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# Metabolizable energy, nitrogen balance, and ileal digestibility of amino acids in quality protein maize for pigs

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## Abstract

**Background:** To compare the nutritional value and digestibility of five quality protein maize (QPM) hybrids to that of white and yellow maize, two experiments were carried out in growing pigs. In experiment 1, the energy metabolizability and the nitrogen balance of growing pigs fed one of five QPM hybrid diets were compared against those of pigs fed white or yellow maize. In experiment 2, the apparent and standardized ileal digestibility (AID and SID, respectively) of proteins and amino acids from the five QPM hybrids were compared against those obtained from pigs fed white and yellow maize. In both experiments, the comparisons were conducted using contrasts.

**Results:** The dry matter and nitrogen intakes were higher in the pigs fed the QPM hybrids ( $P < 0.05$ ) than in the pigs fed white or yellow maize. Energy digestibility ( $P < 0.001$ ) and metabolizability ( $P < 0.01$ ) were higher in the pigs fed the white and yellow maize diets than in those fed the QPM diets. The AID of lysine was higher ( $P < 0.01$ ) in the QPM diets than in the white and yellow maize. The AIDs of leucine, isoleucine, valine, phenylalanine, and methionine were lower in the QPM diets than those of maize (white and yellow) (all  $P < 0.05$ ). Maize (white and yellow) had greater SIDs of leucine, isoleucine, valine, phenylalanine, glutamic acid, serine, alanine, tyrosine, and proline ( $P < 0.05$ ).

**Conclusions:** Based on these results, it was concluded that QPM had a lower metabolizable energy content and a higher amount of digestible lysine than normal maize.

**Keywords:** Amino acids, Energy balance, Maize, Nitrogen balance, Pigs

## Background

Maize (*Zea mays*) is the most widely harvested cultivar in the world, and it is often used as the principal source of energy for pigs. It is also an important source of protein and amino acids in finishing pigs [1]. There are varieties of maize, other than yellow maize, that contain different nutrient concentrations [2]. Owing to these differences in nutrient density and/or composition, new types of maize such as quality protein maize (QPM) may offer nutritional advantages over the conventional yellow maize varieties.

Maize protein is deficient in the amino acids, lysine and tryptophan, which limits its value for monogastric animals [3]. Mertz et al. [4] first reported the mutant maize called Opaque 2 in 1963, which has a higher

content of these amino acids. However, maize Opaque 2 had a soft endosperm that made it susceptible to pests and crop storage problems, for which its production was ceased. Subsequent conventional breeding efforts by the International Maize and Wheat Improvement Centre (CIMMYT) generated numerous cultivars with improved agronomic characteristics, collectively referred to as QPM [5], a type of maize with a hard endosperm rich in lysine and tryptophan due to a change in the ratio of 19- and 22-kD  $\alpha$  zeins, an increment of 27 kD  $\gamma$  zein [6], and a non zein protein called elongation factor 1 $\alpha$  (EF-1  $\alpha$ ) [7]. The substantial reduction in synthesis of  $\alpha$ -zeins results in smaller, less numerous protein bodies and a concomitant increase in non-zein endosperm proteins [3].

The metabolizable energy (ME) and amino acid (AA) digestibility of yellow maize have been extensively investigated and summarized in previous publications [8,9]. This allows for the accurate formulation of yellow maize-based diets for pigs to meet ME and digestible AA

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requirements. However, there is currently limited information about the nutritive value of QPM.

Therefore, the first aim of the current study was to compare the energy metabolizability and nitrogen balance of QPM to those of yellow and white maize, and the second aim was to compare the apparent and standardized ileal digestibility (AID and SID, respectively) of protein and AAs in QPM hybrids to those of yellow and white maize.

## Material and methods

This study was approved by the Scientific Associate Technical Group Committee of CENID Physiology. Two experiments were conducted at the experimental farm of CENID-Physiology (INIFAP, México). The experimental animals were treated according to the guidelines of the International Guiding Principles for Biomedical Research Involving Animals [10] and the Official Mexican Standard for production, care and use of laboratory animals [11].

### Raw materials

The following QPM hybrids were evaluated: 538Ta, 537Ta, 537Ig, 334Ce, and QPM1. Evaluation was also conducted on one white and one yellow maize obtained from a commercial supplier in the state of Guanajuato, Mexico. The chemical composition of these materials is shown in Table 1.

### Energy metabolizability and nitrogen balance (Experiment 1)

Four consecutive groups of seven Landrace x Duroc pigs with a mean weight of  $61.2 \pm 2.6$  kg were used (28 pigs in total, four replicates by treatment). The pigs were placed in individual metabolic cages equipped with a self-feeder and a low-pressure drinking nipple connected to a watering system that controlled the water supply. Screens were placed under the floors, which allowed for total collection of feces and urine. The room temperature ranged from 19–22°C.

The pigs were fed twice daily at 0800 h and 1800 h. The experimental diets (Table 2) were prepared with one QPM or one maize (white or yellow) as the sole protein and energy source. The diets contained calcium carbonate, dicalcium phosphate, salt, and premixed vitamins and minerals. Chromic oxide (3 g/kg of feed) was included as an indigestible marker. The feed intake of the pigs was 2.5 times their digestible energy (DE) requirement for maintenance ( $460 \text{ kJ/kg BW}^{0.75}$  [12]) as recommended by Adeola [13] for pigs weighing >50 kg. The water container in each metabolic cage was filled just before each meal to restrict water intake to 2.5 L/kg of dry matter (DM) intake [13].

The experimental period lasted for 10 d for each consecutive group: 5 d for adaptation and 5 d for the

total collection of feces and urine. The feces were frozen and kept at  $-20^\circ\text{C}$ . At the end of the experimental period, the feces were defrosted and homogenized to obtain 10% of the weight as a final sample for lyophilizing. Urine was collected via funnels underneath the urine collection tray. This collection system included a glass wool mat to avoid contamination with feed or feces. To reduce urine pH and avoid  $\text{NH}_3$  volatilization, 40 mL of HCl 6 mol/L were added to each urine container twice a day. The urine was removed twice a day, weighed, and filtered again through four layers of cheesecloth into a clean container. Then a 5% aliquot was taken and kept at  $-20^\circ\text{C}$  until analysis. Three urine subsamples of 20 mL per pig were lyophilized to measure energy [14].

### Ileal digestibility (Experiment 2)

Seven Landrace x Duroc pigs with a mean weight of  $62.3 \pm 4.9$  kg at the time of data collection were used. When the pigs weighed 45 kg, a T-cannula was fitted at the terminal ileum [15]. After surgery, the pigs were placed in individual metabolism cages that included a self-feeder and a low-pressure drinking nipple. The room temperature ranged from 19–22°C.

The post-operative period lasted for 21 d. During this period, the pigs were fed a grower diet (160 g of CP/kg) twice daily at 0800 h and 1800 h. The amount fed was increased 100 g/d until the level of intake was the same as that prior to surgery.

During the experimental period, the pigs received one of the experimental diets (Table 3). The diets were formulated using maize as the sole source of dietary protein. To avoid the effect of the level of the dietary protein on protein apparent digestibility [16], maize starch was used to ensure that the experimental diets consisted of the same protein level despite the different protein levels of the maize. All experimental diets contained calcium carbonate, dicalcium phosphate, and salt. To reduce dust, 20 g/kg of maize oil was included. Vitamins and minerals exceeded the NRC [8] requirements. Chromic oxide (3 g/kg of feed) was included as an indigestible marker. The feed intake of the pigs was 2.5 times their digestible energy (DE) requirement for maintenance,  $460 \text{ kJ/kg BW}^{0.75}$  [12]. The animals had free access to water.

The four experimental periods lasted seven d each: 5 d for adaptation and 2 d for digesta collection. Ileal digesta were collected continuously over a period of 10 h (0800 h–1800 h) using plastic bags (11 cm × 5 cm) that contained 10 mL of a 0.2 mol/L HCl solution to inhibit bacterial activity and were attached to the cannula using a rubber band. When the collecting bags were full, the ileal digesta was transferred to a container and frozen at  $-20^\circ\text{C}$  until lyophilisation.

**Table 1 Chemical composition of maize (g/kg)<sup>1</sup>**

Nutrient, g /kg	QPM <sup>2</sup>					Maize	
	QPM1	334Ce	537Ta	537Ig	538Ta	White	Yellow
Dry Matter	910.7	911.4	893.0	909.0	911.2	911.0	910.8
Protein	85.0	84.0	86.0	85.0	87.0	80.0	75.0
NDF	114.0	91.0	102.0	84.0	89.0	104.0	94.0
ADF	48.0	39.0	40.0	38.0	35.0	34.0	27.0
Ether Extract	34.0	34.0	47.0	39.0	52.0	39.0	37.0
Ash	17.0	14.0	16.0	17.0	17.0	11.0	12.0
Gross Energy, MJ/kg	16.7	16.6	16.4	16.0	16.6	16.5	16.5
Amino acids							
Alanine	5.1	4.9	4.9	5.0	4.2	5.9	5.6
Arginine	5.9	3.5	5.3	5.3	4.9	3.8	3.6
Aspartic acid	6.7	4.6	6.5	6.4	5.4	5.4	5.3
Cystine	3.5	3.5	2.4	3.5	3.4	3.0	3.3
Glutamic acid	14.1	12.6	13.6	14.4	11.4	15.3	14.2
Glycine	4.1	2.9	3.9	4.0	3.5	3.1	3.0
Histidine	3.5	2.7	3.4	2.5	2.6	2.5	2.1
Isoleucine	2.6	2.0	2.5	2.4	2.1	2.6	2.5
Leucine	7.3	7.7	7.1	7.3	5.8	9.6	9.1
Lysine	3.1	2.7	2.9	2.7	2.7	2.0	2.0
Methionine	1.8	1.8	2.2	2.3	2.3	1.5	2.1
Phenylalanine	3.9	3.1	3.4	3.9	2.9	4.2	3.9
Proline	8.4	7.4	9.4	8.0	7.9	9.2	8.9
Serine	3.7	3.1	3.6	3.9	3.1	3.7	3.5
Threonine	3.3	2.4	3.0	3.5	2.7	2.8	2.6
Tyrosine	2.6	2.2	2.4	2.2	2.1	2.6	2.5
Valine	4.7	3.1	5.4	4.4	4.6	3.9	4.3

<sup>1</sup>As fed basis.

<sup>2</sup>Quality Protein Maize.

At the end of the experiment, narcosis was induced by CO<sub>2</sub> inhalation, followed by euthanasia by exsanguination. A post-mortem examination for fistula along the length of the small intestine was performed to verify its integrity.

#### Chemical analysis

The raw materials, diets, digesta, and feces were ground using a laboratory mill (Arthur H. Thomas Co., Philadelphia, PA) to pass through a 0.5 mm mesh sieve. DM and nitrogen analysis methods, 934.01 and 976.05 [17], respectively, were performed. Chromium analysis was performed as described by Fenton and Fenton [18]. Gross energy was estimated using an adiabatic bomb calorimeter (model 1281, Parr, Moline, IL). Samples from the raw materials, diets, and digesta were hydrolyzed at 110°C for 24 h in 6 mol/L HCl to use in AA analysis method 994.12 [17]. For methionine and cystine analyses, oxidation with performic acid was carried out before acid

hydrolysis [17]. Tryptophan was not estimated. AA analysis was performed using reverse phase HPLC (1100 HPLC Hewlett Packard), according to Henderson *et al.* [19]. Nitrogen in the liquid urine was estimated according to AOAC [17] method 976.05. Energy in the lyophilized urine was estimated according to Le Bellego [14].

#### Calculations

The AID or apparent total tract digestibility (ATTD) was estimated using the equation proposed by Fan and Sauer [20]:

$$AID = [1 - ((ID \times AF) / (AD \times IF))] \times 100$$

where AID = apparent digestibility (ileal or total tract) of a nutrient in the diet, **ID** = concentration of the marker in the diet (mg/kg DM), **AF** = concentration of the nutrient in ileal digesta or feces (mg/kg DM),

**Table 2 Composition of the experimental diets (g/kg): experiment 1<sup>1</sup>**

Ingredient, g/kg	QPM <sup>2</sup>					Maize	
	QPM1	334Ce	537Ta	537Ig	538Ta	White	Yellow
QPM1	967.5						
334Ce		967.5					
537Ta			967.5				
537Ig				967.5			
538Ta					967.5		
White						967.5	
Yellow							967.5
Calcium Phosphate	18.5	18.5	18.5	18.5	18.5	18.5	18.5
Calcium Carbonate	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Salt	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Minerals <sup>3</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamins <sup>4</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Chemical composition <sup>5</sup>							
Dry Matter	906.6	903.3	891.1	904.6	901.4	905.6	904.8
Protein	82.2	81.3	83.2	82.2	84.2	77.4	72.6
Gross Energy, MJ/kg	16.5	16.2	16.1	15.9	16.1	16.0	16.0

<sup>1</sup>As fed basis.

<sup>2</sup>Quality Protein Maize.

<sup>3</sup>Furnished by kg of feed: Cl 1.65 g, Na 0.87 g, Cu 7.7 mg, Fe 89.25 mg, Mn 19.98 mg, Se 0.087 mg, I 0.053 mg.

<sup>4</sup>Furnished by kg of feed: Vitamin A 6,600 IU, D 660 IU, E 100 IU, Choline 350 mg, Niacin 54 mg, Pantothenic acid 13.15 mg, Riboflavin 2.2 mg, B<sub>12</sub> 36 µg.

<sup>5</sup>Analyzed values, on an as fed basis.

**AD** = concentration of the nutrient in the diet (mg/kg DM), and **IF** = concentration of the marker in the ileal digesta or feces (mg/kg DM).

The SID was estimated using the formula proposed by Furuya and Kaji [21]:

$$SID = [AID + (Endogenous/Dietary Content)] \times 100$$

where Endogenous = endogenous losses of a nutrient in mg/kg DM intake, and Dietary content = amount of nutrient consumed in mg/kg DM intake. The calculations were performed using endogenous values reported by Mariscal-Landin and Reis de Souza [22].

The ME was obtained using the formula proposed by Adeola [13]:

$$ME = [(GE - FE - UE) / GE] \times 100$$

where ME = metabolizable energy in MJ/d, **GE** = gross energy intake in MJ/d, **FE** = fecal energy output in MJ/d, and **UE** = urine energy output in MJ/d.

## Statistical analyses

### Experiment 1

Data were analyzed as a randomized complete block design [23] with four blocks of seven animals each and using

the GLM procedure in SAS v9.2 [24]. The variables were the DM amount, nitrogen intake, energy intake, the amount of nitrogen and energy excreted in feces and urine, the apparent total tract digestibility of DM, nitrogen, and energy, nitrogen balance, and energy metabolizability. An alpha value of 0.05 was used to assess significance.

### Experiment 2

Data were analyzed using a Latin square with additional columns, or a 4 × 7 “Youden square” [23], which included seven animals, seven treatments, and four experimental periods. The experimental variables were the protein and amino acids AID and SID of the maize. Data were analyzed using the GLM procedure in SAS v9.2 [24]. An alpha value of 0.05 was used to assess significance.

In both experiments, the means were compared using Duncan’s method and the QPM (QPM1, 334Ce, 537Ta, 537Ig, or 538Ta) and normal maize (white or yellow) were compared using contrasts [23].

## Results

### Energy metabolizability and nitrogen balance

#### Energy metabolizability, QPM vs normal maize

Energy intake was similar ( $P > 0.05$ ) between the treatments: 29.5 MJ/d of QPM or normal maize. Apparent

**Table 3 Composition of the experimental diets (g/kg): experiment 2<sup>1</sup>**

Ingredient, g/kg	QPM <sup>2</sup>					Maize	
	QPM1	334Ce	537Ta	537lg	538Ta	White	Yellow
QPM1	845.5						
334Ce		845.5					
537Ta			825.5				
537lg				835.5			
538Ta					816.5		
White						887.5	
Yellow							947.5
Maize starch	102.0	102.0	122.0	112.0	131.5	60.0	
Calcium Phosphate	18.5	18.5	18.5	18.5	18.5	18.5	18.5
Calcium Carbonate	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Maize oil	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Salt	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Minerals <sup>3</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamins <sup>4</sup>	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Chemical composition <sup>5</sup>							
Dry matter	905.6	901.3	894.1	864.2	899.2	906.5	902.8
Protein	71.1	70.8	70.6	71.0	71.3	70.5	71.6
Gross Energy, MJ/kg	15.8	15.7	15.6	15.2	15.7	15.7	15.6
Alanine	4.70	4.81	4.45	4.32	4.01	5.55	6.08
Arginine	5.36	3.47	4.86	4.57	4.70	3.59	3.93
Aspartic acid	6.10	4.54	5.83	5.59	5.04	5.04	5.69
Cystine	3.22	3.47	2.19	3.13	3.25	2.82	3.53
Glutamic acid	12.87	12.47	12.38	12.62	10.85	14.43	15.41
Glycine	3.71	2.85	3.56	3.56	3.33	2.90	3.24
Histidine	3.22	2.67	3.08	2.54	2.48	2.39	2.36
Isoleucine	2.39	1.96	2.19	1.61	1.96	2.39	2.65
Leucine	6.60	7.57	6.39	6.10	5.55	9.05	9.81
Lysine	2.80	2.67	2.59	2.54	2.48	1.88	2.16
Methionine	1.65	1.16	2.02	1.10	1.45	1.45	2.26
Phenylalanine	3.55	3.12	3.08	2.80	2.82	3.93	4.22
Proline	6.35	6.14	8.50	4.91	7.52	8.71	9.62
Serine	3.38	3.03	3.24	3.47	2.90	3.50	3.73
Threonine	2.97	2.40	2.75	3.39	2.56	2.65	2.85
Tyrosine	2.39	2.23	2.19	2.12	1.96	2.48	2.65
Valine	4.29	3.03	4.86	2.71	4.36	3.59	4.71

<sup>1</sup>As fed basis.

<sup>2</sup>Quality Protein Maize.

<sup>3</sup>Furnished by kg of feed: Cl 1.65 g, Na 0.87 g, Cu 7.7 mg, Fe 89.25 mg, Mn 19.98 mg, Se 0.087 mg, I 0.053 mg.

<sup>4</sup>Furnished by kg of feed: Vitamin A 6,600 IU, D 660 IU, E 100 IU, Choline 350 mg, Niacin 54 mg, Pantothenic acid 13.15 mg, Riboflavin 2.2 mg, B<sub>12</sub> 36 µg.

<sup>5</sup>Analyzed values, on an as fed basis.

total tract digestibility was lower in the pigs fed QPM (89.8) than in those fed normal maize (91.0,  $P < 0.001$ ). While UE was similar (0.58 MJ/d,  $P > 0.05$ ) between all

the treatments, the metabolizability of energy was lower in the pigs fed QPM (87.9) than in those fed normal maize (88.9) (Table 4).

**Table 4 Energy metabolizability and nitrogen balance: experiment 1<sup>1</sup>**

Traits	QPM <sup>2</sup>					Maize		SEM <sup>3</sup>	Contrast		Prob <sup>4</sup>
	QPM1	334Ce	537Ta	537Ig	538Ta	White	Yellow		QPM	Normal	
Dry Matter, kg/d											
Intake	1.67 <sup>a</sup>	1.66 <sup>a</sup>	1.66 <sup>a</sup>	1.68 <sup>a</sup>	1.64 <sup>a</sup>	1.66 <sup>a</sup>	1.55 <sup>b</sup>	0.010	1.66	1.60	0.05
Energy, MJ/d											
Intake	29.9 <sup>a</sup>	29.7 <sup>a</sup>	29.7 <sup>a</sup>	30.2 <sup>a</sup>	29.5 <sup>a</sup>	29.7 <sup>a</sup>	27.7 <sup>b</sup>	0.17	29.8	28.7	0.63
In feces	3.1 <sup>a</sup>	3.2 <sup>a</sup>	3.0 <sup>a</sup>	2.9 <sup>ab</sup>	2.9 <sup>ab</sup>	2.6 <sup>b</sup>	2.6 <sup>b</sup>	0.04	3.0	2.6	0.001
Digestible	26.8 <sup>a</sup>	26.5 <sup>a</sup>	26.7 <sup>a</sup>	27.3 <sup>a</sup>	26.5 <sup>a</sup>	27.1 <sup>a</sup>	25.1 <sup>b</sup>	0.16	26.7	26.1	0.01
In Urine	0.50	0.58	0.53	0.60	0.62	0.60	0.61	0.013	0.57	0.60	0.21
Metabolizable	26.3 <sup>a</sup>	25.9 <sup>a</sup>	26.2 <sup>a</sup>	26.7 <sup>a</sup>	25.9 <sup>a</sup>	26.5 <sup>a</sup>	24.5 <sup>b</sup>	0.16	26.2	25.5	0.01
Nitrogen, g/d											
Intake	22.4 <sup>b</sup>	24.6 <sup>a</sup>	24.5 <sup>a</sup>	24.6 <sup>a</sup>	24.4 <sup>a</sup>	22.0 <sup>b</sup>	22.1 <sup>b</sup>	0.13	24.0	22.0	0.001
In feces	3.6	4.1	3.8	3.7	3.8	3.4	3.5	0.08	3.82	3.46	0.32
Digestible	18.7 <sup>b</sup>	20.5 <sup>a</sup>	20.6 <sup>a</sup>	20.4 <sup>a</sup>	20.5 <sup>a</sup>	18.5 <sup>b</sup>	18.6 <sup>b</sup>	0.35	20.1	18.6	0.001
In Urine	10.2 <sup>a</sup>	12.6 <sup>b</sup>	13.2 <sup>b</sup>	11.9 <sup>b</sup>	12.0 <sup>b</sup>	12.1 <sup>b</sup>	12.2 <sup>b</sup>	0.21	11.97	12.2	0.74
Retained	8.5 <sup>a</sup>	7.9 <sup>ab</sup>	7.5 <sup>ab</sup>	8.5 <sup>a</sup>	8.5 <sup>a</sup>	6.5 <sup>ab</sup>	6.4 <sup>b</sup>	0.24	8.18	6.44	0.19
Dry matter, MJ/kg											
Digestible energy	16.11 <sup>cd</sup>	15.96 <sup>d</sup>	16.12 <sup>bcd</sup>	16.29 <sup>ab</sup>	16.18 <sup>abc</sup>	16.34 <sup>a</sup>	16.22 <sup>abc</sup>	0.007	16.13	16.28	0.01
Metabolizable energy	15.59 <sup>ab</sup>	15.38 <sup>b</sup>	15.59 <sup>ab</sup>	15.70 <sup>a</sup>	15.55 <sup>ab</sup>	15.74 <sup>a</sup>	15.61 <sup>ab</sup>	0.009	15.56	15.68	0.09
Coefficient											
DM Digestibility	88.7 <sup>c</sup>	88.2 <sup>c</sup>	88.9 <sup>c</sup>	90.1 <sup>ab</sup>	89.2 <sup>bc</sup>	90.8 <sup>a</sup>	90.2 <sup>a</sup>	0.12	89.0	90.5	0.001
E Digestibility	89.6 <sup>bc</sup>	89.1 <sup>c</sup>	89.8 <sup>bc</sup>	90.4 <sup>ab</sup>	90.1 <sup>bc</sup>	91.3 <sup>a</sup>	90.6 <sup>ab</sup>	0.14	89.8	91.0	0.001
Metabolizability	87.9 <sup>b</sup>	87.2 <sup>b</sup>	88.0 <sup>b</sup>	88.4 <sup>ab</sup>	88.0 <sup>b</sup>	89.3 <sup>a</sup>	88.4 <sup>ab</sup>	0.15	87.9	88.9	0.01
N Digestibility	83.7	83.3	84.3	84.7	84.3	84.3	84.2	0.30	84.1	84.3	0.72
N retention as percentage of											
Intake <sup>5</sup>	38.2 <sup>a</sup>	32.1 <sup>ab</sup>	30.5 <sup>ab</sup>	35.2 <sup>ab</sup>	35.0 <sup>ab</sup>	29.6 <sup>ab</sup>	28.8 <sup>b</sup>	1.01	34.2	29.2	0.28
Absorbed <sup>6</sup>	45.7 <sup>a</sup>	38.5 <sup>ab</sup>	36.1 <sup>ab</sup>	41.6 <sup>ab</sup>	41.3 <sup>ab</sup>	35.0 <sup>b</sup>	34.2 <sup>b</sup>	1.14	40.3	34.6	0.20

<sup>1</sup>All data are reported on a DM basis.

<sup>2</sup>Quality Protein Maize.

<sup>3</sup>Standard error of the mean.

<sup>4</sup>Contrast Probability.

<sup>5</sup>(N retained/N intake)\*100.

<sup>6</sup>(N retained/N absorbed)\*100.

<sup>abc</sup>different letters in the same line differ ( $P < 0.05$ ).

#### Energy metabolizability, means comparison

Energy intake was lower in the pigs fed yellow maize (27.7 MJ/d) than in the pigs fed white maize (29.8 MJ/d,  $P < 0.05$ ). Apparent total tract digestibility was higher in the pigs fed white maize (91.3) and lower in the pigs fed 334Ce QPM (89.1,  $P < 0.05$ ). The metabolizability of energy was higher in the pigs fed white maize (89.3) than in the pigs fed QPM1 (87.9), except for those fed QPM 537Ig (88.4; Table 4).

#### Nitrogen balance, QPM vs normal maize

The DM intake was higher in the pigs fed QPM, compared to those fed normal maize (1.66 vs 1.60 kg/d,  $P < 0.05$ ), and the DM total tract digestibility was lower in the QPM pigs, compared to those fed normal maize (89.0 vs 90.5,

$P < 0.001$ ). The daily nitrogen intake was higher in the pigs fed QPM (24.0 g/d) than in the pigs fed normal maize (22.0 g/d,  $P < 0.001$ ); however, nitrogen digestibility was similar between the treatments (84.1,  $P > 0.05$ ). Consequently, the digestible nitrogen intake was higher in the pigs fed QPM. Urinary nitrogen excretion was similar between treatments (12.0 g/d,  $P > 0.05$ ). Nitrogen retention as a proportion of nitrogen intake or nitrogen absorption was also similar ( $P > 0.05$ ) between the treatments (Table 4).

#### Nitrogen balance, means comparison

Although the nitrogen intake in the pigs fed QPM1 was lower (22.4 g/d) than in the pigs fed the other hybrid QPMs (24.5 g/d,  $P < 0.001$ ) and similar to the pigs fed normal maize (22.0 g/d), the pigs fed QPM1 retained

more nitrogen as a percentage of nitrogen intake (38.2%) or nitrogen absorption (45.7%) than the pigs fed yellow maize (28.8% and 34.2%, respectively,  $P < 0.05$ ; Table 4).

### Ileal digestibility

#### Apparent ileal digestibility, QPM vs normal maize

The DM digestibility was lower in the pigs fed QPM (78.7) than in those fed normal maize (80.0,  $P < 0.05$ ). The CP digestibility was similar in all of the diets (mean 73.0,  $P > 0.05$ ).

Lysine digestibility was higher in the pigs fed QPM than in the pigs fed normal maize ( $P < 0.05$ ; Table 5). The digestibility of leucine, isoleucine, phenylalanine, glutamic acid, alanine, tyrosine, proline, valine, serine, and methionine was lower in the QPM pigs than in those fed normal maize (all  $P < 0.05$ ; Table 5).

#### Apparent ileal digestibility, means comparison

In general, QPM 537Ig had the lowest digestibility, except for that for arginine, threonine, serine, and glycine; for these AAs, QPM 334Ce had the lowest digestibility (Table 5). The white maize diet demonstrated lower AID of the sulphur AAs than the yellow maize diet. The

digestibility of leucine, alanine, and tyrosine was lower in all of the QPM diets than in the normal maize diets (Table 5).

#### Standardized ileal digestibility, QPM vs normal maize

The digestibility of CP, lysine, arginine, histidine, methionine, threonine, aspartic acid, glycine, and cystine was similar between QPM and normal maize (all  $P > 0.05$ ; Table 6). The digestibility of glutamic acid, tyrosine, leucine, isoleucine, phenylalanine, alanine, valine, serine, and proline was lower in the QPM diets than in the normal maize diets (all  $P < 0.05$ ; Table 6).

#### Standardized ileal digestibility, means comparison

The SID of QPM 537Ig was consistently the lowest among the QPMs. The digestibility of glutamic acid and tyrosine was lower in the QPM diets than in the normal maize diets (Table 6). Methionine was less digestible in the white maize diet than in the yellow maize diet (Table 6).

## Discussion

### Energy metabolizability and nitrogen balance

The total tract digestibility of energy in the QPM diets was 1.3% lower, on average, than the normal maize diets.

**Table 5 Apparent ileal digestibility of the maize: experiment 2**

Traits	QPM <sup>1</sup>					Maize		SEM <sup>2</sup>	Contrast		Prob <sup>3</sup>
	QPM1	334Ce	537Ta	537Ig	538Ta	White	Yellow		QPM	Normal	
Dry Matter	77.5 <sup>c</sup>	77.6 <sup>c</sup>	81.9 <sup>a</sup>	77.7 <sup>c</sup>	78.6 <sup>bc</sup>	80.9 <sup>ab</sup>	79.1 <sup>abc</sup>	0.33	78.7	80.0	0.05
Protein	72.0	74.3	73.4	71.4	74.3	71.8	74.1	0.32	73.1	72.9	0.77
Amino acids											
Alanine	73.2 <sup>b</sup>	72.7 <sup>b</sup>	71.6 <sup>b</sup>	69.0 <sup>b</sup>	70.6 <sup>b</sup>	79.8 <sup>a</sup>	80.1 <sup>a</sup>	0.67	71.4	79.9	0.001
Arginine	82.1 <sup>a</sup>	73.8 <sup>b</sup>	82.3 <sup>a</sup>	81.3 <sup>a</sup>	85.5 <sup>a</sup>	80.0 <sup>a</sup>	82.7 <sup>a</sup>	0.72	81.0	81.4	0.79
Aspartic acid	75.2	68.4	75.9	71.5	71.9	75.4	75.6	0.61	72.6	75.5	0.06
Cystine	72.9 <sup>c</sup>	78.9 <sup>ab</sup>	66.9 <sup>d</sup>	74.9 <sup>bc</sup>	78.0 <sup>abc</sup>	75.1 <sup>bc</sup>	80.9 <sup>a</sup>	0.56	74.3	78.0	0.07
Glutamic acid	83.3 <sup>b</sup>	83.1 <sup>b</sup>	83.1 <sup>b</sup>	81.5 <sup>b</sup>	82.1 <sup>b</sup>	86.9 <sup>a</sup>	86.9 <sup>a</sup>	0.29	82.6	86.9	0.001
Glycine	55.1 <sup>ab</sup>	49.8 <sup>b</sup>	58.9 <sup>a</sup>	54.6 <sup>ab</sup>	51.9 <sup>ab</sup>	55.8 <sup>ab</sup>	57.0 <sup>ab</sup>	0.91	54.0	56.4	0.83
Histidine	87.2 <sup>ab</sup>	83.8 <sup>bc</sup>	88.3 <sup>a</sup>	82.6 <sup>c</sup>	85.4 <sup>abc</sup>	86.1 <sup>abc</sup>	84.8 <sup>abc</sup>	0.45	85.4	85.4	0.93
Isoleucine	75.4 <sup>ab</sup>	69.9 <sup>b</sup>	74.3 <sup>ab</sup>	60.2 <sup>c</sup>	71.8 <sup>b</sup>	79.4 <sup>a</sup>	79.2 <sup>a</sup>	0.66	70.3	79.3	0.001
Leucine	82.4 <sup>b</sup>	83.2 <sup>b</sup>	83.1 <sup>b</sup>	80.5 <sup>b</sup>	82.1 <sup>b</sup>	89.0 <sup>a</sup>	89.4 <sup>a</sup>	0.43	82.3	89.2	0.001
Lysine	77.2 <sup>a</sup>	76.7 <sup>a</sup>	77.1 <sup>a</sup>	73.8 <sup>ab</sup>	75.1 <sup>ab</sup>	69.0 <sup>b</sup>	72.4 <sup>ab</sup>	0.72	76.0	70.7	0.01
Methionine	80.5 <sup>b</sup>	74.2 <sup>c</sup>	85.5 <sup>a</sup>	70.7 <sup>d</sup>	75.5 <sup>c</sup>	76.8 <sup>c</sup>	84.3 <sup>a</sup>	0.36	77.3	80.5	0.05
Phenylalanine	82.7 <sup>b</sup>	80.6 <sup>bc</sup>	81.9 <sup>b</sup>	77.3 <sup>c</sup>	82.6 <sup>b</sup>	88.0 <sup>a</sup>	87.6 <sup>a</sup>	0.46	81.0	87.7	0.001
Proline	51.2 <sup>cd</sup>	61.6 <sup>bc</sup>	72.5 <sup>ab</sup>	45.8 <sup>d</sup>	61.5 <sup>bc</sup>	71.6 <sup>ab</sup>	80.3 <sup>a</sup>	1.63	58.5	75.9	0.001
Serine	71.0 <sup>ab</sup>	67.8 <sup>b</sup>	72.0 <sup>ab</sup>	70.4 <sup>ab</sup>	69.4 <sup>b</sup>	76.8 <sup>a</sup>	76.6 <sup>a</sup>	0.70	70.1	76.6	0.01
Threonine	60.4 <sup>ab</sup>	54.6 <sup>b</sup>	61.6 <sup>ab</sup>	64.1 <sup>a</sup>	60.1 <sup>b</sup>	64.8 <sup>a</sup>	62.4 <sup>ab</sup>	0.96	60.1	63.6	0.39
Tyrosine	77.9 <sup>b</sup>	76.8 <sup>b</sup>	77.7 <sup>b</sup>	73.8 <sup>b</sup>	74.7 <sup>b</sup>	83.7 <sup>a</sup>	83.9 <sup>a</sup>	0.57	76.2	83.8	0.001
Valine	77.6 <sup>a</sup>	70.4 <sup>b</sup>	81.7 <sup>a</sup>	63.3 <sup>c</sup>	80.9 <sup>a</sup>	78.5 <sup>a</sup>	82.8 <sup>a</sup>	0.18	74.8	80.6	0.01

<sup>1</sup>Quality Protein Maize.

<sup>2</sup>Standard error of the mean.

<sup>3</sup>Probability of contrast.

<sup>abcd</sup>different letters in the same line differ ( $P < 0.05$ ).

**Table 6 Standardized ileal digestibility of the maize: experiment 2**

Traits	QPM <sup>1</sup>					Maize			Contrast		Prob <sup>3</sup>
	QPM1	334Ce	537Ta	537lg	538Ta	White	Yellow	SEM <sup>2</sup>	QPM	Normal	
Protein	86.7	88.0	88.2	85.7	88.2	86.9	88.1	0.32	87.4	87.5	0.98
Amino acids											
Alanine	80.7 <sup>ab</sup>	80.0 <sup>bc</sup>	79.4 <sup>c</sup>	77.1 <sup>c</sup>	79.4 <sup>c</sup>	86.1 <sup>a</sup>	85.8 <sup>ab</sup>	0.66	79.3	86.0	0.01
Arginine	87.9 <sup>ab</sup>	82.8 <sup>b</sup>	88.8 <sup>ab</sup>	88.2 <sup>ab</sup>	92.2 <sup>a</sup>	88.8 <sup>ab</sup>	90.8 <sup>a</sup>	0.72	88.0	89.8	0.65
Aspartic acid	85.2	81.9	86.4	82.4	84.0	87.5	86.4	0.61	84.0	86.9	0.06
Cystine	77.1 <sup>cd</sup>	82.8 <sup>ab</sup>	73.1 <sup>c</sup>	79.2 <sup>bc</sup>	82.1 <sup>abc</sup>	79.9 <sup>abc</sup>	88.7 <sup>a</sup>	0.56	78.9	82.3	0.10
Glutamic acid	88.7 <sup>b</sup>	88.7 <sup>b</sup>	88.7 <sup>b</sup>	87.1 <sup>b</sup>	88.6 <sup>b</sup>	91.7 <sup>a</sup>	91.4 <sup>a</sup>	0.28	88.3	91.6	0.001
Glycine	73.8	74.2	78.3	74.2	72.7	79.7	78.4	0.91	74.6	79.0	0.26
Histidine	92.0 <sup>ab</sup>	89.5 <sup>ab</sup>	93.3 <sup>a</sup>	88.6 <sup>b</sup>	91.5 <sup>ab</sup>	92.5 <sup>ab</sup>	91.3 <sup>ab</sup>	0.46	91.0	91.9	0.39
Isoleucine	86.3 <sup>ab</sup>	83.3 <sup>b</sup>	86.3 <sup>ab</sup>	76.4 <sup>c</sup>	85.0 <sup>ab</sup>	90.3 <sup>a</sup>	89.0 <sup>ab</sup>	0.66	83.4	89.7	0.01
Leucine	89.2 <sup>bc</sup>	89.1 <sup>bc</sup>	90.2 <sup>abc</sup>	87.9 <sup>c</sup>	90.2 <sup>abc</sup>	94.0 <sup>a</sup>	94.0 <sup>a</sup>	0.44	89.3	94.0	0.01
Lysine	88.1	88.2	88.9	85.9	87.5	85.3	86.6	0.72	87.7	85.9	0.18
Methionine	84.4 <sup>b</sup>	79.7 <sup>b</sup>	88.7 <sup>a</sup>	76.4 <sup>d</sup>	79.8 <sup>c</sup>	81.1 <sup>c</sup>	87.1 <sup>ab</sup>	0.36	81.8	84.1	0.30
Phenylalanine	89.5 <sup>bcd</sup>	88.4 <sup>cd</sup>	89.8 <sup>bcd</sup>	86.0 <sup>d</sup>	91.3 <sup>abc</sup>	94.2 <sup>a</sup>	93.3 <sup>ab</sup>	0.46	89.0	93.7	0.01
Proline	70.2 <sup>c</sup>	81.2 <sup>abc</sup>	86.7 <sup>ab</sup>	70.3 <sup>c</sup>	77.5 <sup>bc</sup>	85.4 <sup>ab</sup>	92.8 <sup>a</sup>	1.63	77.2	89.1	0.02
Serine	84.6 <sup>ab</sup>	82.9 <sup>b</sup>	86.2 <sup>ab</sup>	83.6 <sup>ab</sup>	85.2 <sup>ab</sup>	89.9 <sup>a</sup>	88.9 <sup>ab</sup>	0.70	84.5	89.4	0.03
Threonine	77.7	76.0	80.2	79.2	80.2	84.1	80.4	0.95	78.6	82.3	0.35
Tyrosine	83.2 <sup>b</sup>	82.5 <sup>b</sup>	83.4 <sup>b</sup>	79.8 <sup>b</sup>	81.1 <sup>b</sup>	88.8 <sup>a</sup>	88.7 <sup>a</sup>	0.57	82.0	89.7	0.001
Valine	86.0 <sup>ab</sup>	82.3 <sup>b</sup>	89.1 <sup>a</sup>	76.6 <sup>c</sup>	89.1 <sup>a</sup>	88.5 <sup>a</sup>	90.4 <sup>a</sup>	0.58	84.6	89.5	0.02

<sup>1</sup>Quality Protein Maize.

<sup>2</sup>Standard error of the mean.

<sup>3</sup>Probability.

<sup>abcd</sup>different letters in the same line differ ( $P < 0.05$ ).

While urinary energy was similar between the diets, metabolizability was also lower in the QPM diets than in the white maize diet.

The starch type of QPM may explain its lower digestibility in the current study; amylose is negatively correlated with average daily gain [25]. Furthermore, the starch of waxy sorghums, which are low in amylose, is more digestible than the starch of non-waxy sorghums [26]; this has also been demonstrated with maize starch in ducks [27]. Although QPM has a vitreous endosperm phenotype, it is rich in a no-crystalline amylopectin that forms bonds with  $\gamma$  zein (27-kDa) [28].

The negative effect of fiber on energy digestibility [29,30] could provide another feasible explanation; dietary fiber is less digestible than other nutrients such as starch, sugars, fat, or protein (<50% vs 80-100%) [31]. Moreover, corn fiber is essentially insoluble [32]; QPM had a higher ADF content than the normal maize (40 g/kg QPM vs 30 g/kg maize), and this could have resulted in considerable effects on digestibility given that maize fiber, in general, is poorly digested by growing pigs [31]. Moreover, the ME:DE ratio in the current study was 0.98, which is similar to the 0.96 estimated by Noblet and van Milgen [31].

Generally, 50% of the nitrogen that is absorbed is retained in the body, and the other 50% is excreted in urine [29]. The retention of nitrogen in the current study was lower (37.5%), which could be attributed to a diet of low protein quality. Maize protein is deficient in lysine and tryptophan [3], and it is well-known that nitrogen retention in growing pigs is related to the lysine level in the diet as lysine is the first limiting AA [33]. Lysine digestibility was higher in the pigs fed QPM1 maize, and these same pigs retained 26% more nitrogen (1.7 g/d). These results are consistent with previous reports based on animal studies [1,34,35], as well as in humans where the consumption of QPM by children resulted in a 12% increase in weight [34] and a 9% increase in height and weight [36].

#### Ileal digestibility

The average AA profile of the proteins in the QPM was different from that of yellow and white maize. QPM had more lysine (45%), arginine (37%), histidine (31%), glycine (23%), methionine (19%), threonine (13%), aspartic acid (13%), valine (10%), and cystine (7%) than white and yellow maize. In contrast, QPM had less leucine (-23%), alanine (-14%), phenylalanine (-13%), glutamic acid (-8%),

tyrosine (−8%), and proline (−7%) than white and yellow maize. Other studies have also reported that QPM is rich in lysine [5,37,38], while the low leucine and proline content is associated with a decrease in zein protein [6].

Dietary protein content affects apparent digestibility [16,39,40]. To avoid this effect, the experimental diets were iso-nitrogenous, resulting in similar protein digestibility in all of the diets. However, the differences in amino acid content may explain the differences in digestibility. It has been previously reported that the high lysine content in QPM results in a higher AID [34,35], as was found in the current study. Similarly, the high leucine, phenylalanine, glutamic acid, alanine, tyrosine, and proline content of normal maize (white and yellow) could explain the high AID that was observed. Moreover, the low AID observed for threonine may be caused by its richness within the endogenous protein [22,41,42].

Although the use of the estimate from one endogenous protein in each experiment has been recommended to estimate the SID [39,40], it is also true that the SID has been estimated from previously published AID data and corrected with an endogenous protein that was estimated later [9,43,44] or previously [45,46]. This supports the use of a “standard” endogenous protein to correct the AID. The SID removes the effect of nutrient level on the digestibility value by correcting for basal endogenous losses [39,43,47]. The SID of lysine was similar in all of the maize diets. Additionally, when SID was estimated, threonine reached similar values to those of the other amino acids; this may be related to its richness within the endogenous protein. The SID coefficients estimated for maize in the present work were similar to those reported in previous studies [8,9,43]. However, no values for SID have been reported previously for QPM.

## Conclusions

The energy furnished by QPM was used less efficiently (metabolizability) than the energy furnished by normal maize. The AID of lysine was higher in the QPM than in the normal maize; however, the SID of lysine was similar between QPM and normal maize. The current study provides additional data about the nutrient composition, AA digestibility, and nitrogen utilization of QPM.

## Abbreviations

CIMMYT: International maize and wheat improvement centre; QPM: Quality protein maize; EF-1  $\alpha$ : Elongation factor 1 $\alpha$ ; DM: Dry matter; DE: Digestible energy; ME: Metabolizable energy; AA: Amino acid; ADF: Acid detergent fiber; AID: Apparent ileal digestibility; SID: Standardized ileal digestibility; ATTD: Apparent total tract digestibility.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

GML conceived and designed the study; ERR carried out the lab analysis; TCRS contributed to data analysis; GML and TCRS wrote the manuscript. All authors read and approved the final manuscript.

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