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# Effects of xylanase supplementation on between-bird variation in energy metabolism and the number of *Clostridium perfringens* in broilers fed a wheat-based diet

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*Abstract.* Three experiments were conducted to examine: (*a*) the apparent metabolisable energy (AME) contents, the level of non-starch polysaccharides (NSP), and the extract viscosity value of 9 wheat samples; (*b*) the efficacy of a xylanase in reducing between-bird variation for AME; and (*c*) the effect of a xylanase on the number of *Clostridium perfringens* in broilers fed a low AME wheat diet. Experiment 1 revealed that the AME value of wheat varied from 11.5 to 13.6 MJ/kg dry matter (DM), which negatively correlated with the total NSP level ($r = -0.97$). Experiment 2 involved a 48-cage individual bird trial, where xylanase increased ($P < 0.05$) the AME from 12.51 to 13.09 MJ/kg DM and reduced ($P < 0.05$) its standard deviation from 1.13 to 0.52. The standard deviation for feed conversion ratio, ileal viscosity, and faecal viscosity was also reduced. In this experiment, there was a strong correlation between AME and excreta viscosity ($r = -0.81$) and the caecal xylanase activity of birds fed the control diet was positively correlated with AME ($r = 0.72$). In Expt 3, xylanase reduced the number of *Clostridium perfringens*, the causative agent for necrotic enteritis, to a non-detectable level in the ileum and caeca of broilers fed a low-ME wheat diet.

*Additional keywords:* non-starch polysaccharides, gut microflora.

## Introduction

Wheat is one of the major feed ingredients in poultry diets, but the nutritive value of wheat is highly variable. It has been reported that the apparent metabolisable energy (AME) content of Australian wheat ranges from 10.35 to 15.9 MJ/kg dry matter (DM) (Mollah *et al*. 1983; Rogel *et al*. 1987), with about 30% of Australian wheat having AME values <13 MJ/kg DM. When chickens are fed diets based on wheat with a low AME, performance and feed-use efficiency are often poor (Annison and Choct 1991). Studies with broilers (Choct 1995; Hughes *et al*. 1996; Hughes and Choct 1997) have further confirmed the variability of AME between wheat varieties (10–15 MJ/kg DM). More importantly, Hughes and Choct (1997) assayed the metabolisable energy (ME) value of a known low-ME wheat using 40 replicates. The wheat had an average AME value of 12.1 MJ/kg DM, but it ranged from 8.8 to 14.9 MJ/kg DM. This large between-bird variation in AME is also seen with new-season grains with low initial AME values. However, such an extreme variability does not occur when diets based on maize, sorghum, or high-ME wheat varieties are used, suggesting that the factor eliciting this response in ME is related to the feed.

It is known that the low AME of wheat is a result of the high levels of non-starch polysaccharides (NSP), such as soluble arabinoxylans. The viscous nature of these polysaccharides has a direct influence on nutrient absorption as well as interacting with the gut microflora and modifying the physiological function of the gut. High levels of soluble NSP raise digesta viscosity in the intestine of chickens, leading to reduced starch, protein, and lipid digestion (Choct and Annison 1990; Annison 1991; Choct *et al*. 1992; Philip *et al*. 1995). The consequences of an altered rate of nutrient digestion in the gut may be manifested in the number and type of microorganisms present in the gut (Vahjen *et al*. 1998). An increased amount of NSP in the digesta has been demonstrated to adversely influence the gut microflora of birds (Choct *et al*. 1996).

It is well documented that the addition of commercial feed enzymes to wheat-based broiler diets generally results in a significant improvement in performance and a reduction in


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the occurrence of wet and sticky droppings (Annison 1992; Choct *et al*. 1994). Enzymes such as xylanases reduce the intestinal viscosity by degradation of soluble arabinoxylans. Studies by Choct *et al*. (1995, 1996) demonstrated that supplementation of a low-ME wheat diet with a commercial glycanase preparation increased the AME by 24% and the feed conversion efficiency by 25% in 3–4-week-old broiler chickens. The addition of soluble NSP in broiler chicken diets significantly elevated fermentation in the small intestine. Subsequent *in vivo* depolymerisation of the soluble NSP using glycanases almost completely overcame this problem. The current study was conducted to examine the effect of a xylanase on individual bird variability of AME and the number of *Clostridium perfringens*, the bacterium responsible for necrotic enteritis, in broiler chickens.

## Materials and methods

*Bird husbandry*

Day-old male broiler chicks (Cobb strain) were obtained from the Baiada Hatchery, Tamworth, New South Wales, and were raised on commercial broiler starter crumbles containing 12.5 ME MJ/kg and a minimum of 20% crude protein (Ridly AgriProducts, Tamworth, NSW), in standard chick brooders for 21 days. During experimentation, all birds were transferred to wire-mesh ME cages situated in well-ventilated rooms. During the winter months, the rooms were heated using electric radiators. All experimental diets were cold-pelleted and offered *ad libitum* and water was freely available. Birds were grown under continuous fluorescent lighting. The Animal Ethics Committee of the University of New England approved this study. Health and husbandry practices complied with The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 1997.

*Experiment 1: AME bioassay of 9 wheat samples*

*Wheat samples and apparent metabolisable energy trial*

Six tonnes each of 9 wheat varieties were obtained shortly after harvest and stored as per commercial practice. The AME was measured within 1 month of harvest and all the assays were completed within 2 months of harvest. The standard basal diet was formulated using the ingredients and proportions shown in Table 1. First, the minor ingredients were mixed in a small rotary mixer and then were pooled with the major ingredients for thorough mixing. All experimental diets were identical in composition and were cold-pelleted.

Two hundred and sixteen one-day-old broiler chicks were raised on commercial starter to Day 21 and were transferred to 36 metabolism cages at 6 birds per cage. The first 4 days served as an adaptation period and for the last 4 days, excreta were collected daily, dried overnight in a forced-drought oven at 80°C, and pooled for determination of gross energy. The amount of feed consumed during the excreta collection period was recorded. The AME value of the major ingredient (cereal) was obtained using a pre-determined AME value (20.1 MJ/kg DM) for casein (Annison *et al*. 1994). The calculation of the AME of the grain is as follows:

$$AME_{grain} = (AME_{diet} - AME_{casein} \times \text{dry casein level})/\text{dry grain level}$$

*Non-starch polysaccharides and extract viscosity*

The NSP fractions were analysed using the Uppsala Method (Theander *et al*. 1995). Extract viscosity was determined by extracting 2 g of finely ground sample at 40°C for 2 h. The mixture was centrifuged (12 000 *G* 10 min at 20°C) and the viscosity was determined on 0.5 mL of supernatant using a Brookfield Model DV-III Rheometer 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.

**Table 1. Composition of the basal diet**

| Ingredient | g/kg |
|---|---|
| Wheat | 820 |
| Casein | 134 |
| Dicalcium phosphate | 26 |
| Calcium carbonate | 11 |
| Trace mineral-vitamin premix[A] | 5 |
| NaCl | 3.6 |
| Choline chloride (50%) | 0.4 |

[A]The active ingredients contained in each kg premix were as follows: retinol 3.03 mg, cholecalciferol 0.09 mg, all-*rac*-α-tocopherol acetate 20 mg, menadione 6.3 mg, riboflavin 8 mg, pyridoxine hydrochloride 5 mg, biotin 0.01 mg, niacin 30 mg, thiamine 1.5 mg, D-calcium pantothenate 15 mg, folic acid 2 mg, ethoxyquin 125 mg, Mn 75 mg, Fe 20 mg, Cu 5 mg, I 1 mg, Co 0.3 mg, Se 0.5 mg, Mo 0.16 mg, cyanocobalamin 0.15 mg.

*Dry matter and gross energy*

DM of the diets was determined using a drying oven at 105°C for 6 h. Gross energy contents of diets and excreta were determined using a Parr isoperibol calorimeter (Parr Instrument Co., Moline, IL, USA) at the South Australian Research and Development Institute (SARDI) and using a DDS 500 isoperibol calorimeter (Digital Data Systems, Johannesburg, South Africa) at the University of New England. Approximately 1 g of digesta or 2 g of feed were pelleted and then accurately weighed. The sample pellet was burned in the calorimeter and the heat given off was determined as the gross energy content of the sample.

*Experiment 2: Influence of xylanase on AME variability*

*The enzyme*

The enzyme used in this experiment was supplied by Novozymes Australia and registered under the commercial name Ronozyme WX (old trading name Bio-Feed Wheat). It was derived from *Thermomyces lanuginosus* and each g of product contained 1000 fungal xylanase units (FXU). There were no side activities.

*Apparent metabolisable energy trial*

Two diets were formulated with a low AME wheat (Harvey variety). The diet formulation was exactly as per Table 1. One diet was used as control, but the other diet was supplemented with xylanase at a rate of 200 g/t. Day-old male broilers were fed a commercial starter diet to 21 days. Then 48 healthy birds were chosen and allocated to the control or enzyme diet. Each diet was replicated 24 times in individual cages. The AME was measured as per Expt 1.

*Ileal and excreta viscosity*

After the last collection, the birds were killed and their ileal and caecal contents collected. Approximately 2 g of fresh ileal digesta or 5 g of fresh excreta were centrifuged (12 000 *G* 10 min at 20°C) and the viscosity was determined on 0.5 mL of supernatant using a Brookfield Model DV-III Rheometer 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.

*Ileal and caecal xylanase and β-glucanase activity*

Ileal and caecal xylanase and β-glucanase activities were determined by adding 0.2 mL of digesta supernatant to 2 mL of arabinoxylan or β-glucan solution (with a viscosity of approx. 10 mPa.s). The mixture was incubated at 37°C for 1 h with stirring. The viscosity of the mixture was then determined. The drop in viscosity was taken as an indicator of enzyme activity and the relative enzyme activity was expressed as:

$$\text{Enzyme activity} = (\text{viscosity of control}/\text{viscosity of mixture}) \times 100$$

*Experiment 3: Influence of xylanase on* Clostridium perfringens *trial*

This experiment was designed to examine the effect of xylanase supplementation of a wheat-based diet on *Clostridium perfringens* and on the total anaerobes in broilers. Broilers were raised on a commercial starter to Day 17 and then were switched to 2 experimental diets (same as the basal diet shown in Table 1), one of which contained xylanase (2.5 g RONOZYME WX/kg). One bird from each diet was killed every alternate day to Day 39. The contents of the ileum and caeca were collected in sterile McCartney tubes for culturing on freshly prepared perfringens agar medium (OPSP CM543, Oxoid, England) for enumeration of *C. perfringens*. Total anaerobes were counted using the Basal Medium 10 (BM 10) (Caldwell and Bryant 1966).

*Statistical analysis*

All data were analysed using Statgraphics (Manugistics, Inc., MD, USA). Analysis of variance was used to determine the significance of the main effects and the simple regression analysis was performed to determine correlations. Duncan's multiple-range test was used to separate means when significant main effects ($P < 0.05$) were detected by analysis of variance.

## Results

The results of Expt 1 are shown in Table 2. The wheat that had the highest NSP level and the lowest AME was Harvey. This wheat was subsequently used in Expts 2 and 3 as low-ME wheat. As expected, the total NSP values were inversely correlated with the AME ($r = –0.9664$; $P < 0.001$). Extract viscosity values obtained by simple water extraction of the whole wheat flour did not relate to the AME well due to one sample (BFB) having a very high extract viscosity but a reasonably high AME value. If that sample was excluded, the predictability of AME by extract viscosity became strong ($R^2 = 0.74$), but the sample repeatedly gave similar values. Similar outliers in extract viscosity occurred in other studies at the University of New England and this suggests that protein and starch characteristics may also contribute to extract viscosity in some wheat samples.

**Table 2. Non-starch polysaccharide (NSP) concentration, extract viscosity, and apparent metabolisable energy (AME) in 9 Australian wheat varieties**

| Wheat | NSP (g/kg DM) | | | Extract viscosity (cP) | AME[B] (MJ/kg DM) |
|---|---|---|---|---|---|
| | Soluble | Insoluble | Total | | |
| BFB | 16.1 | 69.1 | 85.2 | 6.8 | 13.1 (0.98) |
| Cocamba | 17.9 | 68.3 | 86.2 | 5.7 | 12.7 (0.55) |
| Currawong | 16.1 | 71.9 | 88.0 | 4.3 | 12.7 (0.67) |
| Dollarbird | 17.2 | 66.7 | 83.9 | 3.9 | 13.6 (0.28) |
| Finley | 17.8 | 71.7 | 89.5 | 5.8 | 12.3 (0.74) |
| Harvey[A] | 17.6 | 75.6 | 93.2 | 6.4 | 11.5 (0.99) |
| Matong | 15.1 | 71.8 | 86.9 | 4.7 | 13.0 (0.44) |
| Parsons | 16.4 | 71.4 | 87.8 | 5.0 | 12.5 (0.36) |
| Rosella | 16.0 | 73.1 | 89.1 | 5.7 | 12.5 (0.78) |
| Average | 16.7 | 71.1 | 87.8 | 5.4 | 12.7 |
| Minimum | 15.1 | 66.7 | 83.9 | 3.9 | 11.5 |
| Maximum | 17.8 | 75.6 | 93.2 | 6.8 | 13.6 |

[A] The low AME wheat selected for the follow-on experiment.
[B] Each diet consisted of 4 replicates of 6 birds per replicate; numbers in parentheses are standard deviation of the means.

Table 3 summarises the data from Expt 2. Supplementation of xylanase increased ($P < 0.01$) the AME of the wheat from a control value of 12.5 (s.d. = 1.13) to 13.1 (s.d. = 0.52) MJ/kg DM and decreased ileal viscosity from 58.6 to 9.5 mPa.s. The excreta from the control birds had a viscosity of 14.8 mPa.s, whereas that from birds fed the enzyme-supplemented diet had a viscosity of 4 mPa.s. Xylanase and β-glucanase activities were clearly detectable in the caeca of birds fed both diets. Furthermore, the caecal xylanase activity in birds fed the control diet was significantly correlated ($r = 0.72$; $P < 0.001$) with AME, but there was no correlation between

**Table 3. Effect of xylanase on apparent metabolisable energy (AME, MJ/kg DM), feed conversion ratio (FCR for Week 4), ileal and excreta viscosity (mPa.s), and xylanase and β-glucanase activity (viscosity xylanase units) in the caeca of broilers fed wheat**

Within columns, means followed by the same letter are not significantly different at $P = 0.05$

| Diet | AME | FCR | Ileal viscosity | Excreta viscosity | Caecal xylanase[A] | Caecal β-glucanase |
|---|---|---|---|---|---|---|
| Control | 12.51a | 2.191a | 58.6a | 14.8a | 5.2 | 13.6a |
| Range | 10.1–13.7 | 1.791–4.151 | 17.1–150.0 | 2.2–39.8 | | |
| s.d. | 1.13 | 0.503 | 34.1 | 30.3 | 2.2 | 11.9 |
| Xylanase | 13.09b | 1.984a | 9.5b | 4.0a | – | 17.0a |
| Range | 11.6–13.6 | 1.703–2.446 | 5.8–29.2 | 2.1–9.6 | | |
| s.d. | 0.52 | 0.255 | 5.3 | 2.0 | – | 14.9 |

[A] Xylanase activity in the caeca was only measured for the control birds because the xylanase supplemented group had activity levels not suitable for the viscometric assay used in measuring enzyme activities.

caecal β-glucanase activity and AME. Neither enzyme was detectable in the ileum of birds fed the control diet. The AME correlated closely ($r = –0.81$; $P = 0.001$) with excreta viscosity, but did not correlate with ileal viscosity in the current experiment.

Experiment 3 determined whether supplemental xylanase had an effect on the gut microflora of broilers fed a low-ME wheat diet. The experiment showed that the total counts of bacteria in the caeca of broilers fed wheat with or without a xylanase did not differ significantly ($P < 0.05$). However, there was a sudden increase in the total number of caecal bacteria of birds fed the control diet 5 days after introduction of the diet (increasing from $7 \times 10^9$ to $3 \times 10^{10}$). There was no such increase in birds fed the xylanase-containing diet. The numbers of *C. perfringens* increased from $>10^5$ to $4 \times 10^7$ three days after introduction to the diets, with a steady decline thereafter, although levels were maintained at $>10^5$ in birds fed the control diet. The xylanase reduced ($P < 0.05$) the number of *C. perfringens* to less than $10^4$ after Day 5, and they remained low to the end of the experiment (Figs 1 and 2, respectively).

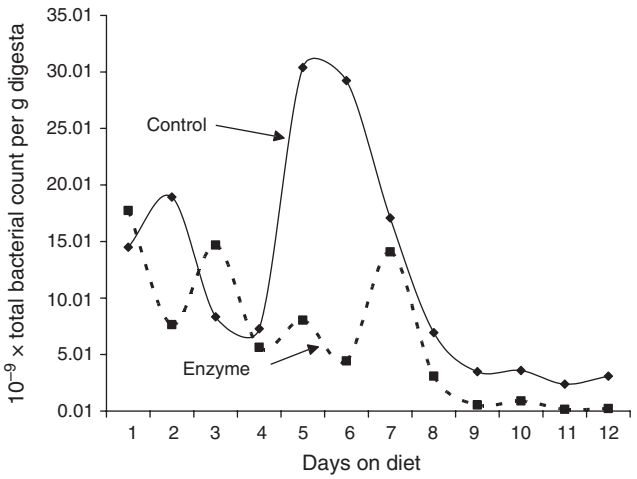


**Fig. 1.** Total counts of bacteria in the caecal digesta of broilers fed wheat with or without xylanase.


**Fig. 2.** The number of *C. perfringens* in the caecal digesta of broilers fed wheat with or without xylanase.

## Discussion

The results clearly demonstrate that individual bird variation in the AME of low-ME wheat is wide and the use of appropriate xylanases cannot only decrease this variability but also increase the AME significantly. This finding in itself is not new as there have been numerous reports demonstrating such an effect in poultry (Classen and Campbell 1990; Choct *et al*. 1999). However, the current study has also examined the possible role of gut microflora in the occurrence of this variation. Thus the data show that the viscosity values for the excreta were much lower than for the ileal digesta, suggesting that the gut microflora of the chicken do produce some NSP-degrading enzymes. The complicating factor may be that each individual bird appears to produce different amounts of the fibre-degrading enzymes even though they are fed identical diets under the same housing and management conditions. The amounts of the enzymes, e.g. xylanase and β-glucanase, produced in the caeca, are small compared with their supplemental counterparts, but seem enough to cleave the NSP polymers at least at a few places to cause a reduction in their viscosity. In the case of wheat, the ability of the xylanase produced in the caeca of broilers to reduce excreta viscosity may be related to the individual variation observed for AME values. This relationship between excreta viscosity, AME, and caecal xylanase activity has been shown for the first time to our knowledge and it may shed light on the mechanism whereby individual birds exhibit extreme variations in their AME values when they are given viscous grains. Furthermore, no clear relationship between ileal viscosity and AME was demonstrated in the current study. It is hypothesised that the large variation observed in the ileal viscosity values is probably related to various degrees of retrograde movement of caecal digesta into the ileum at the time of digesta sampling.

The gut microflora of the chicken, especially at a young age, is highly variable and changes rapidly in response to changes in husbandry conditions and feeding regimens. According to a study by Köhler (2000), feed is the most important source of *C. perfringens* infection and both outbreaks and the severity of outbreaks of necrotic enteritis in chickens are related to the concentration of *C. perfringens* in feed or in the gut. A routine change of diets therefore may introduce different types and amounts of organisms to the bird. The current study demonstrates that the numbers and the types of the microflora can indeed change drastically when new feed is introduced. Of particular interest is the increase in *C. perfringens* in the gut of the chicken when a wheat-based diet is introduced. Supplementation with xylanase not only kept the total bacterial number low, but more importantly, it reduced the number of *C. perfringens* and maintained the performance of the birds.

It is concluded that the high between-bird variation observed when a low-ME wheat diet is fed to young broilers

appears to be related to the ability of the hindgut microflora to produce xylanase. The excreta viscosity may be a usable measurement to predict this variation, although it must be borne in mind that the variation in excreta viscosity is high and the finding requires further verification. Appropriate enzymes in diets based on viscous grains such as wheat can modify the gut microflora of the bird, which appears to suppress the number of undesirable organisms such as *C. perfringens* in the caeca.

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## References

Annison G (1991) Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolisable energy of wheats assayed in broiler chickens. *Journal of Agricultural and Food Chemistry* **39**, 1252–1256. doi: 10.1021/jf00007a011

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. doi: 10.1016/0377-8401(92)90096-O

Annison G, Choct M (1991) Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. *World's Poultry Science Journal* **47**, 232–242. doi: 10.1079/WPS19910019

Annison G, Choct M, Hughes RJ (1994) AME determination and its application to lupins. *Proceedings of the Australian Poultry Science Symposium* **6**, 92–96.

Caldwell DR, Bryant MP (1966) Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Applied Microbiology* **14**, 794–801.

Choct M (1995) Role of soluble and insoluble fibre in broiler nutrition. Final Report to Chicken Meat Research and Development Council on Project CSN 2CM. CSIRO Division of Human Nutrition, Majors Road, O'Halloran Hill, SA.

Choct M, Annison G (1990) Anti-nutritive activity of wheat pentosans in broiler diets. *British Poultry Science* **31**, 811–822.

Choct M, Annison G, Trimble RP (1992) Soluble wheat pentosans exhibit different anti-nutritive activities in intact and cecectomized broiler chickens. *Journal of Nutrition* **122**, 2457–2465.

Choct M, Hughes RJ, Annison G (1999) Apparent metabolisable energy and chemical composition of Australian wheat in relation to environmental factors. *Australian Journal of Agricultural Research* **50**, 447–451. doi: 10.1071/A98155

Choct M, Hughes RJ, Trimble RP, Angkanaporn K, Annison G (1995) Non-starch polysaccharide-degrading enzymes increase the performance of broiler chickens fed wheat of low apparent metabolizable energy. *Journal of Nutrition* **125**, 485–492.

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

Choct M, Hughes RJ, Wang J, Bedford MR, Morgan AJ, Annison G (1996) Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science* **37**, 609–621.

Classen HL, Campbell GL (1990) Improvement of feed utilisation through the use of enzyme products. *Proceedings of the Australian Poultry Science Symposium* **2**, 1–8.

Hughes RJ, Choct M (1997) Low-ME wheat or low-ME chickens?—Highly variable responses by birds on the same low-ME wheat diet. *Proceedings of the Australian Poultry Science Symposium* **9**, 138–141.

Hughes RJ, Kocher A, Acone L, Langston P, Bird JN (1996) Attainable AME and physico-chemical characteristics of xylanase-supplemented wheat. In 'Proceedings of the 10th Australian Poultry and Feed Convention'. pp. 232–235. (Australian Chicken Meat Federation: Melbourne, Vic.)

Köhler B (2000) *Clostridium perfringens* intoxication affects bird performance. *World Poultry* **16**, 57–59.

Mollah Y, Bryden WL, Wallis IR, Balnave D, Annison EF (1983) Studies on low metabolisable energy wheats for poultry using conventional and rapid assay procedures and the effects of processing. *British Poultry Science* **24**, 81–89.

Philip JS, Gilbert HJ, Smithard RR (1995) Growth, viscosity and beta-glucanase activity of intestinal fluid in broiler chickens fed on barley-based diets with or without exogenous beta-glucanase. *British Poultry Science* **36**, 599–603.

Rogel AM, Annison EF, Bryden WL, Balnave D (1987) The digestion of wheat starch in broiler chickens. *Australian Journal of Agricultural Research* **38**, 639–649. doi: 10.1071/AR9870639

Theander O, Åman P, Westerlund E, Andersson R, Pettersson D (1995) Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the Uppsala method): collaborative study. *Journal of the Association of Analytical Chemists International* **78**, 1030–1044.

Vahjen W, Gläser K, Schäfer K, Simon O (1998) Influence of xylanase-supplemented feed on the development of selected bacterial groups in the intestinal tract of broiler chicks. *Journal of Agricultural Science* **130**, 489–500. doi: 10.1017/S0021859698005498

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