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Influence of the Type and Dietary Level of Non-Starch Polysaccharide on the Endogenous Nitrogen Flow in Chickens

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Abstract: The influence of two types of soluble non-starch polysaccharides (barley â-glucan and maize arabinoxylan) at two inclusion levels (30 and 60 g/kg) on endogenous ileal nitrogen flow in broiler chickens was investigated, using the peptide alimentation method. Ileal nitrogen contents (as g/kg titanium marker) and endogenous nitrogen flow (as μg/g dry matter intake) were numerically increased with the addition of non-starch polysaccharides, but the differences were not statistically significant ($P>0.05$). The results indicate that the type and level of soluble non-starch polysaccharides may influence the extent of ileal flow of nitrogen in chickens.

Key words: Non-starch polysaccharides, Endogenous nitrogen losses, Peptide alimentation, Broiler chickens

Introduction

Non-starch polysaccharides (NSP) assume considerable practical significance in poultry nutrition due to the anti-nutritive effects elicited by soluble pentosan components (arabinoxylan and â-glucan) and their effects on bird performance (Choct, 1997). The detrimental effects of NSP are believed to be due to its viscous nature that has physiological, morphological and microbiological effects on the digestive tract. These events negatively impact nutrient digestion and also cause wet and sticky droppings in poultry (Bedford and Schulze, 1998).

It is well established that soluble NSP in wheat influence the apparent ileal digestibility of nutrients, including nitrogen (Choct and Annison, 1992). The reduction in nitrogen digestibility can be attributed to impaired digestion, inhibition of amino acid absorption or increased secretion of endogenous protein derived from gut secretion and sloughed off epithelium, or increased mucin (glycosaminoglycan) secretion. Using the guanidation method, Angkanaporn *et al*. (1994) found that the addition of isolated pentosans equivalent to 15 and 35 g wheat arabinoxylans per kg diet significantly increased endogenous amino acid flow and lowered overall digestibility of amino acids. In the present study, the influence of different types and levels of NSP on endogenous ileal nitrogen losses in broiler chickens was examined, using the peptide alimentation method (Moughan *et al*., 1990). Two types of soluble NSP, namely barley â-glucan (viscous) and maize arabinoxylan (non-viscous), were compared.

Materials and Methods

Five experimental diets were prepared, including a control diet and test diets that contained 30 and 60 g/kg purified maize arabinoxylan or barley â-glucan extract (Glucagel™). The composition of the diets is shown in Table 1. All diets contained 180 g/kg enzymatically hydrolysed casein (EHC) as the source of amino acids and peptides, and 5 g/kg titanium oxide as an inert internal marker.

Day-old male broiler chicks (Ross) was obtained from a local hatchery and reared in floor pens on commercial starter diets till 21 days of age. On day 21, the birds were weighed individually and those with relatively high or low body weights were discarded. A total of 100 birds (of uniform body weight range) were chosen and distributed into 25 colony cages of four birds each so that average weights per pen was nearly equal. The cages were then assigned at random to the five dietary treatments so that there were five replicates per group.

The cages were housed in an electrically heated grower shed (22 – 24 ^{0}C) during the trial. All birds had *ad libitum* access to feed and water. The birds were given a mash commercial-type diet till Day 27. Following overnight fasting on Day 27, a casein-based diet was introduced and fed for the next three days. The casein-based diet was similar to the control diet (Table I) except that casein was used in place of EHC. The use of casein-based diet enables the birds to get adjusted to the change-over from the commercial mash diet to a purified diet. Previous studies in our laboratory have shown that this adjustment is necessary to maintain satisfactory feed intake levels when EHC-diets are introduced. The casein-based diet was withdrawn on the evening of Day 30 and the test diets (Table I) were introduced on the morning of Day 31. The test diets were offered for 36 hours and records of feed intake during this period were maintained. The birds were then euthanased by an intracardial injection of sodium pentabarbitone and the contents of the lower half of the ileum were collected by gently flushing with distilled water into plastic containers. The ileum was defined as that portion of the small intestine extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-caecal junction. The digesta were pooled from birds within a pen, frozen

immediately after collection and subsequently freeze-dried. Diet and dried ileal digesta samples were ground to pass through a 0.5 mm sieve and subsequently analysed for titanium and nitrogen. Nitrogen was determined following Kjeldahl digestion by colorimetric auto-analysis (Technicon, 1973). Titanium was analysed according to the procedures of Short *et al.* (1996).

Apparent ileal dry matter digestibility coefficients were calculated from the dietary ratio of dry matter to titanium relative to the corresponding ratio in the ileal digesta.

The ileal nitrogen flow (related to the ingestion of 1 g of dry matter; the units are μg/g dry matter intake) was calculated using the following equation (Moughan *et al.*, 1992).

$$\text{Ileal nitrogen flow} = \text{Nitrogen concentration in digesta} \times \frac{\text{Titanium concentration in diet}}{\text{Titanium concentration in digesta}}$$

Results and Discussion

In the peptide alimentation method (Moughan *et al.*, 1990), the animal is fed a purified diet containing enzymically

Table 1: Ingredient composition (g/kg) of the experimental diets.

Ingredient	Control	30 g/kg arabinoxylan	60 g/kg arabinoxylan	30 g/kg β-glucan	60 g/kg β-glucan
Maize starch	569.7	539.7	509.7	539.7	509.7
Dextrose	100.0	100.0	100.0	100.0	100.0
EHC[1]	180.0	180.0	180.0	180.0	180.0
Arabinoxylan	-	30.0	60.0	-	-
â-glucan	-	-	-	30.0	60.0
Cellulose	35.0	35.0	35.0	35.0	35.0
Vegetable oil	50.0	50.0	50.0	50.0	50.0
Dicalcium phosphate	24.0	24.0	24.0	24.0	24.0
Dipotassium hydrogen phosphate	14.3	14.3	14.3	14.3	14.3
Sodium bicarbonate	12.0	12.0	12.0	12.0	12.0
Magnesium oxide	2.0	2.0	2.0	2.0	2.0
Salt	2.0	2.0	2.0	2.0	2.0
Vitamin-mineral premix[2]					
Titanium oxide	5.0	5.0	5.0	5.0	5.0

[1]Enzymatically hydrolysed casein (Sigma Chemical Company, St. Louis, MO). Molecular weight, < 5000 Da. According to the manufacturer, the molecular weight profile (%) was as follows: 1-500 Da, 90; 500-1000 Da, 9; 1000-2500 Da and > 2500 Da, 0.

[2] Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

Table 2: Influence of type and level of NSP on endogenous nitrogen flow in broilers[1]

	Dry matter digestibility	Ileal nitrogen (g/g titanium)	Endogenous nitrogen flow (μg/g DMI)
Control	0.878[a]	0.457	2383
30 g/kg Arabinoxylan	0.882[a]	0.552	2549
60 g/kg Arabinoxylan	0.879[a]	0.605	2625
30 g/kg â-glucan	0.884[a]	0.506	2372
60 g/kg â-glucan	0.856[b]	0.535	2700
Pooled SEM	0.006	0.055	269

[a, b] Values in the same column with different superscripts are significantly different ($P<0.05$).

[1] Each mean represents data from five replicate pens.

hydrolysed casein (composed of free amino acids and peptides with a molecular weight of less than 10,000 Da) as the sole source of nitrogen. The digesta are then collected from the animal and the endogenous protein (molecular weight, > 10,000 Da) is separated from unabsorbed free amino acids and peptides by centrifugation and ultrafiltration. In the present study, because of the presence of soluble NSP, difficulties were experienced with the ultrafiltration step. The endogenous flow was therefore calculated assuming a digestibility value of 100% for EHC. This assumption is based on previous work where digestion of amino acids in EHC by broiler chickens was found to be almost close to 100% (Ravindran, unpublished data). A similar approach has been previously used by Leterme *et al.* (1994) who employed the peptide alimentation method, but without the ultrafiltration of digesta. It should be noted, however, this approach may result in overestimation of the ileal flow if the EHC is not completely digested. The results are summarised in Table 2. Dry matter digestibility was not influenced by the dietary inclusion of arabinoxylan or 30 g/kg â-glucan, but was lowered ($P<0.05$) with 60 g/kg inclusion of â-glucan. Ileal nitrogen contents (as g/g titanium) and endogenous nitrogen flow (as μg/g dry matter intake) were numerically increased with the addition of soluble NSP, but the differences were not statistically significant ($P>0.05$) due to high variation among replicates. The ileal endogenous nitrogen flows determined for broiler chickens in the present study were similar than those reported by Ravindran and Hendriks. (2004), and Ravindran *et al.* (2004b). These researchers also determined the flows using the peptide alimentation method.

The lack of significant effects of NSP in our study on endogenous nitrogen flow was in contrast to the results of Angkanaporn *et al.* (1994) who found marked increases in endogenous amino acid losses when birds were fed diets containing levels of wheat arabinoxylans lower (15 and 35 g/kg diet) than those used in the present study. Guanidination technique, based on the homoarginine marker (Siriwan *et al.*, 1994 and Ravindran and Bryden, 1999), was used by Angkanaporn *et al.* (1994), whereas the peptide alimentation technique was used in the present study. However, the methodologies employed could not have caused the observed discrepancy since it has been shown that, under similar dietary protein intakes, these two techniques produce comparable results in terms of endogenous nitrogen and amino acid flow in the pig (Hodgkinson, 1999) and chickens (Ravindran *et al.*, 2004b). Differences in viscosity in the NSP extracts may explain, at least part of, this discrepancy. It is more likely that the effect of barley â-glucan extract on intestinal digesta viscosity may have been lower than that assumed. Although *in vivo* digesta viscosity was not determined in our study, previous *in vitro* studies in our laboratory (Maqueda, 1999) have shown only small increases in extract viscosity with diets containing 50 g/kg barley â-glucan extract.

The present data suggest that soluble NSP may influence the extent of the increase in ileal flow of nitrogen in chickens. The exact causes of the increased nitrogen flow with high NSP levels are not known. It could be due to increased secretion of endogenous proteins into the gut, decreased reabsorption of endogenous proteins or a combination of both effects. As suggested by Angkanaporn *et al.* (1994), it is also possible that soluble NSP interacts with the gut wall modifying the action of peptide hormones, which regulate gut functions including stimulation of secretion of endogenous protein. Finally, the increased nitrogen flow may also reflect, in part, the increased secretion of mucins as an allergic/antigen response, and this may help to explain the high variability found within treatments. Further studies are clearly required to examine the influence of sources and levels of NSP on mucin secretion in the gut. A proportion of the unexplained variation may arise from differences in extract and intestinal digesta viscosities, and these should be examined in future studies.

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