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Effects of Soluble and Insoluble Non-starch Polysaccharides Isolated from Wheat Bran on Endogenous Amino Acid Loss at the Terminal Ileum of Growing Rats

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Abstract: The objective of this study was to investigate the effects of soluble and insoluble non-starch polysaccharides isolated from wheat bran on endogenous amino acid flow at the terminal ileum of growing rats using the protein-free method. In experiment 1, 20 Sprague-Dawley rats (200 ± 0.9 g), were fed either a protein-free, low fiber diet based on cornstarch, sucrose and soybean oil or a similar diet containing 50 g kg^{-1} of soluble non-starch polysaccharides added at the expense of cornstarch. In experiment 2, 40 Sprague-Dawley rats (198 ± 0.8 g), were randomly allotted to either the protein-free, low fiber diet or similar diets containing either 50 g kg^{-1} insoluble non-starch polysaccharides, 50 g kg^{-1} soluble non-starch polysaccharides or a combination of 50 g kg^{-1} soluble and 50 g kg^{-1} insoluble non-starch polysaccharides added at the expense of cornstarch. Chromic oxide (6 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. Compared with the protein-free basal diet, adding 50 g kg^{-1} soluble non-starch polysaccharides significantly ($p<0.01$) increased the endogenous flow of amino acids and nitrogen at the terminal ileum (both experiment 1 and 2). Including 50 g kg^{-1} of insoluble non-starch polysaccharides also significantly increased ($p<0.05$) the ileal flows of amino acids and nitrogen. The effects of the soluble non-starch polysaccharides were 22-85% greater than those of the insoluble non-starch polysaccharides.

Key words: Ileal endogenous amino acid loss, wheat, 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[1]. Values for endogenous loss are needed to correct apparent to true estimates of digestibility and are an important component in the factorial approach to calculating amino acid requirements[2].

Sauer and Ozimek[3] 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 ileal digesta. The impact of different fiber types depends on their solubility, chemical structure and sugar composition[4]. Insoluble cellulose has little water holding capacity and because of its low viscosity, it exerts a relatively minor influence on amino acid digestibility[5,6]. However, soluble non-starch polysaccharides have been reported to significantly reduce apparent amino acid digestibility and utilization rate of amino acids in pigs[7], broilers[8,9] and in rats[10].

Previous research has indicated that a wheat middling or bran based diet significantly ($p<0.001$) increased ileal endogenous nitrogen loss in pigs compared with a wheat-based diet[11]. However, the increase was the result of the combined effects of soluble and insoluble non-starch polysaccharides in the wheat by-products, and it is impossible to distinguish the separate effects of soluble or insoluble non-starch polysaccharides on endogenous amino acid loss when traditional feedstuffs are fed. The objective of this study was to evaluate the effects of purified insoluble and soluble non-starch polysaccharides isolated from wheat bran on endogenous amino acid loss at the terminal ileum of growing rats using the protein-free method.

MATERIALS AND METHODS

Extraction of wheat bran soluble and insoluble non-starch polysaccharides: Insoluble and soluble non-starch polysaccharides were extracted from wheat bran according to the methods of Choct and Annison[12] by treating wheat bran with thermal stable á-amylase (Termamy SC, Novozymes China) and protease (Alcalase 2.4L FG, Novozymes, China), and then obtaining the precipitate and

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Table 1: The ingredient composition and nutrient levels of protein-free diets fed with and without 50 g kg^{-1} soluble non-starch polysaccharides isolated from wheat (Experiment 1)

	Diets	
Ingredients (g kg^{-1} diet)	Basal	Isolated soluble non-starch
Corn starch†	777.0	727.0
Sucrose	100.0	100.0
Soybean oil	70.0	70.0
Vitamin mixture‡	10.0	10.0
Mineral mixture§	35.0	35.0
	2.0	2.0
Choline	0.0	50.0
Isolated soluble non-starch polysaccharides	6.0	6.0
Chromic oxide		
Nutrient values (g kg^{-1}, as-fed basis)		
Dry matter	901.7	906.7
Crude protein	3.5	10.9
Ash	32.9	36.7
Neutral detergent fibre	7.0	16.1
Acid detergent fibre	1.4	5.2

† Purchased from Beijing Redstar Starch Co. Ltd. with Gross energy 15.66 MJ/kg, Dry matter 893 g, Crude protein 0.67 g, Calcium 0.7 g and Total phosphate 0.07 mg kg^{-1} diet. ‡ Vitamin premix provided the following per kilogram of diet, retinal acetate 0.125 mg, cholecalciferol 0.5 mg, alpha-tocoperol 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. § 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 ingredient composition and nutrient levels of protein-free diets containing insoluble or soluble non-starch polysaccharides alone or in combination (Experiment 2)

Ingredients	Diets			
	Basal	Insoluble NSP	Soluble NSP	Insoluble NSP and soluble NSP
Corn starch	777.0	727.0	727.0	677.0
Sucrose	100.0	100.0	100.0	100.0
Soy oil	70.0	70.0	70.0	70.0
Vitamin mixture†	10.0	10.0	10.0	10.0
Mineral mixture†	35.0	35.0	35.0	35.0
Choline	2.0	2.0	2.0	2.0
Isolated insoluble NSP	0.0	50.0	0.0	50.0
Isolated soluble NSP	0.0	0.0	50.0	50.0
Chromic oxide	6.0	6.0	6.0	6.0
Nutrient levels (g kg^{-1} diet, analyzed as-fed basis)Dry matter	897.4	899.6	899.8	897.7
Crude protein	3.7	7.5	9.7	13.2
Ash	33.0	32.1	41.9	45.7
Neutral detergent fibre	¡¡7.2	¡¡46.1	¡¡15.8	49.2
Acid detergent fibre	4.2	15.6	5.9	19.1
Total non-starch polysaccharides	17.2	49.4	47.3	94.5
Insoluble non-starch polysaccharides	3.0	23.7	2.0	21.8
Soluble non-starch polysaccharides	14.2	25.7	45.1	72.7
Cellulose	0.4	6.6	0.4	6.8
Klason lignin	0.2	3.3	1.0	3.2
Total dietary fibre	17.4	52.7	48.3	97.7
Water holding capacity (g/g DM)	2.0	2.2	1.9	2.0

†The vitamin premix and mineral premix was the same as Table 1.

supernatant by centrifugation. The precipitate, containing the insoluble non-starch polysaccharides, was washed three times with distilled water and then oven dried at 50°C. The supernatant, containing the soluble non-starch polysaccharides, was precipitated with 60% (v/v) ethanol and then also oven dried at 50°C.

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 in Beijing, China. Experiment 1 utilized 20 rats weighing 200 ± 0.9 g while experiment 2 utilized 40 rats weighing 198 ± 0.8 g. The trials were conducted in an environmentally controlled room at 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 steel stainless metabolic cages (20 cm × 17.5 cm × 19.5 cm). The room temperature was set at 22 ± 2°C, the relative humidity was set at 50 ± 10%

and the photoperiod was 12 h light and 12 h dark. The rats were given a 7 d acclimation period to adapt to their housing conditions prior to initiation of the experiment.

For experiment 1, the rats were fed either a protein-free, low fiber diet based on corn starch, sucrose and soybean oil or a similar diet containing 50 g kg^{-1} of soluble non-starch polysaccharides added at the expense of corn starch (Table 1). For experiment 2, four diets were fed including the basal diet used in experiment 1 or three experimental diets supplemented with 50g kg^{-1} of insoluble non-starch polysaccharides, 50 g kg^{-1} soluble non-starch polysaccharides, or a combination of 50 g kg^{-1} insoluble and 50 g kg^{-1} soluble non-starch polysaccharides added at the expensive of corn starch (Table 2). Chromic oxide (6 g kg^{-1} diet) was included in all diets as an indigestible marker. The rats were fed 1.25 g of feed every hour, eight times a day (total 10 g) from 08:00 to 15:00. Any feed remaining in the feed trough was recorded and the amount subtracted in order to determine the daily feed intake. 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 killed between 12:00 to 16:00 on d 8, by an overdose of ether. The abdomen was opened and the final 20 cm of the ileum was dissected. The digesta was flushed out with distilled water with an enema syringe and immediately frozen at -20°C. The digesta of the two rats from a cage was mixed and then freeze-dried (Dura-top Bulk Tray Dried, FTS Systems, Stone Ridge, New York) for approximately 30 h for chromium, nitrogen and amino 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[13]. Neutral detergent fiber and acid detergent fiber were determined using a Fiber Analyser (Andom Technology, Fairport, NY). The water holding capacity of the diets was determined as described as Leterme *et al.*, [14]. The non-starch polysaccharides were analyzed by gas chromatography (Agilent 6890, Wilmington, American) according to the method of Englyst *et al.*,[15]. Nitrogen bound to neutral detergent fiber was determined as described by Schulze *et al.*,[16]. 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.*,[17]. All samples were analyzed in duplicate.

Statistical Analysis: All endogenous amino acid flows were tested for homogeneity using Bartlett's and Levene's test, and non-homogenous data were transformed by $\log_{10}$, and then subjected to the General Linear Model (GLM) procedure of SAS software[18]. The mean differences between the treatments were compared by the least significant difference procedure.

RESULTS

Composition of isolated non-starch polysaccharides: The composition of the soluble and insoluble non-starch polysaccharides isolated from wheat bran is presented in Table 3. There was 112 and 70 g kg^{-1} crude protein in the soluble and insoluble materials. The isolated soluble non-starch polysaccharides had 8 g kg^{-1} insoluble non-starch polysaccharides and 815 g kg^{-1} soluble non-starch polysaccharides. The isolated insoluble non-starch polysaccharides had 711 g kg^{-1} insoluble non-starch polysaccharides and 49 g kg^{-1} soluble non-starch polysaccharides.

Table 3: Chemical composition (g kg^{-1} DM) of soluble and insoluble non-starch polysaccharides isolated from wheat bran

	Soluble NSP	Insoluble NSP
Dry matter	939	903
Crude protein	112	70
Neutral detergent fibre	-	683
Acid detergent fibre	-	206
Nitrogen bound to neutral detergent fibre	-	9
Arabinose	225	215
Xylose	384	407
Glucose	91	826
Uronic acid	12	21
Klason lignin	-	79
Cellulose	-	111
Insoluble non-starch polysaccharides	8	711
Soluble non-starch polysaccharides	815	49
Total dietary fibre	823	838

Table 4: The weight gain and feed intake of rats fed 50 g kg^{-1} isolated soluble non-starch polysaccharides (Exp. 1)

	Diets†		
	Basal	Soluble NSP	SEM
Initial body weight (g)	216.2	214.9	1.3
Final body weight (g)	194.1	194.7	1.0
Weight gain (g/day)	-2.8	-2.5	0.1
Feed intake (g/day)	9.5	9.2	0.1†

No significant differences between treatments for any variable (P>0.05)

Table 5: The weight gain and feed intake of rats fed protein-free diets containing soluble or Insoluble non-starch polysaccharides alone or in combination (Exp. 2)

	Diets†				
	Basal	Insoluble NSP	Soluble NSP	Insolube NSP and Soluble NSP	SEM
Initial body weight (g)	226.5	224.1	223.5	222.7	1.0
Final body weight (g)	208.1	204.2	202.8	203.8	1.2
Weight gain (g/day)	-2.3	-2.5	-2.6	-2.4	0.1
Feed intake (g/day)	9.4	9.4	9.2	9.3	0.1†

No significant differences between the treatments for any variable (.p>0.05)

Table 6: Endogenous amino acid and nitrogen flows (μg/g freeze dry matter intake) at the terminal ileum of rats fed wheat isolated soluble non-starch polysaccharides (Exp. 1).

	Diet			
Item	Basal	Soluble NSP	SEM	P-Value
Essential amino acids				
Arginine	220	394	13	***
Histidine	171	295	8	***
Isoleucine	161	289	9	***
Leucine	310	547	22	**
Lysine	284	514	21	**
Phenylalanine	240	405	10	***
Threonine	387	611	13	***
Valine	363	627	15	***
Non-essential amino acids				
Alanine	267	480	13	***
Aspartic acid	571	913	21	***
Glutamic acid	698	1269	32	***
Glycine	694	1194	39	***
Proline	340	524	11	***
Serine	311	548	11	***
Nitrogen	1261	2257	41	***

** Indicates a significant difference at p<0.01. ***Indicates a significant difference at p<0.001

Table 7: Endogenous amino acid and nitrogen losses (μg/g freeze dry matter intake) at the terminal ileum of rats fed protein-free diets containing soluble or insoluble non-starch polysaccharides alone or in combination (Exp. 2)

	Diet composition†					
	Basal	Insoluble NSP	Soluble NSP	Insoluble NSP and soluble NSP	SEM	P-Value
Essential amino acidsArginine	178^{c}	266^{bc}	408^{ab}	495^{a}	20	***
Histidine	146^{c}	235^{b}	403^{a}	402^{a}	14	***
Isoleucine	164^{c}	274^{b}	359^{ab}	443^{a}	11	***
Leucine	294^{b}	515^{a}	701^{a}	791^{a}	23	***
Lysine	225^{c}	410^{b}	582^{ab}	659^{a}	21	***
Phenylalanine	263^{c}	431^{b}	560^{ab}	665^{a}	18	***
Threonine	363^{c}	488^{bc}	679^{ab}	811^{a}	22	***
Valine	401^{c}	623^{b}	798^{ab}	623^{a}	24	***
Non-essential amino acidsAlanine	245^{c}	483^{b}	606^{ab}	713^{a}	18	***
Aspartic acid	523^{c}	757^{b}	1094^{a}	1230^{a}	31	***
Glutamatic acid	638^{c}	1064^{b}	1477^{ab}	1734^{a}	43	***
Glycine	606^{c}	935^{b}	1728^{a}	1655^{a}	49	***
Proline	277^{b}	432^{a}	539^{a}	662^{a}	18	***
Serine	286^{c}	441^{b}	611^{ab}	719^{a}	17	***
Nitrogen	1198^{c}	1912^{b}	2340^{ab}	3285^{a}	102	***

†Values in the same row with different superscript letters are significant different (p<0.01)

Feed consumption and body weight: All rats remained healthy through the entire experimental period and feed intake and body weight of the rats did not differ between the treatment groups in experiment 1 (Table 4) or experiment 2 (Table 5). The rats consumed 9.2-9.5 g feed, and lost 2.3-2.8 g body weight during the two trials.

Terminal ileal endogenous losses of amino acid and nitrogen: Compared with the basal diet, the diet ontaining 50 g kg^{-1} soluble non-starch polysaccharides had significantly (p<0.01) increased ileal endogenous flows of amino acid and nitrogen in experiment 1 (Table 6) and experiment 2 (Table 7). Adding 50 g kg^{-1} insoluble non-starch polysaccharides also significantly improved (p<0.05) the ileal flows of amino acid and nitrogen in experiment 2 (Table 7). The amino acid flows for rats fed soluble non-starch polysaccharides were up to 25-85% higher than those of rats fed insoluble non-starch polysaccharides, and nitrogen flow was 22% higher.

DISCUSSION

In this study, we used the laboratory rat to evaluate the effects of purified insoluble and soluble non-starch polysaccharides on endogenous amino acid loss at the terminal ileum of growing rats 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[19].

It has been reported that ileal endogenous lysine loss in rats fed a protein-free diet without anti-nutritional factors is in the range of 172 to 249 ìg kg^{-1} freeze dry matter intake[19,20]. Similar data were obtained in our experiment (284, 225 ìg kg^{-1} freeze dry matter intake). Reports indicate that the terminal ileum nitrogen flow in rats fed a nitrogen-free diet is in the range of 950 to 1103 ìg kg^{-1} freeze dry matter intake[19,20] which is in the range of the values reported in the current experiment (1261, 1198 µg kg^{-1} freeze dry matter intake).

Our finding that feeding insoluble non-starch polysaccharides increases the endogenous flow of amino acids and nitrogen at the terminal ileum agrees with previous research. Schulze *et al.*,[16] found that insoluble neutral detergent fiber isolated from wheat bran reduced apparent ileal nitrogen and amino acid digestibility in pigs. Insoluble fiber extracted from barley bran[21] and from pea [14,22]has also been shown to exert significant effects on the ileal endogenous amino acid loss in pigs. Our finding that feeding soluble non-starch polysaccharides increases the endogenous flow of amino acids and nitrogen at the terminal ileum also agrees with previous research[10,23].

We observed that ileal flows of amino acids and nitrogen were 22-85% greater when rats were fed soluble as opposed to insoluble non-starch polysaccharides. This agrees with previous research that has shown that insoluble non-starch polysaccharides have fewer anti-nutritional effects than soluble non-starch polysaccharides such as pectin, carboxylmethylellulose and guar gum[10,24]. Burkhalter *et al.*,[25] observed a quadratic response for amino acid digestibility in the ileum of dogs fed a soybean hull based diet with increasing ratios of insoluble to soluble fiber.

There are a number of mechanisms that could explain the increase in endogenous amino acid flow due to the presence of the two types of non-starch polysaccharides in the diet. First, a high water holding capacity such as that observed with the insoluble non-starch polysaccharide diet increases the ileal flow of both endogenous and bacterial nitrogen compounds[26]. It also increases the velocity of the propagated activity of the small intestine and reduces the retention time of the digesta[27] with less time for endogenous nitrogen and amino acid to be reabsorbed as a possible consequence [14]. The presence of insoluble fiber sources in pig diets increases epithelial cell proliferation rates and the number of epithelial cells exhibiting DNA fragmentation, indicating cell death[28]. This would increase the proportion of sloughed cells in the ileal digesta[14].

Microbial fermentation is increased in the presence of soluble fibers[29,30] and volatile fatty acids produced by the microbes increases epithelial cell proliferation and thus the proportion of sloughed cells in the ileal digesta[14]. The presence of soluble fiber in the diet also increases the secretion of mucus into the small intestine[31]. Neutra and Forstner[32] reported that aspartic acid, glutamic acid and glycine were found in high concentration in mucin protein. Therefore, our finding that these amino acids were the most abundant in the endogenous secretions of the rats fed the diet containing soluble fiber supports the suggestion of an increase in mucin production. The presence of soluble fiber in the diet also increases pancreatic secretions[33], the number of goblet cells[34].

The difference in the extent to which the two fibers affect the ileal flows of amino acids and nitrogen is most likely due to their different chemical structure and physical composition as well as physicochemical properties. The insoluble non-starch polysaccharides have high water holding capacity and little viscosity while the soluble non-starch polysaccharides from wheat bran have high viscosity[23]. Larsen *et al.*, [20]reported that increased dietary fiber viscosity was associated with a significant increase in endogenous nitrogen and amino acids at the terminal ileum of growing rats. It is possible that the increased viscosity reduces the ability of the animal to reabsorb endogenous amino acids by impairing their diffusion to the mucosal surface for absorption.

CONCLUSIONS

The soluble non-starch polysaccharides and insoluble non-starch polysaccharides extracted from

wheat bran dramatically influenced terminal ileal endogenous amino acid loss in rats. The effects of soluble non-starch polysaccharides were greater than those of insoluble non-starch polysaccharides.

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