de}	99.00^{e}	185.00^{a}	146.50^{bc}	127.00^{cd}	163.00^{b}	107.50^{de}	133.00^{c}	138.50^{c}	5.43	0.0001	≤330**
ALT (U/L)	21.00^{bc}	17.50^{c}	29.50^{a}	29.00^{a}	20.00^{bc}	22.50^{bc}	19.00^{bc}	24.50^{ab}	21.00^{bc}	0.96	0.0044	
T. Chol (mg/L)	1008.50^{bc}	1303.00^{a}	1219.00^{ab}	928.00^{c}	1104.00^{c}	1075.00^{abc}	1018.50^{bc}	1287.50^{a}	1068.50^{abc}	32.30	0.0332	
Trig. (mg/L)	1206.00^{abc}	1140.50^{bc}	1329.00^{a}	1161.50^{bc}	1100.00^{bc}	1182.50^{bc}	1229.00^{ab}	1223.00^{ab}	1071.00^{c}	18.30	0.0209	
Uric Acid (mg/L)	121.50^{bc}	113.00^{cd}	279.00^{a}	104.00^{cd}	261.00^{ab}	110.00^{cd}	176.00^{bc}	59.50^{d}	136.00^{cd}	16.40	0.0018	44.6-45.40*
ALP (U/L)	34.00^{a}	29.00^{ab}	25.50^{bc}	24.50^{bc}	25.00^{bc}	30.50^{ab}	23.00^{bc}	20.50^{c}	29.00^{ab}	1.03	0.0293	20–50***
HDL (mg/L)	534.50^{c}	730.50^{a}	610.50^{abc}	492.50^{c}	579.00^{bc}	611.50^{abc}	532.00^{c}	721.50^{ab}	535.50^{c}	20.10	0.0145	
VLDL (mg/L)	241.50^{abc}	228.00^{bc}	266.00^{a}	232.00^{bc}	222.00^{bc}	236.50^{bc}	246.00^{ab}	244.50^{ab}	214.50^{c}	3.70	0.0211	
LDL (mg/L)	232.50^{bc}	344.50^{a}	342.50^{a}	203.50^{c}	303.00^{ab}	227.00^{bc}	240.50^{bc}	321.50^{ab}	318.50^{ab}	13.20	0.0160	

a,b,c,d,e; Means with different superscript along the same row are significantly different ($p < 0.05$)
SEM: Standard Error of Mean
AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, T. Chol: Total cholesterol, Trig.: Triglyceride, HDL: High Density Lipoprotein, VLDL: Very Low Density Lipoprotein, LDL: Low Density Lipoprotein
3P0E: 3 mm feed particle with 0 g/kg multienzyme, 3P1E: 3 mm feed particle with 1 g/kg multienzyme, 3P2E: 3 mm feed particle with 2 g/kg multienzyme, 4P0E: 4 mm feed particle with 0 g/kg multienzyme, 4P1E: 4 mm feed particle with 1 g/kg multienzyme, 4P2E: 4 mm feed particle with 2 g/kg multienzyme, 5P0E: 5 mm feed particle with 0 g/kg multienzyme, 5P1E: 5 mm feed particle with 1 g/kg multienzyme, 5P2E: 5 mm feed particle with 2 g/kg multienzyme
* Wikivet (2012); ** Miesle (2011); *** Nanbol et al. (2016)

Table 11. Growth performance of broiler chickens fed varying particle sizes

Parameters	Particle size (mm)				
	3	4	5	SEM	p-value
Starter					
FW (g/b)	503.04[ab]	521.93[a]	474.19[b]	12.53	0.0122
WG (g/b)	378.66[ab]	397.34[a]	348.85[b]	12.55	0.0107
FI (g/b)	852.62[c]	872.28[b]	916.58[a]	5.01	0.0001
FCR	2.28[b]	2.20[b]	2.65[a]	0.08	0.0003
Mortality (%)	2.23	1.12	4.45	1.44	0.1256
Finisher					
FW (g/b)	1747.86	1819.76	1762.14	45.47	0.3363
WG (g/b)	1248.14	1320.05	1262.42	45.47	0.6008
FI (g/b)	2071.48[b]	2071.77[b]	2212.67[a]	11.55	0.0001
FCR	1.66	1.57	1.79	0.07	0.1824
Mortality (%)	0.00	0.00	0.00	0.00	NS

a,b,c; Means with different superscript along the same row are significantly different ($p < 0.05$)
SEM: Standard Error of the Mean
FW: Final weight, WG: Weight gain, FI: Feed intake, FCR: Feed conversion ratio

Table 12. Correlation between growth performance and haematological parameters of broiler chickens fed varying particle sizes at both starter and finisher phases

		PCV	Hb	RBC	WBC	HET	LYM	Het:Lym	EOS	BAS	MONO	MCV	MCH	MCHC
Starter														
FW	r[1]	−0.008	0.013	0.019	−0.066	−0.033	0.074	−0.052	−0.322	0.04	0.222	−0.197	−0.041	0.186
	p-value	0.970	0.949	0.924	0.743	0.871	0.713	0.797	0.102	0.843	0.265	0.325	0.838	0.353
WG	r	−0.014	0.006	0.014	−0.058	−0.029	0.068	−0.048	−0.321	0.049	0.222	−0.203	−0.047	0.186
	p-value	0.945	0.975	0.944	0.773	0.884	0.737	0.813	0.103	0.807	0.267	0.310	0.817	0.354
FI	r	−0.123	−0.090	−0.150	−0.409*	−0.327	0.376	−0.343	0.361	−0.250	−0.212	0.279	0.405*	0.337
	p-value	0.542	0.656	0.456	0.034	0.096	0.053	0.080	0.064	0.208	0.289	0.158	0.036	0.085
FCR	r	0.013	0.004	−0.015	−0.072	−0.036	0.028	−0.026	0.323	−0.128	−0.223	0.215	0.115	−0.088
	p-value	0.948	0.985	0.940	0.722	0.858	0.891	0.896	0.100	0.525	0.263	0.282	0.569	0.662
Finisher														
FW	r	0.045	0.038	0.062	−0.088	−0.103	0.133	−0.118	0.069	0.161	−0.087	−0.104	−0.117	−0.053
	p-value	0.825	0.849	0.759	0.664	0.610	0.510	0.558	0.731	0.422	0.666	0.606	0.561	0.793
WG	r	−0.006	−0.018	−0.015	−0.075	−0.093	0.082	−0.093	0.035	0.138	−0.062	0.071	0.002	−0.128
	p-value	0.978	0.928	0.941	0.709	0.643	0.685	0.643	0.862	0.494	0.759	0.724	0.992	0.525
FI	r	−0.541**	−0.551**	−0.562**	−0.122	0.238	−0.326	0.275	−0.404*	−0.629**	−0.022	0.272	0.150	−0.178
	p-value	0.004	0.003	0.002	0.543	0.232	0.097	0.164	0.037	0.000	0.913	0.170	0.454	0.374
FCR	r	−0.182	−0.177	−0.178	0.000	0.213	−0.188	0.216	−0.213	−0.378	−0.013	0.014	0.021	0.019
	p-value	0.364	0.377	0.374	0.999	0.285	0.348	0.278	0.287	0.052	0.948	0.946	0.916	0.925

PCV: Packed Cell Volume, Hb: Haemoglobin, RBC: Red Blood Cell, WBC: White Blood Cell, Het: Heterophil, Lym: Lymphocyte, Eos: Eosinophil, Bas: Basophil, Mono: Monocyte, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration
1r: Pearson correlation coefficient
** Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed).

highest for birds fed 3 mm particle size supplemented with 2 g/kg multienzyme, 4 mm particle size supplemented with 0 g/kg multienzyme, and 5 mm particle size supplemented with 1 g/kg multienzyme. Birds fed diets of 4 mm particle size recorded increased ($p < 0.05$) ALP and HDL as multienzyme supplementation increased. Birds on diets of 5 mm particle size had increased ($p < 0.05$) values for albumin, AST and VLDL as multienzyme supplementation increased while in those fed the 5 mm particle size with 1 g/kg multienzyme (5PE1) a critically low value for uric acid, compared with other treatments was recorded.

Correlation between growth performance and blood indices

The MCH of the birds was observed to have a significantly positive correlation ($p = 0.036$, $r = 0.405$) with FI at the starter phase (Table 12). However,

Table 13. Correlation between growth performance and serum parameters of broiler chickens fed varying particle sizes at both starter and finisher phases

		T. Pro	Alb	Glob	Gluc.	U. A	AST	ALT	T. Chol.	TRIG	CREAT	ALP	HDL	VLDL	LDL
Starter															
FW	r[1]	–0.070	–0.130	–0.045	0.057	–0.209	–0.240	–0.150	0.305	–0.157	–0.318	–0.340	0.295	–0.159	0.328
	p-value	0.730	0.517	0.822	0.779	0.296	0.228	0.454	0.122	0.433	0.107	0.083	0.136	0.429	0.095
WG	r	–0.071	–0.129	–0.050	0.062	–0.205	–0.245	–0.153	0.308	–0.151	–0.309	–0.338	0.295	–0.152	0.333
	p-value	0.726	0.521	0.806	0.760	0.304	0.217	0.445	0.118	0.454	0.116	0.084	0.135	0.449	0.090
FI	r	0.233	0.241	0.230	0.163	0.152	0.095	0.111	–0.365	–0.289	–0.138	–0.085	–0.257	–0.289	–0.393*
	p-value	0.243	0.226	0.249	0.417	0.448	0.638	0.583	0.061	0.144	0.491	0.672	0.195	0.144	0.043
FCR	r	0.076	0.144	0.057	–0.017	0.151	0.220	0.116	–0.332	0.037	0.225	0.268	–0.293	0.039	–0.360
	p-value	0.705	0.474	0.777	0.932	0.454	0.269	0.565	0.090	0.854	0.260	0.177	0.138	0.848	0.065
Finisher															
FW	r	0.192	0.210	0.160	0.114	–0.020	0.154	–0.035	–0.230	0.006	–0.046	0.131	–0.151	0.006	–0.336
	p-value	0.337	0.294	0.424	0.570	0.921	0.444	0.863	0.248	0.977	0.821	0.515	0.453	0.978	0.086
WG	r	0.152	0.310	0.082	0.165	–0.012	0.246	–0.007	–0.212	–0.031	–0.038	0.019	–0.163	–0.031	–0.262
	p-value	0.448	0.116	0.683	0.411	0.951	0.215	0.970	0.289	0.877	0.850	0.926	0.416	0.877	0.187
FI	r	–0.503**	0.033	–0.546**	0.407*	–0.023	–0.132	–0.080	0.108	–0.073	–0.314	–0.335	0.045	–0.070	0.216
	p-value	0.007	0.871	0.003	0.035	0.911	0.511	0.692	0.592	0.718	0.111	0.087	0.824	0.728	0.280
FCR	r	–0.357	–0.227	–0.324	–0.027	0.042	–0.263	–0.045	0.186	–0.001	–0.091	–0.154	0.110	0.000	0.289
	p-value	0.068	0.255	0.099	0.893	0.836	0.185	0.824	0.352	0.995	0.650	0.442	0.585	0.999	0.144

T. Pro: Total protein, Alb: Albumin, Glob: Globulin, Gluc: Glucose, U. A. Uric acid, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, T. Chol: Total cholesterol, Trig.: Triglyceride, HDL: High Density Lipoprotein, VLDL: Very Low Density Lipoprotein, LDL: Low Density Lipoprotein
[1]r: Pearson correlation coefficient
** Correlation is significant at the 0.01 level (2-tailed) *Correlation is significant at the 0.05 level (2-tailed)

at the finisher phase, the FI had a significantly negative correlation with PCV ($p = 0.004$, r = –0.541), haemoglobin ($p = 0.003$, r = –0.551), RBC ($p = 0.002$, r = –0.562), eosinophil ($p = 0.037$, r = –0.404), and basophil ($p = 0.000$, r = –0.629).

The Pearson correlation coefficients of growth performance and serum indices at both the starter and finisher phases are presented in Table 13. The LDL of the birds significantly decreased ($p = 0.043$, r = –0.393) as the FI of the birds increased during the starter phase. At the finisher phase, however, the FI recorded a significantly negative correlation with total protein ($p = 0.007$, r = –0.503) and globulin ($p = 0.003$, r = –0.546), and positively correlated with the glucose ($p = 0.035$, r = 0.407) values of the broiler chickens.

DISCUSSION

Studies have shown that serum and haematological profiles of birds can be used to understand their health and nutritional status (Adeyemi et al. 2000; Attia et al., 2014b). The effect of multienzyme in the current study, observed on the monocyte, MCH and MCHC of the birds at the starter phase conforms to the study of Attia et al. (2014b; 2020). Monocyte and MCHC values recorded for the birds were observed to lie within the reference range for healthy birds reported by Miesle (2011) and Wikivet (2013), respectively, while the MCH was higher than the recommended range value of Wikivet (2013). Although the MCH was higher than the recommendation, its decreased value with an increase in multienzyme supplementation could indicate that an increase in the supplementation to 2 g/kg regulates the haemoglobin in the blood of the chicks, since MCH is reported by Abdulazeez et al. (2016) to be an indicator of the haemoglobin carrying ability of the RBC. It could also indicate adequate haemoglobin content in the birds. MCHC was observed to decrease with an increase in multienzyme supplementation in the diets of the broiler chicks. The particle sizes of feed had been reported to affect several variables of the broiler chickens, but literature on its effect on blood indices is nearly non-existent. The pattern of increase and decrease of the haematological indices of the birds at their starting phase followed no particular trend, except for MCH which was significantly increased with an increase in the feed particle sizes. WBC, heterophil, lymphocyte, eosinophil and basophil counts fell within the normal blood ranges recommended by Swenson (1970) and Miesle (2011), respectively. The physiological WBC recorded could indicate that the feed particle size caused no infections in the chickens, as white blood cells (WBC is an important part of the body's defence against disease (Peter, 2002). The heterophil:lymphocyte ratio serves as a stress indicator in the blood profiles of broiler chickens, and the lower its value, the less stressed the birds seemed to be. The significantly reduced heterophil:lymphocyte ratio (0.41) recorded with birds fed 5 mm feed particles could be a result of the reduced period it took the birds to get the large particles they so desire since birds are reported to have a higher preference for larger particles (Nir et al., 1994b; Amerah et al., 2007). The WBC and heterophils of the birds as a result of the interaction between feed particle size and multienzyme supplementation fell within the recommended range value of Swenson (1970) while the eosinophil and basophil followed the recommendation of Miesle (2011). This is an indication of an absence of or reduced disease occurrence in the birds as a result of the diet. The haemoglobin content fell within the physiological range of blood indicators for healthy chicks reported by Nanbol et al. (2016) while the MCV and MCH recorded were higher than the recommended values of Wikivet (2013). MCH and MCHC according to Etim et al. (2013) indicate blood level conditions, its low level is an indication of anaemia (Aster, 2004). Hence, the high level exhibited in this study indicates that the birds were not anaemic. The reduced heterophil:lymphocyte ratio recorded across all treatments shows that the birds were not subjected to much stress as a result of the diets, since the heterophil:lymphocyte is a good index for stress in farm animals (Vleck et al., 2000; Attia et al., 2020).

The effect of multienzyme supplementation showed that the PCV fell within the normal haematological range reported by Nanbol et al. (2016) at the finisher phase, and this could imply better transportation of nutrients and oxygen as Packed Cell Volume reported by Isaac et al. (2013) to be involved in the transport of oxygen and absorbed nutrients. Lymphocyte and MCV were above the normal recommended range of Swenson (1970) and Wikivet (2013) respectively. This could indicate increased haemoglobin carrying capacity of the red blood cells (Abdulazeez et al., 2016) and reduced disease occurrence as a result of increased lymphocyte count as a result of multienzyme supplementation. The reason for the reduced packed cell volume (PCV), haemoglobin, RBC, eosinophil, and basophil with an increase in the feed particle size is a phenomenon that cannot be explained at the moment. However, the reduced PCV could be due to reduced nutrient intake by the birds, since it is said that packed cell volume (PCV) is involved in the nutrient transportation in the blood (Isaac et al., 2013). The lowest heterophil and heterophil:lymphocyte ratio recorded with birds fed the 3P2E diets due to the interaction between feed particle sizes and multienzyme could be an indication that the birds were less stressed compared to birds fed other diets.

Increased globulin which is above the recommended range of Wikivet (2012) observed in this study could be a result of dehydration as opined by Sabater and Forbes (2015) and could also be a result of adequate protein intake (Hochleithner, 2013; Al-Harthi et al., 2019). Supplementation of multienzyme can be said to have no hyper/hypoglycaemic effects on the birds since the serum glucose falls within the recommended range of Nanbol et al. (2016). Sabater and Forbes (2015) noted that elevated concentrations of serum glucose may be attributed to factors such as stress, postprandial starvation, pancreatitis, iatrogenic causes such as glucocorticosteroids or progesterone, or diabetes mellitus. Conversely, lowered serum glucose concentration could be associated with hepatic dysfunction, starvation in small birds, neoplasia, aspergillosis, or septicaemia. Some studies (Whitehead and Griffin, 1984; Whitehead et al., 1986) regarded plasma VLDL as a useful variable to infer the degree of fatness in chickens. These authors also stated that decreasing plasma VLDL, by any means, causes decreasing abdominal fat in broiler chickens. The lowest VLDL recorded with birds fed diets supplemented with 1 g/kg multienzyme could indicate the optimal level of inclusion for abdominal fat control in broiler chickens. Significant differences existed among the values recorded across the varying feed particle sizes for albumin, glucose, creatinine, uric acid, and alkaline phosphatase. An increase in ALP in broiler chickens has been associated (Sabater and Forbes, 2015) with increased osteoblastic activity (traumatic, neoplastic and/or infectious). Hence, the fact that the ALP values recorded in this study fall within the normal recommended range of Nanbol et al. (2016) indicates that the feed particle sizes evoked no traumatic or infectious reactions in the birds. Serum total protein and globulin measured for the broiler chickens were above the recommended range of Wikivet (2012). However, albumin fell below the recommended range of Wikivet (2012) for broiler chickens except for the birds fed diet 5PE0 which was within the normal range. A decrease in albumin according to Sabater and Forbes (2015) may be due to several reasons such as hepatic insufficiency, and renal disease (glomerulonephritis/sclerosis); however, the most probable reason could be the reduction in protein absorption as a result of fairly large particles and absence of protease in the multienzyme used. Glucose at all treatment levels was also in line with the recommendation of Wikivet (2012) with the only exception being the birds fed the 4PE0 diet. Hochleithner (2013) stated that several factors can influence blood concentration and increases may not be of clinical importance. The increased feed intake recorded in this study could have triggered the deposition of more fat that could be stored as triglyceride in adipose tissue of fat depots as stated by Crespo and Esteve-Garcia (2002). This could have been the reason for the increased triglyceride values above the recommended range of Nanbol et al. (2016).

At the finisher phase, the increase in albumin that occurred with increased multienzyme supplementation could be an indication that the supplement evoked better utilisation as it increased. The ALT recorded was within the recommendation of Miesle (2011). The cholesterol concentration had been reported by Sabater and Forbes (2015) to have an inconsistent significance in birds. Elevated concentration of this substance may stem from nutritional factors such as excessive dietary fatty acids, obesity, or postprandial effects, as well as egg yolk-related causes. On the other hand, decreased levels may be linked to conditions like starvation, liver disease, intestinal malabsorption/maldigestion, and *Escherichia coli* endotoxemia (Harr, 2006). Although the recommended cholesterol concentration for normal broiler chicken is not known, the birds did not seem to exhibit high abdominal fat when slaughtered, which could be an indication of normal serum cholesterol. Deposition of high body fat in broiler chickens may result in an economic loss to producers (Loh et al., 2011), as it is inefficient in terms of energy metabolism and overall feed utilization (Gaya et al., 2006). The lowest LDL recorded with birds fed diets of 4 mm particle size could indicate that 4 mm particle size evokes reduced the "bad cholesterol" concentration. The reason for recording the highest values of total protein, albumin, glucose, AST, ALT, triglyceride, uric acid, and LDL with birds fed diets of 3PE2 is unknown. Hochleithner (2013) reported that creatinine production is usually constant and is not affected much by the breakdown of dietary or tissue proteins. However, the observed values for the birds in this study were higher than the normal creatinine range of 0.88–0.95 mg/dl reported by Wikivet (2012), except for those fed the 3PE2, 4PE1, 4PE2, and 5PE1 diets. The elevated uric acid concentration recorded in this study may indicate reduced utilisation of protein as the particle size of the birds' diet increased. Furthermore, Hochleithner (2013) reported that ALT and AST are important enzymes involved in the interconversion of amino acids and oxoacids. AST activity is high in liver, skeletal muscle, heart, brain, and kidney cells. The activities of AST in this study were within the physiological range reported by Miesle (2011), indicating that the liver and muscles of the birds were healthy Hochleithner (2013).

Out of the four growth performance indicators considered for correlation with the blood indices, only the feed intake of the birds was observed to be significantly correlated with some of the blood indices. This could be an indication that as the feed particle sizes influence the feed intake, the feed intake in turn influences the blood chemical profile, hence making it a good health indicator. The increased MCH with increased feed intake indicates that haemoglobin concentration of the chickens (Etim et al., 2013) will increase with increased FI. The increased FI observed by Kareem et al. (2022) at the starter phase indicates that even if the feed particle is coarse, it would not impact the oxygen carrying capacity of the blood negatively.

The decrease in PCV and haemoglobin with increased FI could infer that although the birds consume more feed, they would not be able to utilise the nutrients if the particle size is too coarse, hence leading to reduced nutrient and oxygen intake (Isaac et al., 2013), and consequently reduced blood total protein. Although there may be reduced nutrient intake as a result of coarse feed particles, the birds still need to consume enough nutrients to continue normal bodily functions, hence it is understandable that the basophil and eosinophil counts will decrease with increased FI. Sabater and Forbes (2015) opined that increases in serum glucose may be due to stress or other factors. The increased glucose recorded with increased FI could indicate that the birds are stressed when they increase their FI as a result of the coarse feed particles. It is expected that increased FI resulting from the coarse feed particles should lead to increased LDL; however, the correlation coefficient value states otherwise. Perhaps the decrease in feed utilisation with increased feed particle size reduced the rate of fat metabolism, thus reducing the occurrence of blood LDL.

CONCLUSIONS

Increased multienzyme supplementation at the starter phase consistently decreased total cholesterol, high-density lipoprotein, and low-density lipoprotein concentrations of broiler chickens while the increase and decrease of the serum profile caused by the particle sizes of feed were inconsistent. It could therefore be concluded that supplementing coarse particles with multienzyme in young birds' diets could improve their blood profile. Furthermore, an increase or decrease in feed intake as a result of an increase or decrease in feed particle sizes has a significant impact on the birds' blood biochemical composition and consequently the birds' health.

CONFLICT OF INTERESTS

The authors declare no competing interest.

ETHICAL COMPLIANCE

The procedures and methodologies implemented during this study were in agreement with the guidelines endorsed by the Project Review Committee of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State.

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