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Apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen balance of amaranth grains for Japanese quails (*Coturnix coturnix japonica*)

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ABSTRACT

The objective was to determine the values of apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn) of roasted and in natura amaranth grains. A breeding stock of 108 female Japanese quails (*Coturnix coturnix japonica*) with an initial age of 29 days was used. The birds were distributed in a completely randomized design with three treatments and six replications, with six quails per experimental unit. The treatments used were reference diet, reference diet + 30% in natura amaranth and reference diet + 30% roasted amaranth. The variables evaluated were feed intake, body weight variation, retained nitrogen, retained crude protein, dry matter digestibility and crude protein. Higher values of AME and AMEn were verified for the roasted amaranth grains, in relation to the in natura grains. There was no verification regarding the influence of amaranth grains, both roasted and in natura, for the variables feed intake, body weight variation, retained nitrogen, retained crude protein, dry matter and crude protein digestibility. It was concluded that the in natura amaranth grains provided 3888 kcal/kg of AME and 3352 kcal/kg of AMEn, while the roasted amaranth grains provided 4044 kcal/kg of AME and 4028 kcal/kg of AMEn.

Keywords: Alternative food, Cereal, Gross energy, Laying bird

Among the alternative foods with potential use in animal nutrition, we can mention amaranth grains, which in recent years have shown growth in their use in human and animal nutrition due to their great nutritional potential. Amaranth stands out for its nutritional profile (Ferreira *et al*. 2007), with a high content of proteins of high biological value and relatively rich in amino acids such as tryptophan and lysine (Singh *et al.* 2022), with a lysine content considerably higher than that of most cereals (Oszvald *et al.* 2009). In addition, it has a high fibre content (Lamothe *et al.* 2015), high starch content (Kong *et al.* 2009), as well as other limiting amino acids for quail (Costa and Borges 2005) and bioactive compounds, such as flavonoids that show antioxidant activity in this cereal (Escudero *et al.* 2011).

Due to the nutritional quality of this food, amaranth has great potential to be incorporated into quail feed. However, the consumption of birds is regulated according to the energy density of the feed and the nutritional requirement of the same (Moura *et al.* 2010), therefore, it is essential to have a detailed knowledge of the energy contained in the food in order to provide an adequate balance of the diets according to the animal's requirements.

The studies on the use of amaranth in animal feed are scarce, and so little on the effect of thermal processing has been investigated. This justifies the fact that this food has been little explored and used in the feeding of laying birds, mainly because its use in diets has not yet been clearly established (Króliczewska *et al.* 2008).

The objective was to determine the values of apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen balance of roasted and in natura amaranth grains with Japanese quail.

MATERIALS AND METHODS

The experiment was carried out at the Experimental Farm, in the Coturniculture Sector of the Federal University of Mato Grosso - UFMT, located in the city of Santo Antônio do Leverger-MT in the Cuiabana lowland region, with the predominant semi-humid tropical climate. The project was approved by the Ethics Committee for the Use of Animals (CEUA/UFMT) under protocol number 23108.048925/2019-57.

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Diets, animals and experimental design: A total of 240 laying quails (*Coturnix japonica*) were used, weighing 169.6±0.005 g at 40 weeks of age with an initial egg production of 79.18±3.32%. A completely randomized design was used with five diets and six replications containing eight quails per experimental unit. The studied digestible arginine: lysine ratios were: 110%, 120%, 130%, 140% and 150% for 63 days divided into three periods of 21 days each.

The study lasted 10 days, with 5 days of diet adaptation and 5 days of total excreta collection according to the methodology described by Sakomura and Rostagno (2016). A total of 108 female Japanese quails (*Coturnix coturnix japonica*) with an initial age of 29 days were used in a completely randomized design with three treatments, six replications, with six quails per experimental unit.

The birds were housed in galvanized wire cages measuring 50 × 38 × 21 cm (length × width × height), with a density of 190 cm^2/bird. For the collection of excreta, under the cages, steel trays, properly plasticized with white plastic, were arranged individually per experimental plot. Each experimental diet consisted of natural matter, 70% of the reference diet and 30% of the tested food.

The treatments used were reference diet, reference diet + 30% in natura amaranth and reference diet + 30% roasted amaranth (Table 1). Ferric oxide (2%) was used as a marker of excreta, indicating the beginning and end of collections. Experimental feed and water were provided *ad lib*. The birds were exposed to 24 h of daily light, natural plus artificial ones, in order to stimulate the consumption of the diet.

For the toasting of amaranth grains (*Amaranthus cruentus L.* BRS-Alegria), an experimental prototype continuous roaster was used, with a temperature of 160°C and a residence time of 5 min. The reference diet (Table 1) was formulated based on corn and soybean bran, according to the recommendations of Rostagno *et al.* (2017) and Lima *et al.* (2018), and the nutritional composition of roasted and in natura amaranth grains according to Table 2.

Temperatures and relative humidity were monitored twice a day, in the morning and afternoon, using a digital thermo hygrometer, positioned in the center of the cages, at the height of the birds' back.

Samples collection: After the adaptation period, the excreta were collected twice a day, with an interval of 12 h (06:00 and 18:00 h), to avoid fermentation and material losses. During the collection phase, the excreta were placed in plastic bags and stored in a freezer (-18°C) until the end of the total collection period. Subsequently, the excreta were thawed, homogenized and weighed, a sample of 450 g of each repetition was weighed and destined for drying in a forced ventilation oven until constant weight at 60°C.

Feed consumption was evaluated according to the amount of feed consumed as a function of the number of quails in each treatment during the 5 days of collection. For body weight variation, all birds were weighed at the beginning and end of the experimental phase, determining the weight gain of the quails. The amount of excreta of the quails fed with the reference diet and with the experimental diets was registered for the accounting of the digestibility.

Table 1. Ingredient composition and calculated nutrient content of the basal experimental diet

Ingredient	(%)
Corn	52.92
Soybean meal (45%)	34.50
Limestone	6.8
Dicalcium phosphate	1.05
Salt	0.25
Vitamin and mineral supplement[1]	1.80
Soybean oil	2.28
L-Lysine HCl (78,84%)	0.10
DL-Methionine (99%)	0.30
Nutritional composition calculated	
Metabolizable energy (kcal/kg)	2800
Crude protein (%)	18.92
Calcium (%)	0.73
Available phosphorus (%)	0.31
Sodium (%)	0.15
Crude fibre (%)	2.85
Digestible amino acids (%)	
Digestible lysine	1.15
Digestible methionine + Cystine	0.94
Digestible tryptophan	0.24
Digestible threonine	0.70

[1]Vitamin-mineral supplement composition: Calcium (min) 80 g/kg, Calcium (max) 100 g/kg, Phosphorus (min) 37 g/kg, Sodium (min) 20 g/kg, Methionine (min) 21.5 g/kg, Lysine (min) 18 g/kg, Vitamin A (min) 125000 UI/kg, Vitamin D3 (min) 25000 UI/kg, Vitamin E (min) 312 UI/kg, Vitamin K3 (min) 20 mg/kg, Vitamin B1 (min) 20 mg/kg, Vitamin B2 (min) 62.5 mg/kg, Vitamin B6 (min) 37.5 mg/kg, Vitamin B12 (min) 200 mcg/kg, Folic acid (min) 6.25 mg/kg, Pantothenic acid (min) 125 mg/kg, Biotin (min) 1.25 mg/kg, Choline (min) 1700 mg/kg, Niacin (min) 312 mg/kg, Copper (min) 125 mg/kg, Iron (min) 680 mg/kg, Iodine (min) 8.75 mg/kg, Manganese (min) 937 mg/kg, Selenium (min) 3.75 mg/kg, Zinc (min) 500 mg/kg, Fluorine (max) 370 mg/kg.

Chemical analysis: The dry samples were weighed and ground in a knife mill using a 1mm sieve to perform the analysis of dry matter, total nitrogen and crude protein at the Laboratory of Animal Analysis and Nutrition (LANA) of the Federal University of Mato Grosso de according to the methodology proposed by Silva and Queiroz (2002). The gross energy values of diets and excreta were quantified using a PARR calorimetric bomb.

Once the results of the laboratory analysis of the ingredients, the reference feed and the excreta were obtained, the values of apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn) were calculated, using the equations proposed by Matterson *et al.* (1965).

Table 2. Chemical composition per 100 g of *Amaranthus cruentus* grain flour[1]

Nutrient	Amaranth flour	
	In nature	Toasted
Crude protein	14.53±0.03	14.63±0.06
Ether extract	6.46±0.1	7.71±0.05
Total carbohydrates	76.81±0.17	75.46±0.09
Ash	2.19±0.09	2.20±0.04
Total fibres	9.37±0.14	9.13±0.07
Insoluble fibres	8.10±0.13	7.41±0.04
Soluble fibres	1.27±0.04	1.72±0.04
Calcium	0.13±0.002	0.13±0.003
Magnesium	0.19±0.001	0.2±0.006
Copper	0.3±0.015	0.31±0.020
Zinc	2.89±0.105	2.92±0.035
Iron	7.51±0.279	7.75±0.170

[1]Values expressed in dry matter (Bianchini *et al.* 2014).

For the digestibility coefficients (DC) of dry matter and crude protein, the following formula was used:

$$\text{DC (\%)} = \frac{\left[\left(\frac{\text{dry matter}}{\text{CPingested}} - \frac{\text{dry matter}}{\text{CPexcreted}}\right)\right] \times 100}{\frac{\text{DM}}{\text{CPexcreted}}}$$

For the coefficients of nitrogen retained and retinal crude protein, it was calculated through the difference between nitrogen and protein ingested and the loss of nitrogen and crude protein in the excreta.

Statistics: The parameters were evaluated using analysis of variance software Sisvar version 5.6 (Ferreira 2019). The F test at 5% probability was used to compare the treatment with the inclusion of in natura and roasted amaranth.

RESULTS AND DISCUSSION

The maximum and minimum temperatures and relative humidity were checked daily, during the morning and afternoon, throughout the experimental period and are shown in Table 3.

Table 3. Maximum and minimum average temperatures and relative air humidity recorded inside the installation

Temperature and relative humidity	Morning	Afternoon
Maximum temperature (°C)	28.66	30.30
Minimum temperature (°C)	22.98	23.08
Maximum humidity (%)	64.80	67.80
Minimum humidity (%)	45.80	49.40

The thermal comfort range of adult quails is between 18 and 22°C and the relative humidity between 65 and 70% (Oliveira 2007). In view of this information, it is possible to observe that the values recorded during the experimental phase for the minimum and maximum temperature are above the temperature range of thermal neutrality of the quails, so it is possible to infer that, during the experimental period, the quails were in a situation of heat stress.

There was a significant difference for the values of apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn) of foods (P<0.05) (Table 4).

Table 4. Values of apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen balance (AMEn) of roasted and in natura amaranth grains

Variable[1]	Amaranth grains		CV (%)[2]	P value[3]
	In natura	Roasted		
AME (kcal/kg)	3888[a]	4044[b]	10.35	0.0144
AMEn (kcal/kg)	3352[a]	4028[b]	10.44	0.0125

[1]AME, apparent metabolizable energy; AMEn, apparent metabolizable energy corrected for nitrogen balance; [2]CV, coefficient of variation; [3]Values > 0.05 are considered non-significant. Means followed by a different letter on the line differ statistically from each other by the F test, at 5% probability.

Cereals commonly used as an energy source in poultry diets are corn, wheat, sorghum and barley. Knowledge of the particular metabolizable energy content of the source used is critical to its efficiency, proper utilization and accurate formulation (Khalil *et al.* 2021).

Several factors affect the metabolizable energy values of poultry feed, including the age of the birds, the test feed (Tancharoenrat *et al.* 2013), calcium and phosphorus levels, the level of inclusion of the test ingredient, the rate of consumption, the methodology used to determine ME and the anti-nutritional factors in foods.

The metabolizable energy values of the sources are found in the literature using different techniques, such as bioassays with live birds (Tyagi *et al.* 2008, Yegani *et al.* 2013), use of values provided in tables according to prediction equations (Janssen 1989, Sauvant *et al.* 2004, CVB 2009, Rostagno *et al.* 2017), *in vitro* analyses (Gehring *et al.* 2012, Zhao *et al.* 2014) and near-infrared reflectance spectrometry (NIRS) (Valdes and Leeson 1994, Garnsworthy *et al.* 2000, Black *et al.* 2009).

However, within the same method, the metabolizable energy of the ingredients may vary depending on the formulation of the basal diet (Wu *et al.* 2020). Available energy in poultry feed or feed ingredients can be measured by different systems, and despite several limitations (Mateos *et al.* 2019, Wu *et al.* 2020), apparent metabolizable energy is the most accepted system for measuring the energy available in feed for birds (Hill and Anderson 1958, Sibald 1982).

Another factor that can interfere in the coefficients of digestibility and nutritional quality are the existing grain processing methods. The roasting of grains is a thermal mechanism aimed at destroying undesirable factors (Emily *et al.* 2009), in addition to causing the cell wall to rupture, releasing the protein into the extracellular medium (Costa *et al.* 2013).

Thus, the use of grain processing, in addition to eliminating anti-nutritional factors, contributes to improving the efficiency and performance of animals due to increased digestibility and availability of some nutrients. In the present study, the roasted amaranth grains stood out, presenting higher values of AME and AMEn. This fact can be justified by the beneficial effects of the roasting process of amaranth grains.

In view of this information, it is worth mentioning the benefits of roasting amaranth grains, in which Bianchini (2011) when working with amaranth grains with different heat treatments, found that roasting the grains stood out as the best method of heat treatment of the grains, as it brought significant improvement in their nutritional quality.

The roasting of amaranth grains provides a significant improvement in their amino acid profile, increasing leucine by 4.8%, phenylalanine by 3.8%, isoleucine by 3.4%, valine by 2.2% and methionine by 7.3% (Bianchini 2011). It still provides an improvement in lipid levels, being 16.21% higher than in natura grains, not altering the total fibre values, however, it provides changes in the fibre, protein and carbohydrate fractions, and the heat treatments caused a reduction in the content of insoluble fibres and the increase of soluble fibres in relation to in natura amaranth (Bianchini 2011).

Also, according to Bianchini (2011), the roasting of amaranth grain does not significantly affect the content of ash and the minerals magnesium, zinc, iron and phosphorus, except for calcium, since roasted amaranth grains have a 3% higher calcium content than in natura amaranth grains. However, in absolute values, according to the results of the authors, phosphorus has a content of 4.80% higher than in natura amaranth grains. It is important to note that this research used the same principles of toasting and conservation of amaranth grains used by Bianchini (2011).

According to the results obtained in this research, variations were observed in the energetic values of the amaranth grains in relation to those found in the consulted literature. This can be caused by several reasons, among them are the level of feed intake that can directly affect the metabolizable energy content of the feed, as well as the nutrient content, type of processing, age of the birds and levels of inclusion of test food in the diet (Borges *et al.* 2004).

Close values for the AME of in natura amaranth were observed by Ravindran *et al.* (1996) in broilers with 35 days of life, with a trial of total excreta collection, the authors found AME values of 3830 kcal/kg and different values found in this research for amaranth with heat treatment, being 3131 kcal/kg ME.

Tillman and Waldroup (1988) when carrying out a research with broilers in the growth phase, evaluated the effects of the incorporation of in natura amaranth grains in the diet of the birds, using extruded feed to determine the energetic values of AME and AMEn having found the values of 3646 kcal/kg and 3522 kcal/kg, respectively.

The AME values analyzed were higher than the AMEn values, which can be explained by the positive balance of nitrogen retained by the Japanese quails in the assay. This fact was also observed by Tillman and Waldroup (1988) and Ravindran *et al.* (1996).

Some authors report the presence of anti-nutritional factors in in natura amaranth grains, however, there are still few studies with amaranth that confirm these anti-nutritional factors. The literature is outdated in the use of amaranth grains in animal nutrition.

As shown in Table 5, there were no differences for the parameters of nitrogen and retained crude protein, dry matter and crude protein digestibility, feed intake and body weight variation (P>0.05), with a positive nitrogen balance being observed for quails fed with in natura and roasted amaranth.

Bianchini (2011) when carrying out research with amaranth grains, observed trypsin inhibitory activities in in natura grains, noting that roasting the grains provided an 84% reduction in the activity of trypsin inhibitors. However, in view of the results found for the digestibility coefficients of crude protein, the activity of trypsin inhibitors in in natura amaranth grains did not influence the digestibility coefficients of quails fed with the grains. However, in absolute values, it is possible to observe a small improvement in crude protein digestibility for birds that received the diet containing roasted amaranth.

However, in absolute values, the animals that received the diet with in natura amaranth presented values of crude protein retained 5% lower than the animals that received the diets with roasted amaranth, which proves the trypsin inhibitory activities of the in natura amaranth grains, which even though it is not able to significantly alter the crude protein digestibility and retention coefficients for Japanese quails fed with these grains, it is present. It is known that the trypsin inhibitor binds to the trypsin enzyme responsible for the digestion of proteins, presenting specificity of inhibiting the proteolytic enzymes and consequently reducing the protein digestion of food and for this reason, reductions in the weight gain and growth of the animals

Table 5. Nitrogen and retained crude protein, dry matter and crude protein digestibility, and performance of laying Japanese quails fed with in natura and roasted amaranth

Variable	Amaranth grains		CV (%)[1]	P value[2]
	In Natura	Tostado		
Retained nitrogen (g/ave/dia)	0.068	0.064	12.38	0.446
Retained crude protein (g/ave/dia)	0.402	0.420	11.92	0.495
Dry matter digestibility (%)	68.31	67.42	14.15	0.620
Crude protein digestibility (%)	16.28	16.85	3.5	0.595
Feed intake (g/ave/dia)	16.73	14.85	10.38	0.063
Body weight variation (g)	0.015	0.014	8.69	0.486

[1]CV, coefficient of variation; [2]Values > 0.05 are considered non-significant from each other by the F test, at 5% probability.

and the increase in the weight of the pancreas of birds are observed (Miura 2005).

According to the results found in the present work, it can be affirmed that the roasted amaranth provided higher values of AME and AMEn, without altering the digestibility coefficients of dry matter and crude protein. In natura amaranth and roasted amaranth also provided a positive nitrogen balance in the diets, without altering the retinal crude protein by the birds.

In conclusion, the in natura amaranth grains provided 3888 kcal/kg of apparent metabolizable energy and 3352 kcal/kg of apparent metabolizable energy corrected for nitrogen balance, while the roasted amaranth grains provided 4044 kcal/kg of of apparent metabolizable energy and 4028 kcal/kg of apparent metabolizable energy corrected for nitrogen balance.

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