

Short Note [Nota corta]



ESTIMATION OF METABOLIZABLE AND DIGESTIBLE ENERGY OF RAMON (*Brosimum alicastrum* Swartz) SEED IN BROILERS UNDER TROPICAL CONDITIONS †

[ESTIMACIÓN DE LA ENERGÍA METABOLIZABLE Y DIGESTIBLE DE LA SEMILLA DE RAMÓN (*Brosimum alicastrum* Swartz) EN POLLOS DE ENGORDA EN EL TRÓPICO]

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SUMMARY

Background. The current maize production in México is insufficient to supply both human and animal consumption. As maize is the main ingredient used in the formulation of poultry diets, each year, the importation of this cereal rises, thereby, the replacement of this conventional input can be made with alternative resources, such as the Ramón (*Brosimum alicastrum* Swartz) seed. **Objective.** To determine the chemical composition of Ramon seed meal (RSM), together with the estimation of the apparent metabolizable energy (AME), true metabolizable energy (TME), their digestible coefficients, as well as the apparent ileal digestible energy (AIDE) and apparent ileal digestibility coefficient (AIDC) of gross energy (GE). **Methodology.** Two experiments were performed using Cobb broilers; in the first experiment AME, TME and the digestible coefficients were calculated using 24 broilers (twelve 3-week-old and twelve 6-week-old) eight of them were randomly selected for determination of endogenous losses (EL). The sixteen remaining were precision-fed a single dose of RSM and total excreta collection was used. In the second experiment, three diets: 1) 100% maize; 2) 40% RSM-60% maize and; 3) 60% RSM-40% maize) were made to determine the AIDE and AIDC of each ingredient using the difference method. Diets were randomly assigned to a total of 51 7-week-old broilers, distributed in six, six and five replicates respectively (three broilers per replicate). **Results.** No differences were found for the AME (1863 and 1909 kcal/kg for 3 and 6 weeks, respectively) and TME (2234 and 2271 kcal/kg, for 3 and 6 weeks, respectively) values of the RSM. The AIDE and AIDC of RSM at 40 and 60% inclusion (2408 and 2538 kcal/kg, and 0.64 and 0.67, respectively) were found to be lower than that of maize (3179 kcal/kg and 0.81). **Implications.** These results provide information regarding the incorporation of ramon as an energy resource in tropical poultry diets. **Conclusion.** The estimated value of RSM in broilers was 1886 Kcal/kg for AME, 2252.5 Kcal/kg for TME and 0.476 and 0.569 for their digestibility coefficients of GE, respectively. For the AIDE, the estimated value was 2408.8 and 2538.7 Kcal/kg at 40 and 60% inclusion of RSM, with 0.640 and 0.674 AIDC, respectively.

Key words: AME; TME; AIDE; broilers; *Brosimum alicastrum* Sw.

RESUMEN

Antecedentes. La actual producción de maíz en México es insuficiente para suministrar tanto al consumo humano como al pecuario. Como el maíz es el principal ingrediente empleado en la formulación de dietas para aves, cada año la importación de este cereal aumenta, por lo tanto, la sustitución de este insumo convencional puede darse con recursos alternativos como lo es la semilla de ramón (*Brosimum alicastrum* Swartz). **Objetivos.** Determinar la composición química de la harina de semilla de ramón (HSR), además de estimar la energía metabolizable aparente (EMA), energía metabolizable verdadera (EMV), sus coeficientes de digestibilidad, así como la energía digestible ileal aparente (EDIA) y el coeficiente de digestibilidad ileal aparente (CDIA).

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Metodología. Se llevaron a cabo dos experimentos utilizando pollos de engorda Cobb, en el primer experimento, para determinar la EMA, EMV y sus coeficientes de digestibilidad, ocho pollos de un total de 24 (12 de 3 semanas y 12 de 6 semanas) se seleccionaron aleatoriamente para determinación de pérdidas endógenas. A los dieciséis restantes se les administró una sola dosis de HSR mediante alimentación forzada empleando el método de colecta total de excretas. En el segundo experimento, se elaboraron tres dietas: 1) 100% maíz, 2) 40% HSR-60% maíz y; 3) 60% HSR-40% maíz) para la determinación de EDIA y CDIA de cada ingrediente utilizando el método de diferencias. Las dietas se asignaron aleatoriamente a un total de 51 pollos de 7 semanas de edad, distribuidos en 6, 6 y 5 repeticiones, respectivamente (tres pollos por replica). **Resultados.** No se encontraron diferencias entre los valores de EMA (1863 y 1909 Kcal/kg para 3 y 6 semanas, respectivamente) y EMV (2234 y 2271 Kcal/kg para 3 y 6 semanas, respectivamente) de la HSR. La EDIA y el CDIA de la HSR con 40 y 60% de inclusión (2408 y 2538 Kcal/kg y 0.64 y 0.67, respectivamente) se encontraron ser menores comparados con los del maíz (3179 Kcal/kg y 0.81). **Implicaciones.** Este trabajo presenta información para la incorporación de la semilla de ramón como fuente de energía de origen tropical a las dietas de aves. **Conclusión.** El valor estimado de la HSR en pollos de engorda fue de 1886 Kcal/kg EMA y 2252.2 Kcal/kg EMV, con 0.476 y 0.569 de coeficientes de digestibilidad, respectivamente. Para la EDIA, el valor estimado fue 2408.8 y 2538.7 Kcal/kg para el 40 y 60% de inclusión de HSR, respectivamente, con 0.640 y 0.674 de CDIA, respectivamente.

Palabras clave. EMA; EMV; EDIA; pollos de engorda; *Brosimum alicastrum* Swartz.

INTRODUCTION

In Mexico, the current maize production is scarce to meet consumption needs for both the human population and the livestock industry. In 2021, approximately 45 million tons of maize were consumed in this country, from which 27.4 million tons were produced nationally, and 17.4 million tons were imported. In relation with data from the previous years, the volume of imported maize increased in almost 1 million tons, given that, only human consumption and livestock industry comprise almost 35 million tons. This indicates that while the demand for maize increases, the production decreases (CONAFAB, 2022). As maize is the main cereal grown in the world used as primary component for poultry diets (Szcurek *et al.*, 2020), the incorporation of alternative feedstuffs in developing countries is a viable option to substitute conventional energy sources, such as maize and other cereals. An alternative resource is the seed from Ramon (*Brosimum alicastrum* Sw.) tree because the protein and carbohydrate contents, specifically starch, are similar to that of maize (Subiria-Cueto *et al.*, 2019).

The Ramon tree can produce approximately 95.5 kg of seed per tree per year, yielding 38.2 ton per ha per year in a plantation of 400 trees per ha (Olguin-Maciél *et al.*, 2017). This is found to be much higher when compared to the average maize yield for Mexico (8.51 ton per ha) (SIAP, 2018). Because the use of Ramon seeds (RS) in poultry feed is not common, its metabolizable and digestible energy values has not been determined. Therefore, the objective of this study was to estimate the apparent metabolizable (AME) and true metabolizable energy (TME) values in broilers at 2 different ages, as well as the apparent ileal

digestible energy (AIDE) and apparent ileal digestibility coefficient (AIDC) of ramon seed meal (RSM).

MATERIALS AND METHODS

The proximal composition of RS and maize was analyzed following the official AOAC procedures: dry matter (method 930.15), nitrogen content (method 954.01) using factor 6.25 to obtain crude protein content, crude fiber (method 962.09), fat (method 920.39), and ash (method 923.03), (AOAC, 1990). The total carbohydrate content was estimated as nitrogen-free extract (NFE) by difference. Gross energy (GE) was estimated using an adiabatic bomb calorimeter (IKA, mod. c2000).

The study was carried out at the experimental area of the Department of Animal Nutrition of the Faculty of Veterinary Medicine and Animal Science of the Universidad Autonoma de Yucatán (FMVZ-UADY), México (20°58'N and 89°37'W). RS were obtained from two different harvests, one for each experiment, and were provided by local producers. Seeds were then dried (at 60°C for 48 hours) and milled (fine mill IKA ® MF10 basic at 3 mm) for obtaining RSM. Two experiments were carried out using Cobb broilers, in the first experiment, a total of 24 birds, twelve 3-week-old and twelve 6-week-old weighing 0.82 ± 0.068 kg and 2.81 ± 0.16 kg, respectively, were used to determine AME and TME of RSM by total excreta collection. From each group of age, four broilers were randomly selected for determination of endogenous losses (EL) receiving glucose solution only. The remaining eight animals received a single precision-feeding dose either 25 or 40 g, after four days of adaptation (for 3- and 6-week-old,

respectively) of RSM. Each bird was considered a replicate.

In the second experiment, fifty-one 7-week-old broilers weighing 3.23 ± 0.49 kg were randomly divided into 3 groups (6, 6, 5 replicates per group, respectively, with 3 broilers per replicate) for determination of the AIDE and AIDC values of RSM using the difference method. Each group received a diet formulated with maize and RSM at different levels of inclusion, the first group received a diet consisting of 100% maize (diet 1), group two a diet consisting of 40% RSM-60% maize (diet 2) and group three a diet consisting of 60% RSM-40% maize (diet 3). TiO₂ (5 g/kg) was incorporated into the diets as an indigestible marker for digestibility determination (Anwar *et al.*, 2018).

Birds from both experiments were kept in individual metabolic cages (40 x 50 cm); cages in experiment one had aluminum trays wrapped with polyethylene bags underneath for excreta collection (Silva *et al.*, 2012). After four days of adaptation to diets and 12 h of individual housing, all broilers received glucose solution (50% w/v) (25 ml for 3-week-old and 50 ml for 6 and 7-week-old). Following 8 and 16 h, all birds were given their corresponding diet (or glucose) using Sibbald's precision-feeding technique (Sibbald, 1986). Samples for experiment one were collected over the course of 48 h every 12 h, subsequently they were oven-dried at 60°C for 48 h and frozen for GE analysis. For experiment two, birds were sacrificed by manual decapitation four hours after the last feeding and eviscerated to obtain the ileal samples. The ileal contents from three birds were deposited in a 4x4 cm² plastic container and frozen for TiO₂ and GE analyses.

Calculations

For experiment one, the direct method was applied for the AME, TME and digestibility coefficients of gross energy (GE) for the 3- and 6-week-old broilers using the formula:

$$ADC = (GE \text{ intake} - GE \text{ excreta}) / (GE \text{ intake})$$

$$TDC = (GE \text{ intake} - (GE \text{ excreta} - GE \text{ from EL})) / (EG \text{ intake})$$

Where, ADC is Apparent Digestibility Coefficient, TDC is True Digestibility Coefficient, GE is Gross Energy and EL is Endogenous Losses.

$$AME \left(\frac{\text{Kcal}}{\text{kg}} \right) = ADC * GE \text{ diet}$$

$$TME \left(\frac{\text{Kcal}}{\text{kg}} \right) = TDC * GE \text{ diet}$$

For experiment two, the indirect method was used to calculate the AIDC and AIDE of GE of the test diets as described, where Ti is TiO₂:

$$AIDC \left(GE \right) \left(\frac{\text{Kcal}}{\text{kg}} \right) = ((GE/Ti)_{\text{diet}} - (GE/Ti)_{\text{ileal}}) / (GE/Ti)_{\text{diet}}$$

$$AIDE \left(GE \right) \left(\frac{\text{Kcal}}{\text{kg}} \right) = AIDC(GE) * GE \text{ diet}$$

The Digestible Energy (DE) of each ingredient of the diets (maize or RSM at 40% or 60% inclusion) was calculated by the difference method according to Anwar *et al.* (2018) using the following equation:

$$DE \left(\frac{\text{Kcal}}{\text{kg}} \right) = [(T \times Tp) - (B \times Bp)] / Ap$$

Where, Ap is the proportion of the component of RSM in assay diet contributed by test ingredient, B is the digestibility coefficient of RSM in the basal diet, Bp is the proportion of the component of RSM in assay diet contributed by the basal diet, T is the digestibility coefficient of RSM in the test diet and Tp is Ap + Bp.

Finally, the AIDC of GE of RSM at 40 or 60% inclusion:

$$AIDC \left(GE \right) \left(\frac{\text{Kcal}}{\text{kg}} \right) = DE \text{ RSM} * GE \text{ RSM}$$

Statistical analyses

In experiment one, to assess the age effect in both groups of birds, the means of AME, TME, ADC and TDC of RSM were analyzed using a two-sample t-test (Minitab, 20129) in a completely randomized design. Means were considered significant at $p < 0.05$. For experiment two, data from the direct and difference methods were analyzed by analysis of variance using the General Linear Model procedure (Minitab, 2019) in a completely randomized design. Means that were considered significant at $p < 0.05$ were then analyzed using a Tukey test.

RESULTS

The values of the chemical composition of the RSM from each experiment are presented in Table 1. Results of dry matter (DM), crude protein (CP),

crude fiber (CF) and Ash, from experiment two were higher compared to those for experiment one, except for the GE values. Ether extract (EE) and nitrogen free extract (NFE) were only determined in experiment one. There was no age effect ($p = 0.792$) found in AME and ADC values (1863 Kcal/kg and 0.471 for 3-week-old vs. 1909 Kcal/kg and 0.482 for 6-week-old broilers, respectively) of the GE of RSM between both group of ages. The same was observed for the TME and TDC values (2234 Kcal/kg and 0.564 for 3-week-old vs. 2271 Kcal/kg and 0.574 for 6-week-old broilers, respectively) ($p = 0.835$) of RSM from experiment one (Table 2). For experiment two, the values obtained for the AIDC of GE of the three test diets showed no differences ($p = 0.078$) between them (0.812, 0.745 and 0.739 for diet 1, 2 and 3, respectively). Meanwhile, the analysis of variance for AIDE of the GE of the test diets showed a significant difference ($p = 0.020$). When using the Tukey test to compare means, the AIDE of the GE of the 100% maize diet (3179.6 Kcal/kg) was similar to diet 2 (2871.3 Kcal/kg). On the other hand, AIDE of GE of diet 3 had the lowest value (2795.1 Kcal/kg), and it appeared to be similar compared with diet 2. However, diet 1 and diet 3 showed statistical differences ($p = 0.020$).

Table 1. Chemical composition of RSM from both experiments.

Component	Experiment 1 (%)	Experiment 2 (%)
Dry matter	89.41	91.10
Crude protein	11.45	13.63
Crude fiber	3.04	4.48
Ether extract	0.69	ND
Ash	2.72	4.15
Gross energy (Kcal/kg)	3955	3763

Kcal/kg, kilocalories per kilogram;
ND, not determined

As for the DE of each ingredient obtained by the difference method, maize, as stated before, had the highest value and was similar to RSM at 60% inclusion. On the other side, RSM at 40% inclusion had the lowest value, however, it showed no differences ($p > 0.05$) between those from RSM at 60% inclusion. The AIDC of maize had higher value when compared to RSM at 40 and 60% inclusion, yet this difference was not significant ($p = 0.064$) (Table 2).

DISCUSSION

The chemical composition of RSM from experiment one had lower values for DM, CP, CF and Ash, while the GE was higher when compared to the results of experiment two. The values for CP, CF, EE and NFE of RSM reported for experiment one had minor differences when compared to those described by Subiria-Cueto *et al.* (2019) (11.5%, 3.04%, 0.6%, 81.2%, respectively), meanwhile DM (86.7%) and GE (3955 Kcal/kg) values were higher and only ash (3.4%) values were lower for experiment one, when compared to those reported by Subiria-Cueto *et al.* (2019).

Table 2. AME, TME, DE and digestible coefficients of gross energy of RSM from experiments 1 and 2.

Variable	Experiment one	Experiment two	
		RSM at 40% inclusion	RSM at 60% inclusion
AME (Kcal/kg)	1886	-	-
ADC of GE	0.476	-	-
TME (Kcal/kg)	2252.5	-	-
TDC of GE	0.569	-	-
DE (Kcal/kg)	-	2408.8	2538.7
AIDC of GE	-	0.640	0.674

AME, apparent metabolizable energy; TME, true metabolizable energy; ADC, apparent digestibility coefficient; TDC, true digestibility coefficient; DE, digestible energy; AIDC, apparent ileal digestibility coefficient; GE, gross energy; RSM, ramon seed meal; Kcal/kg, kilocalories per kilogram

In experiment two, the DM, CP, CF, ash and GE values of RSM were higher when compared to those found by Subiria-Cueto *et al.* (2019). Although Peters and Pardo-Tejada (1982) reported higher values for CP and GE of RSM than Subiria-Cueto *et al.* (2019), (12.8% and 3610 Kcal/kg, respectively). These results are still lower to those obtained in experiment two (13.6% and 3763 Kcal/kg, respectively). Whereas Peters and Pardo-Tejada (1982) reported a higher CF value (4.6%) than for experiment one (4.4%), the results for Ash were lower (3.2 vs 4.25%, respectively).

These differences in their chemical composition could partly explain the disparity between maize and RSM GE values, since it ranges from 500 to 700 Kcal/kg approximately, while for AME, TME and DE, this disparity was even higher (from 1122.5 to 1624.1 Kcal/kg). Since RSM has a lower content of EE (Ullah *et al.*, 2016) and a higher content of CF and ash compared to maize. Fat and soluble carbohydrates (NFE) represent the main sources of energy in poultry diets due to their higher energy yield compared to protein and fiber (Stevens, 1996; Wu *et al.*, 2020). Given that, CF has been shown to negatively affect metabolizable energy (ME) values of feedstuffs as reported by Hill *et al.* (1960) and Dale (1996). This is due mainly to the non-starch polysaccharides content (NSP), which escape nutrient digestion and absorption because of the lack of endogenous enzymes in the bird for hydrolysis of these polysaccharides (Mtei *et al.*, 2019; Kumar and Kyun, 2021; Tejeda and Kim, 2021). On the other hand, Ash has no energy content, as and therefore metabolizable and digestible energies, are significantly lower (McDonald *et al.*, 2010).

Regarding NFE, maize possesses similar amounts of starch (62.48%), amylose (27.3%) and amylopectin from starch (72.6%) as RSM (61%, 25.3% and 74.6%, respectively) (Rostagno *et al.*, 2005; Perez-Pacheco *et al.*, 2014; Olguin-Maciél *et al.*, 2017). Even though starch is the main source of energy in grains, the energetic values obtained were not as expected, and therefore, a difference between maize and RSM, was found. This could be attributed to differences reported by Perez-Pacheco *et al.* (2014) and Moo-Huchin *et al.* (2015) between the morphological and physicochemical properties of maize and RS starches, which reverberate in their functionality. These include starch granule size, amylose-to-amylopectin ratio and purity (10 μm , 1:2.94 and 92.57% for RS starch and 15 μm , 1:2.65 and 98.86% for maize starch). The latter is related with the physicochemical properties, as the Ash and protein content of RS starch were stated to be higher (0.47% and 0.12%, respectively) than that from maize starch (0.02% and 0.03%, respectively). These properties are related to their water absorption capacity, hydration, and their swelling capacity therefore, to reach viscosities at lower temperatures and have a higher gelatinization capacity (Tang *et al.*, 2004; Perez-Pacheco *et al.*, 2014; Moo-Huchin *et al.*, 2015; Cornejo-Ramirez *et al.*, 2018).

However, starch present in both RSM and maize used in the current experiments, was raw, so it is more difficult to be dissolved in cold water (Ratnayake and Jackson, 2009). For this, several

authors mention that starch needs to be submitted to thermal processes such as steaming, extrusion cooking, baking, parboiling, among others, and thus it is modified structurally and become more digestible (Sajilata *et al.*, 2006). Although, both ingredients were offered raw, the results showed that maize had better digestibility than RSM, which lead us to assume that RSM may contain a higher proportion of slowly digestible or resistant starch than maize. This is due to the fact that resistant starch acts as fiber by escaping enzymatic hydrolysis in the small intestine (Amoako and Awika, 2016).

The results of the age effect in experiment one agreed with those of Huang *et al.* (2005) and Yang *et al.* (2020) who reported increased ME values and feed digestibility with the age of birds. Also, Ten Doeschate *et al.* (1993) indicate that 6, 4 and 2-week-old broilers showed minimal differences in the ME from the diets offered, since at 2 weeks of age, birds reach their maximum nutrient absorption capacity (Batal and Parsons, 2002; Huang *et al.*, 2005; Wu *et al.*, 2020). Therefore, as no differences in AME and TME in experiment one were found between the two ages of broilers, the results indicate that since the age of 3 weeks, birds have already reached their absorptive capacity and are able to digest and metabolize RSM adequately.

This is the first time, that AME, TME and DE values of RSM in broilers have been published, thus, there are no references from other authors (to the best of the authors knowledge), to compare these values obtained.

CONCLUSIONS

There was not effect of age of broiler on AME and TME values, which leads us to assume that birds can be fed with RSM since 3 week of age. With dietary inclusion of 40 and 60% RSM, the DE had lower values when compared with maize, it could be attributed to the differences in the chemical composition between RSM and maize, given that maize grain has higher EE and lower CF and ash contents. Nevertheless, differences in the morphological and physicochemical properties of starch from both feedstuffs should be investigated.

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Conflict of interest. The authors declare that there is no conflict of interest.

Compliance with ethical standards. Experiment protocols were reviewed and approved by the Internal Bioethics Committee of the Campus of Biological and Agricultural Sciences for the correct handling and care of the animals (CB-CCBA-Docencia-2019-001).

Data availability. Data are available with S. Montfort-Grajales, sofiaa.mg@hotmail.com

Author contribution statement (CRediT). **S. Montfort-Grajales** – Conceptualization, data curation, investigation, methodology, software, writing – original draft. **L. Sarmiento-Franco** – Data curation, formal analysis, methodology, software, supervision, visualization, writing – review & editing. **R. Urtecho-Novelo** – Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft. **C.A. Sandoval-Castro** – Funding acquisition, project administration, resources, validation, writing – review & editing.

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