Addition of an enzymatic complex to a sorghum mixture with different granulometries and its effect on nitrogen digestibility and metabolizable energy of birds

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In order to evaluate the effect of the addition of an enzymatic complex in a sorghum mixture on the true digestibility of nitrogen (TND) and metabolizable energy (TME) of roosters, an experiment with 56 roosters of Bovans Brown breed were used. Three levels of granulometry (G) were established (1, 3 and 5 mm). Six treatments were developed: T1) sorghum mixture without enzymatic complex + G 1 mm; T2) sorghum mixture with enzymatic complex + G 1 mm; T3) sorghum mixture without enzymatic complex + G 3 mm; T4) sorghum mixture with enzymatic complex + G 3 mm; T5) sorghum mixture without enzymatic complex + G 5 mm and T6) sorghum mixture with enzymatic complex + G 5 mm. A completely randomized design, with a factorial arrangement of 3 x 2 and 8 roosters (repetition) per treatment, was used. There were no statistical differences for factors of granulomtry and presence or not of the enzymatic complex in the TME (16.04 MJ/kg DM). For TND, there was interaction with the between the enzimatic complex and the granulometry ($P < 0.001$). T4 had the highest digestibility (95.82 ± 1.66 %) and T2 had the lowest (83.43 ± 2.71 %). It can be concluded that adding the enzymatic complex do not affect the evaluated variables. However, it is recommended to use the sorghum mixture with granulometry of 3 mm, because it maintains the TME and increases the TDN.

Key words: exogenous enzymes, diets, rosters, particle size, tannins

Feeding birds in some Latin American countries like Venezuela have had an intake pattern based on cereals (maize), with scarce and low productivity. Consequently, there is a marked external dependence. It is important to consider that, in Venezuela, diets for poultry production are processed through the importing of their main basic ingredients, like maize and soy. High prices of these essential ingredients for diets and demands of the current poultry production lead to search for nutritional alternatives that allow to take advantage of the production of other available cereals. Grain sorghum (*Sorghum bicolor* L. Moench.) is a widely used agricultural crop, with perspectives and high adaptability to different climatic conditions (Sandoval and Valencia 2005 and Chuck-Hernandez *et al.* 2011). However, it presents condensed tannins (CT) among its chemical constituents. They have the responsibility of reducing the nutritional value of the grain, when it is used in diets for non-ruminant animals (Jaramillo 2008 and Farfán 2010).

Under these conditions, it is necessary to study compounds that allow, somehow, to stop or diminish the antinutritional factors of raw matters. This is the case of exogenous enzymes, which help with food digestibility, like the enzymatic complex, obtained through the technique of fermentation in solid state (Selle and Ranvindran 2008, Méndez *et al.* 2009 and Selle *et al.* 2010).

Another alternative for an efficient use of sorghum is the variation of grain granulometry. This procedure is based on studies that confirm the benefits of grinding cereals in grains. According to Kilburn and Edwards (2004) and Mateos *et al.* (2005), the beneficial effect of grinding is more important with ingredients like sorghum because its external fibrous protection is difficult to degrade in the gizzard and to be affected by endogenous enzymes of gastrointestinal system.

The objective of this study was to evaluate the addition of an enzymatic complex to a sorghum mixture at three levels of granulometry in the TDN and TME.

Materials and Methods

The research was carried out in the facilities of the Instituto de Producción Animal, in the Laboratorio Sección de Aves, from the Facultad de Agronomía at the Universidad Central de Venezuela. This institution is located in Maracay, Aragua state, at 10º 17´ 5" N, and 64º 13´ 28" W, at 480 m o.s.l. Its mean temperature is 25 ºC and its relative humidity is 75 % (INIA 2012).

An amount of 56 roosters, from Bovans Brown breed and 36 weeks old, were used, according to methodology described by Sibbald (1976). Roosters were kept in individual metallic cages, of 43 cm high, 47 cm deep and 26 cm wide. These cages had a cup drinker and a tray for collecting feces. Their dimensions were 35 x 55 cm, to assure an effective collection of feces. A completely randomized design was used, with a factorial arrangement of 3 x 2, corresponding to the treatments applied (sorghum mixture with three levels of granulometry 1, 3 and 5 mm) and presence or not of the enzymatic complex. Seven groups were established. Six groups corresponded to the applied treatments and another group was created with roosters for collecting feces generated by the endogenous, with the objective of quantifying fecal metabolic excretion. Eight roosters were used per treatment, and each rooster represented an experimental unit.

For preparing treatments, grains of sorghum mixture from Criollo I, Chaguarama III, Himeca 101, and Himeca 400 varieties were used. These are kept in silos of industrial storage of raw matters for livestock use. A sample of grains of sorghum mixture was taken to determine their chemical composition (table 1), according to AOAC (2000). Grains were ground in a Nogueira® mil, DPM 4 model, with three levels of granulometry (1, 3 and 5 mm). The enzimatic complex was produced through fermentation in solid state and was directed, mainly, to the rupture of non-starch polysaccharide chains, with a composition reported by the manufacturer: 300 units of phytase/g, 100 units of xylanase/g, 700 units protease/g, a minimum of 40 units of cellulase/g, 30 units of amylase/g, 4,000 units of pectinase/g and a minimum of 200 units of β-glucanase/g. The recommended addition of CE to the diets was 0.02 % (Allzyme SSF®. Alltech Inc., Kentucky USA).

Table 1. Chemical composition of sorghum mixture used in treatments applied to roosters

Variables	Sorghum mixture
Dry matter (%)	88.41
Crude protein (%)	10.96
Gross energy (MJ/kg)	16.61
Gross fat (%)	3.20
Crude fiber (%)	2.54
Ash (%)	1.23
Phosphorous (%)	0.23
Condensed tannins (% CE)	0.74

For managing excretions and determining true digestibility of nitrogen (TDN) and true metabolizable energy (TME), a diet was considered for the group of endogenous roosters, composed by maize starch (48 %), sugar (48 %) and a pre-mixture of minerals and vitamins (4 %) (Bourdillon *et al.* 1990). Total collection of feces per experimental unit was carried out in trays for 48 h. Later, they were placed in aluminum trays, where they were weighed and dried in an oven at 65 ºC for 48 h (Sibbald 1976). Feces were ground in a Thomas® lab mil with a sieve of 1 mm. Gross energy was determined in an adiabatic calorimetric pump, Parr® brand. Total nitrogen was determined according to Kjeldahl (AOAC 2000), while the uric nitrogen was determined according to the method of direct spectrophotometry, described by Marquardt (1983). Once the GE and excreted nitrogen without uric nitrogen (total nitrogen-uric nitrogen) from the used diets were determined, TDN and TME of each treatment were calculated regarding the following formulas:

Determination of TDN:

$$\text{TDN (\%)} = \frac{\text{Nc (g)} - [\text{ENWUN (g)} - \text{FMN (g)}]}{\text{Nc (g)}} \text{x100}$$

where:

Nc: nitrógeno consumido

ENWUN: excreted nitrogen without uric nitrogen

FMN: fecal metabolic nitrogen, quantified trough rooster established for collecting the endogenous.

Determinacion of TME, according to Sibbald (1978):

$$\text{TME} = \frac{(\text{IDM x GEfo}) - [(\text{excDM x GEfe}) - (\text{endDM x EGEendx})]}{\text{IDM}}$$

Where:

IDM: ingested dry matter

excDM: excreted dry matter

endDM: dry matter excreted by the endogenous

GEfo: gross energy of food

GEfe: gross energy of feces

EGEendx: excreted gross energy, quantified trough the roosters established for collecting the endogenous.

Data of evaluated variables (TDN and TME) were analyzed according to a completely random design, with a 3 x 2 factorial arrangement and 8 roosters (repetitions) per treatment. The Statistic program, version 8.0, was used. Values for each variable were expressed through the means and their standard errors. Statistical differences were considered at the < 0.05 probability level. The mean test was applied according to the Tukey procedure (Steel *et al.* 1997).

Results and Discussion

There was no effect of factors and interaction after analyzing the TME of a sorghum mixture, with different granulometries and the addition of an enzymatic complex (table 2). The maximum value was 16.07 MJ/kg DM and the minimum was 15.51 MJ/kg DM. According to these results, there was no variation of the energy with the addition of the enzymatic complex. This additive has the ability of increasing the energy contribution in cereals and, specially, in raw matters with secondary metabolites (Selle and Ranvindran 2008 and Méndez *et al.* 2009). The TME in roosters did not vary with the used of granulometries, considering that using a thin one (1 mm), there is no better exposure to the surface of sorghum mixture for the endogenous and exogenous enzymes to act on, with the objective of improving the energy metabolism of the birds. According to studies of Kilburn and Edwards (2004) and Mateos *et al.* (2005), a granulometry higher than 5.61 mm in poultry production is, probably, more convenient than smaller sizes, because gross particles can be retained in the gizzard for more time than the fine ones. A superior time of retention in the proximal portion of the digestive tract favors the solubility of particles and the later enzymatic access (Mateos and Grobas 1993). This way, an excessively fine grinding can reduce digestive motility and contact between nutrients and enzymes (Mateos *et al.* 2005). Therefore, there could also be a reduction of TME in finely ground diets. This fact was demonstrated by Capdevila (1993), who obtained values of TME for diets in meals and granules of 13.31 and 13.74 MJ/kg, respectively.

In similar studies, Tavernari *et al.* (2008) did not found significant effects with the addition of the enzymatic complex in sunflower meals. The same was reported by Fuente *et al.* (1995), who evaluated an enzymatic complex in diets based on barley. These authors referred that the TME had no significant improvement. This response could be regulated by an effect of contact time among diet, exogenous enzyme and adequate substrate on diet for the enzymatic complex to act properly. In other studies, Wu *et al.* (2004) evaluated a phytase of fermentation in solid state in a diet for poultry and reported that the effect of the enzyme on apparent metabolizable energy was not significant. In addition, Fuente *et al.* (1995) assessed an enzymatic complex on diets based on barely, and the metabolizable energy had no significant improvements. Olukosi *et al.* (2007), in a similar study, stated that there was no effect of TME when using an enzymatic complex in diets based on maize and soy. Therefore, these authors concluded that there could be a deficiency on the action of the complex because there was no additive effect of enzymes that were part of it.

The previous results evidence that energy present in the sorghum mixture, used in this study, is not totally available when adding the enzymatic complex, probably due to the content of tannins, which form complexes with the ability of inhibiting the activity of amylase and compromise the starch digestibility (Taylor 2005 and Pérez-Maldonado and Rodríguez 2007). This study coincides with the results reported by Selle *et al.* (2010), who referred that there is a negative correlation between the metabolizable energy and the concentration of tannins from sorghum grains, because the varieties of sorghum with 0.45 %, catechin equivalents (CE), decrease the ME of the grain in 3 %, regarding diets with sorghum grains of 0.15 % CE. This study confirmed a similar performance when it included diets with sorghum mixtures and tannin contents of 0.74 % CE.

Regarding the TDN in roosters, there were statistical differences ($P < 0.01$) for different granulometries and the interaction between granulometry and enzymatic complex ($P < 0.001$). T4 had the highest TDN, corresponding to the granulometry of 3 mm, and T2 presented the lowest TDN. There was a difference of 12.39 % of digestibility among the treatments. This agrees with the analysis of granulometry simple factor, where the highest TDN is for MSO in 3 mm, with 93.31 %, being statistically different to the MSO, with 1 mm (88.31 %). It is possible that the diet flow within the animal would increased with the lowest granulometry (1 mm) and there is less activity in the gizzard and proventriculus for beginning to degrade nutrients of raw matters, which negatively affects digestibility. The presence of the enzymatic complex do not represented statistical differences. Out of these results, it is evident that the sorghum mixture with 3 mm of granulometry favors digestibility and confirms the reports of Wiseman (1993), who refers that diminishing the granulometry through grinding, a nutritional benefit is obtained, if the decrease is considered between 5 mm and 3 mm. Mateos and Grobas (1993) expressed that the reduction of particle size, reached after grinding, has low nutritional value for birds. This effect is observed with granulometry of 1 mm.

Values of TDN in roosters obtained in this study were slightly superior to those reported by Farfán (2010). This author obtained a TDN of 78.81 and 81.52 % in diets with 100 % of sorghum mixture without enzymatic complex and 100 % of sorghum mixture with enzymatic complex, respectively. Likewise, there was no significant variation in the addition of the enzymatic complex. Similarly, Jaramillo *et al.* (1991) obtained 55 % of TDN of sorghum. This value was affected

Table 2. True metabolizable energy in treatments applied to roosters

	TME (MJ/kg DM)
Granulometry (G)	
1 mm	15.79
3 mm	16.07
5 mm	15.74
SE±	0.22
Presence of enzymatic complex	
With enzymatic complex	15.68
Without enzymatic complex	16.01
SE±	0.18
Probability	
Granulometry	0.6780
Presence of enzymatic complex	0.2047
Granulometry by presence of enzymatic complex	0.7454

Table 3. True digestibility of nitrogen (TDN) in treatments provided to roosters

Treatments	TDN (%)
T1 = sorghum mixture without enzymatic complex + G 1 mm	93.19[a]
T2 = sorghum mixture with enzymatic complex + G 1 mm	83.43[b]
T3 = sorghum mixture without enzymatic complex + G 3 mm	90.81[ab]
T4 = sorghum mixture with enzymatic complex + G 3 mm	95.82[a]
T5 = sorghum mixture without enzymatic complex + G 5 mm	91.99[ab]
T6 = sorghum mixture with enzymatic complex + G 5 mm	92.31[ab]
SE	1.66***

[ab] Different letters express statistical differences. *** ($P < 0.001$)

by the content of tannins within the sorghum, which was considered as high. After evaluating the TDN is important to consider the levels of condensed tannins, because, according to Latorre and Calderón (1998), there is a considerable decrease, when comparing a low level of condensed tannins (0.17 % CE) with a high level (3.24 % EC), obtaining a TDN of 69.54 and 50.85 %, respectively. Ravindran *et al.* (1999) evaluated the addition of phytase to diets based on sorghum, and the digestibility improved. Something similar happened in studies of Jiménez (2000), in which the enzymatic supplementation of diets based on sorghum and maize increased the fecal apparent digestibility of crude protein. Wayne and Xiuhua (2010) indicated that adding the protease and phytase+protease to diets based on sorghum increases the TDN in 82 and 83 % respectively, and there is an evident effect, as it was expected in this study, regarding the use of enzymatic complex.

Under the conditions of this experiment, the different granulometries used and the addition of the enzymatic complex did not affect the true metabolizable energy. However, there was effect of the granulometries and their interaction with the enzymatic complex on the true metabolizable energy. Therefore, it is recommended the use of the sorghum mixture with granulometries of 3 mm, because it maintains the true metabolizable energy and increases nitrogen digestibility.

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