

Short communication: Estimation of metabolizable and digestible energy of Ramon (*Brosimum alicastrum* Swartz) seed in broilers under tropical conditions

Sofia Montfort-Grajales

Universidad Autónoma de Yucatán: Universidad Autonoma de Yucatan

Luis Sarmiento-Franco (✉ luis.sarmiento@correo.uady.mx)

Universidad Autonoma de Yucatan <https://orcid.org/0000-0003-1612-0675>

Rosalinda Urtecho-Novelo

Universidad Autónoma de Yucatán: Universidad Autonoma de Yucatan

Carlos Sandoval-Castro

Universidad Autónoma de Yucatán: Universidad Autonoma de Yucatan

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Abstract

The objective of this study was to estimate the apparent metabolizable energy (AME), true metabolizable energy (TME), their digestible coefficients, as well as the apparent ileal digestible energy (AIDE) and apparent ileal digestible coefficient (AIDC) of Ramon (*Brosimum alicastrum* Sw.) seed meal (RSM). Two experiments were performed using Cobb broilers, in the first experiment, eight birds from a total of 24 broilers (twelve 3-week-old and twelve 6-week-old) were randomly selected for determination of endogenous losses (EL). The sixteen remaining were tube-fed a single dose of RSM and total excreta collection was used. In the second experiment, three diets (100% maize; 40% RSM-60% maize and 60% RSM-40% maize) were made to determine the AIDE and AIDC of each ingredient using the difference method by incorporating titanium dioxide (TiO₂) at 5 g/kg. Diets were randomly assigned to a total of 51 7-week-old broilers, distributed in 6, 6 and 5 replicates respectively (three broilers per replicate). No age effect ($p > 0.05$) was found for both 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) 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 ($p < 0.05$) than that of maize (3179 kcal/kg and 0.81). It was concluded that the main factor contributing to the decrease in AME, TME, AIDE and AIDC values for RSM was the difference between the chemical composition of this feedstuff and maize, as they have almost the same starch amounts.

Introduction

In Mexico, the current maize production is scarce to meet consumption needs for both the human population and the livestock industry. In 2018, approximately 13.4 million tons of maize were produced, and 11.3 million tons were imported in the country. Regarding the previous years, this volume represents an increase of 11%, which indicates that while the demand for maize increases, the production decreases (CONAFAB, 2018). 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 Ramon (*Brosimum alicastrum* Sw.) tree seed, because the protein and carbohydrate contents, specifically starch, are similar to that of maize (Subiria 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 maize national yielding (8.51 ton per ha) (SIAP, 2018). Because the use of Ramon seeds in poultry feed is slightly known, their metabolizable and digestible energy values has not been determined. Therefore, the objective of this study was to estimate the AME and TME values at 2 different ages, as well as the AIDE and AIDC of RSM in broilers.

Materials And Methods

The study was carried out in the experimental area of the Department of Animal Nutrition of the Faculty of Veterinary Medicine of the Universidad Autonoma de Yucatán (FMVZ-UADY), México (20°58'N and 89°37'W). RSM was obtained by the harvesting, drying (at 60°C for 48 hours) and milling (fine mill IKA® MF10 basic at 3 mm) of Ramon seeds. 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 ± 0.68 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 tube-feeding dose either 25 or 40 g (for 3- and 6-week-old, respectively) of RSM

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 the same seed 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, group two a diet consisting of 40% RSM-60% maize and group three a diet consisting of 60% RSM-40% maize. TiO₂ (5 g/kg) was incorporated into 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) with aluminum trays wrapped with polyethylene bags underneath the cages for excreta collection. (Silva et al., 2012). After 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). At 20 and 28 h, all birds were given their corresponding diet (or glucose) using Sibbald's force-feeding technique (Sibbald, 1986). Samples for experiment one were collected for 48 h every 12 h and were oven-dried at 60°C for 48 h and then frozen for gross energy (GE) analysis. For experiment two, birds were sacrificed by manual decapitation four hours after the last feeding and eviscerated to obtain the ileum. The ileal contents of 3 birds were deposited in a 4x4 cm² plastic container and frozen for TiO₂ and GE analysis.

Calculations

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

$$ADC = (GE_{intake} - GE_{excreta}) / (GE_{intake})$$

$$TDC = (GE_{intake} - (GE_{excreta} - GE_{fromEL})) / (EG_{intake})$$

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

$$AME = ADC * GE_{diet}$$

$$TME = TDC * GE_{diet}$$

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:

$$\text{AIDC} = ((\text{GE}/\text{Ti})_{\text{diet}} - (\text{GE}/\text{Ti})_{\text{ileal}}) / (\text{GE}/\text{Ti})_{\text{diet}}$$

$$\text{AIDE} = \text{AIDC} \times \text{GE}_{\text{diet}}$$

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:

$$\text{DE} = [(T \times T_p) - (B \times B_p)] / A_p$$

Where, A_p 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, B_p 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 T_p is $A_p + B_p$.

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

$$\text{AIDC} = \text{DERSM} \times \text{GERSM}$$

Statistical analyses

In experiment one, to assess the age effect of 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 and were considered significant at $p < 0.05$. For experiment two, data from the direct and difference methods were analyzed by an 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 of each experiment are presented in Table 1. Results of Dry Matter (DM), CP, CF and A, from experiment two were higher compared to those for experiment one, except for the GE values. EE and NFE could only be 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 the GE of RSM (Table 2). The values obtained for the AIDC of GE of the 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 AIDE of the GE of the maize diet had the highest values (3179.6 kcal/kg) and were similar to those from diet 2 (2871.3 kcal/kg). On the other hand, AIDE of the GE of diet 3 (2795.1 kcal/kg) had the lowest ($p = 0.020$) values, however they showed no differences ($p > 0.05$) to those from diet 2 (Table 2).

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
Ashes	2.72	4.15
Gross energy (Kcal/kg)	3955	3763
Kcal/kg, kilocalories per kilogram; ND, not determined		
Source: Animal Nutrition Laboratory (FMVZ-UADY)		

Table 2
AME, TME, DE and it's digestible coefficients of gross energy of RSM from experiment one and two.

Variable	Experiment one	Experiment two	
		RSM at 40% inclusion	RSM at 60% inclusion
No. of observations	8	6	5
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 digestible coefficient; TDC, true digestible coefficient; DE, digestible energy; AIDC, apparent ileal digestible coefficient; GE, gross energy; RSM, ramon seed meal; Kcal/kg, kilocalores per kilogram			

For the DE of each ingredient obtained by the difference method, maize had the highest values and were similar to RSM at 60% inclusion, while RSM at 40% inclusion had the lowest values ($p = 0.025$) and showed no differences ($p > 0.05$) between those from RSM at 60% inclusion. The AIDC of maize had higher values when compared to RSM at 40 and 60% inclusion, yet this difference was not significant ($p = 0.064$).

Discussion

The chemical composition of RSM from experiment one had lower results for DM, CP, CF and A, while the value of 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 et al. (2019) (11.5%, 3.04%, 0.6%, 81.2%, respectively), meanwhile DM and GE values were higher and only A values were lower for experiment one, compared to those reported by Subiria et al. (2019), (86.7%, 3955 Kcal/kg, 3.4% respectively).

In experiment two, the DM, CP, CF, A and GE values of RSM were higher when compared to those found by Subiria et al. (2019). Although Peters and Pardo-Tejada (1982) reported higher values for CP and GE of ramon seed than Subiria et al. (2019), (12.8% and 3610 Kcal/kg, respectively). These results are still below 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 A were lower (3.2 vs 4.25%, respectively).

Maize possesses similar amounts of starch (62.48%), amylose from starch (27.3%) and amylopectin from starch (72.6%), than RSM (61%, 25.3% and 74.6%, respectively) (Rostagno et al., 2005; Perez-Pacheco et al., 2014; Olguin-Maciel et al., 2017). As starch is the main source of energy in grains, we could assume their GE values would be very similar among them (Meng and Solminski, 2005). However, the difference between the GE in maize and RSM ranges from 500 to 700 Kcal/kg approximately, while for AME, TME and DE, the difference between them was even higher (from 1122.5 to 1624.1 Kcal/kg). This could be due to the differences in their chemical composition, as RSM has a lower content of EE (Ullah et al., 2016) and a higher content of CF and A. Since fat and carbohydrates 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).

Crude fiber has been shown to be negatively related to ME values of feedstuffs (Hill et al, 1960; Dale, 1996). Hill et al. (1960) mentioned that oat and barley hulls and wheat bran had very low ME values, which is consistent with Dale (1996) for wheat by-products, in which, as samples increase in CF content, their TME value decreases. This is due to the fiber content, mainly non-starch polysaccharides (NSP), which reduces the ME values of the feed, starch digestibility and nitrogen retention because the lack of endogenous enzymes in the bird for hydrolysis of these polysaccharides. Similarly, soluble fiber encapsulates nutrients within the cell walls of plant-based inputs, negatively affecting digestibility. This is due to the increased viscosity of the digesta, which impairs the breakdown by endogenous enzymes and nutrient transport, leading to reduce digestion and absorption of nutrients (Mtei et al., 2019; Szczurek et al., 2020; Kumar and Kyun, 2021; Tejada and Kim, 2021). As for ashes, as they have no nutritive benefit, their GE value, and therefore metabolizable and digestible energies as well, are significantly low (McDonald et al., 2010).

In addition, Perez-Pacheco et al. (2014) and Moo-Huchin et al. (2015) reported differences between the morphological and physicochemical properties of maize and Ramon seed (RS) starch, which affects their functionality. These include starch granule size (10 µm for RS starch and 15 µm for maize starch),

granule pH (9.1 for RS starch and 5.9 for maize starch) and amylose-to-amylopectin ratio (1:2.94 for RS starch and 1:2.65 for maize starch). These properties are related to their water absorption capacity, their swelling capacity, to reach viscosities at lower temperatures and to 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 the RSM and maize used in the current experiments, was given 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 modify them 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 RS may contain a higher proportion of slowly digestible or resistant starch than maize. This because, resistant starch acts as fiber by escaping enzymatic hydrolysis in the small intestine (Amoako and Awika, 2016).

Results of the age effect corresponding to experiment one are in agreement with those of Huang et al. (2005) and Yang et al. (2020) where they report that ME values and feed digestibility increases with age of broiler. 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 were found between the two ages of broilers, the results indicate that at the age of 3 weeks the birds have already reached their absorptive capacity and are able to digest and metabolize RSM adequately.

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

Declarations

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CONFLICT OF INTEREST

The authors declare that there are not conflict of interest.

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).

The manuscript does not contain clinical studies or patient data.

Competing interests

The authors have no relevant financial or non-financial interest to disclosure.

Author contributions

All authors contributed to the study conception and design. SMG and RUN performed material preparation, conducted experiments, data collection and analysis. The first drafts of the manuscript was written by SMG and RUN, and LSF and CSC contributed with previous versions of the manuscript and new analytical tools. All authors read and approved the final manuscript.

Data availability

The dataset generated during and analysed during the current study are available in the “Dato Digestibilidad Ramon” folder, in the following link: <https://1drv.ms/u/s!Atf-Ji3F2AXNmXPStHJda8eGCL1D?e=XY2Mbp>

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