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Energy values and metabolizability coefficients of maize kernels with different specific gravities

Valores energéticos e coeficientes de metabolizabilidade de grãos de milho com diferentes densidades específicas

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Abstract
The aim of this study was to examine the influence of the specific gravity of maize kernels on physicochemical traits, energy values, and metabolizability coefficients. Pearson's correlations were evaluated between specific gravity (kg/m³) and crude protein (%); ether extract (%); crude fiber (%); gross energy (%); presence of fumonisins (ppb) and aflatoxins (ppb); and kernel quality (good, rotten, weevil-damaged, broken, and shriveled kernels, %). A metabolism trial was conducted with diets containing maize fractions of different specific gravities for male broilers from 14 to 21 days of age. Apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), and the metabolizability coefficients of dry matter (MC_{DM}), crude protein (MC_{CP}), ether extract (MC_{EE}), calcium (MC_{Ca}), and phosphorus (MC_{P}) were evaluated. The experiment consisted of five treatments (reference diet and diets with 40% replaced with maize at five specific gravities (740, 740, 760, or 800 kg/m³). Eight replications were used, totaling 400 broilers chickens. The Scott-Knott test was applied and regression equations were fitted to compare the treatments. Specific gravity had moderate correlations with good and broken kernels and low-magnitude correlations with chemical parameters. Increasing specific gravities caused AME and AMEn to increase linearly when analyzed on an as-is basis; and to respond quadratically when expressed on a dry-matter basis. The specific gravity of 780 kg/m³ provided the lowest MC_{DM}, MC_{CP}, MC_{Ca}, and MC_{P} values, whereas the lowest MC_{EE}, was found at the lowest density. It was not possible to determine the best nutritional composition or the best metabolizability coefficients.
Keywords: apparent metabolizable energy; chemical analysis; correlation; regression.

Resumo
Objetivou-se avaliar a influência da densidade do grão de milho sobre características físico-químicas, valores energéticos e de metabolizabilidade aparente. Avaliou-se a correlação entre a densidade específica (kg/m³) e os parâmetros: proteína bruta (%); extrato etéreo (%); fibra bruta (%); energia bruta (%); presença de fumonisinas (ppb) e aflatoxinas (ppb); umidade (%); grãos bons, quebrados, chochos, carunchados e ardidos, em Percentagem. Um ensaio de metabolismo com dietas contendo diferentes densidades específicas do grão de milho, para frango de corte, no período de 14 a 21 dias de idade, foi conduzido para avaliar a energia metabolizável aparente (EMA) e a corrigida pelo balanço de nitrogênio (EMAn), os coeficientes de metabolizabilidade: matéria seca (CM_MS), proteína bruta (CM_PB), extrato etéreo (CM_EE), cálcio (CM_Ca) e fósforo (CM_P). O experimento foi composto por cinco tratamentos (dieta referência e dietas substituindo 40% desta com milhos de densidades específicas: 740 kg/m³, 760 kg/m³, 780 kg/m³ e 800 kg/m³), com oito repetições, totalizando 400 frangos machos. As médias foram comparadas pelo teste de Scott-knott e estimada as equações de regressão nas diferentes densidades específicas das frações de milho. As correlações entre a densidade específica e grãos bons e quebrados foram de moderada magnitude e com os parâmetros químicos foram de baixa magnitude. Com o aumento da densidade específica para EMA e EMAn na matéria natural obteve-se resposta linear decrescente, enquanto na matéria seca, foi quadrático. Para CMMS, CMPB, CMCA e CMP a densidade de 780 kg/m³ apresentou os menores valores, enquanto para CM_EE, o menor valor foi para a menor densidade específica. Não foi possível determinar a fração de milho com melhor composição nutricional e melhores coeficientes de metabolizabilidade.
Palavras-chave: análise bromatológica; correlação; energia metabolizável aparente; regressão

1. Introduction

Maize is the most produced cereal in the world and has great potential for use in animal feeds in most countries, constituting 50 to 80% of their composition. In nutritional terms, maize provides on average 65% of the metabolizable energy and 20% of the protein content of broiler diets (1). Given the importance of this grain to the various existing animal production systems, industries

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must define its quality according to the purpose of its use [2]. Factors affecting the quality of maize can vary widely according to the cultivar as well as with climatic conditions, soil fertility, and the processes involved from planting to harvest, transport, and storage [2,3]. The quality of the maize kernel can be assessed considering its physical, chemical, and nutritional characteristics. When it comes to production, kernel quality is related to its integrity aspects, such as the presence of cracks, holes, impurities, and mycotoxins, in addition to moisture content, fungal rot, and specific gravity [2]. These variables may imply changes in nutritional and energy characteristics, which have an impact on the formulation of the animal diet and, consequently, on production costs, such as broiler feed expenses [4].

The nutritional and energy values of feedstuffs can be measured by direct and indirect methods. In the direct approaches, laboratory analyses are conducted to evaluate the chemical composition in terms of protein, ether extract, and crude fiber contents as well as the presence of contaminants such as mycotoxins, which can be determined traditionally by the Weende method (or by near-infrared spectrometry - NIRS). Another option is *in vivo* evaluation with animal metabolism trials, which can measure the digestibility of feedstuffs. These methods are accurate, but incur time and costs for analysis [5,6]. Indirect methods, on the other hand, use the values available in feed composition tables, e.g. the Brazilian Tables for Poultry and Swine [7], helping to increase feed formulation efficiency. However, despite the updates, the chemical and energy composition of an ingredient is known to vary according to several factors related to its cultivation and especially with the selection and development of new varieties, in the case of maize. Another highlighted indirect method are prediction equations for the nutritional and energy values of ingredients [7,8], whose estimates help to increase the accuracy of feed formulations. These equations mainly consider physical and chemical quality characteristics of the kernels, constituting an important practical tool for correcting the nutrient matrix of ingredients used locally in feed factories [9].

In the routine of feed factories, loads of maize are received and classified primarily by their visual aspect, aiming to assess the physical integrity of the kernel and its density, which can be measured by apparent specific weight or by hectoliter weight [10]. Various factors can influence density, such as time of planting, incidence of sunlight or excessive shading, temperature, planting density, harvest time, transport, drying, and storage [11,12]. Several authors have attempted to better understand the influence of kernel density on its energy and nutritional content [8,10,13,14]. Variations in the nutritional composition of kernels can impact the production potential of chickens, e.g. by altering their feed conversion ratio [15]. Classification methodologies are based on physical aspects of maize, and do not relate the chemical composition and digestibility of this ingredient [1]. Therefore, by evaluating the correlation between the chemical and physical variables of maize, it is possible to determine the nutritional impact of these variables, making the work of nutritionists and feed factory managers more efficient.

In view of the foregoing, the present study proposes to correlate the physical and chemical variables of maize to examine the influence of the density of different maize fractions, in broiler diets, on apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) values and the metabolizability coefficients of dry matter (MC_{DM}), crude protein (MC_{CP}), ether extract (MC_{EE}), calcium (MC_{Ca}), and phosphorus (MC_{P}).

2. Material and methods

The metabolism trial was conducted in the experimental poultry facilities at the School of Veterinary and Animal Science, Federal University of Goiás (UFG), Samambaia campus. The research project was approved by the Ethics Committee on the Use of Animals (CEUA) of UFG (approval no. 019/16). Maize samples were harvested over three years, at the time of harvest and during the off-season, at a commercial company located in the southeast of the state of Goiás, Brazil. The samples were obtained from routine collections made with each bulk load received, in accordance with Normative Instruction no. 60, of December 22, 2011 [16].

First, these samples were manually classified into four fraction types based on the specific gravity (weight/volume ratio) of the maize kernels (740, 760, 780, and 800 kg/m³), characterizing the treatments. In total, 1,049 samples were collected during the evaluation period. Chemical analyses were carried out to determine the ether extract (EE), crude protein (CP), crude fiber (CF) contents and the presence of mycotoxins such as fumonisins and aflatoxins (in ppb). The following physical parameters were also analyzed: gravity (kg/m³); kernel moisture; and percentage of broken, weevil-damaged, rotten, shriveled, and good kernels.

After chemical and physical analyses, the samples were classified and separated, into four fractions according to specific gravity, namely 740, 760, 780, or and 800 kg/m³. Then, they were stored to be later used in the production of test diets for the metabolism trial, which involved additional chemical and physical analyses of the kernels. The gross energy of the maize fractions used in the test diets was analyzed using a standard bomb calorimeter from the Animal Nutrition Laboratory (Department of Animal Science, School of Veterinary and Animal Science, UFG).

The consistency of the obtained data was investigated after removing values lower and higher than three standard deviations from the mean. Next, Pearson's correlation analyses were performed between the physical and chemical variables of the maize kernels to check the degree of relationship between these variables. The correlation coefficients were classified into: perfect (r=1), high (r>0.75 and <0.99), moderate (r>0.5 and <0.74), low (r<0.5), and non-existent (r=0). Significance (p value) was considered high when p<0.01, moderate at p<0.05, and inexistent (not significant) for correlations with p>0.05.

The metabolism trial consisted of five treatments and eight replicates, involving 400 14-day-old male broiler chickens of the Cobb 500® strain with an average expected initial live weight of 459 g. A period of three days was used for the birds to acclimatize to the diets, followed by four days of total excreta collection, totaling seven days of experiment. The treatments were formed by replacing 40% of the reference diet (Table 1) with the specified maize fractions, according to standard methodology [17], as follows:

T1 = Reference diet;

T2 = 60% reference diet + 40% maize density 740 kg/m³;

T3 = 60% reference diet + 40% maize density 760 kg/m³;

T4 = 60% reference diet + 40% maize density 780 kg/m³; and

T5 = 60% reference diet + 40% maize density 800 kg/m³.

The metabolism trial was carried out in batteries located in the experimental poultry house of the Department of Animal Science, School of Veterinary and Animal Science, UFG. First, the day-old chicks (average weight of 40 g) were housed in a battery where they received a standard commercial feed for broilers. At 14 days (beginning of the metabolism trial), the birds were allocated to battery cages with four floors and five cages per row, in a completely randomized design. Eight animals were used per experimental unit (cage).

Table 1. Reference diet used in the metabolism trial

Feed composition	Inclusion level	Calculated nutritional composition		Nutritional level
Ingredient	kg/t	Apparent metabolizable energy	kcal	2900
Ground maize	632.04	Crude protein	%	19
Soybean meal 45%	303.000	Digestible arginine	%	1.157
Soybean oil	9.000	Digestible lysine	%	1.000
Dicalcium phosphate	20.000	Digestible methionine	%	0.433
Fine limestone 39%	11.000	Digestible methionine + cystine	%	0.660
Salt	2.900	Digestible tryptophan	%	0.200
Microencapsulated organic acids and essential oils[1]	2.000	Digestible threonine	%	0.632
Soybean meal 45%	7.826	Digestible leucine	%	1.345
Sodium bicarbonate	2.470	Digestible valine	%	0.753
Methionine powder 99%	2.100	Digestible histidine	%	1.103
L-lysine 78%	1.160	Crude fat	%	4.443
L-threonine 98%	0.045			
Choline chloride 60%	1.370	Crude fiber	%	3.100
Prebiotic [2]	0.400	Calcium	%	1.000
Probiotic	0.200	Available phosphorus	%	0.480
Tylosin 25%	0.220			
BHT	0.100			
Adsorbent [3]	1.500			
Mineral premix[4]	1.200			
Vitamin premix[5]	1.000			
Total (kg)	1000.000			

[1]Gallinat+; [2]Actigen; [3]Esterified glucomannan; [4] Mineral premix per kilogram of feed: manganese 90 mg; zinc 75 mg; iron 60 mg; copper 9.75 mg; iodine 1.20 mg. [5]Vitamin Premix per kg of feed: selenium 0.30 mg; vitamin A 10,000 IU; vitamin D3 2,500 IU; vitamin E 25 mg; vitamin K3 2 mg; vitamin B1 2.50 mg; vitamin B2 6.50 mg; vitamin B6 3.50 mg; vitamin B12 18 mcg; folic acid 1.20 mg; pantothenic acid 15 mg; niacin 42 mg; biotin 80 mcg, ethoxyquin 166 mg.

The apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) values were determined by the total excreta collection method [6]. The metabolizable energy values of the ingredients were obtained using the standard formulae and equations [17], adjusted based on nitrogen retention. Excreta were collected twice a day, at 08h00 and 16h00, then weighed and stored in plastic packages under refrigeration (-5 ºC) throughout the experimental period. The diets provided to the birds, as well as their respective leftovers, were weighed and recorded per cage at the beginning and end of the experimental period.

At the end of the collections, the samples were thawed, weighed, and homogenized. Aliquots were taken from each experimental unit and pre-dried in a forced-air oven at 55 ºC for 72 h. After drying, the individual samples were weighed and ground in a hammer mill with 1-mm sieves to determine the DM content at 55 ºC, and three aliquots were separated per experimental unit.

Analyses of gross energy (GE) in the pre-dried excreta and feed samples were performed using a Parr adiabatic bomb calorimeter at the Animal Nutrition Laboratory (Department of Animal Science, School of Veterinary and Animal Science, UFG). Excreta and feed samples were analyzed for levels of N, EE, CF, Ca, P, and DM at 105 ºC, following the method by Silva and Queiroz [18], at the Specialized Laboratory of Animal Nutrition of the Nutron Alimentos company (LABTRON) located in Campinas, SP.

The laboratory results were then used to calculate the apparent metabolizable energy on the fresh ("as is") and dry matter bases (AME_{FM} and AME_{DM}, respectively) as well as the nitrogen-corrected apparent metabolizable energy (fresh and dry matter bases, $AMEn_{FM}$ and $AMEn_{DM}$), by applying the equations below [17]:

AME of TD or RD (kcal/kg DM) = $(GE_{int} - GE_{out}) / DM_{int}$ (Eq. 1),

AME of test ingredient (M) (kcal/kg DM) = AME RD + (AME TD – AME RD) / (% inclusion M/100) (Eq. 2),

AMEn of TD or RD (kcal/kg DM) = $(GE_{int} - GE_{out}) \pm 8.22*NB / DM_{int}$ (Eq. 3),

AMEn (M) (kcal/kg DM) = AMEn RD + (AMEn TD – AMEn RD) / (% of inclusion of M/100) (Eq. 4)

Where GE_{int} = gross energy intake; GE_{out} = gross energy output; DM_{int} = dry matter intake; NB = nitrogen balance (N intake – N output); M = maize; TD = test diet; and RD = reference diet.

The apparent metabolizability coefficients of dry matter (MC_{DM}), crude protein (MC_{CP}), ether extract (MC_{EE}), calcium (MC_{Ca}), and phosphorus (MC_{P}) were obtained by the following formula:

MC_{DM} (%) = (Intake (DM) – Output (DM)) / (Intake (DM)).

The formula was used to obtain the other metabolizability coefficient values, only replacing the DM with variables such as crude protein, ether extract, calcium, and phosphorus. Pearson's correlation analyses were performed between the physical and chemical parameters of maize kernel quality. To compare the treatments based on the specific gravity, analysis of variance was performed. Means were compared by the Scott-Knott test and the regression values of the different maize gravity categories were estimated. In the case of the significant regression, plots were constructed for the AME and AMEn variables (expressed on both the DM and FM bases), and MC_{DM}, MC_{CP}, MC_{EE}, MC_{Ca}, and MC_{P}. All statistical analyses were performed using R statistical software [19].

3. Results and discussion

Based on the physical and chemical results of the kernels (Table 2), the collected samples are within the quality standard established by Normative Instruction no. 61[16] for both moisture (below 14%) and kernel integrity, characterizing the maize in general terms as Type-1.

Table 2. Mean, minimum, maximum, and standard deviation values of the physicochemical variables of the maize kernel samples used for correlation analysis

	N samples	Mean	Maximum	Minimum	SD	CV (%)
Moisture	854	12.55	14.5	9.40	1.16	9.24
Good kernels (%)	664	96.44	98.76	93.34	1.06	1.09
Rotten kernels (%)	810	1.34	2.63	0.24	0.56	4.15
Damaged kernels (%)	670	0.45	1.21	0.10	0.3	68.67
Shriveled kernels (%)	563	0.43	0.68	0.06	0.12	54.07
Broken kernels (%)	805	1.63	4.14	0.32	0.69	42.15
Gravity (kg/m³)	790	738.80	794.20	700.20	26.59	3.37
Ether extract	839	3.79	4.4	3.34	0.25	6.78
Crude protein	664	7.41	8.14	6.94	0.34	4.7
Crude fiber	823	1.73	2.09	1.40	0.13	7.85
Aflatoxins (ppb)	103	2.49	4.00	2.00	0.74	29.69
Fumonisins (ppb)	320	1845	5800	0.45	2040	110.5

SD = standard deviation; and CV % = coefficient of variation.

Correlation analysis (Table 3) revealed that the correlations between the physical and chemical traits were overall of low magnitude (0.19 to 0.4) or not significant. Therefore, it is of paramount importance to evaluate both the physical and chemical quality of maize kernels at each load received.

The highest correlation found was between weevil-damaged kernels and the presence of fumonisin, with a positive coefficient of 0.81 ($P<0.01$), indicating deterioration in quality with increasing presence of weevils in the maize. Mallmann et al. [20] examined the quality of maize hybrids for chickens and found a correlation between damaged kernels and low-magnitude mycotoxins. Besides their toxicity to birds, which can result in damage to the liver and locomotor system [21], the importance of evaluating mycotoxins lies in that it will guide the formulation of the feed according to the intensity of the presence of aflatoxin and fumonisin, requiring the inclusion of adsorbent additives, which affects the feed cost [20].

Table 3. Pearson's correlation between physical and chemical variables of maize kernels

	MOIST	CF	CP	GOOD	DAM	ROT	SHR	BRK	GRV	AFL	FUM
EE	0^{ns}	-0.2**	0.36**	-0.11**	-0.12**	0.33 ^{ns}	0^{ns}	0.19**	-0.15**	0.11^{ns}	0.36**
MOIST		0.05 ^{ns}	-0.21**	-0.22**	0.02 ^{ns}	0.16 ^{ns}	0.03 ^{ns}	0.32**	-0.76**	-0.3**	-0.40**
CF			-0.13**	0.05 ^{ns}	0 ^{ns}	-0.41*	-0.03^{ns}	-0.04 ^{ns}	0.01 ^{ns}	-0.26**	-0.27*
CP				0.07 ^{ns}	-0.23**	0.20 ^{ns}	0.02 ^{ns}	0.04 ^{ns}	0.08 ^{ns}	0.07 ^{ns}	0.63*
GOOD					-0.40**	-0.13 ^{ns}	-0.38**	-0.70**	0.33**	-0.06^{ns}	0.01 ^{ns}
DAM						-0.30 ^{ns}	0.29**	0^{ns}	-0.05 ^{ns}	0.01^{ns}	0.01 ^{ns}
ROT							0.33^{ns}	0.13 ^{ns}	-0.09^{ns}	-0.16^{ns}	0.81**
SHR								0.20**	-0.07^{ns}	0.13^{ns}	0.06 ^{ns}
BRK									-0.41**	0.02^{ns}	-0.05^{ns}
GRV										0.28**	0.43**
AFL											0.26**

**$P<0.01$, *$P<0.05$, ns: not significant; MOIST: moisture; CF: crude fiber; CP: crude protein; GOOD: good kernels; DAM: damaged kernels; ROT: rotten kernels; SHR: shriveled kernels; BRK: broken kernels; GRV: specific gravity; AFL: aflatoxin; FUM: fumonisin. All variables are expressed in %, except GRV (kg/cm³), AFLA (ppb) and FUM (ppb).

The presence of fumonisins can moderately affect the CP content ($r= 0.63$; $p<00.1$) and specific gravity ($r=0.43$; $p<00.1$) of the kernel, as a result of weight loss and a reduction in weight/volume ratio, consequently increasing its protein content [22]. The presence of aflatoxins (ppb) showed low correlations ($p< 0.01$) with density (0.28) and moisture (-0.30). In the study by 036 the authors also found that lower-density whole or broken kernels had an impact on the nutritional and energy value of the ingredient. The authors stressed that 74% of the samples were positive for fumonisins, which shows the potential damage that can be associated with low-quality kernels.

Density and moisture exhibited a high-magnitude antagonistic correlation ($r= -0.76$, $p<0.01$). Stringhini et al. [19] and Lopes et al. [20] found that storing loads for more than 60 days, combined with high temperatures and relative humidity, may lead to fungal proliferation, consumption of nutrients in the kernel mass, and a consequent reduction in its density.

In the analysis of the correlation between density and physical characteristics, low coefficients were also found with good and broken kernels (0.33 and -0.41, respectively) ($p<0.01$). In general, kernels with a higher specific gravity have higher quality [10]. The correlation between density and EE was low and negative (-0.15), similar to the findings of Silva et al. [14], who described that density did not imply important variations in the EE content of the kernels. In another evaluation, a positive correlation was observed with the variable presence of aflatoxin (0.28; $P<0.01$), indicating a low influence of density on these variables. Hess et al. [23] described similar results, stating that they did not find the presence of this toxin at important levels that could indicate deleterious effects on health or nutritional effects for chickens. The other variables showed no significant correlations with kernel density ($P<0.05$). According to Rodrigues et al. [4], the variation in density alone does not necessarily result in changes in the chemical composition of the kernels, a fact that was also elucidated in the present study. Nonetheless, as reported by Silva et al. [14], density can result in important variations in the energy content of maize kernels.

Although the correlations between specific gravity and chemical parameters were shown to have little or no significance (Table 3), different authors have reported differences in the chemical composition of maize kernels with different densities [8,13,14]. Thus, to evaluate the impact of maize density and kernel quality on nutritional and energy values, we conducted a metabolism trial. Before producing the diet, we divided the kernels into four fractions according to their density (740, 760, 780, and 800 kg/m³) and evaluated the chemical, energy, and physical composition and mycotoxin content, as shown in Table 4.

Table 4. Chemical analysis (wet method) of the maize kernels used in the metabolism trial treatments

Variable	Reference diet	Maize specific gravity (kg/m³)			
		740	760	780	800
Gross energy (kcal/g)	3866	3893	3912	3895	3982
Ether extract (%)	4.4	3.51	4.1	3.96	3.68
Dry matter (%)	89.09	90.00	89.00	89.40	88.05
Crude fiber (%)	1.4	1.89	1.54	1.48	1.62
Crude protein (%)	7.97	7.58	7.85	7.74	7.64
Good kernels (%)	96.36	95.54	97.04	95.37	96.02
Rotten kernels (%)	2.42	2.48	1.45	1.91	2.87
Damaged kernels (%)	0	0.83	0.42	0	0
Shriveled kernels (%)	0.65	0.42	0.29	0.25	0.12
Impurities (%)	0.02	0.06	0.03	0.17	0.12
Broken kernels (%)	0.47	0.42	0.51	2.26	0.84
Density (kg/m³)	761.6	741.6	763.9	781.9	791.1
Aflatoxins (ppb)	2	2	2	2	0
Fumonisins (ppb)	4600	2800	4400	5300	4600

According to exploratory analysis of the quality of the maize kernels used in the diets prepared for the experiment, the density fractions were close to expected; in the reference diet, this value was 761 kg/m³. Chemical composition and physical quality did not change linearly between the fractions used, except for DM, which decreased linearly with increasing density, and GE, which increased with density (Table 4). This linear and directly proportional relationship between the two variables was expected, since the DM content decreases as density increases, in contrast to the GE level [(4,24)].

The CP levels of the different maize fractions were lower than those recommended by Rostagno et al. [(7)], with the highest value detected in the fraction of greatest density (Table 4). The CP level can be influenced by regional factors, especially by the type of nitrogen fertilization applied during the cultivation of the kernels. There was no linearity in the relationship between CP and the different fractions, contrary to the linear and inversely proportional response reported in the literature [(10,13)]. This finding was expected due to the decreasing concentration of starch in the kernels with lower specific weight, which resulted in a higher concentration of other nutrients[14].

Regarding the CF and EE values, the small variations did not reflect a possible relationship with the densities of the selected kernels. As regards the AME_{DM} values, there was a significant difference, but they did not follow the expected trend of linear increase according to the specific gravities (Table 5). However, AME_{DM} showed a quadratic behavior, with the lowest estimated level found at the specific gravity of 769 kg/m³ (Table 4, Figure 1). Studies found in the literature describe an increase in AME values directly proportional to the specific gravity of kernels [(10,13,14)]. The highest GE value, which was found in the kernels with the highest specific gravity (Table 5), did not convert into higher AME values when the specific gravity of the maize fractions increased.

Nonetheless, Baidoo et al. [(24)] reported that large reductions in kernel specific gravity (-20%) could result in small reductions in energy values (-4.3%). In the present study, a small variation (-8.1%) was found in the specific gravity of the kernels, which might not have been sufficient to reproduce other results found in the literature. This small reduction in energy values directly influenced the AME results expressed on a fresh-matter basis, when the DM content was adjusted for the different maize fractions. The fraction of greatest density was expected to show the highest AME_{FM} values, but its value was lower than that obtained by the lower-density treatments.

The obtained AME_{FM} values did not demonstrate the proportionality found in other studies [(10,13,14)], varying significantly between the increasing densities in an inversely proportional behavior (Figure 1). This fact can be explained by the variation in the DM content of the different maize fractions (Table 3), which decreased as specific gravity increased.

All treatments showed higher AME than AMEn values, on both the fresh and dry matter bases (Table 5). When the nitrogen balance is positive, AME is higher than AMEn, indicating nitrogen retention. In a case of negative nitrogen balance, AME is lower than AMEn, indicating muscle tissue degradation. Growing birds have greater nitrogen retention due to the deposition of protein tissue [(25)]. It is always important to correct the nitrogen balance to determine variations that can occur between the AME values of the feed. These values can change depending on the species, line, and age, since birds with different degrees of nitrogen retention excrete different amounts of energy consuming the same feed.

The $AMEn_{DM}$ values varied significantly and responded in a positive quadratic manner to the increase in kernel specific gravity (Figure 1), reaching the lowest level at the density of 769 kg/m³. Vieira et al. [(26)] evaluated 45 varieties of maize hybrids and obtained $AMEn_{DM}$ values of 3563 to 4013 kcal/kg, which are higher than the results found in the present study (Table 5).

Regarding $AMEn_{FM}$, the same behavior displayed by AME_{FM}, i.e. significant linear inversion in proportionality (P<0.05; Table 4, Figure 2), occurred due to the decreasing DM content of the kernels as their specific gravity increased. The $AMEn_{FM}$ values found in the present study were lower than those cited in the literature [4,8,14] (3247 to 3562 kcal/kg), but higher than the 2937 kcal/kg found by Silva et al. [14] in maize fractions of low specific gravity.

As for the metabolizability coefficients (Table 5), the specific gravity of the kernels influenced MC_{DM}, whose highest value was obtained by the treatment with the lowest density, in a decreasing linear relationship (Figure 2). Rodrigues et al. [4] found MC_{DM} values from 80.37 to 84.39% across different maize kernel fractions and a moderate correlation between this coefficient and density (0.55).

The MC_{CP} values found did not differ statistically between the maize specific gravity fractions (P>0.05) (Table 5). The MC_{CP} values and CP levels (Table 3) were lower than those recommended in the Brazilian food composition table[7] (87% MC_{CP}), but higher than the mean value of 57.3% found by Rodrigues et al. [8] in different maize fractions. These results suggest that, in the present study, the higher CP level found at the lowest specific gravity fraction, resulting from the lower starch concentration in these kernels, did not influence the levels of digestible amino acids or low-nutritional-value zein proteins [10].

Table 5. Apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn) on the dry (DM) and fresh (FM) matter bases (kcal/kg) and apparent metabolizability coefficients (%) of the tested treatments, considering different specific gravities (kg/m³)

	Maize specific gravity (kg/m³)				CV (%)	Regression equation	R^2 adjusted	Max/Min (Y)	Critical value (X)
	740	760	780	800					
AME_{DM}	3694.11^a	3694.74^b	3694.11^b	3698.22^a	0.06	$Y = 6289.181 - 6.74635x + 0.004384x^2$	0.36	3693.98	769.36
$AMEn_{DM}$	3506.49^a	3503.45^b	3502.55^b	3507.29^a	0.06	$Y = 6383.393 - 7.49033x + 0.004869x^2$	0.47	3502.51	769.22
AME_{FM}	3327.88^a	3288.31^c	3302.53^b	3256.28^d	0.06	$Y = 4065.916 - 1.002806x$	0.73		
$AMEn_{FM}$	3155.84^a	3118.07^c	3131.28^b	3088.17^d	0.06	$Y = 3854.075 - 0.949x$	0.75		
MC_{DM}	74.36^a	74.30^b	74.26^b	74.29^b	0.08	$Y = 75.27513 - 0.001256x$	0.13		
MC_{CP}	65.15	65.11	65.13	65.05	0.18	$Y = 66.24825 - 0.001475x$	0.04*		
MC_{EE}	79.75^b	79.87^a	79.95^a	79.87^a	0.12	$Y = 1.441 + 0.201794x - 0.00013x^2$	0.31	79.93	778.00
MC_{Ca}	42.72^a	42.61^a	41.96^b	42.57^a	0.37	$Y = 315.117 - 0.703288x + 0.000453x^2$	0.39	42.22	776.04
MC_P	33.71^a	33.60^a	32.90^b	33.55^a	0.58	$Y = 321.2755 - 0.742275x + 0.000478x^2$	0.36	33.18	776.23

Values followed by common letters in the same row do not differ (p>0.05) by the Scott-Knott test. * Non-significant equation, P>0.05.

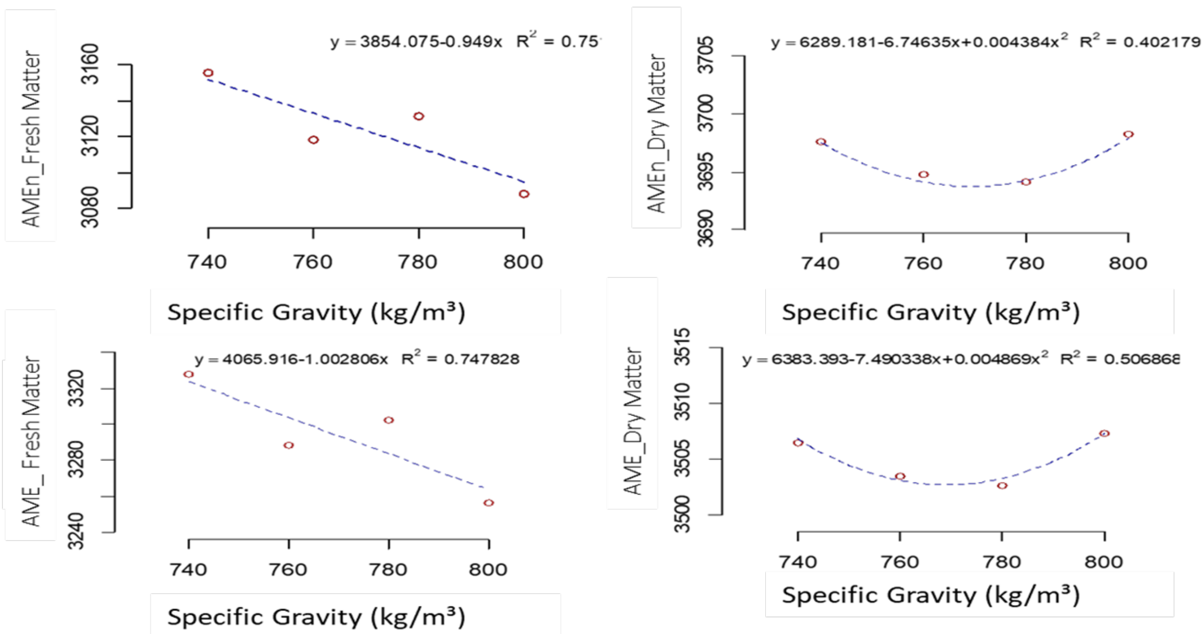

Figure 1. Plots of regression analysis for the significant variables (p<0.05) of apparent metabolizable energy (AME) and nitrogen-corrected AME (AMEn), on the dry and fresh matter bases (kcal/kg), considering the treatments based on specific gravity (kg/m³).

The lowest MC_{EE} value was detected at the lowest density level ($p<0.05$). The MC_{EE} values found in the present study were higher than those found by Rodrigues et al. [8], who reported a mean of 59.8% among the different maize fractions analyzed. However, the value recommended in the Brazilian Feed Composition Table for Poultry and Swine [7] is higher (92%) than the results found in this study. The metabolizability coefficient of EE showed a quadratic response to density, increasing up to a maximum estimated level of 79.93% at the specific gravity of 778 kg/m³ and decreasing thereafter, as gravity increased (Table 5, Figure 2).

Finally, the lowest MC_{Ca} and MC_P values were found in the maize fractions with the specific gravity of 780 kg/m³ (Table 4). Rostagno et al. [7] observed a MC_{Ca} of 40.8%, which is similar, and a MC_P of 24%, which is lower than the results found in this study. Both variables showed a quadratic response to the density of maize, reaching their maximum estimated values at 776 kg/m³ (Figure 2).

According to the obtained results, the variation in kernel density was not sufficient to allow for determining the maize fraction with the best nutritional composition and metabolizability coefficients. Therefore, all the physical and chemical variables of maize kernels must be evaluated in general, as they can correlate with each other strongly influencing the nutritional composition of the kernels, as opposed to assessing only specific gravity in isolation from other characteristics of the product.

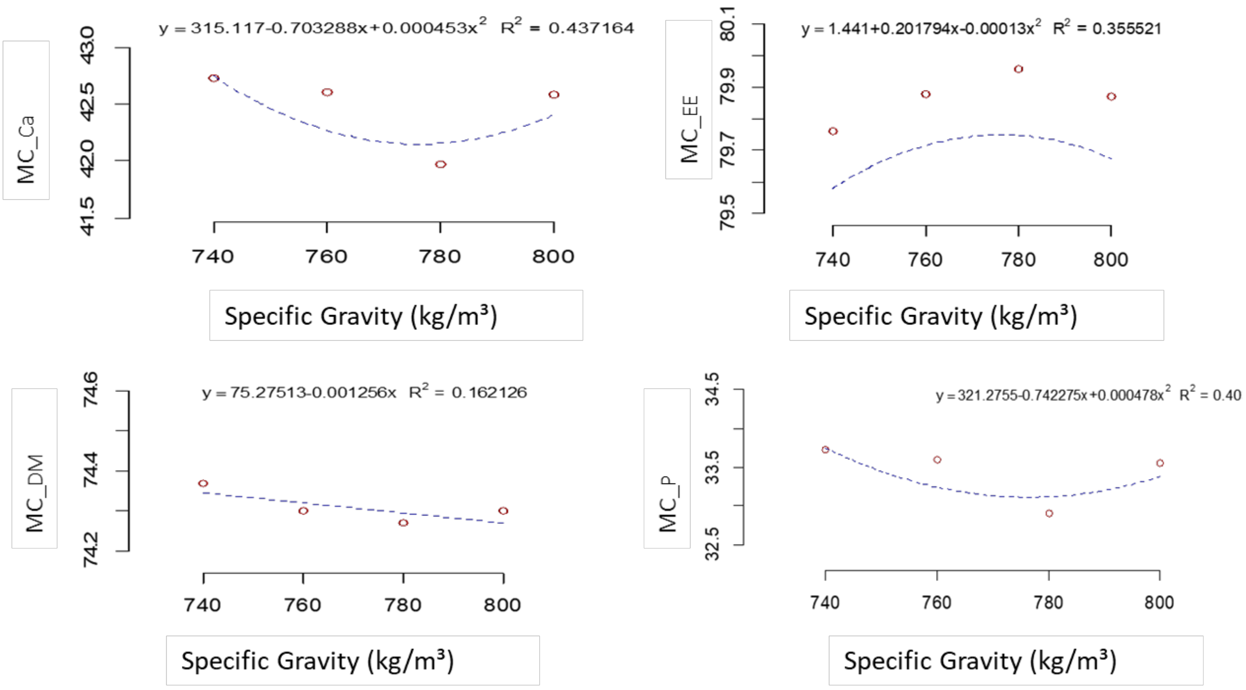

Figure 2. Plot of regression analysis for the significant variables ($p<0.05$) of apparent metabolizability coefficients (%) of dry matter (MC_{DM}), ether extract (MC_{EE}), calcium (MC_{Ca}), and phosphorus (MC_P) considering the treatments based on specific gravity (kg/m³).

4. Conclusion

The low correlations found between the physical and chemical characteristics highlighted the importance of evaluating the collected maize samples both physically and chemically in order to enhance the nutritional adjustment of broiler diets. Some important variations were observed in the different maize specific gravity fractions analyzed; however, when analyzed in isolation, as in the present study, the variations were not sufficient to determine which would be the best-quality fraction of maize. These findings demonstrate the importance of analyzing maize kernels not only regarding their gravity but considering all their physical and chemical quality traits.

Interest conflicts

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: J. H. Stringhini and L.F. Batista. *Data curation*: J. H. Stringhini and L.F. Batista. *Formal analysis*: E. Arnhold. *Methodology*: J.H. Stringhini, M.B. Café and E. Arnhold. *Supervision*: J.H. Stringhini. *Writing (original draft, proofreading and editing):* J. H. Stringhini, E. F. Viana, C.D.S. Leite and L.F. Batista.

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