



Inclusion of Foliage Meal of *Moringa oleifera* Lamark in Free-Range Layer Diets. Impact on Bioproductive Behaviour

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Received: August 29, 2025

Published: September 17, 2025

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Abstract

In order to evaluate the inclusion of *Moringa oleifera* foliage meal (HFMO) in free-range layer diets and its impact on bio-productive indicators, a study was developed that considered 200 30-week-old k53 line hens housed in 2 m² quarters (5 birds/m²), and distributed in a completely randomized design with five replications, with inclusion levels (0, 6, 8 and 10 %) being considered as treatments. The following variables were considered: Viability (%); Feed intake (kg); Live weight/bird (g); egg/bird (U); laying intensity (%); broken eggs (U), cracked eggs (U) and dirty eggs (U). To determine the indicators of external egg quality, the following variables were considered: egg weight (g), width (cm), egg equator with a caliper and egg length (cm) between the poles, shell thickness, porosity, and for internal quality, the variables height, yolk diameter and white (mm). Data were compared using the Duncan Twelfth for the comparison of means at a 95% confidence level. The consumption of HFMO showed differences between treatments ($P \leq 0.05$), increasing by 15.60 g/bird/day. In the live weight of the free-range layers there were no differences for $p \geq 0.05$; in initial measurements (2,200 and 2,198 g) at the end of the first week of evaluation; with discrete differences (2,202 and 2,199 g) after the experiment. Concluding that up to 8% showed the best results in the diet of layers without affecting bioproductive indicators; however, employment up to 10% is possible for non-specialized breeding.

Keywords: Alternative Feed; Productive Quality; Free-Range Layers

Introduction

Providing food for a growing global population is one of the great challenges for the coming decades [8]. A particular challenge will be to produce sufficient proteins, especially of animal origin [26]. Meat and eggs are, in addition to milk, the most valuable sources for human nutrition [44].

The United Nations (UN) predicts that the world's population will reach 9.7 billion people by 2050, driving an increase in demand for food, which requires an expansion of production. This implies a better use of natural resources, and considering within production systems the adoption of sustainable production models to guarantee the preservation of the environment. Likewise, it

must implement the greater use of natural resources, demanding new production models focused on sustainability [8]. To meet this growing demand for food, it is estimated that an increase of about 60% in global agricultural production and a 70% increase in meat and dairy production will be necessary [36].

Rural poultry farming is an activity of social, cultural and economic importance, as it is a source of food for peasant families that helps to complement nutritional requirements, it is also an important animal genetic resource, the breeding of small animals becomes the savings bank of rural producers, due to its contribution to family resources based on the production of low inputs [41]. Eggs and meat from free-range poultry that are raised in the open field freely, with leftover organic and ecological foods such as grains, grasses from the area and other resources that are produced on a plot, are a food option for poultry producers and their by-products.

However, poultry feed and nutrition is a problem that affects many producers due to the lack of feeds that meet nutritional requirements and provide a diet of low economic value and high nutritional content [3]. In this sense, alternatives such as the use of by-products from the food industry [6] medicinal plants [28], and other natural resources [12] have been considered for poultry feed. Within the natural resources, mention is made of grains that contribute to the food of man, such as corn, sorghum, oats, wheat, among others that are used for the production of flour for animal feed, however, there are other alternatives that have been little addressed despite having dismembered their impact. such is the case of *Moringa oleifera* Lamark.

The scientific evidence of the properties of *Moringa oleifera* makes this plant a firm candidate in the search for medicinal, chemical, industrial, livestock, agricultural and nutritional alternatives that meet the specific deficiencies of each sector [9], as a raw material, the foliage of this plant is capable of providing antioxidants and protein of high biological value; in addition to being an alternative to the use of antibiotics in birds [1,39], however, the type of ecosystem in which the plant grows and develops determines the composition of the nutritive and non-nutritive elements that are of interest in *Moringa* for animal feeding and health [39].

The objective of this research is to evaluate the inclusion of *Moringa oleifera* foliage meal (HFMO) in the diets of free-range layers and its impact on bioproductive indicators.

Materials and Methods

The study with free-range birds was carried out on the farm "La Auxiliadora" Consejo Popular la Aguada, in the municipality of Bayamo, Granma, located at coordinates 20°19'10 LN and 76° 45'35 LW, 8 km from the provincial capital. The average relative humidity was 78%, the average minimum temperature was 23.3°C and the average maximum temperature was 33.7°C [14].

Experimental design

200 male free-range crossbred hens σ k5 x φ k3, 30-week line k53, were distributed according to a completely random design, the mathematical model for the development of ANOVA according to experimental design was as follows: $y_{ij} = \mu + \tau_i + \epsilon_{ij}$ with 4 treatments consisting of inclusion levels 0 (T1) control without addition of Moringa flour, 6% (T2), 8% (T3) and 10% (T4) each with five replications. Each repetition consisted of a 2 m² (5 birds.m²) quarter with 10 hens.

Where

- Y_{ij} is the response variable;
- μ is the overall average of the experiment;
- τ_i is the effect of the i -th level of moringa;
- ϵ_{ij} is the experimental error

Study design distribution scheme

The birds were reared in an intensive rice-husk bedding system, with rustic palm plank nests and a 5-nest nest/layer ratio, artificial lighting was offered on a 14½ hour light and 9½ hour darkness regime each day. Without vaccinations or veterinary treatments for the duration of the experiment, the birds received water at will in rustic bamboo drinkers.

The animals underwent two weeks of adaptation to the new food as recommended by Rugel and Emén [34]. The concentrate was offered restricted as regulated by Pampín *et al.* [27], consumed

156 g of feed/bird/day for ♀ weeks 30 to 39 because it is the period where the plateau and peak laying occurs.

The diets were formulated considering the chemical composition of the raw materials to make poultry feed, based on corn and

soybean cake, in addition to taking into account the nutritional requirements described by Fumero *et al.* [10]. It should be noted that the concentrate was acquired at the “Eduardo Vailly” Feed Factory in Bayamo, Granma. Where it met the standards established by the UECAN [42]. The marketing company reported the chemical composition shown (Table 1).

Ingredients	HFMO Inclusion Levels (%)			
	0	6	8	10
Corn flour (7.0% CP)	62.1342	57.7275	57.0523	55.8062
Soybean meal (47.5% CP)	25.3558	22.9575	22.4087	21.9481
Moringa meal (28.34% PB)	---	6	8	10
Vegetable oil	1.4992	2.5549	2.2100	2.6035
Choline chloride 60%	0.0100	0.0100	0.0100	0.0100
DL-Methionine	0.186	0.163	0.159	0.138
Calcium carbonate	8.661	8.420	8.105	7.263
Monocalcium phosphate 21	1.573	1.587	1.475	1.650
Common salt	0.280	0.280	0.280	0.280
N. Vitaminic Rep. Avic. ¹	0.150	0.150	0.150	0.150
N. Mineral2	0.150	0.150	0.150	0.150
Calculated contributions (% DM)				
PB	16.502	16.640	16.900	17.160
IN ^{Mj.kg-1}	11.500	11.500	11.500	11.500
FB	2.290	3.030	3.217	3.521
FND	8.840	10.190	11.270	12.810
Total Fat	5.010	6.100	6.720	6.850
Linoleic acid	2.570	1.980	2.090	2.490
Calcium	3.500	4.500	4.598	4.607
Assimilable phosphorus	0.453	0.400	0.434	0.456
Lysine	0.746	0.600	0.630	0.704
Methionine and cystine	0.655	0.550	0.532	0.515
Threonine	0.548	0.626	0.587	0.505
Tryptophan	0.200	0.170	0.180	0.180

Table 1: Diets and Calculated Inputs for HFMO-Fed Free-Range Layers (30-39 Weeks of Age).

¹Active product/t: vit. A, 10 x 10⁶ I.U.; vit. D3 (calciferol), 1.5x 10⁶ I.U.; vit. K3, 2100 mg; vit. E (tocopherol), 10000 mg; vit. B1 (thiamine), 800 mg; vit. B2 (riboflavin) 2500 mg; pantothenic acid, 10000 mg; vit. B₆ (pyridoxine), 2500 mg; folic acid, 250 mg; biotin, 100 mg; vit. B₁₂ (cyanocobalamin), 1500 mg.

²Active product /t: manganese, 60000 mg; copper, 8000 mg; iron, 60000 mg; zinc, 50000 mg; selenium, 200 mg; iodine, 800 mg; cobalt, 500 mg; Antioxidant 100%, 125000 mg.

Bio-productive indicators

To evaluate its effect on the variables: Viability (V, %); Feed intake (AC, g); Live weight of birds (BP, kg); Egg per bird (HA, U); Laying intensity (PI, %); Broken eggs (RH, %), cracked (HC, %) and dirty (HS, %).

External and internal egg quality

At week 35, 20 eggs were collected for each experimental treatment. To determine the indicators of external and internal egg quality. The variables evaluated were: Egg Weight (PH, g), was determined with a SARTORIUS model BL 1500 digital scale with accuracy ± 0.1 g. Egg Width (AH, mm), Egg Length (LH, mm), Shell Thickness (GC, mm) and Porosity (P, pores/cm²), height and diameter of the yolk and white.

The (LH) between the poles and the (HA) were quantified at the equator of the egg with a caliper (Stefanelli resolution 0.05 mm accuracy). To determine the thickness of the shell, caliper was used (Stefanelli resolution 0.05 mm accuracy) and the equator and mean of the egg poles were considered to obtain an average value. To quantify porosity, the egg was immersed in a solution of 10% cobalt chloride up to the pink hue of the shell, followed by 1 cm² of shell and using a microstereoscope to count the amount of pore, according to the methodology of Narushin (2005).

The width of the white and yolk was determined by breaking the egg on a glass with graph paper; both structures were delimited and the grids were counted. The shape index (IF) was determined according to the formula: $IF = (\text{Smaller diameter} / \text{major diameter}) \times 100$. To calculate the volume, the egg was immersed in a 1 L graduated test tube according to the methodology of Narushin [23].

The height of the dense white and yolk was measured with a height gauge with an accuracy of ± 0.01 mm. The records of the Haugh Unit (UH) were calculated by the relationship between the weight of the egg (W), the latter was determined with a SARTORIUS digital scale (model BL 1500 with accuracy ± 0.1 g). The height of the yolk and white were measured with a spherometer

and diameter with graph paper in flat transparent glass, the yolk index was determined by the formula: $IY = \text{height of the yolk} / \text{average diameter of the yolk} \times 100$; the clearance index by the formula $IC = \text{height of the gap} / \text{average diameter of the white} \times 100$. The Haugh unit was determined by the following formula: $UH = 100 \log (H + 7.75 - 1.5W^{0.37})$, where H is the height in mm of the white and W is the weight of the egg, according to the methodology of Narushin [23].

Statistical analysis

The data were analyzed using simple variance (ANOVA) and for the comparison of means, the Duncan Twelfth (1955) was used at a 95% confidence level. Prior to the analysis of variance, the normality of the data was checked with the Kolmogorov-Smirnov test [18] and the Bartlett test [4] for the homogeneity of variance between treatments. All analyses were performed using the SPSS version 23 statistical package.

Results and Discussion

Viability and consumption of free-range layers when including 6, 8 and 10% of HFMO in the diet.

The viability and consumption of the commercial concentrate showed differences between treatments (Table 2), suggesting that the inclusion of up to 10% of HFMO in free-range layers does not generate high mortality, which coincides with what was reported by Valdivi   *et al.*, [43]. These authors reported that the use of 20% or higher levels of forage meal is assimilable in poultry.

Likewise, the diet with 10% inclusion of HFMO stimulated ($P \leq 0.05$) consumption by 40 and 20%, compared to levels with 6 and 8% of HFMO, respectively.

According to Romero-Yerena *et al.* [32], the incorporation of Moringa in 3% and 6% does not affect food consumption between treatments and control ($818.7 \text{ g} \pm 19.1$, $881.4 \text{ g} \pm 25.2$ and $801.3 \text{ g} \pm 32.4$, for the 0%, 3% and 6% levels of moringa), these results differ from those reported by Shad and Xiang, [38] who mention that

Indicators	% inclusion of HFMO				EE ±
	0	6	8	10	
Feasibility (%)	100	100	100	100	-
Total Consumption (g/bird/day)	156	156	156	156	-
Food Intake Total Weekly (kg)	54.53 ± 0.07 ^a	54.46 ± 0.14 ^b	54.30 ± 0.26 ^c	54.05 ± 0.52 ^d	0.07
Total HFMO Consumption Weekly (kg)	0	3.17 ± 0.83 ^a	4.24 ± 0.49 ^b	5.04 ± 0.61 ^c	0.01

Table 2: Effect of HFMO on viability and feed intake in free-range layers.

^{abcd}Values with uncommon letters in the same row differ according to Duncan (1955) (p < 0.05).

the addition of 10% and 20% of moringa flour in the diet of laying hens, As a substitute for sunflower seed meal, it increases feed intake, these authors attribute this to a possible decrease in energy and protein digestibility as an effect of the addition of moringa.

Similar results were obtained by Martínez-Gutiérrez *et al.* [22] by reporting no variations in viability and feed consumption when using protein plants in the feed of commercial laying hens with the inclusion of 20 and 30 % of *M. oleifera foliage* meal in feed. On the other hand, Jin *et al.* [15] indicated that, in layers, some breeding firms offer strains more adapted to intensive productions (Lohm-

ann tradition, ISA plein air), whose genetic potential differs little from that of conventional ones; however, very often results are obtained below the standards, especially in free-range hens and organic backyard poultry. Therefore, the potential impact of alternative ingredients, nutritional levels, condition of the beds, herd management must be taken into account.

Weight behavior of free-range layers fed with 6, 8 and 10% HFMO.

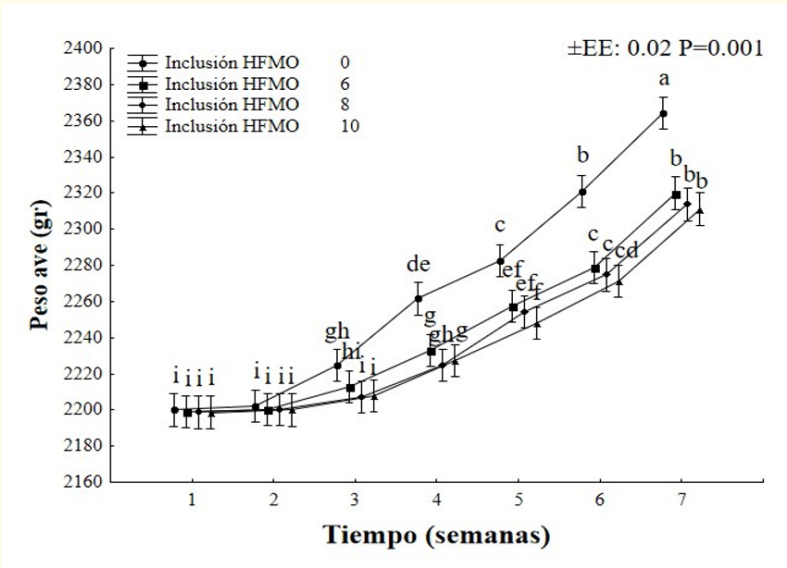


Figure 1: Effect of HFMO inclusion on the weight of free-range layers.

a,b,c,d,e,f,g,h,i Different superindices suggest significant differences according to Duncan at a 95% confidence level. Significance was achieved by transformation $\sqrt{x+5}$.

The productive response of the weight of free-range layers when including 6, 8 and 10 % of HFMO remained in correspondence to the parameters recorded for line k_{53} , observing statistical differences between experimental treatments, with respect to the control and coincides with what was proposed by Fernández-Ruelas *et al.* (9) which ensure that the increase in live weight of laying hens in the peak laying period if there are no changes in feed intake, the live weight remains similar without altering production indices.

On the other hand, Valdivié *et al.* [43] conclude that levels of incorporation of 0%, 10% and 20% of moringa in the diet of laying hens aged between 34 weeks and 50 weeks, do not affect their live weight, it is shown that the use of this feeding alternative ensures the correct levels of nutrients for the category in question.

The weight of the birds reported until day 14 of the research, for the commercial concentrate, increases to 2025 g, in the same way, the maximum weight obtained for 10% of moringa meal, was close to 2000 g. According to information from Sánchez and Barreto [35], in another study it was observed that weight differs by treatments and when 20% moringa flour is used, the referred weight was 1971 g, and in this same sense, when using 30% moringa flour, 1958 g was obtained. This shows us that using higher levels of moringa flour deteriorates feed conversion rates, there is less use of the ration and therefore negatively influences the weight gain of the layer, since there is a lower concentration of MS

in the diet and the bird can mobilize energy from its biological reserves to maintain productive rates.

Intensity of laying

Table 3 states that the inclusion of 8% HFMO increased the laying rate and eggs per bird of free-range layers compared to the control treatment and increasing levels of HFMO (Table 3). This result shows that the nutritional requirements of these non-specialized birds were met and that it seems that the secondary metabolites supplied in small concentrations could have a biological promoting effect, in addition, a non-pathological increase of crude fiber in the dietary intake can favor gastrointestinal transit, digestibility and the use of the nutrients of the ration. which translates into higher productivity of the bird [37].

Other authors such as Guerrero *et al.* [13] recommended that the inclusion of between 3-5% of HFMO should not cause productive problems in laying hens, as well as the internal, external quality and shelf life of the commercial egg. Also, Bidura *et al.* [5] found the best results by including between 5 and 8% inclusion of HFMO for laying hens, however, inclusion with 10 and 15% reduced laying intensity, due to the decrease in feed intake and lower availability of nutrients for egg production. It is known that free-range birds have a higher hardiness and acceptance of fiber and secondary metabolites than specialized birds Teteh *et al.* (2016), this implied that the inclusion of up to 8% of HFMO had the same response as the control treatment. In this sense, free-range birds are able to produce eggs with few inputs and with unconventional sources and maintain Nguyen *et al.* (2023).

Indicators	% HFMO Inclusion				± EE
	0	6	8	10	
Laying index (%)	62.5 ± 2.81 ^b	61.4 ± 2.18 ^c	60.6 ± 2.17 ^c	60.1 ± 2.97 ^a	1.14
Eggs per bird (U)	2.78 ± 1.42 ^a	2.24 ± 1.02 ^c	2.28 ± 1.06 ^c	1.82 ± 1.14 ^b	0.16
Huevos rotos (%)	1.43 ± 0.20 ^a	0.89 ± 0.01 ^c	0.87 ± 0.01 ^c	1.09 ± 0.14 ^b	0.03
Dirty eggs (%)	1.43 ± 0.20 ^a	0.89 ± 0.01 ^c	0.87 ± 0.01 ^c	1.09 ± 0.14 ^b	0.03
Cracked eggs (%)	0.71 ± 0.02 ^a	0.89 ± 0.04 ^b	0.87 ± 0.04 ^b	0	0.01

Table 3: Effect of HFMO inclusion levels on laying index (%) and egg classifications in free-range layers.

^{abcd} Values with uncommon letters in the same row differ according to Duncan (1955) (p < 0.05).

The indicator of egg per bird did not present significant differences between the control and the first treatment (1.44 and 1.40), which show correspondence with the technological week evaluated for this line of semi-heavy layers, where significant differences were evidenced in treatments two and three (1.32 and 1.24) in the same period, which corroborates that when reducing MS in the diet, posture and therefore the quality of the production system are affected. although in backyard conditions it is usually an efficient alternative due to the availability of this material, it coincides with studies carried out by Rocha (2020), Valdivié [43] assures that this response in lines with greater specialization can present a deterioration of the indicator when energy levels in the diet are not properly managed.

External and internal egg quality

In the external and internal quality of eggs from free-range layers fed with HFMO (Table 4) there were significant differences, the egg weight with the lowest value for T4 with 56.93g, shows that the biological quality of the diet plays a preponderant role in the

production indices, as well as a higher porosity value (151) for T3. The thickness of the shell did not show significant differences. In the length of the egg, there were no differences between T1 and T2 (54.44 and 54.51 mm), T3 and T4 showed no difference between them (55.03 and 55.08 mm) as they corresponded to what was expected, on the other hand, there was a significant difference between the first two treatments and the last two. The width showed significant differences between T1, T2 and T3, where T1 maintains similarity with T4.

The values shown by width of the egg white showed significant differences between treatments, with a greater impact on T3 and T4 (72, 88 and 72.76 respectively), where the behavior of this variable is favored by the amount of carotenes and selenium present in the diet, this result coincides with [21] that show similar values when moringa leaf flour is used (7 and 10%), which in turn influences the width of the bud and its coloration, which in this study describes differences ($p < 0.05$) for cases T2 and T3 in correspondence with T1, where T4 obtained the least suggestive values.

Parameter	% HFMO Inclusion				± EE
	0	6	8	10	
Egg weight (g)	57.25 ± 1.21 ^b	57.33 ± 1.18 ^b	57.86 ± 1.32 ^a	56.93 ± 1.01 ^c	0.051
Width of the egg white (mm)	72.44 ± 0.65 ^c	72.56 ± 0.68 ^c	72.88 ± 0.89 ^a	72.76 ± 0.72 ^b	0.047
Height of the egg white (mm)	5.61 ± 0.22 ^c	5.67 ± 0.48 ^b	5.73 ± 0.67 ^a	5.60 ± 0.21 ^c	0.078
Egg Clear Index	7.73 ± 1.57 ^{bc}	7.80 ± 1.61 ^b	8.10 ± 1.79 ^a	7.63 ± 1.54 ^c	0.117
Yolk width (mm)	39.15 ± 1.12 ^b	39.33 ± 1.28 ^a	39.33 ± 1.28 ^a	38.98 ± 1.02 ^c	0.076
Yolk height (mm)	18.75	18.73	18.72	18.70	0.001
Yolk index	47.89	47.86	47.84	47.97	0.006
Shell thickness (mm)	0.332 ± 0.3 ^{bc}	0.333 ± 0.3 ^{bc}	0.341 ± 0.8 ^a	0.330 ± 0.3 ^c	0.013
Porosity (poros.cm ²)	149 ± 1.1 ^b	149 ± 1.1 ^b	151 ± 1.3 ^a	147 ± 1.02 ^c	0.115
Egg length (mm)	54.44 ± 0.23 ^c	54.51 ± 0.27 ^c	55.03 ± 0.33 ^b	55.08 ± 0.37 ^a	0.051
Egg width (mm)	41.18 ± 0.85 ^b	41.80 ± 0.93 ^a	41.73 ± 0.90 ^a	41.08 ± 0.83 ^b	0.064
Shape Index	75.62	75.60	75.58	75.56	0.017
Haugh Units	86.48	86.35	86.60	86.68	0.011

Table 4: Effect of HFMO inclusion on external and internal egg quality in free-range layers (week 35).

^{abc}Values with uncommon letters in the same row differ according to Duncan (1955) ($p < 0.05$).

The weight of the egg is related to the weight of the birds. Romera, *et al.* [31] evidenced a similar study when evaluating the dynamic behavior of egg weight in free-range hens selected by productive indicators at sexual maturity, they also stated that the early choice of birds that begin their laying with greater age and body weight on a regular basis, heavier and more uniform eggs are obtained, would be accompanied by a favorable dynamic pattern of egg mass.

In studies, Ahmad *et al.*, [2] pointed out that the properties of moringa leaf and pod meal as an additive favor the increase in weight of eggs, as well as the structure of their shells, by improving their quality in laying. In correspondence to this study, Gayathri *et al.*, [11] stated that the structural relationship of the egg depends on the body weight of the animal and its quality of life or general health, the incorporated values of moringa must be less than 10% to guarantee an adequate productive response. In addition, internal quality indicators related to egg freshness are considered [24], a parameter that positively influences egg incubation, embryonic development, embryo vitality, and quality of the future chick.

The quality of the eggshell is very important, both for the hatching and for the one intended for consumption by the population, because on the one hand it participates in the conservation of the components inside it and on the other hand it allows greater resistance to manipulation. Conversely, a thinner shell causes a change in fracture force and decreases egg quality [21].

In studies carried out by Mabusela *et al.*, [20] assures that values between 5 and 7% of Moringa Flour, directly influence the quality of the shell with values that do not differ significantly in terms of weight and thickness of the same, but evidence an increase in the coloration of the yolk, in that sense it shows an increase in the height and thickness of the albumin, as well as the presence of an important group of saturated fatty acids.

On the other hand [33] shows that the use of Moringa Flour in Hy Line Brown hens, in values of 2.5 and 4.5 %, improves the quality indices of the shell and yolk, this corresponds to the presence of sulfur amino acids and selenium in the leaves of the same,

the same reports showed Rajesh *et al.*, [29], which in turn states that levels above 10% of moringa in the diet deteriorate production results due to the increase in anti-nutritional factors and the palatability of the food offered, as well as the increase in unwanted flavors and excessive pigmentation in the yolk.

Conclusions

The inclusion of HFMO up to 8% showed the best results in the diet of free-range layers, did not affect the indicators and evidenced the best bioproductive contributions; however, the use of up to 10% as alternative feed in these birds registered significant production rates for non-specialized rearing systems.

Acknowledgements

To the Granma Poultry Company for its support in the preparation of the balance for the completion of the experiment, to the group of workers of the producer Juan Ramón Ortiz who maintained the responsibility of the work, to the University of Granma for the project management for the analysis of the samples.

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