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Original Research

Carbohydrase enzymes improve performance of broilers fed both nutritionally adequate and marginal wheat-based diets

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Summary

A study was conducted to examine the effects of a multi-carbohydrase enzyme complex on the nutritive value of wheat in diets differing in nutrient density. It was hypothesised that response to enzyme inclusion would be greater in diets with lower nutrient density. The study was conducted using 1008 Ross 308 male broiler chicks (four treatments with seven replicate pens of 36 chicks). A 2 × 2 factorial arrangement of treatments was employed. Factors were adequate or low nutrient density with or without enzyme supplementation. The wheat-soybean meal based positive control (PC) diet was formulated to be nutritionally adequate in energy and digestible amino acids according to local industry recommendations. A negative control (NC) was formulated to have 80 kcal/kg less ME and 1.5% less digestible amino acids as compared to the PC. A multi-carbohydrase complex containing 19 carbohydrase activities derived from *Penicillium funiculosum* was added in both the PC and NC diets (Rovabio® Excel LC, Adisseo Asia Pacific Pte Ltd., Singapore). Birds fed the NC had 3.7 points ($P < 0.05$) poorer FCR than the PC. Across the diet type, enzyme supplementation increased body weight by 3.2% ($P < 0.05$) and improved FCR by 5.2 points ($P < 0.01$). There was no nutrient density x enzyme interaction ($P > 0.05$), indicating that performance improvement was independent of nutrient density. Apparent ileal digestibility of crude protein followed a similar trend, showing a 4.9% enhancement ($P < 0.01$) with the inclusion of the enzyme product in either diet. Enzyme supplementation reduced ileal viscosity by 39.0% ($P < 0.05$). It was concluded that multi-carbohydrase could overcome the negative effect in broiler performance brought by nutrient reduction, however, there was no indication that nutrient density affected bird response to supplementation of multi-carbohydrase.

Key words: **Carbohydrase: digestibility: nutrient density: wheat: performance**

Introduction

The use of non-starch polysaccharide (NSP)-degrading enzymes in poultry diets has previously been shown to improve performance in birds fed wheat-based diets. These enzymes mainly exert their performance-promoting effect by partially degrading water soluble and viscous arabinoxylans, which inhibit nutrient digestion and absorption by raising intestinal viscosity (Bedford and Classen, 1992). In addition, the breakdown of cell wall polysaccharides possibly releases entrapped starch and protein (Theander *et al.*, 1989; Wiseman *et al.*, 2000). The NSP content of feed ingredients is highly variable. Wheat contains 5–8% arabinoxylans which consist of β 1–4 xylan backbone to which single arabinose residues are linked through O-2, O-3 or O-2,3 positions. In addition, up to 1% of β-glucans and 2–3% of cellulose are present (Choct *et al.*, 2004). The total NSP content in soybean meal is around 23–25% (Choct *et al.*, 2010) and canola meal contains 17.9% (Slominski and Campbell, 1990), mostly in the form of cellulose and pectic polysaccharides (Knudsen, 1997). Given that more plant ingredients containing different forms of NSP are being used in the poultry industry, it is reasonable to speculate that enzyme combinations with additional activities, each differing in substrate affinity, may deliver further enhancement in nutrient utilisation than a single xylanase alone

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can achieve. In addition, nutritional adequacy of the diet has been determined to be an important factor affecting the response to a dietary enzyme (Cowieson, 2010). Zhou *et al.* (2009) reported that addition of xylanase, amylase, and protease had greater effects on increasing AME of corn-soy diets with lower energy contents compared with diets with higher energy levels fed to broiler chickens. Kocher *et al.* (2003) found that when enzymes were included in a lower-energy corn-soybean meal basal diet, the combination of pectinase, protease, and amylase significantly improved AMEn in comparison to the unsupplemented basal diet. However, a subsequent experiment with a higher energy and protein basal diet failed to show the same improvement when enzymes were added. Thus, responses to exogenous enzyme products would be expected to be greater in diets with lower nutritional quality. This study was conducted to examine the hypothesis that the nutrient density of the diet would affect the response to exogenous carbohydrase supplementation in terms of bird performance and nutrient digestibility.

Materials and methods

Enzyme preparation

The multi-carbohydrase complex (Rovabio® Excel LC, Adisseo Asia Pacific Pte Ltd., Singapore) is a concentrated solution with the main enzymatic activities of xylanase (1,100 visco unit/kg), and β-glucanase (100 AGL units/kg). This product is made from a fermentation broth of *Penicillium funiculosum*. The multi-carbohydrase complex was applied post-pelleting at a dose rate of 200 ml/tonne of feed. The enzyme recovery from grower diets was analysed by Adisseo Asia Pacific Pte Ltd (PC + multi-carbohydrase: 1163 visco units/kg feed; NC + multi-carbohydrase: 891 visco units/kg feed).

Bird husbandry

A total of 1008 male broiler chicks (1-d-old; Ross 308) were obtained from the Baiada Hatchery, Tamworth, New South Wales and reared in floor pens with hardwood litter with eight nipple drinkers per pen. There were 36 birds per pen, which was 1.5 m x 1.5 m in dimension. Birds were fed 500 g of 1–2 mm starter crumbles followed by 1500 g of a 5 mm pelleted grower feed and the remainder as 5 mm pelleted finisher feed. Birds were weighed on d 10, 24 and 42, and were given starter and grower feed for slightly longer than these time points. Residual feed was weighed on days 10 and 24 was placed back into the feeders. The next phase (pre-weighed) was placed into the feeder on top of the previous phase. Diets and water were offered *ad libitum*. The lighting program was 24 h of light during the first 3 d, 23 h of light from 4 d to 7 d, 20 h of light from 8 d to 10 d, and 18 h of light thereafter until the end of the experiment. The animal ethics committee of the University of New England approved the study protocol (AEC 11/032).

Diets and experimental design

The positive control (PC) was a nutrient adequate wheat-soybean meal based diet formulated to meet the local industry recommendations, varying according to starter, grower and finisher phases. Nutrient levels in the negative control (NC) were 80 kcal/kg ME and 1.5% digestible amino acid (DAA) lower compared to the PC. The diet composition and nutrient content are shown in Table 1. Diets were mixed in a 250 or 500 kg mixer as appropriate and pelleted without steam conditioning at 65 °C. A multi-carbohydrase complex containing 19 carbohydrase activities (Rovabio® Excel LC) was supplemented to both diets to give a 2×2 factorial trial arrangement of treatments, i.e. PC or NC with and without enzyme, with seven pen replicates per treatment. Starter feed pellets were crumbled through a roller mill (1 to 2 mm). Grower and finisher diets were fed as 5 mm intact pellets. Pellets were spread onto a clean surface to a depth of 3 cm for cooling. The multi-carbohydrase (liquid form) was sprayed post-pelleting at a rate of 200 ml/tonne feed. The spray was applied as a diluted spray (250 x dilution) of received product to cooled pellets. Each batch of pellets was sprayed evenly twice, turning the pellets over in between applications to ensure even coverage.

Sample collection and laboratory analysis

Three birds per pen were removed at both d 27 and d 42 and euthanised by cervical dislocation. Ileal contents were immediately collected from Meckel's diverticulum to the ileo-caecal junction. Approximately 2 g of pooled fresh digesta was centrifuged (12000 *g*, 10 min at 20 °C) and viscosity was determined on 0.5 ml supernatant fraction using a Brookfield Model DV-III Rheometer (Brookfield Engineering Laboratories Corp., Stoughton, MA, USA) at 25 °C with a CP40 cone and shear rate of 5–500/s. The samples did not exhibit shear thinning at these shear rates. The remaining samples were placed in plastic tubes with covers and frozen

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Table 1. Diet composition, nutrient content, ingredient prices used and diet costs

Ingredient (g/kg)	Starter		Grower		Finisher	
	PC	NC	PC	NC	PC	NC
Wheat	573	600	623	647	660	683
Soybean meal	303	291	196	187	110	102
Meat meal	40	40	40	40	40	40
Canola oil	38	22	45	31	53	39
Canola meal	20	20	70	70	120	120
Limestone	7.2	7.3	56.6	5.7	5.0	5.1
Dicalcium phosphate	5.8	5.8	2.5	2.4	1.2	1.1
Sodium chloride	3.05	3.03	2.37	2.35	1.78	1.76
Na bicarbonate	1.34	1.34	1.50	1.50	1.50	1.50
DL-Methionine	2.83	2.71	2.30	2.19	1.49	1.39
L-Lysine	1.80	1.83	2.46	2.46	2.35	2.35
L-Threonine	1.06	1.04	1.16	1.12	0.89	0.85
Vitamins[1]	0.75	0.75	0.50	0.50	0.50	0.50
Minerals[2]	1.25	1.25	0.75	0.75	0.75	0.75
Choline Cl (70%)	0.82	0.82	0.42	0.41	0.44	0.43
Monensin (12%)	0.50	0.50	0.50	0.50	0.50	0.50
Zn-bacitracin (15%)	0.33	0.33	0.33	0.33	0.33	0.33
TiO_2	–	–	5	5	–	–
Nutrients (g/kg)						
ME (kcal/kg)	2,950	2,865	3,025	2,940	3,100	3,015
CP	232	230	207	205	189	188
Crude fibre	29.6	29.7	32.2	32.4	35.3	35.5
Crude fat	59.1	44.3	67.2	53.1	76.0	62.0
Dig[3] Arg	13.4	13.2	11.3	11.2	9.8	9.6
Dig Lys	12.4	12.2	11.0	10.8	9.5	9.4
Dig Met	5.99	5.85	5.20	5.07	4.24	4.12
Dig M + C	8.93	8.79	8.03	7.91	7.03	6.92
Dig Thr	8.06	7.94	7.26	7.15	6.37	6.27
Dig Trp	2.55	2.52	2.20	2.17	1.94	1.92
Dig Ile	8.31	8.18	7.72	7.65	6.83	6.77
Dig Val	10.05	9.95	8.95	8.88	8.19	8.14
Na	2.2	2.2	2.0	2.0	1.8	1.8
Cl	3.0	3.0	2.6	2.6	2.2	2.2
Linoleic	16.4	13.2	17.9	14.9	19.6	16.6
Choline (mg/kg)	1.85	1.85	1.50	1.50	1.40	1.40
Ca	0.84	0.84	0.72	0.72	0.68	0.68
Total P	0.69	0.69	0.63	0.63	0.63	0.63
Available P	0.42	0.42	0.36	0.36	0.34	0.34

[1] Vitamins supplied the following per kilogram of diet in starter, grower and finisher, respectively: vitamin A, 12000 IU, 8000 IU, 8000 IU; cholecalciferol, 5,000 IU, 3333 IU, 3333 IU; vitamin E, 75 IU, 50 IU, 50 IU; vitamin K, 3 mg, 2 mg, 2 mg; vitamin B_{12}, 16 mcg, 11 mcg, 11 mcg; riboflavin, 8 mg, 5 mg, 5 mg; pantothenic acid, 13 mg, 9 mg, 9 mg; nicotinic acid, 55 mg, 37 mg, 37 mg; folic acid, 2 mg, 1.3 mg, 1.3 mg; biotin, 0.2 mg, 0.13 mg, 0.13 mg.
[2] Mineral mix supplied the following per kilogram of diet in starter, grower and finisher, respectively: Mn, 120 mg, 71 mg, 71 mg; Zn, 100 mg, 59 mg, 59 mg; Fe, 40 mg, 24 mg, 24 mg; Cu, 16 mg, 9 mg, 9 mg; Se, 0.3 mg, 0.17 mg, 0.17 mg; I, 1.25 mg, 0.74 mg, 0.74 mg.
[3] Dig = digestible

at −20 °C. Proventriculus, gizzard, liver, pancreas and small intestine were collected for the calculation of the relative organ weight (organ weight g/live bird weight kg).

The Dumas combustion method was applied to determine the crude protein content [N × 6.25] of both diet and ileal digesta samples (Etheridge *et al.*, 1998). Nitrogen liberated by combustion at high temperature in pure oxygen was measured by thermal conductivity detection and converted to equivalent protein. Starch was determined colorimetrically as glucose using a glucose oxide and proxidase method (Megazyme Total Starch Kit, Megazyme International Ireland Ltd., Bray, Ireland). Titanium dioxide was determined based on the method of Short *et al.* (1996). The free sugar, soluble NSP and insoluble NSP contents of ileal digesta was tested by the Uppsala method (Theander and Westerlund, 1993)

Ileal digestibility values were calculated using the following equation

$$AND_x = 1 - \left[\left(T_i/T_o\right) \times \left(X_o/X_i\right)\right]$$

Here, AND_x is apparent nutrient digestibility for ileal samples expressed as a percentage; T_i is the concentration of TiO2 present in the diet; T_o is the concentration of TiO2 present in the ileal digesta; X_o is the nutrient concentration present in the ileal digesta; and X_i is the nutrient concentration present in the diet. All values

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for T_i, T_o, X_o, X_i were expressed as a percentage of dry matter.

Statistical Analysis

All data were analysed using SPSS software. One-way ANOVA and the general linear model procedure were used to determine the significance of the main effects and any interactions, where appropriate. Duncan's multiple-range test was used to separate means when significant main effects ($P < 0.05$) were detected by one-way ANOVA.

Results

From 0 to 42 d diet had no effect on body weight (BW), feed intake, and livability ($P > 0.05$). However, feeding the NC diet negatively impacted FCR ($P < 0.01$) by 3.7 points compared to birds fed the PC diets. Across diet types, the inclusion of multi-carbohydrase increased BW by 3.2% ($P < 0.05$) and improved FCR by 5.2 points ($P < 0.001$) but had no effect on feed intake or livability. No interaction was observed between the two factors. Results are shown in Table 2.

Viscosity values of ileal contents from birds sampled at d 24 and d 42 are presented in Table 3. Viscosity was significantly lower in birds fed diets containing multi-carbohydrase at d 42 ($P < 0.05$). There was a tendency for reduced viscosity in birds fed diets supplemented with multi-carbohydrase at d 24 ($P = 0.068$).

Table 2. Performance of broilers from 0 to 42 d

Treatment means	Body weight, g	Feed intake, g	FCR, g/g	Livability, %
PC[1]	3246	5227	1.633	92.6
PC + enz[2]	3343	5212	1.580	93.1
NC[3]	3235	5320	1.669	96.6
NC + enz	3345	5336	1.617	93.1
SEM	22.8	33.3	0.01	1.08
Main effects				
Diet				
PC	3295	5220	1.606[b]	92.9
NC	3290	5328	1.643[a]	94.9
Enz				
–	3240[b]	5274	1.651[a]	94.6
+	3344[a]	5274	1.599[b]	93.1
Significance				
Diet	0.907	0.117	0.004	0.371
Enz	0.025	0.992	< 0.001	0.521
Diet * enz	0.878	0.820	0.943	0.371

[a,b] means within a column with different superscripts are significantly different ($P < 0.05$)
[1]PC = positive control
[2]Enz = multi-carbohydrase complex
[3]NC = negative control

Table 3. Ileal digesta viscosity in birds with or without multi-carbohydrase (mPa.s)

Treatment Means	Viscosity d 27	Viscosity d 42
PC[1]	7.50	4.28
PC + enz[2]	5.71	3.24
NC[3]	8.45	5.63
NC + enz	6.00	3.72
SEM	0.55	0.47
Main effects		
Diet		
PC	6.60	4.50
NC	7.22	4.67
Enz		
–	7.97	5.70[a]
+	5.85	3.48[b]
Significance		
Diet	0.579	0.858
Enz	0.068	0.029
Diet * enz	0.772	0.750

[a,b] means within a column with different superscripts are significantly different ($P < 0.05$)
[1]PC = positive control
[2]Enz = multi-carbohydrase complex
[3]NC = negative control

As shown in Table 4, diet did not affect apparent ileal digestibility of protein. However, the multi-carbohydrase improved digestibility of crude protein ($P < 0.01$). There was no diet by enzyme interaction ($P > 0.05$). No main effects of diet, enzyme, or their interaction were detected on starch digestibility.

Table 5 shows the relative selected organ weights. The addition of enzyme reduced the relative gizzard weight ($P < 0.05$) and tended to reduce the relative small intestine weight ($P = 0.097$) at d 42. The liver ($P < 0.01$) and small intestine ($P < 0.05$) of birds fed the NC diet were heavier than that of birds fed the PC diet, respectively. An interaction was detected between nutrient density and multi-carbohydrase for relative weight of liver ($P < 0.05$).

Supplementation of multi-carbohydrase significantly increased the free sugar concentration compared to the non-supplemented group ($P < 0.05$), and tended to decrease the insoluble NSP concentration ($P = 0.095$) regardless of the nutrient density. Birds fed NC diet had higher soluble NSP concentration than those fed PC diet ($P < 0.05$). There was no interaction on free sugar, soluble NSP, or insoluble NSP concentration (Table 6).

Discussion

The mechanisms by which supplemental xylanases improve the nutritive value of wheat-based diets for

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Table 4. Apparent ileal digestibility of crude protein and starch at d 27

Treatment Means	Digestibility of crude, protein %	Digestibility of starch, %
PC[1]	73.7	90.7
PC + enz[2]	75.9	93.8
NC[3]	74.4	94.9
NC + enz	79.3	95.0
SEM	0.55	0.78
Main effects		
Diet		
PC	74.8	92.2
NC	76.8	94.9
Enz		
–	74.0[b]	92.8
+	77.6[a]	94.4
Significance		
Diet	0.086	0.101
Enz	0.005	0.317
Diet * Enz	0.242	0.354

[a,b] means within a column with different superscripts are significantly different (P < 0.05)
[1]PC = positive control
[2]Enz = multi-carbohydrase complex
[3]NC = negative control

Table 6. Amounts of free sugar, soluble NSP and insoluble NSP (mg/g digesta) in ileal digesta at d 27

Treatment Means	Free sugar, mg/g digesta	Soluble NSP, mg/g digesta	Insoluble NSP, mg/g digesta
PC[1]	79.5	32.8	242.8
PC + enz[2]	92.7	32.7	194.0
NC[3]	79.1	37.8	213.3
NC + enz	91.0	40.1	208.9
SEM	2.85	1.30	7.38
Main effects			
Diet			
PC	86.1	32.8[b]	218.4
NC	85.0	38.9[a]	211.1

[a,b] means within a column with different superscripts are significantly different (P < 0.05)
[1]PC = positive control
[2]Enz = multi-carbohydrase complex
[3]NC = negative control

poultry may be well-documented, but research concerning the effect of nutrient density on responses of birds to carbohydrase supplementation remains scant. It has been suggested the inherent ileal digestibility of starch, protein and lipid in a corn/soy-based diet varies from around 70% to over 95%, and this explains up to 90% of the variance in enzyme response (Cowieson, 2010). In addition, responses to exogenous enzyme addition are expected to be greater in diets with lower nutritional quality (Kocher, *et al.*, 2003; Cowieson and Ravindrani, 2008; Zhou, *et al.*, 2009). Zhou *et al.* (2009) reported that supplementation of xylanase, amylase and protease led to an additional increase on AME of corn-soy diets with lower energy contents compared to diets with higher energy levels fed to broiler chickens. Kocher *et al.* (2003) found that the combination of pectinase, protease, and amylase significantly improved AMEn in comparison to the unsupplemented basal diet in a lower-energy corn–SBM diet. However, a subsequent experiment with a higher energy and protein basal diet failed to deliver the same improvement when enzymes were added. However, in the current study, there was no indication that nutrient density affected bird response to supplementation with multi-carbohydrase. Instead, the enzyme improved performance regardless of nutrient density. There was no diet x enzyme interaction for performance

Table 5. The relative organ weight (organ weight/ live bird weight, g/kg) at d 42

Treatment means	Proventriculus	Gizzard	Liver	Pancreas	Small intestine
PC[1]	2.15	10.01	18.9[c]	1.51	25.6
PC + enz[2]	2.28	8.78	20.7[bc]	1.55	24.2
NC[3]	2.48	9.84	23.0[a]	1.77	29.1
NC+ enz	2.19	8.33	21.5[ab]	1.56	26.8
SEM	0.05	0.26	0.37	0.04	0.54
Main effects					
Diet					
PC	2.22	9.42	19.8[b]	1.53	24.9[b]
NC	2.34	9.08	22.3[a]	1.67	28.0[a]
Enz					
–	2.32	9.95[a]	21.0	1.64	27.4
+	2.24	8.55[b]	21.1	1.56	25.5
Significance					
Diet	0.248	0.517	0.003	0.137	0.010
Enz	0.437	0.012	0.874	0.365	0.097
Diet * enz	0.056	0.824	0.042	0.179	0.672

[a-c] means within a column with different superscripts are significantly different (P < 0.05)
[1]PC = positive control
[2]Enz = multi-carbohydrase complex
[3]NC = negative control

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variables. This is in agreement with the finding of Neto et al. (2015), where they tested the effect of graded levels of DAA on performance of broilers fed corn-based diets, and found that DAA reduction linearly reduced all performance parameters, but the addition of a multi-carbohydrase improved BW gain and FCR regardless of nutritional reduction levels. Moreover, it is possible that the lack of effect of nutrient density on enzyme response may be related to the grain source. Neto et al. (2015) reported that when birds were fed wheat-based diets with different reduction levels of DAA, the addition of a multi-carbohydrase significantly improved growth performance and the deposition of energy and protein regardless of the DAA levels. Unlike the situation in corn-soy diets, supplementation with multi-carbohydrase might be expected to reduce digesta viscosity of wheat-based diets regardless of nutrient density. Wheat pentosans are known to increase the secretion of endogenous amino acids in broiler chicks (Angkanaporn *et al.*, 1994). While the nutrient improvements observed with supplemental enzyme may be dependent on the nutritional quality of the basal diet, the ability of carbohydrases to reduce viscosity and ameliorate anti-nutritive properties may not be confined to the nutrient density of the basal diet as supplemental enzymes not only modify the feed, but also the way feed is digested.

The changes brought about by exogenous enzymes on gut physiology, secretion and absorption, and microbial population may be less tangible but their effects on animals should not be overlooked. Such physiological effect might remain irrelevant from a practical nutrition view point (Cowieson, 2010). In the current study, birds fed the NC diet had a heavier liver and a smaller intestine compared to those fed the PC diet. The addition of multi-carbohydrase decreased the relative weight of gizzard, and tended to reduce the relative weight of small intestine. A diet x enzyme interaction was observed for liver weight, because birds fed NC diet with no enzyme had a heavier liver compared to those fed PC diet without enzyme. With enzyme supplementation there was no difference in the relative weight of liver between birds fed PC and NC diet. This may indicate that the birds fed the NC diet without enzyme produced more bile in order to facilitate the digestion of fat, whereas supplementation improved the mixing of the digestive enzymes with their substrates. In the case of fat digestion, this would increase the effectiveness of micelle formation, leading to the restoration of the normal digestive efficiency and eliminating the requirement for additional bile secretion. While the liver and gut account for only 3% of the BW of chickens, they may contribute up to 26% of the total heat production, which demonstrates that a relatively small GIT size could lead to a significant saving in maintenance energy (Spratt *et al.*, 1990). Therefore, the effect of the multi-carbohydrase on the relative weights of small intestine, liver, and gizzard may have been effective in enhancing growth performance by sparing endogenous energy loss, and this sparing effect should be independent from the nutritional quality of the basal diet.

In the current experiment, the ileal viscosity at d 42 was reduced by enzyme supplementation, regardless of the diet type. The effect on intestinal viscosity is generally believed to be a primary mechanism through which NSP-degrading enzymes exert their beneficial effects (Annison, 1992; Choct, 1994). In addition, the enzyme improved protein digestibility. This may be due to disruption of the cell wall of wheat, leading to the release of previously encapsulated protein as well as a more effective enzyme-substrate mix in the lumen due to a more fluid, less viscous digesta.

The current study demonstrated that multi-carbohydrase supplementation elevated the ileal free sugar concentration. The extra released free sugars, along with oligosaccharides and low molecular weight polysaccharides, would be available for microbial fermentation in the hindgut of broilers, leading to the generation of volatile fatty acids that can be absorbed and utilised as an energy source. The soluble NSP levels in the ileal digesta of birds fed the NC diet was greater than samples from birds fed PC. This may be due to the higher inclusion of wheat in the NC diet. The addition of multi-carbohydrase tended to reduce the insoluble NSP concentration in the ileal digesta, which may partially explain the increased crude protein digestibility.

Conclusions

In summary, it is not surprising that nutritive value of wheat-based diets was enhanced by dietary multi-carbohydrase supplementation, but it is interesting to see that the effect of the enzyme was independent from nutrient density of the diet in this study. The reason may be that the difference between the PC and NC diets was not large enough to demonstrate an effect of diet density x enzyme interaction. The performance of the birds fed the NC diet plus the enzyme was actually superior to that of the PC, which suggested that the nutrient

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uplift due to enzyme supplementation in the NC diet was actually greater than the 80 kcal/kg and 1.5% reduction in digestible amino acids.

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Declaration of interest

Y.G. Liu is an employee of Adisseo Asia Pacific Pte Ltd.

References

Angkanaporn K., Choct M., Bryden W. L., Annison E. F. and Annison G. (1994). Effects of wheat pentosans on endogenous amino-acid losses in chickens. *Journal of the Science of Food and Agriculture*, **66**: 399–404.

Annison G. (1992). Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolisable energy, starch and pentosan digestibilities in broiler-chickens. *Animal Feed Science and Technology*, **38**: 105–121.

Bedford M. R. and Classen H. L. (1992). Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate-composition of the intestinal aqueous phase and results in improved growth-rate and food conversion efficiency of broiler chicks. *Journal of Nutrition*, **12**: 560–569.

Choct M., Dersjant-Li Y., McLeish J. and Peisker M. (2010). Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. *Asian-Australasian Journal of Animal Sciences*, **23**: 1386–1398.

Choct M, H. R., Trimble R.P. and Annison G. (1994). The use of enzymes in low-AME wheat broiler diets: effects on bird performance and gut viscosity. *Proceedings of the 6th Australian Poultry Science Symposium*, Sydney, pp. 83–87.

Choct M., Kocher A., Waters D. L. E., Pettersson D. and Ross G. (2004). A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. *British Journal of Nutrition*, **92**: 53–61.

Cowieson A. J. (2010). Strategic Selection of Exogenous Enzymes for Corn/soy-based Poultry Diets. *Journal of Poultry Science*, **47**: 1–7.

Cowieson A. J. and Ravindrani V. (2008). Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. *British Poultry Science*, **49**: 37–44.

Etheridge R. D., Pesti G. M. and Foster E. H. (1998). A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. *Animal Feed Science and Technology*, **73**: 21–28.

Knudsen K. E. B. (1997). Carbohydrate and lignin contents of plant materials used in animal feeding. *Animal Feed Science and Technology*, **67**: 319–338.

Kocher A., Choct M., Ross G., Broz J. and Chung T. K. (2003). Effects of enzyme combinations on apparent metabolisable energy of corn-soybean meal-based diets in broilers. *Journal of Applied Poultry Research*, **12**: 275–283.

Neto R. M., Cozannet P., and Preynat A. (2015). Effect of the addtion of a carbohydrase complex on performance ad carcass yield of broiler chickens fed wheat-based diets. *Proceedings of the 20th European Symposium on Poultry Conference*, Prague, pp. 339–341.

Neto R. M., Cozannet P., Rouffineau F., and Preynat A. (2015). Effet of a carbohydrase complex in feeds with different metablolisable energy and available amino acids levles for broilers reared under a hot climate condition. *Proceedings of the 20th European Symposium on Poultry Conference*, Prague, pp. 336–338.

Short F. J., Gorton P., Wiseman J. and Boorman K. N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology*, **59**: 215–221.

Slominski B. A. and Campbell L. D. (1990). Nonstarch polysaccharides of canola-meal - quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. *Journal of the Science of Food and Agriculture*, **53**: 175–184.

Spratt R. S., McBride B. W., Bayley H. S. and Leeson S. (1990). Energy-metabolism of broiler breeder hens .2. contribution of tissues to total heat-production in fed and fasted hens. *Poultry Science*, **69**: 1348–1356.

Theander O., and Westerlund E. (1993). Determination of individual components of dietary fiber. In A. G. Spiller (Ed.), *Dietary Fiber in Human Nutrition* (2nd ed., pp. 77–98). Boca Raton, FL: CRC Press, Inc.

Theander O., Westerlund E., Aman P. and Graham H. (1989). Plant-cell walls and monogastric diets. *Animal Feed Science and Technology*, **23**: 205–225.

Wiseman J., Nicol N. T. and Norton G. (2000). Relationship between apparent metabolisable (AME) values and in vivo/in vitro starch digestibility of wheat for broilers. *Worlds Poultry Science Journal*, **56**: 305–318.

Zhou Y., Jiang Z., Lv D. and Wang T. (2009). Improved energy-utilizing efficiency by enzyme preparation supplement in broiler diets with different metabolisable energy levels. *Poultry Science*, **88**: 316–322.

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