

## Endogenous Amino Acid Loss at the Terminal Ileum of Rats Fed Water-Extractable or Alkali-Extractable Soluble Non-Starch Polysaccharides Obtained from Wheat Bran When Fed with and Without Xylanase

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**Abstract:** The objective of these studies was to determine how the endogenous amino acid flow at the terminal ileum of rats is affected by feeding diets containing water extractable or alkali extractable soluble non-starch polysaccharides obtained from wheat bran. In addition, the effect of xylanase on endogenous amino acid flow was studied. In experiment one, 40 Sprague-Dawley rats ( $190.9 \pm 2.0$  g) were fed one of four protein-free diets, based on cornstarch, sucrose and soybean oil that contained 0, 20, 30, or  $40 \text{ g kg}^{-1}$  of water-extractable soluble non-starch polysaccharides. In experiment two, 40 Sprague-Dawley rats ( $204.3 \pm 1.6$  g), were randomly allotted to one of four protein-free diets in a  $2 \times 2$  factorial design with the diets containing  $30 \text{ g kg}^{-1}$  of either water-extractable or alkali-extractable soluble non-starch polysaccharides. The diets were fed with or without xylanase ( $2500 \text{ FTU/g}$ ) added at the expense of cornstarch. Chromic oxide ( $6 \text{ g kg}^{-1}$  diet) was included in all diets as an indigestible marker. Both experiments lasted 8 d. The rats were killed on day 8 and the digesta contained in the final 20 cm of the ileum was obtained for assay. The results of experiment one revealed that adding graded levels of water-extractable soluble non-starch polysaccharides significantly increased (linear and quadratic effect;  $p < 0.01$ ) the endogenous flows of amino acid and nitrogen at the terminal ileum. The endogenous loss of nitrogen increased by  $19.11 \text{ mg}$  for every  $\text{g kg}^{-1}$  increase in water-extractable soluble non-starch polysaccharides in the diet. Putrescine and spermine levels in the ileum increased (linear and quadratic effect;  $p < 0.05$ ) as the level of water-extractable soluble non-starch polysaccharides in the diet increased. Acetate, propionate and butyrate levels also increased (linear and quadratic effect;  $p < 0.05$ ) with increasing levels of water-extractable non-starch polysaccharides. In experiment two, with the exception of glycine, dietary supplementation of alkali-extractable non-starch polysaccharides significantly increased the endogenous amino acid loss at the terminal ileum compared with water-extractable non-starch polysaccharides ( $p < 0.05$ ). The addition of xylanase significantly decreased ileal endogenous amino acid loss in rats. The overall results of these experiments indicate that the presence of soluble non-starch polysaccharides increases endogenous amino acid loss at the terminal ileal, which may explain the reduction in the digestibility of crude protein and amino acids commonly observed in animals fed diets containing a high concentration of soluble non-starch polysaccharides.

**Key words:** Ileal endogenous amino acid loss, wheat, soluble non-starch polysaccharides, rat

### INTRODUCTION

The measurement of endogenous amino acid flow at the terminal ileum of animals is of fundamental importance to animal nutritionists<sup>[1]</sup>. Values for endogenous loss are needed to correct apparent estimates of amino acid digestibility to true estimates of amino acid digestibility and are an important component in the factorial approach to calculating amino acid requirements<sup>[2]</sup>.

Sauer and Ozimek<sup>[3]</sup> stated that the level and source of dietary fiber are the two most important factors influencing the amount of endogenous nitrogen and

amino acids present in the ileal digesta. The impact of different types of non-starch polysaccharides depends on their solubility, chemical structure and sugar composition<sup>[4]</sup>. In a previous study, we reported that both soluble and insoluble non-starch polysaccharides, isolated from wheat bran, increased ileal flows of amino acids but the effects of the soluble non-starch polysaccharides were 22-85% greater than those of the insoluble non-starch polysaccharides<sup>[5]</sup>.

Soluble non-starch polysaccharides come in two forms, namely water-extractable non-starch polysaccharides and alkali-extractable non-starch

polysaccharides. Choct and Annison<sup>[6]</sup> and Choct *et al.*<sup>[7]</sup> found that water-extractable non-starch polysaccharides had less of an effect on nutrient digestibility than alkali-extractable non-starch polysaccharides when fed to broiler chickens. However, the effects of these different types of soluble fiber on nutrient digestibility have not been investigated in other species.

Mammals and birds do not produce the necessary endogenous enzymes required to break down cell wall non-starch polysaccharides<sup>[8]</sup>. As a result, there is a negative relationship between dietary fiber and the digestibility of the nutrients contained in feeds, particularly crude protein and amino acids. Yin *et al.*<sup>[8]</sup> reported significant improvements in the ileal digestibility of nitrogen and some amino acids associated with the inclusion of the enzyme xylanase in the diet of pigs fed wheat by-products. Therefore, the objective of this study was to evaluate the effects of alkali and water-extractable non-starch polysaccharides on endogenous amino acid loss at the terminal ileum of growing rats and to determine if the negative effects of non-starch polysaccharides from wheat can be overcome by xylanase supplementation.

## MATERIALS AND METHODS

**Extraction of water-extractable and alkali-extractable non-starch polysaccharides from wheat bran:** Non-starch polysaccharides were extracted from wheat bran according to the methods of Choct and Annison<sup>6</sup> by treating wheat bran with thermal stable  $\alpha$ -amylase (Termamy SC, Novozymes, China) and protease (Alcalase 2.4 L FG, Novozymes, China), followed by centrifugation. The supernatant was precipitated with 60% (v/v) ethanol and then oven dried at 50°C, to obtain the water-extractable non-starch polysaccharides. The precipitate was re-suspended in 0.2 M sodium hydroxide (1:10) at 20°C, neutralized with hydrochloric acid to pH 4.8 and centrifuged. The supernatant was precipitated with 60% (v/v) ethanol and then oven dried at 50°C, to obtain the alkali-extractable non-starch polysaccharides.

**Animals and diets:** Two experiments were conducted using Sprague-Dawley rats obtained from the Institute of Genetics and Developmental Biology of the China Academy of Science (Beijing, China). The trials were conducted in an environmentally controlled room located in the Laboratory Animal Unit of the Ministry of Agriculture Feed Safety and Bio-availability Evaluation Center, China Agricultural University (Beijing, China). The rats were housed in pairs in stainless steel metabolic cages (20 × 17.5 × 19.5). The room temperature was set at 22±2°C, the relative humidity was set at 50±10%

and the photoperiod was maintained at 12 h light and 12 h dark.

For experiment one, 40 rats (190.9±2.0 g) were fed one of four protein-free diets based on cornstarch, sucrose and soybean oil. The four experimental diets were produced by supplementing the basal diet with 0, 20, 30, or 40 g kg<sup>-1</sup> of soluble, water-extractable non-starch polysaccharides added at the expense of cornstarch (Table 1). In experiment two, 40 rats (204.3±1.6 g), were used in a factorial design experiment where four protein-free diets based on cornstarch, sucrose and soybean oil were fed (Table 2). The diets were formulated to contain 30 g kg<sup>-1</sup> of either water-extractable or alkali-extractable soluble non-starch polysaccharides and were fed with or without xylanase (2500 FTU/g; Pentopan, Novozymes, China). Chromic oxide (6 g kg<sup>-1</sup> diet) was included in all diets as an indigestible marker.

Both experiments lasted 8 d. The rats were weighed at the start and end of the experimental period in order to calculate weight gain. All rats had free access to water throughout the trial. The rats were fed 1.25 g of diet every hour, eight times a day from 0800 to 1500 h. The weight of any feed that remained in the feed trough was recorded and that amount was subtracted in order to determine the daily feed intake.

The rats were killed between 1200 and 1600 h on d 8, by an overdose of ether. The abdomen was opened and the final 20 cm of the ileum was dissected and opened. The digesta was flushed out with distilled water, using a syringe and immediately frozen at -20°C. The ileum, without digesta, was freeze-dried for polyamine assay. Approximately 0.5 g of cecal content was transferred to plastic tubes and stored at -80°C for volatile fatty acid assay.

**Analytical methods:** The dry matter, nitrogen and ash content of the diets were determined according to the procedures of the Association of Official Analytical Chemists<sup>[9]</sup>. Neutral detergent fiber and acid detergent fiber were determined using a Fiber Analyzer (Ankom Technology, Fairport, New York).

The non-starch polysaccharides were analyzed by gas chromatography (Agilent 6890, Wilmington, DE) according to the method of Englyst *et al.*<sup>[10]</sup>. The apparent viscosity of the soluble non-starch polysaccharides was determined using a Rotational Digital Viscometer (Fungilab Elite L Viscometer, Barcelona, Spain). In order to determine viscosity, the non-starch polysaccharide samples were dissolved in water at 10 g L<sup>-1</sup>, at 20°C and subjected to a shear rate of 122.36 radians/s (Determined by Beijing Shinetek Instruments, Beijing, China).

For chromium, nitrogen and amino acid assay, the digesta of the two rats previously housed together in a

Table 1: The ingredient composition and nutrient levels of protein-free diets fed graded water-extractable non-starch polysaccharides isolated from wheat (Experiment 1)

	Levels of water-extractable non-starch polysaccharides in the diets			
	0%	2%	3%	4%
Ingredients (% as-fed)				
Cornstarch	72.7	70.7	69.7	68.7
Sucrose	10.0	10.0	10.0	10.0
Soy oil	7.0	7.0	7.0	7.0
Vitamin mix <sup>a</sup>	1.0	1.0	1.0	1.0
Mineral mix <sup>b</sup>	3.5	3.5	3.5	3.5
Choline	0.2	0.2	0.2	0.2
Wood pulp cellulose	5.0	5.0	5.0	5.0
Water-extractable non-starch polysaccharides	0.0	2.0	3.0	4.0
Chromic oxide	0.6	0.6	0.6	0.6
Nutrient levels (g kg <sup>-1</sup> , as-fed)				
Dry matter	897.4	899.6	899.8	897.7
Crude protein	4.1	7.2	8.4	11.6
Ash	33.0	32.1	41.9	45.7
Neutral detergent fiber	7.2	46.1	45.8	49.2
Acid detergent fiber	4.2	15.6	15.9	19.1

<sup>a</sup>Vitamin premix provided the following per kilogram of diet, retinal acetate 0.125 mg, cholecalciferol 0.5 mg, alpha-tocopherol 100 mg, menaquinone 1.72 mg, cyanocobalamin 25 µg, riboflavin 6 mg, pantothenate acid 15 mg, nicotinic acid 30 mg, pyridoxine 6 mg, thiamin 5 mg, folic acid 2 mg, biotin 0.2 mg. <sup>b</sup>Mineral premix provided the following per kilogram of diet, calcium 5000 mg, phosphorus 3000 mg, magnesium 511 mg, sodium 1033 mg, potassium 3600 mg, chloride 1613 mg, iron 45 mg, zinc 35 mg, manganese 10 mg, copper 6 mg, iodine 0.2 mg, selenium 0.17 mg

Table 2: The ingredients composition and nutrient levels of protein-free diets containing soluble non-starch polysaccharides with xylanase or not (Experiment 2)

	Water-extractable non-starch polysaccharides		Alkali-extractable non-starch polysaccharides	
	-Xylanase	+Xylanase	-Xylanase	+Xylanase
Ingredients <sup>a</sup> (% as-fed)				
Cornstarch	69.7	69.6	69.7	69.6
Sucrose	10.0	10.0	10.0	10.0
Soy oil	7.0	7.0	7.0	7.0
Vitamin mix	1.0	1.0	1.0	1.0
Mineral mix	3.5	3.5	3.5	3.5
Choline	0.2	0.2	0.2	0.2
Wood pulp cellulose	5.0	5.0	5.0	5.0
Water-extractable non-starch polysaccharides	3.0	3.0	0.0	0.0
Alkali-extractable non-starch polysaccharides	0.0	0.0	3.0	3.0
Chromic oxide	0.6	0.6	0.6	0.6
Xylanase	-	0.1	-	0.1
Nutrient levels (g/kg, as-fed)				
Dry matter	896.2	909.1	897.2	895.3
Crude protein	9.8	9.7	8.6	8.9
Ash	32.4	32.2	34.0	32.3
Neutral detergent fiber	46.5	47.5	47.3	47.0
Acid detergent fiber	15.4	16.4	16.7	16.1

<sup>a</sup>The vitamin premix and mineral premix are the same as Table 1

cage were mixed and then freeze-dried (Dura-to p Bulk Tray Dried, FTS Systems, Stone Ridge, New York) for approximately 30 h. Nitrogen content of digesta was determined with the method of Dumas (AOAC, 990.03), using an Elementar Analysensysteme GmbH, Rapid N III (Hanau, Germany). Amino acids were assayed using ion-exchange chromatography with an automatic Amino Acid Analyzer (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan) after hydrolyzing with 6 mol/L HCl for 24 h at 110°C. The chromium content was determined with an atomic absorption spectrophotometer (Hitachi Z-5000 Automatic Absorption Spectrophotometer, Tokyo, Japan) according to the procedures given by Williams *et al.*<sup>[11]</sup>.

The polyamine content of the ileum, without digesta, was determined by the procedure of Bardocz and White<sup>[12]</sup>

and was analyzed by high performance liquid chromatography (LC-10A, Shimadzu, Kyoto, Japan) following the methods of Seiler and Knödgen<sup>[13]</sup>. Volatile fatty acid in cecal content was determined by gas-liquid chromatography (Agilent 6890, Wilmington, DE) following the method of Wang *et al.*<sup>[14]</sup>.

**Statistical analysis:** All endogenous amino acid flows were tested for homogeneity using Bartlett and Levene's test and non-homogenous data were transformed by log10. Data in experiment one were analyzed as a one-way ANOVA and the effects of the graded levels of water-extractable non-starch polysaccharides were tested for linear and quadratic components by polynomial contrasts. Data in experiment two were analyzed as a 2×2 factorial with the factors in the model consisting of non-starch

polysaccharide type (water-extractable and alkali extractable), xylanase supplementation (with and without) and their interaction. All statistics were conducted using SAS software<sup>[15]</sup>. The mean differences between the treatments were compared by the least significant difference procedure. Differences with  $p < 0.05$  were considered significant.

## RESULTS

**Composition of isolated non-starch polysaccharides:** The composition of the two soluble non-starch polysaccharides isolated from wheat bran is presented in Table 3. The water-extractable material contained 122 g kg<sup>-1</sup> crude protein while the alkali-extractable material contained 73 g kg<sup>-1</sup> crude protein. There was 815 and 823 g kg<sup>-1</sup> of soluble and total non-starch polysaccharides in the water-extractable non-starch polysaccharides while the alkali-extractable non-starch polysaccharides had 859 and 864 g kg<sup>-1</sup> of soluble and total non-starch polysaccharides.

**Terminal ileal endogenous losses of amino acid and nitrogen:** Adding graded levels of soluble, water-extractable non-starch polysaccharides significantly increased (linear and quadratic effects;  $p < 0.05$ ) the endogenous flows of all amino acids and nitrogen at the terminal ileum (Table 4). The endogenous loss of nitrogen increased by 19.1 mg for every g kg<sup>-1</sup> increase in dietary non-starch polysaccharides in rats fed the water-extractable non-starch polysaccharides diet (Fig. 1).

The addition of graded levels of soluble water-extractable non-starch polysaccharides significantly increased (linear and quadratic effects;  $p < 0.05$ ) the content of putrescine and spermine, but not spermidine  $p > 0.05$  at the terminal ileum (Table 5). The addition of soluble water-extractable non-starch polysaccharides also significantly increased (linear and quadratic effect;  $p < 0.01$ ) the content of acetate, propionate, butyrate and total volatile fatty acids in the cecal contents (Table 6).

In experiment two, with the exception of glycine, supplementation of alkali-extractable non-starch

Table 3: Chemical composition (g kg<sup>-1</sup> DM) of soluble and insoluble non-starch polysaccharides isolated from wheat bran

	Water-extractable non-starch polysaccharides	Alkali-extractable non-starch polysaccharides
Dry matter	939	945
Crude protein	122	73
Viscosity, cP	28-32	58-60
Arabinose	225	241
Xylose	384	429
Glucose	91	120
Uronic acid	12	6
Insoluble non-starch polysaccharides	8	8
Soluble non-starch polysaccharides	815	859
Total non-starch polysaccharides	823	864

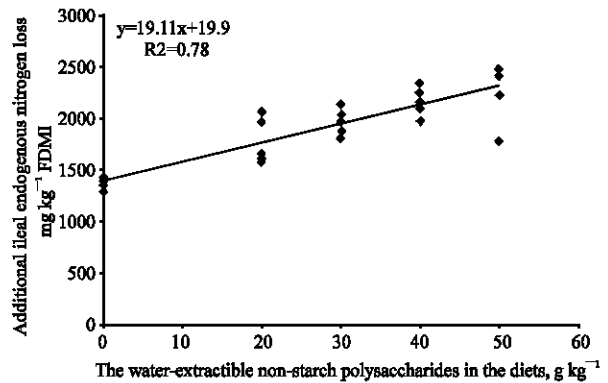


Fig. 1: Effects of graded levels of water-extractable non-starch polysaccharides on the ileal endogenous nitrogen flow in rats. Values for the 50 g kg<sup>-1</sup> water-extractable non-starch polysaccharide diet were previously reported by He *et al.*<sup>[5]</sup>

polysaccharides resulted in higher endogenous amino acid loss at the terminal ileum than did the water-extractable non-starch polysaccharides (Table 7). Adding xylanase to the soluble non-starch polysaccharide containing diets significantly reduced ( $p < 0.05$ ) ileal endogenous amino acid loss in rats. There was no interaction ( $p > 0.05$ ) between the effects of non-starch polysaccharide type and xylanase treatment (Table 7).

## DISCUSSION

In this study, we used the laboratory rat to evaluate the effects of soluble non-starch polysaccharides on endogenous amino acid loss at the terminal ileum of growing rats determined using the protein-free method. The laboratory rat is a suitable animal model for the growing pig and offers a rapid and relatively inexpensive approach to determining ileal amino acid digestibility<sup>[16]</sup>.

Water-extractable non-starch polysaccharides isolated from wheat bran had a significant influence on endogenous amino acid loss, which is accordance with the effects exerted by other soluble non-starch polysaccharides such as carboxymethylcellulose<sup>[17]</sup>, wheat alkali-extractable non-starch polysaccharides<sup>[18]</sup> and pectin<sup>[19]</sup>. However, it would appear that soluble fiber has a greater effect on endogenous loss of nitrogen than does insoluble fiber. In the current experiment, the endogenous loss of nitrogen increased by 19.1 mg for every g kg<sup>-1</sup> increase in total non-starch polysaccharide in the diet. In contrast, Schulze *et al.*<sup>[20]</sup> reported that endogenous nitrogen loss was only increased 8 mg for every g kg<sup>-1</sup> increase in insoluble fiber

Table 4: Endogenous amino acid and nitrogen flows ( $\mu\text{g g}$  freeze dry matter intake) at the terminal ileum of rats fed wheat isolated soluble non-starch polysaccharides (Exp. 1)

	Level of water-extractable non-starch polysaccharides <sup>a</sup>				SEM <sup>b</sup>	P-value	
	0%	2%	3%	4%		Linear	Quadratic
<b>Essential amino acids</b>							
Arginine	210 <sup>c</sup>	299 <sup>b</sup>	341 <sup>ab</sup>	361 <sup>a</sup>	16	< 0.01	< 0.01
Histidine	162 <sup>c</sup>	197 <sup>b</sup>	236 <sup>a</sup>	257 <sup>a</sup>	10	< 0.01	< 0.01
Isoleucine	188 <sup>b</sup>	243 <sup>a</sup>	264 <sup>a</sup>	278 <sup>a</sup>	10	< 0.01	< 0.01
Leucine	303 <sup>c</sup>	393 <sup>b</sup>	427 <sup>ab</sup>	467 <sup>a</sup>	23	< 0.01	< 0.01
Lysine	233 <sup>c</sup>	333 <sup>b</sup>	420 <sup>a</sup>	467 <sup>a</sup>	24	< 0.01	< 0.01
Phenylalanine	241 <sup>c</sup>	278 <sup>bc</sup>	309 <sup>b</sup>	357 <sup>a</sup>	12	< 0.01	< 0.01
Threonine	378 <sup>c</sup>	474 <sup>b</sup>	539 <sup>ab</sup>	552 <sup>a</sup>	19	< 0.01	< 0.01
Valine	362 <sup>d</sup>	416 <sup>c</sup>	458 <sup>b</sup>	564 <sup>a</sup>	18	< 0.01	< 0.01
<b>Non-essential amino acids</b>							
Alanine	236 <sup>c</sup>	304 <sup>b</sup>	398 <sup>a</sup>	414 <sup>a</sup>	19	< 0.01	< 0.01
Aspartic acid	556 <sup>c</sup>	676 <sup>b</sup>	827 <sup>a</sup>	825 <sup>a</sup>	29	< 0.01	< 0.01
Glutamic acid	639 <sup>c</sup>	877 <sup>b</sup>	1119 <sup>a</sup>	1170 <sup>a</sup>	55	< 0.01	< 0.01
Glycine	612 <sup>c</sup>	735 <sup>bc</sup>	833 <sup>ab</sup>	964 <sup>a</sup>	37	< 0.01	< 0.01
Proline	290 <sup>b</sup>	311 <sup>b</sup>	446 <sup>a</sup>	491 <sup>a</sup>	21	< 0.01	< 0.01
Serine	300 <sup>c</sup>	388 <sup>b</sup>	446 <sup>a</sup>	468 <sup>a</sup>	17	< 0.01	< 0.01
Nitrogen	1357 <sup>c</sup>	1765 <sup>b</sup>	1946 <sup>ab</sup>	2132 <sup>a</sup>	72	< 0.01	< 0.01

<sup>a</sup>Values in the same row with different superscript letters are significant different ( $p < 0.05$ ). <sup>b</sup>SEM, standard error of the means

Table 5: Polyamine contents ( $\mu\text{mol g}^{-1}$  wet weight) of ileum tissue in rats fed graded levels of soluble water-extractable non-starch polysaccharides (Exp. 1)

	Level of water-extractable non-starch polysaccharides <sup>a</sup>				SEM <sup>b</sup>	p-value	
	0%	2%	3%	4%		Linear	Quadratic
Putrescine	81.1	92.4	85.7	136.4	8.1	0.04	0.04
Spermidine	1100.0	1220.4	1204.1	1219.4	35.9	0.23	0.42
Spermine	320.9	371.8	398.6	451.2	18.4	0.01	0.03

<sup>a</sup>SEM, standard error of the means

Table 6: Volatile fatty acids (VFA,  $\mu\text{mol/g}$ ) content in the cecum of rats fed graded levels of soluble water-extractable non-starch polysaccharides (Exp. 1)

	Level of water-extractable non-starch polysaccharides <sup>a</sup>				SEM <sup>b</sup>	p-value	
	0%	2%	3%	4%		Linear	Quadratic
Acetate	13.3	16.4	21.2	28.3	1.3	< 0.01	< 0.01
Propionate	2.9	5.2	5.3	5.9	0.0	< 0.01	< 0.01
Butyrate	2.0	3.1	4.6	7.3	0.5	< 0.01	< 0.01
Total VFA	18.9	25.5	32.4	42.4	2.0	< 0.01	< 0.01

<sup>a</sup>SEM, standard error of the means

obtained from wheat. Our study is supported by the research of Choct and Annison<sup>[6]</sup> as well as He *et al.*<sup>[5]</sup> who indicated that soluble fiber has a greater effect than insoluble fiber on endogenous amino acid loss.

Previous research has indicated that feeding soluble non-starch polysaccharides significantly reduces apparent amino acid digestibility of amino acids in pigs<sup>[21]</sup>, broilers<sup>[7]</sup> and rats<sup>[17]</sup>. Our finding of an increased endogenous amino acid loss at the terminal ileum, as a result on dietary inclusion of soluble non-starch polysaccharides, provides an explanation as to why apparent amino acid digestibility declines for feeds containing a high soluble non-starch polysaccharide content.

The putrescine, spermidine and spermine content of ileal tissue increased with increasing levels of soluble water extractable non-starch polysaccharides. These compounds, commonly referred to as polyamines, are thought to be involved in a variety of cellular processes such as replication and transcription, as well as cell growth and differentiation, due to their interaction with nucleic acids. Rapidly proliferating cells have higher levels of polyamine than do quiescent or slowly growing

cells<sup>[22]</sup>. The intestinal and mucosal hypertrophy induced by polyamine<sup>[23]</sup> may account for the increase in ileal endogenous nitrogen loss due to the presence of soluble non-starch polysaccharides.

We observed a significant increase in the content of acetate, propionate, butyrate and total volatile fatty acids in the cecum. Soluble water-extractable non-starch polysaccharides are used as fermentable substrates by cecal microflora<sup>[7]</sup>. The increase in volatile fatty acid content could increase epithelial cell proliferation and thus the proportion of sloughed cells in the ileal digesta<sup>[24]</sup>, which again could account for the increase in ileal endogenous amino acid loss due to the presence of soluble non-starch polysaccharides.

An important aspect of the present experiment was the finding that alkali-extractable non-starch polysaccharides caused a greater increase in endogenous amino acid loss at the terminal ileum than water-extractable non-starch polysaccharides. This finding supports the findings of Choct and Annison<sup>[6]</sup> and indicates that knowledge of the type of water soluble non-starch polysaccharide present in a feedstuff could be valuable in assessing the potential negative impact of a

Table 7: Endogenous amino acid and nitrogen losses ( $\mu\text{g g}^{-1}$  freeze dry matter intake) at the terminal ileum of rats fed protein-free diets containing water extractable and alkali-extractable soluble non-starch polysaccharides with or without xylanase (Exp.2)

Items	Alkali-extractable non-starch polysaccharides	Water-extractable non-starch polysaccharides	Without Xylanase	With Xylanase	SEM*	p-value		
						Non-starch polysaccharides	Xylanase	Non-starch polysaccharides $\times$ Xylanase
<b>Essential amino acids</b>								
Arginine	379	341	408	311	12	<0.01	<0.01	0.16
Histidine	225	191	229	187	10	0.01	<0.01	0.74
Isoleucine	237	210	244	203	8	0.02	<0.01	0.60
Leucine	427	398	435	390	11	0.01	<0.01	0.15
Lysine	422	385	430	377	10	0.01	<0.01	0.07
Phenylalanine	305	290	214	281	6	0.04	<0.01	0.13
Threonine	482	436	495	429	14	0.05	<0.01	0.98
Valine	483	349	484	441	8	<0.01	<0.01	0.85
<b>Non-essential amino acids</b>								
Alanine	381	349	389	341	9	0.04	<0.01	0.74
Aspartic acid	799	730	814	715	20	0.04	0.01	0.58
Glutamic acid	1278	1201	1331	1148	29	0.05	<0.01	0.98
Glycine	895	804	921	778	34	0.16	0.03	0.95
Proline	362	328	375	315	10	0.02	<0.01	0.26
Serine	401	351	412	340	14	0.04	0.01	0.70
Nitrogen	1724	1591	1839	1477	49	0.01	<.01	0.09

\*SEM, standard error of the means

particular feedstuff on apparent nitrogen and amino acid digestibility. The fact that alkali-extractable non-starch polysaccharides are more detrimental than water-extractable non-starch polysaccharides could also impact decisions on the best processing methods used to alleviate the effects of the presence of non-starch polysaccharides in feeds.

The addition of the enzyme xylanase to diets containing soluble non-starch polysaccharides reduced endogenous amino acid loss. This result could explain the increase in the apparent digestibility of amino acids and nitrogen observed by Yin *et al.*<sup>[6]</sup> in diets containing wheat by-products supplemented with xylanase. Xylanase could degrade the soluble non-starch polysaccharides in the hindgut of animals, which would reduce the population of anaerobic microflora and thus decrease the volatile fatty acid content of the hindgut and therefore decrease epithelial cell proliferation and thus the proportion of sloughed cells in the ileal digesta<sup>[24]</sup>. This would account for the decrease in ileal endogenous amino acid loss observed when xylanase supplemented diets are fed.

### CONCLUSION

The overall results of these experiments indicate that the presence of soluble non-starch polysaccharides increases endogenous amino acid loss at the terminal ileal, which could explain, at least partially, the reduction in the digestibility of crude protein and amino acids commonly observed in animals fed diets containing a high concentration of soluble non-starch polysaccharides. Alkali-extractable non-starch polysaccharides are more detrimental than water-extractable non-starch polysaccharides. Therefore, knowledge of the type of soluble non-starch polysaccharide present in a feedstuff could be valuable in assessing the potential negative impact of a particular feedstuff on apparent nitrogen and

amino acid digestibility. The addition of xylanase to diets containing high concentrations of soluble non-starch polysaccharides reduced endogenous amino acid loss and could be an effective means of improving the apparent digestibility of amino acids and nitrogen in diets containing high concentrations of soluble non-starch polysaccharides.

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