J. Agric. Food Chem. 1986, 34, 468–469

Effect of Phytic Acid Reduction on Rapeseed Protein Digestibility and Amino Acid Absorption

Lilian U. Thompson* and Maria R. Serraino

This study determined the effect of phytic acid reduction on the in vivo digestibility of proteins and absorption of amino acids in rapeseed flour (RF). Weanling rats were fed a high-phytate (1.26%) vs. a low-phytate (0.41%) diet in which the 10% protein was supplied by a high- (5.7%) vs. a low- (2.4%) phytate RF. Rats fed a protein-free diet served as a control for metabolic protein losses. Diet and fecal samples were analyzed for protein and amino acids. No significant differences were observed between the high- vs. low-phytate diet groups in protein digestibility and amino acid absorption.

INTRODUCTION

Phytic acid (myoinositol, 1,2,3,4,5,6-hexakis(dihydrogen phosphate)), a major component of all plant seeds, has a tremendous potential for binding positively charge molecules such as cations or proteins. The interaction between phytic acid and minerals such as zinc, calcium, magnesium, etc., leads to the formation of complexes that are insoluble at intestinal pH and, hence, biologically unavailable for absorption (Erdman, 1979). In addition, complex formation of phytic acid with proteins may inhibit the enzymatic digestion of the protein (Barre, 1956; Singh and Krikorian, 1982).

The possible adverse nutritional effects of phytic acid have to some extent limited the use of many plant proteins. This is particularly true of rapeseed protein, which has a well-balanced amino acid composition and is especially rich in lysine and the sulfur-containing amino acids (Gillberg and Tornell, 1976). Atwal et al. (1980) reported that growth rate, diet consumption, and efficiency of protein utilization were lower for rats fed rapeseed protein that provided 1.24% phytic acid in the diet than for those fed casein but improved as the phytate content of the diet was reduced to 0.41%. Others (McDonald et al., 1978b), however, found no evidence that phytate had any effect on nutritive value of rapeseed protein in vivo.

Methods of removing phytic acid from rapeseed flour and protein concentrate have been developed and reported previously by Serraino and Thompson (1984). Reduction of phytic acid in rapeseed flour by such methods did not improve the in vitro rate of protein digestibility but did change the rate of release of many amino acids (Serraino et al., 1985). The in vitro system used, nevertheless, made no direct implication regarding physiological availability or absorption of the amino acids. Therefore, the objective of this study was to determine the effect of phytate reduction from rapeseed flour on the in vivo protein digestibility and amino acid absorption. The effect of phytic acid on amino acid absorption has not been previously reported.

MATERIALS AND METHODS

Ten percent protein diets were prepared by using as the protein source either dehulled, solvent-extracted, non-heat-treated rapeseed flour (RF), provided by Dr. J. D. Jones, Food Research Institute, Ottawa, Canada, or low-phytate RF prepared by the previously described method (Serraino and Thompson, 1984; Serraino et al., 1985), i.e. dialysis of the 5% (w/v) aqueous RF dispersion at pH 5.15 for 3 days followed by freeze drying. A protein-free diet was also prepared to determine metabolic nitrogen and amino acid excretion.

The diets contained 5% moisture, 8% corn oil (Mazola), 5.47% total fiber, 1% vitamin mix (Teklad Test Diets), and 4% Bernhart and Tomarelli modified mineral mix (ICN Biochemicals Co.) and corn starch to make 100%. Since low-phytate RF contributed 5.47% total dietary fiber in the diet, the high-phytate RF diet and the protein-free diet were prepared with the same amount of dietary fiber by the addition of cellulose (Teklad Test Diets).

A total of 18 male weanling Wistar rats (Woodlyn Farms, Guelph, Ontario, Canada; 71.9 ± 0.7 g mean body weight) were randomly divided into three experimental groups of six rats each and fed one of the above diets for seven days. Diets and deionized water were provided ad libitum. Rats were individually housed in stainless-steel metabolic cages in a room kept at 20–23 °C and on a 12-h light–dark cycle. Diet intakes were measured daily, and fecal samples were collected on days 5, 6, and 7, freeze-dried, and weighed. The 3-day fecal samples of each rat were pooled and ground before analysis.

The low- and high-phytate RF diets and fecal samples were analyzed in duplicate for moisture, nitrogen (AOAC, 1980), phytic acid (Latta and Eskin, 1980), and amino acids. Total dietary fiber (Prosky et al., 1985) and fat (AOAC, 1980) were also determined in the flour samples. All amino acids were measured on a Beckman amino acid analyzer after acid hydrolysis of the samples with 6 N HCl for 24 h at 110 °C.

Protein digestibility and amino acid absorption were calculated as follows:

$$\% \text{ true digestibility or absorption} = [[\text{intake} - (\text{fecal losses} + \text{metabolic losses})]/\text{intake}] \times 100$$

Apparent digestibility or absorption was also calculated as above except that no correction for metabolic losses was done.

Statistical comparisons were made by using Student's t-test.

RESULTS AND DISCUSSION

The chemical composition of RF and low-phytate RF is given in Table I. Dialysis of RF at pH 5.15 for 3 days (Serraino and Thompson, 1984; Serraino et al., 1985) reduced the phytic acid by 50% and increased the concentration of fat, total dietary fiber, and protein probably due to loss of soluble compounds such as minerals, sugars, pigments, phenolics, and nonprotein nitrogen in addition to phytic acid. This method of preparing low-phytate RF, however, did not affect the amino acid composition of the flour.

Significantly greater overall diet intake and weight gain were observed for the low-phytate diet (Table II). This

Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

0021-8561/86/1434-0468$01.50/0

Table I. Chemical Composition of High- and Low-Phytate Rapeseed Flour (RF)[a]

component	high-phytate RF	low-phytate RF
moisture, %	6.1 ± 1.0	1.0 ± 0.0
fat, %	5.8 ± 0.2	8.9 ± 0.1
total dietary fiber, %	21.3 ± 0.3	31.5 ± 0.2
protein, %	45.4 ± 0.8	57.8 ± 0.3
phytic acid, %	5.7 ± 0.4	2.4 ± 0.2
amino acids, g/16 g N[b]		
lysine	6.7 ± 0.4	6.8 ± 0.0
threonine	5.2 ± 0.5	4.6 ± 0.1
valine	3.7 ± 0.2	3.6 ± 0.2
methionine	2.5 ± 0.2	2.3 ± 0.4
cystine	1.7 ± 0.1	1.8 ± 0.3
isoleucine	2.9 ± 0.3	2.9 ± 0.5
leucine	7.5 ± 0.6	7.8 ± 0.7
phenylalanine	4.1 ± 0.5	3.9 ± 0.6
tyrosine	2.7 ± 0.0	2.4 ± 0.8
aspartic acid	8.5 ± 0.4	8.2 ± 0.0
serine	6.1 ± 0.3	6.3 ± 0.2
glutamic acid	18.0 ± 4.8	21.5 ± 0.4
proline	6.5 ± 0.3	6.9 ± 0.3
glycine	6.4 ± 0.2	6.6 ± 0.0
alanine	5.6 ± 0.2	5.8 ± 0.1
histidine	3.2 ± 0.0	3.2 ± 0.2
arginine	6.4 ± 0.6	5.6 ± 0.4

[a] Mean ± standard error of mean ($n = 2$). [b] Tryptophan not analyzed.

may be due partly to the improvement of palatability and acceptability of the diet as a result of the losses during dialysis of phenolic compounds with adverse flavor potentials (Kozlowska et al., 1975). It was not due to mineral deficiency in the rats fed the high-phytate diet since mineral availabilities in both diets did not differ significantly (unpublished data). Neither was the difference due to glucosinolates in rapeseed since both diets had nondetectable concentrations of these substances.

True and apparent protein digestibilities (Table II) were not significantly different between the high-phytate and low-phytate diets. Rapeseed has previously been found to contain 85–90% digestible protein (Bell et al., 1976; McDonald et al., 1978b; Nwokolo et al., 1976), and the results of this study (86.7 and 85.6% for high-phytate and low-phytate RF, respectively) fall in this range. Obviously, phytic acid reduction failed to improve the digestibility of rapeseed protein, in agreement with our in vitro digestibility data (Serraino et al., 1985).

While phytic acid affected the in vitro rate of amino acid release (Serraino et al., 1985), it did not affect the in vivo absorption of any of the amino acids except proline and phenylalanine (Table II). The reason for the lower absorption of phenylalanine and proline with the low-phytate diet remains uncertain.

Kinetic and thermodynamic studies with human serum albumin indicate that phytic acid binds first with α-amino terminal groups, followed by ϵ-amino group of lysine, and then histidine and guanidyl groups of arginine (Barre et al., 1965; Cheryan, 1980). Thus, these amino acids that are rich in rapeseed, if complexed by negatively charged phytic acid, would have been expected to become readily available upon phytate removal. Amino acid absorption, however, was lower, although not significantly, for many of the amino acids in the low-phytate diet. Thus, contrary to expectations, phytic acid does not seem to play a role in reducing the absorption of the amino acids in rapeseed protein. These findings support studies that find phytate to have no effect on protein quality and conclude that rapeseed protein is of comparable if not superior quality to casein and soybean (Lieden and Hambraeus, 1977; McDonald et al., 1978a, 1978b).

Table II. Weight Gain and Diet Consumption of Rats, Protein Digestibility, and Amino Acid Absorption

determin	high-phytate RF diet	low-phytate RF diet
diet consumption, g	66.5 ± 2.1	93.2 ± 1.3[d]
wt gain, g	22.6 ± 1.1	45.9 ± 1.8[d]
true protein digestibility, %	86.7 ± 0.4 (82.4 ± 0.2)[c]	85.6 ± 0.6 (82.5 ± 0.7)
true amino acid abs, %		
lysine	87.5 ± 1.3 (83.2 ± 1.2)	85.0 ± 1.3 (81.9 ± 1.4)
threonine	83.8 ± 0.2 (78.2 ± 0.2)	81.2 ± 0.8 (76.6 ± 0.8)
valine	82.6 ± 1.3 (78.2 ± 1.6)	84.5 ± 1.5 (81.2 ± 1.5)
methionine	84.5 ± 0.6 (79.9 ± 0.3)	82.5 ± 0.8 (79.0 ± 0.9)
cystine	90.1 ± 1.0 (86.3 ± 0.8)	90.0 ± 1.2 (86.9 ± 1.9)
isoleucine	82.5 ± 1.9 (78.6 ± 2.2)	83.2 ± 1.7 (80.4 ± 1.7)
leucine	85.4 ± 0.2 (81.7 ± 0.3)	85.2 ± 0.8 (82.7 ± 1.0)
phenylalanine	83.6 ± 0.6 (77.3 ± 0.4)	76.9 ± 0.8[d] (72.1 ± 1.0)[d]
tyrosine	82.0 ± 1.0 (77.8 ± 0.7)	79.5 ± 1.3 (76.0 ± 1.4)
aspartic acid	83.4 ± 1.1 (77.3 ± 0.7)	80.1 ± 2.5 (75.5 ± 2.4)
serine	84.0 ± 1.1 (79.3 ± 0.8)	83.9 ± 1.0 (80.6 ± 1.0)
glutamic acid	90.3 ± 0.7 (86.9 ± 0.5)	91.7 ± 0.8 (89.7 ± 0.8)
proline	92.7 ± 0.7 (89.5 ± 0.7)	88.5 ± 0.5[d] (86.3 ± 0.6)[d]
glycine	85.7 ± 1.3 (81.0 ± 1.0)	86.0 ± 0.8 (82.6 ± 0.7)
alanine	82.8 ± 1.4 (77.2 ± 1.0)	81.6 ± 0.8 (77.7 ± 0.8)
histidine	91.6 ± 0.1 (89.1 ± 0.2)	91.8 ± 0.5 (90.0 ± 0.5)
arginine	91.3 ± 0.4 (88.9 ± 0.4)	89.9 ± 0.7 (87.9 ± 0.7)
mean	86.1 ± 0.8 (81.8 ± 1.1)	84.8 ± 1.0 (81.6 ± 1.3)

[a] Mean ± standard error of mean ($n = 6$). [b] Tryptophan not analyzed. [c] Values in brackets are either apparent protein digestibility or amino acid absorption. [d] Differs significantly ($p \leq 0.01$) from the corresponding value for high-phytate RF diet.

In conclusion, phytate reduction in rapeseed flour has no significant effect on in vivo protein digestibility and amino acid absorption.

Registry No. Phytate, 83-86-3.

LITERATURE CITED

AOAC "*Official Methods of Analysis*", 13th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.
Atwal, A. S.; Eskin, N. A. M.; McDonald, B. E.; Vaisey-Genser, M. *Nutr. Rep. Int.* **1980**, *21*, 257.
Barre, R. *Ann. Pharm. Fr.* **1956**, *14*, 182.
Barre, R.; Nguyen-van Hout, N. *Bull. Soc. Chim. Biol.* **1965**, *47*, 1399.
Bell, J. M.; Giovanetti, P.; Sharby, T. F.; Jones, J. D. *Can. J. Anim. Sci.* **1976**, *56*, 763.
Cheryan, M. *CRC Crit. Rev. Food Sci. Nutr.* **1980**, *13*, 297.
Erdman, J. W. *J. Am. Oil Chem. Soc.* **1979**, *56*, 736.
Gillberg, L.; Tornell, B. *J. Food Sci.* **1976**, *41*, 1063.
Kozlowska, H.; Sabir, M. A.; Sosulski, F. W. *Can. Inst. Food Sci. Tech. J.* **1975**, *8*, 160.
Latta, M.; Eskin, M. *J. Agric. Food Chem.* **1980**, *28*, 1315.
Lieden, S; Hambraeus, L. *Nutr. Rep. Int.* **1977**, *16*, 367.
McDonald, B. E.; Lieden, S. A.; Anjou, K. *Nutr. Rep. Int.* **1978a**, *18*, 51.
McDonald, B. E.; Lieden, S. A.; Hambraeus, L. *Nutr. Rep. Int.* **1978b**, *17*, 49.
Nwokolo, E.; Bragg, D. B.; Kitts, W. D. *Poult. Sci.* **1976**, *55*, 2300.
Prosky, L.; Asp, N. G.; Furda, I.; Devries, J. W.; Schweizer, T. F.; Harland, H. *J. Assoc. Anal. Chem.* **1984**, *67*, 1044.
Serraino, M. R.; Thompson, L. U. *J. Agric. Food Chem.* **1984**, *32*, 38.
Serraino, M. R.; Thompson, L. U.; Savoie, L.; Parent, G. *J. Food Sci.* **1985**, *50*, 1689.
Singh, M.; Krikorian, A. D. *J. Agric. Food Chem.* **1982**, *30*, 199.

Received for review August 2, 1985. Accepted January 13, 1986. The study was funded by the Natural Sciences and Engineering Research Council of Canada.