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Apparent ileal digestibility of the protein in broilers partially fed with Sacha Inchi (*Plukenetia volubilis*) cake

Digestibilidad ileal aparente de la proteína, en pollos de engorde alimentados parcialmente con torta de Sacha Inchi (*Plukenetia volubilis*)

José L. Alcívar-Cobeña*[1], Madeleidy Martínez-Pérez[2], María F. Figueroa-Toalombo[1] and Lizeth V. Salazar-Salazar[1]

[1]*Facultad de Ciencias Naturales y de la Agricultura, Universidad Estatal del Sur de Manabí, Jipijapa, Ecuador.*
[2]*Instituto de Ciencia Animal, San José de Las Lajas, Mayabeque, Cuba.*

*Corresponding author: jose.alcivar@unesum.edu.ec

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Abstract

This research was conducted at the experimental farm "Andil" of the State University of the South of Manabí, Cantón Jipijapa, Manabí, Ecuador, with the aim of evaluating the ethological behavior of the apparent ileal digestibility of dry matter (DM) and crude protein (CP) of Cobb 500 chickens in individual cages. 36 male chickens were used which were distributed in four treatments with different inclusion levels of sacha Inchi cake (SIC): T1 = control, without SIC, T2 = 10% SIC, T3 = 20% SIC and T4 = 30% SIC. A Completely Randomized Design (DCA) was used for the statistical analysis, where each treatment had 12 repetitions, locating one animal per experimental unit. The results determined that chickens had a good response in quickly adapting to the cages, in addition to not reporting mortality and accepting food. The apparent ileal digestibility of DM did not show a difference between the treatments, while the N differed between the control group and the inclusion of T4 with 30% (SIC), which was higher for the latter (85.20 vs 90.23%). The results allow to conclude that the ethological behavior of the animals is not affected and good indices of ileal digestibility of DM and N are obtained when adding SIC in the diet of broilers.

Keywords: Animal physiology, adaptability, animal nutrition, metabolism.

Resumen

El presente trabajo experimental se desarrolló en la finca experimental "Andil" de la Universidad Estatal del Sur de Manabí, Cantón Jipijapa, Manabí, Ecuador, con el objetivo de evaluar el comportamiento etológico y la digestibilidad ileal aparente de la materia seca (MS) y la proteína bruta (PB) de pollos Cobb 500 en jaulas individuales. Se utilizaron 36 pollos machos que se distribuyeron en cuatro tratamientos con diferentes niveles de inclusión de torta de sacha Inchi (TSI): T1 = testigo, sin TSI, T2 = 10% TSI, T3 = 20% TSI y T4 = 30% TSI. Para el análisis estadístico se empleó un Diseño Completamente Aleatorizado (DCA), donde cada tratamiento contó con 12 repeticiones, ubicando un animal por unidad experimental. Los resultados determinaron que los pollos tuvieron buena respuesta en adaptarse rápidamente a las jaulas, además de no reportar mortalidad y de aceptar el alimento. La digestibilidad ileal aparente de la MS no mostró diferencia entre los tratamientos, en tanto que el nitrógeno (N) difirió entre el grupo testigo y la inclusión del T4 con 30% (TSI), el cual fue superior para este último (85,20 vs 90,23%). Los resultados permiten concluir que no se afecta el comportamiento etológico de los animales y se obtienen buenos índices de digestibilidad ileal de la MS y el N con la inclusión de TSI en la dieta de pollos de ceba.

Palabras clave: Fisiología animal, adaptabilidad, nutrición animal, metabolismo.

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Orcid IDs:

José Luis Alcívar-Cobeña: https://orcid.org/0000-0002-6648-3864
Madeleidy Martínez-Pérez: https://orcid.org/0000-0003-1585-2858
María Figueroa-Toalombo: https://orcid.org/0000-0002-3590-6051
Lizeth Salazar-Salazar: https://orcid.org/0000-0002-8798-6938

1 Introduction

The growing food demand requires looking for an appropriate nutritional model, using functional, novel and efficient resources to be applied in livestock production. Poultry farming in Ecuador involves agricultural production to obtain raw materials and/or by-products for poultry feed, for subsequent productive conversion (Vargas González, 2016).

When non-traditional feeds are to be included in animal feed, knowledge of nutrient concentrations is not enough, but it is also necessary to study their availability (Adedokun et al., 2011). According to Atchade et al. (2019) nutrient digestibility refers to bioavailability and depends on the animal, the composition of the feed and the treatment to which it may be subjected. When one wants to study the nutritional value of protein sources for poultry, ileal digestibility is used because these animals have different anatomical characteristics in the large intestine, so the study of fecal digestibility can be influenced by the fermentation of the microorganisms that inhabit this portion (Denbow, 2015).

Soybean (*Glycine max*) provides excellent energy concentration and availability, amino acids and essential fatty acids, fat content (18 to 20%) and protein (37 to 38%), satisfying the nutritional needs of modern poultry (Dei, 2011). It presents high digestibility (82%) and good amino acid balance (García-Rebollar et al., 2016). In broiler feeding, it is used as the main source of protein in diets (Stefanello et al., 2016). However, it has high prices in the international market, hence alternative sources are sought that allow its substitution in rations.

Sacha Inchi (SI) (*Plukenetia volubilis*) is a plant species with excellent yields, crude protein content (29%), amino acids, essential fatty acids (54%) and vitamin A and E (Romero et al., 2019). After oil extraction, its seeds have high protein values (59.13%) and fat of 6.93% (Kodahl and Sørensen, 2021). According to Romero, Valdiviezo and Bonilla (2019) the percentage of oil extraction by extrusion is lower (26.92%) when compared to that obtained in physicochemical analysis (42.03%). The residual product known as Sacha Inchi cake (SIC) has been added in the diet of some monogastrics (Robles-Huaynate et al., 2014). Although its leucine and lysine levels are lower than those of soybean, they are equal to or higher than in other seeds (Wang et al., 2018). Sacha Inchi is currently an important crop in the Ecuadorian Amazon that could replace traditional crops in the area (Andino Nájera et al., 2018). Sacha Inchi seeds that do not meet the established standards for commercialization could be considered an alternative for animal feed (Viamonte et al., 2020).

SI seeds contain anti-nutritional factors that affect digestibility and inhibit enzymatic activity. These factors could be inactivated by chemical or thermal treatment (Reátegui and Reiner, 2012; Alayón and Echeverri, 2016). Thermally processed SIC is considered a high-quality input, as it has a high level of protein and crude energy (Alcívar-Cobeña et al., 2020a).

From the nutritional point of view, research has emerged on new techniques and food sources with the available resources that can supply the traditional ones, which allows competing in quality/price in the market, being Sacha Inchi by-products a good alternative (Castro et al., 2017). The aim of the paper is to evaluate the ethological behavior in individual cages and the apparent digestibility of dry matter (DM) and crude protein (CP) of male Cobb 500 broilers fed with SIC (*Plukenetia volubilis*) at three levels as a partial replacement of Soy (*Glycine max*).

2 Methodology

2.1 Research methodology

The analytical method was used, which consists of separating the whole, decomposing it into its parts or elements to observe the causes, nature and effects. In this case, the ethological behavior of each chicken per cage was observed. Likewise, the review of scientific literature related to the topic of feeding with cakes of different oilseeds, including *Sacha inchi*, was included. The descriptive method: quantitative and qualitative data (physiological data) were also used.

2.2 Location of the study

The study was carried out at the Andil experimental farm of Universidad Estatal del Sur de Manabí, located in the Andil commune, San Lorenzo parish,

Jipijapa canton, in the province of Manabí. The coordinates of the study site are: North $1^{\circ}\ 15^{'}\ 54^{''}$ latitude South and $80^{\circ}\ 41^{'}\ 24^{''}$ longitude West and an average annual temperature of 24-26°C.

2.3 Sample population

Thirty-six male broilers of the Cobb 500 line from 7 to 42 days of age were used for the study. This line was selected because of its high resistance to diseases in the study area and its rapid conversion into muscle mass.

2.4 Treatments

The treatments consisted of: T1= control without SIC, T2= 10% SIC, T3= 20% SIC and T4= 30% SIC. The diets evaluated in each treatment were offered from day 7 and according to physiological stages such as initial (0-14 days), growth (15-28 days) and fattening (29-42 days). The diets were the same as those used by Alcívar-Cobeña et al. (2020b).

2.5 Experimental design

A Completely Randomized Design (CRD) composed of 4 treatments and 9 replicates was used, where three animals are considered as the experimental unit for analyzing ileal digestibility of DM and N. Tukey's dyzyme at $P< 0{,}05$ probability was used for detecting differences where necessary, and the statistical package InfoStat was used for the statistical processing (Di Rienzo et al., 2012).

2.6 Management of the study

2.6.1 Facilities

Prior to the arrival of the birds in the house, the house was cleaned and fumigated, and the floor was disinfected with lime. One day prior to the arrival of the birds, the brooder was turned on, optimizing a temperature between 30 and 32°C. The litter was built with rice shavings, and was removed daily to maintain adequate cleanliness conditions, where the birds remained for 7 days. In the second week, the litter was replaced by 48 galvanized wire cages of $40 \times 40 \times 80$ cm, equipped with their own drinking and feeding troughs, in which each bird was placed individually. Asepsis, washing and disinfection measures were applied daily.

2.6.2 Feed management

During the last week that corresponds to the fattening stage (35-42 days of age), the animals were fed with 200g of feed per day. They were divided into two rations: 100g in the morning and the same amount in the afternoon. Feed consumption was calculated from the supply and refusal.

2.6.3 Sampling

The ethology of the animals was studied from day 7 to day 14 according to (Cebrián et al., 2011). This moment coincided with the adaptation to the change from floor rearing to individual cages, located one meter above ground level together with the supply of feed that was formulated by adding SIC in different percentages. The time spent on food and water consumption and excretion and the manifestations of stress due to the management conditions were recorded. With these data, the percentage dedicated to each activity was calculated considering the total observation time (period between 8:00 am and 6:00 pm), as well as the ingestion-excretion ratio (IER). For the latter, the Equation 1 was used.

$$RIE, \% = \frac{No.\,consuming\,food}{No.\,sometimes\,excreting} * 100 \qquad (1)$$

The scale used in each case was from 1 to 100% where low values represent stress/rejection; medium values neutrality and high values or close to 100 mean good adaptation and acceptance inside the cages. After this time, animals were sacrificed for studying apparent ileal digestibility of dry matter and crude protein. Both components were determined in the diet and ileal content according to AOAC (2019) analytical techniques. Samples were taken by manually extracting the contents in the intestinal section by sliding the index finger and thumb along the ileum and were put on Petri dishes (Sebastian et al., 1997). The ileal contents of three chicks were taken as a replicate. Samples were stored at -20 °C until further processing.

2.6.4 Slaughter of the animals and calculation of apparent ileal digestibility of nutrients

The birds were sacrificed, using electric shock to stun them and reduce the level of stress. Subsequently, the abdominal cavity was opened and the

ileum was identified, which is defined as the portion of the small intestine that extends from Meckel's diverticulum to the ileo-cecal junction (Castro et al., 2020). Once the samples were processed, the apparent ileal digestibility of DM and CP was calculated by the direct method according to the Equation 2. Where $(DM \vee CP)$ *consumed* and $(DM \vee CP)$ *ileum* represent the content of both nutrients in the feed consumed and in the ileal content, respectively.

$$Ileal\ digestibility, \% = \frac{(DM \vee CP)\ consumed - (DM \vee CP)\ ileum}{(DM \vee CP)\ consumed} \quad (2)$$

3 Results and Discussion

3.1 Ethological analysis of the birds in cages

A high level of stress was observed during the first two days in the ethological descriptive analytical study (Table 1). The birds showed restlessness, so they were orally administered (dissolved in the drinker) a commercial stress regulator "Trolvit", which also contained electrolytes, amino acids and vitamins. This contributed to a faster adaptation to management.

Good responses were obtained in the ethological analysis in all variables between days 6 and 7, valued on a percentage scale from 0 to 100 %, which showed a satisfactory adaptation level during the days of the study and a good sanitary control from the cages. The results are recorded in Table 1. The use of individual cages gave good results and the birds began to show their adaptation to this environment two days after their establishment. The results agree with those obtained by Martínez-Pérez et al. (2008), who proposed an adaptation time to the cages and feed of 7 days, when metabolic studies are carried out in individual cages for broilers.

Table 1. Descriptive analysis of chick ethology.

DC	CV	RCM	RIE
1	30 %	30 %	50 %
2	40 %	40 %	40 %
3	60 %	60 %	60 %
4	70 %	70 %	60 %
5	75 %	75 %	70 %
6	90 %	95 %	80 %
7	100 %	100 %	100 %

DC day of rearing, CV voluntary feed and water consumption, RCM response to management conditions, RIE ingestion-excretion ratio.

3.2 Apparent ileal digestibility of nutrients using Sacha inchi cake

Table 2 shows the analysis of variance of the ileal digestibility of DM. The p value was not significant for p< 0,05. The means by treatments are shown in Table 3. The ileal digestibility coefficients were high in all cases, showing good SIC digestion by the animals. Chickens have an anatomically and physiologically adaptable gastrointestinal tract according to the type of food provided (Flórez and Osorio, 2013), which favored the consumption of the cake without causing unfavorable changes for the animal.

Table 2. Analysis of Variance of Apparent Ileal Digestibility of dry matter.

F.V.	SC	Gl	CM	F	p-value
Tratamientos	0.33	3	0.11	0.10	0.9585^{NS}
Error	8.79	8	0.11		
Total	9.11	11			

NS: Not significant.

The analysis of variance for the apparent ileal digestibility of crude protein is shown in Table 4. The p-value in this case was significant, and differences between treatments were found when comparing the means shown in Table 5. The control group and the inclusion of 30 % SIC show differences between them, and their coefficients were the lowest and highest, respectively. The 10 and 20 % SIC were similar to the rest of the treatments. It seems that the quality of the protein provided by the Sacha inchi cake is high, so there is an eminent utilization by the animal, hence it is excreted in lower concentration and therefore it increases the digestibility when

included in the poultry rations.

Despite the differences observed, the coefficients were generally high. These results do not coincide with those observed by Woyengo et al. (2017) when using camelina cake (around 75.30%). The differences could be explained by the chemical composition of both ingredients after the oil extraction process to which the oilseeds were subjected. These authors reported CP and FDN values of 39.80 and 38.30%, respectively, while in the Sacha inchi cake used in this study.

Table 3. Analysis of means by Tukey's test for Apparent Ileal Digestibility of Dry Matter (DIAMS).

Variable (%)	Treatments	Means	No.	E.E ±
DIAMS	T4	82.59	3	0.61
	T3	82.51	3	0.61
	T2	82.50	3	0.61
	T1	82.16	3	0.61

Alcívar-Cobeña et al. (2020a) obtained values of 41.49 and 16.64%, respectively. The fact of having a higher protein concentration and lower fiber content favors its digestibility; several authors mention that fiber content has a direct incidence on the digestive process (Zając et al., 2020).

On the other hand, according to Woyengo et al. (2017) products resulting from oil extraction in oilseeds generally contain anti-nutritional factors that can limit the use of nutrients by the animal. Sacha inchi cake shows presence of alkaloids, saponins and α-amino groups (Alcívar-Cobeña et al., 2020a), however, the concentration of these components does not seem to be very high, since they did not unfavorably impact ileal digestibility of DM and CP.

Palpa (2009) determined the metabolizable nutrients and apparent metabolizable energy for precooked and pre-cooked extruded sacha inchi cake in broilers by collecting fecal samples. The metabolizable dry matter was between 73.95% and 74.78%; the metabolizable crude protein between 42.97% and 44.26% and the apparent metabolizable energy between 4 570.0 and 4 578.91 kcal/kg, respectively. Since the amount of protein and fat in SIC is high, its application could optimize the resources available to producers since soybean cake has high prices in the international market and livestock activity imports approximately one hundred thousand tons per year (Benítez et al., 2018).

Table 4. Analysis of Variance for Apparent Ileal Digestibility of CP.

F.V.	SC	Gl	CM	F	p-value
Treatments	37.96	3	12.65	7.43	0.0107
Error	13.62	8	1.70		
Total	51.58	11			

Table 5. Analysis of means by Tukey's test for Apparent Ileal Digestibility of CP (DIAPB).

Variable (%)	Treatments	Means	No.	E.E±	
DIAPB	T4	90.23	3	0.75	A
	T3	87.79	3	0.75	AB
	T2	87.74	3	0.75	AB
	T1	85.20	3	0.75	B

Means with different letters differ significantly.

4 Conclusions

Ethologically, the Cobb 500 chickens obtained a positive response in adapting satisfactorily to the individual cages. During the process of the study, there was no mortality, and better control of feed and drinking water intake was achieved. Sanitary control was better compared with rearing in litter.

Good indices of ileal digestibility of DM and N were obtained when using SIC the diet of broilers, so it can be used as a partial substitute for soybean in the conventional feed for Cobb 500 broilers. Further research with greater variability in the experiments is recommended for its application in other breeds.

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Author contribution

Conceptualization, methodology and discussion: J.L.A.C.; Experimental design and statistics: M.M.P.; Management of field research, laboratory analysis: M.F.F.T.; Writing and bibliography: L.V.S.S.

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