Impact of combined xylanase and β -glucanase enzymes on digesta transit time, short-chain fatty acids and caecal thermal profile of broilers fed corn-soy-based diets

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Complete List of Authors:	<p>Pasquali, Guilherme; São Paulo State University, Department of Breeding and Animal Nutrition</p> <p>Santos, Tatiane; São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Department of Breeding and Animal Nutrition</p> <p>Zanetti, Leonardo; Universidade Estadual Paulista - UNESP, Melhoramento e nutrição Animal</p> <p>Dornelas, Livia; São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Department of Breeding and Animal Nutrition</p> <p>Cruvinel, Jéssica; São Paulo State University, Department of Breeding and Animal Nutrition</p> <p>Muro, Everton; Universidade Estadual Paulista Julio de Mesquita Filho - Campus de Botucatu, Breeding and Animal Nutrition</p> <p>Araújo, Robert; Universty Estadual Paulista - UNESP, Animal Science</p> <p>Ferreira Netto, Raimundo; São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Department of Breeding and Animal Nutrition</p> <p>Denadai, Juliana Célia ; São Paulo State University</p> <p>Pezzato, Antonio; Universidade Estadual Paulista Julio de Mesquita Filho - Campus de Botucatu, Breeding and Animal Nutrition</p> <p>Sartori, José Roberto; São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Department of Breeding and Animal Nutrition</p>
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Impact of combined xylanase and β -glucanase enzymes on digesta transit time, short-chain fatty acids and caecal thermal profile of broilers fed corn-soy-based diets

Guilherme A. M. Pasquali^{1*}, Tatiane S. dos Santos¹, Leonardo H. Zanetti¹, Livia C. Dornelas¹,
Jéssica M. Cruvinel¹, Everton M. Muro¹, Robert G. A. C. Araujo¹, Raimundo G. F. Netto¹,
Juliana C. Denadai¹, Antonio C. Pezzato¹, José R. Sartori¹

¹Department of Breeding and Animal Nutrition, College of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, São Paulo 18618-970, Brazil.

*Corresponding author: ogamp@msn.com

Abstract: A study was conducted to evaluate the effects of including a xylanase + β -glucanase enzyme product in corn-soy-based diets on performance, caecal short-chain fatty acids and thermal profile, ileal digestibility and intestinal kinetics of broiler chickens. A total of 744 d-old male chicks were randomly allotted to 24 floor pens and distributed in a 2×2 factorial arrangement with low or standard energy level $\times$ without or with 100 grams of the enzyme per ton of feed. Enzyme supplementation improved bodyweight gain from 1 to 21 d ($P < 0.05$). An increased caecal concentration of acetic acid was observed when the enzyme was added to the low-energy diet ($P < 0.05$). The pH of the caecal content was reduced ($P < 0.01$) and the caecal temperature was increased by low-energy diets ($P < 0.05$). The apparent ileal digestibility of energy was improved by the enzyme ($P < 0.01$). The addition of the enzyme increased the mean retention time in the distal ileum ($P < 0.05$). In summary, the addition of a xylanase + β -glucanase enzyme product in corn-based diets increases the retention time of digesta in the distal ileum and the caecal acetic acid concentration, improves ileal digestibility of energy and performance from 1 to 21 d in broiler chickens fed corn-soy-based diets.

Keywords: Carbohydrase, NSP-enzymes, Thermography

Introduction

The inclusion of non-starch polysaccharides degrading enzymes (NSP-enzymes) such as xylanase and β -glucanase in broiler diets is a common practice in the poultry industry, particularly in diets based on cereal grains rich in soluble NSPs, such as barley and rye (Viveros et al., 1994; Shakouri et al. 2009). More recently, studies investigating the use of NSP-enzymes in corn-based diets have been extensively published by researchers. However, there is a certain lack of consistency when it comes to positive responses on performance and nutrient digestibility in studies where NSP-enzymes are included to corn-based diets (Cengiz et al., 2012; Coppedge et al., 2012; Gehring et al., 2013). One of the key factors involved in the responsiveness of NSP-enzymes is the energy level of the diet and the amount of added fat. A lack of benefits can be observed when the dietary fat and energy level is high or not appropriately adjusted (Zakaria et al., 2008; Zhou et al., 2009). The inherent digestibility of nutrients such as starch, protein and lipid can also play a role in the response to NSP-enzymes, as high digestible diets, such as corn-based diets, tend to respond less to a xylanase/ β -glucanase supplementation (Cowieson, 2010).

There are three main mechanisms of action attributed to NSP-enzymes (viscosity reduction, cell wall degradation and prebiotic effect) but the prebiotic mechanism is probably the most relevant mode of action by which NSP-enzymes play a role in the responses observed in poultry (Bedford, 2018).

Xylanase can breakdown arabinoxylans into arabinoxyloligosaccharides which can be fermented by commensal bacteria present in the gut, change the short-chain fatty acid profile in the caeca (Courtin et al., 2008) and stimulate the release of peptide YY (Singh et al., 2012), a gut hormone that can slow down intestinal transit and gastric emptying (Taylor, 1993).

Therefore, this study investigated the effects of a xylanase/ β -glucanase enzyme product on the intestinal and caecal characteristics, ileal digestibility and performance of broilers fed energy-varying corn-based diets.

Materials and Methods

Birds were cared for in accordance with the guidelines established by São Paulo State University Ethical Committee for Animal (02/2015-CEUA).

Experimental design and dietary treatments

The trial was completely randomized in a 2×2 factorial design - standard or low metabolizable energy $\times$ inclusion or not of an enzyme product (Natugrain TS[®], BASF Germany) composed by endo-1,4- β -xylanase (5600 TXU/g) and endo-1,4- β -glucanase (2500 TXU/g), produced by *Aspergillus niger*. Standard energy diets were formulated to contain 3025 (1 to 21d) and 3125 kcal of AMEn/kg (22 to 35d) whereas low energy diets contained 2925 (1 to 21d) and 3025 kcal of AMEn/kg (22 to 35d) (Table 1). Chromic oxide was added (0.1%) to the grower diet (22 to 35d) as an indigestible marker to assess digestibility and mean retention time (MRT). All diets were fed in mash form. The standard energy diets were formulated to meet or exceed nutritional requirements of male broilers with standard performance (Rostagno et al., 2011)

Birds and Housing

A total of 744 male day-old broiler chicks (Cobb-500) were randomly housed in 24 floor pens (2.5 m²) at the rate of 31 chicks per pen. The floor of each pen was covered with wood shavings at 10 cm thickness. The birds had unlimited access to water and feed, throughout the experimental period.

Sample collection, preparation and chemical analysis

Broilers were weighed on a pen basis on d 1, 21, and 35. Feed intake was determined by pen and mortality was checked three times a day. The weight of dead birds was used to adjust the feed conversion ratio.

After weighing out the birds at day-35 post-hatch, four birds per experimental unit were kept in the floor pen for 24 hours until slaughter. All the four birds of each pen were then sacrificed by cervical dislocation and within 30 s of death the excised caeca of each bird were imaged using a Testo 875-1i Thermal Imaging Camera (Testo®, Germany). The images were processed by using Testo IR Soft 4.2, to determine the mean surface temperature of the caeca (Cowieson and Masey O'Neill, 2013).

The pH values of the caecal content were determined in two birds per pen (12 birds per treatment). The pH meter probe was inserted directly into the caecal content and the measurement was taken three times, by inserting the probe in different points within the caeca, ensuring the pH electrode did not touch the caecal wall (adapted from Morgan et al., 2014) and the mean of the three measured values was calculated. At each measurement, the probe was rinsed with distilled water. The caecal content of the four birds of each pen was pooled and homogenized and then two grams of the pooled content were collected and diluted in 4 mL of formic acid (17%). The concentration of acetic, butyric and propionic acids was determined in the sample supernatants after centrifugation at $12000 \times g$ for 10 minutes, by gas chromatography (GC Focus Series®, Thermo Scientific, Milan, Italy).

The small intestine was removed and the entire digesta contents were collected from the proximal jejunum, distal jejunum, proximal ileum and distal ileum, which were demarcated by the end of the duodenal loop, Meckel's diverticulum and the ileocecal

junction and their midpoints. Samples from the four birds in the same pen were pooled, homogenized, lyophilized and weighed. Feed intake over 24 h before sampling was recorded to calculate the MRT by the following equation:

$$\text{MRT (min)} = \frac{1440 \times C \times W}{I}$$

where 1440 equals min per day; C is the Cr_2O_3 concentration in the digesta (mg/g); W is the weight of the dry digesta content (g) and I is the Cr_2O_3 intake over 24 h (mg) (Weurding et al., 2001).

The nitrogen concentration and gross energy of feed and distal ileum content were determined by the micro-Kjeldahl method and by adiabatic bomb calorimeter (C-200, IKA®, Werke), respectively. Chromium was analyzed by atomic absorption spectroscopy (GFAAS) after acid mineralization of the samples. Briefly, 100 mg samples were transferred to 100 mL glass tubes, in which 3 mL of nitric acid 65 % m/m (HNO_3) was added. After complete oxidation of the organic matter, 1 mL of 70 % m/m perchloric acid (HClO_4) was added. The acid extracts were then diluted to 50 mL with ultrapure water.

Apparent ileal digestibility of gross energy and nitrogen was calculated according to the following formula:

$$\text{AID (\%)} = 100 - [100 \times (\text{Nutrient}_{\text{digesta}} \times \text{Cr}_2\text{O}_3_{\text{diet}}) / (\text{Nutrient}_{\text{diet}} \times \text{Cr}_2\text{O}_3_{\text{digesta}})]$$

The relative weights of empty proventriculus, gizzard, pancreas, duodenum, jejunum and ileum were assessed in two birds per pen (12 birds per treatment) and determined by the relation between organ weight and live body weight of each bird. The lengths of duodenum, jejunum and ileum were measured in two birds per pen (12 birds per treatment).

Statistical analysis

Data were subjected to analysis of variance ($P < 0.05$) with two factors (two-way ANOVA) using the GLM procedure of SAS version 9.3 (SAS Institute) to determine the main effects (dietary energy and enzyme) and their interaction. Mean separation was adjusted by Tukey's test with $P < 0.05$ as the significance level.

Results

There was no interaction ($P > 0.05$) between energy level and enzyme supplementation on performance (Table 2). The broiler chickens fed standard energy diets showed an improved performance throughout the trial ($P < 0.05$). Including the enzyme increased BWG from 1 to 7 d ($P < 0.05$), and 1 to 21 d post-hatch ($P < 0.05$) but did not affect overall performance from 21 to 35 d and from 1 to 35 d post-hatch ($P > 0.05$). Feed intake from 1 to 21 d was increased by enzyme ($P < 0.05$).

The addition of enzyme to low-energy diets increased the molar proportion of acetic acid, whereas no effects were observed when the enzyme was included to standard energy diets, resulting in a significant ($P < 0.05$) energy $\times$ enzyme interaction (Table 3). The caecal pH of chickens fed a low-energy diet was reduced in comparison to those fed a standard energy diet (< 0.01). Broilers fed low-energy diets showed an increased caecal surface temperature ($P < 0.05$).

There was no interaction ($P > 0.05$) between the dietary energy and the inclusion of the enzyme on the energy and nitrogen ileal digestibility coefficients (Table 4). The ileal digestibility coefficients of gross energy ($P < 0.01$) and nitrogen ($P < 0.05$) were improved by standard energy diets. Including the enzyme increased the ileal digestibility coefficient of gross energy ($P < 0.01$).

There was no interaction ($P > 0.05$) between the dietary energy and the inclusion of the enzyme on the MRT in the different sites of the small intestine (Table 5). The MRT

in the distal jejunum was increased by low-energy diets ($P<0.05$). The addition of the enzyme increased the MRT in the distal ileum ($P<0.05$).

In the absence of enzyme, broilers fed a standard energy diet showed a reduced relative weight of the ileum when compared to those fed a low-energy diet ($P<0.05$), resulting in an energy $\times$ enzyme interaction (Table 6).

There was no interaction ($P>0.05$) between the dietary energy and the inclusion of the enzyme on the length of different sites of the small intestine. Jejunum length of broilers fed a standard energy diet was reduced ($P<0.05$) in comparison to those fed a low-energy diet (Table 7).

Discussion

The supplementation of the enzyme product resulted in an increased bodyweight gain from 1 to 7 d (+ 6.8%), and 1 to 21 d (+ 3%), however no effect on performance was observed from 21 to 35 and from 1 to 35 d showing that the response to exogenous NSP-enzymes is more pronounced in young birds at the early stages of life. As young birds are more susceptible to the presence of anti-nutrients in the feed (Masey O'Neill et al., 2012), it is possible that the response to NSP-enzymes is greater during the initial stages of growth. Furthermore, inconsistent results with NSP enzymes in studies have been associated to the low soluble NSPs content in corn and to the variable NSPs composition of the raw materials used in the feed (Kocher et al., 2002; Bach Knudsen, 2014), but it is also related to the inclusion level of the enzymes and the variation in the composition of the enzyme products utilized in the trials (Masey O'Neill et al., 2014a). Barekatain et al. (2013), Lee et al. (2017) and Montanhini Neto et al. (2017) also observed improved broiler performance only in the first weeks post-hatch when NSP-enzymes were included to the diets. Cengiz et al. (2012) and Coppedge et al. (2012) found similar results as the

inclusion of various NSP-enzymes products in corn-based diets did not affect the performance of broiler chickens fed a corn-based diet considering the overall period of growth. The increased feed intake with enzyme from 1 to 21 d (+3.7%) may be a consequence of the faster growth of broilers fed enzyme-supplemented diets, as also shown by other studies when NSP-enzymes were included to broiler diets (Liu et al., 2011; Latham et al., 2016).

Broilers fed standard energy diets showed improved performance over the whole period of growth, demonstrating that a reduction of 100 kcal/kg of AMEn could lead to an energy deficit and hence impair the performance of chickens. Shirmohammad and Mehri (2011) found similar results when the performance of chickens was decreased by the reduction of 120 kcal/kg in dietary AMEn.

The addition of the enzyme increased the MRT of the digesta in the distal ileum by 44.6% (from 86.3 min to 124.8 min) regardless of the dietary energy level. It has been suggested that xylanases can release oligomers such as xylo-oligosaccharides which are fermented to short-chain fatty acids in the caeca (Courtin et al., 2008) in such concentrations that a neuro-hormonal response is triggered and results in a stimulation of peptide YY release (Singh et al., 2012). Peptide YY is a neuropeptide produced by intestinal endocrine cells located in the distal ileum and in the colon (Taylor, 1993), and is responsible for regulating the gastric emptying and intestinal transit rate. The foregoing mechanism describes a possible explanation for the increased MRT in the distal ileum when the enzyme was added to the diet. In the present study, the inclusion of xylanase in a low-energy diet increased the percentage of acetic acid in the caeca. Lee et al. (2017) showed that the inclusion of exogenous xylanase in broiler chickens diets can increase the population of *Bifidobacterium* spp. in the caecal content, which is a well-known group of acetate- and lactic-acid-producing bacteria. Kiarie et al. (2014) and Masey O'Neill et

al. (2014b) also observed an increase in the concentration of acetic acid in the caecal content of broilers with the addition of a xylanase in corn-based diets.

Based on these findings, it is likely that the benefits arising from the inclusion of NSP-enzymes, such as xylanase and β -glucanase, in diets for broilers are indirectly related to the regulation of the intestinal transit rate, especially in the distal part of the ileum, which results in an increased digestibility of energy, as observed in the present study. In our study, there was a positive correlation between MRT of digesta in the distal ileum and ileal digestibility of energy ($R = 0.54$; $P < 0.01$). In addition, the use of NSP-enzymes in commercial practice is usually associated with the application of an energy matrix value, which in general results in a reduction in the oil or fat content of the feed formulation. As the oil and the fat have extra caloric effects such as controlling and reducing the intestinal transit rate (Mateos et al., 1982), the fat removal from the diet could lead to an increased passage rate and decreased ileal digestibility. Based on that, it is possible that NSP-enzymes can compensate that at least partially.

The MRT of digesta in the distal jejunum was 42% longer in broilers fed a standard energy diet when compared to those fed a low-energy diet (56.9 min vs. 40.1 min). This effect may be related to a greater amount of vegetable oil in the standard energy diet, as additional oil slows down the rate of feed passage through the gastrointestinal tract, allowing more time for an enhanced digestion of nutrients (Mateos et al., 1982). A longer retention time in the jejunum contributes to an improved digestibility of nutrients as seen in the present study by the increased energy and protein ileal digestibility when standard energy diets were fed.

The lower pH of the caecal content in broilers fed a low-energy diet in comparison to those fed a standard energy diet (6.55 vs. 6.15), associated with the higher caecal temperature in broilers fed the low-energy diet in comparison to those fed a standard

energy diet (35.1 °C vs. 34.4 °C) is indicative of a higher fermentative activity in the caeca (Cowieson and Masey O'Neill, 2013). Despite this, in the present study, no effect of the enzyme on the temperature of the caeca was observed. Masey O'Neill et al. (2014b) also found no effects of NSP-enzymes on the caecal temperature of chickens fed wheat- or corn-based diets. On the other hand, Cowieson and Masey O'Neill (2013) concluded that supplementing a xylanase in wheat-based diets can raise the caecal temperature up to 1.4 °C.

Therefore, the improved ileal digestibility of energy by NSP-enzymes such as xylanase and β -glucanase may be related to the breakdown of the vegetable cell wall and the consequent release of encapsulated nutrients (Khadem et al., 2016) but also to the indirect effect on the digestive transit rate, which, in the present study, was evaluated as the MRT of digesta in different sites of the small intestine. Together, these effects appear to be the most relevant mechanisms by which NSP-enzymes benefit modern broilers fed corn-based diets. An increased digestibility of nutrients in broilers fed diets with NSP-enzymes is also associated to the upregulation of genes involved with nutrient transporters in the jejunum (Saleh et al., 2018).

The apparent ileal digestibility of energy and protein was 18.4% and 7% higher, respectively, when the standard energy diet was fed. The increased digestibility with standard energy diets may be related to the higher oil inclusion in the feed, as oil can regulate the digesta transit rate and thus may improve the digestion of nutrients (Mateos et al., 1982), as seen by the increased MRT in the present study.

The relative weight of the ileum was reduced when standard energy diets without enzyme was fed in comparison to the low-energy diets without xylanase, as shown by the interaction effect. In this study, broilers fed standard energy diets showed an improved ileal digestibility of energy and nitrogen. An increased uptake of nutrients will likely

reduce the amount of nutrients reaching the distal part of the intestinal tract, which could be used by pathogenic microorganisms. Furthermore, a reduced growth of pathogenic microorganisms is related to a mitigated cell proliferation in the intestinal epithelium since the relative weight of the intestine is an indicative of the epithelial cells proliferation rate (Brenes et al., 2002). When the enzyme was included to the diet, however, no impact of the energy level on the relative weight of the ileum was observed, as shown by the interaction effect, and that might be related to the improved energy digestibility observed when the enzyme was included, leading to a reduced amount of nutrients for microorganisms in the distal part of the digestive tract.

The jejunum length was 6.9% longer in chickens fed a low-energy diet, compared to those fed a standard energy diet (77 cm vs. 72 cm). The increased jejunum length may be associated with the reduced digestibility of nutrients in low-energy diets, as observed in the present study, in a tentative to digest the increased quantity of undigested nutrients remaining in the small intestine. An increased amount of undigested nutrients or nutrients resistant to digestion can lead to a longer intestine and intestinal hypertrophy, especially in the jejunum, where significant part of the digestive process takes place in chickens. Viveros et al. (1994) and Jørgensen et al. (1996) observed an increased length of the small intestine and caeca when high-fiber diets were fed to chickens, and those diets also showed a reduced digestibility of nutrients.

Conclusion

The addition of an enzyme product containing xylanase and β -glucanase activities improved performance of broiler chickens from 1 to 21 d post-hatch regardless of dietary energy level, which may be due to an increased caecal acetic acid proportion, a longer

retention time of digesta content in the distal ileum and an improved ileal digestibility of energy.

Competing interests

The authors declare there are no competing interests.

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References

- Bach Knudsen, K.E. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. *Poult. Sci.* **93**: 2380–2393. doi:10.3382/ps.2014-03902
- Barekattain, M.R., Choct, M., and Iji, P.A. 2013. Xylanase supplementation improves the nutritive value of diets containing high levels of sorghum distillers' dried grains with solubles for broiler chickens. *J. Sci. Food Agric.* **93**: 1552–1559. doi:10.1002/jsfa.5992
- Bedford, M. R. 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. *Br. Poult. Sci.* **59**: 486–493. <https://doi.org/10.1080/00071668.2018.1484074>
- Brenes, A., Marquardt, R.R., Guenter, W., and Viveros, A. 2002. Effect of enzyme addition on the performance and gastrointestinal tract size of chicks fed lupin seed and their fractions. *Poult. Sci.* **81**: 670–678. <https://doi.org/10.1093/ps/81.5.670>
- Cengiz, Ö., Hess, J.B., and Bilgili, S.F., 2012. Feed enzyme supplementation does not

- ameliorate foot pad dermatitis in broiler chickens fed on a corn-soyabean diet. *Br. Poult. Sci.* **53**: 401–407. doi:10.1080/00071668.2012.711467
- Coppedge, J.R., Oden, L.A., Ratliff, B., Brown, B., Ruch, F., and Lee, J.T. 2012. Evaluation of nonstarch polysaccharide-degrading enzymes in broiler diets varying in nutrient and energy levels as measured by broiler performance and processing parameters. *J. Appl. Poult. Res.* **21**: 226–234. doi:10.3382/japr.2011-00329
- Courtin, C.M., Broekaert, W.F., Swennen, K., Lescroart, O., Onagbesan, O., Buyse, J., Decuypere, E., Van de Wiele, T., Marzorati, M., Verstraete, W., G., H., and Delcour, J.A. 2008. Dietary inclusion of wheat bran arabinoxyloligosaccharides induces beneficial nutritinal effects in chickens. *Cereal Chem.* **85**: 607–613. doi:10.1094/CCHEM-85-5-0607
- Cowieson, A.J. 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. *The Journal of Poultry Science*, **47**: 1-7.
- Cowieson, A.J., and Masey O'Neill, H.V. 2013. Effects of exogenous xylanase on performance, nutrient digestibility and caecal thermal profiles of broilers given wheat-based diets. *Br. Poult. Sci.* **54**: 346–354. doi:10.1080/00071668.2013.780200
- Gehring, C.K., Bedford, M.R., and Dozier, W.A. 2013. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. *Poult. Sci.* **92**: 979–991. doi:10.3382/ps.2012-02769
- Jørgensen, H., Zhao, X.-Q., Knudsen, K.E.B., and Eggum, B.O. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. *Br. J. Nutr.* **75**: 379–395. doi:10.1079/BJN19960141
- Khadem, A., Lourenço, M., Delezie, E., Maertens, L., Goderis, A., Mombaerts, R., Höfte, M., Eeckhaut, V., Van Immerseel, F., and Janssens, G.P.J. 2016. Does release of

- encapsulated nutrients have an important role in the efficacy of xylanase in broilers? Poult. Sci. **95**: 1066–1076. doi:10.3382/ps/pew002
- Kiarie, E., Romero, L.F., and Ravindran, V. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. Poult. Sci. **93**: 1186–1196. doi:10.3382/ps.2013-03715
- Kocher, A., Choct, M., Porter, M.D., and Broz, J. 2002. Effects of feed enzymes on nutritive value of soyabean meal fed to broilers. Br. Poult. Sci. **43**: 54–63. doi:10.1080/00071660120109890
- Latham, R. E., Williams, M. P., Flores, C., O'Neill, H. M., York, T. W., and Lee, J. T. 2016. Impact of variable corn nutrient content, AME prediction, and xylanase inclusion on growth performance. J. Appl. Poult. Res. **25**: 338-351.
- Lee, S.A., Apajalahti, J., Vienola, K., González-Ortiz, G., Fontes, C.M.G.A., and Bedford, M.R. 2017. Age and dietary xylanase supplementation affects ileal sugar residues and short chain fatty acid concentration in the ileum and caecum of broiler chickens. Anim. Feed Sci. Technol. **234**: 29–42. doi:10.1016/j.anifeedsci.2017.07.017
- Liu, N., Ru, Y. J., Tang, D. F., Xu, T. S., and Partridge, G. G. 2011. Effects of corn distillers dried grains with solubles and xylanase on growth performance and digestibility of diet components in broilers. Anim. Feed Sci. Technol. **163**: 260-266.
- Masey O'Neill, H.V.M., Liu, N., Wang, J.P., Diallo, A., and Hill, S. 2012. Effect of xylanase on performance and apparent metabolisable energy in starter broilers fed diets containing one maize variety harvested in different regions of china. Asian-Australasian J. Anim. Sci. **25**: 515–523. doi:10.5713/ajas.2011.11314
- Masey O'Neill, H. V., Smith, J.A., and Bedford, M.R. 2014a. Multicarbohydrase

- enzymes for non-ruminants. *Asian-Australasian J. Anim. Sci.* **27**: 290–301.
doi:10.5713/ajas.2013.13261
- Masey-O'Neill, H.V., Singh, M., and Cowieson, A.J. 2014b. Effects of exogenous xylanase on performance, nutrient digestibility, volatile fatty acid production and digestive tract thermal profiles of broilers fed on wheat- or maize-based diet. *Br. Poult. Sci.* **55**: 351–359. doi:10.1080/00071668.2014.898836
- Mateos, G.G., Sell, J.L., and Eastwood, J.A. 1982. Rate of food passage (transit time) as influenced by level of supplemental fat. *Poult. Sci.* **61**: 94–100.
doi:10.3382/ps.0610094
- Montanhini Neto, R., N'Guetta, E., Gady, C., Francesch, M., and Preynat, A. 2017. Combined effect of using near-infrared spectroscopy for nutritional evaluation of feed ingredients and non-starch polysaccharide carbohydrase complex on performance of broiler chickens. *Anim. Sci. J.* **88**: 1979–1986.
doi:10.1111/asj.12822
- Morgan, N.K., Walk, C.L., Bedford, M.R., and Burton, E.J. 2014. The effect of dietary calcium inclusion on broiler gastrointestinal pH: Quantification and method optimization. *Poult. Sci.* **93**: 354–363. doi:10.3382/ps.2013-03305
- Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C., Oliveira, R.F., Lopes, D.C., Ferreira, A.S., Barreto, S.L.T., and Euclides, R.F. 2011. Brazilian tables for poultry and swine: feed composition and nutritional requirements. 3rd ed. Universidade Federal de Viçosa, Viçosa, MG, Brazil. 119 pp.
- Saleh, A.A., El-Far, A.H., Abdel-Latif, M.A., Emam, M.A., Ghanem, R., and Abd El-Hamid, H.S. 2018. Exogenous dietary enzyme formulations improve growth performance of broiler chickens fed a low-energy diet targeting the intestinal nutrient transporter genes. *PLoS One*, **13**: e0198085.

<https://doi.org/10.1371/journal.pone.0198085>

- Shakouri, M.D., Iji, P.A., Mikkelsen, L.L., and Cowieson, A.J. 2009. Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. *J. Anim. Physiol. Anim. Nutr. (Berl)*. **93**: 647–658. doi:10.1111/j.1439-0396.2008.00852.x
- Shirmohammad, F., and Mehri, M. 2011. Effects of dietary supplementation of multi-enzyme complex on the energy utilization in rooster and performance of broiler chicks. *African J. Biotechnol.* **10**: 7541–7547. doi:10.5897/AJB10.2260
- Singh, A., Neill, H.V.M.O., Ghosh, T.K., Bedford, M.R., and Haldar, S. 2012. Effects of xylanase supplementation on performance, total volatile fatty acids and selected bacterial population in caeca, metabolic indices and peptide YY concentrations in serum of broiler chickens fed energy restricted maize–soybean based diets. *Anim. Feed Sci. Technol.* **177**: 194–203. doi:10.1016/j.anifeedsci.2012.08.005
- Taylor, I.L. 1993. Role of peptide YY in the endocrine control of digestion. *J. Dairy Sci.* **76**: 2094–2101. [https://doi.org/10.3168/jds.S0022-0302\(93\)77544-X](https://doi.org/10.3168/jds.S0022-0302(93)77544-X)
- Viveros, A., Brenes, A., Pizarro, M., and Castaño, M. 1994. Effect of enzyme supplementation of a diet based on barley, and autoclave treatment, on apparent digestibility, growth performance and gut morphology of broilers. *Anim. Feed Sci. Technol.* **48**: 237–251. doi:10.1016/0377-8401(94)90175-9
- Weurding, R.E., Veldman, A., Veen, W.A.G., Aar, P.J. van der, and Verstegen, M.W.A. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. *J. Nutr.* **131**: 2329–2335. <https://doi.org/10.1093/jn/131.9.2329>
- Zakaria, H.A.H., Jalal, M.A.R., and Jabarin, A.S. 2008. Effect of exogenous enzymes on the growing performance of broiler chickens fed regular corn/soybean-based diets and the economics of enzyme supplementation. *Pakistan J. Nutr.* **7**: 534–539.

<https://dx.doi.org/10.3923/pjn.2008.534.539>

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For Review Only

Table 1. Composition and calculated nutrient content of the experimental diets.

Item (g/kg)	Starter (1 – 21d)		Grower (22 – 35d)	
	Standard Energy	Low Energy	Standard Energy	Low Energy
Corn	565.25	588.82	613.52	637.09
Soybean meal 45% CP	362.80	358.56	308.32	304.08
Soybean oil	31.46	12.08	37.86	18.48
Dicalcium phosphate	18.18	18.15	15.97	15.94
Limestone	10.63	10.65	11.05	11.07
Sodium chloride	4.57	4.56	4.32	4.31
DL-Methionine	2.69	2.67	2.66	2.64
L-Lysine HCl	1.56	1.64	2.24	2.32
L-Threonine	0.21	0.21	0.51	0.51
Vitamin + mineral premix*	2.00	2.00	2.00	2.00
Choline chloride	0.55	0.55	0.45	0.45
Inert (kaolin)	0.10	0.10	0.10	0.10
Chromium oxide	0.00	0.00	1.00	1.00
Calculated specifications				
AMEn, kcal/kg	3025	2925	3125	3025
Crude protein	211.7	211.7	191.7	191.7
Calcium	9.5	9.5	9.0	9.0
Available phosphorus	4.5	4.5	4.0	4.0
Sodium	2.0	2.0	1.9	1.9
Digestible Lys	11.6	11.6	10.9	10.9
Digestible Met	5.5	5.5	5.3	5.3
Digestible SAA	8.4	8.4	7.9	7.9
Digestible Thr	7.4	7.4	7.0	7.0
Digestible Trp	2.4	2.4	2.1	2.1
Digestible Arg	13.4	13.4	11.9	11.8
Digestible Ile	8.3	8.3	7.4	7.4
Analyzed composition				
Dry matter	-	-	912.2	885.7
Gross energy, kcal/kg	-	-	4169	4005
Crude protein	-	-	186.1	183.4
Analyzed enzyme activity				
Xylanase	-Enzyme	<2000	<2000	<2000
(XU/kg) ^a	+Enzyme	7040	9220	10400
β-glucanase	-Enzyme	2780	1190	3060
(GU/kg) ^b	+Enzyme	8700	<1000	4360

*Vitamin + mineral premix provided per kg of feed: vitamin A, 9000 IU; vitamin D3, 2500 IU; vitamin E, 14 IU; vitamin K3, 2 mg; vitamin B1, 2.5 mg; vitamin B2, 6.2 mg; vitamin B6, 4 mg; vitamin B12, 14 µg; niacin 40 mg; panthotenic acid, 15 mg; folic acid, 1 mg; Cu, 10 mg; Fe, 50 mg; I, 1.2 mg; Mn, 80 mg; Se, 0.2 mg; Zn, 60 mg.

^a One XU is defined as the amount of enzyme that produces 1 nmol reducing sugars from birchwood xylan in one second at 50°C and pH 5.3.

^b One BU is the amount of enzyme which liberates 1 nmol of reducing sugars from barley beta-glucan per second at 50 °C and pH 4.8.

For Review Only

Table 2. Effects of a xylanase and β -glucanase enzyme product on the performance of broiler chickens fed corn-based diets.

Energy	Enzyme	BWG (g)				FI (g)				FCR			
Main effects for energy		1 to 7d	1 to 21d	21 to 35d	1 to 35d	1 to 7d	1 to 21d	21 to 35d	1 to 35d	1 to 7d	1 to 21d	21 to 35d	1 to 35d
Low		102	803	1027	1830	140	1150	1953	3103	1.374	1.447	1.927	1.711
Standard		109	867	1031	1898	137	1185	1929	3114	1.271	1.383	1.907	1.666
SEM		2.24	7.54	13.10	16.40	2.32	11.90	14.40	23.40	0.03	0.01	0.02	0.01
Main effects for enzyme													
	No	102	823	1034	1857	135	1147	1945	3092	1.320	1.414	1.914	1.684
	Yes	109	848	1024	1872	142	1189	1935	3124	1.326	1.416	1.919	1.690
SEM		2.24	7.54	13.10	16.40	2.32	11.90	14.40	23.40	0.03	0.01	0.02	0.01
Low	No	97	794	1028	1823	135	1135	1978	3113	1.377	1.444	1.922	1.702
Low	Yes	106	812	1026	1838	144	1166	1925	3091	1.372	1.450	1.932	1.718
Standard	No	106	851	1040	1890	135	1158	1909	3067	1.263	1.384	1.907	1.669
Standard	Yes	112	884	1022	1906	139	1212	1941	3153	1.279	1.381	1.906	1.662
SEM		3.17	10.70	18.50	23.20	3.27	16.80	20.40	31.50	0.04	0.01	0.02	0.01
p-value													
Energy		0.025	<0.01	0.825	<0.01	0.472	0.054	0.240	0.810	0.023	<0.01	0.344	<0.01
Enzyme		0.038	0.026	0.600	0.505	0.066	0.021	0.399	0.354	0.891	0.822	0.819	0.729
Energy × Enzyme		0.641	0.484	0.696	0.990	0.499	0.494	0.988	0.118	0.811	0.587	0.807	0.350

SEM: pooled standard error of the mean.

Table 3. Effects of a xylanase and β -glucanase enzyme product on the molar proportion of caecal short-chain fatty acids (SCFA, mol/100 mol), pH of caecal content and mean caecal temperature ($^{\circ}\text{C}$) of broiler chickens fed corn-based diets.

Energy	Enzyme	Acetic	Propionic	Butyric	pH	Temp.
Main effects for energy						
Low		66.19	19.24	14.57	6.15	35.05
Standard		66.41	20.20	12.97	6.55	34.39
SEM		0.84	1.18	0.69	0.09	0.21
Main effects for enzyme						
	No	65.59	20.37	14.05	6.31	34.70
	Yes	67.06	19.08	13.50	6.39	34.72
SEM		0.84	1.18	0.69	0.09	0.21
Low	No	64.12b	20.31	15.57	6.17	34.82
Low	Yes	68.26a	18.17	13.57	6.13	35.27
Standard	No	67.06ab	20.42	12.52	6.46	34.59
Standard	Yes	66.59ab	19.98	13.42	6.65	34.17
SEM		1.19	1.67	0.97	0.12	0.30
p-value						
Energy		0.891	0.571	0.114	<0.01	0.022
Enzyme		0.249	0.449	0.577	0.568	0.969
Energy $\times$ Enzyme		0.025	0.615	0.151	0.396	0.127

a,b: means within columns followed by different lowercased letters are significantly different ($P < 0.05$);

SEM: pooled standard error of the mean.

Table 4. Effects of a xylanase and β -glucanase enzyme product on the coefficients of apparent ileal digestibility of gross energy and nitrogen of broiler chickens fed corn-based diets.

Energy	Enzyme	Gross energy (%)	Nitrogen (%)
Main effects for energy			
Low		52.5	66.6
Standard		62.2	71.3
SEM		1.70	1.38
Main effects for enzyme			
	No	53.8	68.0
	Yes	60.9	69.9
SEM		1.70	1.38
Low	No	48.9	65.5
Low	Yes	56.1	67.7
Standard	No	58.6	70.5
Standard	Yes	65.8	72.1
SEM		2.40	1.96
p-value			
Energy		<0.01	0.026
Enzyme		<0.01	0.345
Energy $\times$ Enzyme		0.991	0.895

SEM: pooled standard error of the mean.

Table 5. Effects of a xylanase and β -glucanase enzyme product on the mean retention time (min) in proximal jejunum, distal jejunum, proximal ileum and distal ileum of broiler chickens fed corn-based diets.

Energy	Enzyme	Proximal jejunum	Distal jejunum	Proximal Ileum	Distal Ileum	Total
Main effects for energy						
Low		13.34	40.05	81.89	103.36	242.33
Standard		13.35	56.86	81.52	107.81	247.85
SEM		2.73	4.63	6.40	11.50	16.60
Main effects for enzyme						
	No	13.22	47.14	83.58	86.34	229.69
	Yes	13.47	49.13	79.68	124.82	261.89
SEM		2.73	4.63	6.40	11.50	16.60
Low	No	12.75	40.69	85.16	79.92	218.69
Low	Yes	13.94	39.41	78.63	126.79	265.96
Standard	No	13.70	53.59	82.00	92.75	240.69
Standard	Yes	13.00	60.79	80.95	122.86	256.79
SEM		3.86	6.55	9.05	16.30	23.5
p-value						
Energy		0.999	0.019	0.964	0.788	0.795
Enzyme		0.952	0.665	0.687	0.031	0.211
Energy $\times$ Enzyme		0.808	0.535	0.771	0.613	0.530

SEM: pooled standard error of the mean.

Table 6. Effects of a xylanase and β -glucanase enzyme product on the relative organs weight (g/kg of bodyweight) of broiler chickens fed corn-based diets.

Energy	Enzyme	Proventriculus	Gizzard	Pancreas	Duodenum	Jejunum	Ileum
Main effects for energy							
Low		3.45	17.33	2.49	7.69	13.88	10.48
Standard		3.53	17.44	2.38	7.31	12.92	10.24
SEM		0.12	0.42	0.07	0.61	0.43	0.29
Main effects for enzyme							
	No	3.42	16.87	2.37	7.34	13.54	10.31
	Yes	3.56	17.90	2.50	7.66	13.26	10.42
SEM		0.12	0.42	0.07	0.61	0.43	0.29
Low	No	3.48	16.89	2.44	7.76	14.39	10.85a
Low	Yes	3.43	17.77	2.53	7.64	13.37	10.11ab
Standard	No	3.37	16.86	2.29	6.99	12.70	9.76b
Standard	Yes	3.69	18.03	2.48	7.70	13.14	10.72ab
SEM		0.17	0.59	0.10	0.86	0.61	0.41
p-value							
Energy		0.662	0.841	0.339	0.367	0.121	0.555
Enzyme		0.443	0.088	0.194	0.449	0.639	0.783
Energy $\times$ Enzyme		0.280	0.805	0.608	0.291	0.235	0.041

a,b: means within columns followed by different lowercased letters are significantly different ($P<0.05$);

SEM: pooled standard error of the mean.

Table 7. Effects of a xylanase and β -glucanase enzyme product on the length (cm) of duodenum, jejunum, ileum, and small intestine (duodenum+jejunum+ileum) of broiler chickens fed corn-based diets.

Energy	Enzyme	Duodenum	Jejunum	Ileum	Small intestine
Main effects for energy					
Low		33	77	79	189
Standard		33	72	76	182
SEM		0.69	1.64	1.75	3.17
Main effects for enzyme					
	No	33	76	79	188
	Yes	33	72	77	183
SEM		0.69	1.64	1.75	3.17
Low	No	33	81	81	195
Low	Yes	33	73	77	183
Standard	No	33	72	76	181
Standard	Yes	34	72	76	184
SEM		0.97	2.31	2.48	4.48
p-value					
Energy		0.802	0.039	0.226	0.144
Enzyme		0.802	0.097	0.453	0.321
Energy $\times$ Enzyme		0.617	0.068	0.515	0.102

SEM: pooled standard error of the mean.