REMOVAL OF ANTINUTRITIVE SUBSTANCE FROM RAPESEED AND NUTRITIVE PROPERTIES OF PROTEINS

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INTRODUCTION

Various constituents in rapeseed have been reported to exhibit antinutritive properties in both growing and pregnant rats. Enzyme hydrolyzed glucosinolates in rapeseed are thyrotoxic and hepatotoxic (1). However, unhydrolyzed glucosinolates are readily water-soluble following heat inactivation of enzymes, and the resulting rapeseed protein is of high quality (2). Phytic acid, present in major proportions in rapeseed, is not easily extractable, and several antinutritive properties have been ascribed to this compound. Phytic acid predisposes zinc deficiency in rats (3), and growth depression and reduced protein utilization have been reported in growing and reproducing animals (3-6). A non-phytate related substance has been shown to be anorexic in rats (7). In some cases with growing animals this characteristic has been reversed by added dietary zinc (6). Other constituents, including fiber (8) and tannins (9), may be antinutritive in their adverse effect on mineral availability and protein utilization.

This investigation examined methods for removal of proven and suspected antinutritive substances in rapeseed, as well as determining their influence on such factors as food intake, growth, protein quality evaluations, and mineral (Zn and Fe) availability.

METHODS

Antinutritive substances were removed from rapeseed during aqueous extraction followed by centrifugation or dialysis, as previously described (7). Extraction by methanol, chloroform or diethyl ether was accomplished by washing and either filtration or centrifugation. Subsequent to extraction, proteins were separated from the antinutritive material by gelfiltration. Biological value of proteins and antinutritive properties were assessed using routine methods on growing and pregnant rats. Basal diets were formulated to contain nutrients at recommended levels. Standard methods of statistical analysis of variance were employed to determine differences between means.

RESULTS

Removal of Antinutritive Substances

Glucosinolate. Optimum conditions for the processing of rapeseed should avoid glucosinolate hydrolysis, since the intact compound is much more easily removed than resulting hydrolytic products. The activity of glucosinolate-splitting enzymes was effectively minimized in this study by (a) treatment in tap water at 100°C, (b) treatment in dilute HCl at 0°C, (c) treatment with dilute NaOH at room temperature, or by (d) treatment in distilled water at 0°C. Glucosinolates were readily removed from the alkaline solution by gelfiltration, and from the other treatments by repeated washings. Subsequent feeding trials with growing rats, including gross pathological examination, were used to establish the successful removal of glucosinolates and derived hydrolytic products.

Phytic acid. Extraction of crushed rapeseed with distilled water at 0°C resulted in removal of 64 % of the phytic acid. Substitution of tap water for distilled water, however, completely inhibited extraction, while treating crushed rapeseed for 5 minutes in boiling distilled water prior to distilled water extraction at 0°C yielded only 24 % phytic acid. Gelfiltration of alkali extracted proteins was 100 % effective in removing phytic acid from the high molecular weight and neutral proteins (pI for main components ranged from 3.8-7.1). Low molecular weight proteins, reported to

be strongly basic, contained only 5 % of the total phytic acid. Extraction of crushed seed with delute HCl at 0°C completely removed phytic acid.

Some obvious differences were observed in studying the influence of different treatments on the in vitro enzymatic digestibility of phytic acid using 3 % wheat phytase at 55°C and pH 4.5. Phytic acid, insoluble in tap water at 0°C without prior heating, was completely resistant to digestion, while 39 % was digested following treatment in hot distilled water. At the same time, phytic acid was completely digestible subsequent to distilled water extraction at 0°C. In general, the less extractable the phytic acid, the less digestible it proved to be under conditions of this investigation. Zinc deficiency was observed only on growing rats fed rapeseed protein which included unextractable and undigestible phytic acid.

Ester. Even though acid-extracted rapeseed was phytic acid-free, pregnant rats developed anorexia on this preparation as well as on heated preparations containing phytic acid. When extraction temperature was 0°C, food consumption was normal and equal to that of rats on gelfiltered proteins. Normal feed consumption could also be obtained using 65°C methanol extraction of acid-extracted or heated preparations. A major precipitate of the methanol extract obtained at -75°C and included in a casein diet, depressed both feed consumption and serum zinc. Separation of the filtrate was accomplished by column chromatography on LH-20, and subsequent IR analysis indicated aliphatic and ester structures for all fractions. Mass spectroscopy established an average molecular weight of 800. All esters proved to be very soluble in chloroform, but less soluble in diethyl ether and methanol. One of the two major fractions (E_I), almost solid, was not as soluble in chloroform as the more viscous fraction (E_{II}). Fraction E_I predominated in heated and acid-washed preparations, and only small quantities were extractable from cold water-washed preparations. This might imply a relationship between biological activity, anorexia and reduced serum zinc and fraction E_I.

If defatted rapeseed (hexane treated at 15°C) was extracted with boiling chloroform (61°C) at atmospheric pressure in a soxhlet apparatus, both fractions E_I and E_{II} were recoverable from the extract, but when extraction took place at reduced pressure (10°C), only E_{II} was recovered.

Trace quantities of an unidentified substance were eluted in the E_I position, and further extraction at atmospheric pressure (61°C) resulted in the removal of only minor additional quantities of both fraction E_{II} and the unidentified trace component. This may indicate the possibility that fraction E_I was not present in the original rapeseed, but was produced by heat treatment. This coincides with the previous observation that heated and hot water-extracted rapeseed products cause anorexia and depressed serum zinc in reproducing rats, conditions not observed with cold water-extracted rapeseed.

Fiber. Rapeseed fiber quantitatively remaining in cold distilled water-extracted seed exhibited no influence on growth, protein utilization, or serum Fe and Zn in either growing or pregnant rats.

Phenolic compounds. Hot tap water extraction of crushed whole rapeseed had no influence on protein utilization, even though such treatment could be expected to remove appreciable quantities of phenolic compounds.

Nutritive Properties of Proteins

Rapeseed proteins would seem to contain no appreciable quantities of non-water soluble antinutritive factors since protein efficiency ratio (PER) and weight gain (g/day) for native proteins extracted in distilled water at 0°C and lyophilized, were 3.41 and 4.95 respectively. These values were both significantly greater ($P<0.001$) than the 2.90 and 3.14 values observed for casein. Commercial soy flour exhibited a PER of 2.64 and 3.34 g/day weight gain, thus indicating the superiority of rapeseed protein over soy as well. The true digestibility (TD) of rapeseed protein averaged 94.4, which was significantly higher than that for soy flour (90.8), but not significantly

different from casein (97.8). A minor protein fraction located in the hull (4-6 % of total protein) was largely unavailable to the rat.

The average biological value (BV) of 83.6 for rapeseed preparations was considerable greater than the 67.8 for casein, or the 64.8 for soy flour. Net protein utilization (NPU) of 79.5 for rapeseed was significantly ($P<0.001$) better than the 66.1 for casein or 58.8 for soy. When used as a 33 % replacement of the protein in a ground meat mixture, rapeseed protein improved the nutritive quality significantly ($P<0.05$). BV and NPU values were elevated from 76.3 to 86.3. As a means of comparison, similar replacements with textured soy flour had no appreciable effect on protein quality.

CONCLUSIONS

In addition to earlier well documented antinutritive components of glucosinolate hydrolysates, it is probable that both phytic acid and aliphatic esters present in rapeseed function as antinutrients through interference with bioavailability of zinc, protein utilization and growth.

Extraction with distilled water at $0^{o}C$ removed all antinutritive effects of glucosinolates, phytic acid and aliphatic esters on growing and reproducing rats fed diets with basal nutrients (including Zn) supplied at recommended levels. Combined aqueous and organic solvent extraction also eliminated the observed antinutrient effects of these substances. No antinutritive properties were noted for either rapeseed fiber or phenolic compounds.

Nutritional quality of detoxified native rapeseed protein was superior to both soy flour and reference casein, and comparable to high quality animal protein.

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