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Research Article
Effect of Seasonality and Dietary *Moringa oleifera* Leaf Meal on the Quality of Spermatozoa of the Pearl Guinea Fowl Cock

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Abstract
Objective: A study was carried out to determine the effect of graded levels of moringa leaf meal and season on spermatozoa characteristics of the Pearl Guinea fowl cock in Ghana. **Materials and Methods:** Thirty-two (32) cocks and one hundred and twenty-eight (128) hens aged one-day-old were used for the study after been reared to attain sexual maturity. A completely randomized design with a 3 x 4 factorial arrangement 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. **Results:** Result from this study revealed that, the highest sperm motility occurred in birds fed with the control diet (77.12%) while birds fed with 15% *Moringa oleifera* leaf meal (MOLM) had the lowest sperm motility (55.83%). Semen pH, sperm count and normal spermatozoa increased with increasing dietary moringa leaf meal. Semen pH was higher in birds fed with 15% MOLM (8.00) and lowest in birds fed with the control diet (7.70). Sperm count was higher in birds fed with 15% MOLM (4.63×10^9 mL) and lowest in birds fed with 9% MOLM (2.33×10^9 mL). Normal spermatozoa was higher ($p<0.05$) in birds fed with 15% MOLM (85.42%), whereas, 9% MOLM (74.17%) and the control diet (74.58%) had similar ($p>0.05$) but significantly lower values for normal sperm. Season had no significant ($p>0.05$) effect on spermatozoa characteristics except motility and sperm count. Sperm motility was higher ($p<0.05$) in the dry season (74.09%), whereas minor (67.18%) and major rainy (65.93%) seasons had similar ($p>0.05$) but significantly lower values for sperm motility. Sperm count was higher ($p<0.05$) in the major rainy season (4.04×10^9 mL) and lower in the dry season (1.89×10^9 mL). There was no significant ($p>0.05$) effect of moringa leaf meal×season interaction on spermatozoa characteristics. **Conclusion:** This study concluded that spermatozoa quality and quantity increased with increasing levels of MOLM in the diet of Guinea fowl cocks.

Key words: Guinea fowl, moringa leaf meal, semen production, sperm motility, spermatozoa

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The production of Guinea fowls plays a major role in bridging the protein gap in developing countries including Ghana where the average daily consumption is far below recommended levels[1,2]. However, the productivity of Guinea fowl in Ghana has been limited due to higher protein prices, seasonal variation and poor reproductive performance. Seasonal changes can lead to hens not laying at certain times of the year. Short day length affects egg laying because it affects hormonal production and hence reproduction[3]. Poor nutrition can lead to protein deficiency, which can affect fertility, hatchability and sperm production. Protein sources are especially limiting factors for Guinea fowl and poultry feed production in the tropics[4]. Hence, there is the need to search for locally available alternative sources of protein for use as poultry feed ingredient. One possible source of cheap protein for Guinea fowl is the leaf meal of some tropical legume and plants[5]. 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[6,7]. Moringa leaves have quality attributes that make them a potential replacement for soyabean meal or fish meal in non-ruminant diets.

Despite the nutritive and medicinal value of *Moringa oleifera*, there is scanty information regarding its effect on sperm production in Guinea fowls. Guinea fowls are known to be seasonal breeders with little or no eggs during the dry season[3]. The study was carried out to determine the effect of dietary moringa leaf meal and season on semen production of the Pearl Guinea fowl in Ghana.

MATERIALS AND METHODS

The study was conducted at the Poultry Unit of the Department of Animal Science Education, University of Education, Winneba, Mampong-Ashanti. 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. Mampong-Ashanti lies between latitude 07°04′ degree north and longitude 01°24′ degrees west with an altitude of 457 m above sea level. Maximum and minimum annual temperatures recorded during the study period were 30.6 and 21.2°C, respectively[8,9]. Rainfall pattern in the district is bimodal, occurring from April to July (major rainy season) and again from August to November (minor rainy season), with about 1224 mm per annum. The dry season occurs from December to March[10,11].

A total of 160 pearl Guinea fowl pullets were selected from the initial stock obtained from Akate Farms Co. Limited in Kumasi after brooding and used for the study. The birds (32 males and 128 females) were distributed in a completely randomized design in a 3×4 factorial arrangement. The factors that were considered in the experiment 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 (T1, Control), 9% MOLM (T2), 12% MOLM (T3) and 15% MOLM (T4)]. All treatment combinations (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. One male (four months old) was paired with four Guinea hens.

A total of 16 experimental cages were used for rearing the birds, each measuring 1.4 m × 1.34 m and housed 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. In each cage, 4.5 L watering trough was used for supplying water *ad libitum* for the growers. The experimental diets were supplied to the birds *ad libitum* throughout the experimental period (Table 1). Vaccination and other

Table 1: Composition (%) of breeder diet

Attributes	T1 (0% MOLM)	T2 (9% MOLM)	T3 (12% MOLM)	T4 (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

Table 2: Weather records for the municipality for 2017

Variables	Dry season (December-March)	Major rainy season (April-July)	Minor rainy season (August-November)
Temperature (°C)	32.00	30.25	27.00
Rainfall (mm)	27.28	130.00	125.05
Humidity (%)	65.50	81.25	84.50
Cloud cover (%)	37.50	60.00	65.25
Sun hrs (h)	103.10	89.30	62.45

Table 3: Weather records for the municipality for 2018

Variables	Dry season (December-March)	Major rainy season (April-July)	Minor rainy season (August-November)
Temperature (°C)	33.00	29.11	26.30
Rainfall (mm)	28.41	123.12	117.02
Humidity (%)	68.90	82.07	80.11
Cloud cover (%)	38.80	65.21	63.15
Sun hrs (h)	106.20	67.22	85.40

routine poultry practices were also carried out. The weather records for the experimental period are shown in Table 2 and 3.

Spermatozoa characteristics: Mature males were trained for eight weeks to be responsive to release off semen. Semen was collected using the dorso-ventral massage method[12] from birds randomly selected from each of the replicates. The testes located at the dorsum were stroked and massaged until there was protrusion of the cloacae. The semen was then milked and collected using a rubber pipette and transferred to collection vials. Semen was analyzed immediately after collection at the Science laboratory, College of Agriculture Education, University of Education, Winneba, Mampong-Ashanti.

Physical characteristics of spermatozoa: Sperm motility and debris were assessed by examination of a drop of semen (5 µL) under the microscope at 10× magnification as described by Hutt[13] using Olympus BX43-Standard laboratory Microscope manufactured by Mason Technology Company Ltd, India. Sperm motility was determined as the number of cells that were motile in a volume of semen and it was reported as percent cells per millilitre (cells mL^{-1}) or millions of cells per millilitre ($\times 10^6$ mL^{-1})[14]. Semen pH was measured by using a pH meter (specially treated paper blot that changes colour according to the pH of the specimen that it is exposed to)[13]. Sperm count was determined as the number of spermatozoa in millilitre of semen sample by counting the spermatozoa in a counting chamber and was reported as millions of cells per millilitre (M mL^{-1} or $\times 10^6$ cells mL^{-1})[13].

Morphological characteristics of spermatozoa: Sperm morphology was determined as the measurement of the shape of sperm cells and was reported as percentage normal sperm cells and abnormal spermatozoa cells (big head cells, double tail cells). The percent normal and abnormal spermatozoa were counted after preparing smears and staining them with eosin and nigrosin according to the methods described by López-Rull and Gil[14]. Semen volume was measured by using calibrated micro pipette, the semen was aspirated and the volume was reported in millilitres (mL) as described by Burrows and Quinn[12].

Spermatozoa cell differential characteristics: Maximal leucocyte and erythrocyte concentration were determined as number of epithelia cell, white blood cells and red blood cells in milliliter of semen sample and was reported as millions of cells per milliliter (1×10^6 cells mL^{-1})[14].

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 using the probability of difference (PDIFF) procedure of SAS[15].

RESULTS

Proximate composition of MOLM: The proximate components of moringa leaf meal contain higher levels of carbohydrates (26.96±1.52), crude protein (28.91±0.21), metabolizable energy (2043.50±55 kcal kg^{-1}) and appreciable levels of crude fibre (13.34±0.078), dry matter (89.64±0.45), ether extracts (5.32±0.21), moisture (10.36±0.075), nitrogen free extracts (43.85±0.11) and total ash (7.13±0.04) as shown in Table 4.

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 5. 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 4: Results on proximate composition of moringa leaf meal

Parameters	Percentage (%)	Standard deviation
Carbohydrates	26.96	1.52
Crude protein	28.91	0.21
Crude fibre	13.34	0.08
Dry matter	89.64	0.45
Ether extracts	5.32	0.21
Moisture	10.36	0.05
Nitrogen free extracts	33.94	0.21
Total ash	7.13	0.04
ME (kcal kg^{-1})	2043.50	55.00

ME: Metabolizable energy

Table 5: Proximate composition of moringa leaf meal

Parameters	Dry matter (g g^{-1})	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

Proximate composition of the breeder diet: The proximate composition of the breeder diet is shown in Table 6. The crude protein, crude fibre and metabolizable energy in the breeder diet increased with increasing levels of dietary moringa leaf meal. The control treatment recorded the highest levels of moisture, ether extract and dry matter in the breeder diet while the 15% moringa leaf meal level recorded the lowest moisture content, ether extract and dry matter in the diet. The diet met the nutrient requirement for Guinea fowls as suggested by Okyere *et al.*[2].

Effect of dietary Moringa leaf meal and season on semen physical characteristics: Dietary moringa leaf meal and season had no significant ($p>0.05$) effect on sperm debris, however, it showed significant influence ($p<0.05$) on sperm motility, semen pH and sperm count (Table 7). Increasing levels of moringa leaf meal in the diet decreased sperm motility ($p<0.05$). The highest sperm motility occurred in birds that were fed the control diet while birds fed diets that contained 15% MOLM had the lowest sperm motility. The pH of the semen was higher ($p<0.05$) for birds fed the diet that contained 15% MOLM (Table 7) which made it relatively alkaline as compared to the other dietary treatments. Sperm concentration was the highest ($p<0.001$) in birds fed the diet that contained 15% MOLM diet (Table 8). Season of production had influence ($p<0.05$) on sperm motility and sperm count but not semen pH and sperm debris ($p>0.05$) (Table 7). Sperm motility was higher ($p<0.05$) in the dry season whereas, minor and major rainy seasons had similar ($p>0.05$) but significantly lower values for sperm motility.

Sperm count was higher ($p<0.05$) in the major rainy season and lower in the dry season. The highest ($p<0.05$) sperm concentration was recorded in the major rainy season and was followed by minor rainy season with the dry season being the least. Two-way interaction effect of season and dietary moringa leaf meal had little or no influence ($p>0.05$) on sperm motility, semen pH and sperm count.

Effect of dietary Moringa leaf meal and season on spermatozoa morphological characteristics: Dietary moringa leaf meal had no significant ($p>0.05$) effect on the number of abnormal spermatozoa and semen volume (Table 8). Dietary moringa leaf meal influenced ($p<0.05$) the percentage of normal sperm count. Increasing levels of moringa leaf meal in the diet increased the percentage of normal spermatozoa produced. Season had little or no effect ($p>0.05$) on abnormal spermatozoa, normal spermatozoa forms count and semen volume (Table 8). Therefore, similar means were observed across all seasons for those parameters. The combined effect of season and different levels of moringa leaf meal had little or no influence ($p>0.05$) on abnormal sperm, normal spermatozoa forms count and semen volume. Guinea fowl cocks fed diets that contained 15% MOLM produced the highest ($p = 0.003$) normal sperm as compared with other dietary treatments. Guinea fowl cocks fed diets that contained 12% MOLM had higher ($p<0.05$) normal sperm as compared to 9% MOLM and control which had similar values.

Effect of dietary moringa leaf meal and season on spermatozoa cell differential characteristics: Dietary moringa leaf meal improved ($p<0.05$) the level of white blood cells (Table 9). However, no significant effect ($p>0.05$) was observed in epithelial cells and red blood cells (Table 9). The highest white blood cells were recorded in birds fed diet that contained 15% MOLM followed by birds fed diet that contained 12 and 9% MOLM which had similar mean values. Lowest white blood cells were observed among birds fed with the control diet. There was little or no effect ($p>0.05$) of season on red blood cells and white blood cells. However, there was seasonal influence ($p<0.05$) on epithelial cells (Table 9). Epithelial cells were highest ($p<0.05$) in the minor rainy season and lowest in major and minor rainy seasons, since mean values for both seasons were similar ($p>0.05$). Interactions of season and different levels of dietary moringa leaf meal on all traits were not important ($p>0.05$).

Table 6: Proximate composition of the breeder diet

Nutrient composition	0% MOLM	9% MOLM	12% MOLM	15% MOLM
Ash (%)	16.82	17.01	17.06	17.25
Crude protein (%)	14.55	14.65	14.69	14.70
Crude fibre (%)	3.84	3.89	3.92	3.98
Moisture (%)	10.16	10.08	9.89	9.83
Ether extract (%)	4.46	4.41	4.40	4.38
Nitrogen Free Extracts	50.17	49.96	50.04	49.86
DM (%)	89.84	89.92	90.11	90.17
ME (kcal kg^{-1})	2744.00	2710.00	2710.00	2712.00

DM: Dry matter, ME: Metabolizable energy

Table 7: Dietary moringa leaf meal and season on sperm physical characteristics

Variables	Sperm motility (%)	pH	Sperm debris (%)	Sperm count (million mL^{-1})
Moringa leaf meal				
0% MOLM	77.12^{a}	7.70^{c}	47.50	2.76×10^{9b}
9% MOLM	73.75^{b}	7.92^{b}	39.58	2.32×10^{9c}
12% MOLM	69.58^{c}	7.96^{b}	40.83	2.49×10^{9bc}
15% MOLM	55.83^{d}	8.00^{a}	27.91	4.63×10^{9a}
SEM	2.31	0.05	6.04	289.90
p-value	<0.0001	0.001	0.16	0.001
Season				
Major rainy season	65.93^{b}	7.90	35.00	4.04×10^{9}
Minor rainy season	67.18^{b}	7.93	44.68	3.22×10^{9}
Dry season	74.09^{a}	7.83	37.18	$1.89.\times10^{9}$
SEM	2.01	0.04	5.23	251.06
p-value	0.014	0.28	0.39	<.0001

^{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; SEM: Standard error of means, P: Probability of main effect

Table 8: Dietary moringa leaf meal and season on morphological characteristics of spermatozoa

Variables	Abnormal spermatozoa (%)	Normal spermatozoa forms (%)	Volume of semen (µL)
Moringa leaf meal			
0% MOLM	1.58	74.58^{c}	2.83
9% MOLM	1.67	74.17^{c}	3.17
12% MOLM	1.33	79.17^{b}	2.83
15% MOLM	1.50	85.42^{a}	3.75
SEM	0.15	2.23	0.42
p-value	0.44	0.003	0.38
Season			
Major rainy season	1.63	78.75	3.25
Minor rainy season	1.44	78.75	2.59
Dry season	1.50	77.50	3.59
SEM	0.13	1.92	0.36
p-value	0.57	0.87	0.15

^{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, SEM: Standard error of means, P: Probability of main effects

Table 9: Dietary moringa leaf meal and season on spermatozoa cell differential characteristics

Variables	EC (%)	RBC (%)	WBC (%)
Moringa leaf meal			
0% MOLM	3.33	1.50	1.92^{c}
9% MOLM	3.50	1.25	2.75^{b}
12% MOLM	3.08	1.00	2.25^{b}
15% MOLM	4.42	1.42	4.67^{a}
SEM	0.53	0.23	0.46
p-value	0.33	0.43	0.0007
Season			
Major rainy season	3.06^{b}	1.25	2.50
Minor rainy season	4.56^{a}	1.63	3.37
Dry season	3.12^{b}	1.00	2.81
SEM	0.46	0.19	0.39
p-value	0.04	0.09	0.29

^{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, SEM: Standard error of means, P: Probability of main effects, EC: Epithelial cells, RBC: Red blood cells, WBC: White blood cells

DISCUSSION

Proximate composition of moringa leaf meal: Generally, moringa leaf meal was found to accumulate more protein (28.91%) (Table 4). This value is higher (17.01 and 23%) than those reported by Ogbe and Affiku[7] and Olugbemi *et al.*[16]. However, this result is similar to previous studies conducted by Ogbe and Affiku[7] (27.44%) and Kwafo *et al.*[17] (28.50%) but lower (30.65%) than those obtained by Mutayoba *et al.*[18]. The crude protein value obtained confirms that moringa leaf meal is a good source of protein in the diets of birds. The value of crude fibre (13.34%) obtained in this study is lower (16.11%) than those reported by Kwafo *et al.*[17] but higher (13.05 and 10.59%) than those reported by Kwafo *et al.*[17] and Abbas *et al.*[19]. The value of dry matter (89.64%) observed in the present study is lower than those obtained by Kwafo *et al.*[17] (90.21) but higher than those reported by Kakengi *et al.*[20] (86%). The values obtained for ether extracts (5.23%), moisture (10.36%), nitrogen free extracts (43.85%) and total ash (7.13%) were similar to values obtained in previous study[17]. The value of metabolizable energy (2043.5 kcal kg^{-1}) obtained in this study is lower (2086.5 kcal kg^{-1}) than those reported by Ogbe and Affiku[7] and Olugbemi *et al.*[16] but higher (2024.43 MJ kg^{-1}) than those obtained by Kwafo *et al.*[17]. The variations in the proximate composition of the moringa leaf meal observed in this study could be due to age of plant, soil fertility and the season of harvest[6]. The proximate compositions of moringa leaf meal observed in this study indicate that it could be used as feed ingredient.

Phytochemical properties of moringa leaf meal: Phytochemical properties of moringa leaf meal observed in this study are consistent with a previous study conducted by Valdez-Solana *et al.*[21] who reported high levels of chlorogenic acid (286.13±15.09 g g^{-1}), kaempferol (46.43±2.14 g g^{-1}), quercetin (46.18±0.6 g g^{-1}) and luteolin (44.56±2.03 g g^{-1}). The levels of phytochemicals of lipids in the liver and plasma were low and hence, will have little or no negative effect on the health status of the birds. Previous studies have identified phytochemicals like quercetin and kaempferol in moringa leaf meal[21]. Although, 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[7]. Chlorogenic acid and its isomers are esters of quinic acid and caffeic acid 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[22]. The phytochemical in moringa leaves influence the production of hormones as reported by Abou Sekken[22].

Proximate composition of the breeder diet: The crude protein levels in the formulated diet of Guinea fowls (Table 6) were within the recommended levels[23,24]. These values were within 14-16% for pullets[16]. This implies that dietary moringa leaf meal increased the protein level in the diets. Ether extract in the breeder diet showed a decreasing trend with increasing dietary moringa leaf meal, which is in agreement with the findings of Kakengi *et al.*[20]. The decrease in the level of ether extract could be attributed to the decrease in the levels of soya bean in the diets as soya bean is known to have a high fat content[17]. Conversely moringa leaf meal is lower in fat. Crude fibre level in the breeder diet showed an increasing trend in the test diets which was in contrast with Kakengi *et al.*[20] but in agreement with the reports of Kwafo *et al.*[17]. The level of crude fibre in this study were below the recommended levels (5%) for forages and thus the feed had better crude fibre levels, because the higher the crude fibre content, the poorer the diet for Guinea fowls[23,24]. The high level of crude fibre in the test diets was due to slightly high fibre content of moringa leaf meal. Moisture and dry matter in the breeder diet showed a decreasing trend with increasing level of dietary moringa leaf meal which was in contrast with the report of Kakengi *et al.*[20] but in agreement with the reports of Kwafo *et al.*[17]. The energy value of the control diet (Table 6) was slightly higher than that of the test diets. This is because the energy content of moringa leaf meal (Table 4) was observed to be low due to lower NFE in the MOLM used for the breeder diet formulation[23,24]. The implication is that, birds fed with the control diet will not have much energy and protein for growth and reproduction.

Effect of different levels of moringa leaf meal on spermatozoa physical characteristics: Semen of local male Guinea fowls with optimum attributes is required for improved reproductive efficiency[25]. In this study the highest semen pH, sperm count and the lowest sperm mortality was obtained with 15% dietary moringa leaf meal. This could be attributed to the alkalinity of the diet which supports optimum pH for successful reduction in sperm motility. Higher pH value (alkalinity) promotes sperm motility through copulation especially when prostate secretion is absent. Physiologically, the highest semen pH, sperm count and the lowest sperm motility obtained with 15% dietary moringa leaf

meal indicated that increasing moringa leaf meal in the diet increased the levels of essential amino acids such as lysine, phenylalanine, valine, histidine and isoleucine. These amino acids are responsible for high production of thymus (T-cells) and the bursa of fabricius (B-cells)[21]. These cells secrete cytokinines that act as effector cells to enhance their cytotoxic or cytostatic capabilities and increase cell numbers[21]. This explains the reason why birds on 15% dietary moringa leaf meal had the lowest sperm motility and the highest sperm count. The average pH (7.8) recorded in this study falls within the range reported by Abu *et al.*[26]. Increasing dietary moringa leaf meal significantly decreased sperm motility[27] in indigenous Zambian chickens but there was no significant relationship between moringa leaf meal and semen volume. Sperm quality parameters such as pH, concentration and motility tend to follow an upward trend in indigenous Zambian cocks that received 4% moringa leaf meal as compared to birds fed on the control diet[25]. The result of this study is consistent with the findings of Abu *et al.*[26] who observed higher sperm pH, sperm count and the lowest sperm mortality in cocks fed *Moringa oleifera* leaf meal.

Effect of season on spermatozoa physical characteristics: In this study, sperm motility was higher in the dry season. However, sperm counts was higher in the major rainy season while sperm motility was lower during that period. These findings are supported by earlier study conducted by McDaniel *et al.*[28] who reported that ambient temperature above 31°C caused high sperm motility despite the higher semen production. In this study the mean ambient temperature recorded in the dry season was 32.0°C which was above the ambient temperature (31°C) reported by McDaniel *et al.*[28]. The mean ambient temperature recorded for major (30.25°C) and minor (27.00°C) rainy seasons were lower than the 31°C reported by McDaniel *et al.*[28]. According to Deeb *et al.*[25] environmental temperature at ejaculation has significant effect on sperm motility, hence, cold seasons are ideal to reduce sperm motility and increased productivity. Optimum temperature (36-39°C) stimulates testicular growth and production and reduced sperm motility[29]. The improvement in sperm count and reduction in sperm motility in the cold seasons as compared to the dry season could be attributed to the period of rainfall as rainfall increases pituitary release of gonadotropins, luteinizing hormone and follicle stimulating hormone which enhance semen production[30].

Effect of dietary moringa leaf meal and season on semen morphological characteristics: The inclusion of 15% MOLM in the diet influenced the percentage of normal spermatozoa forms count but not on abnormal sperm and semen volume. It indicates that dietary protein in the 15% moringa leaf meal was effectively and efficiently utilized to support growth and maturation of spermatozoa. Increase in dietary moringa leaf meal significantly increased the quantity of normal forms and reduced spermatozoa abnormality[25]. Similarly, this result agrees with the findings of Andrew[27] who reported that MOLM had significant effect on the quantity and quality of semen produced. According to Abu *et al.*[26], nutrition appears to mediate its effect through increasing the frequency of pulses LH and FSH which influence semen quality and quantity. Hence, moringa leaf meal increased amino acid profile of the diet thereby increased semen quality and quantity. Results of the current study agree with the findings of Abu *et al.*[26] who recorded high normal sperm forms as compared to the abnormal forms.

Increasing dietary moringa leaf meal did not increase abnormal sperm, normal forms count and semen volume. The result of this study is supported by Abu *et al.*[26] who reported no increase in spermatozoa concentration, abnormal sperm, normal forms count and semen volume in male rabbits fed varied levels of moringa leaf meal. This report agrees with the observation made by Brun *et al.*[31] who reported similar trends in male rabbit breeders' semen volume.

Effect of dietary moringa leaf meal and season on semen cell differential characteristics: Increasing dietary moringa leaf meal significantly increased the levels of white blood cells[25]. The highest WBC (4.67%) observed with 15% moringa leaf meal was lower than 10-20% which has been identified as major cause of infertility in males[32]. Similarly, the highest WBC (4.67%) observed in this study is lower than those reported by Okyere *et al.*[33] who recorded 5.45% in pearl Guinea fowls. These significant differences could be due to the level of MOLM which supplied all the essential amino acids for semen production and to maintain the healthy spermatozoa for normal function. The WBC in semen has the potential to damage sperm function and ovum penetration and has been associated with decreased sperm number, reduced sperm velocity and impaired sperm fertility[27]. In general, the lower level of WBC observed in this study indicates high sperm production, increased sperm velocity and improved sperm fertility. The presence of high WBC level indicates poor semen quality. Quality semen may have only leucocytes and epithelial cell[30]. The present result is also in agreement with Okyere *et al.*[34] who evaluated the effect of *Moringa oleifera* leaf meal on egg fertility and spermatozoa quality of Potchefstroom koekoek indigenous chicken and recorded lower mean value (4.33%) for WBC.

Epithelial cells were higher during the minor rainy season and similar values were observed in the major rainy and the dry seasons but not for red blood cells and white blood cells. The significant differences could be explained that moderate ambient temperature enhances maximum production of gonadotropin hormones which stimulate high epithelial cells' production. Hence, this is possible during the minor rainy season where temperatures are not too high and also not too low. This result agrees with the findings of Brun *et al*.[31] who reported high production of epithelial cells in cold seasons as compared to the dry seasons. According to Okyere *et al*.[34] hot dry season affect reproduction ability of cocks due to heat stress[30], reducing feed intake, inhibiting release or response of GnRH, FSH and LH which are hormones that support spermatogenesis. These hormones, especially, LH are inhibited by increasing level of plasma corticosteroids due to heat stress in the dry season and trigger decline of thyroxin secretion to reduce feed intake and metabolism and causing reduction in quality of semen produced[33,35].

CONCLUSION

Spermatozoa quality and quantity increased with increasing levels of moringa leaf meal (up to 15%) in the diet of Guinea fowl cocks. Semen is higher during the major rainy season with low mortality rate. This study recommends that, farmers and breeders should consider 15% dietary MOLM inclusion level for optimum spermatozoa production. This study further recommends that Guinea fowl breeding should be carried out in the major and minor rainy seasons.

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