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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (7): 325-331, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.325.331

Health Status of Birds Fed Diets Containing Three Differently Processed Discarded Vegetable-bovine Blood-rumen Content Mixtures

D.A. Ekunseitan, O.O. Balogun, O.M. Sogunle, A.O. Yusuf, A.A. Ayoola, L.T. Egbeyale, O.A. Adeyemi, I.B. Allison and A.I. Iyanda
Department of Animal Production and Health, Federal University of Agriculture, PMB 2240, Abeokuta, Nigeria

Abstract: This study was conducted to determine the effect of feeding three differently processed mixtures on health status of broilers. A total of 1080 day-old Marshal broilers were fed; discarded vegetable-fresh bovine blood-fresh rumen digesta (P1), discarded vegetable-ensiled bovine blood-fresh rumen digesta (P2) and discarded vegetable-fresh bovine blood-ensiled rumen digesta (P3) at three levels of inclusion (0, 3 and 6%). Data on blood parameters was taken and were subjected to 3×3 factorial arrangements in a completely randomized design. Birds fed P1 had least values ($p<0.05$) of serum glucose, total protein, globulin, uric acid and creatinine at starter phase. Birds fed diets containing 3 and 6% level of inclusion recorded the highest ($p<0.05$) Packed cell volume, Haemoglobin, White blood cell and Red blood cell values. However, those fed at 0% level of inclusion recorded the highest albumin value. At finisher phase, birds fed P2 and P3 had the highest glucose, uric acid and creatinine values. 6% level of inclusion significantly ($p<0.05$) increased the total protein and albumin values. Therefore, for enhanced performance and without comprising the health condition of birds; broiler chickens could be fed diets containing discarded vegetable-fresh bovine blood-ensiled rumen digesta (P3) up to 6% level of inclusion.

Key words: Health status, discarded vegetable, bovine blood, rumen content mixtures, processing methods

INTRODUCTION

Haematobiochemical indices are of immense importance in animal production, most especially in nutrition. The blood in the body (Ahmad *et al.,* 2003) mainly maintains physiological equilibrium but many physiological conditions may alter this equilibrium. Blood contains myriad of constituents provides a valuable medium both for clinical investigations and nutritional evaluation of the organism (Aderemi, 2004). Haematological and biochemical indices can be affected by nutrition, age, sex, genetics, physiological status, housing, starvation, environmental factors, stress, disease and transportation (Opara *et al.,* 2006). These effects can be evaluated by measuring physiological response variables because environmental and nutritional conditions are known to predispose animals to disease incidence and reduced productivity of animals. These deviations from normal healthy state alters body constituents of animals especially body fluids and health risk condition can well be understood by evaluation of blood components and its constituents. Incidence of disease and malnutrition are diagnosed by the deviation from the normal reference values of the various serum biochemical and haematological parameters measured depending on the case being investigated. Evaluation and interpretation of the results obtained are dependent on the reference values for each animal species, in those regions under existing environmental conditions (Otto *et al.,* 2000). These values are used as reference point for comparing and interpretation of metabolic state or condition of animals (Babatunde *et al.,* 1992). In Nigeria, the poultry industry is faced with scarcity of conventional feedstuffs. The culprit ingredient, soybean meal occupies an important place in the poultry industry. Its importance lies in the fact of being the reference standard for all protein source-ingredients. A shift in its availability will result in partially collapse in feedstuff production and a need in finding a suitable alternative. Therefore, evaluating the nutritional and potential of wholesome mixtures of the three waste materials with the aim of replacing and serving as an input in animal feedstuff production was inevitable. The vector relationship between animals, its environment and nutritional status is of great importance when setting up an animal production system. This study was aimed at

Corresponding Author: D.A. Ekunseitan, Department of Animal Production and Health, Federal University of Agriculture, PMB. 2240, Abeokuta, Nigeria

determining the level of the novel ingredients that commercial birds can tolerate when included in their diet without detrimental effect on their health status.

MATERIALS AND METHODS

Area depiction: The study was carried out from July-November 2011 at Alao Farms, Tanke-Akata, Ilorin, Kwara state, Nigeria located at an altitude of 1412 ft, longitude 4° 35'N and latitude 8° 30'E .

Experimental birds and management: A total of 1080, day old Hubbard strain broiler were used for the study. The birds were sourced from Obasanjo Farms, Igboora, Ogun state. The experiment was a 3×3 factorial experimental layout with factors due to processing methods and dietary levels of inclusion of mixtures in the diets. The inclusion of the three differently processed mixtures in the diet meal was at 3 levels: 0, 3 and 6% giving rise to 9 dietary treatment groups of 120 birds each and 3 replicates of 40 birds each.

Collection and preparation of processed mixtures: Discarded vegetables (*Amaranthus* sp.) which has wilted and undergoing decomposition was obtained from markets. Fresh blood and rumen content were collected from several cattle which have been physically examined and ascertained fit for slaughtering at Ipata market slaughter slab in Ilorin, Kwara state.

Preparation of mixtures: Discarded vegetable, fresh bovine blood and fresh rumen digesta (P1) The chopped *Amaranthus,* fresh bovine blood and bovine rumen digesta were weighed out (weight for weight) at a ratio of 1:1:1 and mixed. The mixture was cooked and stirred intermittently for duration of 2 h until the mixture was semi-dried. Stirring was done to prevent burning. After cooking, the mixture was sun dried to constant moisture content.

Discarded vegetable, ensiled bovine blood and fresh rumen digesta (P2): Freshly collected blood was covered up in a hermetic container for 3 days. It was then mixed with chopped *Amaranthus* and fresh bovine rumen digesta at a ratio of 1:1:1 in a drum. The mixture was subjected to similar cooking procedure as P1 above.

Discarded vegetable, fresh bovine blood and ensiled rumen digesta (P3): Freshly collected bovine rumen was obtained from bowels of cattle and ensiled in an hermetic container for 3 days. Ensiled rumen content was then mixed with chopped *Amaranthus* and fresh bovine blood at ratio 1:1:1 and subjected to similar cooking method as P1 above.

Experimental diets and management of birds: Nine experimental diets were formulated and used throughout the duration of the study. This experimental diets is shown in Table 1 and 2. The processed mixtures were used to replace soybean meal in diet to meet the nutrient requirement of birds at the starter and finisher phases. The birds were fed *ad libitum* and managed intensively throughout the duration of the experiment with necessary routine medications provided according. Housing

Table 1: Composition of broiler starter diets

	Replacement levels of soybean meal								
	1	2	3	4	5	6	7	8	9
Ingredient	0%	3%	6%	0%	3%	6%	0%	3%	6%
Maize	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
Wheat offal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Groundnut cake	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	17.50
Soybean meal	20.00	17.00	14.00	20.00	17.00	14.00	20.00	17.00	14.00
P1	-	3.00	6.00	-	-	-	-	-	-
P2	-	-	-	-	3.00	6.00	-	-	-
P3	-	-	-	-	-	-	-	3.00	6.00
Fish meal (72%)	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
*Vit./Min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Palm-Oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analysis									
Crude protein (%)	21.09	20.97	21.16	21.09	20.71	20.59	21.09	20.70	20.69
Crude fibre (%)	4.42	4.48	4.53	4.42	4.63	4.82	4.42	4.60	4.77
Ether extract (%)	6.78	4.23	6.03	6.78	4.43	4.71	6.78	4.81	7.41
**ME(MJ kg^{-1})	12.60	11.39	11.74	12.60	11.54	11.43	12.60	11.65	12.07

*Premix composition per kg diet: Vit A : 400000IU, Vit D: 80000IU, Vit E: 40000 ng, Vit k3: 800 mg, Vit B1:1000 MG, Vit B2: 6000 mg, Vit B6: 500 mg, VitB12: 25 mg, Niacin: 6000mg, Panthothenic acid: 2000 mg, Folic acid: 200 mg, Biotin: 8 mg, Manganese:300000 g, Iron: 8000 mg, Zinc: 20000 g, Cobalt:80 mg, Iodine: 400 mg, Selenium: 40 mg, Choline: 800000 g, **Estimated using the formula by Pauzenga (1985) i.e: ME(kcal kg^{-1}) = (37.7×CP%)+(81.8×EE%)+(35.5×NFE%). ME(MJ kg^{-1}) = ME(kcal kg^{-1})* (4.18/1000)

Table 2: Composition of broiler finisher diets

	Replacement levels of soybean meal								
	1	2	3	4	5	6	7	8	9
Ingredient	0%	3%	6%	0%	3%	6%	0%	3%	6%
Maize	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00
Wheat offal	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Groundnut Cake	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	15.00	12.75	10.50	15.00	12.75	10.50	15.00	12.75	10.50
P1	-	2.25	4.50	-	-	-	-	-	-
P2	-	-	-	-	2.25	4.50	-	-	-
P3	-	-	-	-	-	-	-	2.25	4.50
Fish meal (72%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
*Vit./Min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Palm oil	2.50	2.50							
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analysis									
Crude protein (%)	22.57	21.28	21.43	22.57	21.07	21.00	22.57	21.08	21.03
Crude fibre (%)	3.98	4.02	4.05	3.98	3.43	4.28	3.98	4.11	4.23
Ether extract (%)	4.05	5.85	6.40	4.05	4.65	4.92	4.05	5.71	5.71
**ME (MJ kg^{-1})	12.95	10.76	9.51	12.95	11.65	11.61	12.95	10.70	10.51

*Premix composition per kg diet: Vit A : 400000IU, Vit D: 80000IU, Vit E: 40000 ng, Vit k3: 800 mg, Vit B1: 1000MG, Vit B2: 6000 mg, Vit B6: 500 mg, VitB12: 25 mg, Niacin: 6000 mg, Panthothenic acid: 2000 mg, Folic acid: 200 mg, Biotin: 8 mg, Manganese: 300000 g, Iron: 8000 mg, Zinc: 20000 g, Cobalt: 80 mg, Iodine: 400 mg, Selenium: 40 mg, Choline: 800000 g, *Estimated using the formula by Pauzenga (1985) i.e: ME (kcal kg^{-1}) = (37.7×CP%)+(81.8×EE%) +(35.5×NFE%). ME (MJ kg^{-1}) = ME (kcal kg^{-1})* (4.18/1000)

protocols, handling the animals as well as study design were pre-approved by the faculty ethics committee of the Federal University of Agriculture, Abeokuta.

DATA COLLECTION

Blood Analysis: At the 4th and 8th weeks of the experiment, blood samples (2 mL each) were collected from each of 3 birds into Ethylene Diamine Tetra-Acetate (EDTA) bottles for haematological analysis and heparine tube for serum constituents. Blood samples were analyzed for Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC); Haemoglobin (Hb) concentration was determined using improved Neubauer haemocytometer, Wintrobes microhaematocrit and colorimetry cyanomethaemoglobin method respectively (Coles, 1986). All these methods are standard methods used for haematological parameters (Schalm, 1986). Serum total protein, albumin and globulin were analyzed colorimetrically using diagnostic reagent kit.

The mean corpuscular haemoglobin concentration, or MCHC, is a measure of the concentration of haemoglobin in a given volume of packed red blood cells. It was calculated using the formula (Van Beekvelt *et al.*, 2001):

$$\text{MCHC (g L}^{-1}) = \frac{\text{Hb}}{\text{PCV}}$$

The mean corpuscular haemoglobin (MCH), is the average mass of haemoglobin per red blood cell in a sample of blood. It was calculated using the formula (Van Beekvelt *et al.*, 2001):

$$\text{MCH (g Cell}^{-1}) = \frac{\text{Hb}}{\text{RBC}}$$

The Mean Corpuscular Volume (MCV), is a measure of the average red blood cell volume that is reported as part of a standard complete blood count. It was calculated using the formula (Tonnesen *et al.*, 1986):

$$\text{MCV (L Cell}^{-1}) = \frac{\text{PCV}}{\text{RBC}}$$

RESULTS

The result of the main effect of mixtures from the different processing methods and levels of inclusion on haemaological and serum biochemical parameters presented in Table 3 revealed no significant ($p>0.05$) differences recorded for birds fed the processed mixtures on most parameters measured except in glucose, total protein, uric acid and globulin values. Highest values (224.19, 48.56, 0.97 and 24.50 g dL^{-1}) were recorded for birds fed P3 for glucose, total protein, creatinine and globulin. However, uric acid was highest for birds fed P2

Table 3: Main effect of mixtures from the different processing methods and inclusion level on haematological and serum biochemical parameters of broiler chickens at starter phase

	Processed mixtures				Levels of inclusion				
Parameters	P1	P2	P3	SEM	0	1	30	SEM	P×LI
Packed cell volume (%)	28.33	27.44	22.89	2.94	19.67^{b}	28.33^{a}	30.67^{a}	2.37	NS
Haemoglobin (g dL^{-1})	9.76	9.96	7.79	0.84	7.51^{b}	9.58^{ab}	10.41^{a}	0.75	NS
White blood cell (cumm$^{2}\times10^{3}$)	26.33	25.59	26.58	0.85	23.38^{b}	27.09^{a}	28.03^{a}	0.49	NS
Red blood cell (X10^{12} L^{-1})	2.43	2.46	1.97	0.90	1.96^{b}	2.32^{ab}	2.58^{a}	0.18	NS
Glucose (mg dL^{-1})	158.50^{b}	177.52^{b}	224.19^{a}	9.77	187.19	174.03	199.03	12.65	NS
Total protein (g dL^{-1})	43.90^{b}	45.78^{b}	48.56^{a}	0.85	45.93	45.43	46.87	1.07	NS
Albumin (g dL^{-1})	23.99	23.04	24.06	0.53	24.84^{a}	22.87^{b}	23.47^{b}	0.41	NS
Globulin (g dL^{-1})	19.91^{b}	22.73^{ab}	24.50^{a}	0.36	21.09	22.66	23.40	0.35	NS
Uric Acid (g dL)	5.92^{b}	7.09^{a}	6.10^{ab}	1.05	6.54	5.91	6.66	1.19	NS
Creatinine (mg dL^{-1})	0.87^{b}	0.91^{ab}	0.97^{a}	0.03	0.90	0.91	0.93	0.02	NS
Cholesterol (mg dL^{-1})	175.07	181.19	186.07	3.46	185.76	176.26	180.31	3.31	*
Mean corpuscular volume (fl)	1.15	1.14	1.13	0.44	1.01	1.22	1.19	0.00	*
Mean corpuscular haemoglobin concentration (g dL^{-1})	35.24	39.28	34.18	2.13	40.96	33.77	33.97	1.67	NS
Mean corpuscular haemoglobin (pg)	4.01	4.20	3.87	0.24	3.89	4.14	4.04	0.20	*

Means in the same row by factor with different superscripts differ significantly ($p<0.05$), SEM: Standard Error Mean, P1: Discarded vegetable, fresh bovine blood and rumen digesta, P2: Discarded vegetable, ensiled blood and fresh rumen digesta, P3: Discarded vegetable, fresh bovine blood and ensiled rumen digesta, P×LI: Interaction of Processed mixtures and levels of inclusion, *: Significant, NS: Not significant

Table 4: Details of interaction between mixtures from the different processing methods and levels of inclusion on the haematological and serum biochemical parameters of broiler chickens at starter phase

	Processed mixtures									
	P1			P2			P3			
Parameter	0	3	6	0	3	6	0	3	6	SEM
Packed cell volume (%)	20.67^{ab}	29.67^{ab}	34.67^{a}	21.00^{ab}	31.33^{ab}	30.00^{ab}	17.330^{b}	24.00^{ab}	27.33^{ab}	4.78
Haemoglobin (g dL^{-1})	7.67^{ab}	9.87^{ab}	11.73^{a}	8.930^{a}	10.73^{ab}	10.20^{ab}	5.930^{b}	8.13^{ab}	9.30^{ab}	1.44
White blood cell (cumm$^{2}\times10^{3}$)	23.00^{c}	27.82^{a}	28.28^{a}	22.78^{c}	26.60^{ab}	27.38^{a}	24.350^{a}	26.85^{ab}	28.53^{a}	0.90
Red blood cell (X10^{12} L^{-1})	1.89^{ab}	2.45^{ab}	2.96^{a}	2.330^{ab}	2.56^{ab}	2.50^{ab}	1.660^{b}	1.95^{ab}	2.29^{ab}	2.99
Glucose (mg dL^{-1})	187.67^{d}	140.17^{d}	149.67^{cd}	1810^{bcd}	160.30^{cd}	190.47^{bcd}	193.970^{bc}	221.63^{ab}	256.97^{a}	15.58
Total protein (g dL^{-1})	45.37^{abc}	42.67^{c}	43.67^{bc}	45.07^{ab}	45.23^{abc}	47.03^{abc}	47.370^{abc}	48.40^{ab}	49.90^{a}	1.56
Albumin (g dL^{-1})	24.73^{abc}	23.00^{ab}	24.20^{a}	25.00^{a}	21.10^{b}	23.00^{ab}	24.700^{a}	24.20^{a}	23.20^{ab}	0.74
Globulin (g dL^{-1})	20.63^{ab}	19.67^{b}	19.43^{b}	20.00^{b}	24.13^{ab}	24.07^{ab}	22.630^{ab}	24.17^{ab}	26.70^{a}	1.88
Uric acid (g dL^{-1})	6.40^{ab}	5.17^{b}	6.20^{ab}	6.700^{ab}	7.37^{a}	7.20^{ab}	6.530^{ab}	5.20^{ab}	6.75^{ab}	0.64
Creatinine (mg dL^{-1})	0.90^{ab}	0.83^{b}	0.87^{ab}	0.870^{ab}	0.93^{ab}	0.93^{ab}	0.930^{ab}	0.97^{ab}	1.00^{a}	0.94
Mean corpuscular haemoglobin concentration (g dL^{-1})	38.72^{ab}	33.16^{b}	33.84^{b}	49.57^{a}	34.26^{b}	34.02^{b}	34.580^{b}	33.90^{b}	34.08^{b}	4.64

Means in the same row with different superscripts differ significantly($p<0.05$), SEM: Standard Error Mean

(7.09 g dL^{-1}). Although there was no significant difference ($p<0.05$) in cholesterol value, P3 recorded the highest value of 186.07 mg dL^{-1}. The levels of inclusion had significant effect ($p<0.05$) on PCV, haemoglobin, WBC, RBC and albumin. There were significant increases in haemoglobin, WBC and RBC values as the level of inclusion of mixtures increased with the least values of all three parameters obtained in birds fed at 0% inclusion and highest values obtained at 6% level of inclusion.

Table 4 shows the details of interaction between mixtures from the different processing methods and levels of inclusion on haematological and serum biochemical parameters of broiler chickens at the end of the starter Phase. All parameters measured were significantly ($p<0.05$) influenced by interaction between processed mixtures and levels of inclusion except the cholesterol level. Values obtained for PCV, WBC and Hb increased as the level of inclusion of each mixtures increased. Glucose and Serum total protein were statistically highest in birds placed on diet containing 6% inclusion of P3 (256.97 mg dL^{-1}, 49.90 g dL^{-1}) while the lowest values for the two indices were obtained at 3 % inclusion of P1. In addition, Serum total protein, creatinine and uric acid values were elevated as the level of inclusion of P2 and P3 increased from 0 to 6 % but a decline was observed in globulin as the level of inclusion of P1 was increased while a reverse trend was obtained in birds on P3.

Table 5 shows the main effect of mixtures from the different processing methods and levels of inclusion on haematological and serum biochemical parameters of broiler chickens at finisher phase. Processed mixtures had no significant effect ($p>0.05$) on all parameters measured except glucose, total protein, uric acid and creatinine. Values obtained for glucose, uric acid and creatinine were higher and comparable for P2 and P3 with birds fed P1 having lowest values for the three indices (226.58, 8.49

Table 5: Main effect of mixtures from the different processing methods and levels of inclusions on haematological and serum biochemical parameters of broiler chickens at finisher phase

	Processed mixtures				Levels of inclusion				
Parameter	P1	P2	P3	SEM	0%	3%	6%	SEM	P×LI
Packed cell volume (%)	25.89	23.56	26.44	2.66	25.22	28.78	21.89	2.03	*
Haemoglobin (g dL^{-1})	8.81	8.10	9.09	0.90	8.58^{ab}	9.92^{a}	7.50^{b}	0.77	*
White Blood Cell(cumm^2×10^3)	30.90	28.30	28.80	1.25	28.50^{b}	27.70^{b}	31.80^{a}	1.13	*
Red Blood Cell(X10^{12} L^{-1})	2.14	2.04	2.32		2.20	2.36	1.95	0.17	*
Glucose (mg dL^{-1})	226.58^{b}	271.34^{a}	267.16^{a}	10.21	263.79	238.69	262.60	12.04	*
Total Protein (g dL^{-1})	49.09^{c}	53.52^{b}	57.37^{a}	0.97	52.59^{b}	52.13^{b}	55.26^{a}	1.43	*
Albumin (g dL^{-1})	30.18	31.11	30.88	0.78	30.89^{ab}	29.28^{b}	32.00^{a}	0.68	NS
Globulin (g dL^{-1})	18.91	22.41	26.49	0.80	21.70	22.86	23.26	1.30	NS
Uric acid (g dL^{-1})	8.49^{b}	11.26^{a}	12.06^{a}	0.63	9.64	10.67	11.49	0.79	*
Creatinine (mg dL^{-1})	1.04^{b}	1.36^{a}	1.51^{a}	0.06	1.22	1.29	1.40	0.09	*
Cholesterol (mg dL^{-1})	212.16	212.37	198.27	7.30	212.40	211.03	208.36	8.38	*
Mean corpuscular volume(fl)	1.19	1.13	1.12	0.04	1.14^{ab}	1.22^{a}	1.09^{b}	0.00	NS
Mean corpuscular haemoglobin concentration (g dL^{-1})	34.00	34.44	34.40	0.22	34.05	34.54	34.25	0.17	*
Mean corpuscular haemoglobin (pg)	4.08	3.91	3.88	0.01	3.89^{ab}	4.23^{a}	3.76^{b}	0.00	*

Means in the same row by factor with different superscripts differ significantly at $p<0.05$, SEM: Standard Error Mean, P×LI: Processed mixtures by Levels of inclusion interaction, *: Significant, NS: Not significant

Table 6: Details of interaction between mixtures from the different processing methods and levels of inclusion on the haematological and serum biochemical parameters of broiler chickens at finisher phase

	Processed mixtures									
	P1			P2			P3			
Parameter	0	3	6	0	3	6	0	3	6	SEM
Packed cell volume (%)	23.00^{ab}	29.00^{ab}	25.67^{ab}	18.00^{b}	31.00^{ab}	21.67^{ab}	34.67^{a}	26.33^{ab}	18.33^{b}	3.91
Haemoglobin (g dL^{-1})	7.80^{ab}	9.93^{ab}	8.67^{ab}	6.13^{b}	10.57^{ab}	7.60^{ab}	11.77^{a}	9.27^{ab}	6.23^{b}	1.32
White blood cell (cumm^2×10^3)	31.00^{abc}	27.80^{bcd}	33.80^{a}	29.90^{abcd}	26.20^{cd}	28.70^{abcd}	24.50^{d}	28.90^{abcd}	33.00^{ab}	1.75
Red blood cell(X10^{12} L^{-1})	1.91^{bc}	2.28^{abc}	2.25^{abc}	1.57^{c}	2.63^{ab}	1.93^{bc}	3.11^{a}	2.16^{abc}	1.68^{bc}	0.28
Glucose (mg dL^{-1})	244.43^{ab}	197.47^{b}	238.83^{ab}	276.30^{a}	244.60^{ab}	293.13^{a}	270.63^{a}	275.00^{a}	255.83^{a}	17.24
Total Protein(g dL^{-1})	49.67^{de}	46.30^{e}	51.30^{cd}	53.67^{bcd}	53.53^{bcd}	53.37^{bcd}	54.43^{bc}	56.57^{b}	61.10^{a}	1.35
Uric acid (g dL^{-1})	7.30^{c}	7.83^{bc}	10.33^{abc}	10.67^{abc}	11.67^{a}	11.43^{ab}	10.97^{abc}	11.67^{a}	11.43^{a}	1.14
Creatinine (mg dL^{-1})	0.97^{c}	0.97^{c}	1.20^{bc}	1.20^{bc}	1.30^{abc}	1.57^{a}	1.50^{ab}	1.60^{a}	1.43^{ab}	0.10
Cholesterol (mg dL^{-1})	212.53^{ab}	200.20^{ab}	214.47^{ab}	214.47^{ab}	241.33^{a}	208.30^{ab}	210.20^{ab}	191.03^{ab}	193.57^{ab}	13.68
Mean corpuscular haemoglobin concentration (g dL^{-1})	34.10^{b}	34.31^{b}	33.61^{b}	34.10^{b}	34.13^{b}	35.09^{a}	33.94^{b}	35.19^{a}	34.05^{a}	0.29
Mean corpuscular haemoglobin (pg)	4.06^{ab}	4.36^{a}	3.81^{ab}	3.76^{ab}	4.04^{ab}	3.94^{ab}	3.85^{ab}	4.28^{ab}	3.52^{b}	0.00

Means in the same row with different superscripts differ significantly ($p<0.05$), SEM: Standard Error Mean

and 1.04 mg dL^{-1}). Serum Total protein value was highest for birds on P3 (57.37 g dL^{-1}) followed by those on P2 (53.52 g dL^{-1}) with the least value obtained on P1 (49.09 g dL^{-1}). In addition, cholesterol level though not different ($p>0.05$) in birds on the three processed mixtures was lowest in birds on P3 (198.27). On the other hand, the levels of inclusion had a significant ($p<0.05$) effect on Hb, WBC, Total protein, MCV and MCH.

The result of effect of interaction between processed mixtures and levels of inclusion on haematological and serum biochemical parameters is presented in Table 6 revealed significant ($p<0.05$) effect on most of the parameters except albumin and MCV. Values for PCV and haemoglobin were within similar range except at 0% inclusion level of P2 and 6% inclusion of P3. Birds fed at 3% inclusion of P1 were highest in MCH but recorded least value in total protein, creatinine, glucose and globulin. Similarly, red blood cell count value was significantly highest at 0% inclusion of P3 (3.11×10^{12}/l) and least (24.50 cumm^2×10^3) in white blood cell at similar level.

DISCUSSION

Blood parameters are good indicators of physiological, pathological and nutritional status of an animal and changes in haematological parameters have the potential of being used to elucidate the impact of nutritional factors and additives supplied in diet on any living creature. Processed mixtures had no effect on haematological parameters but significantly affected serum parameters except albumin and cholesterol values at the starting phase of growth. This indicates that the test additives had no any beneficial effect on haematological status of birds and are nearly similar to each other in haematological parameters. There was a significant increase in the erythrocytic parameters (except Erythrocyte indices: MCV, MCH and MCHC) with increasing level of inclusion with the least value obtained in birds fed at 0% inclusion. This is supported by haematological studies in birds carried out by a number of authors previous work that RBC and other parameters such as HBC and ESR of a bird vary among species and are affected by diet contents (Odunsi *et al*., 1999; Kurtoglu *et al.,* 2005) and several factors including physiological (Alodan and Mashaly, 1999) and environmental conditions (Vecerek *et al*., 2002; Graczyk *et al*., 2003).

The single effect of processed mixtures revealed no significant on erythrocytic parameters, WBC and serum biochemistry at finishing phase except glucose, total protein, uric acid and creatinine. Values for total protein increased across the processed mixtures with the highest value obtained in P3 (57.37 g dL^{-1}). This is as a result of high protein digestibility since high protein serum is an indicator of protein adequacy (Ahamefule *et al*., 2006). Therefore P3 could be capable of increasing the protein level in birds feed diets containing it. The total proteins and albumin contents observed in this study were within the normal ranges reported by Mitruka and Rawnsley (1977). In addition, the absence of variations in the albumin content could be attributed to the comparable protein intake across the groups of mixtures. The uric acid value significantly higher in P3 (12.06 g dL^{-1}) was comparable to that obtained in birds fed diet containing P2 (11.26 g dL^{-1}). This indicates that birds better utilised the P3 and P2 than P1 where the lowest value (8.49 g dL^{-1}) was obtained. Uric acid metabolism is influenced by the amount and quality of protein in the diet (Ward *et al.,* 1974). The varying uric acid value was worthy of comparison with reference values reported by Simaraks *et al*. (2004).The lower values recorded in creatinine indicates that no muscular wastage occurred in the birds as reported in the findings of Umit *et al.* (2011). Awosanya *et al.* (1999) also observed the dependence of blood protein and creatinine on the quality of dietary protein. The levels of inclusion had significant influence on Hb, WBC, RBC, TP, MCH, albumin and MCV. Haemoglobin concentration is mainly influenced by the efficiency of bleeding during slaughter and volume of blood collected. The details of interaction of mixtures from the different processing methods and level of inclusion revealed significant effect on haemagram indices (except MCV and MCHC) and serum metabolites (except albumin and globulin). PCV being the proportion of the volume comprised of erythrocytes in the whole blood value was lowest in birds fed 0% inclusion in P2 though comparable with that on 6% inclusion which resulted in a concomitant decrease in Hb concentration values obtained. Haemoglobin concentration is mainly influenced by the efficiency of bleeding during slaughter. The cholesterol content of the blood also showed significant difference among the treatment groups but was highest at 3% inclusion level of P2 (241.33 g dL^{-1}). The higher cholesterol level (at 3% inclusion level in P2) could be attributed to the high content of fat as measured by the amount of abdominal fat pad in birds fed at this inclusion level when birds were slaughtered. The varying Cholesterol levels of birds were found to be within the range previously determined in various avian species including pheasant (Arslan *et al.,* 2001; Lloyd and Gibson, 2006), broiler (Arslan *et al.,* 2001) and turkey (Bounous *et al*., 2000). It was reported that wide variation of cholesterol and triglyceride levels among avian species may depend more on the effect of diet (Palomeque *et al*., 1991; Harr, 2002). Both compounds are influenced by diet and animal-protein-rich diets causing low plasma cholesterol and triglyceride levels (Arslan *et al.,* 2001).

CONCLUSION AND FUTURE RECOMMENDATION

There was no detrimental effect of processed mixtures in diets fed to birds. The highest values were recorded for birds fed P3 for blood glucose, total protein, creatinine and globulin at starter phase. Glucose and Serum total protein were statistically highest in birds placed on diet containing 6% inclusion of P3. The values obtained for glucose, uric acid and creatinine were higher and comparable for P2 and P3. Serum Total protein value was highest for birds on P3.Cholesterol level though not different in birds on the three mixtures was lowest in birds fed diet containing P3 at finisher phase. Also worthy of note is that the range of values obtained for the haematological and serum parameters was within normal limits for avian species.

Based on the findings of this study, the followings could be recommended: birds could be fed diet containing P3 (discarded vegetable-fresh bovine blood-ensiled rumen content) at up to 6% inclusion levels for enhanced performance and for reduced cholesterol concentration P3 could be recommended.

Further research should be carried out to access the amino-acid profile and also standardize the chemical constituents of processed mixtures and inclusion in diets of poultry birds.

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