Carbohydrase and protease supplementation increased performance of broilers fed maize-soybean-based diets with restricted metabolizable energy content

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Abstract

A trial was conducted to evaluate whether the addition of commercial enzyme preparations containing carbohydrases and a protease would increase the available metabolizable energy (ME) of maize-soya-based broiler diets. Seven thousand five hundred and sixty (7560) day-old Ross 788 chicks were randomly allocated to 60 pens (126 chicks per pen). The trial consisted of five dietary treatments, with 12 replicates per treatment. A negative control feed and three treatment feeds were formulated to contain a lower level of ME than a standard (positive control) feed. Two commercial enzyme preparations, one a mixture of amylase, xylanase and protease; and the other a β-mannanase product, were added separately or in combination to the treatment diets. Broiler performance was recorded over a 35-day period. Broilers fed the lower energy (negative control) diet performed significantly worse than all other treatments in terms of growth and feed conversion, but not mortality. The addition of the enzyme preparations to the energy-restricted diets significantly improved performance. Cumulative feed conversion ratio (FCR) on day 35 for the negative control birds was 12% higher than that of the positive control, while diets supplemented with single enzyme preparations resulted in 2.4% and 3.0% higher FCRs, respectively. Combining the two products caused significantly higher bodyweights and feed intakes than the positive control, while cumulative FCR was only 1.2% higher than for the positive control. A positive synergistic effect was evident when combining the two enzyme products. Performance gains in the enzyme-supplemented broilers were most pronounced during early growth.

Keywords: Amylase, Avizyme 1502, β-mannanase, Hemicell, maize, protease, soybeans, xylanase
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Introduction

Broiler diets containing cereals with high non-starch polysaccharide (NSP) levels, such as barley and wheat, typically result in high intestinal viscosity (Annison, 1991; Choct, 2006). The positive effect of exogenous carbohydrases in these types of diets is well recognized (Oloffs, *et al*., 1999; Steenfeldt, 2001; Wu *et al*., 2004) with the main mechanism of action being the reduction of digesta viscosity. Although maize-based diets do not induce such high intestinal viscosity as other cereals, it was shown that these diets could benefit from carbohydrase supplementation when fed to broilers (Cowieson, 2010). Results, however, are somewhat inconsistent (Zanella *et al*., 1999; Centeno *et al.*, 2006; Singh *et al.*, 2012). Several possible mechanisms of action were proposed, which included breakdown of starch or cell walls that encapsulate nutrients (Meng & Slominski, 2005), modulation of intestinal microbiotica by producing fermentable oligosaccharides (Bedford & Cowieson, 2012) and reduced endogenous nitrogen losses (Cowieson & Bedford, 2009).

The inclusion of exogenous proteases in maize-soya-based diets increases protein digestion by improving hydrolysis of protein (Caine *et al*., 1998; Romero *et al*., 2013) or by destroying antinutrients such as lectins and trypsin inhibitors (Ghazi *et al*., 2002). Starch digestibility may also be improved by proteases through a disruption of the starch-protein crosslinks (Han & BeMiller, 2008).

Avizyme 1502 is a commercially available product (DuPont Danisco, Copenhagen, Denmark) that combines the enzymes amylase and xylanase and a protease (subtilisin). The manufacturer claims that the

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product increases the apparent metabolizable energy (ME) value of maize by up to five per cent, therefore about 0.4 MJ/kg of a typical broiler feed containing 60% maize.

Hemicell (ChemGen Corp., Gaithersburg, MD, USA) is an enzyme product containing β-mannanase, which, according to the manufacturers, would increase the apparent ME value of the feed by at least 0.5 MJ/kg. This was confirmed by studies in which the addition of β-mannanase to a broiler diet increased energy availability by at least 0.5 MJ ME/kg feed (Li *et al.*, 2010) to 0.6 MJ ME/kg feed (McNaughten *et al.*, 1998).

This study investigated whether the enzyme products, Avizyme 1502 and Hemicell, would release additional energy when included in a maize-soybean diet. It was hypothesized that the performance of broilers would not be affected when given a feed with a reduced ME value, but supplemented with these enzymes. A possible synergistic effect was also tested by measuring broiler performance on a feed containing both enzyme products. The ME content of the test diets was reduced by 0.45 MJ/kg based on average values recommended by manufacturers.

Materials and Methods

The study was approved by the Animal Use and Care Committee of the University of Pretoria (EC EC067-12).

Seven thousand five hundred and sixty (7560) day-old Ross 788 chicks were randomly allocated to five treatments with 12 replicates per treatment. A positive control was formulated to meet or exceed the recommendations of the NRC (1994) for all nutrients, except for a slightly lower apparent ME to ensure that energy is the first limiting nutrient. A negative control feed contained 0.45 MJ ME/kg less than the positive control feed. Three enzyme treatment feeds, all with similar ME levels to the negative control, were included in the study. The commercial enzyme preparations, Avizyme 1502 (DuPont Danisco, Copenhagen, Denmark) and Hemicell (ChemGen Corp., Gaithersburg, MD USA), were added to two treatment diets, and a combination of Avizyme and Hemicell was added to the third treatment diet. Avizyme was included at a level of 0.05% and Hemicell at 0.0125%. The ingredient inclusion and nutrient levels for the diets are shown in Table 1.

The trial was conducted in two environmentally controlled broiler houses. Each house was divided into three blocks, each containing 10 pens with a surface area of 6 m^2/pen, with 126 chicks per pen. Each treatment was randomly replicated twice in each of the three blocks per house.

Each house was fitted with a boiler to control the temperature. Heated air was distributed by a hot air tunnel that ran in the centre through the length of the house. To minimize possible house and temperature effects on results, each house was divided into three blocks, based on distance from the boiler. Houses had curtains on the sides to control ventilation. The houses had solid concrete floors, and pens were covered with a layer of wood shavings. At first, the chicks were fed from a combination of pan and tube feeders, and water was supplied by both nipple and fountain drinkers. After five days, the pan feeders and fountain drinkers were removed. Feeders were kept full at all times to ensure that feed intake was not affected by low feed levels.

Broiler performance was measured over a 35-day period. Mortality was recorded daily, and total bodyweight and feed intake per pen were measured weekly. Average bodyweight, weekly bodyweight gain, cumulative feed intake, weekly feed intake, cumulative and weekly feed conversion ratio and cumulative mortality were calculated for each pen.

Representative samples of the different diets were analysed for dry matter, ash and crude fat (ether extract) according to AOAC (2000) procedures. Crude protein was determined by the Dumas combustion procedure (Leco FP-428; Leco Corporation, St. Joseph, MI, USA) and crude fibre using the Fibre-Tech apparatus (Robertson & Van Soest, 1981). Calcium, potassium and sodium analyses were done by using the Perkin Elmer Atomic Spectrophotometer (Giron, 1973). Phosphorus (P) analysis was done with the Spekol 1300 apparatus using the spectrophotometric method (AOAC 2000). Representative samples of each feed were also analysed for Avizyme activity and Hemicell activity by Danisco Animal Nutrition (Wiltshire, UK) and ChemGen Corporation (Gathersburg, USA), respectively.

The treatments in this trial were not structured, so simple analysis of treatment means, using the generalised linear model (GLM) function in Statistical Analysis Systems (SAS, 1989; 1994), was used in preference to the balanced ANOVA, so that post hoc multiple comparison tests could be run on the treatment means when the GLM found significant differences in performance between treatments. Fischer's protected test was used for the post hoc multiple comparison test. Repeated tests were included in the model. The confidence level was set at 95%. The variations because of block and house effects were accounted for by including house and block nested in house as fixed effects in the model. Initial bodyweight was tested as a covariant.

Results and Discussions

The weekly and cumulative performance parameters for this trial are presented in Tables 3 - 5. No significant differences in mortality were noted between any of the experimental groups. From day 7 onwards, the negative control group performed significantly worse than the positive control group. On day 35, bodyweight of the negative control group was 24% lower than that of the positive control group and

Table 1 Raw material inclusions and calculated and analysed nutrient values (% as fed basis) of the high-energy (positive control) and low-energy (negative control and enzyme-treated) feeds

	Starter		Grower		Finisher	
	High ME	Low ME	High ME	Low ME	High ME	Low ME
Raw materials (% inclusion)						
Maize	58.15	58.75	61.73	63.35	63.55	66.13
Soybean oilcake	22.73	21.00	16.68	14.90	14.73	14.50
Fullfat soya	7.00	7.00	10.00	10.00	10.00	10.00
Sunflower oilcake	4.00	7.00	4.00	6.05	4.00	4.00
Vegetable oil	2.16	0.70	2.92	0.98	3.43	1.09
Fish meal	1.80	1.38	1.00	1.00	1.08	1.00
Limestone	1.50	1.58	1.30	1.30	1.15	1.15
MCP	1.42	1.43	1.19	1.17	0.97	0.97
Salt	0.365	0.370	0.385	0.380	0.373	0.373
Lysine HCl	0.235	0.277	0.249	0.279	0.255	0.260
DL methionine	0.271	0.128	0.207	0.199	0.194	0.189
L threonine	0.042	0.045	0.041	0.043	0.041	0.039
Sodium bicarbonate	0.06	0.06	0.02	0.03	-	-
Vit. & mineral premix	0.12	0.12	0.11	0.11	0.10	0.10
Coccidiostat and AGP[1]	0.16	0.16	0.16	0.16	0.16	0.16
Calculated nutrients (%)						
AME for chicks (MJ/kg)	11.80	11.35	12.40	11.95	12.66	12.21
Crude protein	20.96	20.86	18.81	18.84	18.08	18.12
Crude fibre	3.24	3.85	3.07	3.49	3.04	3.08
Crude fat	6.05	4.58	7.44	5.55	7.97	5.71
Lysine[2]	1.10	1.10	0.98	0.98	0.94	0.94
Methionine[2]	0.57	0.43	0.47	0.47	0.45	0.45
Retainable P	0.40	0.40	0.34	0.34	0.30	0.30
Chloride	0.30	0.30	0.30	0.30	0.29	0.29
Analysed nutrient values (%)						
Dry matter	90.98	90.51	90.32	89.72	89.67	89.68
Crude protein	19.74	19.46	16.98	17.59	16.77	16.32
Crude fibre	3.82	4.62	4.15	4.58	3.32	4.13
Ether extract	5.73	5.43	6.68	4.67	7.98	5.47
Calcium	0.92	0.92	0.81	0.82	0.71	0.75
Total phosphorus	0.63	0.68	0.62	0.60	0.57	0.60
Potassium	0.89	0.90	0.81	0.75	0.77	0.74
Sodium	0.18	0.19	0.17	0.18	0.17	0.20

[1] Stafac 4% and Salinomycin 12%.
[2] Apparent digestible for poultry.
ME: metabolizable energy; MCP: monocalcium phosphate; P: phosphorus.

cumulative feed conversion ratio (FCR) 12% higher. Energy was thus seemingly a limiting factor for broilers receiving the low ME diet. However, the 35-day bodyweights of the Avizyme and the Hemicell treatment groups, which received diets with the same ME content as the negative control group, did not differ significantly from the bodyweight of the positive control group. Cumulative feed intake was significantly higher for Avizyme up to 28 days of age and for Hemicell up to 35 days (P <0.05) compared with the positive control, resulting in a cumulative FCR that was worse (P <0.05) for both the Avizyme (2.4%) and Hemicell (3.0%) groups.

These results indicated that the addition of Avizyme and Hemicell to a maize-soya-based broiler feed increased the ME content by almost 0.45 MJ ME/kg. The higher cumulative feed intake of the Avizyme and Hemicell groups could suggest a marginal energy deficit compared with the positive control, as the broilers attempted to obtain more of the limiting nutrient (Burnham *et al*., 1992) to grow at genetic potential (Emmans, 1981; 1989). However, the negative control group apparently did not increase feed intake as expected. Gous (2007) explained that Emmans' (1981; 1989) theory is valid only for marginally deficient feeds. Where the limiting nutrient is severely deficient, growth rates and subsequently the capacity for feed intake will decrease when measured over a fixed time. Also, the environmental temperature measured in the houses was consistently 1 - 2 ºC higher than target temperatures (temperature profiles not shown). This could have prevented a sufficient increase in feed intake for the negative control birds because they would not be able to lose excess heat to the environment (Gous, 2007).

Table 2 Analysed activity values for Avizyme and Hemicell in all experimental diets

Treatments	Avizyme activity (amylase units/ton)[6]	Hemicell (million β-mannanase units/ton)[7]
Starter diets*		
Positive control[1]	0.00	9.92
Negative control[2]	<100	5.79
Avizyme treatment[3]	1069.59	9.89
Hemicell treatment[4]	<100	133.78
Combination treatment[5]	1443.18	155.19
Grower diets*		
Positive control[1]	0.00	6.50
Negative control[2]	<100	6.46
Avizyme treatment[3]	971.48	2.53
Hemicell treatment[4]	<100	188.58
Combination treatment[5]	1412.64	214.80
Finisher diets*		
Positive control[1]	<100	3.77
Negative control[2]	0	5.92
Avizyme treatment[3]	1201.75	20.55
Hemicell treatment[4]	330.07	141.15
Combination treatment[5]	1561.22	180.93

[1] Positive control with no exogenous enzymes added.
[2] Negative control with 0.45 MJ less energy than the positive control. No exogenous enzyme was included;
[3] Formulated to have 0.45 MJ less energy than the positive control; Avizyme included at 0.05%;
[4] Formulated to have 0.45 MJ less energy than the positive control; Hemicell included at 0.0125%;
[5] Formulated to have 0.45 MJ less energy than the positive control; Avizyme and Hemicell included at 0.05% and 0.0125%, respectively;
[6] Avizyme treated diets should have an amylase activity of at least 400 units/ton;
[7] Hemicell treated diets should have a Hemicell activity of at least 90 million β-mannanase units/ton;
* Untreated diets should have a Hemicell activity of less than 15 MM units/ton.

Table 3 Least square means (± standard error of the mean) of the weekly bodyweight (g/bird) of broilers that received different treatments from day 0 until day 35

Treatments	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Body weight						
Positive[1]	42.8^{b}	156.5^{c}	371.9^{b}	754.7^{b}	1292^{b}	1876^{ab}
Negative[2]	43.0^{ab}	119.8^{d}	226.5^{c}	434.1^{c}	887^{c}	1431^{c}
Avizyme[3]	43.3^{a}	160.0^{bc}	372.9^{b}	754.2^{b}	1282^{b}	1858^{b}
Hemicell[4]	43.4^{a}	163.7^{ab}	377.0^{b}	756.9^{b}	1290^{b}	1855^{b}
Combination[5]	42.9^{b}	165.1^{a}	388.2^{a}	773.2^{a}	1322^{a}	1893^{a}
SEM	0.15	1.37	2.40	4.78	9.30	9.60

^{abcd} Column means with same superscript did not differ significantly for the treatments least square means (*P* >0.05).
[1] Positive control was formulated to meet or exceed the recommendations of the NRC (1994) for all nutrients, except or a slightly lower apparent metabolizable energy to ensure that energy is the first limiting nutrient;
[2] Formulated to have 0.45 MJ less energy than the positive control. No exogenous enzyme was included;
[3] Formulated to have 0.45 MJ less energy than the positive control. Avizyme included at 0.05%;
[4] Formulated to have 0.45 MJ less energy than the positive control. Hemicell included at 0.0125%;
[5] Formulated to have 0.45 MJ less energy than the positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Broilers that received a combination of Avizyme and Hemicell in their feed had a significantly higher (*P* <0.05) bodyweight at day 35 than the broilers receiving only one of these enzymes. As with the individual enzyme applications, the combination treatment resulted in significantly higher feed intakes, but the cumulative FCR on day 35 was only 1.2% higher than the positive control. Thus, the two enzymes showed a positive synergistic effect on each other.

Studies relating to the effect of enzyme activity on broiler performance have been contradictory. Some studies have found that the addition of exogenous enzymes to maize-soya-based broiler diets had no effect on parameters such as digestibility, viscosity (Mahagna *et al*., 1995; Zanella *et al*., 1999; Saleh *et al*., 2003), bodyweight gain, feed consumption and FCR (Singh *et al*., 2012). The improved broiler performance measured in the current study is similar to the results found by other researchers (McNaughten *et al*., 1998; Centeno *et al*., 2006; Karimi *et al*., 2007). Kocher *et al*. (2000) also recognised the high degree of variability in broiler responses to enzyme inclusion in maize-soya-based diets and suggested that since enzymes may improve energy digestibility, their beneficial effects are more likely to be observed when applied to nutritionally marginal diets than diets without any limiting nutrient.

Furthermore, much of the variation might be explained by the starch structure and composition (Oates, 1997), such as the ratio of amylose and amylopectin, which vary between cultivars of maize. Amylose is considered less digestible than amylopectin. The linear structure of amylose in comparison with the branched structure of amylopectin creates a smaller surface area per molecule for enzymes to attach to the molecule for digestion. Amylose also contains hydrogen bonds between glucose chains, which make this structure less susceptible to enzymatic hydrolyses. Increasing the broiler's capacity to digest amylose by the supplementation of exogenous amylase in the feed could possibly overcome the negative effects of high amylose content in the starch (Cowieson, 2005).

Kocher *et al*. (2000) pointed out that the specific enzymes and their activities in the feeds contribute to varying broiler responses to enzyme inclusion in maize-soya-based diets. For instance, an enzyme preparation containing β-mannanase will improve hydrolysis of β-mannans, a group of closely related compounds in soybeans associated mainly with the hull and fibre fractions. The β-mannans are highly viscous (Odetallah *et al*., 2002; Li *et al*., 2010), which causes a partial blockage of receptor sites on intestinal surfaces, thereby decreasing the utilization of carbohydrates, resulting in poor FCR (Li *et al*., 2010).

Non-starch polysaccharides in cereals and soybeans serve not only as physical barriers that inhibit enzyme accessibility to starch granules, but associations between the starch granules and protein and cell wall structures in the feed can hinder the digestion of starch (Abdollahi *et al*., 2013). The protein matrixes around the starch granules, as well as the NSP fraction, reduce the accessibility of enzymes to the starch granules (Abdollahi *et al*., 2013). The protein matrix and NSP will delay or even inhibit complete starch digestion in the small intestine. An exogenous protease enzyme supplementation could assist in digesting the protein matrix and making the starch granules more accessible to amylolytic enzymes (Han & BeMiller, 2008).

Table 4 Least square means (± standard error of the mean) of the weekly bodyweight gain (g/bird/day), feed intake (g/bird/day) and feed conversion ratio (FCR; g feed intake/g bodyweight gain) of broilers that received different treatments from day 0 until day 35

Treatments	Day 0 - 7	Day 7 - 14	Day 14 - 21	Day 21 - 28	Day 28 - 35
Body weight gain					
Positive[1]	14.2^{c}	30.8^{b}	54.7^{a}	76.7^{a}	83.4^{a}
Negative[2]	9.6^{d}	15.2^{c}	29.7^{b}	64.7^{b}	77.7^{b}
Avizyme[3]	14.6^{bc}	30.4^{b}	54.5^{a}	75.4^{a}	82.3^{a}
Hemicell[4]	15.0^{ab}	30.5^{b}	54.3^{a}	76.1^{a}	80.8^{ab}
Combination[5]	15.3^{a}	31.9^{a}	55.0^{a}	78.3^{a}	81.6^{ab}
SEM	0.17	0.21	0.52	1.15	1.28
Feed intake					
Positive[1]	18.9^{b}	42.1^{b}	79.4^{a}	124.4^{c}	153.2^{a}
Negative[2]	18.9^{b}	34.1^{c}	44.8^{b}	126.0^{bc}	136.5^{b}
Avizyme[3]	20.4^{a}	41.8^{b}	82.0^{a}	126.7^{ac}	153.0^{a}
Hemicell[4]	19.8^{ab}	44.2^{ab}	81.8^{a}	128.5^{a}	152.3^{a}
Combination[5]	19.3^{ab}	46.3^{a}	80.7^{a}	128.2^{ab}	154.2^{a}
SEM	0.42	0.95	1.04	0.87	1.29
Feed conversion ratio					
Positive[1]	1.33^{bc}	1.37^{c}	1.45^{b}	1.62^{b}	1.84^{ab}
Negative[2]	1.97^{a}	2.24^{a}	1.51^{a}	1.98^{a}	1.78^{b}
Avizyme[3]	1.40^{b}	1.37^{bc}	1.51^{ab}	1.68^{b}	1.86^{ab}
Hemicell[4]	1.32^{bc}	1.45^{bc}	1.51^{a}	1.69^{b}	1.89^{a}
Combination[5]	1.27^{c}	1.45^{b}	1.47^{ab}	1.64^{b}	1.89^{a}
SEM	0.030	0.029	0.019	0.024	0.034

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means ($P > 0.05$).
[1] Positive control was formulated to meet or exceed the recommendations of the NRC (1994) for all nutrients, except for a slightly lower apparent metabolizable energy to ensure that energy is the first limiting nutrient;
[2] Formulated to have 0.45 MJ less energy than the positive control. No exogenous enzyme was included;
[3] Formulated to have 0.45 MJ less energy than the positive control. Avizyme included at 0.05%;
[4] Formulated to have 0.45 MJ less energy than the positive control. Hemicell included at 0.0125%;
[5] Formulated to have 0.45 MJ less energy than the positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

In general, the improvements in growth associated with enzyme supplementation were most pronounced during the early growth period (0 - 21 days of age). Differences between weekly bodyweight gain of the negative control and enzyme-treated birds during the second week were 52% or less, compared with 5.5% or less for the last week of the growth period. The results obtained in this trial are supported by a study by Classen (1996), who stated that exogenous enzyme addition to the feed is more effective in younger broilers, since younger broilers have fewer endogenous enzymes. Caecal populations in older birds are also larger and more varied, which should improve fermentation responses to cell wall fragments with age (Wang *et al*., 2005).

Bedford & Cowieson (2012) discussed the effects of exogenous enzymes on intestinal microbiota. Carbohydrases that act on NSPs provide fermentable oligosaccharides to host microbial populations. Singh *et al*. (2012) found that xylanase supplementation of energy-restricted maize-soya-based diets induced a prebiotic effect that stimulated an increase in serum peptide YY concentration. Peptide YY is a hormone produced by enteroendocrine cells in the distal ileum and colon that slows down gastric emptying and intestinal transit time, thereby improving digestibility.

In this regard, a limitation of the present study was the higher fat content of the positive control (high ME) diets. This in itself could have slowed down the digesta transit time with a positive effect on pre-caecal digestibility of nutrients (Singh *et al*., 2012).

Table 5 Least square means (± standard error of the mean) of the cumulative feed intake (g/bird) and feed conversion ratios (FCR; g feed intake/g bodyweight gain) of broilers that received different treatments from day 0 until day 35

Treatments	Day 0 - 7	Day 0 - 14	Day 0 - 21	Day 0 - 28	Day 0 - 35
Feed intake (cumulative)					
Positive[1]	151.1[b]	445.7[c]	1001.8[b]	1872[b]	2945[b]
Negative[2]	151.2[b]	390.2[d]	704.0[c]	1586[c]	2542[c]
Avizyme[3]	163.5[a]	456.2[bc]	1030.0[a]	1917[a]	2988[ab]
Hemicell[4]	158.6[ab]	467.9[ab]	1040.3[a]	1940[a]	3006[a]
Combination[5]	154.4[ab]	478.8[a]	1043.8[a]	1941[a]	3020[a]
SEM	3.36	6.50	6.81	10.40	17.7
Feed conversion ratio					
Positive[1]	1.33[bc]	1.36[b]	1.41[c]	1.50[c]	1.61[d]
Negative[2]	1.97[a]	2.13[a]	1.80[a]	1.89[a]	1.83[a]
Avizyme[3]	1.40[b]	1.38[b]	1.45[b]	1.55[b]	1.65[bc]
Hemicell[4]	1.32[bc]	1.40[b]	1.46[b]	1.56[b]	1.66[b]
Combination[5]	1.27[c]	1.39[b]	1.43[bc]	1.52[bc]	1.63[c]
SEM	0.030	0.020	0.011	0.016	0.009

[abcd] Column means with the same superscript did not differ significantly for the treatments least square means (*P* >0.05).
[1] Positive control was formulated to meet or exceed the recommendations of the NRC (1994) for all nutrients, except for a slightly lower apparent metabolizable energy to ensure that energy is the first limiting nutrient;
[2] Formulated to have 0.45 MJ less energy than the positive control. No exogenous enzyme was included;
[3] Formulated to have 0.45 MJ less energy than the positive control. Avizyme included at 0.05%;
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[5] Formulated to have 0.45 MJ less energy than the positive control. Avizyme and Hemicell included at 0.05% and 0.0125%, respectively.

Conclusions

The addition of Avizyme (containing the enzymes amylase, and xylanase and a protease) and Hemicell (a β-mannanase product) to an energy-deficient maize-soya-based diet increased the energy available to broilers, which resulted in significant improvements in production performances. The broilers on the enzyme treatments had slightly higher feed intakes than the standard control and a worse cumulative FCR at day 35, and it is suggested that the feed was marginally deficient in available energy. It is therefore concluded that the enzyme preparations contributed slightly less than 0.45 MJ ME/kg feed. Combining the two enzyme preparations in a maize-soya-based broiler feed indicated a positive synergistic effect of the enzymes on each other, especially for the younger broiler.

References

Abdollahi, M.R., Ravindran, V. & Svihus, B., 2013. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. Anim. Feed Sci. Technol. 179, 1-23.

Annison, G., 1991. Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheat assayed in broiler chickens. J. Agric. Food Chem. 39, 1252-1256.

AOAC, 2000. Official Methods of Analysis. 17th ed. The Association of Official Analytical Chemists. Arlington, V.A., USA

Bedford, M.R. & Cowieson, A.J., 2012. Exogenous enzymes and their effects on intestinal microbiology. Anim. Feed Sci. Technol. 173, 76-85.

Burnham, D., Emmans G.C. & Gous R.M., 1992. Isoleucine responses in broiler chickens. Interactions with leucine and valine. Br. Poult. Sci. 33, 71-87.

Caine, W.R., Verstegen, M.W.A., Sauer, W.C. Tamminga, S. & Schulze, H., 1998. Effect of protease treatment of soybean meal on content of total soluble matter and crude protein and level of soybean trypsin inhibitors. Anim. Feed Sci. Technol. 71, 177-183.

Centeno, M.S.J., Ponte, P.I.P., Ribeiro, T., Prates, J.A.M., Ferreira, L.M.A., Soares, M.C., Gilbert, H.J. & Fontes, C.M.G.A., 2006. Galactanases and mannanases improve the nutritive value of maize and soybean meal based diets for broiler chicks. J. Poult. Sci. 43, 344-350.
Choct, M., 2006. Enzymes for the feed industry: past, present and future. Wrlds Poult. Sci. J. 62, 5-15.
Classen, H.L., 1996. Cereal grain starch and exogenous enzymes in poultry diets. Anim. Feed Sci. Technol. 62, 21-27.
Cowieson, A.J., 2005. Factors that affect the nutritional value of maize for broilers. Anim. Feed Sci. Technol. 119, 293-305.
Cowieson, A.J., 2010. Strategic selection of exogenous enzymes for maize/soy-based poultry diets. J. Poult. Sci. 47, 1-7.
Cowieson, A.J. & Bedford, M.R., 2009. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complementary mode of action? Wrlds Poult. Sci. J. 65, 609-624.
Emmans, G.C., 1981. A model of the growth and feed intake of ad libitum fed animals, particularly poultry. In: Computers in Animal Production. Eds: Hillyer, G.M., Whittemore, C.T. & Gunn, R.G., British Society of Animal Production, Edinburgh. Occasional publication no. 5. pp. 103-110.
Emmans, G.C., 1989. The growth of turkeys. In: Recent Advances in Turkey Science. Eds: Nixey, C. & Grey, T.C., Butterworths, London. pp. 135-166.
Ghazi, S., Rooke, J.A., Galbraith, H. & Bedford, M.R., 2002. The potential for the improvement of the nutritive value of soybean meal by different proteases in broiler chicks and broiler cockerels. Br. Poult. Sci. 43, 70-77.
Giron, H.C., 1973. Perkin Elmer Atomic Spectrophotometer. Atomic Absorption Newsletter 12, 28.
Gous, R.M., 2007. Predicting nutrient responses in poultry: Future challenges. Animal 1, 57-65.
Han, J.A. & BeMiller, J.M., 2008. Effects of protein on crosslinking of normal maize, waxy maize, and potato starches. Carbohydr. Polym. 73, 532-540.
Karimi, A., Bedford, M., Kamyab, A. & Moradi, M., 2007. Comparative effects of xylanase supplementation on broiler, broiler breeder and layer chick performance and feed utilization on wheat based diet. J. Poult. Sci. 44, 322-329.
Kocher, A., Choct, M., Porter, M.D. & Broz, J., 2000. The effect of enzyme addition to broiler diets containing high concentrations of canola or sunflower meal. Poult. Sci. 79, 1767-1774.
Li, Y., Chen, X., Chen, Y., Li, Z. & Cao, Y., 2010. Effects of β-mannanase expressed by Pichia pastoris in corn-soybean meal diets on broiler performance, nutrient digestibility, energy utilization and immunoglobulin levels. Anim. Feed Sci. Technol. 159, 59-67.
Mahagna, M., Nir, I., Larbier, M. & Nitsan, Z., 1995. Effect of age and exogenous amylase and protease on development of digestive tract pancreatic enzyme activities and digestibility of nutrients in young meat-type chicks. Reprod. Nutr. Dev. 35, 201-212.
McNaughten, J.L., Hsiao, H., Anderson, D. & Fodge, D.W., 1998. Corn / soy / fat diets for broilers, β-mannanase and improved feed conversion. Poult. Sci. 77 (Suppl.), 1153 (Abstr.).
Meng, X. & Slominski, B.A., 2005. Nutritive values of corn soybean meal, canola meal and peas for broiler chickens as affected by a multicarbohydrase preparation of cell wall degrading enzymes. Poult. Sci. 84, 1242-1251.
Oates, C.G., 1997. Towards an understanding of starch granule structure and hydrolysis. Trends Food Sci. Technol. 8, 375-382.
Odetallah, N.H., Ferket, P.R., Grimes, J.L. & McNaughton, J.L., 2002. Effect of mannan-endo-1,4-β-mannosidase on the growth performance of turkeys fed diets containing 44 and 48% crude protein soybean meal. Poult. Sci. 81, 1322-1331.
Oloffs, K., Samli, E. & Jeroch, H., 1999. Effect of supplementation of non-starch polysaccharide (NSP) hydrolyzing enzymes and wheat variety on prececal and faecal digestibility as well as metabolisable energy of laying hens. Proceedings 12th European Symposium on Poultry Nutrition. WPSA - Dutch Branch, Veldhoven, The Netherlands, 256.
Romero, L.F., Parsons, C.M., Utterback, P.L., Plumstead, P.W. & Ravindran, V., 2013. Comparative effects of dietary carbohydrases without or with protease on the ileal digestibility of energy and amino acids and AME_n in young broilers. Anim. Feed Sci. Technol. 181, 35-44.
Saleh, F., Ohtsuka, A., Tanaka, T. & Hayashi, K., 2003. Effect of enzymes of microbial origin on in vitro digestibilities of dry matter and crude protein in maize. J. Poult. Sci. 40, 274-281.
Singh, A., Masey O'Neill, H.V., Ghosh, T.K., Bedford, M.R. & Haldar, S., 2012. Effects of xylanase supplementation of 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.

SAS, 1989. Statistical Analysis Systems user's guide (4th ed.). SAS Institute Inc., Raleigh, North Carolina, USA.
SAS, 1994. Statistical Analysis Systems user's guide (6th ed.). SAS Institute Inc., Raleigh, North Carolina, USA.
Steenfeldt, S., 2001. Improvements in nutrient digestibility and performance of broiler chickens fed a wheat- and rye-based diet supplemented with enzymes. J. Anim. Feed Sci. 10, 143-157.
Wang, Z.R., Qiao, S.Y., Lu, W.Q. & Li, D.F., 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. Poult. Sci. 84, 875-881.
Wu, Y.B., Ravindran, V. & Hendriks, W.H., 2004. Influence of exogenous enzyme supplementation on energy utilization and nutrient digestibility of cereals for broilers. J. Sci. Food Agric. 84, 1817-1822.
Zanella, I., Sakomura, N.K., Silversides, F.G., Fiqueirdo, A. & Pack, M., 1999. Effect of enzyme supplementation of broiler diets based on maize and soybeans. Poult. Sci. 78, 561-568.