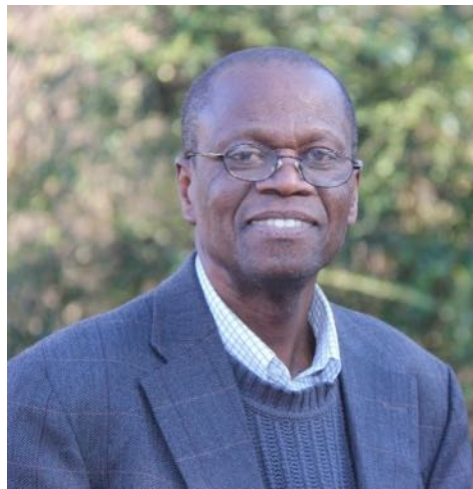


INSECT-BASED PROTEIN FEED FOR GUINEA FOWL: GROWTH AND EGG LAYING PERFORMANCE UNDER DIFFERENT FEED REGIMES

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ABSTRACT

Expansion of guinea fowl flock has been a challenge and one of the reasons is the low feed availability, especially in the dry season. The objective of this study was to contribute to the improvement of the nutritional situation of local guinea fowl with house fly larvae as a protein source. A total of 144 guinea fowls (*Numida meleagris*) of approximately 16 weeks old were grouped in batches of 12 and randomly distributed to four feeding treatments with three replications per treatment. Pre-egg laying and egg laying feed rations were formulated with different levels of sun-dried fly larvae and standard pre-egg laying and egg laying ration containing fishmeal as controls. The pre-egg-laying rations were served for 11 days, while the egg-laying rations were served for 58 days. The chemical composition of the formulated rations, individual Daily Feed Intake (DFI), Average Daily Gain (ADG), Feed Conversion Ratio (FCR), and number of eggs and eggs characteristics (weight, large diameter and shape index) were recorded for each treatment. The dried larvae-based feed showed the highest crude protein content compared to the control feed that was fishmeal-based. In addition, the results showed a relationship between the incorporated quantities of dried fly larvae and the resulting guinea fowl performances. For example, the highest DFI (63.35 g/bird; $p \leq 0.05$) and FCR (11.94; $p \leq 0.05$) were obtained in the group fed the pre-egg laying and egg-laying rations containing 25 % and 13 % fly larvae, respectively. However, the group fed the ration containing 16.67 % and 12.94 % of fly larvae in the pre-egg laying and egg laying phases, respectively, showed a DFI of 57.05 g/bird and an FCR of 9.09. These were similar to the values obtained for the control group (57.19 g/bird/day and 11.94, respectively), with a better ADG (2.91 g against 1.71 g). Egg laying performance (number of eggs) and egg characteristics (large diameter) were significantly improved ($p < 0.05$) in favor of treatments including dried fly larvae with values proportional to incorporation quantities. Findings from the current study suggest that the incorporation of fly larvae in pre-egg-laying and egg-laying feeds significantly increased the performance of guinea fowls.

Key words: insect protein, feed formulation, circularity, Burkina Faso, food security



INTRODUCTION

The livestock sector is an important developmental pillar that contributes to food and nutrition security in Sub-Saharan Africa. However, despite this importance and the efforts made by various governments to develop this sector, it remains essentially traditional and faces many barriers hindering its development [1, 2]. Among the barriers are the inadequacies of feed resources [3 - 5]. Inadequate protein source for feed production is particularly pronounced. In contrast, available conventional feed inputs (such as fish meal, soybean meal and cottonseed meal) are either of poor quality or very expensive and difficult to access by farmers, mostly in rural areas [5, 6]. To overcome this difficulty, insects appear to be a sustainable and ecologically viable solution [7, 8]. They live everywhere, reproduce quickly and are relatively available in all seasons [5, 8, 9]. Among edible insects, house fly larvae are of growing interest as a good substitute for fishmeal and soybean in the feed of monogastric animals, particularly poultry. The use of fly larvae, has a positive impact on production performances [10, 11]. Additionally, it is possible to reduce the costs of poultry feed but also adds value to organic waste (animal manure and cereal residues) that are used as a substrate for fly larvae production [5]. Also, fly larvae exhibit a nutrient profile and remarkably high growth and feed conversion rates [5, 10].

House fly larvae have been the subject of several animal nutrition trials. They have been successfully introduced into the feed of local chickens, for which zootechnical performance has been increased tenfold [5, 12]. We have not found any studies conducted to assess the effects of fly larvae on the performance of local guinea fowl. The closest study found focused on free range scavenging with a mix of whatever insects the birds could find. The general objective of this study was to contribute to improving the nutrition and production of local guinea fowl by developing efficient feed formula based on fly larvae as protein source in the feed. Specifically, we aimed to (i) assess the effect of fly larvae on growth and egg-laying characteristics of local guinea fowl and (ii) determine the dried fly larvae quantity required for optimal feeding of local guinea fowls.

MATERIALS AND METHODS

Location of the study site

The study was carried out from March 14 to May 21, 2020, on the experimental site of the non-governmental organization (NGO), Africa's Sustainable Development Council (A.SU.DE.C), located in Gampéla (Latitude-12.431 and Longitude-1.372), about ten kilometers east of Ouagadougou on the



Ouagadougou-Fada N'Gourma axis. The pedoclimatic conditions are similar to those of Ouagadougou, the capital of Burkina Faso. The climate is North Sudanese type with a dry season from October to May and a rainy season from June to October. The average rainfall over the past ten years was 860 mm with an average temperature of 35 °C. The vegetation comprises a wooded savanna with shrubs of variable density and is generally sparse with a dominance of woody species. The most widespread soils are of the tropical ferruginous type, generally sandy-clay or gravelly, with low agronomic potential with a higher silt and clay content in the depressions. However, in other places, they are completely bare and appear in the form of a glaze or “zipélé.”

Production and drying of fly larvae

Fly larvae were produced and harvested according to the methods described by Sanou *et al.* [9]. Poultry manure was used as substrate and was proportionally mixed with water and exposed in 1 m x 1 m x 0.3 m concrete boxes. After 24 hours of exposure to egg-laying of flies, the mixture was covered with bags that allowed air circulation for the development of the larvae. The larvae harvesting was carried out on the 5th day after the mixture, using a 2 to 3mm diameter hole sieve. Larvae were cleaned and sun-dried on a plastic bag one day after harvesting. This delay allowed the larvae to digest all the contents in their digestive tract and become ‘cleaner.’

Materials for feeding trials

Birds were managed in a pens (36 m x 6.40 m), divided into 12 sub-rooms, with each sub-room having two windows and a little scavenging space of 3.2 m x 3 m. The ventilation of the poultry house was natural, and its floor was covered with rice husks for bedding. Water and feed were given to the birds ad-libitum. A mechanical balance (CONSTANT brand) with a capacity of 50 kg (precision 0.1 kg) was used for weighing the substrate and water while a second electronic balance (SF-400 brand) with a capacity of 5 kg (precision 1 g) was used for weighing the quantities of feed served as well as the weights of eggs and live birds. Eggs' dimensions (length and diameter) were measured with a 1 mm precision caliper.

Experimental animals

A total of 144 local guinea fowls (*Numida meleagris*) (Figure 1A) of approximately 16 weeks old were used for the experiment. They were obtained from two private farms. The birds received comprehensive on-site prophylaxis against the most common guinea fowl diseases in the area. The birds were distributed randomly into four (4) treatments with three (3) replications per treatment and twelve (12) guinea



fowls per replication (9 females and 3 males), giving a total of twelve (12) poultry houses (Figure 1B).

A



B



Figure 1: Guinea fowl and housing

Left (A) – flock of local guinea fowl similar to the birds used in the study with a few chickens visible in the picture, a practice common among Burkina Faso smallholder farmers raising birds in a backyard style that mixes chicken and guinea fowl husbandry
Right (B) – picture of some of the twelve poultry pens used in the study with entrances through the metallic door. The grass mat used for insulation on top of the nearest room is visible

Feed formulation

Four different feeds (treatments, T) were formulated for the pre-egg laying and the egg-laying phases. For each phase, one of the four feeds was the control with 0 % fly larvae (T(0; 0)) and containing only fishmeal as an animal source protein, with 16.67 % in the pre-egg laying and 8% in the egg-laying rations. The second feed (T(8.33; 6)) contained an equal level of both fishmeal and fly larvae as animal source protein in the rations of the two phases (8.33 % of fishmeal and fly larvae in the pre-egg laying ration and 6 % of fishmeal and fly larvae in the egg laying ration). The third feed (T(16.67; 12.94)) contained only fly larvae as animal source protein in the two-phase feeds, with 16.67 % in the pre-egg laying ration and 12.94 % in the egg laying ration. The fourth feed (T (25; 13)) was formulated with 25 % fly larvae in the pre-egg laying ration and 13 % in the egg-laying ration. The formulations were based on maintaining isoprotein and isogenic conditions informed by poultry nutrition standards. Tables 1 and 2 show the feed formulation for the two phases. Fly larvae were mixed with the other feed ingredients, and the whole mix was ground together for each ration. A fixed daily amount of 80 g of

feed/bird was fed to the birds during the experiment. The birds were fed the control rations for two weeks before the start of the experiment to bring them to the same starting point. The pre-egg laying rations were applied for 11 days, while the egg-laying rations were provided to the guinea fowls for 58 days, for a total of 69 days of experimentation.

Nutritional quality of fly larvae and formulated feed rations

The chemical compositions of the dried fly larvae, as well as the formulated feeds, were determined. Samples of 100 g of sun-dried fly larvae and each feed were collected and sent to the Animal Nutrition laboratory of INERA (National Institute for the Environment and Agricultural Research) in Kamboincé, Ouagadougou, for chemical analysis. The analysis included dry matter (DM), ash, organic matter (OM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL), as well as calcium (Ca) and phosphorus (P) contents determined according to AOAC standard methods [13].

Calculation of performance parameters

The following parameters were calculated according to formulas previously used by others [1, 2]:

- The daily feed intake (DFI) per bird was calculated according to the following formula for each bird house:
$$\text{DFI (g)} = (\text{QD} - \text{QR}) / \text{number of birds in a house,}$$
where QD = quantity of feed distributed and QR = quantity of feed not consumed.
- The live weight of the birds (LW) was measured weekly from the first day until the 5th week of the experiment, then every two weeks (to reduce stress) until the end of the experiment. These live weights were used to calculate the average daily gain (ADG) according to the formula: $\text{ADG (g)} = (\text{LW}_j - \text{LW}_i) / \Delta T$ where “j” is the starting day, “i” is the final day and ΔT = number of days between the weighing dates i and j.
- The feed conversion ratio (FCR) was calculated by dividing DFI by ADG.
- The number (N) of eggs laid per bird was calculated by dividing the total number of eggs per house by the number of females in the house.
- The length and large diameter of the eggs were measured as well as the weight.

Egg laying data was collected for thirty-two days.



Statistical analysis

Microsoft Excel software (2013 version) was used for data entry, calculations and making of graphics. MINITAB statistical software (version 16) was used for basic descriptive analysis. ANOVA was then performed using GLM procedure and pairwise comparisons of means were done using Tukey test for post-hoc analysis at 95 % confidence interval.

RESULTS AND DISCUSSION

Nutritional quality of fly larvae and formulated feeds

Chemical composition of the dried fly larvae showed 95 % of dry matter and 49.3 % of crude protein contents. Calcium and phosphorus contents were 1.2 % and 1.47 %, respectively. Table 3 shows the chemical analysis data of the fly larvae. Dry matter contents of the different feed rations varied from 94.74 to 95.92 % and similar values for pre-egg laying and egg-laying feed rations. In addition, the two control feeds showed the lowest CP levels (15.9 % for pre-egg laying and 16.2 % for egg laying rations). The pre-egg laying rations containing 8.33 %, 16.67 %, and 25 % of fly larvae showed similar CP levels of 23.17, 23.60, and 23.59 %, respectively. Proximate composition of the feed rations are presented in Table 4.

The crude protein content of dried fly larvae (49.33 %) recorded in the present study is close to that reported in previous work, where levels of 49.65 % (oven-dried fly larvae) and 48.85 % (sun-dried fly larvae) from Benin and Burkina Faso, respectively [5, 14]. In comparison to crude protein content of fishmeal (39 %) reported previously, fly larvae appear to be an alternative animal source protein for feeding monogastric animals in general [4]. Commercial fishmeal available in landlocked countries such as Burkina Faso is often of poor quality and very expensive. Therefore, using dried fly larvae will significantly reduce protein ingredient costs in feed formulation.

Effect of feeds on guinea fowl growth

During the experiment (pre-egg laying and egg laying trials), daily feed intake (DFI) was 57.19 g/bird for the group that was fed the control feeds (R0) containing fishmeal as animal source protein during pre-egg laying as well as egg laying trial (T(0; 0)). See Figure 2 caption for meaning of trial numbers in brackets). DFI of the experimental birds were 53.08, 57.05, and 63.35 g/bird, for T (8.33; 6), T (16.67; 12.94) and T (25; 13) treatments, respectively. There was a significant difference ($p < 0.05$) between the treatments except for T (0; 0) compared to T (16.67; 12.94), where the difference was not statistically significant (Table 5).



Average Daily Gains (ADGs) recorded were 1.71, 2.51, 2.91, and 2.60 g/bird/day for guinea fowl under treatments T (0; 0), T (8.33; 6), T (16.67; 12.94), and T (25; 13) treatments, respectively. There was a significant difference between T (0; 0) and T (16.67; 12.94) treatments ($p < 0.05$) (Table 5). No statistically significant difference ($p > 0.05$) was found between the T (8.33; 6) and T (25; 13) treatments.

Average Daily Gains (ADGs) fluctuated widely from the first week to the last week of experiment (Figure 2). The T (0; 0) and T (8.33; 6) treatments showed increasing ADGs at the end of the trial, while ADGs of the treatments including higher fly larvae (T (16.67; 12.94) and T (25; 13)) were lower at the end of the experiment.

Feed conversion ratio was significantly ($p < 0.05$) higher in the group that was fed the ration containing 8.33 % of larvae in the pre-egg laying trial followed by the ration containing 6 % of larvae during the egg laying trial (T (8.33; 6), in comparison to the other treatments and the control (Table 5). The T (0; 0) and T (16.67; 12.94) treatments presented similar FCR (11.94 and 9.09 respectively), while T (25; 13) treatment yielded the lowest FCR of 7.36, which is the best in this study. Lower FCR values are desirable as they suggest better feed conversion.



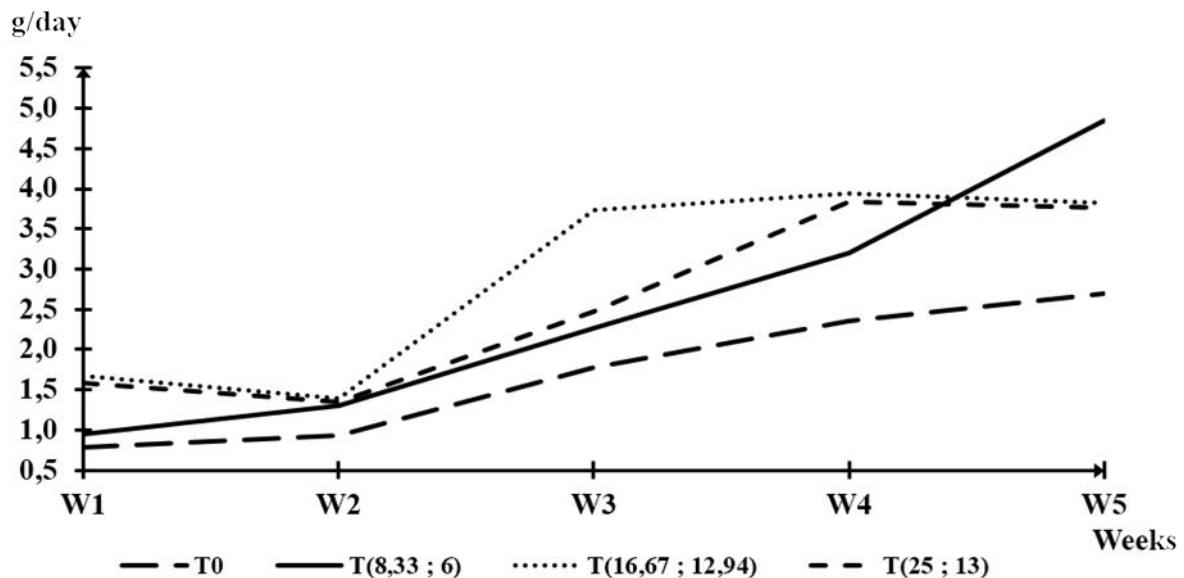


Figure 2: Average daily weight gain (g/day/bird) of guinea fowls in response to different feed rations (Treatments, T), whose fish-based protein source was substituted with domestic fly larvae protein source. The experiment was conducted for four weeks. Treatments abbreviations – the first and second numbers in brackets are percentage of fly larvae used in the pre-egg laying and egg laying rations, respectively

T0 = control– (16.67 % fishmeal; 8 % fishmeal), T (8.33; 6) = (8.33 % fishmeal + 8.33 % fly larvae; 6 % fishmeal + 6 % fly larvae), T (16.67; 12.94) = (16.67 fly larvae; 12.94 fly larvae), T (25; 13) = (25 % fly larvae; 13 % fly larvae)

The DFI values from this study were lower than values from egg laying guinea fowl of 123 g/bird/day from a Nigerian study [15]. The data on DFI of the present study were also lower, compared to the findings from another study conducted in central Burkina Faso that recorded DFI of 75.6 g/day with finishing guinea fowl layers [14]. The lower DFI in the present study could be explained by the young age of the fowl and the duration of the experiment. DFI was recorded at the end of “pullet phase” and 5 weeks after the onset of egg laying, where feed consumption is still relatively low according to body size. However, this low consumption could also be explained by the heat because the experiment was carried out during the hot, dry season when the mean temperature is often more than 38 °C in the study area [16]. It is known that heat causes thermal stress in poultry, resulting in reduced feed intake just for survival [17]. Indeed, high temperatures lead to a considerable

deterioration in zootechnical performance with respect to DFI, ADG, and FCR [18, 19].

The ADGs values from this study were low compared to those from a Benin (5.68 g) and a Burkina Faso (5.34 g to 5.64 g) studies with adult guinea fowl, all in controlled environments [20, 2]. An ADG of 12.5 g for growing guinea fowl was also reported [21]. The low ADGs obtained in the present study may be the result of the low feed intake reported above. FCR was low (the lower the FCR, the better the feed conversion) for the T (16.67; 12.94) and T (25; 13) treatments, where the larvae levels were high, with the latter treatment showing the lowest FCR (7.36), which is the best number from this study. This suggests a better valorization of the feed containing high fly larvae levels for guinea fowl, which could be related to the good protein balance of these rations. However, FCR for guinea fowl from all the treatments were significantly higher (indicating poor feed conversion) than that previously reported and this difference could be due to the young bird age, short duration of the study, and the heat stress as explained above [14].

Effect of feed on guinea fowl egg-laying performances

The first egg was seen in the group fed the T (25; 13) rations, followed by the T (16.67; 12.94) treatment. The onset of egg laying was late in the control group. Total number of eggs was also higher in the groups that started egg laying earlier compared to the others. The number of eggs obtained in the T (25; 13) of 18 eggs/bird was approximately equal to one from T (16.67; 12.94) treatment. The T (0; 0) and T (8.33; 6) treatments, birds laid lower numbers of eggs of 11 and 13 eggs/bird, respectively. A significant ($p < 0.05$) difference was found in the number of eggs between treatments T (25; 13) and T (16.67; 12.94) compared to T (0; 0) and T (8.33; 6) treatments. However, no significant difference was found in the number of eggs between T (25; 13) compared to T (16.67; 12.94), and between T (0; 0) and T (8.33; 6). A similar trend was also observed in egg weight and length. Eggs' large diameter and shape index from T (25; 13) treatment differed. The large diameter of the eggs varied from 37.4 mm (T (0; 0)) to 37.90 mm (T (25; 13)). As for the shape index, the lowest value was recorded from birds on T (25; 13) treatment. Average egg weights were 39.45 g, 40.59 g, 39.47 g and 41.14 g, from T (0;0), T (8.33; 6), T (16.67; 12.94) and T (25; 13), respectively, with a relatively higher value from birds on T (25; 13) treatment (Table 6).

Egg-laying performance appears to have increased in proportion to the amount of fly larvae incorporated into the rations. Indeed, fly larvae is a source of protein and fat, and this might have a positive impact on the reproduction of the guinea fowl. These fly larvae-based feed rations might have also provided other essential



nutrients for egg laying fowls, boosting their reproductive performance. It has been shown that the inclusion of fly larvae in the feed of chickens made it possible to begin the egg laying in the absence of male birds, and that the opposite situation was observed when the supplementation was stopped [3, 12]. Egg dimensions (length and large diameter) in the present study were slightly larger than those previously reported (average length of 47.1 ± 1.9 mm and a large diameter of 36.5 ± 1.3 mm) [14]. Mean egg weights found in this study were slightly higher than the previously reported of 29.2 ± 1.7 g in the first cycle and 37.8 ± 3.5 g in the second egg laying cycle [14]. However, these weights were in the 35 - 40 g range identified as the category of heavy eggs in guinea fowl [14].

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

This study showed that dried fly larvae, as a protein source, could be used as a substitute for fishmeal, in local guinea fowl feed. Better ADG (Average Daily Gain) was observed with the birds fed rations containing 16.67 % of dried fly larvae in the pre-egg laying period and then receiving a ration containing 12.94 % of fly larvae in the egg laying period [T (16.67; 12.94)]. Better FCR (Feed Conversion Ratio) and egg-laying performances were observed from birds fed the T (25; 13) ration. The incorporation of fly larvae in the pre-egg-laying and egg-laying feeds significantly increased the zootechnical performance of the guinea fowl in this study. Further studies are needed to investigate the level of incorporation of dried fly larvae in growing guinea fowl feeds in the range of 16.67 to 25 % fly larvae for pre-egg laying in combination with more than 13 % fly larvae in egg laying rations.

Conflicts of Interest

All authors declare no conflicts of interest with this paper.

Contributions from Authors

KBA, SP, SS, AJN, WSK and AKA were involved in the design and implementation of the experimental protocol. SP and AJN formulated the feed rations while KBA performed data collection. KBA, SP, SS and WSK analyzed the results and wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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(Assurance #: D16-00276/A3437) approvals from the University of Georgia Institutional Review Board. Parallel approvals in Burkina Faso were obtained from AVIS DE LA COMMISSION (Code Agreement #: CE-U01/2019-03). We also thank Ibrahima Traore for internal review of the manuscript.



Table 1: Pre-egg laying feed rations formulation (%)

Ingredients	Rations			
	R ₀	R _{8.33}	R _{16.67}	R ₂₅
Maize	67.16	63.33	60.50	58.00
Wheat bran	12.50	11.67	9.17	15.00
Fishmeal	16.67	8.33	0.00	0.00
Fly larvae	-	8.33	16.67	25.00
Soya	0.83	3.00	4.17	0.42
Cottonseed cake	1.67	4.17	8.33	0.42
NaCl	0.83	0.83	0.83	0.83
Premix	0.13	0.13	0.13	0.13
Iron	0.21	0.21	0.21	0.21
Total	100	100	100	100

Table 2: Egg laying feed rations formulation (%)

Ingrédients	Rations			
	R ₀	R ₆	R _{12.94}	R ₁₃
Maize	69.06	66.58	64.00	63.85
Wheat bran	6.00	9.00	9.50	10.00
Fishmeal	8.00	6.00	-	-
Fly larvae	-	6.00	12.94	13.00
Soya	3.00	2.50	3.00	3.00
Cottonseed cake	6.00	2.00	3.00	3.00
Oyster shell	7.00	7.00	7.00	6.50
Methionine	0.02	-	0.02	-
NaCl	0.50	0.50	0.50	0.50
Premix	0.15	0.15	0.02	0.15
Lysine	0.02	0.02	0.02	-
Iron	0.25	0.25	-	-
Total	100	100	100	100

Table 3: Proximate composition of sun-dried house fly larvae (% of dry matter)

DM	Ash	OM	CP	CF	NDF	ADF	ADL	Ca	P
95.64	16.12	83.88	49.33	12.20	44.44	12.90	1.80	1.20	1.47

Abbreviations: Dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) as well as the calcium (Ca) and phosphorus (P)

Table 4: Proximate composition of formulated feed rations (% of dry matter)

Feeds	Nutrients									
	DM	Ash	OM	CP	CF	NDF	ADF	ADL	Ca	P
Pre-egg laying feed rations										
R ₀	94.74	13.09	86.91	15.96	7.29	29.72	6.79	1.60	3.12	1.03
R _{8.33}	94.95	6.69	93.31	23.17	7.24	30.70	7.07	1.34	0.59	1.00
R _{16.67}	94.97	6.20	93.80	23.60	8.13	28.10	7.64	1.51	0.21	0.76
R ₂₅	95.04	6.43	93.57	23.59	7.42	29.91	7.20	1.54	0.23	0.79
Egg laying feed rations										
R ₀	95.11	14.47	85.53	16.18	5.52	25.30	5.08	1.20	3.19	0.80
R ₆	95.15	15.41	84.59	18.79	6.10	25.18	5.35	1.09	3.38	0.91
R _{12.94}	95.64	12.31	87.69	19.23	6.42	26.68	6.17	1.21	2.85	0.79
R ₁₃	95.92	11.70	88.3	20.12	6.85	26.24	6.49	1.23	2.17	0.90

Abbreviations: Dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) as well as the calcium (Ca) and phosphorus (P)

Table 5: Daily feed intake (DFI), average daily gain (ADG), and feed conversion ratio (FCR) of local guinea fowl, fed feed rations with different level of fly larvae (M±SEM)[#]

Feeds	DFI (g)	ADG (g/day/bird)	FCR
T _(0;0)	57.19 ± 1.34 ^b	1.71±0.54 ^c	11.94 ^{ab}
T _(8.33;6)	53.08 ± 2.73 ^c	2.51±0.72 ^b	16.67 ^a
T _(16.67;2.94)	57.05 ± 2.20 ^b	2.91±0.66 ^a	9.09 ^{ab}
T _(25;13)	63.35 ± 4.37 ^a	2.60 ± 0.65 ^b	7.36 ^c
P-Value	0.015	0.001	0.035

[#]In the same column, numbers with different superscript letters are significantly different at 5% alpha-level

Table 6: Effect of feed on guinea fowl egg-laying performance

Laying parameters	Feed Ration Treatments [#]			
	T _(0; 0)	T _(8.33; 6)	T _(16.67; 12.94)	T _(25; 13)
Total number of eggs	286	358	481	485
Eggs (number/bird)	10.59±2.67 ^c	13.26±2.32 ^b	17.81±3.48 ^a	17.96±3.19 ^a
Percentage of lay	33.10±8.36 ^c	41.44±7.25 ^b	55.67±10.87 ^a	56.13±9.96 ^a
Egg weight (g)	39.45 ± 3.54 ^b	40.59 ± 4,10 ^a	39.47 ± 4.32 ^b	41.14 ± 3.73 ^a
Egg length (mm)	49.20 ± 0.26 ^b	49.90 ± 0,25 ^a	49.00 ± 0.25 ^b	50.90 ± 2.16 ^a
Large diameter (mm)	37.40 ± 0.13 ^a	37.80 ± 0,20 ^a	37.50 ± 0.18 ^a	37.90 ± 0.19 ^b
Shape index (SI)	0.76 ± 0.04 ^a	0.76 ± 0,05 ^a	0.7 ± 0.05 ^a	0.74 ± 0.06 ^b

[#]In the same rows numbers with different superscript letters are significantly different at 5 % alpha-level

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