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Effect of moringa leaf meal and season on blood and hormonal profile of the pearl guinea fowl (*Numida meleagris*)

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Abstract

This study was undertaken to determine the effect of moringa leaf meal and season on blood and hormonal profile of the Pearl Guinea fowl (*Numida meleagris*) in the Middle belt of Ghana. Thirty-two (32) males and one hundred and twenty-eight (128) female Pearl Guinea fowls aged one-day-old were used for the study. A 3 x 4 factorial experimental design was used for the experiment. Data collected were analyzed using General Linear Model (GLM) procedure of Statistical Analysis System (SAS for Windows, version 7) and means were separated by the probability of difference (PDIFF) procedure of SAS (2008). Mean cell volume was highest (175.39 fl) among Guinea fowls fed with diet containing 12 % moringa leaf meal level. Guinea fowls fed with diet containing 15 % moringa leaf meal had the highest (3.44 $x10^{12}/L$) red blood cells production. Eosinophil level was highest (3.95 $x10^{12}/L$) among Guinea fowls fed with diet containing 9 % moringa leaf meal. Birds fed with the moringa diets recorded the highest (P= 0.022) WBC values as compared to the control diet. Triglycerides, high density lipoprotein and low-density lipoprotein levels increased (P<0.05) with increasing levels of dietary moringa leaf meal in the diet. The highest (P= 0.0025) level of progesterone was observed among birds fed with diet containing 12 and 15 % moringa leaf meal inclusion levels. The level of sodium was highest (166.69 nmol/l) among Guinea fowls fed with diet containing 12 % moringa leaf meal. The major and minor rainy seasons recorded the highest (P<0.05) mean cell hemoglobin, red blood cells, albumin and oestrogen levels. Platelets, follicle stimulating hormone, luteinizing hormone, prolactin and chlorine levels were highest (P<0.05) in the dry season while basophil level was highest in the major rainy season. Cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and potassium levels were highest (P<0.05) in the major rainy season. This study concludes that feeding Guinea fowls with moringa leaf meal had positive effect on some haematological, biochemical and hormonal parameters.

Keywords: Moringa leaf meal; Season; Erythrocytes; Platelets; Leukocytes; Hormonal; Metabolites

1. Introduction

In many parts of the world, Guinea fowls are raised mainly for their gamey flesh and eggs. Guinea fowl has a taste similar to other game birds and has many nutritional qualities that make it a worthwhile addition to the diet. The meat of a young Guinea fowl is tender and of especially fine flavour, resembling that of wild game [1]. Guinea fowl meat has a higher protein content of approximately 28 % compared to 20 % for domestic fowl [2]. Guinea fowls play a significant role in the lives of people in Northern Ghana, ranging from socio-cultural to economic and religious purposes [3]. With the realization for increased animal source of protein in the diets of the average Ghanaian, it is becoming increasingly important to adopt efficient methods to improve animals' performance. This will involve changes in management practices and provision of adequate and appropriate health schemes [4].

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Guinea fowl production in Ghana is constrained by the levels of hormonal production, low body weight at first maturity, out of season breeding and seasonal changes. The number of eggs laid depends on hormonal production, nutrients levels in the diet, breeding stock and health status of the bird [5]. Seasonal changes can lead to Guinea fowl hens not laying at certain times of the year. Short-day length affects egg laying because it affects hormonal production and hence reproduction [6]. Poor nutrition can lead to inadequate protein and other nutrients in the diet which can affect fertility, hatchability, growth performance and sperm production.

Recently, there has been interest in the utilization of moringa (*Moringa oleifera*) commonly called horseradish tree or drumstick tree, as a protein source for livestock [7, 8]. Moringa leaves have quality attributes that make it a potential replacement for soyabean meal or fish meal in non-ruminant diets. *Moringa oleifera* is in the group of high-yielding nutritious browse plants with every part having food value [8]. The leaves, flowers and pods are used as good sources of vitamins A, B and C, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and alpha-tocopherol [9].

The use of moringa in livestock ration is limited by the presence of anti-nutritional factors [7]. However, the presence of these anti-physiological agents in moringa products is removed via air drying, soaking or boiling in water [8]. AbouSekken [9] reported that growth-promoting effect in chicken may be due to its direct nutritional and immune-stimulating actions of its phytochemicals. The positive and negative impact of phytochemicals properties in moringa leaf meal have been reported by Ogbe [8] and AbouSekken [9]. According to the work done by Ogbe [8], phytochemicals in moringa leaf meal have significant effect on the blood constituent of broiler chicken. A phytochemical analysis in moringa leaf meal conducted by Ogbe [8] revealed that chlorogenic acid and its isomers are esters of quinic and caffeic acids that have abilities to inhibit oxidation and also promote various pharmacological activities such as antiobesity, reduction of plasma and liver lipids, and inhibition of acute lung injury.

The present investigation was undertaken to study the effect of moringa leaf meal and season on blood and hormonal profile of the Pearl Guinea fowl (*Numida meleagris*) in Ghana.

2. Material and methods

2.1. Study location and duration

The study was conducted at the Poultry Unit of the Department of Animal Science Education, University of Education, Winneba, Mampong-Ashanti, Ghana. Mampong-Ashanti lies in the transitional zone between the Guinea savanna zone of the north and the tropical rain forest of the south of Ghana. The climatic, vegetation and demographic characteristics of Mampong-Ashanti have been described by [10]. Mampong-Ashanti lies between latitude 07^{o} 04’ degree north and longitude 01^{0} 24’ degrees west with an altitude of 457m above sea level. Maximum and minimum annual temperatures recorded during the study period were 30.6 ^{o}C and 21.2 ^{o}C, respectively [11, 12]. Rainfall pattern in the district is bimodal, occurring from April to July (major rainy season) and again August to November (minor rainy season), with about 1224mm per annum. The dry season occurs from December to March [13]. The vegetation is transitional savanna woodland, which guarantees proper poultry keeping on free range basis.

2.2. Experimental birds, treatment and design

One hundred and sixty (160) birds were used for the study. This comprised thirty-two (32) males and one hundred and twenty-eight (128) females. A 3 x 4 factorial experimental design was used. The factors considered included; Factor I Season at 3 levels (Dry season -December-March, Major rainy season - April-July and Minor rainy season -August-November) and Factor II: Different levels of Moringa leaf meal (MOLM) (moringa leaf meal at four levels: 0 % MOLM, 9 % MOLM, 12 % MOLM and 15 % MOLM). All treatment combination (Season and different levels of moringa leaf meal combination: 0 % MOLM: Dry season, Major rainy season and Minor rainy season, 9 % MOLM: Dry season, Major rainy season and Minor rainy season, 12 % MOLM: Dry season, Major rainy season and Minor rainy season, 15 % MOLM: Dry season, Major rainy season and Minor rainy season) were used. Each treatment was replicated four times and had ten birds per replicate. The birds in each replicate were housed in one pen. At four (4) months old, a male was paired with four Guinea hens based on National Research Council 2006 recommendation for Guinea fowl [14].

2.3. Housing, Feeding and Medication

A total of sixteen (16) experimental pens were used for rearing the birds, each measuring 1.4 m × 1.34 m and housed ten (10) birds. The floor was concreted, and wood shavings were used as litter for the birds. Removable wooden feeding troughs measuring 0.8 m × 0.04 m × 0.03 m were used for feeding the growers.

Table 1 Ingredient and composition (%) of Starter diet

Attributes	0% MOLM	9% MOLM	12% MOLM	15% MOLM
Moringa	0.00	9.00	12.0	15.0
Maize	57.5	54.0	52.5	52.0
Wheat bran	11.0	8.50	8.50	6.00
Soya bean meal	8.50	8.00	7.00	7.00
Tuna fish meal	8.00	6.50	6.00	6.00
Anchovy fish meal	12.0	11.0	11.0	11.0
Oyster shell	1.50	1.50	1.50	1.50
Dicalcium phosphate	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50

Table 2 Ingredient and composition (%) of Grower diet

Attributes	0% MOLM	9% MOLM	12% MOLM	15% MOLM
Moringa	0.00	9.00	12.0	15.0
Maize	63.0	60.5	56.0	58.0
Wheat bran	22.0	18.5	16.0	17.0
Soya bean meal	3.00	2.00	3.00	2.00
Tuna fish meal	4.00	3.00	4.00	2.00
Anchovy fish meal	5.00	4.00	6.00	3.00
Oyster shell	1.50	1.50	1.50	1.50
Dicalcium phosphate	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50

Table 3 Ingredient and composition (%) of Breeder diet

Attributes	0% MOLM	9% MOLM	12% MOLM	15% MOLM
Moringa	0.00	9.00	12.0	15.0
Maize	55.0	50.0	50.0	50.0
Wheat bran	19.5	18.5	16.5	14.5
Soya bean meal	4.00	3.00	2.50	2.00
Tuna fish meal	4.50	4.50	4.00	3.00
Anchovy fish meal	8.00	6.00	6.00	6.50
Oyster shell	7.50	7.50	7.50	7.50
Dicalcium phosphate	0.50	0.50	0.50	0.50
Vitamin premix	0.50	0.50	0.50	0.50

A 4.5-litre watering trough was used for supplying water *ad libitum* for the growers in each pen. The experimental diets (Table 1, 2 and 3) were supplied to the birds *ad libitum* throughout the experimental period, vaccination and other routine poultry practices were also carried out. The weather records for the 2017 and 2018 are shown in Tables 4 and 5.

Table 4 Average Weather Records for the Experimental Location in the first year

Variables	Dryseason (December-March)	Major Rainy Season (April-July)	Minor Rainy Season (August-November)
Temperature (˚C)	32.0	30.25	27.0
Rainfall (mm)	27.28	130.0	125.05
Humidity (%)	65.5	81.25	84.5
Cloud cover (%)	37.50	60.00	65.25
Sun Hours (hr.)	103.10	89.30	62.45

Table 5 Weather Records for the Experimental Location in the second year

Variables	Dry season (December-March)	Major Rainy Season (April-July)	Minor Rainy Season (August-November)
Temperature (˚C)	33.0	29.11	26.30
Rainfall (mm)	28.41	123.12	117.02
Humidity (%)	68.9	82.07	80.11
Cloud cover (%)	38.80	65.21	63.15
Sun Hours (hr.)	106.2	67.22	85.40

2.4. Parameters Measured

Data on haematological, biochemical and hormonal profile were recorded. Blood samples from each bird were collected from the armpit (under the wing) of the bird for haematological and biochemical analysis using a sterilized disposable syringe and needles [15]. Blood samples were collected in the morning. A cotton swab soaked in methylated spirit was used to dilate the veins and to prevent infection. Blood samples were obtained by puncturing the brachial vein of the underside of the web of the wing of each of the hens using needles and syringes. An initial three (3) ml blood was taken into lablelled sterile universal bottle containing Ethylene-Diamine-Tetra-Acetic Acid (EDTA) as anticoagulant [15]. This was used to determine the haematological components within an hour of sample taken. Another three (3) ml of blood was collected from the same birds into labelled sterile sample bottle without anticoagulant and used to determine the biochemical components.

2.4.1. Haematological parameters

Haematological parameters were determined in full blood count (FBC) by using the Sysmex Haematological Auto-Analyser (Shenzhen Mindray Bio-medical electronics Co., Ltd, China) and included erythrocytes (Haemoglobin (Hb), Red blood cells (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC)) and Leukocytes and platelet (White blood cells, neutrophil, lymphocytes, eosinophil, basophil and platelet) [15].

2.4.2. Biochemical parameters

Biochemical parameters analyzed include total serum protein, albumin, globulin and cholesterol concentrations. The serum was used for the analysis as follows; the total protein (TP) was determined using Biuret method as described by Keller [16]. Blood albumin was determined using the Bromocresol Green (BCG) method [17]. Total cholesterol (TC) was estimated using the CHOP-PAP method and the globulin level was also calculated [17]. The globulin content was determined by subtracting albumin from the total protein [16]. Again, blood samples were obtained from birds by the

same procedure mentioned above and drawn into plain vacutainer tubes to determine the triglyceride, High-density Lipoprotein (HDL), Low-density Lipoprotein (LDL) levels and glucose as described by Keller [16].

2.4.3. Hormonal parameters

The assay procedure was performed on Mindray® microplate reader *MR* 96 A (Shenzhen Mindray Bio-medical electronics Co., Ltd, China) and hormones determined included Progesterone, Estradiol, Prolactin, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) [15].

2.5. Data Analysis

Data collected were analyzed using General Linear Model (GLM) procedure of Statistical Analysis System (SAS for Windows, version 7). The means were separated by using the probability of difference (PDIFF) procedure of SAS [18].

3. Results

3.1. Proximate composition of moringa leaf meal

Results of proximate composition of Moringa leaf meal (MOLM) are presented in Table 6. The proximate components of moringa leaf meal contains higher levels of crude protein (28.91 **%**), metabolizable energy (8.55 Kcal/kg) and appreciable levels of crude fibre (13.34 **%**), dry matter (89.64 **%**), ether extracts (5.32 **%**), moisture (88.15 **%**), nitrogen free extracts (43.85 **%**) and total ash (7.13 **%**).

Table 6 Proximate composition of moringa leaf meal

Attributes	Percentage (%)	Standard deviation
Moisture	88.15	0.05
Crude fibre	13.34	0.08
Dry matter	89.64	0.45
Ether extracts	5.32	0.21
Crude protein	28.91	0.21
Nitrogen Free Extracts	43.85	0.11
Total Ash	7.13	0.04
ME (Kcal/kg)	8.55	0.07

3.2. Phytochemical properties in moringa leaf meal

A phytochemical analysis was performed to determine the major class of compounds present in moringa leaf meal and the results are shown in Table 7. The results revealed the presence of high levels of chlorogenic acid as compared to all the other parameters. Kaempferol, quercetin and luteolin were observed to be moderate. However, apigenin was observed to be the lowest among all the parameters measured.

Table 7 Proximate composition of moringa leaf meal

Parameter	*µ*g /g Dry matter	Standard deviation
Apigenin	25.37	2.19
Chlorogenic acid	295.87	11.41
Kaempferol	51.23	1.86
Luteolin	45.36	2.02
Quercetin	48.49	1.80

3.3. Effect of moringa leaf meal and season on erythrocytes and platelets

The results of this study showed that moringa leaf meal had little or no effect (P>0.05) on the values of erythrocytes and platelets except mean cell volume and red blood cells (P<0.05) (Table 8). Mean values for mean cell volume was highest (175.39 fl) among Guinea fowls fed with diet containing 12 % moringa leaf meal and lowest (163.93 fl) among birds fed with the control and 9 % moringa leaf meal diets. Mean values for red blood cells were highest (3.44 $x10^{12}/L$) among Guinea fowls fed with diet containing 15 % moringa leaf meal and lowest (2.51 $x10^{12}/L$) among birds fed with the control and 9 % moringa leaf meal diets. The major and minor rainy seasons had the highest (P<0.05) mean cell haemoglobin and red blood cells, whilst the lowest haemoglobin and red blood cells values were recorded in the dry season. The highest (23.69 $x10^{9}/L$) platelets value was recorded in the dry season whereas the major (19.16 $x10^{9}/L$) and minor (19.13 $x10^{9}/L$) rainy seasons had the lowest mean values. Interactions of seasons with moringa leaf meal had little (P>0.05) effect on erythrocytes and platelets traits except mean cell volume (P<0.05).

Table 8 Effect of dietary moringa leaf meal and season on erythrocytes and platelets

Variables	Hb (*g/dL*)	MCH (*pg*)	MCHC (*g/dL*)	MCV (*fL*)	PCV (*%*)	Plat ($x10^{9}/L$)	RBC ($x10^{12}/L$)
Moringa leaf meal							
0% MOLM	11.08	62.53	42.31	163.93^{c}	31.84	22.10	2.51^{c}
9% MOLM	11.06	62.37	45.02	165.89^{c}	33.87	20.66	2.60^{c}
12% MOLM	11.35	66.48	43.06	175.39^{a}	34.94	20.54	2.87^{b}
15% MOLM	11.53	60.61	42.80	170.88^{b}	34.59	19.34	3.44^{a}
SEM	0.19	4.21	1.02	2.91	0.90	0.952	0.20
P-value	0.2805	0.7927	0.2792	0.0381	0.0890	0.2600	0.0125
Season							
Major Rainy Season	11.26	71.12^{a}	43.33	169.03	34.33	19.16^{b}	3.04^{a}
Minor Rainy Season	11.30	72.44^{a}	43.55	168.91	34.46	19.13^{b}	3.19^{a}
Dry Season	11.21	45.44^{b}	43.01	169.13	32.64	23.69^{a}	2.34^{b}
SEM	0.16	3.65	0.88	2.51	0.78	0.82	0.17
P-value	0.9305	<.0001	0.9088	0.9980	0.2031	0.0004	0.0036

^{abcd}= Means bearing different superscripts in the same column are significantly different (P < 0.05); * = p < 0.05, ns =not significant, % = Percent; MOLM = Moringa Leaf Meal; S. E = Standard error of means; P = probability of main effects; Dry S= Dry Season; Maj. R. S. = Major Rainy Season; Min. R. S. =Minor Rainy Season, Hb=Haemoglobin; MCV = Mean cell volume; MCH= Mean cell haemoglobin; MCHC= Mean cell haemoglobin concentration; PCV= Packed cell volume; Plat= Platelets; RBC = Red blood cells.

3.4. Effect of dietary moringa leaf meal and season on leukocytes

Dietary moringa leaf meal had influence (P<0.05) on eosinophil and white blood cells (WBC) (Table 9). Eosinophil level was highest (3.95$x10^{9}/\mu l$) among Guinea fowls fed with diet that containing 9 % MOLM and lowest (2.06$x10^{9}/\mu l$) among birds fed with the control diet. Birds fed with diet contained 9, 12 and 15 % MOLM had similar mean values for white blood cells and these were higher than that of the control. Season of production had positive effect (P= 0.01) on basophil levels. However, no significant (P>0.05) effect was observed in all the other parameters. The highest (2.00 $x10^{9}/\mu l$) basophil level was produced in the major rainy season and was followed by minor rainy season (1.56 $x10^{9}/\mu l$). The dry season had the lowest (1.37 $x10^{9}/\mu l$) basophil levels (Table 9). Dietary moringa leaf meal × season interaction did not show significant (P>0.05) effect on all leukocyte's parameters.

Table 9 Effect of moringa leaf meal and season on leukocytes

Variables	Bas *(x10⁹/µl)*	Eos *(x10⁹/µl)*	Lymph *(x10⁹/µl)*	Mon *(x10⁹/µl)*	Neu *(x10⁹/µl)*	WBC *(x10⁹/L)*
Moringa leaf meal						
0% MOLM	1.83	2.06[c]	46.41	4.53	47.00	158.80[b]
9% MOLM	1.83	3.95[a]	42.28	6.04	47.65	166.29[a]
12% MOLM	1.33	2.70[b]	46.97	5.16	45.09	167.13[a]
15% MOLM	1.58	2.40[b]	48.47	4.16	46.73	167.12[a]
SEM	0.16	0.32	3.25	0.50	2.93	2.51
P-value	0.1035	0.0014	0.5813	0.0673	0.9370	0.0218
Season						
Major Rainy Season	2.00[a]	2.75	47.23	4.96	45.68	161.63
Minor Rainy Season	1.56[b]	2.75	47.23	4.96	45.68	161.29
Dry Season	1.37[c]	2.83	43.63	5.02	48.50	164.73
SEM	0.13	0.27	2.81	0.43	2.54	2.17
P-value	0.0099	0.9757	0.5863	0.9916	0.6687	0.4775

[abc]= Means bearing different superscripts in the same column are significantly different (p <0.05);* = p < 0.05, ns =not significant, % = Percent; MOLM = Moringa Leaf Meal; S. E = Standard error of means; P = probability of main effects; Dry S= Dry Season; Maj. R. S. = Major Rainy Season; Min. R. S. = Minor Rainy Season, Bas = Basophil; Eosi = Eosinophil; Lymph= Lymphocytes; Mon=Monocytes; Neu= Neutrophil; WBC=White blood cell.

3.5. Effect of dietary moringa leaf meal and season on biochemical parameters

Triglycerides, high density lipoprotein and low-density lipoprotein levels increased (P<0.05) with increasing levels of dietary MOLM (Table 10). However, all other biochemical parameters measured were not (P>0.05) affected.

Table 10 Effect of moringa leaf meal and season on biochemical parameters

Variables	Alb *(g/L)*	Chol (mmol/l)	Glob *(g/L)*	HDL (mmol/l)	LDL (mmol/l)	TRIG (mmol/l)	TSP *(g/L)*
Moringa leaf meal							
0% MOLM	19.84	3.98	31.75	1.51[d]	1.73[d]	0.81[a]	49.10
9% MOLM	18.67	3.84	31.17	1.99[c]	2.41[c]	0.66[b]	49.86
12% MOLM	16.77	3.78	26.70	2.37[b]	2.89[b]	0.65[b]	43.48
15% MOLM	16.90	3.69	31.20	2.58[a]	3.21[a]	0.55[c]	48.11
SEM	1.43	0.08	1.70	0.12	0.44	0.13	2.36
P-value	0.3810	0.1052	0.1472	<.0001	<.0001	0.013	0.2429
Season	**Alb *(g/L)***	**Chol (mmol/l)**	**Glob *(g/L)***	**HDL (mmol/l)**	**LD (mmol/l)**	**TRIG (mmol/l)**	**TSP *(g/L)***
Major Rainy Season	19.77[a]	4.09[a]	28.57[b]	2.44[a]	2.88[a]	0.78[a]	47.41
Minor Rainy Season	19.77[a]	3.89[b]	28.57[b]	2.11[b]	2.17[b]	0.65[b]	47.41
Dry Season	14.60[b]	3.50[c]	33.49[a]	1.75[c]	1.89[c]	0.52[c]	48.09
SEM	1.24	0.07	1.47	0.14	0.55	0.11	2.04
P-value	0.0073	<.0001	0.0349	<.0001	<.0001	0.0014	0.9638

[abc]= Means bearing different superscripts in the same column are significantly different (P<0.05); * = p < 0.05, ns =not significant, % = Percent; MOLM = Moringa Leaf Meal; S. E = Standard error of means; P = probability of main effects; Dry S = Dry Season; Maj. R. S. = Major Rainy Season; Min. R. S. = Minor Rainy Season, Alb=Albumin; Chol= Cholesterol; Glob = Globulin; TSP= Total serum protein, TRIG = Triglycerides; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein.

Mean values for total serum protein observed was not influenced by season (P= 0.2429). The mean albumin values observed was similar (P>0.05) in both major and minor rainy seasons which was significantly (P= 0.0073) higher than that in the dry season. Season had significant (P<0.05) effect on cholesterol and globulin levels. Cholesterol, triglycerides, high density lipoprotein and low-density lipoprotein levels were highest in the major rainy season, followed by minor rainy season and lowest in the dry season in that order. The highest (P<0.05) value for globulin was observed in the dry season and similar in both major and minor rainy seasons. Dietary moringa leaf meal × season interaction did not show significant (P>0.05) effect on all biochemical parameters observed.

3.6. Effect of dietary moringa leaf meal and season on hormonal levels

Progesterone and oestrogen were extracted from females only while testosterone was extracted from males only. However, all other traits were observed from males and females. Different levels of moringa leaf meal showed little or no effect (P>0.05) on hormonal levels considered in this study except progesterone (P= 0.0025) (Table 11). The highest level of progesterone was observed among birds fed with diet containing 12 and 15 % moringa leaf meal inclusion levels and lowest among birds on the control diet. Seasonal variation influenced (P<0.05) the levels of follicle stimulating hormone, luteinizing hormone, oestrogen and prolactin. However, no positive (P>0.05) effect was observed for both progesterone and testosterone. The highest mean values for follicle stimulating hormone, luteinizing hormone and prolactin were observed in the dry season. Oestrogen level was highest (P= 0.0042) in the major (45.40 pg/ml) and minor (48.46 pg/ml) rainy seasons and lowest (27.80 pg/ml) in the dry season. Interactions of different levels of dietary moringa leaf meal with seasons were important to influence (P<0.05) the level of prolactin. However, interactions on all other traits were not significant (P>0.05)

Table 11 Effect of moringa leaf meal and season on hormonal parameters

Variables	FSH *(IU/ml)*	LH *(IU/ml)*	Oestrogen (*pg/ml*)	Prog *(ng/dl)*	Prolactin *(ng/ml)*	Testosterone *(ng/dl)*
Moringa leaf meal						
0% MOLM	0.47	0.67	33.25	1.37^{c}	12.38	1.91
9% MOLM	0.55	0.73	44.08	2.99^{b}	11.36	1.60
12% MOLM	0.61	0.81	45.07	3.04^{a}	9.22	1.96
15% MOLM	0.58	1.72	39.80	3.28^{a}	10.10	2.14
SEM	0.09	0.60	5.05	0.36	0.97	0.38
P-value	0.7104	0.5744	0.3505	0.0025	0.1290	0.7996
Season						
Major Rainy Season	0.34^{b}	0.41^{b}	45.40^{a}	2.97	8.39^{b}	1.86
Minor Rainy Season	0.34^{b}	0.41^{b}	48.46^{a}	3.01	8.39^{b}	1.86
Dry Season	0.98^{a}	2.13^{a}	27.80^{b}	2.03	15.52^{a}	1.99
SEM	0.07	0.52	4.37	0.31	0.84	0.33
P-value	<.0001	0.0394	0.0042	0.0568	<.0001	0.9515

^{abcd}= Means bearing different superscripts in the same column are significantly different (P<0.05); * = p < 0.05, ns =not significant, % = Percent; MOLM = Moringa Leaf Meal; S. E = Standard error of means; P = probability of main effects; Dry S= Dry Season; Maj. R. S = Major Rainy Season; Min. R. S = Minor Rainy Season, FSH = Follicle Stimulating Hormone; LH = Luteinizing Hormone; Prog = Progesterone

3.7. Effect of dietary moringa leaf meal and season on metabolites

The effect of different levels of moringa leaf meal on metabolites is presented in Table 12. Different levels of moringa leaf meal showed no significant (P>0.05) effect on calcium, chlorine and potassium levels. However, significant (P<0.05) effect was observed on sodium. The highest (166.69 nmol/l) level of sodium was observed among birds fed with diet that contained 12 % MOLM and lowest (154.84 nmol/l) among birds fed with diet containing 9 % moringa leaf meal. The control and 15 % MOLM treatments had similar (P>0.05) sodium levels. The levels of chlorine and potassium were influenced (P<0.05) by seasonal variation. However, calcium and sodium levels were not affected by seasonal variation (Table 12). The highest level of chlorine was observed in the dry season and lowest in the major and minor rainy

seasons. Potassium level was significantly (P<0.05) highest in the major rainy season followed by minor rainy season and lowest in the dry season. Interactions of seasons with moringa leaf meal had no influence (P>0.05) on metabolites except (P<0.05) potassium.

Table 12 Graded levels of moringa leaf meal and season on metabolites

Variables	Ca^{2+} (nmol/L)	Cl^{-} (nmol/L)	K^{+} (nmol/L)	Na^{+} (nmol/L)
Moringa leaf meal				
0% MOLM	4.00	112.18	3.80	158.79^{b}
9% MOLM	4.40	113.40	4.21	154.84^{c}
12% MOLM	4.67	114.66	4.19	166.69^{a}
15% MOLM	3.96	107.52	3.55	159.30^{b}
SEM	0.22	2.65	0.20	1.98
P-value	0.0960	0.2656	0.0771	0.0018
Season				
Major Rainy Season	4.40	108.37^{b}	4.77^{a}	161.00
Minor Rainy Season	4.31	108.52^{b}	4.35^{b}	159.35
Dry Season	4.06	118.93^{a}	2.70^{c}	159.37
SEM	0.19	2.29	0.17	1.71
P-value	0.4469	0.0030	<.0001	0.7411

^{abc}= Means bearing different superscripts in the same column are significantly different (P < 0.05); * = p < 0.05, ns = not significant, % = Percent; MOLM = Moringa Leaf Meal; S. E = Standard error of means; P = probability of main effects; Dry S = Dry Season; Maj. R. S = Major Rainy Season; Min. R. S = Minor Rainy Season, Ca = Calcium; Cl = Chlorine; K = Potassium; Na= Sodium.

4. Discussion

4.1. Proximate composition and phytochemical properties of moringa leaf meal

Generally, moringa leaf meal was found to contain considerable protein (28.91) (Table 6). This value is higher than the value of 17.01 % and 23 % reported by Ogbe and Afiku [19] and Makkar and Becker [20] respectively. However, the result is comparable to the 27.44 % reported by Olugbemi *et al*. [21] and 28.50 % reported by Kwafo *et al*. [22] but lower than the 30.65 % obtained by Mutayoba *et al*. [23]. The protein value (28.91) recorded in this study indicates that the nutrient content of the moringa gives an indication of its usefulness as an important feed resource for feeding Guinea fowls. The crude fibre value of 13.34 % obtained in this study appears lower than the 16.11 % reported by Richter *et al*. [24] but was higher than 13.05 % and 10.59 % obtained by Kwafo *et al*. [22] and Abbas *et al*. [25]. The crude fibre value obtained in this study is an indication that adding MOLM to the diet of Guinea fowls will not affect digestibility. The value for dry matter (89.64 %) was observed to be lower than the 90.21 obtained by Kwafo *et al*. [22] but was higher than 86 % reported by Kakengi *et al*. [26]. The values obtained for ether extracts (5.23 %), moisture (88.15 %), nitrogen free extracts (43.85 %) and total ash (7.13 %) were similar to values obtained in previous studies [19, 21, 25].

Results on phytochemical properties of this study are consistent with those reported by Valdez-Solana *et al*. [27] who reported high levels of chlorogenic acid (286.13 ± 15.09 μg/g), kaempferol (46.43 ± 2.14 μg/g), quercetin (46.18 ± 0.6 μg/g) and luteolin (44.56 ± 2.03 μg/g). The levels of phytochemicals observed in this study indicate that the levels of lipids in the liver and plasma will be low and will not have any negative effect on the health status of the birds. Previous phytochemical investigations have identified quercetin and kaempferol phytochemicals in moringa leaf meal [28]. Though MOLM is known to contain quercetin and kaempferol however, traceable amounts of chlorogenic acid and derivatives have been detected within the leaves from Ghana, Senegal, and Zambia [8]. Chlorogenic acid and its isomers are esters of quinic and caffeic acids that have abilities to inhibit oxidation and also promote various pharmacological activities such as antiobesity, reduction of plasma and liver lipids, and inhibition of acute lung injury [9]. The levels of phytochemical properties in moringa leaves influences the production of hormones as reported by AbouSekken [9].

4.2. Effect of dietary moringa leaf meal and season on erythrocytes and platelets

The mean cell volume and red blood cells values recorded in this study compared to standard values in birds. Similarly, the RBC values of birds observed in this study fall within the normal reference range (1.85-3.5) reported by Aengwanich *et al.* [29]. The highest (P<0.05) mean values for mean cell volume and red blood cells observed in the dietary moringa leaf meal as compared to the control diet could be explained that moringa leaves are excellent source of vitamin B [30, 31], calcium, protein, potassium and essential micronutrients with antioxidant activity - Selenium which influences cell volume and the production of more red blood cells in farm animals [30]. Red Blood Cells (RBC) are responsible for the transportation of oxygen and carbon dioxide in the blood as well as manufacture of haemoglobin hence, higher (P= 0.0125) values observed in this study (Table 8) indicate a greater potential for this function and a better state of health among Guinea fowls fed with dietary MOLM as compared with Guinea fowls fed the control diet. The results of this study again demonstrated that Guinea fowl birds fed with dietary moringa leaf meal indicate greater potential for the transportation functions and hence such birds could be predicted to be healthy as compared to birds on the control treatment. According to Amevor [31] the major role of red blood cells is to transport oxygen from the lungs of the birds to the body tissues and transport carbon dioxide from the tissue to the lungs of the bird. In general, the higher levels of mean cell volume and red blood cells values observed in this study corroborates with the findings of [32, 33] who reported that birds supplemented with moringa leaf meal had improved levels of mean cell volume and red blood cells.

Highest (P<0.05) mean cell haemoglobin and red blood cells during minor rainy season could be attributed to the favourable environmental conditions. The importance of maintaining a comfortable and stress-free environment for Guinea fowls cannot be underestimated. Favourable environmental conditions remove excess heat in the pens thereby reducing the rate at which birds are exposed to heat stress. Similarly, Ashizawa and Sano [34] and Korankye *et al.* [35] reported that, the season of production had significant improvement on cell haemoglobin, lymphocyte, monocytes and red blood cells. The result of this study corroborates with the findings of Tutubalang [36] who observed enhanced mean cell volume, WBC and red blood cells in Potchefstroom koekoek indigenous chicken. Platelet was observed to be higher in the dry season and this could be attributed to the higher temperature recorded in the dry season. This could be explained that temperatures above 30 ˚C cause marked activation of platelets in birds leading to higher production in the dry season. This result agrees with the observation made by Aderinola [37] who reported that birds supplemented with moringa leaf meal had better feed conversion ability in the dry season hence had higher values for platelets and PCV. Results of the study agree with earlier report that hot and cold periods exert detrimental effect on mean cell haemoglobin and red blood cells [38, 39].

4.3. Effect of moringa leaf meal and season on leukocytes

In both human and animals, WBC produces antibodies in the blood which help in the development of immunity thus, protecting the body against disease causing microorganisms [40, 41]. Eosinophil is also responsible for strong immunity which protects birds against diseases and infections. The increase in eosinophil and white blood cells observed among the moringa leaf meal diets as compared to the control treatment could be explained that dietary moringa leaf meal increased the levels of all the necessary essential nutrients needed for rapid growth and body building which protect birds against infections. In general, the higher level of eosinophil and white blood cells values observed in this study corroborates with the findings of Mohan *et al.* [32].

Basophil was higher during the major rainy season followed by minor rainy season and lower in the dry season. This could be explained that moderate ambient temperature enhances maximum production of gonadotropin hormones which stimulate basophil production hence; this is possibly during the major rainy season where temperatures are relatively low as compared to the dry season. The results of this study corroborate with the findings of Elagib *et al.* [42] who reported higher levels of epithelial cells and basophil in cold seasons as compared to the dry seasons.

4.4. Effect of dietary moringa leaf meal and season on biochemical parameters

The improvement in the levels of triglycerides, high density lipoprotein and low-density lipoprotein indicates that the diet was relatively of good quality and that birds fed the diet were more efficient in protein metabolism and utilization. The high- and low-density lipoprotein values observed with increasing levels of dietary moringa leaf meal reflects the protein levels in the test diets. The values of triglycerides in the dietary treatments numerically decreased from 0.81-0.13 as the MOLM inclusion level increased in the diet. This showed that the Guinea fowls fed with dietary moringa leaf meal efficiently utilized the carbohydrate content of the diets. These findings are in line with the work done by Kout *et al.* [43] who stated that the total protein was significantly improved in both the control and MOLM treated diets (2 %, 4 % and 6 % MOLM).

The non-significant differences observed on other biochemical traits could be explained that the presence of polyphenols and flavonoids in moringa leaf meal affect tissue bio-efficiency and metabolites in birds which affect biochemical traits [44]. The biochemical values obtained in this study compared to standard values in birds. Similar findings were reported by Makanjuola *et al*. [45] who reported that 0.2 %, 0.4 % and 0.6 % moringa leaf meal inclusion in the diet did not influence the serum total protein, albumin and globulin. Amevor [31] also reported that, moringa leaf meal showed no significant effect on cholesterol and triglyceride levels. However, the author observed significant effect on albumin, globulin, total serum protein, high density lipoprotein and low-density lipoprotein. The result is also in agreement with [29, 46] who reported no significant effect on albumin, cholesterol, globulin and triglyceride levels.

The result of this study corroborates with earlier studies conducted by Nalubamba *et al*. [47] who observed highest levels of cholesterol, triglycerides, high density lipoprotein and low-density lipoprotein levels during the rainy season and lowest in the dry season. This is because during the rainy season, moringa leaf meal contained high levels of essential plant nutrients as compared to the dry season, hence when fed to birds there was high improvement in biochemical parameters. The results of this study corroborate with the findings of Elagib *et al*. [42] who reported significant effect of season on albumin and cholesterol levels. Research conducted by Yokus *et al.* [48]; Jesuyon and Salako [49] indicates that mean values for total protein, albumin, and globulin had been found to be significantly lower due to exposure of the animals to high ambient temperature thereby affecting the biochemical traits in birds. According to the reports of Korankye *et al.* [35] plasma total proteins decreased significantly when laying hens were exposed to above 36 ˚C environmental temperature than moderate temperature regime. This finding agrees with the report made by Elaroussi [50] who reported that at higher temperatures, plasma albumin decreased significantly during hot seasons and may be attributable to changes in body temperature that causes a shift in tissue fluids and thus cause a change in the concentration of plasma proteins.

4.5. Effect of dietary moringa leaf meal and season on hormonal parameters

Birds fed with diet that contained 15 % MOLM had the highest progesterone secretion level possibly due to high levels of phytochemicals in the diet. The level of progesterone secretion in this study is similar to the report of Renema *et al.* [51] on broiler hens fed diet that contained graded levels of MOLM. According to the work done by Bocchinfuso and Hammond [52], high levels of amino acids in the diet trigger high levels of steroid hormones such as progesterone production in the body during the breeder stage. The results of this study showed no significant increase in follicle stimulating hormone, luteinizing hormone, oestrogen, prolactin and testosterone. This corroborates with earlier report of Becker [53] that dietary moringa leaf meal consumption had no direct relationship with follicle stimulating hormone, luteinizing hormone, oestrogen, prolactin and testosterone concentration in female in the reproductive stage. The results are also in agreement with the report made by [29, 46] that there was no significant effect on follicle stimulating hormone, luteinizing hormone and oestrogen.

Seasonal variations in poultry production and reproductive activities are generally associated with gonadotropin secretion, especially follicle stimulating hormone, luteinizing hormone and prolactin [54]. In this study, higher levels of follicle stimulating hormone, luteinizing hormone and prolactin were observed in the dry season where there is adequate sunshine and long day length. According to the report made by Balog *et al.* [55] at higher temperatures and long day length there is high rate of gonadotropin secretion as compared to the rainy seasons in birds. Dunn and Sharp [56] reported that when male quails were transferred from short day length to long day lengths, the level of gonadotropins rose substantially during the first week of photo stimulation, testicular growth and steroid genesis begin and maturity reached as compared to short day lengths. This indicates that long day length enhances steroid genesis leading to high production of follicle stimulating hormone and luteinizing hormone.

4.6. Effect of dietary moringa leaf meal and season on metabolites

Dietary MOLM significantly influenced ($P<0.05$) sodium secretion but had little effect on calcium, chlorine and potassium levels. Birds fed with 12 % MOLM had the highest ($P<0.05$) level of sodium. The level of sodium observed in this study is higher than the normal serum sodium values which range from 135 to 145 nmol/L. The result indicates that the birds were very healthy. Sodium is the major extracellular cation which regulates the total amount of water in the body [57]. Reduction in sodium levels in birds, results in decrease in osmotic pressure and consequent disturbance of acid-based balance in the body. Sodium deficiency results in heart failure, decreased blood pressure and increased hematocrit and decreased elasticity of subcutaneous tissue as well as low adrenal gland function leading to elevated levels of uric acid in the blood and subsequent shock and death [58]. The result is in agreement with the report obtained by [29, 46] who reported no significant effect on calcium and potassium levels. However, the authors reported significant effect on sodium levels.

Seasonal variations in poultry production and reproductive activities are generally associated with hormonal and metabolites secretion [54]. In this study, higher levels of chlorine and potassium were observed in the cold seasons where temperatures were relatively low indicating that cold temperature increased feed intake and feed utilization which increased elasticity of subcutaneous tissue as well as high adrenal gland functioning in the body. Currently, there is limited literature on the effect of season on metabolites.

4.7. Interaction effects of fixed factors on blood and hormonal profile of the Pearl Guinea fowl

Moringa leaf meal × season interactions observed on mean cell volume, prolactin and potassium (K^+) in this study suggest that the combine sets of the two factors should be considered in farm animal genetic improvement. This means that in the absence of moringa leaf meal × season interactions the blood components of the Guinea fowls will be underestimated. Interactions on all other traits had no considerable effect. There has never been a study of moringa leaf meal and season interaction on blood profile of the Guinea fowl. The importance of interactions of season and age factors has been recognized in farm animal genetic improvement. Age × season interaction was found to be significant on white blood cells, red blood cells, mean cell volume and prolactin of white leghorn [42].

5. Conclusion

This study concludes that Guinea fowls fed with diet containing 12 % and 15 % moringa leaf meal increased red blood cells, mean cell volume and sodium levels. Guinea fowls fed with diet containing 9 % moringa leaf meal produced more eosinophil. Increasing dietary moringa leaf meal in the diet of Guinea fowls increased the production of progesterone. Minor rainy season recorded the best mean cell haemoglobin and red blood cells in the blood. Platelets, globulins, follicle stimulating hormone, luteinizing hormone, prolactin and chlorine production were highest in the dry season. Major rainy season stimulated higher production of basophil, cholesterol and potassium levels. Mean cell volume, prolactin and potassium levels were influenced by the interaction effects of moringa leaf meal and season.

Recommendation

This study recommends that farmers and breeders should consider 15 % dietary MOLM inclusion level for optimum productivity. This study further recommends that Guinea fowl health management should be intensified in the major and minor rainy seasons to avert health challenges resulting from seasonal variations especially, during the brooding stage to reduce keet mortalities

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

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