

Effects of *Moringa oleifera* Leaf Meal on Local Guinea Fowl Breeder Hens' Performance, Egg Quality, and Blood Parameters

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ABSTRACT

Breeding local guinea fowl has the potential to address protein malnutrition and alleviate poverty in West African countries. The current study aimed to examine the effects of incorporating *Moringa oleifera* leaf meal into the diet on hematology and biochemical parameters as well as the productive performance of local guinea fowl breeders in Togo. Thus, 512 local guinea fowls (22 weeks of age with an average weight of 1176.7 ± 2.9 g), comprising 384 females and 128 males, were examined for 31 weeks. The fowls were randomly assigned to four dietary groups, namely M0, M1, M2, and M3 containing 0%, 0.5%, 1%, and 1.5% of *Moringa oleifera* leaf meal in diets, respectively. Each group had 4 replicates of 32 fowls. During the study, feed intake, body weight, egg-laying rate, and feed conversion ratio were weekly recorded. Blood samples for hematology and biochemical analysis were taken from 12 females (3/replicate) at 34 and 50 weeks of age. Eggs were collected for the quality evaluation. Results showed that feed intake was comparable across all groups during the rearing period (23 to 33 weeks of age). However, it significantly decreased in fowls of the M2 and M3 during the laying period (34 to 50 weeks of age). The live weight of fowls in M3 was significantly higher than other groups before the laying period. The egg production, yolk ratio, and shell ratio of the birds in the M1 were like that of M0 and higher than that of M2 and M3. The feed conversion ratio was not significantly different between groups during the laying period. However, the albumen ratio and haugh unit were improved by feeding *Moringa* leaves especially at level 1.5%. The level of white blood cells and lymphocytes decreased by feeding the *Moringa* leaves at 1%. Neutrophils and platelet levels were comparable across groups. Total proteins, albumin, and transaminases increased in *Moringa* groups (M1, M2, M3), especially in M3. It was concluded that the use of *Moringa oleifera* leaves at 0.5% improved egg-laying performance in local guinea fowl. Moreover, 1% and 1.5% of incorporation improved the quality of eggs.

Keywords: Blood parameter, Breeder, Guinea fowl, Egg quality, *Moringa oleifera*

INTRODUCTION

The rapid human population growth has led to an increase in meat consumption (Boko et al., 2012; Popkin et al., 2012). The breeding of local guinea fowls (*Numida meleagris*) is one of the animal production sectors that contributes to meeting the animal protein requirements of the population in West African countries (Konlan et al., 2011; Sodjedo et al., 2022). Local guinea fowl production is considered in West African countries, especially in

Togo, as a sector for poverty reduction (Konlan et al., 2011; Sodjedo et al., 2022). It originates from Africa (Ikani and Dafwang, 2004), and guinea fowls have also a cultural significance (Koné et al., 2018). However, guinea fowl production still faces many difficulties such as low growth and availability of post-hatch juveniles and their high mortality rate during the start (Sanfo et al., 2008; Houndonougbo et al., 2017). Also, laying in local guinea

fowl is in cycles (about 75 to 110 eggs per year) and coincides with the rainy season (Konlan et al., 2011; Sodjedo et al., 2022). However, Sodjedo et al. (2022) reported that guinea fowl can lay throughout the year if they are fed adequate feed *ad libitum*. Thus, feed is one of the major factors in developing local guinea fowl breeding (Lombo et al., 2018).

Several studies have shown that the nutritional composition of feed consumed by chickens influences egg laying, egg quality, and hatchability (Teteh et al., 2016; Voemesse et al., 2019; N'nanle et al., 2020). The productivity of animals can be enhanced by utilizing natural feed additives derived from herbs, spices, or other plants. These additives exert positive effects on digestibility, nutrient absorption, and the control of parasites in the digestive tract (Sadr et al., 2022; Eftekhari Hasan Abad and Ghaniei, 2023; Nyembo et al., 2023). *Curcuma longa*, incorporated at the levels of 0.5% and 1% in layer mash has improved egg weight, shell thickness, and yolk ratio (Radwan et al., 2008). Houndonougbo et al. (2012) reported significant improvement in egg weight, albumen, and yolk ratio of guinea fowls at 21 and 32 weeks of age when incorporating dried leaves of *Manihot esculenta* into their layer mash.

Moringa oleifera is a leguminous plant and a feed additive used in animal production (Ahmed and Lohakare, 2021; Giang et al., 2023). Its leaves are rich in carbohydrates, lipids, proteins, minerals, salts, and vitamins (Kashyap et al., 2022; Ahmed et al., 2023; Yang et al., 2023a). *Moringa oleifera* is known to have antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, cholesterol-reducing, and hepatoprotective properties (Khalid et al., 2023; Kashyap et al., 2022; Ntshambiwa et al., 2023). The leaves contain also antinutritional substances such as tannins, saponins that limit their use (Khalid et al., 2023; Kashyap et al., 2022). In breeding, several studies have shown an improvement in the production performance of cows (Mendieta-Araica et al., 2011), small ruminants (Fadiyimu et al., 2010; Sultana et al., 2015), rabbits (Abubakar et al., 2015) with the use of *Moringa oleifera* leaves. In poultry, Voemesse et al. (2019) and Ahmed and Lohakare (2021) reported an increase in the production and weight of eggs when *Moringa* leaves are incorporated into the diet of laying hens. Yang et al. (2023b) have shown an improvement in feed conversion ratio (from hatching to 4 weeks of age), laying rate, average egg weight, and feed conversion rate in laying ducks fed with *Moringa* flavonoid meal diets. The leaves of *Moringa Oleifera* improved the performance (feed conversion ratio, laying rate, average egg weight) of

Sasso breeder hens and the internal quality of hatching eggs (N'nanle et al., 2020). *Moringa oleifera* also improves broiler chicken growth performance (Body weight gain, feed conversion ratio), carcass characteristics, and cecal microbial structure (El-Tazi 2014; Zhang et al., 2023).

Despite numerous studies and scientific reports on the effects of *Moringa oleifera* leaves, research on the incorporation of *Moringa oleifera* leaf meal in enhancing the productive performance of local guinea fowl breeders remains limited. Therefore, the current study aimed to evaluate the effects of *Moringa oleifera* leaf meal on hematology and biochemical parameters as well as the productive performance of local guinea fowl breeders.

MATERIAL AND METHODS

Ethical approval

The animal care guidelines recommended by the Animal Ethics Committee of the University of Lomé in Togo were followed (008/2021/BC-BPA/FDS-UL).

Study area

The Regional Center of Excellence in Poultry Sciences (CERSA) of the University of Lomé in Togo served as a framework for the study. The manipulations were conducted in the Poultry Production Techniques laboratories of the Regional Center of Excellence in Poultry Sciences and the laboratories of the Higher School of Biological and Feed Techniques (ESTBA) of the University of Lomé. The experimental management of the breeders lasted 31 weeks, from November 2021 to June 2022. The guinea fowl were provided by the Poultry Production Techniques laboratories of the Regional Center of Excellence in Poultry Sciences.

Preparation of *Moringa oleifera* leaves and diet formulation

Moringa oleifera leaves were collected from rural areas of Togo (in Togoville: 6°14'28.68"N and 1°29'06.43"E; in the prefecture of Vo), spread out on a clean surface and dried with natural ventilation. The dried leaves were milled into powder form before their incorporation into feed. The experimental diets were formulated to contain 0% (control), 0.5% (500g added to 100kg of diet), 1% (1000g added to 100kg of diet), and 1.5% (1500g added to 100kg of diet) of dry matter of *Moringa oleifera* leaves. All diets were iso-nitrogenous and iso-caloric. The compositions of the experimental diets are shown in Table 1.

Table 1. Composition (%) of experimental diets according to *Moringa oleifera* treatments during 22-31 weeks of age and 32-50 week of age (laying period) in guinea fowl

Ingredient	Feed composition according to age and group							
	22-31 weeks of age				32-50 weeks of age			
	M0	M1	M2	M3	M0	M1	M2	M3
Maize	54	53.5	53.5	53	59	59	59	59
Wheat bran	17	17	17	17	13	12.5	12	12
Roasted soybean	19	19	18.5	18.5	18	18	18	17.5
Laying concentrate	2	2	2	2	2	2	2	2
oyster shell	7	7	7	7	7	7	7	7
Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
<i>Moringa oleifera</i> leaf	0	0.5	1	1.5	0	0.5	1	1.5
Total	100	100	100	100	100	100	100	100
Calculated analysis								
ME (Kcal/Kg)	2787	2791	2795	2799	2849	2860	2870	2873
Crude protein (%)	17.72	17.67	17.45	17.40	17.06	17.13	17.21	17.13
Calcium (%)	2.27	2.26	2.26	2.26	2.27	2.28	2.29	2.30
Phosphorus (%)	0.53	0.52	0.52	0.52	0.49	0.48	0.48	0.48
Methionine (%)	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Lysine (%)	1.07	1.07	1.05	1.05	1.03	1.03	1.03	1.03
Methionine + Cystine (%)	1.02	1.02	1.01	1.01	1	1	0.99	0.99

ME: Metabolizable energy; M0, M1, M2, M3: Treatments with 0%, 0.5%, 1%, and 1.5% *Moringa oleifera* leaves in diet, respectively. The study lasted 28 weeks.

Study design and animal management

For this study, a total of 384 female local guinea fowl breeders and 128 males, all aged 22 weeks, were randomly divided equally into four dietary treatments, each with four replicates. This distribution entailed 24 females and 8 males per replicate following a study by Sodjedo et al. (2022). In each replicate, including the control group (M0) and three other groups (M1, M2, M3), guinea fowls were fed accordingly. The control group was provided with a basal diet devoid of *Moringa oleifera* leaves. The other treatment groups received the same basal diet, with the addition of 0.5% (M1), 1% (M2), and 1.5% (M3) dry matter of *Moringa oleifera* leaves. After one week of acclimatization, the four experimental diets were randomly assigned and fed to the guinea fowls *ad libitum*. Guinea fowls were reared in an open henhouse, wood partitioned (2.7 m × 2 m) with a deep litter floor housing system at a density of 6 per square meter (Nahashon et al., 2006). Water was offered *ad libitum* throughout the experiment and natural light (dry season with 25°C on average and rainy season with 27.3°C on average) was used as the source of lighting. All treatments followed the same prophylaxis program, including anti-infection prevention with oxyfuran 4® (1g/l of water) for days 1 to 4, anti-Newcastle vaccine with Hitchner B1 for day 3, coccidiosis prevention with Amprolium 20%® (3g/5l of water) for days 12 to 16, deworming with Pipérazine citrate® (2g/l of water for 1 day) and anti-Newcastle vaccine with Cevac® for week 3, deworming with VSP® (¼ tablet for 1 day) for week 8, anti-infection prevention with oxyfuran 4® (1g/l

of water for 5 days) for week 10, an anti-Newcastle vaccine with Ita-New for week 14, deworming with VSV® for week 17, coccidiosis prevention with Amprolium 20%® (3g/5l of water for 5 days) for week 22, and deworming with VSV® for week 26 (Hien et al., 2002; Lombo et al., 2011).

Data collection and calculated parameters

Throughout the experiment, the amount of feed offered to the breeders each day was measured. Feed intake (FI) was calculated weekly as the difference between feed offered and leftover for each replication. Feed conversion ratio (FCR) was calculated as the ratio of total feed consumed to total body weight gain (BWG) before laying (total FI/total BWG, Voemesse et al., 2018). During the laying period, eggs were collected daily, and the laying rate was registered weekly (sum of daily egg number × 100/sum of daily bird number). Feed conversion ratio was calculated with average egg laying rate, egg weight, and FI as grams of egg mass per gram of feed consumed (daily FI/[laying rate × average egg weight]) following N'nanle et al. (2020).

At the onset of the experiment and then every week before the egg-laying period, the fowls underwent weighing to ascertain BWG corresponding to their respective treatment groups. Body weight gain was computed by subtracting the initial weight from the successive body weight measurements during the experimental (final weight-initial weight). Additionally, daily weight gain (DWG) was calculated as the ratio of BWG to the duration of the

experimental period (in days). Mortalities recorded were utilized to calculate the mortality rate.

At 38, 42, 46, and 50 weeks of breeders' age, 32 eggs per treatment were weighed and broken delicately to collect shell, albumen, and yolk (N'nanle et al., 2020). These components were also weighed to determine their ratio (component weight \times 100/absolute egg weight, N'nanle et al., 2020) and Haugh Unit (HU) with the following relationship, $HU = 100 \times \log (Hb - 1.7W^{0.37} + 7.6)$, where, Hb denotes the height of thick albumen and W is egg weight (N'nanle et al., 2020).

At the end of the experiment, abdominal fat and organs (heart, liver, kidney, intestine, and gizzard), randomly taken from 8 females per treatment were weighed to determine their ratio (abdominal fat or organs weight \times 100/live weight, N'nanle et al., 2020). Fowls were stunned and slaughtered for the samples.

At 33 and 50 weeks of breeders' age, venous blood samples (wing vein, approximately 2 ml) were taken from 12 females (chosen at random) per treatment. These blood samples were collected in tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) for blood count and blood formula (NFS) and in dry tubes, without anticoagulant, for assay of biochemical parameters. For hematological parameters, the blood samples were used on Mindray BC-3000 Auto Hematology Analyzer, from Mindray Buiding, Keji 12th Road South, High-tech Industrial Park, Nanshan, Shenzhen 518057, P.R. China. For biochemical parameters, blood samples were centrifuged at 3000 rpm. Serum was collected for total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, uric acid, urea, triglycerides, total cholesterol, and HDL-Cholesterol measuring. Blood parameters were determined by colorimetric method using Mindray BS Auto-biochemical analyzer from China.

Statistical analysis

All data collected were analyzed using ANOVA with GraphPad Prism 8.1 software followed by Tukey's test for comparisons between treatments. Data obtained were expressed as mean \pm standard deviation of the mean and as a percentage. Results were statistically significant when $p < 0.05$.

RESULTS

Feed intake

The effect of *Moringa oleifera* leaves on FI before and during lay is presented in Table 2. According to the

result of the study, there was no significant difference in FI across treatments before laying (23 to 33 weeks of age, $p > 0.05$). However, during the laying (34 to 50 weeks of age), guinea fowls in M2 and M3 had significantly lower FI, compared to those in other groups ($p < 0.05$).

Daily weight gain

Before egg-laying, the daily weight gain was comparable between treatments M0, M1, and M2, but significantly lower than that of the M3 treatment, as indicated in Table 3 ($p < 0.05$).

Egg laying rate and egg weight

Figure 1 shows the effect of *Moringa oleifera* leaves on the improvement of the laying rate according to the age of guinea fowls. Egg laying rates were similar among treatments from week 34 to week 39 of breeders' age ($p > 0.05$). However, starting from week 40, the laying rate of groups M0 and M1 exceeded that of other groups, except for week 49 when laying rates remained similar between treatments ($p < 0.05$).

The average laying rate of groups M0 and M1 was higher than that of groups M2 and M3, as shown in Table 3 ($p < 0.05$).

The average egg weight increased with breeders' age in each group and there was no significant difference between groups ($p > 0.05$) although M3 had numerically the highest egg weight (Table 3).

Feed conversion ratio

The FCR, before and during the laying period, is shown in Table 2. Before laying, FCR values of groups M0, M1, and M2 were not significantly different ($p > 0.05$) but significantly higher than that of group M3 ($p < 0.05$). During laying, FCR values of all treatments were insignificantly different ($p > 0.05$). However, M2 and M3 groups recorded numerically the highest FCR.

Mortality rate

The mortality rate was relatively low in all groups. Group M0 recorded a higher rate of mortality ($p < 0.05$) and there was no significant difference between the M1 and M2 groups ($p > 0.05$). The M3 treatment recorded the lowest mortality rate (Table 3).

Average weight of organs and abdominal fat

Concerning gizzard weight the difference between treatments was insignificant ($p > 0.05$). The weight of the liver and the intestine of the guinea fowls of group M3 was significantly higher than that of the M0, M1, and M2

groups ($p < 0.05$). For these same parameters, the difference was not significant between M1 and M2 groups ($p > 0.05$). The M0 group recorded the lowest values ($p < 0.05$) of liver and intestine weight. The absolute heart weight values of groups M2 and M3 were higher than those of M0 and M1 ($p < 0.05$). Abdominal fat and carcass weight were higher in groups M0 and M1 than in groups M2 and M3, although the difference was not significant in the fat abdominal area ($p > 0.05$), as shown in Table 4.

Egg components and haugh unit

Table 5 shows the yolk ratio albumen ratio and shell ratio. Concerning the yolk ratio and shell ratio the difference was insignificant between all groups. The albumin ratio of groups M3 and M2 was significantly higher than that of M0 and M1 groups, especially at 38 and 50 weeks of age ($p < 0.05$).

Concerning the Haugh Unit, there was no significant difference across all groups at week 38 ($p > 0.05$). However, at 42, 46, and 50 weeks of age, the Haugh Unit of eggs from M3 breeders was significantly higher compared to the other groups, while the group M0 recorded the lowest values, as shown in Figure 2 ($p < 0.05$).

Hematological parameters

The level of white blood cells (WBC) and lymphocytes detected in guinea fowls of M0 and M1 treatments were higher than that of M2 and M3 treatments

($p < 0.05$) at 34 and 50 weeks of age. Regarding the red blood cell (RBC) count, there were insignificant differences between treatments ($p > 0.05$). At 34 weeks of age, blood platelets and neutrophils showed no significant differences among all treatments ($p > 0.05$). However, at 50 weeks of age, blood platelet levels decreased with the incorporation of *Moringa oleifera* leaves in the M2 and M3 groups (Table 6).

Biochemical parameters

Serum parameter concentrations are summarized in Table 7. Total protein level at 50 weeks of age was significantly higher in treatments M1, M2, and M3, compared to the control group ($p < 0.05$). At 34 weeks of age, the levels of alanine aminotransferase and uric acid were significantly higher in groups M1, M2, and M3, compared to the control ($p < 0.05$). At 50 weeks of age, the levels of albumin, aspartate aminotransferase, alanine aminotransferase, and uric acid were significantly higher in groups M1, M2, and M3, compared to the control ($p < 0.05$). Triglyceride levels were lower in M1, M2, and M3 compared to M0 at 34 weeks of age ($p < 0.05$). At 50 weeks of age, triglyceride decreased in M2 and M3, compared to M0 and M1 ($p < 0.05$). Total cholesterol level decreased in M1, M2, and M3, compared to M0 at 34 weeks of age. There was no significant difference between treatments concerning total cholesterol and HDL-CH levels at 50 weeks of age ($p > 0.05$).

Table 2. Feed intake and feed conversion ratio according to *Moringa oleifera* treatments and age of guinea fowls

Age	Groups	Parameters	M0	M1	M2	M3	P-value
23 - 33		FI	60.3 ± 1.8	60.58 ± 1.07	59.36 ± 0.97	61.37 ± 1.4	0.7752
		FCR	13.08 ± 0.46 ^a	13.05 ± 0.23 ^a	14.44 ± 0.37 ^a	11.07 ± 0.14 ^b	0.0085
34 - 50		FI	89.76 ± 1.02 ^a	89.57 ± 0.58 ^a	82.62 ± 0.75 ^b	84.04 ± 0.41 ^b	0.0044
		FCR	4.44 ± 0.18	4.48 ± 0.11	4.71 ± 0.2	4.55 ± 0.23	0.7536

M0, M1, M2, M3: Treatments having received respectively 0%, 0.5%, 1% and 1.5% *Moringa oleifera* leaves in the diet; FI: Feed intake FCR: Feed conversion ratio; ^{a,b,c} Within row, values not sharing the same letters are significantly different ($p < 0.05$).

Table 3. Daily weight gain (23-33 weeks of age), laying rate and egg weight (34-50 weeks of age), mortality rate (22-50 weeks of age) of guinea fowls according to *Moringa oleifera* treatments

Parameters	Groups	M0	M1	M2	M3	P-value
DWG (g)		4.20 ± 0.08 ^b	4.23 ± 0.11 ^b	4.04 ± 0.04 ^b	4.88 ± 0.13 ^a	0.0123
Laying rate (%)		50.53 ± 2.02 ^a	49.94 ± 1.88 ^a	43.04 ± 1.74 ^b	44.62 ± 1.58 ^b	0.0188
Egg weight (g)		39.14 ± 0.40	39.26 ± 0.43	39.26 ± 0.41	39.75 ± 0.48	0.9143
Mortality rate (%)		15 ± 0.78 ^a	13.33 ± 0.57 ^{ab}	13.33 ± 0.48 ^{ab}	11.67 ± 0.24 ^b	0.0473

M0, M1, M2, M3: Treatments having received respectively 0%, 0.5%, 1%, and 1.5% *Moringa oleifera* leaves in the diet; DWG: Daily weight gain; ^{a,b,c} Within row, values not sharing the same letters are significantly different ($p < 0.05$).

Table 4. Average weight and ratio of organs and abdominal fat of guinea fowls at 50 weeks of age according to *Moringa oleifera* treatments

Parameters		Groups	M0	M1	M2	M3	p-value
Average weight (g)	Heart		6.67 ± 0.24 ^b	6.45 ± 0.26 ^b	7.4 ± 0.24 ^{ab}	8 ± 0.2 ^a	0.0023
	Liver		27.33 ± 0.33 ^c	31.33 ± 1.2 ^b	31.67 ± 0.88 ^b	38.67 ± 0.33 ^a	<0.0001
	Kidney		6 ± 0.41 ^{ab}	7.25 ± 0.25 ^a	5.88 ± 0.31 ^b	6.5 ± 0.29 ^{ab}	0.0403
	Gizzard		27 ± 1.23	27 ± 1.78	27.5 ± 1.85	27.75 ± 1.11	0.9796
	Intestine		100.75 ± 0.75 ^c	111 ± 1.08 ^b	119.75 ± 1.25 ^b	141 ± 4.45 ^a	<0.0001
	MPS		73.5 ± 2.22 ^c	92 ± 2.16 ^a	80.25 ± 0.63 ^{bc}	82 ± 2.04 ^b	<0.0001
	Abdomina fat		11.50 ± 0.65	11.75 ± 0.48	10.25 ± 0.48	10.5 ± 0.46	0.1678
	Carcass		1386.75 ± 34.4 ^{ab}	1465.25 ± 9.6 ^a	1247.5 ± 19.4 ^c	1300.5 ± 31.9 ^{bc}	0.0003
Ratio (%)	Heart		0.4 ± 0.007 ^b	0.37 ± 0.004 ^b	0.48 ± 0.009 ^a	0.51 ± 0.006 ^a	0.0033
	Liver		1.64 ± 0.1 ^b	1.82 ± 0.114 ^b	2.07 ± 0.13 ^{ab}	2.48 ± 0.1 ^a	0.0002
	Kidney		0.36 ± 0.015	0.42 ± 0.011	0.38 ± 0.007	0.42 ± 0.013	0.0923
	Gizzard		1.62 ± 0.03 ^{ab}	1.57 ± 0.02 ^b	1.8 ± 0.045 ^a	1.78 ± 0.053 ^{ab}	0.0496
	Intestine		6.03 ± 0.12 ^c	6.44 ± 0.15 ^c	7.82 ± 0.18 ^b	9.05 ± 0.19 ^a	<0.0001
	MPS		4.40 ± 0.11	5.34 ± 0.24	5.24 ± 0.19	5.27 ± 0.27	0.1251
	Abdominal fat		0.69 ± 0.012	0.68 ± 0.025	0.67 ± 0.015	0.67 ± 0.013	0.1977
	Carcass		83.01 ± 0.83 ^{ab}	85.02 ± 1.01 ^a	81.51 ± 0.92 ^c	83.51 ± 0.81 ^{bc}	0.0007

M0, M1, M2, M3: Treatments having received respectively 0%, 0.5%, 1%, and 1.5% *Moringa oleifera* leaves in their diet; MPS: Superficial pectoral muscle; ^{a,b,c} Within row, values not sharing the same letters are significantly different (p < 0.05).

Table 5. Egg components ratio of guinea fowls according to *Moringa oleifera* treatments at different ages

Parameters		Groups	Age (weeks)	M0	M1	M2	M3	p-value
Yolk ratio			38	34.12 ± 0.1	34.30 ± 1.05	36.08 ± 1.36	32.02 ± 1.01	0.1747
			42	31.54 ± 0.87	32.06 ± 1.1	32.35 ± 1.1	32.93 ± 0.5	0.7658
			46	29.95 ± 1.08	31.12 ± 1.85	29.60 ± 1.2	29.54 ± 0.44	0.7947
			50	31.06 ± 0.97	33.12 ± 2.02	30.48 ± 1.08	30.80 ± 2.2	0.6927
Albumen ratio			38	51.44 ± 0.22 ^{bc}	50.40 ± 0.2 ^c	52.42 ± 0.12 ^{ab}	52.99 ± 0.17 ^a	0.0020
			42	51.53 ± 0.5	51.45 ± 0.21	51.97 ± 0.18	52.28 ± 0.38	0.3931
			46	50.59 ± 0.19	51.99 ± 0.56	52.06 ± 1.09	52.78 ± 0.08	0.1376
			50	50.42 ± 0.21 ^c	50.94 ± 0.18 ^{bc}	51.80 ± 0.14 ^b	53.20 ± 0.16 ^a	0.0016
Shell ratio			38	14.46 ± 0.29	13.89 ± 0.23	14.20 ± 1.01	14.67 ± 0.17	0.7719
			42	16.38 ± 0.17	16.02 ± 0.84	16.75 ± 0.21	17.49 ± 0.33	0.8667
			46	18.60 ± 0.70	19.24 ± 0.98	17.41 ± 1.02	17.68 ± 0.79	0.3690
			50	17.66 ± 0.12	16.46 ± 0.67	17.12 ± 0.88	16.60 ± 1.01	0.9327

M0, M1, M2, M3: Treatments having received respectively 0%, 0.5%, 1%, and 1.5% *Moringa oleifera* leaves in the diet; ^{a,b,c} Within row, values not sharing the same letters are significantly different (p < 0.05).

Table 6. Hematology parameters of guinea fowls according to *Moringa oleifera* treatments at different ages

Age (weeks)	Blood parameters	Groups	M0	M1	M2	M3	p-value
34	WBC (10 ³ /μL)		44.85 ± 0.57 ^b	62.80 ± 2.81 ^a	30.88 ± 0.89 ^c	41.04 ± 3.44 ^b	<0.0001
	RBC (10 ⁶ /μL)		2.55 ± 0.16	2.34 ± 0.30	2.19 ± 0.13	2.23 ± 0.13	0.5795
	Hemoglobin (g/dl)		11.17 ± 0.49 ^a	9.17 ± 1.37 ^b	9.20 ± 0.36 ^b	9.13 ± 1.04 ^b	0.0347
	Hematocrit (%)		41.57 ± 3.08 ^a	38.13 ± 5.11 ^{ab}	37.70 ± 2.4 ^b	37.43 ± 3.74 ^b	0.0184
	PLT (10 ³ /μL)		1.67 ± 0.33	1.33 ± 0.33	2.33 ± 0.67	2.33 ± 0.33	0.3437
	Neutrophil (10 ³ /μL)		15.93 ± 3.02	33.54 ± 11.4	20.49 ± 1.8	37.60 ± 18.8	0.5022
	Lymphocyte (10 ³ /μL)		14.06 ± 0.3 ^b	23.39 ± 4.5 ^a	9.29 ± 1.28 ^c	12.28 ± 4.25 ^b	0.0125
	Monocyte (10 ³ /μL)		1.59 ± 1.2	0.45 ± 0.1	0.54 ± 0.07	0.66 ± 0.39	0.6171
	Basophil (10 ³ /μL)		7.04 ± 2.2	5.48 ± 0.5	7.23 ± 0.5	6.15 ± 0.6	0.7224
	50	WBC (10 ³ /μL)		92.01 ± 4.2 ^a	64.53 ± 5.78 ^b	35.80 ± 2.9 ^c	61.97 ± 6.8 ^b
RBC (10 ⁶ /μL)			2.43 ± 0.08	2.35 ± 0.27	2.54 ± 0.26	2.59 ± 0.3	0.8988
Hemoglobin (g/dl)			9.40 ± 0.46	9.43 ± 1.7	9.77 ± 1.5	11 ± 1.6	0.8267
Hematocrit (%)			38.73 ± 0.74 ^b	38.93 ± 7.8 ^b	42.13 ± 5.8 ^{ab}	45.63 ± 5.9 ^a	0.0028
PLT (10 ³ /μL)			1.67 ± 0.33	1 ± 0.58	1 ± 0.58	0.67 ± 0.33	0.5319
Neutrophil (10 ³ /μL)			59.73 ± 19.62 ^a	40.34 ± 26.3 ^b	15.76 ± 1.6 ^c	36.28 ± 9.9 ^b	0.0028
Lymphocyte (10 ³ /μL)			25.16 ± 6.35 ^a	17.81 ± 8.7 ^b	11.41 ± 2.34 ^c	14.14 ± 1.06 ^{bc}	<0.0001
Monocyte (10 ³ /μL)			0.30 ± 0.03	0.25 ± 0.02	0.30 ± 0.1	0.28 ± 0.04	0.9130
Basophil (10 ³ /μL)			6.82 ± 1.6	6.17 ± 1.4	7.33 ± 1.3	7.93 ± 1.7	0.8562

M0, M1, M2, M3: Treatments having received respectively 0%, 0.5%, 1%, and 1.5% *Moringa oleifera* leaves in the diet; WBC: White blood cell; RBC: Red blood cell; PLT: Blood platelets; ^{a,b,c} Within row, values not sharing the same letters are significantly different (p < 0.05).

Table 7. Biochemical parameters of guinea fowls according to *Moringa oleifera* treatment at different ages

		Groups				p-value
Age (weeks)	Blood parameters	M0	M1	M2	M3	
34	Total protein (g/L)	30.9 ± 0.33 ^a	23.7 ± 0.48 ^b	25.85 ± 0.14 ^b	25.95 ± 1.14 ^b	<0.0001
	Albumin (g/L)	7 ± 0.29 ^b	7.1 ± 0.36 ^b	7.2 ± 0.12 ^b	9.13 ± 0.26 ^a	0.0003
	AST (U/L)	251.5 ± 2.87 ^c	251.25 ± 3.04 ^c	271.25 ± 1.7 ^b	300.5 ± 3.86 ^a	<0.0001
	ALT (U/L)	9 ± 0.41 ^c	12 ± 0.41 ^{ab}	11.75 ± 0.32 ^b	13.5 ± 0.35 ^a	<0.0001
	Creatinine (mg/dl)	0.14 ± 0.04	0.21 ± 0.07	0.11 ± 0.03	0.14 ± 0.04	0.5290
	Uric Acid (mg/dl)	6.4 ± 0.24 ^c	7.5 ± 0.38 ^b	6.95 ± 0.27 ^{bc}	8.75 ± 0.36 ^a	0.0249
	Urea (g/L)	0.03 ± 0.007	0.03 ± 0.004	0.03 ± 0.007	0.03 ± 0.004	0.9780
	Triglycerides (g/L)	5.86 ± 0.13 ^a	3.52 ± 0.1 ^c	5.30 ± 0.11 ^b	2.95 ± 0.13 ^d	<0.0001
	Cholesterol (g/L)	1.34 ± 0.12 ^a	0.85 ± 0.04 ^b	0.99 ± 0.04 ^b	1.07 ± 0.04 ^b	0.0034
HDL-CH (g/L)	0.37 ± 0.04	0.45 ± 0.02	0.34 ± 0.04	0.45 ± 0.03	0.1068	
50	Total protein (g/L)	27.53 ± 1.9 ^b	41.35 ± 1 ^a	37.93 ± 0.55 ^a	39.08 ± 0.62 ^a	<0.0001
	Albumin (g/L)	5.38 ± 0.19 ^b	6.73 ± 0.18 ^a	7.28 ± 0.28 ^a	7.23 ± 0.16 ^a	<0.0001
	AST (U/L)	252 ± 4.04 ^c	333.5 ± 10 ^b	381.25 ± 4.42 ^a	356.75 ± 5.2 ^{ab}	<0.0001
	ALT (U/L)	4.5 ± 0.29 ^c	8 ± 0.2 ^a	6 ± 0.41 ^b	7.5 ± 0.29 ^a	<0.0001
	Creatinine (mg/dl)	0.18 ± 0.01 ^b	0.25 ± 0.02 ^{ab}	0.25 ± 0.03 ^{ab}	0.30 ± 0.02 ^a	0.0118
	Uric Acid (mg/dl)	5.45 ± 0.22 ^c	7.49 ± 0.35 ^b	6.59 ± 0.18 ^b	9.38 ± 0.26 ^a	<0.0001
	Urea (g/L)	0.03 ± 0.007	0.03 ± 0.005	0.04 ± 0.008	0.04 ± 0.005	0.3362
	Triglycerides (g/L)	5.37 ± 0.13 ^b	7.20 ± 0.3 ^a	4.44 ± 0.43 ^{bc}	4.12 ± 0.09 ^c	<0.0001
	Cholesterol (g/L)	1.15 ± 0.05	1.31 ± 0.08	1.20 ± 0.19	1.16 ± 0.13	0.8726
HDL-CH (g/L)	0.35 ± 0.06	0.34 ± 0.08	0.35 ± 0.1	0.44 ± 0.1	0.8548	

M0, M1, M2, M3: Treatments having received respectively 0%, 0.5%, 1%, and 1.5% *Moringa oleifera* leaves in the diet; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HDL-CH: High density lipoproteins-cholesterol; ^{a,b,c} Within a row, values not sharing the same letters are significantly different (p < 0.05).

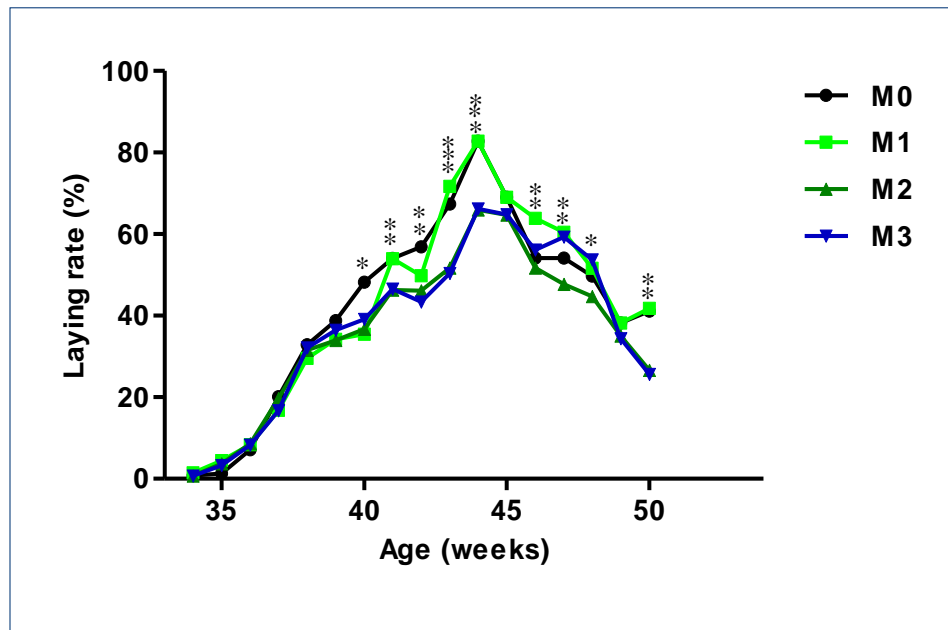


Figure 1. Laying rate according to age (34-50 weeks of age) and *Moringa oleifera* treatment (* show significant difference at 0.05). M0, M1, M2, M3: Treatments having received 0%, 0.5%, 1% and 1.5% *Moringa oleifera* leaves in diet respectively; at each age, significant differences are indicated by * (p < 0,05); ** (p < 0,01); *** (p < 0.001).

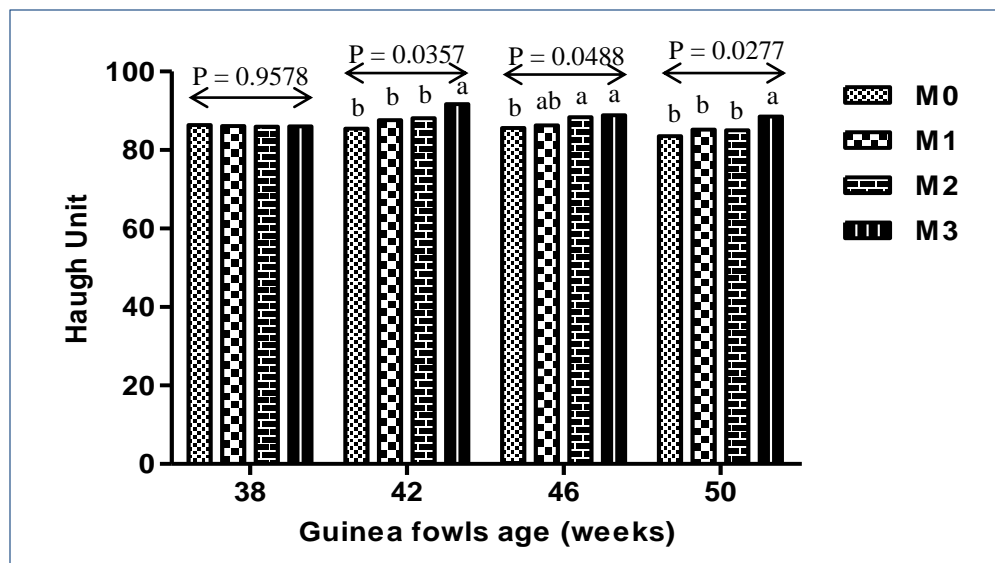


Figure 2. Haugh Unit according to different ages (38, 42, 46, and 50 week of age) and *Moringa oleifera* treatment. M0, M1, M2, M3: Treatments having received respectively 0%, 0.5%, 1%, and 1.5% *Moringa oleifera* leaves in diet; ^{a,b,c} Within a row, values not sharing the same letters are significantly different ($p < 0.05$).

DISCUSSION

The present study demonstrated clearly that the incorporation of *Moringa oleifera* leaves in the local guinea fowl breeder diet affected productive performance, hematological as well as biochemical values, and egg quality. Before the laying period, the similarity in FI is comparable to that reported by Voemesse et al. (2018) in laying chickens during juvenile growth and Teteh et al. (2016) in ISA brown chicks (layer-type). Sanchez et al., (2006) estimated that *Moringa oleifera* leaves did not contain any factors that could limit feed consumption. However, a decrease in feed consumption with 10% of *Moringa oleifera* leaves in one-week-old chicks was obtained by Ashong and Brown (2011). This difference could be explained by the level of incorporation of *Moringa oleifera* leaves, which reached a maximum of 1.5% in this experiment. Additionally, it is important to consider the poultry species used in this study, which was guinea fowl, as opposed to laying chickens or ISA brown chicks examined in the studies referenced. In the laying period, the decrease in feed consumption in M2 and M3 was also reported by N'nanle et al. (2020) on Sasso breeding hens and Voemesse et al. (2019) on laying hens. This result could be explained by the cumulative effect of *Moringa oleifera* leaves with the age of animals, which would increase the level of components such as saponins, tannins, and fibers (Gupa et al., 1989; Kakengi et al., 2007). According to Francis et al. (2001), the presence of these substances creates a feeling of satiety in animals and

slows down the progression of feed in the digestive tract. This situation could lead to a decrease in feed consumption. Furthermore, the higher weight of intestines observed in the *Moringa oleifera* groups could be attributed to a slowing down of feed progression in the digestive tract.

The reduction of FCR in group M3 is consistent with the increase in weight gain of fowls in the same group. The same results were reported by Sarker et al. (2017) and Teteh et al (2013) in broilers and by Teteh (2016) and Voemesse et al. (2018) in laying hens. The high BWG in M3 treatment could be due to the richness and quality of the nutrients contained in *Moringa* leaves (Teteh et al., 2016; Manzo et al., 2016). Likewise, *Moringa oleifera* leaves have antimicrobial (Djakalia et al., 2011; Divya et al., 2014; Ahmed et al., 2023), immunomodulatory (Tété-Bénissan et al., 2013), and antioxidant (Santos et al., 2012; Wiwit et al., 2016) properties that can improve animal health and nutrients uptake. Thus, by reducing intestinal microflora (*Escherichia coli* and *Staphylococcus aureus*), *Moringa oleifera* leaves reduce gut competition for available nutrients (Voemesse et al., 2019; Ahmed et al., 2023). This mechanism contributes to the higher concentration of proteins, such as albumin in blood which is known as one of the main serum proteins serving as an amino acid source for the synthesis of tissue proteins in the growth period of birds (Yaman et al., 2000). All these factors would have contributed to the better BWG obtained by the M3 group. Furthermore, the hematological results at the start of the lay show a significant decrease in

white blood cell counts and lymphocytes with *Moringa oleifera* leaves levels in the diet. However it shows that a high white blood cell count is associated with an infection or the presence of foreign bodies or antigens in the circulating system (Ahamefule et al., 2008). The decrease in these immune cells is therefore synonymous with improving animal health.

The higher laying rate in M0 and M1 treatments confirms the results of Teteh et al. (2016) and Voemesse et al. (2019) in laying hens with the difference that the best level of incorporation of *Moringa oleifera* leaves for laying is 0.5% in the present study against 1% in Teteh et al. (2016) and Voemesse et al. (2019). The same results were reported by N'nanle et al. (2020) in Sasso breeders with 1% of *Moringa oleifera* leaves. This difference would be linked to the species of poultry used for the experiment. The lowest laying performance of M2 and M3 could be related to the high level of *Moringa oleifera* leaves which might increase estrogens level, because of phytoestrogens contained in the leaves (Zade and Dabhadkar 2014). Indeed, Zade and Dabhadkar (2014) obtained in rats a decrease of FSH and LH, associated with phytoestrogens, thus leading to an increase in the duration of the ovarian cycle of rats. In addition, Titi et al. (2013) showed that sterols contained in *Moringa oleifera* leaves are used as precursors for estrogen synthesis. These results confirm the reports of Musa-Azara et al. (2014) who claimed that the intake of some amount of estrogenic substances in *Moringa* leaves inhibits the secretion of LH and FSH and reduces endogenous estrogen and progesterone levels. This reduction may play a role in the low number of follicles and consequently a decrease in the laying rate.

The improvement in egg weight with the level of *Moringa oleifera* leaves is in agreement with that of N'nanle et al. (2020) in Sasso breeders and Teteh et al. (2016) in layers. According to the study of Suk and Park (2001), heavy eggs have proportionally more albumen and less yolk. However, the results of this study showed that the proportion of albumen was higher in the eggs from M2 and M3 treatments compared to M0 and M1. Thus, the amino acids profile of *Moringa oleifera* leaves notably the sulfurous amino acids which are essential for protein synthesis improve egg weight (Bunchasak, 2009). In addition, the phytochemical analysis of *Moringa* leaves revealed a significant presence of selenium (Tarmizi et al., 2023). This component could also justify the high egg weight of M3 treatment according to Attia et al. (2010) who indicated that the use of organic selenium sources improves egg weight.

The increase in albumen ratio, positively correlated with Haugh Unit would be due to the concentration of amino acids, vitamins, mineral salts, and other compounds contained in *Moringa oleifera* leaves (Yang et al., 2006; Moyo et al., 2012; Kashyap et al., 2022). Brought to the breeding hens these components would stimulate the synthesis of albumin by the liver, thus causing the increased serum albumen level and consequently the increase of albumen ratio (N'nanle et al. 2020).

The similar yolk ratio between treatments could be explained by the yolk formation process according to the results of Nys et al. (2011). These authors claimed that during the formation of yolk from embryogenesis, the lipoproteins are transported from the liver to the ovaries where they are deposited in oocytes to form follicles. Thus, nutritional problems related to the feeding of breeders have little influence on the yolk ratio. The maximum rate of 1.5% of *Moringa oleifera* leaves used in this experiment would also be insufficient to reduce the proportion of egg yolk. The same is true for shell ratio which was not influenced by *Moringa* leaves. The anti-nutritional factors such as phytates and oxalates, found in *Moringa oleifera* leaves, which have a less chelating effect on minerals such as calcium (Foidl et al., 2001), making phosphorus and calcium unavailable could explain these results. Moreover, hens have still the capacity to move calcium from bones compensating feed calcium intake for shell calcification (Wright et al., 1990).

The total serum proteins which include the proteins of the nutritional, immune, enzymatic, and inflammatory state (Tété-Bénissan et al., 2013) were low in *Moringa oleifera* leaves groups compared to the control at 34 weeks of age. At 50 weeks of age, the results showed that total proteins were higher in *Moringa oleifera* leaves groups. The increase in albumin level at 34 weeks of age with *Moringa oleifera* leaves would testify to nutritional improvement, especially with increased uric acid and similarity of creatinine. Indeed, uric acid and urea are indicators of an increase in protein catabolism (Donsbough et al., 2010). Thus, the decrease in total proteins at 34 weeks would be related to the decrease in proteins of immunity and inflammation, which, according to Tennant and Center (1997) and Tete-Benissan et al. (2013), would be a consequence of the improvement of animal health.

Results showed that AST and ALT levels were significantly higher in *Moringa oleifera* leaves treatments at 50 weeks of age. Indeed, the liver, involved in metabolic and detoxification, can present lesions, responsible for an increase in gamma-glutamyltransferase

and transaminases (AST and ALT). These lesions can be caused by infections, diet intoxication, large liver, and poisoning (Nkosi et al., 2005; Mega et al., 2021). Thus, the secondary metabolites of diet or products resulting from their metabolism can cause liver damage, especially with age, that can lead to the release of transaminases (Mega et al., 2021). Thus, the results of this study showed that liver and heart weight were higher in groups fed *Moringa oleifera* leaves, compared to the control. The results also noted an increase in creatinine levels with *Moringa oleifera* leaves, at 50 weeks of age, which could also show the effect of these toxins on the kidneys. The nephrons could be damaged by toxins found in *Moringa oleifera* leaves, such as phenols, especially with prolonged use. This could potentially lead to the accumulation of products like creatinine in the blood (Lauriola et al., 2023). These observations are in agreement with Hetland et al. (2003) results which stipulate that prolonged exposure of oat hulls and wood shaving in broilers and layers diet exacerbates the effect of antinutritional factors. The high levels of AST, ALT, and creatinine could explain the high levels of total protein in groups fed *Moringa oleifera* leaves at 50 weeks of age.

Concerning serum lipids parameters, the slight decrease in total cholesterol at 34 weeks of age would be linked to the decrease in LDL-cholesterol because of the similarity of HDL-CH levels between groups. The slight reduction in cholesterol levels could be explained by the low level of *Moringa oleifera* leaves. Indeed, phytosterols and stanols contained in *Moringa* leaves only compete with cholesterol in the formation of micelles necessary for cholesterol absorption if they are present in sufficient quantity in the gut (Serfaty-Lacrosnière et al., 2001). The significant decrease in triglycerides at 34 weeks of age would be due to the presence of some secondary metabolites such as saponins in *Moringa oleifera* leaves (Nweze and Nwafor, 2014). Indeed, the use of feed additives relatively rich in saponins reduced serum triglycerides (Afrose et al., 2010). Furthermore, Francis et al. (2002) showed that saponins are known to form insoluble salts with lipids.

The decrease of white blood cells and lymphocytes with *Moringa oleifera* leaves is in agreement with the results obtained by Voemesse et al. (2019) in laying hens, and could be explain by the antimicrobial properties of *Moringa* leaf (Ahmed et al., 2023). According to Ahamefule et al. (2008), the increase in WBCs is often associated with bacterial infections or the presence of foreign bodies. Authors found that *Moringa oleifera* leaves reduced gut microflora by inhibiting the growth of

some pathogenic germs such as *Escherichia coli* and *Staphylococcus aureus* (Djakalia et al., 2011; Divya et al., 2014; Ahmed et al., 2023), thus causing a reduction in WBC and lymphocytes. This reduction confirms the phyto-therapeutic properties of *Moringa oleifera* leaves mentioned by Tété-Bénissan et al. (2013) and Leone et al. (2015).

Contrary to the decrease in red blood cell and hemoglobin levels with incorporation of *M. oleifera* leaves at 3% observed by Voemesse et al. (2019) in laying hens, the use of these leaves had no significant influence on RBC in guinea fowl, although a slight decrease in hemoglobin level was observed at 34 weeks of age. This difference would be due to the species of poultry used, which was guinea fowl in this study, and the low level of incorporation of the leaves which was 1.5% in this experiment and 3% in a study of Voemesse et al. (2019).

CONCLUSION

This study reveals that the incorporation of *Moringa oleifera* leaves into local guinea fowl breeders' feed improved growth (1.5% of incorporation) and egg production (0.5% of incorporation), probably by the richness and quality of the nutrients contained in *Moringa* leaves as shown by improved albumin levels. From the point of view of egg production, the use of 0.5% of *Moringa oleifera* leaves in the diet should be encouraged. But for growth (23 to 33 weeks of age in this study), egg weight, and egg quality, the best performance is obtained with dietary inclusion of 1% and 1.5% *Moringa oleifera* leaves. Therefore, the investigation is necessary to explore the hatchability of eggs from guinea fowl breeders fed with a diet containing *Moringa oleifera* leaves and post-hatch performance.

DECLARATIONS

Authors' contributions

Komi Nukunu Patrik Atitso designed the protocol, performed the experiments, collected and analyzed data, and prepared the original draft, edited it, and finalized the manuscript. Kafui Amivi Tété-Benissan validated the protocol, supervised the data collection, and critically revised the manuscript. Oumbortime N'nanle and Kokou Voemesse validated the protocol and revised the manuscript. Lamboni Laré and Komi Attivi supervised the data collection and revised the manuscript. The authors confirmed the final version of the manuscript.

Availability of data and materials

All data presented in this study are available upon request from the corresponding author.

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Competing interests

The authors have declared that no competing interests exist.

Ethical considerations

This manuscript does not contain plagiarized sentences and has not been published or accepted for publication elsewhere or under editorial review elsewhere. The data are not fabricated or falsified.

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