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**Carla Cristina Bauermann Brasil
(Organizadora)**

ALIMENTOS, NUTRIÇÃO E SAÚDE



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APRESENTAÇÃO

A presente obra “Alimentos, Nutrição e Saúde” publicada no formato *e-book*, traduz o olhar multidisciplinar e intersetorial da Alimentação e Nutrição. Os volumes abordarão de forma categorizada e interdisciplinar trabalhos, pesquisas, relatos de casos e revisões que transitam nos diversos caminhos da Nutrição e Saúde. O principal objetivo desse *e-book* foi apresentar de forma categorizada e clara estudos desenvolvidos em diversas instituições de ensino e pesquisa do país em quatro volumes. Em todos esses trabalhos a linha condutora foi o aspecto relacionado à avaliação antropométrica da população brasileira; padrões alimentares; avaliações físico-químicas e sensoriais de alimentos e preparações, determinação e caracterização de alimentos e de compostos bioativos; desenvolvimento de novos produtos alimentícios e áreas correlatas.

Temas diversos e interessantes são, deste modo, discutidos nestes volumes com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela área da Alimentação, Nutrição, Saúde e seus aspectos. A Nutrição é uma ciência relativamente nova, mas a dimensão de sua importância se traduz na amplitude de áreas com as quais dialoga. Portanto, possuir um material científico que demonstre com dados substanciais de regiões específicas do país é muito relevante, assim como abordar temas atuais e de interesse direto da sociedade. Deste modo a obra “Alimentos, Nutrição e Saúde” se constitui em uma interessante ferramenta para que o leitor, seja ele um profissional, acadêmico ou apenas um interessado pelo campo das ciências da nutrição, tenha acesso a um panorama do que tem sido construído na área em nosso país.

Uma ótima leitura a todos(as)!

Carla Cristina Bauermann Brasil

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EFFECT OF CRICKET MEAL (*Gryllus assimilis*) AS A POTENTIAL SUPPLEMENT ON EGG QUALITY AND PERFORMANCE OF LAYING HEN

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ABSTRACT: This study was conducted to inquire using *Gryllus assimilis* protein in laying hen feeding and its effects on egg quality and laying hen productivity. In setting the diets, a Cricket meal was added at 2.5%, 5%, and 7.5% combining with other ingredients. The physical-chemical quality, nutritional quality, and sensory analysis by hedonic tests with effective judges in the laboratory were performed as the response variable. Results indicated that 7.5% addition of *G. assimilis* meal brought out protein increase, higher mineral and carbohydrate content of the egg, as well as higher laying potential of the birds. However, with regards to physical-chemical properties and sensory acceptance of the egg, no changes were found. At the same time, through an acute toxicity test, *G. assimilis* flour was deemed safe food and an excellent source of fortification.

KEYWORDS: Egg quality; Feed additive; Insect meal; Protein supplement.

1 | INTRODUCTION

Constant world population growth is estimated to reach 8.6 billion in 2030 so providing enough food, energy, and necessary nutrients

concern the food industry (Machado *et al.*, 2019) while laying hens which have 70-weeks years old perform weakly (low rate of laying and poor egg quality) (Gan *et al.*, 2019) so using supplements in the poultry industry is inevitable leading to increase productivity and reduce costs. Currently, seeking long term animal production sustainability through conventional alternative ingredients is an option to minimize time and costs production (Khan *et al.*, 2016) so poultry meat and eggs are considered as a vital source of energy, protein, and essential nutrients to humankind (Sharma *et al.*, 2019). Consequently, the physical and chemical quality of the egg is strongly related to laying hen nutrition and because of the probability of adjustment, producers apply the feed additives, the aim of which is not only to enhance the stock productivity but also to fulfill the quality of eggs (Batkowska *et al.*, 2020).

There is a great interest in the use of edible insects are considered as a valuable resource of proteins in terms of rapid reproduce, and high rate of growth (Soares Araújo *et al.*, 2019) and feed conversion leading to be a source of food nutrients due to its high protein content (Van Huis *et al.*, 2013) more than 77% in some species like Cricket and grasshoppers (Rumpold & Schl 2013). The use of cricket flour as a protein source especially in nutritional diets development is an economical and sustainable alternative (Norhidayah, 2016; Lee *et al.*, 2017; Irungu *et al.*, 2018; Taufek *et al.*, 2018; Alfaro *et al.*, 2019; Lokman *et al.*, 2019; Kovitvadhi *et al.*, 2019; Permatahatia *et al.*, 2019; Van Huis; 2020). The first reason to use insects in the hen diet is the lower emission of CO₂ by respiration due to the high rate of feeding conversion, low metabolism, and less fecal losses, compared to regular livestock. Also, insects could be a fascinating protein basis for growing birds, and that chitin arouses their immune response. However, the exact amount of insect meal should be examined to ensure animal health and productive performance. On the other hand, few experiments show the effects of insect meals for laying hens (Bovera *et al.*, 2018)

The main component of the insect skeleton is chitin comprising a significant amount of nitrogen. The nutrition composition of 100 grams of *Gryllus assimilis* powder on a dry basis is about 62 grams protein, 21 grams lipids, 8.5 grams fibers, 3 grams ash, and 10 grams moisture. Besides, it is a basis to establish new sources of nutrients with high biological value, and also it is indicated cricket powder (CP) is safe for feeding and human consumption (Machado *et al.*, 2019). Therefore, the objective of this research was to develop different diets fortified with *Gryllus assimilis* protein for laying hen feeding and to assess the effects on the physical, chemical, nutritional, and sensory quality of the egg.

2 | MATERIAL AND METHODS

2.1 Preparation of raw material

The study was carried out in the Poultry Section of the Institute of Biotechnology, at the National University of Agriculture, located in the department of Olancho, Honduras. *Assimilis* species of wild cricket was used as raw material and was fed with fruits and

pastures *ad libitum*, until its harvest and flour preparation. CP was developed using the methodology proposed by Alfaro *et al.*, 2019, and Marcía *et al.*, 2020; the process stemmed by subjecting 10 kg of fresh state *G. assimilis* to dehydration at 50 ° C for 75 h, in an oven (Digitronic-TFT series, Spain) with the convective flow of hot air. Subsequently, they were ground and sieved at 212 µm particle size. Finally, it was stored at room temperature (35 °C ± 5 °C) until formulations design and analyze time.

2.2 Formulation design

Formulation of diet development was done according to Mendoza's (2016) methodology with mild modifications which is the mixture of cornflour, soy flour, molasses, and CP, among others (Table 1).

Ingredient	Diets			
	T1 (%)	T2 (%)	T3 (%)	T4 (%)
Corn flour	66.69	68.17	65.23	59.57
Soy flour	16.31	13.47	14.43	17.74
Calcium carbonate	10.41	10.47	10.51	10.55
Molasses	3.00	3.00	3.00	3.00
CP	0.00	2.50	5.00	7.50
Calcium phosphate	0.96	0.97	0.83	0.75
Oil	1.52	0.42	0.00	0.00
Salt	0.35	0.36	0.36	0.36
Methionine	0.29	0.29	0.23	0.12
Premix	0.20	0.20	0.20	0.20
Binder	0.20	0.20	0.20	0.20
Lysine	0.04	0.00	0.00	0.00

Table 1. Formulation development (%)

2.3 *Gryllus assimilis* toxicology evaluation

The OECD regulation, 423 (2001) was used; for the development of analysis of Acute Oral Toxicity (OAT) from female Wistar mice, with a body mass between 150 and 200g through limit tests of 2000 mg/kg of CP, by a gastric oral test for 14 test days, in 3 groups in triplicate of mice.

2.4 Poultry management

Forty laying hens of *Hy Brown-Line* line of the age of eighty-three weeks were used. They were in turn segmented into 4 groups in duplicate of 5 hens each (20 hens), which were kept in gable-roof cage structures, with a feeder and a Nipple-type drinker. The formulations

were supplied for each group for 16 days.

2.5 Bromatological and mineralogical quality evaluation of the different formulations

Nutritional quality determination of the formulations was carried out using the Marcía *et al.*, (2019) methodology, with slight modifications; protein content, fiber, energy value, calcium, phosphorous, sodium, arginine, lysine, methionine, threonine, tryptophan, and linoleic acid were determined from 100g sample from each treatment. The bromatological analyzes were developed at the Pan-American School, El Zamorano, Honduras.

2.6 Eggs Physico-chemical and nutritional evaluations between treatments

As pointed earlier, the derived physical-chemical and nutritional quality of eggs from each treatment were determined according to the methodology proposed by Marcía *et al.*, (2020). Determinations consisted of the application of a random selection of 100 units for each treatment. Moisture, lipids, carbohydrates, ash, protein, fiber, and energy value were determined according to AOAC (2005). Furthermore, to laying percentage, pH, viscosity, egg weight, and density (Zumbado, 2004) were studied. As inclusion criterion, fresh eggs with a laying differential were taken amongst treatments of less than one day. The physical-chemical analysis was developed at the Institute of Biotechnology of the National University of Agriculture, Honduras, and the nutritional quality was developed at the Pan American School, El Zamorano, Honduras.

2.7 Eggs sensory quality determination between treatments

The sensory quality was determined by the methodology proposed by Espinoza (2014); using a hedonic scale of 7 points, through 60 affective type consumers; and was evaluating flavor, color, odor, texture, and overall acceptability. The samples were prepared in triplicate using 10g of each through an unflavored cookie working as a flavor eraser.

2.8 Statistical analysis

For data analysis IBM SPSS Statistics V25.0 was used. The tools employed were the mean, standard deviation, ANOVA, and Tukey's tests of multiple comparisons.

3 | RESULTS

3.1 Cricket meal preparation

There were six boxes with 1,815 crickets each. The insect weight average in the boxes ranges from 0.28 to 0.45g per insect (Table 2). Also, the insect weight loss in the dehydration process of obtaining the flours ranged from 0.03 to 0.19g per insect.

Box	live weight (g)	Dehydration-weight (g)	Losses (g)
1	0.40	0.22	0.18
2	0.35	0.24	0.11
3	0.33	0.30	0.03
4	0.34	0.25	0.09
5	0.45	0.26	0.19
6	0.28	0.19	0.09

Table 2. Weight (means) of crickets ready for flour preparation

3.2 The chemical quality between treatments determination

Chemical nutrition-quality of the different treatments is presented in Table 3. Producing modifications in the content of tryptophan, fiber, and linoleic acid, between treatments.

Nutrient	T1 %	T2 %	T3%	T4 %
Energy (kcal 100 g-1)	2.83	2.83	2.83	2.83
Protein (g 100 g-1)	14.00	14.00	14.00	14.00
Calcium (g 100 g-1)	4.30	4.30	4.30	4.30
Sodium (g 100 g-1)	0.16	0.16	0.16	0.16
Arginine (g 100 g-1)	0.93	0.911	1.00	1.18
Lysine (g 100 g-1)	0.72	0.72	0.72	0.72
Methionine (g 100 g-1)	0.42	0.42	0.40	0.36
Met-Cist (g 100 g-1)	0.64	0.64	0.64	0.64
Threonine (g 100 g-1)	0.54	0.54	0.54	0.54
Tryptophan (g 100 g-1)	0.17	0.17	0.20	0.24
Fiber (g 100 g-1)	2.18	2.11	2.17	2.27
Linoleic acid (g 100 g-1)	1.67	1.59	1.49	1.38

Table 3. The nutritional composition between different formulations

3.3 Acute Oral Toxicity (OAT) evaluation of CP

From 2000 mg/kg limit dose in Wistar mice, according to the OECD-423 Normative standard for OAT, the toxicity of CP in limit dose was ruled out.

3.4 Eggs physic-chemical evaluation between treatments

The eggs' viscosity between treatments was determined using a rotational viscometer PCE-RVI-1 (USA) as it can be seen on Table 4 and eggs weight, laying percentage, pH (Using a Eutech Instruments Potentiometer, Germany, with a scale of 0 to 14 pH, the hydrogen potential (pH) of the yolk, white and whole fresh egg was determined.), the density of the

egg (in the fresh state was determined using a Gay-Lussac model immersion densimeter with a scale of 1000 to 1500 kg/m³, from 100 randomized units of each treatment), and sensory evaluation (using a 7-point hedonic scale with 60 effective consumers so for the organoleptic test there were beaten and grilled and nothing was added.) are indicated in Figure 3, Figure 4, Table 5, Table 6 and Figure 5 respectively.

Treatment	Yolk viscosity(cP)	White viscosity (cP)
T1	2401.00 ± 3.5a	251.29 ± 3.2a
T2	2249.57 ± 2.1a	174.86 ± 4.3b
T3	1380.71 ± 1.0b	183.20 ± 2.5b
T4	1300.71 ± 5.0b	230.39 ± 3.1a

^{a-b}Values labeled with different letters in the same column are significantly different ($p>0.05$).

Table 4. Egg viscosity between treatments.

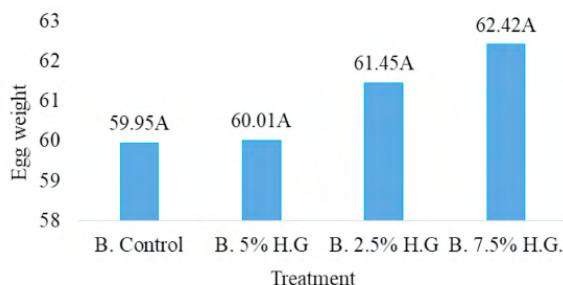


Figure 1. Egg final weight between treatments.

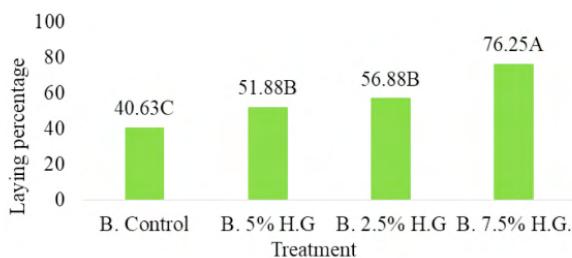


Figure 2. Laying percentage between treatments.

Treatment	Yolk pH	White pH	Whole egg pH
T1	6.01 ± 0.19 ^a	8.60 ± 0.23 ^b	8.9 ± 0.63 ^b
T2	6.40 ± 0.21 ^a	8.31 ± 0.43 ^b	8.8 ± 0.37 ^b
T3	6.47 ± 0.10 ^a	8.51 ± 0.25 ^b	8.47 ± 0.29 ^b
T4	6.34 ± 0.50 ^a	8.67 ± 0.31 ^b	8.40 ± 0.50 ^b

^{a-b} Values labeled with different letters in the same column are significantly different ($p>0.05$).

Table 5. Egg pH between treatments.

Treatment	Density (kg.m ⁻³)
T1	1038 ± 2.0
T2	1038 ± 3.0
T3	1039 ± 2.0
T4	1036 .0

Table 6. Egg density between treatments.

3.5 The comparison between fresh eggs from laying hen fed with traditional diets and laying hen fed with diets fortified with a 7.5% cricket meal.

The chemical quality of eggs of laying hen fed with the controlled and fortified cricket flour at 7.5% is presented in Table 7.

Teste	Method	Control	Diet with 7.5% cricket meal
Moisture (g 100 g-1)	AOAC 950.46B*	74.26	76.00
Ash (g 100 g-1)	AOAC 923.03*	0.6	0.90
Protein (g 100 g-1)	AOAC 2001.11*	12.01	13.36
Fat (g 100 g-1)	AOAC 2003.06*	11.40	7.96
Fiber (g 100 g-1)	AOAC 962.09	0.00	0.00
Carbohydrate (g 100 g-1)	21CFR101.92	1.28	1.78
Total energy (kcal 100g-1)	21CFR101.92	158.50	132.20

Table 7. Chemical analysis of laying hen eggs with traditional diets and diets fortified with a 7.5% cricket meal.

4 | DISCUSSION

The increasing amount of CP from 0 to 7.5% indicates Arginine, Tryptophan, and fiber increase and also decrease in Linoleic acid and Methionine levels. Moreover, CP protein content is higher than other species (Soares Araújo *et al.*, 2019) so the higher Arginine levels in CP, in dried powder, higher production of eggs, and better laying hen performance were observed (Lima *et al.*, 2020). One of the essential amino acids in the laying hen diet is

Tryptophan which is required for protein synthesis and several other metabolic processes, and also it is important for supporting egg production. Besides, vital metabolic pathways are dependent on it, for example, serotonin production which is associated with stress response and appetite regulation. Tryptophan plays a vital role in nicotinamide adenine dinucleotide, niacin, and picolinic acid metabolism. It is suggested that 160 grams of tryptophan per day are needed for white-egg laying hens (Wen *et al.*, 2012). In some studies claimed that lipid content is different because of the different development stages of insects while the amount of the lipids of this species should still be considered as another important point of it (Soares Araújo *et al.*, 2019).

The results of OAT indicates that it did not yield death in mice during 14 days of experimentation. Besides, it generated an increase in rats' weight of in $48.60\text{ g} \pm 5\text{ g}$ between the test groups and didn't show internal and external hazards, which suggests that there are no toxic effects at the systemic level. Not only CP is a safe component, but also can be safe for human consumption like gluten-free bread (Machado *et al.*, 2019).

Figure 3 depicts no statistically significant difference in the relationship of the incorporation of CP in the diet for laying hens and the weight gain of the egg. Classifying them according to their weight as a large fresh egg of B category (Egg Studies Institute, 2009). According to the same results (Bovera *et al.*, 2018), it is proved that insect meal has no effects on feed intake, egg weight, and feed conversion ratio. This shows that CP has the same efficiency among other common meals. From the evaluation of the laying percentage, it was determined that the use of CP in higher concentration positively affects the oviposition cycle of the hen which increased from 40.63% for the control sample to 76.25% for CP meal with 7.5% of insect powder sample, increasing its production. These results could be assessed considering the different levels of the Tryptophan in the hens' diets as well as higher protein amounts. Results indicate that the incorporation of CP in diets in laying hens does not affect the change in pH (Table 5). These values are consistent with the ones exposed by Hernández *et al.*, (2013) and Soler & Fonseca (2011); which expressed that the pH of the egg ranges between 5.85 and 5.92 in the yolk and 8.61 to 8.87 in the white. Furthermore, Posadas *et al.*, (2005) establish that this physical-chemical condition responds to the type of feeding of the bird and also there is no effect on the density of eggs that these results are in tune with the exposed by Quirral *et al.*, (2003); indicating that the density of the egg is $1031\text{ kg/m}^3 \pm 10\text{ kg/m}^3$ and the use of CP in the diet of laying hen do not affect the sensory attributes of eggs (flavor, color, odor, texture, and overall acceptability).

The results indicate that the use of CP increases the protein, carbohydrates, and moisture and reduces the fat and energy content of eggs. The results of the control sympathize with the exposure in the Fifth International Poultry Congress (2008); where similar values were declared in the nutritional chemical content of the fresh egg. As can be seen, a higher amount of protein in eggs leads to an increase in water binding with proteins. It is commonly supposed that these peculiar characteristics of water in the surrounding area

of proteins make biological functionalities to the protein molecule. The important aspect of protein hydration is the H-bonding between protein surface polar groups and hydration water and also moisture is indicated higher than the control sample compared to a diet with 7.5% CP and consequently, protein increase resulted to increase ash (Mallamace *et al.*, 2015).

5 | CONCLUSION

Cricket meal use is a novel alternative to increase the chemical quality of laying hen diets. Its implementation in different concentrations as a mixture enhances posture potential in laying hen. The fortification with cricket meal at 7.5% in food diets formulation design, improves the protein content of the egg, and reduces its calorific intake. The implementation of cricket meal in diets for laying hen does not affect the sensorial quality of the egg texture, color, odor, and flavor attributes. Furthermore, there is no evidence of changes in pH, viscosity, density, and egg weight.

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