

Starch and Fiber Fractions in Selected Food and Feed Ingredients Affect Their Small Intestinal Digestibility and Fermentability and Their Large Bowel Fermentability In Vitro in a Canine Model^{1,2}

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ABSTRACT The digestion of legumes, cereal grains, cereal and potato flours and grain-based foods in dogs was studied using two in vitro model systems. The first simulated the stomach and small intestine through the additions of acid and enzymes and large bowel fermentation through use of fecal inocula from dogs, and the second simulated small intestinal fermentation using canine ileal chyme as the bacterial source. All substrates were analyzed for total dietary fiber (TDF) including insoluble and soluble components, and starch fractions: rapidly digestible starch, slowly digestible starch, resistant starch (RS) and total starch. Legumes had high TDF and RS concentrations (mean 36.5 and 24.7%, respectively), resulting in lower ileal digestible starch and total digestible starch concentrations (mean 21 and 31%, respectively). Seventy-four percent of the TS in the cereal grains group was rapidly digestible starch plus slowly digestible starch compared with the flour group, where the corresponding value was 95%. This related to the processing of cereals to flours, in which TDF and RS concentrations were reduced markedly. This increased ileal digestible starch concentrations in the flour group (65%) versus the cereal grains group (60%). Ileal digestion of starch in grain-based food products like macaroni and spaghetti was high (96 and 92%, expressed as a percentage of TS, respectively). Fermentation of substrates with ileal microflora was influenced by substrate chemical composition, with the flour group exhibiting the highest organic matter disappearance values. The legume group had a high total short-chain fatty acid concentration (7.8 mmol/g organic matter fermented), perhaps as a result of fermentation of TDF as well as starch components. A database such as this one provides information about utilization of foods and feeds in the dog and potentially in humans. *J. Nutr.* 131: 276–286, 2001.

KEY WORDS: • starch • total dietary fiber • digestion • fermentation • in vitro • dog

Starch is the primary digestible carbohydrate found in plants. It is an important source of energy in the diets of humans and animals. Starch is a cost-effective means of supplying dietary energy. It is digested primarily in the small intestine by enzymatic degradation, but some can escape digestion and be fermented in the large bowel.

Although most fermentation occurs in the large bowel, a few studies (Ruseler-van Embden et al. 1992, Zentek 1995) suggest that fermentative activity can occur in the small intestine. Aman et al. (1995) indicated that substantial degradation of mixed-linked β -glucans may occur in ileostomy subjects, presumable due to bacterial fermentation in the small intestine. The ileum of humans has been reported to contain bacteria in concentrations of 10^5 – 10^6 colonies/g of contents (Drasar and Hill 1974). Small intestinal bacteria ostensibly

could affect digestive processes occurring at this site. Relatively little data are available on the effects of starch and fiber fractions in selected food and feed ingredients on small intestinal and large bowel digestibility characteristics.

The objectives of this research were to first compile a starch and fiber fraction database for common food and feed ingredients. The general categories studied were legumes, cereal grains, cereal and potato flours, grain-based food products and reference substrates. Second, in vitro ileal digestible starch (IDS)⁴ and total tract digestible starch (TDS) values were determined using a monogastric starch digestion model. Finally, the ileal disappearance and fermentative characteristics of selected food and feed ingredients were determined using ileal microbes from dogs in an in vitro model. Information gained in this experiment will aid in the understanding of effects of microbes in the distal small intestine on the starch and fiber fraction of food and feed ingredients. The dog was

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⁴ Abbreviations used: CP, crude protein; DM, dry matter; FG, free glucose; I, insoluble fiber; IDS, ileal digestible starch; OM, organic matter; OMD, organic matter disappearance; RDS, rapidly digestible starch; RS, resistant starch; SCFA, short-chain fatty acids; SDS, slowly digestible starch; S, soluble fiber; TDF, total dietary fiber; TDS, total digestible starch; TS, total starch.

used in the *in vitro* experiments as an animal model for humans. Both dogs and humans are omnivorous monogastrics. The lower gastrointestinal tract of the dog, like that of humans, contains numerous endogenous species of bacteria (Balish et al. 1977; Davis et al. 1977) that contribute significantly to colonic fermentation (Banta et al. 1979). The contribution of the large bowel to total digestive tract volume is also similar in the dog (14%) and the human (17%), in contrast to that in the pig (48%) and the rat (61%) (van Soest 1995).

MATERIALS AND METHODS

All experiments were conducted under protocols approved by the Laboratory Animal Care Advisory Committee, University of Illinois, Urbana-Champaign.

Chemical analyses. All substrates (legumes, cereal grains, cereal and potato flours, grain-based food products and reference substrates) were analyzed for dry matter (DM), organic matter (OM), Kjeldahl nitrogen (N) (Association of Official Analytical Chemists 1985) and total dietary fiber (TDF) (Prosky et al. 1984). Insoluble fiber (I) was determined according to the method of Prosky et al. (1992). Soluble fiber (S) was calculated by subtracting the I from the TDF. Total fat content was determined by acid hydrolysis followed by ether extraction according to the American Association of Cereal Chemists (1983) and Budde (1952).

Starch fractions [free glucose (FG), rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS)] of samples were determined according to the methods of Muir and O'Dea (1992 and 1993). Total starch (TS) values were determined according to the method of Thivend et al. (1972). Both starch fractionation and TS assays used dimethyl sulfoxide to disassociate the retrograded amylose (Englyst and Cummings 1984).

Experiment 1: Quantification of starch and fiber fractions and *in vitro* IDS and TDS values for common food and feed ingredients

Substrates. Substrates used in the *in vitro* experiment consisted of seven legumes (black beans, red kidney beans, lentils, navy beans, black-eyed peas, split peas and northern beans) and nine cereal grains (barley, corn, white rice, brewer's rice, brown rice, wheat, millet, oats and sorghum), all purchased from local vendors. The substrates were ground through a 2-mm screen in a Wiley mill. Seven flours (corn, wheat, rice, potato, soy, barley and sorghum) were obtained from a pet food manufacturer. Flours had been prepared according to the normal methods of grinding, fine milling, sieving and steam processing. Other substrates included six prepared grain-based food products (macaroni, spaghetti, corn meal, rice bran, rolled oats and hominy grits) purchased from local vendors. The final set of samples included three reference substrates: corn starch (73% amylopectin, 27% amylose; Sigma Chemical Co., St. Louis, MO), potato starch (approximately 80% amylopectin, 20% amylose; Sigma Chemical Co.) and amylomaize (Crystalean; almost 100% amylose; Opta Food Ingredients, Bedford, MA). These standards were included as part of each fractionation method to validate the efficacy of the experimental conditions imposed (i.e., a database containing information on key response criteria measured in this experiment was available for these standards, and any deviations in results obtained with these standards resulted in invalidation of the entire set of substrates being studied).

Donors. Two mixed-breed purpose-bred mature female ileally cannulated dogs (Walker et al. 1994) with hound bloodlines had ad libitum access twice daily to a commercial diet (Diamond Petfoods, Meta, MO) containing ~21% crude protein (CP) and 12% fat for 14 d before the collection of feces. Major ingredients in the diet included ground corn, poultry by-product meal, chicken fat and beet pulp. Dogs were housed in a temperature-controlled room in 1.2 × 3.1-m solid-floor pens. Free access to water was provided at all times.

Monogastric *in vitro* digestion model. This model represents a combination of three assays used to determine the amount of digestible starch at the ileum and in the total gastrointestinal tract. The

TABLE 1

Composition of medium used for *in vitro* fermentation of food and feed ingredients

Component	Concentration in medium
	mL/L
Solution A ¹	330.0
Solution B ²	330.0
Trace mineral solution ³	10.0
Water-soluble vitamin mix ⁴	20.0
Folate/biotin solution ⁵	5.0
Riboflavin solution ⁶	5.0
Hemin solution ⁷	2.5
Short-chain fatty acid mix ⁸	0.4
Resazurin ⁹	1.0
Distilled H ₂ O	296.0
	g/L
Yeast extract	0.5
Trypticase	0.5
Na ₂ CO ₃	4.0
Cysteine HCl · H ₂ O	0.5

¹ Composition (g/L): NaCl, 5.4; KH₂PO₄, 2.7; CaCl₂ · H₂O, 0.16; MgCl₂ · 6H₂O, 0.12; MnCl₂ · 4H₂O, 0.06; CoCl₂ · 6H₂O, 0.06; and (NH₄)₂SO₄, 5.4.

² Composition: K₂HPO₄, 2.7 g/L.

³ Composition (mg/L): EDTA (disodium salt), 500; FeSO₄ · 7H₂O, 200; ZnSO₄ · 7H₂O, 10; MnCl₂ · 4H₂O, 3; H₃PO₄, 30; CoCl₂ · 6H₂O, 20; CuCl₂ · 2H₂O, 1; NiCl₂ · 6H₂O, 2; Na₂MoO₄ · 2H₂O, 3.

⁴ Composition (mg/L): thiamin · HCl, 100; *d*-pantothenic acid, 100; niacin, 100; pyridoxine, 100; *p*-aminobenzoic acid, 5; vitamin B-12, 0.25.

⁵ Composition (mg/L): folic acid, 10; *d*-biotin, 2; NH₄HCO₃, 100.

⁶ Composition: riboflavin, 10 mg/L in 5 mmol/L of HEPES.

⁷ Hemin, 500 mg/L in 10 mmol/L NaOH.

⁸ 250 mL/L each of *n*-valerate, isovalerate, isobutyrate and DL- α -methylbutyrate.

⁹ Resazurin, 1 g/L in distilled H₂O.

method of Muir and O'Dea (1993) was used to determine the amount of starch digestion in the stomach and small intestine by measuring glucose in the supernatant resulting from acid-enzyme digestion of the substrate. Each substrate in triplicate was exposed to pepsin/hydrochloric acid, amyloglucosidase and α -amylase. Tubes containing reagents but no substrate were run as blanks. Glucose concentrations then were determined on the supernatant. Glucose was measured according to a glucose oxidase method (Glucose Test Kit 510-A; Sigma Chemical Co.). Glucose concentration was determined by reading the absorbance of individual samples at 450 nm on a DU 640 spectrophotometer (Beckman Instruments, Schaumburg, IL) and comparing those values against a glucose standard curve. IDS was determined by subtracting (FG × 0.9) from (total glucose/original sample weight) present in the supernatant after 15 h of digestion. The 0.9 used in the calculation of IDS is a correction factor for the difference in weight between an FG unit and a glucose residue in starch. Because the measurement of glucose is used to determine starch content, the correction factor is needed. The substrate remaining after simulated stomach and small intestinal digestion then was used in a model that simulated large bowel fermentation (Bourquin et al. 1993). Freshly voided feces from two dogs was diluted (1:10) in anaerobic diluting solution. This inoculum was used to inoculate all substrates individually for each dog. Substrates were incubated in an *in vitro* medium (Table 1) at 39°C for 12 h. TS was determined in the pellet that remained after simulated large bowel fermentation according to the method of Thivend et al. (1972) with dimethyl sulfoxide solubilization of amylose. TDS was determined by subtracting [(total glucose/original sample weight) × 0.9] in the remaining sample from the percentage TS.

Experiment 2: Determination of the ileal disappearance and fermentative characteristics of selected food and feed ingredients

Substrates. All substrates were the same as those described for expt. 1.

Donors and collection methods. Six mixed breed purpose-bred female ileally cannulated dogs (Walker et al. 1994) had ad libitum access twice daily to the diet used in expt. 1 for a 14-d period before the collection of ileal effluent. Dogs were housed in a temperature-controlled room in 1.2 × 3.1-m clean-floor pens. Free access to water was provided at all times. Fresh ileal fluid was collected from each dog for 15-min intervals in a Whirlpak bag (Pioneer Container Corp., Cedarburg, WI) until sufficient amounts needed to inoculate all tubes were obtained. At the end of each 15-min period, the bags were removed and replaced with new ones. Bags containing samples were sealed immediately after expressing excess air, placed inside a pre-warmed thermos (37°C) and transported to a laboratory within the same building for processing.

Medium composition and substrate fermentation. The composition of the medium used to culture the ileal microflora is presented in Table 1. All medium components except the vitamin mixes were added before autoclaving. The vitamin mixes were aseptically added after they were filter-sterilized.

On arrival in the laboratory, fresh ileal samples were immediately pooled under anaerobic conditions and diluted 1:10 (v/v) in a 39°C anaerobic dilution solution (Bryant and Burke 1953) by blending for 10 s in a Waring blender. Blended, diluted ileal effluent was filtered through four layers of cheesecloth, and the filtrate was sealed in 125-mL serum bottles under CO₂. Appropriate sample and blank tubes containing 26 mL of medium and 300 mg of substrate were aseptically inoculated with 4 mL of diluted ileal effluent. Tubes were flushed with CO₂ and capped with stoppers equipped with one-way gas release valves. Blank tubes contained 4 mL of inoculum and 26 mL of medium but did not contain any substrate.

Triplicate tubes were placed in a forced air incubator at 39°C with periodic mixing for each fermentation time period (2.5, 5 and 7.5 h). At the appropriate time, tubes were removed from the incubator and processed immediately. A 2-mL aliquot was removed from each tube for short-chain fatty acid (SCFA) and lactate analyses. The remaining 28 mL was combined with 112 mL of 95% ethanol and allowed to set for 1 h to precipitate the soluble polysaccharide fractions. To recover unfermented residues, samples were filtered through tared Whatman 541 filter paper and washed sequentially with 78% ethanol, 95% ethanol and acetone. Samples then were dried at 105°C, weighed, ashed in aluminum weigh boats (500°C) and weighed again to determine OM disappearance (OMD). In vitro OMD (percentage) was calculated as $\{1 - [(OM \text{ residue} - OM \text{ blank}) / \text{original OM}]\} \times 100$, where OM residue is the OM recovered after 2.5, 5 or 7.5 h of fermentation; OM blank is the OM recovered in the corresponding blank after the same fermentation times; and original OM is the OM of the substrate placed in the tube. Corrected OMD was calculated as the 2.5-, 5- and 7.5-h OMD minus the 0-h OMD.

The 2-mL aliquot of fluid removed from the sample tubes for SCFA and lactate analyses was immediately added to 0.5 mL of metaphosphoric acid (250 g/L), precipitated for 30 min and centrifuged at 20,000 × g for 20 min. The supernatant was decanted and frozen at -20°C in microfuge tubes. After freezing, the supernatant was thawed and centrifuged in microfuge tubes at 10,000 × g for 10 min. Concentrations of acetate, propionate and butyrate were determined in the supernatant using a Hewlett-Packard 5890A Series II gas-liquid chromatograph and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H₃PO₄ on 80:100 mesh Chromosorb WAW (Supelco, Bellefonte, PA). SCFA concentrations were corrected for by the blank tube production of SCFA. The supernatants also were analyzed for lactate concentration according to the spectrophotometric method described by Barker and Summerson (1941).

Statistical analysis. The General Linear Models procedures of SAS (1994) were used to analyze data from these experiments. In expt. 1, the experimental design was a randomized complete block design with the two fecal donors serving as blocks. Donor × substrate was used in the statistical model. In expt. 2, the experimental design was a factorial arrangement of substrates within groups (legumes,

cereal grains, cereal and potato flours, grain-based food products and reference substrates) and fermentation times (0, 2.5, 5 and 7.5 h). Arithmetic means are reported along with the SEM for each group of substrates. When significant ($P < 0.05$) differences were detected, individual means were compared using the least significant difference (LSD) method of SAS (1994).

RESULTS AND DISCUSSION

Chemical composition. The chemical composition of substrates is presented in Table 2. Chemical composition of legumes varied widely, corroborating data of Kamath et al. (1980). In our experiment, DM concentrations were similar among legumes, except for navy beans, which were lower in DM. OM concentrations were similar among substrates. CP content ranged from a low for navy beans to a high for lentils and northern beans. The CP concentration of lentils agreed with the reported range of 20.4–30.5% (Salunkhe et al. 1985). The fat concentration of legumes ranged from a low for navy beans to a high for northern beans. Concentrations of TDF were high for the entire legume group, with black beans having the highest TDF content and black-eyed peas having the lowest. The legume group contained mainly I (92.2–100% of TDF), whereas S values ranged from 0 to 7.8% of the TDF.

DM concentrations of the cereal group ranged from a low for corn to a high for sorghum. OM concentrations differed by ~3 percentage units for the cereal group. CP content varied from a low for white rice to a high for wheat. Watson (1953) and Juliano et al. (1964) reported CP values of 11.8 and 11.0% for barley and brown rice, respectively. These values agree with our values of 12.7% for barley and 10.2% for brown rice. Cereal grain fat concentrations ranged from a low for white rice to a high for oats. Total dietary fiber concentrations varied widely, with oats containing ~25 times more TDF than white rice. Cereal grains contain the husk, pericarp and/or bran in varying concentrations, thus providing components that contain fiber, albeit in generally lower concentrations compared with legumes. The majority of TDF found in the cereal group was I, although cereals contained a much greater proportion of S than did legumes.

DM concentrations were similar among the grain and potato flour group. OM concentrations ranged from 92.9% (soy) to 99.6% (sorghum). CP content was highest for soy and lowest for brown rice. Soy flour contained the highest concentration of fat in the flour group. Total dietary fiber concentrations varied from a high for barley flour to a low for sorghum flour. High concentrations of fiber in barley flour may be a result of high concentrations of β -glucans present in the grain (Liljeberg et al. 1992). I concentrations again were higher than S concentrations in the flour group, although large differences in S concentrations occurred among substrates. Overall, grain flours contained less TDF compared with their cereal grain counterparts.

DM concentrations of grain-based food products differed by only 3 percentage units. OM concentrations were similar among substrates except for rice bran, which contained more ash (>10%). CP content ranged from 7.7% (corn meal) to 16.9% (rice bran). Fat concentrations ranged from a high for rolled oats to a low for corn meal. Total dietary fiber varied widely among the prepared grain products, with rice bran being the highest, rolled oats and hominy grits being intermediate, and macaroni, spaghetti and corn meal being the lowest. I concentrations were highest for rice bran and lowest for macaroni. As a percentage of TDF, S concentrations were highest (mean 34.5%) for macaroni, spaghetti, rolled oats and hominy grits.

TABLE 2

Proximate and fiber constituents of selected food and feed ingredients

Substrate	DM ¹	OM	CP	Fat	TDF	I ²	S ²
<i>g/100 g dry matter</i>							
Legumes							
Black beans	89.0	95.5	23.6	3.3	42.6	39.4 (92.5)	3.2 (7.5)
Red kidney beans	89.1	95.6	25.4	3.0	36.8	36.3 (98.6)	0.5 (1.4)
Lentils	90.7	96.6	27.5	2.4	33.1	33.0 (99.7)	0.1 (0.3)
Navy beans	83.7	95.1	22.8	1.6	36.2	36.2 (100.0)	0.0 (0.0)
Black-eyed peas	90.6	96.5	24.4	2.7	32.6	32.4 (99.4)	0.2 (0.6)
Split peas	90.0	96.8	26.1	2.5	33.1	31.3 (94.6)	1.8 (5.4)
Northern beans	89.2	95.3	27.5	4.0	41.1	37.9 (92.2)	3.2 (7.8)
Cereal grains							
Barley	90.3	98.6	12.7	2.8	17.0	12.0 (70.6)	5.0 (29.4)
Corn	86.8	98.6	12.8	4.9	19.6	16.0 (81.6)	3.6 (18.4)
White rice	87.9	99.4	9.1	1.6	1.5	1.2 (80.0)	0.3 (20.0)
Brewer's rice	92.2	99.2	9.6	1.9	2.1	1.5 (71.4)	0.6 (28.6)
Brown rice	87.7	98.6	10.2	4.0	5.7	4.3 (75.4)	1.4 (24.6)
Wheat	94.2	97.7	18.1	3.2	17.0	14.7 (86.5)	2.3 (13.5)
Millet	88.5	98.8	11.2	4.8	5.4	3.1 (57.4)	2.3 (42.6)
Oats	90.0	96.7	14.2	6.9	37.7	33.9 (89.9)	3.8 (10.1)
Sorghum	94.6	98.6	11.1	4.3	4.6	4.2 (91.3)	0.4 (8.7)
Flours							
Corn	92.1	99.4	11.2	2.6	2.8	2.8 (100.0)	0.0 (0.0)
Wheat	91.1	98.3	17.2	3.1	12.1	8.5 (70.2)	3.6 (29.8)
Brown rice	89.1	98.8	7.6	4.2	5.1	3.4 (66.7)	1.7 (33.3)
Potato	94.4	95.3	9.8	1.5	2.1	1.1 (52.4)	1.0 (47.6)
Soy	94.5	92.9	54.5	5.2	15.4	14.7 (95.5)	0.7 (4.5)
Barley	91.0	97.9	11.4	3.8	22.9	20.1 (87.8)	2.8 (12.2)
Sorghum	91.9	99.6	10.6	2.0	1.3	1.1 (84.6)	0.2 (15.4)
Grain-based food products							
Macaroni	89.2	99.0	15.9	2.7	5.6	3.4 (60.7)	2.2 (39.3)
Spaghetti	90.4	99.2	15.5	2.6	5.6	3.7 (66.1)	1.9 (33.9)
Corn meal	89.6	99.7	7.7	1.9	4.4	4.3 (97.7)	0.1 (2.3)
Rice bran	92.1	89.4	16.9	3.6	28.0	27.6 (98.6)	0.4 (1.4)
Rollod oats	91.1	97.9	14.2	9.1	10.0	6.6 (66.0)	3.4 (34.0)
Hominy grits	88.7	99.4	8.4	2.2	11.4	7.9 (69.3)	3.5 (30.7)
Reference substrates							
Corn starch	91.2	99.8	0.6	0.9	0.0	0.0	0.0
Potato starch	90.2	99.9	0.2	0.03	0.0	0.0	0.0
Amylomaize	88.7	99.7	0.9	1.5	5.3	3.2 (60.4)	2.1 (39.6)

¹ DM = dry matter; OM = organic matter; CP = crude protein; TDF = total dietary fiber; S = soluble fiber; I = insoluble fiber.² Numbers in parentheses are soluble (S) or insoluble (I) fiber concentrations expressed as a percentage of TDF.

DM, OM and CP concentrations were similar among the reference substrates. Corn and potato starch contained low fat concentrations. Corn starch and potato starch contained no TDF. Although 5.3% TDF was detected in the amylomaize, this is probably RS rather than fiber.

Starch fractions. Concentrations of starch fractions of substrates are presented in Table 3. FG concentrations were low for all substrates, as expected.

The concentrations of RDS, SDS and RS in the legume group varied widely. RDS concentrations were lowest for red kidney beans and highest for split peas. SDS concentrations varied more than twofold. RS concentrations generally constituted the highest proportion of the starch fractions of legumes. Ring et al. (1988) reported that leguminous starches displayed a C-type pattern of crystallinity. This type of starch is more resistant to hydrolysis than that with an A-type crystallinity pattern and helps explain why legumes have high amounts of RS.

Gee and Johnson (1985) found that there was a relationship between the "half-time starch hydrolysis" (time taken to achieve 50% hydrolysis of the original starch of the substrate) and the dietary fiber content of certain foods. Their data

indicate that legumes such as peas and red kidney beans all had higher half-time hydrolysis rates (60.0 and 58.0 min, respectively), whereas white bread and white rice had much lower values (19.5 and 2.1 min, respectively). In their study, dietary fiber content averaged 27.8% for peas and red kidney beans and only 3.1% for white bread and white rice. McBurney et al. (1988) also found that SCFA production from ileal effluent was significantly correlated with dietary fiber isolates but not whole foods. The authors concluded that dietary fiber isolates, rather than whole foods, could provide the closest estimation of colonic SCFA production. Another possible reason for the higher RS concentrations in legumes could be the relationship between starch and protein. Tovar et al. (1990) found that when red kidney beans were preincubated with pepsin, there was an increase in their susceptibility to amylolytic attack.

TS values of legumes obtained by adding FG, RDS, SDS and RS components closely paralleled those reported from the determination of starch using the method of Thivend et al. (1972), attesting to the accuracy of the Muir and O'Dea (1993) method for quantifying starch fractions. A possible explanation for the higher concentration of TS for substrates such as black beans and black-eyed peas using the Thivend et

TABLE 3

Starch fractions of selected food and feed ingredients

Substrate	% FG ¹	% RDS	% SDS	% RS	TS	Thivend
<i>g/100 g dry matter</i>						
Legumes						
Black beans	0.1 (0.2)	7.5 (17.5)	8.4 (19.6)	26.9 (62.7)	42.9	46.8
Red kidney beans	0.1 (0.2)	4.3 (10.1)	13.7 (32.2)	24.6 (57.7)	42.6	42.9
Lentils	0.1 (0.2)	16.4 (30.8)	11.4 (21.4)	25.4 (47.7)	53.3	51.9
Navy beans	0.1 (0.2)	6.5 (13.2)	16.8 (34.1)	25.9 (52.5)	49.3	50.8
Black-eyed peas	0.1 (0.2)	18.5 (34.3)	18.5 (34.3)	17.7 (32.8)	53.9	57.0
Split peas	0.1 (0.2)	22.3 (34.5)	17.8 (27.5)	24.5 (37.9)	64.7	65.6
Northern beans	0.1 (0.2)	10.9 (21.8)	10.9 (21.8)	28.0 (56.1)	49.9	46.7
Cereal grains						
Barley	0.1 (0.2)	24.9 (45.1)	12.1 (21.9)	18.2 (33.0)	55.2	54.7
Corn	0.2 (0.3)	37.1 (47.6)	15.6 (20.0)	25.2 (32.3)	77.9	80.1
White rice	0.1 (0.1)	32.0 (33.6)	48.9 (51.4)	14.1 (14.8)	95.1	93.0
Brewer's rice	0.0 (0.0)	68.4 (79.4)	14.3 (16.6)	3.5 (4.1)	86.2	83.7
Brown rice	0.0 (0.0)	28.7 (32.5)	44.7 (50.7)	14.8 (16.8)	88.2	87.8
Wheat	0.1 (0.2)	29.9 (58.9)	7.3 (14.4)	13.6 (26.8)	50.8	52.9
Millet	0.1 (0.1)	35.9 (41.6)	37.7 (43.7)	12.6 (14.6)	86.2	90.9
Oats	0.2 (0.5)	35.8 (82.5)	0.3 (0.7)	7.2 (16.6)	43.4	46.5
Sorghum	0.0 (0.0)	29.2 (36.9)	13.9 (17.6)	36.1 (45.6)	79.2	77.8
Flours						
Corn	0.1 (0.1)	73.2 (86.8)	0.0 (0.0)	11.0 (13.0)	84.3	90.8
Wheat	0.0 (0.0)	38.1 (55.4)	29.0 (42.2)	1.7 (2.5)	68.8	73.4
Rice	0.0 (0.0)	57.7 (66.4)	27.6 (31.8)	1.6 (1.8)	86.9	89.3
Potato	0.1 (0.1)	75.5 (93.2)	3.8 (4.7)	1.7 (2.1)	81.0	77.9
Soy	0.1 (1.8)	2.2 (40.0)	2.7 (49.1)	0.6 (10.9)	5.5	8.2
Barley	0.0 (0.0)	37.7 (54.2)	30.6 (44.0)	1.2 (1.7)	69.5	75.6
Sorghum	0.2 (0.2)	63.5 (70.8)	24.6 (27.4)	1.6 (1.8)	89.7	84.8
Grain-based food products						
Macaroni	0.3 (0.4)	60.0 (79.9)	8.9 (11.9)	6.0 (8.0)	75.1	77.2
Spaghetti	0.0 (0.0)	48.9 (67.0)	20.9 (28.6)	3.3 (4.5)	73.0	82.7
Corn meal	0.2 (0.2)	49.3 (56.3)	33.0 (37.7)	5.0 (5.7)	87.5	95.5
Rice bran	0.0 (0.0)	0.1 (0.4)	25.1 (88.1)	3.4 (11.9)	28.5	27.5
Rolled oats	0.0 (0.0)	42.5 (75.9)	4.8 (8.6)	8.5 (15.2)	56.0	60.1
Hominy grits	0.1 (0.2)	36.8 (56.4)	20.4 (31.2)	8.0 (12.3)	65.3	70.9
Reference substrates						
Corn starch	0.0 (0.0)	71.8 (70.0)	22.6 (22.0)	8.1 (7.9)	102.5	101.5
Potato starch	0.0 (0.0)	27.2 (27.9)	3.3 (3.4)	66.9 (68.7)	97.4	99.3
Amylomaize	0.0 (0.0)	30.3 (30.1)	18.2 (18.1)	52.0 (51.7)	100.5	99.8

¹ FG = free glucose; RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch; TS = total starch = (FG × 0.9) + RDS + SDS + RS; Thivend = total starch as measured by the Thivend et al. (1972) method. Numbers in parentheses are FG, RDS, SDS and RS expressed as a percentage of the TS.

al. (1972) method may be the inclusion of sucrose in the measurement. This method enzymatically converts sucrose into monosaccharides and allows for their recovery in the supernatant. The Muir and O'Dea (1993) method does not account for this conversion, so sucrose is not part of the starch value.

Cereal grains varied widely in percentage starch found in each of the starch fractions. RDS and SDS concentrations represented the majority of the TS in the cereal group. Cereal grains have an A-type crystalline form, which is the starch structure least resistant to hydrolysis (Ring et al. 1988). This crystalline form leads to more of the starch being categorized as RDS and SDS. RDS concentrations as a percentage of TS varied from 32.5 to 82.5%. White rice contained the highest concentration of SDS (51.4%) as a percentage of TS. Raw cereals are partially inaccessible to digestion due to the physical form of the cereal itself (Englyst et al. 1992b). Structures like the pericarp and seed coat may impede the efficiency of amylase digestion of starch in cereal grains.

RS concentrations were highest for sorghum and lowest for oats. Four categories of RS have been defined (Brown 1996). The first category (RS1) includes starch granules that are

physically inaccessible and can be found in whole or partially milled grains and legumes. The second category (RS2) refers to native starch granules, whereas the third category (RS3) refers to retrograded starch that is formed during processing. The fourth category of RS (RS4) was only recently described and includes chemically modified starches resistant to enzymatic hydrolysis to some degree.

Flours also varied widely in percentage starch found in each of the starch fractions. Approximately 95% of the TS in flours is RDS and SDS combined. RS concentrations were highest for corn and soy and lowest for barley. Englyst et al. (1992a) reported that white wheat flour contained 49% RDS, 48% SDS and 3% RS as a percentage of TS. Our wheat flour contained 55.4% RDS, 42.2% SDS and 2.5% RS as a percentage of TS, agreeing closely with the values of Englyst et al. (1992a). RS concentrations were low for the flour group as a whole. Cereal flours display an A-type crystalline pattern, which is more readily hydrolyzed than raw cereals that are not as highly processed as flours. Therefore, cereal flours contain more RDS and SDS than RS.

The nutrient profile of cereal grains and their corresponding flours varied considerably. Grain flours are made up pri-

marily of two components: protein and starch. Cereal grains, in contrast, contain the pericarp, aleurone layers and germ portions of the grain that provide lipid and fiber (Hoseney 1994). Cereal grains are processed and milled to flours, thereby altering the chemical composition of the flour compared with the cereal grain. Even DM concentrations varied when cereal grains were compared with their flour counterparts. Except for barley, flours were numerically higher in OM. CP concentrations of flours were 1–3 percentage units lower than that for ground grains. Total dietary fiber concentrations of flours, except for barley, were numerically lower compared with their ground grain counterparts. This reduction in TDF points to how the processing of grains alters their fiber content through removal of the pericarp, aleurone layers and germ. Of interest is how the processing of cereals to flours affects the starch fraction profile. The combined RDS and SDS concentrations of cereal grains were ~74% of TS versus, flours, which were 95% of TS. The RS concentrations were, on average, five times higher in the cereal grains than in the flours.

For the grain-based food products, RDS concentrations, expressed as a percentage of TS, were highest for macaroni and rolled oats; intermediate for spaghetti, corn meal and hominy grits; and lowest for rice bran. SDS concentrations varied, with corn meal having the highest concentration and rolled oats having the lowest. Prepared grain products contained moderate levels of RS (mean 9.6% as a percentage of TS). Hermansen et al. (1986) postulated that starch in foods like spaghetti is more slowly digested because of the densely packed starch in the food. During pasta production, pasta is kneaded and extruded, leading to a tight, entrapped starch granule (Colonna et al. 1990). Again, food ingredients like rice bran with high TDF (28.0%) may experience a lower amount of starch hydrolysis as a result of its fiber content. Corn meal contained the highest concentration of TS, whereas rice bran contained the lowest.

The reference substrates varied widely in their starch fractions. RDS values were highest for corn starch and similar for potato starch and amylomaize. SDS values were similar for corn starch and amylomaize and lower for potato starch. As a percentage of TS, potato starch had the highest RS concentration and corn starch had the lowest. Englyst et al. (1992a) found that raw potato starch contained 75% RS as a percentage of TS. Starches from tubers such as potatoes tend to exhibit B-type crystallinity patterns that are highly resistant to digestion (Englyst et al. 1992a). Amylomaize contains mostly amylose, which has been shown to lower not only digestibility but also blood insulin and glucose values in humans (Behall et al. 1995).

A common characteristic of all foods and feeds studied is that RS is a component of each. This starch fraction is not hydrolyzed and enzymatically digested in the small intestine but rather serves as a substrate for fermentation by microflora either in the ileum and/or large bowel.

In vitro experiment 1

Ileal bacteria. Fermentative events in the nonruminant occur as a result of bacterial activity in the colon and possibly in the ileum of the small intestine. Ruseler-van Embden et al. (1992) found >25 different species of bacteria residing in the small intestine of dogs. Murray et al. (2000) found the following colony-forming units (CFU)/mL of ileal effluent after isolation and plating: 4.2×10^8 total anaerobes, 7.1×10^5 total aerobes, 1.3×10^6 *Escherichia coli*, 1.7×10^8 *Clostridium perfringens*, 1.8×10^8 *Bifidobacteria* and 3.3×10^6 *Lactobacillus*. Finegold et al. (1970) found that in the human ileostomate,

there were 10^7 – 10^8 colonies/g of ileal contents. These values confirm that there may be a substantial bacterial population residing in the small intestine of both dogs and humans. It is uncertain whether ileal microbes are indigenous to this site or whether they emanate from the cecum, finding their way via the ileocecal valve into the small intestine. The contents of the small intestine normally flow rapidly, possibly becoming static for an appreciable period only in the distal small intestine (Drasar and Hill 1974).

IDS and TDS concentrations. IDS concentrations for the legume group were statistically highest ($P < 0.05$) for black-eyed peas and split peas, next highest for lentils and navy beans and lowest for northern beans, black beans and red kidney beans (Table 4). Bjorck et al. (1992) reported that the small intestinal digestibility by rats of a cooked and canned pea product was 70%. As a percentage of TS (i.e., IDS/TS), our ileal digestibility value for split peas (45.7%) was lower and may be due to the raw, unprocessed nature and high RS content of this substrate. Key et al. (1995) also found that as the concentration of cooked haricot beans in the diet of rats increased from 0 to 450 g/kg, ileal digestibility decreased from 87 to 69% for the haricot bean-containing diet.

TDS concentrations were lowest ($P < 0.05$) for black beans, red kidney beans and northern beans and highest ($P < 0.05$) for black-eyed peas and split peas. Biliaderis et al. (1981) found that wrinkled peas contained 55.4% amylose as a percentage of TS, whereas beans contained higher amounts of amylose (mean 59.2%). Higher amylose concentrations in starches are believed to lower digestibility (Borchers 1962). This could explain why the peas displayed higher TDS concentrations and the beans displayed lower TDS concentrations. Goodlad et al. (1992) found that as the proportion of peas doubled from 250 to 500 g/kg in the diet of rats, total tract digestibility was reduced ($P < 0.05$) from 94 to 91%. The TDS concentrations were low for the bean group as a whole, with a possible explanation being the physical entrapment of the starch within fibrous, thick-walled parenchyma cells (Wursch et al. 1986). Also, antinutrients (e.g., enzyme inhibitors, lectins and tannins) have been found in legumes, and that could reduce the digestibility of legume starches. RS and TDF concentrations also were high for the legume group and could affect starch digestion in these substrates.

Starch utilized by the microflora in the large bowel (percent TDS – percent IDS) was numerically highest for split peas and lowest for northern beans.

For the cereal grains group, IDS concentrations were highest ($P < 0.05$) for brewer's rice and lowest for oats. As a percentage of TS, starch in oats and barley was completely digested at the ileum. Englyst and Cummings (1985), using human ileostomates, found that raw oat starch hydrolysis was complete in the small intestine. Barriers to amylase digestion apparently do not impede the starch in oats. Of interest is how white rice and brown rice differ with respect to IDS values. O'Dea et al. (1981) found that relative rates of starch hydrolysis in an in vitro system correlated very closely with in vivo peak glucose responses in humans. In vitro rates of starch hydrolysis (percent hydrolyzed/30 min) for ground brown rice and white rice were 68.2 and 71.8%, respectively. O'Dea et al. (1981) suggest that fiber might act indirectly to slow carbohydrate absorption, restricting access of hydrolytic enzymes to starch from an unrefined source like brown rice. Our brown rice source was higher in TDF content and lower in IDS than white rice. Two other grains have been shown to have structural differences that may contribute to resistance to digestion. Hosney (1994) suggested that the protein-starch matrix of

TABLE 4

In vitro ileal digestible starch (IDS) and total digestible starch (TDS) concentrations of selected food and feed ingredients^{1,2,3}

Substrate	IDS	TDS	TDS-IDS
	%		
Legumes			
Black beans	16.4 ^c (35.0)	22.3 ^c (47.6)	5.9 ^c
Red kidney beans	15.0 ^d (35.0)	21.7 ^c (50.6)	6.7 ^{b,c}
Lentils	20.6 ^b (39.7)	32.8 ^b (63.2)	12.2 ^{a,b}
Navy beans	20.6 ^b (40.6)	33.7 ^b (66.3)	13.1 ^a
Black-eyed peas	30.0 ^a (52.6)	44.4 ^a (77.9)	14.4 ^a
Split peas	30.0 ^a (45.7)	44.5 ^a (67.8)	14.5 ^a
Northern beans	17.0 ^c (36.4)	20.3 ^c (43.5)	3.3 ^c
SEM	0.33	1.93	1.72
Cereal grains			
Barley	54.7 ^d (100.0)	52.3 ^e (95.6)	-2.4 ^h
Corn	59.4 ^c (74.2)	74.2 ^c (92.6)	14.8 ^b
White rice	69.0 ^b (74.2)	80.0 ^b (86.0)	11.0 ^d
Brewer's rice	73.2 ^a (87.5)	79.1 ^b (94.5)	5.9 ^f
Brown rice	61.3 ^c (69.8)	74.3 ^c (84.6)	13.0 ^c
Wheat	52.8 ^d (99.8)	47.1 ^f (89.0)	-5.7 ⁱ
Millet	68.9 ^b (75.8)	86.4 ^a (95.0)	17.5 ^a
Oats	46.5 ^e (100.0)	46.5 ^e (100.0)	0.0 ^g
Sorghum	52.8 ^d (67.9)	62.4 ^d (80.2)	9.6 ^e
SEM	1.07	0.71	0.92
Flours			
Corn	82.3 ^a (90.6)	90.8 ^a (100.0)	8.5 ^{a,b}
Wheat	62.7 ^d (85.4)	73.4 ^f (100.0)	10.7 ^a
Rice	78.6 ^b (88.0)	88.7 ^b (99.3)	10.1 ^a
Potato	70.7 ^c (90.8)	77.6 ^d (99.6)	6.9 ^{b,c}
Soy	2.4 ^e (29.3)	8.2 ^g (100.0)	5.8 ^{b,c}
Barley	69.9 ^c (92.5)	75.2 ^e (99.5)	5.3 ^c
Sorghum	84.8 ^a (100.0)	84.8 ^c (100.0)	0.0 ^d
SEM	0.82	0.33	0.82
Grain-based food products			
Macaroni	73.7 ^c (95.5)	76.7 ^c (99.4)	3.0 ^c
Spaghetti	75.7 ^b (91.5)	82.6 ^b (99.9)	6.9 ^b
Corn meal	83.9 ^a (87.9)	92.3 ^a (96.6)	8.4 ^a
Rice bran	27.5 ^f (100.0)	27.5 ^f (100.0)	0.0 ^d
Rolled oats	60.1 ^e (100.0)	60.1 ^e (100.0)	0.0 ^d
Hominy grits	70.9 ^d (100.0)	63.2 ^d (89.1)	-7.7 ^e
SEM	0.14	0.75	0.31
Reference substrates			
Corn starch	95.0 ^a (93.6)	101.5 ^a (100.0)	6.5 ^c
Potato starch	34.5 ^c (34.7)	64.0 ^b (64.5)	29.5 ^a
Amylomaize	42.5 ^b (42.6)	59.8 ^c (59.9)	17.3 ^b
SEM	0.74	0.38	1.00

¹ IDS determined by subtracting (free glucose $\times$ 0.9) from (total glucose/original sample weight) present in supernate after 15 h of digestion. Numbers in parentheses are IDS concentrations expressed as a percentage of total starch as determined by the Thivend et al. (1972) method.

² TDS determined by subtracting [(total glucose/original sample weight) $\times$ 0.9] in the remaining sample from the percent total starch as determined by the Thivend et al. (1972) method. Numbers in parentheses are TDS concentrations expressed as a percentage of total starch as determined by the Thivend et al. (1972) method.

³ Means in a column within substrate category not sharing superscript letters differ ($P < 0.05$).

sorghum and corn grains was quite strong, making hydrolysis and digestion more difficult.

TDS concentrations for cereals varied from a high ($P < 0.05$) for millet to a low ($P < 0.05$) for oats and barley. TDS concentrations were high, indicating continued digestion of

starch by the microflora once it reached the large bowel. Moore et al. (1980) fed plant-based diets containing one of three grain sources (rice, oats or corn) to dogs. Total tract starch digestibility for the uncooked oat diet (93.8%) was lowest, intermediate for the uncooked corn diet (94.3%) and highest for the uncooked rice diet (98.6%). Our values were 92.6% for corn, 86.0% for white rice and 100.0% for oats. The dog diets in the Moore et al. (1980) study were extruded; this leads to increased susceptibility to amylase and greater starch digestion.

Cereal starch utilization by microflora in the large bowel varied. Millet had the highest ($P < 0.05$) and wheat had the lowest ($P < 0.05$) digestibility values. Of interest is how fermentable each substrate is if any appreciable amount reaches the large bowel. According to Hosney (1994), millet, sorghum and corn starch granules appear to be similar. In our study, their fermentative capabilities were similar, with millet having the highest value.

IDS concentrations for flours were lowest ($P < 0.05$) for soy, which has very low starch concentrations, but higher for wheat, barley, potato and brown rice. The highest ($P < 0.05$) concentrations were noted for corn and sorghum. IDS concentrations were high for most flours. With low TDF and RS concentrations in the flours, there appears to be less of a barrier to digestion of starch. Our wheat flour had a lower IDS concentration compared with all other flours with the exception of soy. (Snow and O'Dea 1981) assayed different flours (rice, barley, rye, white [bleached wheat flour] and wheat) to determine their *in vitro* starch hydrolysis capacity. After 30 min of hydrolysis, all flours were similar in percent starch hydrolyzed (mean 16.1%) except for wheat flour. The authors postulated that an amylase inhibitor may have affected the hydrolysis rate of the wheat flour. Also, wheat starch can contain nonstarch polysaccharides (Topping et al. 1993).

The flour that had the lowest ($P < 0.05$) TDS concentration was soy, whereas corn had the highest ($P < 0.05$) TDS value. All flours were virtually completely digested when TDS concentrations were compared. The flours used were primarily composed of RDS and SDS (mean 95.1%), and as a result of processing, most barriers to digestion are overcome. Murray et al. (1999) found that the starch component of canine diets containing high-starch flours as the main source of carbohydrate was nearly completely digested (>99%).

Starch utilization by microflora (percent TDS - percent IDS) varied numerically in the flour group from a low for sorghum to a high for wheat. Microflora fermented virtually all available remaining starch. Even though the wheat flour IDS concentration was relatively low, large bowel microflora appeared to ferment the remaining starch well. The wheat amylase inhibitor mentioned by Snow and O'Dea (1981) appeared to have no effect on the microflora once the wheat starch was placed in an environment simulating the large bowel.

For the grain-based food products, IDS was lowest for rice bran and highest ($P < 0.05$) for corn meal. Expressed as a percentage of TS, rice bran, rolled oat and hominy grit starches were completely digested at or before the ileum. Macaroni and spaghetti were well digested at the ileum (95.5 and 91.5% as a percentage of TS, respectively), but certain factors can reduce their susceptibility to amylolytic attack. Colonna et al. (1990) found that high-temperature drying of pasta may result in high levels of protein cross-linking, leading to a greater encapsulation of starch and thus decreasing its susceptibility to amylase. There also can be differences ($P < 0.05$) between the digestion of macaroni and spaghetti, as noted in our study. Granfeldt and Bjorck (1991) tested mac-

aroni and spaghetti glucose responses in 10 human subjects. Spaghetti resulted in a glycemic index score of 60.5, whereas macaroni resulted in a score of 78.0. Macaroni had a lower product thickness and a greater surface area that allowed easier access to amylase. Rolled oats were completely digested at the ileum. This corroborates the results of Heaton et al. (1988), where insulin responses were measured in humans fed certain cereal products (corn, wheat or rolled oats). Rolled oats resulted in a higher peak insulin response compared with oat flour. Decreasing the particle size of both corn and wheat seemed to increase digestion rate, but this was not the case for oat products.

When grain-based food products were compared, TDS concentrations were different ($P < 0.05$) among substrates. The highest ($P < 0.05$) TDS value was found for corn meal. Rice bran, rolled oats and hominy grits were completely digested proximal to the terminal ileum. The processing and cooking of rice bran and rolled oats affect their digestion. As mentioned previously, rolling oats appeared to disrupt the structural integrity of the grain, leaving it accessible to enzymatic attack. Processing of the rice kernel through a milling machine produces rice bran and polished rice. The compositions of rice and rice bran vary greatly due to this processing. Rice bran is composed of the aleurone layer and some parts of the endosperm and germ of the rice kernel after milling.

Starch utilization by microflora (percent TDS – percent IDS) again varied for the grain-based food products. Corn meal was highest ($P < 0.05$) compared with all other substrates. A larger percentage of starch was fermented in the large bowel for spaghetti compared with macaroni. This relates to the greater amount of starch escaping digestion in the small intestine, making spaghetti more efficacious if the goal is to supply the large bowel with more starch.

Of the reference substrates, IDS concentrations were lowest ($P < 0.05$) for potato starch, intermediate for amylo maize and highest ($P < 0.05$) for corn starch. High concentrations of RS in potato starch cause its digestion to be limited in the small intestine. Mathers et al. (1997) fed either a raw potato or corn starch diet to rats and found that the digestibility of the corn starch diet was 99% at the ileum, whereas only 28% of the potato starch diet was digested at the ileum. Native potato starch granules are composed of a B-type crystalline pattern. These granules exist as a layer of large blocklets that appear to confer resistance to enzymatic hydrolysis (Gallant et al. 1992). Amylo maize was more digestible than potato starch, possibly due to its lower concentration of RS.

TDS concentrations were lowest ($P < 0.05$) for amylo maize, intermediate for potato starch and highest ($P < 0.05$) for corn starch. Total tract digestibility of potato starch fed to rats at 240 g/kg of the diet was 80%, whereas corn starch at 240 g/kg was 100% (Mathers et al. 1997).

Starch utilization by microflora in the large bowel (percent TDS – percent IDS) was greatest ($P < 0.05$) for potato starch, pointing to its high fermentative capacity. Of interest is that although potato starch was lower in IDS, it was higher in TDS compared with amylo maize. Lajvardi et al. (1993) fed rats either a cooked potato starch, arrowroot starch, high amylose corn starch or raw potato starch diet. Raw potato starch was found to be the most fermentable starch of the four tested. Only raw potato starch was found to significantly prolong gastrointestinal transit time, possibly allowing this substrate a longer time to ferment in the large bowel.

In vitro experiment 2

OMD. This experiment was conducted to determine whether ileal fermentation events, independent of hydrolytic digestion events, affected the disappearance of OM for a widely divergent group of substrates. Although data were collected at 0-, 2.5-, 5- and 7.5-h time periods, only those collected at 7.5 h are reported because they were judged to be most relevant from a biological perspective (i.e., data at the 5- and 7.5-h time points were similar; 7.5 h is about the length of time chyme would be available to ileal microbes).

OMD of substrates is reported in Table 5. All substrate $\times$ time interactions were significant at $P < 0.05$. After correction for solubility, OMD was very low for the legume group as a whole. Solubilization of the substrates at the 0-h fermentation time was high (13–17%), resulting in lower corrected OMD values. Red kidney beans and black beans had the lowest ($P < 0.05$) OMD values of all legumes tested. Schweizer et al. (1990) found, using ileostomates fed a white kidney bean flake-containing diet, that ~10% of the bean starch was not absorbed from the small intestine. Tovar et al. (1992) postulated that the high amylose-to-amylopectin ratio, the physical insulation of starch by thick-walled cells and the presence of amylase inhibitors resulted in a reduction in digestibility of leguminous starches. These physicochemical characteristics of legumes act as direct inhibitors of α -amylase and, thus, starch breakdown.

Solubility of cereal grains in the *in vitro* medium generally was much lower than was the case for legumes. OMD was greatest ($P < 0.05$) for oats and lowest for corn. Again, the starch in certain cereals may be inaccessible due to the physical form of the cereal, resulting in digestion responses lower than expected (Englyst et al. 1992b). Cell walls and encapsulation of starch in a protein matrix of whole grains greatly affect digestion by reducing access of amylase to the starch itself.

Flours as a group were very digestible by ileal microbes. Potato and soy flours had extremely high solubility values. Rice and corn had the highest ($P < 0.05$) OMD values compared with other flours, and wheat flour had the lowest ($P < 0.05$). Interestingly, the two former flours had among the lowest solubility values. Wheat flour was two to three times lower in OMD than all other flours. Murray et al. (2000) also reported a low OMD at 7.5 h for wheat flour (1.9%). In this case, protein may encapsulate the starch granules, thereby reducing the digestibility of the starch (Annison and Topping 1994).

Of particular interest is how processing affects substrate disappearance. For example, barley flour was approximately five times more digestible compared with barley grain. Heaton et al. (1988) compared particle size effects of wheat, corn and oats on human *in vivo* plasma insulin responses and on *in vitro* rate of starch digestion by pancreatic amylase. Insulin responses were as follows: whole grains < cracked grains < coarse flour < fine flour. *In vitro* starch hydrolysis by amylase was faster for grains of smaller particle size. Larger food particles have a lower surface-to-volume ratio, and this might reduce the access of enzymes to the interior of the particle as might the presence of intact cell walls. Processing affects the physical nature of cereals, causing the disruption of the cell matrix and increasing starch digestion.

Grain-based food products ranged in OMD from a low for macaroni to a high for rolled oats. Knudsen et al. (1993) stated that oat bran, a rich source of dietary fiber containing β -glucans, is an easily fermentable energy source for microflora. The

TABLE 5

Zero hour solubilities, organic matter disappearance (OMD) and total short-chain fatty acid (SCFA) and lactate concentrations at 7.5 h of *in vitro* fermentation of selected food and feed ingredients using canine ileal fluid as inoculum¹

Substrate	Solubility ²	Corrected OMD ³ at 7.5 h	Total SCFA ⁴	Lactate
	%		mmol/g OM at 7.5 h	
Legumes				
Black beans	14.2	1.8	7.24	0.04
Red kidney beans	15.2	0.2	7.28	0.05
Lentils	12.5	5.3	8.46	0.05
Navy beans	13.6	3.5	6.79	0.02
Black-eyed peas	15.4	3.4	8.35	0.06
Split peas	13.3	8.1	8.72	0.06
Northern beans	17.4	4.7	7.73	0.05
SEM	1.0	1.0	0.16	0.004
Cereal grains				
Barley	9.5	7.2	8.13	0.10
Corn	10.6	2.7	5.18	0.04
White rice	0.9	3.8	4.72	0.03
Brewer's rice	0.8	8.8	5.84	0.04
Brown rice	4.6	5.4	4.82	0.04
Wheat	2.8	8.9	6.54	0.01
Millet	2.7	15.4	5.48	0.04
Oats	9.0	19.0	7.87	0.07
Sorghum	0.0	8.4	4.70	0.02
SEM	1.1	1.1	0.17	0.01
Flours				
Corn	6.7	35.6	3.64	0.26
Wheat	18.0	11.7	4.59	0.01
Rice	5.9	37.1	2.93	0.26
Potato	58.5	21.7	10.10	0.51
Soy	30.2	25.2	6.55	0.10
Barley	13.6	33.3	4.66	0.30
Sorghum	5.9	28.0	2.45	0.17
SEM	0.9	0.9	0.12	0.01
Grain-based food products				
Macaroni	11.7	13.1	4.39	0.05
Spaghetti	9.8	18.3	4.75	0.07
Corn meal	2.3	22.4	2.27	0.14
Rice bran	13.3	22.0	3.70	0.14
Rolled oats	2.9	59.1	6.04	0.39
Hominy grits	0.0	17.6	2.08	0.07
SEM	1.1	1.1	0.11	0.01
Reference substrates				
Corn starch	0.0	32.5	3.58	0.25
Potato starch	0.0	3.9	0.64	0.02
Amylomaize	0.0	12.9	0.81	0.04
SEM	1.0	1.0	0.07	0.004

¹ The interaction of substrate $\times$ time was significant ($P < 0.05$) for OMD, total SCFA and lactate. The LSD values for separating substrate means are $2.95 \times \text{SEM}$.

² Solubility of substrates in *in vitro* medium at 0 h at 39°C.

³ Values have been corrected for solubility.

⁴ Total SCFA = acetate + propionate + butyrate.

process of rolling would make the fiber more accessible to microbial enzymes during the fermentation process.

OMD was greatest ($P < 0.05$) among reference substrates for corn starch, intermediate for amylomaize and lowest ($P < 0.05$) for potato starch. Potato starch contained the highest concentrations of RS, which influenced its digestion.

Data indicate that small intestinal bacteria ferment cereal grains and flours differently. The flour group had relatively high OMD values (mean 27.5%), whereas the cereal group had relatively low OMD values (mean 8.8%). This relates to the greater amount of processing that resulted in production of the flours. The cereal grain, as a result of this processing, loses TDF and RS components, as was found in this study. The lower concentrations of TDF and RS in flours lead to increased susceptibility to both enzymatic and microbial digestion.

Organic acid production. SCFA and lactate production data at the 7.5-h fermentation time are reported in Table 5. All substrate $\times$ time interactions were significant at $P < 0.05$.

Among leguminous substrates, the greatest ($P < 0.05$) total production of SCFA was for split peas, and the lowest was for navy beans. The high concentrations of total SCFA as a result of pea fermentation point to the ability of this substrate to be more rapidly fermented than beans. Bjorck and Siljestrom (1992) found that 90% of a pea product that reached the large bowel of a rat was fermented. The lower amylose content of peas could lead to higher fermentability by microflora, whether ileal or large bowel in origin. Tovar et al. (1992) reported that lentils contained more potentially available starch than did red kidney beans, corroborating the higher total SCFA concentration.

Lactate production was similar for all legumes. The largest amount of lactate produced was for split peas and black-eyed peas, whereas the lowest lactate production was for navy beans.

Among cereals, barley and oat fermentation resulted in the greatest ($P < 0.05$) total SCFA concentrations. The lowest ($P < 0.05$) total SCFA concentrations were for corn, white rice, brown rice and sorghum. Lactate production was greatest ($P < 0.05$) for barley compared with all other cereal grains.

Butyrate concentrations found in oats and barley (data not shown) were numerically higher compared with the cereal grains group (mean 0.66 mmol/g OM) as a whole. The presence of β -glucans, a soluble dietary fiber found in both oats and barley, may have stimulated butyrate production by ileal microflora.

Potato flour resulted in the highest ($P < 0.05$) total SCFA production compared with all other flours. Murray et al. (2000) also found that potato flour was numerically highest in total SCFA production when comparing six different flours incubated in inoculum containing ileal microorganisms. Processing was suggested as responsible for the increased susceptibility of potato flour to fermentation. The lowest ($P < 0.05$) total SCFA production was for sorghum and corn flours.

Flour fermentation resulted in generally higher lactate concentrations than for the other groups. Average lactate production for flours was 0.23 mmol/g OM. Zentek (1995) performed *in vitro* studies using canine ileal chyme to measure the fermentative capabilities of different substrates. He postulated that ileal fermentation of carbohydrates favored the growth of lactobacilli, which produce lactate as a major metabolic end-product. The high starch levels resulting from extensive processing of flours may have created a favorable environment for the selection of lactobacilli and subsequent production of lactate.

Rolled oats resulted in the highest ($P < 0.05$) total SCFA production compared with all other grain-based food products. Yiu et al. (1987) found raw oat starch to be highly digestible because of the disruption of starch granules due to oat processing. Rolling the oats leads to this disruption of the starch granules in the oat grain. Also, lactate production was highest ($P < 0.05$) for rolled oats, again relating to the high degree of processing and subsequent fermentative capacity of rolled oats.

Total SCFA production for the reference substrates was highest ($P < 0.05$) for corn starch compared with all other substrates. Zentek (1995), using canine ileal chyme, found that after 24 h of in vitro fermentation, corn starch resulted in higher concentrations of total SCFA compared with potato starch (7.11 versus 5.80 $\mu\text{mol/mL}$ of fermentation broth, respectively). This is comparable to our SCFA and lactate data, in which corn starch had the highest ($P < 0.05$) concentrations and potato starch had the lowest. Although both are composed of starch, potato starch contains a much higher concentration of RS (66.9%) than corn starch (8.1%), possibly leading to a reduction in the fermentation of potato starch.

The response criteria used in this experiment to test differences among substrates included OMD and organic acid production. Organic acid production appears to be the more accurate criterion for the determination of fermentative activity, because OMD values are obtained using a gravimetric method with its attendant difficulties. High solubility values in relation to OMD do not appear to equate to high total SCFA concentrations. For example, the average solubility value for the flour group was 19.8% and total SCFA concentrations were only 4.99 mmol/g OM. The cereal grains group, on the other hand, averaged 4.5% solubility but had a total SCFA concentration of 5.92 mmol/g OM. Likewise, there were no statistically significant correlations between OMD and total SCFA concentrations (data not shown).

Of interest to many researchers is the potential fermentation of RS. Although starch is fermentable and believed to favor butyrate production, the data are not entirely consistent (Topping and Clifton 2000). Our data do not point to increased concentrations of butyrate from the fermentation of RS. For example, legumes had high concentrations of RS (mean 24.7%), whereas butyrate concentrations averaged 0.77 mmol/g OM. Flours, low in RS concentrations (mean 2.8%), had similar butyrate concentrations (mean 0.67 mmol/g OM) as the legume group (data not shown).

What is the contribution of ileal bacteria to starch disappearance compared with that resulting from any residual digestive enzymes present in ileal chyme? Using the same ileal in vitro model, Murray et al. (2000) found that fermenting substrates in the presence of sodium azide-treated ileal bacteria resulted in no total SCFA for the first 5 h and minimal amounts at 7.5 h. This points to the minimal effect of residual digestive enzymes on starch disappearance using this in vitro model.

In conclusion, starch and fiber fractions in foods and feeds affect starch digestion in the gastrointestinal tract as assessed using in vitro models. It should be noted that the emphasis of this work was the effect of starch and fiber fractions on intestinal microbial digestion. Gut motility, digestive enzymes and other aspects of gut function will affect digestion in vivo. Greater knowledge of the precise chemical composition and digestive capabilities of starch fractions in foods and feeds will allow for more precise dietary formulations for both humans and companion animals, with implications in both performance and health arenas.

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