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Corn and Soybean Meal Metabolizable Energy with the Addition of Exogenous Enzymes for Poultry

■ Author(s)

Dourado LRB[1,2]
Sakomura NK[2]
Barbosa NAA[2]
Bonato MA[2]
Kawuauchi IM[2]
Fernandes JBK[3]
Costa FGP[4]

[1] Universidade Federal do Piauí – Campus: Profª Cinobelina Elvas
[2] Departamento de Zootecnia Faculdade de Ciências Agrárias and Veterinárias -UNESP. Jaboticabal, SP, Brasil. E-mail: sakomura@fcav.unesp.br
[3] Centro de Aqüicultura da UNESP. Jaboticabal, SP, Brasil.
[4] Universidade Federal da Paraíba - Convênio PROCAD UNESP. Jaboticabal-UFPB. Areia, PB, Brasil.

■ Mail Address

Leilane Barros Dourado
Universidade Federal do Piauí
Campus Profa. Cinobelina Elvas.
BR 135, Km 03, Planalto Horizonte
64.900-000. Bom Jesus, PI, Brasil.

E-mail:leilane@ufpi.br

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ABSTRACT

Two metabolism assays were carried out to determine corn and soybean meal metabolizable energy when enzymes were added. In the first trial, 35 cockerels per studied feedstuff (corn and soybean meal) were distributed in a completely randomized experimental design with four treatments of seven replicates of one bird each. The evaluated treatments were: ingredient (corn and soybean meal) with no enzyme addition, with the addition of an enzyme complex (xylanase, amylase, protease - XAP), xylanase, or phytase. Precise feeding method was used to determine true metabolizable energy corrected for nitrogen balance (TMEn). The use of enzymes did not result in any differences ($p>0.05$) in soybean meal TMEn, but phytase improved corn TMEn in 2.3% ($p=0.004$). In the second trial, 280 seven-day-old broiler chicks were distributed in a completely randomized experimental design with seven treatments of five replicates of eight birds each. Treatments consisted of corn with no enzyme addition or with the addition of amylase, xylanase, phytase, XAP complex, XAP+phytase combination, or xylanase/pectinase/β-glucanase complex (XPBG). Corn was supplemented with macro and trace minerals. Total excreta collection was used to determine apparent metabolizable energy corrected for nitrogen balance (AMEn). Differences were observed ($p=0.08$) in AMEn and dry matter metabolizability coefficient ($p=0.03$). The combination of the XAP complex with phytase promoted a 2.11% increase in corn AMEn values, and the remaining enzymes allowed increased between 0.86% and 1.66%.

INTRODUCTION

The use of enzymes has been proposed to improve nutrient digestibility in poultry diets. Most studies have determined the effect of enzymes on dietary energy (Zanella *et al.*, 1999; Douglas *et al.*, 2000; Kocher *et al.*, 2003; Brito *et al.*, 2006; Olukosi *et al.*, 2007), but just a few were carried out to determine the effect of enzymes on ingredients. The knowledge of the nutritional value of ingredients with the addition exogenous enzymes is essential to establish their nutritional matrix.

Among the methods used to determine ingredient metabolizable energy (ME), the total excreta collection, with the use of a test diet and a reference diet were the most used (Sakomura Rostagno, 2007). However, this technique does not allow the determination of the effect of enzymes exclusively on feed ME, as not only the tested feedstuff, but the entire feed may suffer the action of the enzyme, making it difficult to establish the changes caused in the feedstuff only.

When evaluating individual ingredients using total excreta collection technique with chicks, it is difficult to obtain correct estimations, as the deficiency or excess of some nutrients could be limiting. On the other hand, the technique of precise feeding of cockerels, supplying an exact amount of the ingredient directly in the crop of fasted birds, allows energy determination. Also, the supply of small amounts of a pure ingredient for a short period of time allows isolating the effect of enzymes on the specific substrates of each ingredient. Therefore, the determination of feedstuff energy by the addition of enzymes may be used to improve poultry diet formulation. Bedford (2002) reported the importance of adjusting information relative to the effect of enzymes on nutrient availability or performance, allowing building models that can predict animal responses and a more economical feed formulation.

The objective of this study was to evaluate the effect of the addition of exogenous enzymes on corn and soybean meal metabolizable energy for chickens.

MATERIAL AND METHODS

Two metabolic trials were carried out at the Poultry Sector of the Animal Science Department of the School of Agrarian and Veterinary Sciences (FCAV), UNESP, Jaboticabal, SP, Brazil. The first trial was conducted with cockerels using precise feeding technique, and the second one with broilers chicks, using total excreta collection.

In the cockerel trial, 35 intact Hy-line cockerels were used for each studied ingredient (corn and soybean meal). Birds were distributed in a completely randomized experimental design, with four treatments of seven replicates of one cockerel each. A group of seven cockerels was fasted to determine endogenous losses. Each food constituted an independent assay.In the beginning of the trial, cockerels were individually weighed and distributed to allow similar replicate average weight (2.007±0.043kg). Corn and soybean meal were supplied with or without addition of exogenous enzymes. The following treatments were applied: T1- Ingredient (corn or soybean meal) with no enzyme addition; T2- Ingredient + XAP complex (xylanase, amylase, and protease - 200g/ton); T3- Ingredient + xylanase (100g/ton); T4- Ingredient + phytase (100g/ton).

Birds were submitted to forced feeding by placing a funnel directly in the crop. Ingredients were supplied twice daily: 20g at 07:00am and 20g at 05:00pm. Excreta collecting bags were coupled to a plastic ring fixed in the cloaca after the first supply. Excreta were collected twice daily, and for 48 hours after the last supply. Excreta were weighed, frozen, freeze-dried, ground, and had their nitrogen, gross energy, and dry matter analyzed to calculate true metabolizable energy corrected for nitrogen balance (TMEn), according to Sakomura & Rostagno (2007).

In the chick trial, seven-day-old male Cobb® broilers were distributed in a completely randomized experimental design with seven treatments of five replicates of eight birds each, and housed in metabolic cages. In the beginning of the trial, chicks were individually weighed and distributed to allow similar replicate average weight (138.77±0.51g).

At the corn evaluated corn was evaluated, dicalcium phosphate (1.20%), limestone (1.18%), salt (0.405%), coccidiostat (0.05%), and vitamin (0.10%) and mineral (0.05%) supplements were added in order to supply minimum available phosphorus (0.300%), calcium (0.780%), and sodium (0.180%) levels, as well as adequate vitamins and minerals. Corn was the only energy source with specific substrates for enzymes. The evaluated enzymes were purchased from Danisco Animal Nutrition and were added to corn at the following ratios: T1- Corn with no enzyme addition; T2- Corn with amylase (500g/ton); T3- Corn with xylanase (500g/ton); T4- Corn with phytase (100g/ton); T5- Corn with XAP (xylanase, amylase, and protease - 500g/ton); T6- Corn with XAP (500g/ton)+ phytase (100g/ton); T7- Corn with XPBG (xylanase, pectinase and β-glucanase, 500g/ton).

The method used was total excreta collection. Diets were offered ad libitum for eight days (7 to 14 days of age), with the first four days for diet adaptation, and four for excreta collection. Feeds were weighed in the beginning of the experiment, and feed residues were weighed at the end in order to determine feed intake.

The excreta collected from each experimental unit were weighed, frozen, dried in a force-ventilation oven at 55ºC for 76 hours, and subsequently submitted to nitrogen content, gross energy, and dry matter analyses to calculated apparent metabolizable energy corrected for nitrogen balance (AMEn) and dry matter metabolizability (DMM), according to Sakomura & Rostagno (2007). Ingredient chemical composition and fiber content were also determined.

The data obtained in both trials were submitted to homogeneity and normality analyses. Identified outliers were removed, and data were then submitted to analysis of variance using GLM procedures of SAS, and means were compared by the Duncan's Test at 10% significance level.

RESULTS

Soybean meal and corn TMEn results obtained in the cockerel trial are shown in Table 1. There was no effect (p>0.05) of enzyme addition on soybean meal TMEn. In corn, a significant increase of 95kcal/kg in TMEn was observed with phytase addition as compared to corn with no supplementation. The addition of XAP or xylanase did not influence corn TMEn.

AMEn values and dry matter metabolizability coefficients (DMMC) obtained in the chick trial are presented in Table 2.

Table 1 - Analysis of variance of true metabolizable energy corrected for nitrogen balance (TMEn) of soybean meal and corn with enzyme addition (mean values ± standard deviation).

Treatments	TMEn (kcal/kg de DM)	
	Soybean meal	Corn
Ingredient	3151±96	4011±25 BC
Ingredient + XAP[1]	3186±89	4055±32 AB
Ingredient + xylanase	3011±159	3950±75 C
Ingredient + phytase	3141±102	4106±56 A
Probability[2]	0.2053	0.0044
CV (%)	3.67	1.23

1 - Complex xylanase, amylase, and protease. 2 - Means followed by the same letter in the same column are not different by the Duncan's Test (p>0.10).

Table 2 - Analysis of variance of apparent metabolizable energy corrected for nitrogen balance (TMEn) and dry matter metabolizability coefficient (DMMC) of corn with enzyme addition (mean values ± standard deviation).

Tratamentos	AMEn (kcal/kg de DM)	DMMC (%)
Corn	3504±55 B	77.86±0.81 B
Corn + amylase	3548±17 A	78.89±0.38 A
Corn + xylanase	3557±43 A	78.98±0.85 A
Corn + phytase	3557±21 A	79.06±0.51 A
Corn + XAP[1]	3562±36 A	79.67±1.31 A
Corn + XAP + phytase	3578±31 A	79.54±0.56 A
Corn + XPBG[2]	3534±27 AB	78.81±0.53 A
Probability[3]	0.080	0.023
CV (%)	0.999	0.905

1 - Complex xylanase, amylase, and protease; 2 - Complex xylanase, pectinase, and β-glucanase 3 - Means followed by the same letter in the same column are not different by the Duncan's Test (p>0.10).

All enzymes improved (p<0.05) dry matter metabolizability coefficient, indicating better nutrient utilization by the birds. For AMEn, the addition of the combination XAP + phytase, XAP, phytase, xylanase, and amylase promoted improvements (p<0.10) of 2.11%, 1.66%, 1.51%, 1.51%, and 1.26% in corn AMEn, representing increases of 74, 58, 53, 53, and 44 kcal/kg, respectively. The addition of XPBG improved corn AMEn in 0.86% (30kcal/kg); however, this result was not statistically different from the corn with no supplementation or the other supplemented diets.

The chemical analysis of the ingredients used in both trials is shown in Table 3.

Table 3 - Chemical composition of the ingredients used in both trials.

Ingredients Composition (%)[1]	Corn		Soybean meal[2]
	Trial with cockerels	Trial with chicks	
Dry matter (DM)	86.65	87.53	89.45
Gross energy (Kcal/kg)	3956	3798	44.55
Crude protein (PB)	8.56	9.59	45.24
Fat	2.58	4.32	1.12
NDF	10.35	11.54	13.51
ADF	1.91	2.09	6.51
Celullose	1.51	1.60	5.93
Hemicelullose	8.44	9.45	7.01
Lignin	0.39	0.49	0.57

1 - Values "natural matter". 2 - Trial with cockerels.

DISCUSSION

Phytase improves energy availability of feedstuffs and feeds, as demonstrated in several studies (Kornegay, 2001; Cowieson Adeola, 2005; Olukosi *et al*., 2007; Barbosa *et al*., 2008; Santos *et al*., 2008). This enzyme acts on the molecule phytate, which may complex with cations, carbohydrates, enzymes and amino acids, and inhibits the activity of several digestive enzymes, such as pepsin, α-amylase, and trypsin mainly due to the chelation of phytate with calcium ions (Ca^{++}), which are essential for the activity of these enzymes (Lima, 2005).

The action of phytase on phytate may release starch, enzymes, enzyme co-factors, proteins, and minerals, which will be better digested and absorbed by the birds, consequently improving the use of energy by the birds.

Phytase addition improved only corn TMEn. Managi Coon (2006), working with semi-purified diets and soybean meal as the only vegetable ingredient, and did not find significant differences in energy retention when adding phytase.

Olukosi *et al*. (2007) found that phytase effect on energy availability when dietary metabolizable energy is limiting, suggesting that the lower energy availability of soybean meal as compared to corn in the present study may have interfered with phytase action. Cowieson *et al*. (2006) reported that phytase addition in absence of substrate reduced dry matter and nitrogen digestilibility probably because it stimulated endogenous losses and nitrogen catabolism.

The addition of the XAP complex in the cockerel trial increased corn TMEn in 1.1%, but it was not sufficient to promote significant improvements. On the other hand, in the chick trial, AMEn increased when this enzyme complex was added. Olukosi *et al*. (2007)

reported that phosphorus deficiency can limit energy utilization when carbohydrases (xylanase) are added. This may explain the response to the addition of the enzyme complex only in the trial with chicks, which diet was supplemented with dicalcium phosphate. According to Ávila *et al*. (2006), the addition of vitamin and mineral supplements in assays to determine feedstuff energy must be adjusted to obtain more precise values.

However, the differences in the responses between the two trials may be related to the employed method and bird physiology, as the enzyme system of young birds is still immature, and therefore use exogenous enzymes to better utilize dietary nutrients, resulting in lower energy cost to activate endogenous enzymes, thereby enhancing feedstuff ME.

According to the results obtained in the total excreta collection trial with chicks, enzyme addition significantly increased corn AMEn. The best relative increase (2.11%) was obtained with XAP and phytase combination, which may be explained by the release of nutrients that are embedded in the cell wall by the action of xylanase and consequent better utilization of the other compounds released by the action of phytase, amylase, and protease. According to Olukosi *et al*. (2007), glycosidases are capable of breaking down the layer of non-starch polysaccharides (NSPs) of the cell membrane, allowing the access of phytase to the phytate stored in the cell wall membrane. However, when phytase is used alone, its capacity is limited due to the lack of access to its substrate, which is embedded inside the NSP matrix. In addition, some soluble fiber-phosphorus bonds may be hydrolyzed by glycosidases (xylanase), releasing that mineral for animal utilization, thereby improving animal energy metabolism. Some studies have shown nutrient digestibility and broiler performance improvement with the dietary addition of XAP + phytase (Cowieson & Adeola, 2005; Olukosi *et al*., 2007; Barbosa *et al*., 2008).

The use of xylanase may have beneficial effects as it promotes changes in the cell wall architecture by hydrolyzing the structural arabinoxylans that encapsulate nutrients, enhancing their utilization (Sheppy, 2001; Gracia *et al*., 2003; Yu & Chung, 2004; Hruby & Pierson

, 2005; Lima, 2005; Olukosi *et al*., 2007). Cowieson (2005) stressed that the effect of xylanase can be better evidenced when associated with other exogenous enzymes, such as protease, amylases, and phytase.

The addition of amylase to diets aids the hydrolysis of starch amylose and amylopectin, improving starch digestion in the small intestine and enhancing nutrient use, with consequent higher growth rates (Sheppy, 2001), as it complements the activity of endogenous amylase, reducing its endogenous synthesis by the pancreas (Gracia *et al*., 2003), and therefore saving energy, which becomes more available for growth.

The absence of a significant response to the addition of the complex xylanase, pectinase, and β-glucanase may be related to the low availability of their substrate, as corn has low pectin levels (Malathi & Devegowda, 2001) and corn β-glucan levels have not been reported or are negligible (Knudsen, 1997; Choct, 2006).

In the present study, it was observed that the energy values obtained for corn were different between the two trials. This may be related to the applied methods and to bird age. ME determination techniques have changed throughout the years (Avila *et al*., 2006). The most commonly used methods to determine ME in poultry feedstuffs are total excreta collection and forced feeding, and each has its advantages and disadvantages (Sakomura & Rostagno, 2007).

Due to the problems in isolating enzyme effects, particularly on feedstuffs such as corn or soybean meal, the methods applied in the present study produced results that may contribute to define a nutritional matrix of these ingredients when exogenous enzymes are used. Therefore, the choice of the method must be based on the purpose of the enzymes and the birds' needs for those enzymes, as young animals have different digestive systems and different requirements as compared to adults, and therefore the use of exogenous enzymes may also be different. Therefore, the methods used in the present study can be applied to evaluate metabolizable energy increase in the diets supplemented with exogenous enzymes.

CONCLUSIONS

The addition of phytase, xylanase, amylase, and protease was efficient to increase corn apparent metabolizable energy. No increase in true metabolizable energy of soybean meal was evidenced with the addition of the tested enzymes.

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