Effect of common antinutritive factors and fibrous feedstuffs in pig diets on amino acid digestibilities with special emphasis on threonine[1,2]

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ABSTRACT: Most feedstuffs contain antinutritive factors (ANF) such as insoluble fibers, lignins, tannins, and lectins. Intake of these ANF has the ability to reduce nutrient digestibility and to increase endogenous protein losses, such as through increased intestinal mucus secretion. The objective of this experiment was to determine the apparent ileal digestibilities (AID) of AA of 6 ANF-enriched diets to estimate endogenous protein loss associated with these ingredients in diets for young pigs. Forty-two 10-kg BW pigs fitted with a simple T-cannula at the distal ileum were randomly assigned to 1 of 7 casein-based diets with: no supplement (control), 100 g/kg of canola meal (CM), 100 g/kg of wheat bran (WB), 150 g/kg of barley (BR), 22.5 g/kg of lignin (LG), 15 g/kg of kidney beans [as a lectin (LE) source], and 15 g/kg of tannins (TN). All diets were formulated to be similar in N, indispensable AA, and caloric contents. After a 7-d adaptation to the test diets, N balance was conducted for 5 d, followed by 24 h of collection of digesta for analyses of AA. Pigs fed BR had 17% lower ADG and 15% lower feed conversion ratio ($P < 0.05$) compared with control and CM pigs. Pigs fed diets containing WB and BR had lower N retention as a percentage of absorbed N compared with all other groups ($P = 0.03$). The AID for CP was lower in BR, WB, and LE pigs compared with control. Of the AA, AID of Thr was notably lowest in BR, WB, and TN pigs ($P < 0.05$). The standardized ileal digestibility was lower in WB and BR pigs for most indispensable AA. Altogether, these data suggest that hemicellulose fiber, at concentrations typical in commercial swine diets, reduces AID of AA by increasing endogenous losses. Understanding the differential effects of ANF on endogenous losses of individual dietary AA will improve the accuracy of diet formulation.

Key words: amino acid digestibility, antinutritional factor, fiber, nitrogen balance, pig, threonine

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INTRODUCTION

Cereal grains are the primary ingredients in most swine diets in western Canada and supply 30 to 60% of the total AA requirements. Because AA are an expensive component of pig diets, there have been extensive studies to determine AA digestibility (Sauer et al., 2001) and true availability values in many feedstuffs (Moughan, 2003; Moehn et al., 2005). In particular, much research has attempted to improve availability estimates by correcting AA digestibility estimates for endogenous AA contributions. At present, digestibility values are corrected for endogenous losses using an estimated value, which is used to represent endogenous losses for all feedstuffs (NRC, 1998). However, the variable antinutritional factor (**ANF**) contents of feedstuffs are known to affect intestinal mucus secretion and may affect endogenous AA losses. For example, mucin secre-

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tion is affected by dietary components (Satchithanandam et al., 1990), and its synthesis is correlated to Thr intake (Bertolo et al., 1998), because this indispensable AA is critical to the structure and function of mucin proteins (Lamont, 1992). Because gastrointestinal mucin proteins present in mucus are particularly resistant to digestion and recycling (Allen et al., 1984), an increase in mucus secretion directly leads to increased endogenous losses of AA found in mucins. Gastrointestinal mucin entering the large intestine is associated with a net loss of 20 to 30% of the AA in pigs, with the greatest amounts of AA losses being Thr, Ser, and Pro (Lien 1995).

Therefore, we hypothesized that dietary factors and ingredients that increase mucin losses will result in a greater increase in endogenous Thr losses relative to other indispensable AA. The objective of this study was to determine the effect of common ANF on the ileal recoveries of CP and AA, particularly Thr.

MATERIALS AND METHODS

Animals, Housing, and Surgery

Protocols and procedures for this study were approved by the Animal Policy and Welfare Committee of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta.

Forty-eight barrows (Duroc × Yorkshire) were obtained from the University of Alberta Swine Research and Technology Center at 21 d of age (6 to 7 kg). Young pigs were used, because their small intestine is more sensitive to mucus-stimulating ANF (Huisman and Tolman, 1992). On arrival, the pigs were fed a commercial starter diet (Consultant Feeds, Calmar, Alberta, Canada) for 3 d before surgery. After a 12-h fast, the pigs were surgically fitted with a simple T-cannula at the distal ileum, as described by Sauer (1976) and Li et al. (1993). Cannulas were approximately 3 mm in wall thickness, with a 13-mm internal barrel diameter; wings were 16 mm in diameter and 40 mm in length. Pigs recovered from anesthesia within 2 to 3 h after surgery and were fasted for an additional 24 h to ensure complete healing of the intestinal tissue. Pigs were housed individually in metabolic crates (1.5 × 1.0 m) in a temperature-controlled room.

The next day, pigs were provided with 25 g of the starter diet 4 times daily at 6-h intervals. The daily dietary allowance was gradually increased until the pigs consumed the diet at a rate of 5% of their BW (within 7 d), at which time pigs were fed twice daily. During the recuperation period, the crate temperature was maintained at 30 to 32°C by adjusting the positioning of the infrared heating lamp. On d 1 to 3 after surgery, analgesic (Torbugesic, 0.05 mL/kg of BW, Wyeth Animal Health Canada, Guelph, Ontario, Canada) was administered i.m. From d 3 postsurgery onwards, the animals were washed with warm water twice daily around the cannula area, and zinc oxide cream (Coopers Agropharm Inc., Ajax, Ontario, Canada) was applied under and around the retaining ring to minimize skin irritation.

Diet Regimen

The pigs consumed the starter diet for a total of 12 d. Forty-two pigs were selected based on consistent feed intake and were adapted to the casein diet for 3 d. Pigs (BW 9.42 kg, SD = 0.14) were allocated randomly to either the casein-cornstarch-sugar diet (control) or 1 of 6 test diets, according to a completely randomized design. Test diets were casein-based plus: 100 g/kg of canola meal (CM), 100 g/kg of wheat bran (WB), 150 g/kg of barley (BR), 22.5 g/kg of lignin (LG), 15 g/kg of kidney beans [as a lectin (LE) source], and 15 g/kg of tannins (TN; Table 1). The aim was to supplement the diets with these ingredients at levels that include ANF at concentrations that are commonly present in practical pig diets. For example, the level of lectins found in typical pig diets containing various feedstuffs (Nachbar and Oppenheim, 1980) is approximately 3.0 g/kg. Relative to other beans and legumes, the lectins in kidney beans have been shown to be the most potent of antinutritional factors in these beans (Pusztai et al., 1982; Bond and Smith, 1989); 15 g of red kidney beans provided 3.0 g of lectins/kg to the diet. Barley, sorghum, rapeseed, and millet are particularly rich in tannins, and a typical intake of 20 g of dietary tannins/kg was estimated for pig diets (Bravo, 1998). Because cellulose as a dietary fiber source has been shown to have no effect on ileal endogenous N excretion in pigs (de Lange et al., 1989; Furuya and Kaji, 1992; Leterme et al., 1992), the fibrous constituents other than cellulose in purified NDF (i.e., hemicellulose and lignin) induce the increased endogenous N secretion. After cellulose, lignins are the second most abundant fiber component in most plants and can also be used as a binding agent during pelleting. Because lignin levels of 30 to 60 g/kg are present in most plant food sources (Shah et al., 1982; Chiou et al., 1994), we chose an inclusion level of 22.5 g/kg to reflect a cereal-based pig diet. High, moderate, and low hemicellulose diets were also included to assess the effect of hemicellulose level on endogenous N secretion. Wheat bran was used at an inclusion level of 100 g/kg to reflect a 300 g/kg wheat diet to represent a high hemicellulose concentration (Table 1). Lastly, barley and canola meal are the primary feedstuffs fed to swine in western Canada, so we included 150 g/kg of barley and 100 g/kg of canola meal diets to reflect moderate and low hemicellulose concentrations, respectively. We equalized total dietary cellulose in all diets in an effort to isolate the effects of hemicellulose in these 3 diets (Table 1).

All diets were formulated to meet or exceed intake of all nutrients according to the NRC (1998), which included additions of Cys, Thr, and Trp to all diets. In particular, the indispensable AA concentrations were matched as closely as possible, accounting for the pro-

Table 1. Ingredients and calculated composition of the experimental diets, as-fed basis

	Test diets						
Item	Control	Canola meal	Wheat bran	Barley	Lignin	Lectin	Tannins
Ingredients, g/kg							
Barley[1]	—	—	—	150.0	—	—	—
Canola meal[1]	—	100.0	—	—	—	—	—
Casein	145.0	123.0	140.0	140.0	145.0	142.0	145.0
Chromic oxide[2]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn oil	4.0	22.5	34.0	38.0	24.0	2.0	17.5
Cornstarch	380.0	312.7	274.6	212.6	338.3	376.0	352.3
Dicalcium phosphate	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Kidney beans[3]	—	—	—	—	—	15.0	—
L-Asn[4]	48.0	40.0	44.0	44.0	48.0	48.0	48.0
L-Cys	2.8	2.1	2.5	2.5	2.8	2.8	2.8
Lignin[5]	—	—	—	—	22.5	—	—
Limestone	10.0	10.0	10.0	10.0	10.0	10.0	10.0
L-Thr	0.9	0.5	0.7	0.7	0.9	0.9	0.9
L-Trp	0.3	0.2	0.2	0.2	0.3	0.3	0.3
Mg sulfate	2.0	—	—	2.0	2.0	2.0	2.0
K chloride	5.3	3.0	3.0	4.0	5.3	5.0	5.3
Na bicarbonate	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Solkafloc[6]	15.0	—	5.0	10.0	15.0	10.0	15.0
Sugar (sucrose)[7]	350.0	350.0	350.0	350.0	350.0	350.0	350.0
Tannins[8]	—	—	—	—	—	—	15.0
Vitamin-mineral mix[9]	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Wheat bran[7]	—	—	100.0	—	—	—	—
Total	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Calculated composition							
ME, kcal/kg	3,621	3,628	3,626	3,626	3,621	3,621	3,621
CP, %	19.63	20.07	20.15	20.14	19.62	19.70	19.62
NDF,[10] g/kg	—	21.2	42.1	27.9	22.5	10.6	—
ADF,[10] g/kg	—	17.2	13.0	10.5	22.5	5.6	—
Hemicellulose,[10] g/kg	—	4.0	29.1	17.4	—	5.0	—
Lignin,[10] g/kg	—	2.0	2.5	5.0	22.5	0.4	—
Cellulose,[10] g/kg	—	15.2	10.5	5.5	—	5.2	—
Total cellulose,[11] g/kg	15.0	15.2	15.5	15.5	15.0	15.2	15.0

[1] Barley and canola meal were obtained from the University of Alberta feed mill.

[2] Fisher Scientific (Fair Lawn, NJ).

[3] Kidney beans were used as a source of lectins (Unico Inc., Concord, Ontario, Canada); this inclusion level provided approximately 3.0 g of lectins/kg for the total diet (Nachbar and Oppenheim, 1980; Pusztai et al., 1982).

[4] L-Asparagine added to make all diets isonitrogenous.

[5] Lignin used was sodium lignosulfonate donated by Tembec Chemical & Power Group Inc. (Quebec, Canada).

[6] Provided cellulose.

[7] Sugar and wheat bran were from Rogers Foods Ltd. (Armstrong, British Columbia, Canada).

[8] Grape tannins were used as a source of tannins (Prescott & Co., Mississauga, Ontario, Canada).

[9] Provided the following per kilogram of diet: 215 mg of Ca, 84 mg of digestible P, 10 mg of Mg, 2.0 mg of S, 6.0 mg of Cu (as copper sulfate), 99 µg of I (as potassium iodate), 70 mg of Fe (as ferrous sulfate), 16 mg of Mn (as manganese sulfate), 74 µg of Se (as sodium selenite), 35 mg of Zn (as zinc carbonate), 150 µg of biotin, 130 mg of choline, 0.8 mg of folacin, 6.2 mg of pantothenic acid (as calcium pantothenate), 9.5 mg of niacin, 1.75 mg of riboflavin, 8.8 µg of vitamin B_{12}, 15.5 mg of vitamin E (as DL-α-tocopheryl acetate), 3,000 IU of vitamin A (as vitamin A acetate), and 350 IU of vitamin D_3.

[10] NDF = ADF + hemicellulose; ADF = cellulose + lignin; hemicellulose = NDF − ADF; cellulose = ADF − lignin.

[11] Total cellulose = calculated cellulose + added Solkafloc.

tein contribution and digestibility of the barley and canola meal test ingredients. Diets were designed with Thr as the first-limiting AA (at 100% of the requirement), with other indispensable AA set to at least 105% of the requirements across diets (NRC, 1998). Analyzed chemical and AA compositions of the experimental diets are presented in Table 2. The analyzed values of CP and AA in the experimental diets were very close to the calculated values used to formulate the diets. Chromic oxide was included in the diets at a rate of 0.5% as an indigestible marker. Feed intake was restricted to 5% of BW, with the total daily feed intake of each pig divided in equal amounts and fed twice daily (at 0700 and 1900). Pigs were weighed, and feed intakes were adjusted on a daily basis. Water was provided on an ad libitum basis from a low-pressure drinking nipple.

Table 2. Analyzed composition of the experimental diets (as-fed basis)

Item	Control	Canola meal	Wheat bran	Barley	Lignin	Lectin	Tannins
OM, %	95.60	95.22	95.32	95.47	95.02	95.78	95.79
CP,[1] %	17.80	18.96	18.06	19.27	18.30	18.08	17.81
DM, %	94.65	94.94	94.70	94.71	94.83	94.65	94.52
Ether extract, %	0.19	1.89	2.85	2.75	1.79	0.12	1.13
ME,[1] kcal/kg	3,578	3,540	3,187	3,730	3,097	3,551	3,454
Indispensable AA, %							
Arg	0.36	0.51	0.45	0.44	0.36	0.37	0.36
His	0.32	0.36	0.34	0.34	0.32	0.32	0.32
Ile	0.63	0.66	0.65	0.67	0.63	0.63	0.63
Leu	1.12	1.24	1.22	1.28	1.18	1.18	1.18
Lys	0.92	0.96	0.94	0.95	0.92	0.92	0.92
Met	0.38	0.39	0.39	0.40	0.38	0.38	0.38
Phe	0.61	0.66	0.65	0.69	0.61	0.62	0.61
Thr	0.64	0.67	0.65	0.67	0.64	0.64	0.64
Trp	0.20	0.21	0.21	0.21	0.20	0.20	0.20
Val	0.78	0.83	0.81	0.84	0.78	0.78	0.78
Dispensable AA, %							
Ala	0.36	0.46	0.41	0.42	0.36	0.36	0.36
Asp	0.85	0.97	0.92	0.93	0.85	0.87	0.85
Cys	0.10	0.16	0.13	0.14	0.10	0.10	0.10
Glu	2.68	2.87	2.86	3.07	2.68	2.68	2.68
Gly	0.22	0.36	0.29	0.29	0.22	0.23	0.22
Pro	1.34	1.35	1.39	1.50	1.34	1.33	1.34
Ser	0.66	0.71	0.70	0.72	0.66	0.67	0.66

[1] Crude protein calculated as total N × 6.25; ME calculated from the DE and CP of the diet. The average of the following 3 equations was used to determine ME: ME = DE (1.012 – (0.0019 × %CP) (May and Bell, 1971); ME = DE [0.988 – (0.002 × %CP)] (Noblet et al., 1989); and ME = DE [1.003 – (0.0021 × %CP)] (Noblet and Perez, 1993).

Conduct of the Study

After 7 d of recovery from surgery, the pigs were given 3 d of adaptation to the casein diet. The experimental diets were then fed for 7 d, followed by total urine and feces collection for 5 d, from 0700 on d 6 until 0700 on d 11 of each experimental period. Concentrated (18.4 *M*) sulfuric acid was added (5 to 8 mL) to each urine flask to prevent NH_3 loss. After collection, the volume of urine was measured, and aliquots of urine and feces were frozen at –20°C to determine total N output. After the N balance study, ileal digesta were collected for a total of 24 h, from 0600 to 1800 on d 11 and from 1800 on d 12 to 0600 on d 13 of each experimental period, and stored at –20°C until analyzed. The digesta were freeze-dried, pooled within pig, and mixed before analyses.

After the ileal digesta collection period, the pigs were sedated using a mixture of halothane and O_2. Twenty milliliters of blood was obtained from the heart via needle puncture and then transferred into heparinized tubes and centrifuged, and plasma samples were collected and frozen at –80°C until AA analyses. Pigs were then slaughtered.

Analytical Procedures

Diets, feces, and freeze-dried digesta were analyzed for DM according to the AOAC (1995) and for N using the method described by Sweeney and Rexroad (1987). Nitrogen contents of urine samples were determined by Kjeldahl analysis (Bradstreet, 1965). Amino acid contents of the diets were determined by Degussa AG, Düsseldorf, Germany (Llames and Fontaine, 1994), and in ileal digesta by HPLC after acid hydrolysis in 6 *N* HCl for 24 h (Sedgwick et al., 1991). Chromium analyses on feed, digesta, and fecal samples were performed by the method of Fenton and Fenton (1979).

The apparent digestibility of components in ileal digesta were determined by the following equation: apparent ileal digestibilities (**AID**), % = $[1 - (N_D \times Cr_F)/(N_F \times Cr_D)] \times 100\%$, where N_D = the concentration of the component in ileal digesta; Cr_F = the concentration of Cr in feed; N_F = concentration of the component in the diet; and Cr_D = the concentration of Cr in ileal digesta. The flow of a component through the distal ileum was calculated based on the Cr in the diet and the equation described by Furuya and Kaji (1992), where ileal flow of a component (g/d) = [(concentration of a component in ileal digesta, g/g) × (concentration of Cr in the diet, g/g)/concentration of Cr in ileal digesta (g/g)] × feed intake of component (g/d).

All diets in this experiment were casein-based; as such, the casein-cornstarch-sucrose diet was used as the basal diet to estimate endogenous protein and AA losses. Endogenous component losses (**ECL**) were calculated from the equation provided by Moughan and

Table 3. Apparent ileal digestibility (%) of CP and AA in growing pigs fed diets containing common levels of antinutritional factors[1]

Item	Control	Canola meal	Wheat bran	Barley	Lignin	Lectin	Tannins	SD	*P*-value
CP, %	91.9^{a}	87.2^{ab}	83.2^{b}	85.1^{b}	91.5^{a}	85.3^{b}	87.2^{ab}	7.0	0.01
Indispensable AA, %									
Arg	93.2	90.1	82.1	83.9	91.8	83.8	79.8	2.0	0.06
His	96.1	92.8	87.1	90.3	95.4	91.0	86.7	1.4	0.08
Ile	94.7^{a}	91.2^{ab}	86.9^{b}	87.9^{b}	94.9^{a}	90.6^{ab}	89.3^{b}	2.0	0.02
Leu	95.5	91.9	86.3	88.4	96.2	93.0	90.7	2.1	0.06
Lys	96.0	91.8	86.1	89.4	96.2	92.6	90.7	1.7	0.07
Phe	96.0	92.6	87.7	89.1	95.8	91.3	90.9	2.0	0.07
Thr	90.6^{a}	85.5^{abc}	80.9^{c}	80.2^{c}	89.3^{ab}	83.7^{abc}	82.6^{bc}	2.4	0.05
Val	93.3^{a}	87.6^{abc}	81.1^{c}	84.3^{bc}	95.0^{a}	91.0^{ab}	87.1^{abc}	3.0	0.02
Dispensable AA, %									
Ala	88.5^{a}	85.5^{ab}	70.3^{c}	74.6^{bc}	88.5^{a}	80.0^{abc}	76.2^{abc}	3.7	0.04
Asp	85.5	84.6	79.2	79.0	90.7	84.1	75.3	9.9	0.72
Glu	95.0	93.4	91.0	88.7	94.8	92.5	89.5	2.3	0.21
Gly	79.0	75.7	62.7	67.1	76.3	61.9	52.4	4.1	0.09
Ser	91.2	88.4	84.5	83.4	90.4	84.3	85.7	1.9	0.11
Tyr	97.1^{a}	94.2^{ab}	90.7^{b}	91.0^{b}	96.5^{a}	93.1^{ab}	92.7^{ab}	2.0	0.05

[a-c]Values with different superscripts within the same row are different at $P < 0.05$; n = 6 per dietary treatment.

[1]All diets were casein-based with: no supplement (control), 100 g/kg of canola meal, 100 g/kg of wheat bran, 150 g/kg of barley, 22.5 g/kg of lignin, 15 g/kg of kidney beans (as a lectin source), or 15 g/kg of tannins.

Schuttert (1991), ECL = $N_D \times (Cr_F/Cr_D)$, where N_D = the concentration of a component in ileal digesta and Cr_F and Cr_D = the concentration of Cr in feed and digesta, respectively. Standardized ileal digestibility (**SID**) was determined from the equation of Smiricky et al. (2002), where SID = apparent digestibility + $[(ECL/N_D) \times 100]$.

Statistical Analyses

The experiment used a complete randomized design using the GLM procedure (SAS Inst. Inc., Cary, NC). Differences among diets for ADG, N balance, mucin output, and digesta AA concentrations were assessed by protected LSD multiple-comparisons procedure and were considered statistically significant at $P < 0.05$. An analysis of covariance, using initial BW as the covariate, was tested against BW gain and was not significant.

RESULTS

Growth Performance

The pigs remained healthy and attentive to their environment throughout the trial. Postmortem examinations of the pigs revealed no adhesions or other intestinal abnormalities due to cannulation. The BW of the pigs at the start (9.42 kg, SD = 0.14) and end of the experiment (15.41 kg, SD = 0.77) were not different among diets. All pigs consumed their entire feed ration throughout the trial, so the ADFI of the pigs was not different among diets (0.57 kg, SD = 0.06) but ADG was decreased ($P < 0.05$) for pigs receiving WB (0.31 kg/d) compared with control, CM, and TN diets (all at 0.41 kg/d).

Ileal AA and Protein Digestibility

The AID of CP and AA for the control diet (Table 3) were in close agreement with results reported by other researchers (Kies et al., 1986; Furuya and Kaji, 1989; Chung and Baker, 1992). Of particular note, the AID of CP was decreased for WB, BR, and LE diets relative to control ($P = 0.01$), and AID of Thr, Val, Ile, Ala, and Tyr were decreased for WB and BR diets relative to control ($P < 0.05$; Table 3); AID of Thr and Ile were also decreased compared with control in TN diets ($P < 0.05$). Overall, AID of all indispensable AA tended to be decreased in WB and BR pigs ($P < 0.08$). Comparing endogenous losses among ANF groups, SID of most indispensable AA were decreased in WB, BR, and TN groups compared with other ANF groups ($P < 0.05$); however, SID for Thr and His were not different ($P < 0.12$; Table 4). On average, SID values for AA and protein were 7% greater than the AID values for all diets. Although casein concentrations varied among diets, total CP was constant, and for all ANF groups, casein contributed >97% of total protein, except for the CM diet, where casein contributed >85% of total CP. The effects of these slight variations of casein concentration on total endogenous losses were likely negligible. Furthermore, with respect to the protein contribution of canola meal, wheat bran, barley, and kidney beans, we used true ileal digestibility estimates of AA in these respective feedstuffs. Thus, changes in AID in this study would be attributable to changes in endogenous losses and not variations in digestibility among feedstuffs.

Table 4. Standardized[1] ileal digestibility (%) of protein and AA in growing pigs fed diets containing common levels of antinutritional factors[2]

Item	Canola meal	Wheat bran	Barley	Lignin	Lectin	Tannins	SD	*P*-value
CP, %	93.7	91.6	92.4	99.6	91.1	93.8	7.4	0.27
Indispensable AA, %								
Arg	96.4^{abc}	88.4^{dc}	89.6^{abcd}	99.7^{ab}	88.9^{bcd}	84.5^{d}	10.4	0.04
His	98.6	93.2	95.9	103.4	96.7	90.9	8.3	0.06
Ile	98.1^{abc}	93.9^{c}	94.0^{c}	104.2^{a}	97.6^{abc}	95.8^{bc}	6.3	0.02
Leu	98.3^{abc}	92.8^{c}	93.8^{c}	106.5^{a}	100.8^{abc}	96.9^{bc}	8.0	0.03
Lys	97.5^{abc}	91.9^{c}	94.9^{bc}	105.5^{a}	99.7^{abc}	96.4^{bc}	7.9	0.04
Phe	98.8^{abc}	94.0^{c}	94.1^{c}	104.3^{a}	97.1^{abc}	96.8^{bc}	6.9	0.04
Thr	92.7	88.6	87.1	97.0	90.5	89.2	7.8	0.12
Val	93.9^{bc}	87.7^{c}	90.4^{c}	106.9^{a}	100.5^{ab}	93.7^{bc}	9.7	0.003
Dispensable AA, %								
Ala	92.8^{abc}	76.5^{d}	80.5^{dc}	97.6^{a}	87.1^{abcd}	82.6^{bcd}	13.1	0.02
Asp	91.9	89.9	87.3	101.6	92.5	82.0	15.0	0.49
Glu	101.2	100.1	94.6	104.4	100.3	95.7	6.8	0.15
Gly	81.7^{a}	69.9^{ab}	73.4^{ab}	85.1^{a}	67.7^{ab}	58.1^{b}	17.8	0.05
Ser	96.5	93.4	90.5	98.5	91.0	92.5	6.8	0.20
Tyr	100.2^{ab}	96.5^{b}	95.7^{b}	104.2^{a}	98.6^{ab}	98.2^{ab}	5.7	0.04

$^{a-d}$Values with different superscripts within the same row are different at $P < 0.05$; n = 6 per dietary treatment.

[1] Casein (control) diet was used to determine basal loss and digestibility, according to the formulations of Moughan and Schuttert (1991) and Smiricky et al. (2002).

[2] All diets were casein-based with: no supplement (control), 100 g/kg of canola meal, 100 g/kg of wheat bran, 150 g/kg of barley, 22.5 g/kg of lignin, 15 g/kg of kidney beans (as a lectin source), or 15 g/kg of tannins.

Endogenous Ileal AA and Protein Losses and N Balance

Total ileal flow of protein (Figure 1), Thr (Figure 2), and Ser (Figure 3) were greatest for pigs fed the diets containing wheat bran and barley. The ileal Thr flows (mg/d) for pigs offered the WB (69.1), BR (73.5), CM (56.4), and TN (59.1) diets were all ($P = 0.001$) greater than those for pigs fed the control (37.5) and LG (36.3) diets (Figure 2). Among the diets, the ileal flow of Ser (Figure 3) followed a similar pattern as Thr. Similarly, ileal protein flows (g/d; Figure 1) for pigs offered the WB (6.90), BR (7.53), CM (5.90), and LE diets (5.64) were ($P = 0.01$) greater than for those fed the control diet (3.65).

During the course of the N balance study, N intake and excretion were similar among pigs given the 7 diets (Table 5). However, 2 pigs in the WB group had decreased growth rates in spite of consuming their entire feed ration; because feed intake was restricted according to BW, these pigs had lower absolute N intakes. When corrected for this lower intake, pigs fed diets containing WB and BR had lower N retention as a percentage of absorbed N ($P = 0.03$; Table 5); N retention in grams per day and as a percentage of intake tended to be lower in the WB group ($P = 0.16$ and $P = 0.14$, respectively).

DISCUSSION

Endogenous N and AA recoveries in digesta collected from the distal ileum have traditionally been determined after feeding a protein-free diet. However, this method has been criticized for creating a physiologically abnormal state (Low, 1980). Other studies (Moughan and Rutherford, 1990; Butts et al., 1993) have shown that the presence of dietary protein or peptides in the gastrointestinal tract results in increased recoveries of endogenous N and AA in ileal digesta compared with a protein-free diet. Under most conditions, it is assumed that the endogenous N loss (which includes mucin, along with sloughed cells, enzymes, and other intestinal secretions) is relatively constant (Furuya and Kaji, 1992). However, if a feedstuff contains components that stimulate endogenous secretions, especially mucus, then the amount of mucin, and therefore AA losses, becomes a variable function, dependent upon the dietary ingredients.

The main objective of this trial was to determine the flow of Thr in ileal digesta to measure the total losses per day regardless of source of loss (i.e., endogenous plus dietary losses). All diets were formulated to provide equal amounts of total Thr, with all other AA intakes above requirement and approximately similar among diets (Table 2). In addition, the only Thr sources in the diets were from casein and the experimental feedstuffs (Table 1). Thus, total ileal Thr loss, along with N balance, allows estimates of differences in endogenous Thr loss due to the different diet ingredients. Differences among feedstuffs in endogenous Thr losses have significant implications for the Thr intake necessary to meet the requirement of lean growth.

The highly digestible casein diet in this experiment was used as the basal diet to estimate endogenous protein and AA output. Diets containing highly digestible protein sources, such as casein, are commonly used to

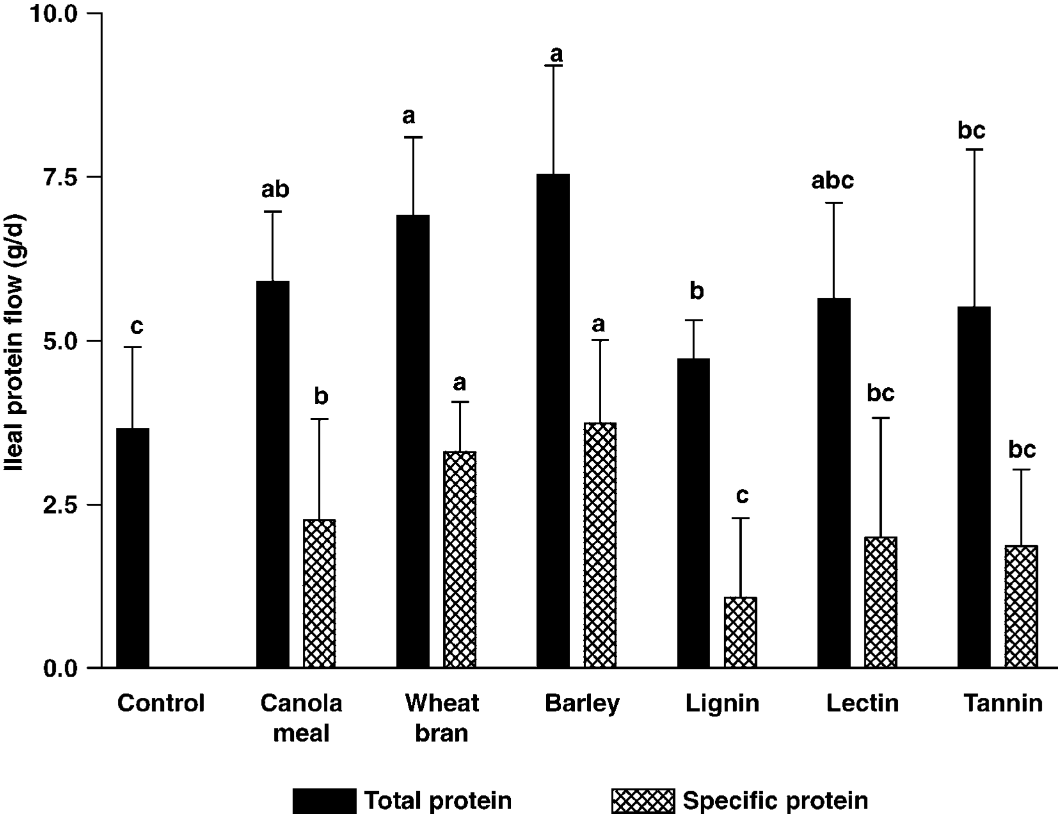

Figure 1. Total and ingredient-specific ileal protein flow (g/d) in growing pigs fed casein-based diets with: no supplement (control), 100 g/kg of canola meal, 100 g/kg of wheat bran, 150 g/kg of barley, 22.5 g/kg of lignin, 15 g/kg of kidney beans (as a lectin source), or 15 g/kg of tannins. Total protein flow represents protein intake minus retention using the apparent ileal digestibility of the protein; ingredient-specific ileal protein flow is corrected for protein flow in pigs fed the casein diet. Each bar represents the mean of 6 pigs. $^{a\text{-}c}P < 0.05$.

determine endogenous protein and AA losses in pigs (Kies et al., 1986; Furuya and Kaji, 1989; Jansman et al., 2002). The true digestibility of CP and AA in casein is usually assumed to be 99%, and casein effectively minimizes specific secretions of endogenous protein and AA (Kies et al., 1986; Jansman et al., 2002). We used Solkafloc (purified cellulose) to balance the diets for total cellulose, and this included some cellulose addition to our casein control diet. However, because purified cellulose does not affect endogenous protein ileal losses (de Lange et al., 1989; Furuya and Kaji, 1992; Leterme et al., 1992), the casein control diet can be used to estimate basal endogenous protein flow. Therefore, the amount, which represents minimal values, and AA composition of endogenous protein can be calculated from studies in which the AID of protein in casein has been determined (Kies et al., 1986; Furuya and Kaji, 1989; Jansman et al., 2002). Indeed, the AID values of CP and AA for the casein diet in the present experiment (Table 3) are in agreement with results reported by other researchers (Kies et al., 1986; Furuya and Kaji, 1989; Chung and Baker, 1992).

The AID of AA are usually used as the basis for calculating the supply to the body. Values for the AID and SID are primarily affected by the level of feed intake, protein intake, and diet characteristics (Souffrant et al., 1993; Sauer et al., 2000). There were no differences in feed and protein intake among the pigs fed the different diets, because these were controlled; therefore, differences in digestibilities must be due to diet characteristics. Of the indispensable AA, AID of Thr was lowest in all pigs, suggesting that endogenous AA losses were greater for Thr than for the other AA. In agreement with studies reviewed by Sauer and Ozimek (1986), these data suggest that a greater proportion of this AA is required for maintenance in the growing pig.

Sève and Hess (2000) discussed dietary conditions that influence the endogenous losses of AA and concluded that the rate of endogenous secretion was primarily dependent on the amount of protein and fiber in the diet. In the current study, ingredient-specific ileal flows of protein (i.e., corrected for flows in casein diet; Figure 1) were affected by dietary components such as dietary fiber, lectins, and condensed tannins, in agreement with studies by Jansman (1993) and Schulze et al. (1994). The length of the pretest period has been reported to affect the amount and AA composition of endogenous protein in ileal digesta of pigs (Leterme et al., 1992; Jansman et al., 1993). In the present experiment, the pigs received the diets for 12 d before

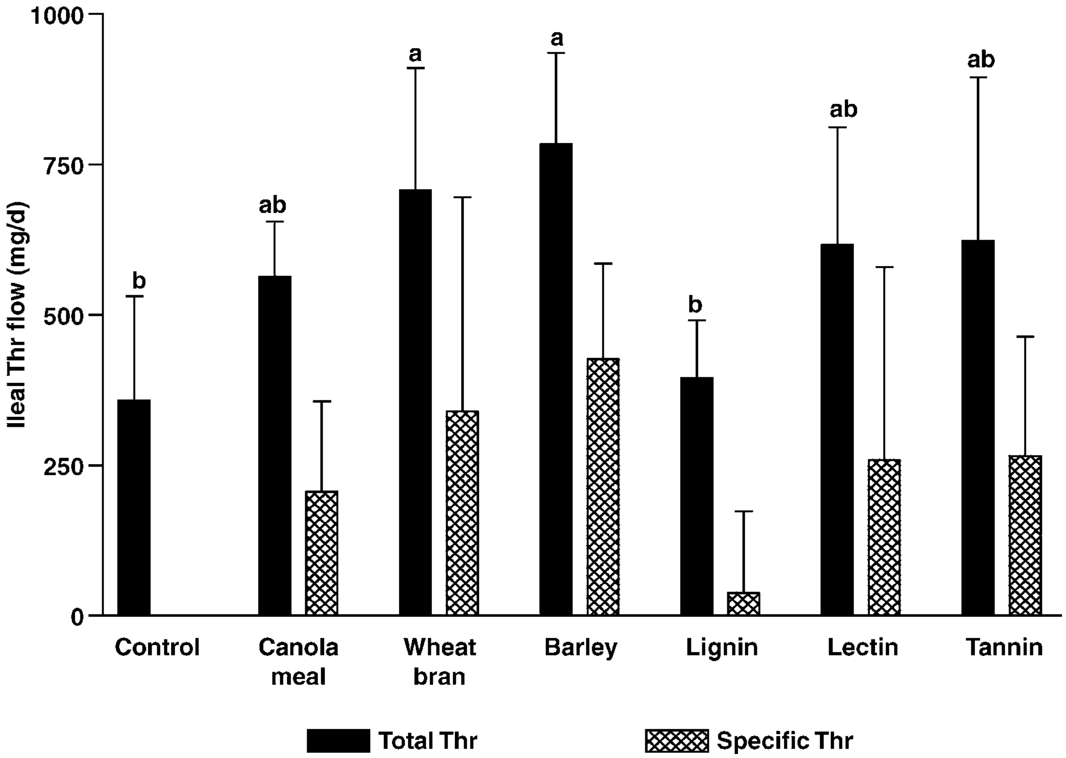

Figure 2. Total and ingredient-specific ileal Thr flow (mg/d) in growing pigs fed casein-based diets with: no supplement (control), 100 g/kg of canola meal, 100 g/kg of wheat bran, 150 g/kg of barley, 22.5 g/kg of lignin, 15 g/kg of kidney beans (as a lectin source), or 15 g/kg of tannins. Total Thr flow represents Thr intake minus retention using the apparent ileal digestibility of Thr; ingredient-specific ileal Thr flow is corrected for Thr flow in pigs fed the casein diet. Each bar represents the mean of 6 pigs. $^{a,b}P < 0.05$.

digesta collection to ensure that the time of adaptation was sufficient to elicit diet-specific responses.

Because cellulose has no effect on ileal endogenous N excretion in pigs (de Lange et al., 1989; Furuya and Kaji, 1992; Leterme et al., 1992), other fibrous constituents in purified NDF (i.e., hemicellulose) induce the increased endogenous N secretion. Indeed, pigs fed the barley (representing moderate hemicellulose) and wheat bran (high hemicellulose) diets had the lowest AID of CP, Thr, Ile, and Val (Table 3). Pigs fed these diets also tended to have the lowest AID for all indispensable AA ($P < 0.08$). The AID and ileal recoveries of AA observed suggest that wheat bran and barley increased endogenous protein and AA losses, probably including an increase in mucus secretion. Lien (1995) determined that mucin represented approximately 5 to 11% of total endogenous protein. In mucin, Thr has the largest concentration of any indispensable AA (28 to 35% of total AA), whereas its concentration in nonmucin endogenous protein is much smaller (Mantle and Allen, 1981; Lien, 1995). Indeed, the barley (moderate hemicellulose) and wheat bran (high hemicellulose) groups had greater total ileal flow of Thr compared with control (no hemicellulose) pigs (Figure 2). Thus, an increase in the rate of Thr loss, relative to other AA, at the distal ileum strongly supports the conclusion that there was an increase in mucin secretion and loss in pigs fed diets containing wheat bran and barley.

The current study showed that condensed tannins (15 g/kg) from grape reduced the AID of CP and Thr (Table 3). Similar findings were reported by Jansman et al. (1993), in piglets fed diets containing field beans with various levels (4 to 14 g/kg) of condensed tannins. Several studies (reviewed by Jansman, 1993) have also shown that tannins in different feedstuffs reduced AID of protein and AA in pigs and other animals. In particular, the inclusion of 15 g/kg of tannins in the current study decreased AID of Thr compared with casein (Table 3, 82.6 vs. 90.6). This suggests that the reduced protein and AA digestibilities resulting from the inclusion of condensed tannins in the diet of pigs is either due to interaction of tannins with dietary proteins or due to an increase in endogenous intestinal secretions (Jansman, 1993).

Because the diet design controlled total protein, ME, cellulose, and Thr availability, AID comparisons demonstrated that wheat bran, barley, and tannins diets, at concentrations typical in swine diets, led to increased endogenous protein (except tannins) and Thr losses compared with the casein control. The SID comparisons were used to determine which of the ANF were most effective at inducing endogenous protein losses. When the control diet was used to determine SID, the data suggested that the wheat bran, barley, and tannins diets significantly induced endogenous losses of most indispensable AA (except for Thr) compared with the

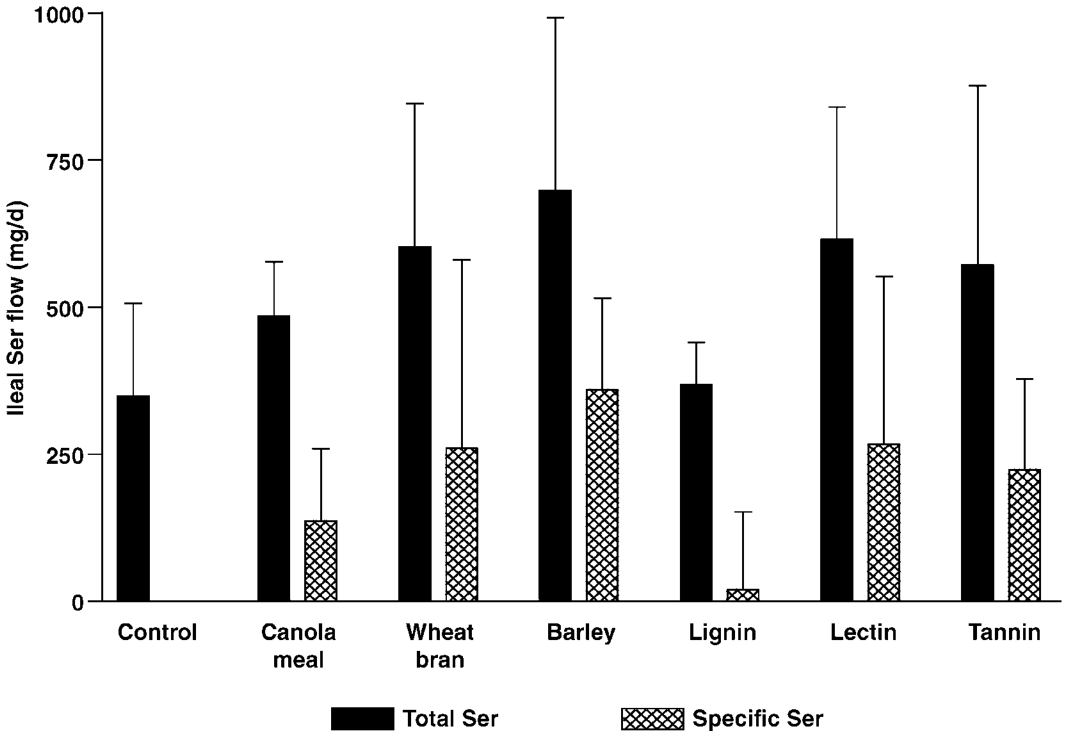

Figure 3. Total and ingredient-specific ileal Ser flow (mg/d) in growing pigs fed casein-based diets with: no supplement (control), 100 g/kg of canola meal, 100 g/kg of wheat bran, 150 g/kg of barley, 22.5 g/kg of lignin, 15 g/kg of kidney beans (as a lectin source), or 15 g/kg of tannins. Total Ser flow represents Ser intake minus retention using the apparent ileal digestibility of Ser; ingredient-specific ileal Ser flow is corrected for Ser flow in pigs fed the casein diet. Each bar represents the mean of 6 pigs.

other ANF. However, most importantly, SID of Thr was lowest of all indispensable AA among ANF groups, suggesting that each of the ANF induced some endogenous Thr losses over control. In particular, the disproportionate loss of Thr suggests that Thr-rich proteins dominated endogenous protein losses.

Pigs receiving the wheat bran (high hemicellulose) and the barley (moderate hemicellulose) diets had lower percentage retention of absorbed N than the other pigs (Table 5), indicating that the greater endogenous losses impacted performance. This lower N retention is probably a result of the greater endogenous protein losses, which would reduce whole-body protein retention and deposition. Souffrant et al. (1993) and Krawielitzki et al. (1994) showed that approximately 25% of the total endogenous protein secretions are present in the ileal digesta of pigs. The endogenous N supply is then replenished at the expense of AA for growth (Fuller and Reeds, 1998). Indeed, in spite of similar feed intakes, pigs fed wheat bran and barley diets had lower BW gains in the second week (results not shown). Despite the slightly lower BW gains for the pigs fed wheat bran- and barley-based diets, there were no differences in G:F among pigs fed the various diets. Although we only used 6 pigs per treatment, our limited interpretation of these performance data is that because of the greater ileal

Table 5. Nitrogen balance in growing pigs fed diets containing common levels of antinutritional factors[1]

Item	Control	Canola meal	Wheat bran	Barley	Lignin	Lectin	Tannins	SD	*P*-value
N Intake, g/d	22.5	22.49	19.47	21.83	21.88	22.34	22.35	2.52	0.28
Fecal N, g/d	2.82	3.51	4.25	3.4	3.26	4.5	3.19	1.79	0.40
Absorbed N, g/d	19.68	18.97	15.21	18.44	18.62	17.86	19.17	3.40	0.40
Urinary N, g/d	5.31	5.49	5.50	6.12	5.55	5.41	4.91	0.85	0.32
N retention, g/d	14.38	13.49	9.71	12.29	13.07	12.45	14.26	2.93	0.16
N retention, % of intake	63.71	59.48	48.80	55.29	59.56	55.03	63.56	9.15	0.14
N retention, % of absorbed N	72.95^{a}	70.70^{a}	62.35^{b}	64.74^{b}	70.13^{a}	69.23^{a}	74.17^{a}	5.35	0.03

[a,b] Values with different superscripts within the same row are different at $P < 0.05$; n = 6 per dietary treatment.

[1] All diets were casein-based with: no supplement (control), 100 g/kg of canola meal, 100 g/kg of wheat bran, 150 g/kg of barley, 22.5 g/kg of lignin, 15 g/kg of kidney beans (as a lectin source), or 15 g/kg of tannins.

nutrient flow and AID of AA, the maintenance demand for AA, particularly Thr for mucin synthesis, was greater in the pigs fed the barley and the wheat bran diets such that there was less AA available for lean tissue protein deposition, resulting in a lower retention of absorbed N and lower BW gain.

Because the pattern of AA required for maintenance is different from that required for growth (NRC, 1998), replenishing endogenous AA losses will alter the pattern of AA available for protein deposition. This shift in AA pattern may, in turn, result in an increase in urinary N excretion (Sève and Henry, 1996). Thus, an increase in endogenous mucin losses would result in less AA such as Thr, Ser, and Pro being available for N retention. If any indispensable AA, such as Thr, becomes limiting, then other AA would be in excess, resulting in greater urinary N losses. Indeed, the wheat bran and barley groups excreted more urinary N as a percentage of absorbed N supporting such a mechanism. The combination of greater endogenous losses of N at the ileum and via urine would lead to lower N retention and lower growth.

In conclusion, these results show that the barley-based diet (representing moderate hemicellulose) lowered the ileal Thr and protein digestibility by 12 and 8%, respectively, compared with the casein diet. Wheat bran (high hemicellulose) diet had the next lowest ileal Thr digestibility, 11% lower than in pigs fed the casein, whereas protein digestibility was 10% lower than in casein. Pigs receiving the wheat bran and the barley diets had the lowest N retentions of all, except for the lignin diet. High-fiber ingredients, primarily hemicellulose content such as in wheat bran and barley, significantly decreased the apparent and true ileal protein and AA digestibilities; Thr was particularly affected. The various antinutritive factors, at concentrations typical in feedstuffs, are known mucin secretagogues and may have stimulated mucus secretion and hence increased endogenous AA losses. Therefore, the values used by NRC (1998) to correct AID values to true ileal digestibility values are not applicable to all ingredients. New values for endogenous losses, particularly for Thr, are required for ingredients containing different levels of antinutritional factors, particularly fiber.

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