



Influence of Dietary Protein on Embryonic Development and Hatching Traits of Indigenous Guinea Fowl (*Numida meleagris*) Breeders in Togo

Lamboni Lare^{1*}, Essodina Talaki^{1,2}, Koffi Francois-Xavier Dzogbema¹, Comla Sodjedo², and Yao Lombo³

¹Centre d'Excellence Régional sur les Sciences Aviaires, Université de Lomé CERSA/UL, 01 B.P. 1515 Lomé, Togo

²Ecole Supérieure d'Agronomie, Université de Lomé ESA/UL, 01 B.P. 1515 Lomé, Togo

³Institut Togolais de Recherche Agronomique, BP : 1163 Lomé, Togo

* Corresponding author's Email: nicody92@gmail.com; ORCID: 0000-0001-7272-3556

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ABSTRACT

The diet of breeding indigenous guinea fowl breeders can influence their reproductive performance. Thus, the current study was designed to investigate the effects of different dietary protein intake levels of breeders on embryonic development. A total of 1152 hatching eggs were collected, numbered, weighed, and allocated to 3 groups (control, high-protein [High-P], and low-protein [Low-P]) with 4 replicates of 96 eggs in each dietary treatment group. The eggs were candled on incubation days 9 and 24. At the end of the hatch, all keets were counted and weighed. Six keets in each group were randomly selected and sacrificed for blood and organ collection. The results showed that the heaviest eggs were for the High-P group and keets at hatch had higher relative hatching muscle weights, compared to the control group. However, the Low-P group had smaller eggs and keets gained lower relative hatching muscle weights compared to the control. Fertility, early mortality rate, and delayed mortality decreased in the High-P group but it increased in the Low-P group, compared to the control. The dietary protein did not influence the incubation time. The maternal diet protein content affected fertility, embryonic development, hatchability, and keet quality. The indigenous guinea fowl breeders need appropriate protein intake to show their potential reproduction performances.

Keywords: Guinea fowl, Hatching, Incubation, Nutrition, Protein

INTRODUCTION

The local guinea fowl or common guinea fowl is the second-highest poultry species after chicken in Togo. It is very important for socio-cultural Togolese events, such as ceremonies, donations, and welcoming of important visitors (Gnassingbe, 1983). The meat of the guinea fowl and its eggs provide immediate satisfaction with protein needs (Sanou, 2005). According to Sanou (2005), the flesh of the guinea fowl is appealing to consumers disgusted by broiler chickens for its "wild" taste. However, the production of guinea fowl has many constraints, such as high keets mortality, the exigency of brooder low fertility, low hatchability, and diet requirement. For the local guinea fowl producers, low fertility, poor hatchability, and poor quality day-old keets are the main impediments.

Poor performance of guinea fowl is linked to the incubation and hatching conditions, age, health, and nutritional status of the parents. Parental nutrition can

significantly influence egg and meat quality (Anton et al., 2006). Besides these factors, nutrition can impact the fertility and hatchability of the eggs. Nutritional deficiency in breeder feed can directly result in the abnormal development of the embryo (Bourin et al., 2011). Indeed, feed protein content affects the feed cost of breeder hens (Wu et al., 2007), their laying performance (Wilson, 1997), and their offspring quality and performance (Calini and Sirri, 2007). According to Moran (2007), the feed protein content of the hen's diet has an influence on embryonic development and hatchability. An excessive protein intake in the cocks feed affects their reproductive performance by reducing fertility (Vieira, 2007).

The effect of chickens breeders' diets on their embryonic development and hatchability has been widely investigated by researchers. However, there are few studies on the impact of the breeding diet of local guinea fowl on embryonic development. Moreover, several studies have focused on improving the nutritional quality

of feed for breeding hens and its optimal effect on offspring (Larbier and Leclercq, 1992). The protein characteristics of feeds are a major nutritional factor in assessing the nutritional value of feeds (Ding et al., 2016) and must be considered when formulating breeder diets (Nideou, 2018). The aim of this study was to assess the impact of dietary protein intake of guinea fowl breeders on their embryonic development.

MATERIALS AND METHODS

Ethical approval

Due to the unavailability of the Animal Care Committee at the University of Lomé at the time of this research, the research was conducted under the supervision of the leader of the animal research team following the guidelines of CCOAC (2009).

Experimental design

A total number of 168 female and 84 male local guinea fowls aged 48 weeks old were allocated into three groups. The fowls were divided into 4 replicates of 14 hens and 7 cocks (Houndonougbo, 2017) according to their dietary treatments. These groups consisted of the control group (control), and the two experimental groups, which included those that received low protein diets (Low-P), and a group, which received high protein diets (High-P). Diet ingredients and calculated nutritive values are shown in Table 1. A total of 1152 eggs were collected randomly within 10 consecutive days and stored at 15°C and 70% relative humidity prior to incubation. Eggs were numbered, weighed, and allocated to four replicates of 96 eggs per each according to the parent's dietary treatments. The eggs were subjected to the same temperature, humidity, and turning conditions. During the first 24 days, the temperature was 37.7°C, the humidity was 60%, and the turning occurred automatically every hour at a 90° angle. On days 9 and 24 of incubation, the eggs were candled to determine fertility and embryonic mortality, respectively. During candling on day 24, only the eggs containing embryos were weighed and transferred into the hatcher at a temperature of 37.4°C with 70% relative humidity during the remaining days. This weighing allowed us to determine the water loss using the following formula:

$$\text{Weight loss} = P_0 - P_{24}$$

Where, P₀ is the initial egg weight and P₂₄ refers to weight at 24 days of incubation (Nideou, 2018).

Incubation time, hatchability and embryonic mortality, and day-old keets body weight determination

From day 26 of incubation, the keets hatched in each group were counted every three hours until the end of the

hatching period. The keets were counted based on dietary treatments and then weighed at the end of the hatch. Unhatched eggs were counted and then broken to identify and count embryonic mortalities. These data were used to determine the incubation time, hatchability, early and late mortality rates, and day-old keets body weight.

Keets weight, carcass weight without the yolk sac, yolk sac weight, and hatching muscle relative weight determination

At the end of the hatch, six keets from each treatment were randomly sampled, weighed, and sacrificed for the blood and the organ collection. The yolk sac and hatching muscle were weighed.

Total proteins, albumin, glucose, triglycerides, and cholesterol levels determination

At post-hatch, blood samples were collected from six local keets from each treatment for the determination of the total protein, albumin, glucose, triglyceride, and cholesterol. The total protein, albumin, triglycerides, and cholesterol concentration of serum was determined using an enzymatic colorimetric method on 25, 10, 10, 10, and 3 µl of serum respectively for the total protein, albumin, glucose triglycerides, and cholesterol. This method is based on the formation of a colored product from which the intensity is proportional to the concentration of the parameter. To validate the test, a standard was assayed and the optical density measurements were made at 546, 630, 500, 490, and 500 nm for the total protein, albumin, triglycerides, and cholesterol, respectively according to the protocols provided by Cypress Diagnostics from Nijverheidsstraat 82235 Hulshout of Belgium reagent (Cypress Diagnostics, 2020).

Hematology determination

Blood samples were taken from six keets per treatment in EDTA-impregnated tubes and used to determine the red blood cells (RBC), white blood cells (WBC), Hematocrit (Hte), hemoglobin (Hb), and lymphocytes by ABX Micros 60, which is a fully automated hematology analyzer (Sysmex Corporation International Company, Japan) according to Nakul et al. (2003).

Statistical analysis

A commercial scientific 2D graphics and statistics software GraphPad Prism software Inc. (version 5.00.288), a privately held California (USA) corporation (Morgan, 1998) was used to analyze the data. Hatchability and embryo mortality were analyzed by the Chi-square test. The ANOVA test was also used for statistical analysis of the other parameters. The comparison between the different groups after ANOVA was done with the Tukey test. The significant level was set at $p < 0.05$.

Table 1. Local guinea fowl breeders raised in Togo diet ingredients and macronutrient levels according to treatment and age

Ingredients	Starter (1-8 weeks)			Growth (9-22 weeks)			Laying (23-60 weeks)		
	Control (%)	Low-P (%)	High-P (%)	Control (%)	Low-P (%)	High-P (%)	Control (%)	Low-P (%)	High-P (%)
Maize	32	34	21.5	53	57.5	50	57	61.5	53.5
Wheat	10	8	7	17	16.5	15	10.5	10	8
Soybeans	25	18	27.5	15.5	11	16.5	17	12	19
Dresh	5	3	5	-	-	-	-	-	-
Fish	7	10	12	5	6.5	7	5	6	6
Millet	15	19	21	-	-	-	-	-	-
Flesh concentrated	5	7	5	5	4	7	-	-	-
Laying concentrated	-	-	-	-	-	-	4	4	7
Shell	1	1	1	4.5	4.5	4.5	6.5	6.5	6.5
Total	100	100	100	100	100	100	100	100	100
Analysis									
ME (kcal/kg)	2893.7	2876	2886	2757.2	2764.53	2755.87	2802	2701	2799
Crude protein	21.34	19.69	23.06	18.04	16.57	19.55	17.13	15.67	18.62
Calcium	0.94	1.09	1.10	1.84	1.88	1.98	2.54	2.58	2.78
Phophorus	0.78	0.87	0.82	0.73	0.72	0.83	0.70	0.70	0.84
Methionine	0.49	0.57	0.56	0.45	0.42	0.53	0.09	0.07	0.09
Lysine	1.1	1.13	1.39	0.93	0.87	1.08	0.36	0.27	0.39
Methionine + cysteine	0.75	0.82	0.84	0.67	0.63	0.78	0.19	0.14	0.19

Low-P: Low protein, High-P: High protein, ME: Metabolizable energy. The diet was balanced according to Yildirim (2012).

Table 2. Effect of protein intake of 48 weeks old local guinea fowl raised in Togo on their egg weight and weight loss during incubation

Parameters	Treatments			Probability
	Control	High-P	Low-P	
Egg weight (g)	38.46 ± 0.26 ^b	40.12 ± 0.36 ^a	35.38 ± 0.29 ^c	0.0001
Water loss (g)	5.54 ± 0.19 ^b	4.83 ± 0.08 ^c	6.00 ± 0.10 ^a	0.0001

^{a, b, c}: Different letters in the same row indicate significant differences ($p < 0.05$). Low-P: Low protein, High-P: High protein

Table 3. Effect of protein intake of 48 weeks old local guinea fowl raised in Togo on embryonic development and hatchability

Parameters	Treatments			Probability
	Control	High-P	Low-P	
Fertility rate (%)	72.49 ± 1.26 ^b	61.14 ± 1.48 ^c	85.79 ± 0.48 ^a	0.0001
Hatch rate (%)	84.17 ± 0.54 ^b	89.11 ± 4.00 ^a	58.92 ± 3.94 ^c	0.0004
Early mortality (%)	2.44 ± 1.24 ^b	0.88 ± 0.88 ^b	12.07 ± 2.05 ^a	0.0116
Delayed mortality (%)	13.38 ± 0.81 ^b	10.03 ± 3.19 ^b	29.01 ± 2.01 ^a	0.0003
Incubation time (hours)	621.00 ± 3.24	619.50 ± 3.35	621.00 ± 3.24	0.9919

^{a, b, c}: Different letters in the same row indicate significant differences ($p < 0.05$). Low-P: Low protein, High-P: High protein

Table 4. Effect of protein intake of 48 weeks old local guinea fowl breeders raised in Togo on their keets weight at hatch, carcass weight without the yolk sac (YFBM), yolk sac weight, relative hatching muscle weight

Parameters	Treatments			Probability
	Control	High-P	Low-P	
Keets weight at hatch (g)	22.69 ± 0.25 ^b	24.38 ± 0.51 ^a	20.44 ± 0.40 ^c	0.0001
Relative weight of hatching muscle (%)	0.011 ± 0.00 ^b	0.014 ± 0.00 ^a	0.010 ± 0.00 ^b	0.0001
Yolk sac weight (g)	1.51 ± 0.06 ^{ab}	1.70 ± 0.09 ^a	1.09 ± 0.09 ^b	0.0004
YFBM (g)	21.00 ± 0.18 ^b	22.74 ± 0.39 ^a	19.13 ± 0.28 ^c	0.0001

^{a, b, c}: Different letters in the same row indicate significant differences ($p < 0.05$). Low-P: Low protein, High-P: High protein

Table 5. Effect of protein intake of 48 weeks old local guinea fowl breeders raised in Togo on serum biochemistry of their keets

Parameters (g/l)	Treatments			Probability
	Control	High-P	Low-P	
Pt	22.62 ± 0.33 ^b	25.71 ± 0.68 ^a	20.54 ± 0.26 ^c	0.0006
Alb	16.22 ± 0.19 ^b	17.44 ± 0.34 ^a	15.03 ± 0.12 ^c	0.0001
Glu	3.19 ± 0.18	3.27 ± 0.17	2.85 ± 0.06	0.1901
Trigl	0.40 ± 0.02 ^b	0.48 ± 0.03 ^a	0.30 ± 0.0 ^c	0.0001
Chol	1.57 ± 0.18	1.65 ± 0.04	1.48 ± 0.03	0.1960

^{a, b, c}: Different letters in the same row indicate significant differences ($p < 0.05$). Low-P: Low protein, High-P: High protein, Pt: Total protein, Alb: Albumin, Glu: Glucose, Triglycerides, Chol: Cholesterol

Table 6. Effect of protein intake of 48 weeks old local guinea fowl breeders raised in Togo on hematological indices of their keets

Parameters (g/l)	Treatments			Probability
	Control	High-P	Low-P	
RBC ($\times 10^9/L$)	2.62 ± 0.06 ^{ab}	2.67 ± 0.07 ^a	2.28 ± 0.06 ^b	0.0015
WBC ($\times 10^{12}/L$)	78.51 ± 0.92 ^b	91.95 ± 0.80 ^a	74.75 ± 1.14 ^c	0.0001
Lym ($\times 10^9/L$)	66.21 ± 0.75 ^a	71.46 ± 3.72 ^a	53.57 ± 2.68 ^b	0.0060
Haemo (g/dl)	18.35 ± 0.52 ^b	18.65 ± 0.27 ^a	17.47 ± 0.41 ^c	0.0001
Hte (%)	35.65 ± 0.36 ^a	37.54 ± 0.49 ^a	33.68 ± 0.65 ^b	0.0004

^{a, b, c}: Different superscripts in the same row indicate significant differences ($p < 0.05$). Low-P: Low protein, High-P: High protein, RBC: Red blood cells, WBC: White blood cells, Lym: Lymphocytes, Hte: Hematocrit, Haemo: Hemoglobin

RESULTS

Effect of protein intake of breeder guinea fowls on egg weight and weight loss

Table 2 shows the weight of the eggs and the weight loss according to the dietary treatment of the parent. In terms of egg weight, keets of the High-P group recorded the highest and the Low-P keets had the lowest eggs weights ($p < 0.05$). For water loss, the results showed that the water loss was significantly higher in the Low-P treatment and lower in the High-P treatment, compared to the control ($p < 0.05$).

Fertility rate, hatch rate, early embryonic mortality rate, delayed embryonic mortality rate, and incubation time

The fertility was significantly lower in the High-P group ($p < 0.05$), compared to the control group. On the other hand, it was significantly higher in the Low-P group ($p < 0.05$), compared to the control. The hatchability was significantly higher in the High-P group than that of the control group ($p < 0.05$). Hatchability was, however, significantly lower in the Low-P group ($p < 0.05$), compared with the control. Reducing the protein content of the feed significantly increased the early mortality rate ($p < 0.05$). However, an increase in protein content did not influence the early mortality rate, compared to the control group. The delayed mortality rate was significantly high in the Low-P treatment ($p < 0.05$) but similar to that of the High-P and control treatments. The feed quality did not affect the incubation time because it was similar across all the treatments (Table 3).

Hatching keets weight, carcass weight without the yolk sac weight, yolk sac weight, and relative weight hatching muscle

The weight of the keets at hatch, the yolk free body mass, the weight of the yolk sac, and the relative weight of the hatching muscle are recorded in Table 4. The weights of the keets from the Control, High-P, and Low-P groups are respectively 22.69 ± 0.25 g, 24.38 ± 0.51 g, and 20.44 ± 0.40 g. The high protein diet significantly increased keets weight at hatch. On the other hand, the reduction in the protein of diet significantly reduced keets weight at hatch ($p < 0.05$). Keets from the High-P group showed significantly higher relative hatch muscle weight ($p < 0.05$), compared to the control group. Nevertheless, the reduction in the protein level did not influence the relative weight of the hatching muscle because they were

comparable to the control ($p > 0.05$). Increasing the protein level of the parents' feed did not affect the yolk sac weight of the keets because their weights were similar to those of the control group ($p > 0.05$). However, the keets from the Low-P group showed a significantly low yolk sac weight, compared to the control. Low-P keets exhibited a low carcass weight without the yolk sac compared to the control group ($p < 0.05$). On the other hand, the High-P group showed greater carcass weight without the yolk sac ($p < 0.05$) compared to the control.

Effect of protein variations of breeder guinea fowl on serum biochemistry of keets

Table 5 shows the serum concentration of the total protein, albumin, glucose, triglycerides, and total cholesterol. The total protein, albumin, and triglycerides concentration of the serum were significantly higher in keets from parents fed by the high protein diets, compared to the control ($p < 0.05$). However, it was lower in keets whose parents consumed a low protein diet compared to the control ($p < 0.05$). On the other hand, they were low ($p < 0.05$) in the keets whose parents consumed a low protein diet compared to the control group ($p < 0.05$). Glucose and cholesterol levels of keets were not affected by maternal diet ($p > 0.05$).

Effect of protein variations of breeder guinea fowl on hematological indices of keets

Keets whose parents were fed a low protein ration had low levels of RBC and hematocrit ($p < 0.05$) while they were similar in keets from the High-P and control parents groups. The Low-P group had the lowest hemoglobin level ($p < 0.05$) and the high protein group had a higher hemoglobin level ($p < 0.05$), compared to the control. The low protein content of the parent's diet negatively affected the concentration of lymphocytes and WBC in their keets while these concentrations were similar in the High-P and control groups (Table 6).

DISCUSSION

The nutritional quality of the breeder diet can influence the quality of eggs, embryonic development, hatchability, hematological and biochemical parameters of their offspring. The obtained results of the present study indicated that the eggs of the Low-P group had more moisture loss than those of the control group. According to Jaiswal *et al.* (2016), this situation could be explained by the high surface-to-volume ratio, that has larger pore areas

and pore diameters, which thus, become more favorable to gas and heat exchanges in these eggs. Indeed, the eggs from this group showed low weight before incubation. The larger size of the eggs from the High-P group explains their low water loss, compared to the control. In addition, a study conducted by [Abanikannda et al. \(2011\)](#) involving three strains of broiler chickens (Anak-361, Marshall-359, and Ross-282) showed that weight loss was lower in larger eggs than smaller eggs. Similarly, [Ulmer-Franco et al. \(2010\)](#) reported that the percentage of weight loss of incubating eggs decreased with increasing egg size in Cobb 500 broiler breeders. This water loss is due to the porosity of the shell because it significantly influences the loss of water during incubation ([Peebles and Brake, 1986](#)). This can be attributed to the fact that there is a mismatch of certain amino acids, such as methionine in the low protein diet, which influences the absorption and retention of calcium. Methionine forms a complex compound with calcium, which slows calcium absorption and increases serum calcium retention. This retention according to [Nideou \(2018\)](#) leads to a greater flow of calcium to the bones and the eggs, resulting in good porosity of the shell and consequently an optimal loss of water by the egg.

The fertility of the High-P group was significantly lower in the present study. This may be due to the excessive supply of nutrients before and during the laying phase period, which increases the deposition of the fat in the reproductive system, thereby blocking the oviduct of the hen to prevent the movement and storage of sperm ([Van Emous et al., 2015](#)). Similarly, [Larbier and Leclercq \(1992\)](#) asserted that excessive protein intake in males during the laying phase affected reproductive performance by reducing fertility. In addition, increased protein intake leads to excessive nitrogen excretion with an increased uric acid rejection, which makes the cloacal environment acidic with the consequence of reducing the quality and motility of sperm in storage ([De Beer, 2009](#)).

The hatchability of the Low-P group was significantly lower than those of the control and High-P groups. This poor performance is linked to the high rate of early and delayed mortality in this group. These high mortalities could be attributed, in part, to the low egg weights and abundant loss of water from their eggs ([Sanfo et al., 2007](#)). On the other hand, a maternal diet low in crude protein may lead to reduced albumin in eggs, which can increase embryonic mortality and decrease the hatching of chicks ([Lopez and Leeson, 1994](#)). Indeed, the free amino acids in the yolk originate largely from the diets and induce the growth of the embryo. They are initially used during the early stages of embryonic

development. Their partial deficiency has adverse implications on embryonic development ([Larbier and Leclercq, 1992](#)). Feeding a diet of 0.21% methionine instead of 0.36% reduced the hatching but injection of methionine into the egg eliminates the effects of the deficiency ([Larbier and Leclercq, 1992](#)). Furthermore, deficiencies of linoleic acid and linolenic acid partially led to a decrease in egg weight, a reduction in the number of yolk lipids, and an increase in embryonic mortality ([Larbier and Leclercq, 1992](#)). [Walsh and Brake \(1997\)](#) demonstrated that the 11% and 14 % crude protein decreased fertility and hatching than the hen diet of 17 % crude protein. Furthermore, a study by [Leeson et al. \(1979\)](#) showed that nutrient deficiency is the cause of embryonic mortality on the eighth day of incubation. The increase in the level of crude protein in the diet could increase the content of methionine, linoleic acid, linolenic acid, biotin, Vitamins A and E, and pantothenic acid which explains the high hatchability of the eggs from the High-P group. In addition, keets from the High-P group exhibited greater relative hatching muscle weights than those from the other groups. Indeed, the hatching muscle is an organ that provides the necessary energy for the chick to break the shell by the dorsal than the lateral movement of the head ([Nwagu, 1997](#)). The shell of the guinea fowl egg is very hard, the consistency of this muscle, therefore, defines the quality of the hatching. Therefore, the Low-P group with a high embryonic death rate had a low hemoglobin level and the High-P group with a low embryonic mortality rate had a high hemoglobin level. This result corroborates the findings of [Harmon \(1936\)](#) who reported that groups with low mortality had higher hemoglobin levels than those with high mortality.

Feeding the parents with the low protein decreased the concentration of lymphocytes and white blood cells of the keets at hatch. This suggests that these keets have low immunity. This result explains the high mortality rate of the embryos of the Low-P group.

The protein intake of the diet did not influence the incubation time, as it was similar in all treatments. [Enting et al. \(2007\)](#) report that decreasing or increasing protein levels in parental diets had no impact on incubation time. The consumption of the high protein diets increased the weight of the keets at hatching in the current study. This difference could be related to the weight of the eggs, which were heavier in this treatment. The high nutrient reserve content of large eggs may explain the high hatching weight of the keets. A study conducted by [Tona et al. \(2003\)](#) demonstrated that chick weight increases with

an increase in egg weight because heavier eggs contain more nutrients than smaller eggs. According to Tullett and Burton (1982), most of the variations in hatching chick weight are associated with the differences in the fresh egg weight and the weight loss during incubation.

The parental diet influenced the serum concentration of the total protein and albumin in keets from parents that consumed high protein feed. In fact, their total serum proteins were significantly higher than those of the control group. This result could be attributed to the high serum concentration of the total protein and albumin of the parents, which strongly influenced the protein profile of the offspring. The early increase in the level of plasma proteins is explained by intense somatic development in keets with a high hatching weight (Nideou, 2018). This increase was also observed in turkeys (Szabo *et al.*, 2005) and Sasso broiler chicks (Nideou, 2018). On the other hand, a low concentration of the total serum proteins was observed in the keets from parents fed a lower content of dietary protein. This result could be attributed to the result of the maternal parents because their parents had low serum total protein concentration. The results of the present study showed that the protein level of the parent feed did not influence the serum cholesterol concentration of the hatched guinea keets. This finding may result from low feed intake in the hepatic synthesis of cholesterol.

The higher serum triglycerides concentration of keets from the parents fed high protein ration is believed to be due to the maternal lipid profile because the parents of the High-P group had a high serum triglycerides concentration. In addition, it was found that these keets had large yolk sacs, which is the unique source of lipid for hatching keets. On the other hand, these types of lipids were low in the serum of keets whose parents consumed a lower content of protein compared to the control group. This could be the consequence of the nutritional deficiency of their parents. According to Zhao *et al.* (2009), a low concentration of triglycerides would be the consequence of a low lipid profile in the blood reflecting an important transport of amino acids and better lipid metabolism with a strong decrease in fat deposits.

The keets from the Low-P group indicated a low level of red blood cells, hemoglobin, and hematocrit. This means that the protein deficiency in the parents' diets resulted in anemia in their hatching offspring. Indeed, some proteins promote the absorption and binding of iron leading to the optimal synthesis of red blood cells (Johnson-Wimbley and Graham, 2011).

CONCLUSION

The dietary protein of breeder guinea fowl affected embryonic development and hatchability. The feed with high protein improved the egg weight, keets weight, and hatchability but reduced the rate of fertility. The feed of guinea fowl breeders with low protein content improved fertility but had a negative effect on embryonic development. The protein level of diet had no impact on hematological and biochemical parameters. It is suggested to pursue this study on offspring through to laying.

DECLARATION

Competing interests

There is no conflict of interest with the authors in this study.

Authors' contributions

This study was carried out with gratitude to the contribution of the following authors. Lamboni Lare designed the protocol, collected and analyzed data, drafted the manuscript, and edited the draft. Essodina Talaki validated the protocol, supervised the data collected, and revised critically the manuscript. Koffi François-Xavier Dzogbema and Comla Sodjedo collected data and revised the manuscript. Yao Lombo revised manuscript. All these authors read and approved the final version of the manuscript.

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Ethical considerations

This manuscript does not contain plagiarized sentences, has not been published or accepted for publication elsewhere or under editorial review for publication elsewhere. The data are not fabricated or falsified henceforth our Institute's Centre d'Excellence Regional sur les Sciences Aviaires of the University of Lomé representative is fully aware of this submission.

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