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Effect of Adding Commercial High Fiber Ingredients on Protein Quality of Casein and Gluten Diets for Rats as Assessed by Bioassays

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Abstract: The food industry has responded to consumer demand for foods with higher fiber contents by developing products with high-fiber ingredients, which have unique properties that raise the fiber level. However, excessive consumption of high-fiber products can affect digestibility and protein quality. This study evaluated the effect of three commercial products used to increase fiber content in diets with casein or gluten as a protein source and Sprague Dawley rats as an experimental animal model. Six synthetic diets were prepared wherein three different fiber sources were added to a casein or gluten protein base: (i) insoluble fiber from wheat bran; (ii) soluble fiber with resistant maltodextrins and (iii) fiber from orange pulp that had similar amounts of soluble and insoluble fiber. Biological indicators such as weight were determined, as were the net protein ratio (NPR) and the percentage of apparent and true digestibility of nitrogen (AND, TND). Results were analyzed using statistical software with 95% significance. AND and TND of casein diets with high fiber ingredients ranged from 85.5 to 91.3% and from 87.8 to 94.21%, respectively. The gluten diets with different fiber sources had AND and TND that ranged from 87.8 to 90.4% and 91.1 to 93.8%, respectively. The NPR results ranged between 1.7 and 2.0. The results demonstrated that the addition of commercial fibers to gluten based diets resulted significant differences in digestibility and NPR.

Key words: High fiber ingredients, dietary fiber, protein quality

INTRODUCTION

The inclusion of fiber in the diet may have beneficial health effects by reducing the risk of developing diseases such as hypertension, diabetes and obesity, as well as certain cardiovascular and gastrointestinal disorders. Fiber-rich diets may also help improve serum lipid levels, lower blood pressure, improve glucose control in diabetes and promote regularity, while aiding in weight loss and enhancing immune system function (Anderson *et al*., 2009). However, consumption of excess dietary fiber can adversely affect food digestion, absorption and protein metabolism, which can diminish protein quality (Wong and Cheung, 2003). Moreover, several studies showed that high fiber diets can also result in increased fecal nitrogen excretion and decreased dietary nitrogen digestibility in both humans and laboratory animals (Mariotti *et al*., 2001; Frias and Sgarbieri, 1998; Shah *et al*., 1982; Jorgensen *et al*., 2003; Feddern *et al*., 2008).

Production of foods with increased amounts of whole grains and fiber has increased dramatically worldwide. In 2002, only 231 types of whole grain-based products were available in the global market, but by 2010 this number had risen to 3,272 (Feng, 2011). Moreover, food producers have responded to consumer demand for foods that are higher in dietary fiber by developing products that include high fiber ingredients with unique properties. Different types of high fiber ingredients, both synthetic and natural, can be used during product development and these may vary from whole grain flour to the outer portion of shellfish (Nelson, 2001). The growing market for high fiber ingredients used to manufacture higher fiber foods could increase fiber intake and promote healthier diets, but, as mentioned above, increasing dietary fiber amounts may also have adverse effects on digestion, absorption and protein metabolism that affect protein quality. This study used bioassays to evaluate the effect of three commercial products used to increase food fiber content on fecal nitrogen excretion and digestibility by rats fed diets with casein or gluten as protein sources.

MATERIALS AND METHODS

Experimental plan: The overall approach was to prepare test diets for white rats that used casein or gluten as the protein source and different types of high fiber ingredients. A casein-based diet without fiber was used for protein quality control. These different diets would allow observations of how different dietary fibers affect protein use in rats. Various measures of protein quality or utilization were obtained by measuring weight gains, total feed and protein intake and fecal total.

Sample descriptions: Six experimental diets were prepared. Casein and gluten were the protein sources and one of three different commercial high fiber ingredients

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was added: (i)insoluble fiber from wheat bran (CAS-INS, GLU-INS); (ii) soluble fiber based on resistant maltodextrins (CAS-SOL, GLU-SOL) and (iii) fiber from orange pulp that has a combination of insoluble and soluble dietary fiber (CAS-IS, GLU-IS). Casein and gluten diets without fiber (WF) were used as control diets (CAS-WF, GLU-WF), while casein and gluten diets with cellulose (CAS-CEL, GLU-CEL) were used for reference. Nitrogen-free diets were also produced using the commercial ingredients described above as fiber sources (FN-INS, FN-SOL and FN-IS). Nitrogen-free diets either with or without fiber (FN-CEL, FN-WF, respectively) were prepared according to the AOAC basal diet (1990).

Three commercial dietary fiber sources were analyzed for their protein content (AACC, 2000) and the total dietary fiber contents were also determined using a TDF-100A kit (Sigma, St. Louis, MO USA) that is based on the method proposed by Prosky *et al.* (1988). Batches of 2 kg were prepared for each diet and stored at <5°C in sealed containers.

Animal care and feeding: Fourteen-day feeding trials were conducted with twenty Sprague-Dawley weanling rats aged 21-23 days and weighing between 45 and 55 g. Rats were supplied by the Experimental Animal Unit of the Department of Food Research and Postgraduate Studies at the University of Sonora. Rats were weighed and randomly distributed into 11 groups with four rats each that were fed one of the experimental diets. The rats were kept in individual stainless steel cages with screen bottoms. Diets and water were supplied *ad libitum* and the laboratory temperature was maintained at 25±2°C with alternating 12 h periods of light and dark. The feeding experiments were replicated four times with the same diet lots in two observations. One group received the nitrogen-free diet for 14 days.

Bioassays: Dry matter digestibility (%DMD) was expressed as the ratio of total food consumed minus the weight of feces to the total food consumed (Church and Pond, 1974).

In vivo protein digestibility was considered as the quantity of protein digested and was calculated by determining the difference between the amount of nitrogen ingested and eliminated by the animal (Pellett and Young, 1978).

The net protein ratio (NPR) was calculated from body weight variations and the efficiency of protein in 'maintaining' and 'increasing' body weight. Diet weight consumption was measured every third day over a 14 day period. NPR values were calculated according to Bender and Doell (1957).

RESULTS AND DISCUSSION

Commercial high fiber ingredients: The protein content of the high fiber ingredients ranged from 0.32% of soluble fiber for resistant maltodextrin (SOL) and 7.65% for fiber obtained from orange pulp that had a combination of both insoluble and soluble dietary fiber (IS) (Table 1).

The dietary fiber content of the fiber obtained from wheat bran (INS) was 94.0% of insoluble fiber and traces of soluble fiber (Table 1). This amount is consistent with a report by Cho *et al.* (1999) that showed the dietary fiber content of ingredients in high fiber wheat bran was up to 90%. Meanwhile, the high fiber ingredient based on resistant maltodextrins had 50% soluble fiber and traces of insoluble fiber (Table 1), which is similar to that reported by Hashizume and Okuma (2009) who found that resistant maltodextrins had 58% dietary fiber. The high fiber ingredient derived from citrus pulp had 36.6% insoluble dietary fiber and 31.4% soluble fiber (Table 1). These values were similar to a study by Nelson in (2001) that reported a range of 50 to 80% total dietary fiber derived from citrus fruits (Table 1).

Dry matter and nitrogen digestibility: The digestibility of dry matter (DMD) is an indicator of a food's nutritional capacity, which provides information on the absorption of all nutrients in the evaluated product and their possible assimilation. In this study consumption of diets with various high fiber ingredients was similar, but the total amount of feces excreted varied (Table 2). Diets with CEL and INS fibers showed the highest amounts of total feces and these exceeded the amount of feces excreted by rats fed the WF diet by about 3-fold. Moreover, the CEL and INS diets had differences in protein quality indicators expressed as DMD (Table 2).

One of the most important biological determinants in food quality is nitrogen digestibility, which is an indicator of a food's nutritional quality in terms of nutrient absorption. Digestibility is also an indicator of protein quality and represents the proportion of the dietary protein that is digested and absorbed. However, determining the digestibility coefficient as the difference between nutrients ingested and excreted overlooks the fact that not all material in the stool is actually undigested food. Some feces components are formed by enzymes and are secreted into the intestine, while epithelial desquamation products are also present in feces. Because these substances do not come directly from food, the actual proportion of absorbed food can be underestimated. Consequently, values obtained in digestibility assays are referred to as apparent digestibility, which differs from the true digestibility, in that the latter considers endogenous nutrients lost by the animal. The true digestibility of nitrogen (TND) is a more accurate measurement than the apparent digestibility of nitrogen (AND), which encompasses both ingested and endogenous nitrogen. To calculate true digestibility, one control group of experimental animals must receive a diet that is free of the nutrient being measured, in this case nitrogen. Determining the nitrogen concentration excreted in the control group feces will allow calculation of endogenous

Table 1: Protein and dietary fiber content of commercial high fiber ingredients

	Protein	IDF	SDF	TDF
Commercial high fiber ingredients	%			
Insoluble fiber (wheat bran)	0.65±0.5[b]	94.0±0.9[a]	0.12±0.05[c]	94.12±0.9[a]
Soluble fiber (Resistant Maltodextrins)	0.32±0.3[b]	0.25±0.06[c]	49.75±0.2[a]	50.0±0.1[c]
Insoluble-soluble fiber (orange pulp)	7.65±0.8[a]	36.6.0±0.2[b]	31.4±0.6[b]	68.0±0.4[b]

Values correspond to average±standard deviation of three measurements. Equal means in each column for each sample are not significantly different (p<0.05). IDF: Insoluble dietary fiber, SDF: Soluble fiber diet, TDF: Total dietary fiber

Table 2: Food intake, total feces, %dry matter digestibility (DMD) and nitrogen digestibility (AND, TND) of casein and gluten diets by rats

Diet	Food intake (g)	Total feces (g)	DMD (%)	Nitrogen intake (g)	Fecal nitrogen (g)	AND (%)	TND (%)
CAS CEL	151.4[a]	10.1[a]	93.3[c]	2.35[ab]	0.26[b]	88.8[a]	91.3[b]
CAS INS	146.5[a]	9.0[a]	93.9[c]	2.42[ab]	0.34[a]	86.0[b]	88.5[c]
CAS SOL	137.5[a]	4.0[c]	97.1[a]	2.12[b]	0.18[c]	91.3[a]	94.2[a]
CAS IS	150.4[a]	6.5[b]	95.6[b]	2.65[a]	0.38[a]	85.5[b]	87.8[c]
CAS WF	121.1	3.0	97.5	2.32	0.18	92.1	94.7
GLU CEL	79.2[b]	6.3[ab]	92.1[b]	1.28[b]	0.13[b]	90.0[ab]	94.7[a]
GLU INS	104.8[a]	8.0[a]	92.3[b]	1.78[a]	0.17[b]	90.4[a]	93.8[a]
GLU SOL	93.2[ab]	4.4[b]	95.3[a]	1.49[b]	0.16[b]	88.9[ab]	93.0[ab]
GLU IS	106.9[a]	6.2[b]	94.2[a]	1.82[a]	0.22[a]	87.8[b]	91.1[b]
GLU WF	90.2	2.5	97.2	1.53	0.10	93.3	97.3

Values are means of eight determinations. Feces weights are on water free basis. Values followed by different letter in the same column are significantly different (p<0.05). CAS: Casein, GLU: Gluten, CEL: Cellulose, INS: Insoluble fiber from wheat bran, SOL: Soluble fiber from resistant maltodextrin, IS: Insoluble and soluble fiber from orange pulp, WF: Without fiber

Table 3: Effect of commercial high fiber ingredients on protein free diets

Basal diets	Food intake (g)	Fecal weight (g)	Fecal nitrogen (%)	Endogenous nitrogen (%)
FN-CEL	51.4[b]	3.55[a]	1.58[b]	0.0564[d]
FN-INS	66.0[a]	3.77[a]	3.35[a]	0.1259[a]
FN-SOL	65.3[a]	3.02[a]	3.33[a]	0.1005[b]
FN-IS	62.8[a]	3.0[a]	3.15[a]	0.0912[c]
FN-WF	61.5	1.73	3.0	0.053

Values are means of eight determinations. Values followed by different letter in the same column are significantly different (p<0.05). FN: Nitrogen-free, CEL: Cellulose, INS: Insoluble fiber from wheat bran, SOL: Soluble fiber from resistant maltodextrin, IS: Insoluble and soluble fiber from orange pulp, WF: Without fiber

Table 4: Weight gain, protein intake and net protein ratio (NPR) of casein and gluten diets by rats

Diets	Weight gain (g)	Protein intake (g)	NPR
CAS CEL	45.7[a]	14.6[bc]	4.20[a]
CAS INS	42.5[a]	15.7[ab]	3.5[b]
CAS SOL	38.5[a]	14.1[abc]	3.8[ab]
CAS IS	48.51[a]	16.85[a]	3.7[ab]
CAS WF	40.5[a]	12.4[c]	4.5[a]
GLU CEL	5.7[b]	8.03[b]	2.14[a]
GLU INS	7.8[b]	11.4[a]	1.7[c]
GLU SOL	5.6[b]	10.3[ab]	1.7[c]
GLU IS	11.16[a]	11.55[a]	2.04[ab]
GLU WF	5.6[b]	9.54[b]	1.8[bc]

Values are means of eight determinations. Values followed by different letter in the same column are significantly different (p<0.05). CAS: Casein, GLU: Gluten, CEL: Cellulose, INS: Insoluble fiber from wheat bran, SOL: Soluble fiber from resistant maltodextrin, IS: Insoluble and soluble fiber from orange pulp, WF: Without fiber

nitrogen loss. Results obtained from rats fed the protein-free diets, containing different sources of dietary fiber are shown in Table 3. Feed consumption was not significantly different among these groups of rats. Also the amount of feces was not different among groups of rats fed with FN-INS, FN-SOL and FN-IS diets. Here, endogenous nitrogen from nitrogen-free diets supplemented with cellulose (FN-CEL), insoluble wheat bran fiber (FN-INS), soluble maltodextrin fiber (FN-SOL) and orange pulp soluble and insoluble fiber (FN-IS) was 0.06, 0.12, 0.10 and 0.09 g, respectively. Endogenous nitrogen, as part of the total fecal nitrogen, was higher in rats fed with the INS diet.

Among the experimental diets, those with IS fiber had the highest values of fecal nitrogen that influenced both AND and TND. The SOL diets had the lowest values of nitrogen in feces and the highest percentage of AND and TND. Meanwhile, the casein diet without fiber showed values that were similar to those reported by Shah *et al*. (1982), who reported an AND of 92.3% for a casein diet without fiber and AND values ranging between 81.4 and 88.7% for fiber diets with casein (Table 2).

Net protein ratio: The net protein ratio (NPR) is based on a linear relationship between the increase in weight and the quality of protein consumed. NPR values for the casein diets ranged from 3.5-3.8 and 1.7-2.0 for gluten diets (Table 4). There were no significant differences in NPR between the casein-based diets that were evaluated and the NPR results were lower than those reported by Shah *et al.* (1982) who determined NPR values of 3.98 for a casein diet without fiber and 3.90 and 3.65 for casein diets with 5% cellulose or 5% guar gum, respectively. However, in this study the inclusion of commercial high fiber ingredients in casein diets did not cause a significant reduction in NPR. Meanwhile, gluten diets with INS and SOL fiber showed significantly different NPR values and the gluten diet with IS fiber had the same NPR as that for the gluten diet with CEL. These different values are likely a direct effect of the diet components and the physicochemical nature of the fibrous material. In addition, the different diets influenced the growth patterns of experimental animals in terms of digestibility as well as dietary and endogenous nitrogen excretion. Another cause of the decline in NPR values seen for casein diets with INS fiber and gluten diets with either INS or SOL fiber could be explained by analyzing dietary nitrogen excretion trends, which suggest that these fibers may have a physical effect on digestion by preventing proper absorption of amino acids and increasing the excretion of dietary nitrogen. Indeed, Bergner *et al.* (1975) reported that diets containing dietary fibers can affect nutrient absorption to cause a decrease in reabsorption of amino acids along the small intestine, inhibiting enzyme activity (Shah *et al.*, 1986) and further hindering amino acid absorption in the intestinal epithelium (Matos and Chambilla, 2010).

Conclusions: The inclusion of different commercial high fiber ingredients in casein and gluten diets had a significant effect on the total amount of feces excreted. Diets with insoluble fiber had a greater effect on the total amount of feces, wherein rats fed this diet excreted about 3-fold greater amounts of feces compared to rats fed the fiber-free diet. The addition of these fibers also caused a decrease in DMD of about 5%. The inclusion of the dietary fibers increased fecal nitrogen excretion such that the nitrogen content of feces for the INS and IS fiber diets was double that of rats fed the diet without fiber. This result shows that consumption of dietary fiber alters both the endogenous nitrogen as well as nitrogen obtained from the diet. Moreover, different commercial high fiber ingredients added to diets to achieve 5% of total dietary fiber reduced the digestibility of nitrogen in growing rats by between 6 and 9%.

Meanwhile, the addition of commercial high fiber ingredients to casein diets did not cause a significant reduction in NPR, while gluten diets with INS and SOL fibers showed significantly lower NPR values.

This study indicates that the consumption of different fiber types reduces the digestibility and nitrogen utilization of the experimental diets and that rat growth was promoted by an increase in both endogenous and fecal nitrogen excretion. The effect of fiber protein quality depends on the quality of the protein in the diet, as evidenced by the larger effect seen when rats were fed a diet with gluten as the sole protein source.

Conflict of interests: The authors have no conflict of interest to declare.

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