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STUDIES OF ANTINUTRITIVE SUBSTANCES IN RYE. IV. EFFECT OF TRYPSIN INHIBITORS IN RYE, WHEAT AND TRITICALE GRAIN ON PROTEIN DIGESTIBILITY

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Trypsin inhibitor (TI*) inactivation and alkylresorcinols extraction by washing with acetone did not improve digestibility of protein from wheat grain and bran. Addition of TI isolated from triticale and rye grain to the diet of experimental rats caused statistically significant reduction of digestibility of protein (TD) from casein at doses over 2 TI units per g of grain. TI isolated from rye added to chicken diet in doses of 0.75, 1.5 and 3.0 TI units/g feed did not lead to changes in weight increment and feed consumption during a 10-day experiment.

The protein of cereal grain is not completely digested leading to substantial losses considering that cereals supply 60-80% of protein for pigs and poultry. Proteins from different cereals are variously digestible, and individual animals species have been observed to digest different cereal cultivars to various extents [4, 15]. One of the reasons for reduced digestibility of protein may be the activity of proteolytic enzymes, mainly trypsin, given the fact that cereals are not thermally treated before consumption by animals. The content of trypsin inhibitors per unit weight is much lower in cereals than in peas, broadbeans and especially soybeans. However, the level of TI per g of protein is similar in cereal grain and leguminous plants seeds (except soybean). Boisen [1] underlines that one of the factors determining the antinutritive effect of enzymatic inhibitors is their susceptibility or resistivity to degradation by pepsin in the acid environment of gastric juices. According to this author, trypsin inhibitors are usually pepsin-resistant [1].

A well known fact is the coupling of genes of the high-lisine Opaque 2 maize cultivar with genes responsible for TI synthesis, and also of the gene responsible

* TI unit amount of trypsin inhibitor deactivating 1 ng of trypsin.

for the high lysine contens in Hiproly barley with the high activity genes of chymotripsin [7]. According to Eggum [4], however, the digestibility of Hiproly barley protein is not different from that of proteins in barley varieties containing less inhibitors.

In their genetical study of the original triticale, Tanner and Reinbergs found that this cereal inherits rye's trypsin inhibitors [18], and they believe that since various ryes contain different amounts of TI, it is possible to select triticale cultivars with lower TI content [19]. The level of antitrypsin in triticale grain is usually between that in rye (higher) and wheat (lower level).

Knoblauch et al. [9] demonstrated a high correlation between TI level and PER of triticale grain in experimental feeding of the vole Microtus pensylvl. In their experiments with pigs Erickson et al. [6] replaced maize with increasing does of triticale and observed a high correlation between average weight increments in piglets and TI content in the diet. However, when selected triticale with a lowered TI content was used in the diet, there was no imporovement in the growth of piglets [5]. These facts may be evidence that trypsin inhibitors (particularly in rye in which they occur in much higher concentrations than in other cereals) may be among the factors inhibiting protein digestibility and animal growth chicken in particular) [14]. Looking for antinutritive factors in rye which may contribute to the (usually poor) digestibility of this cereals protein and the inhibition of animals growth (especially chicks with incompletely active digestive enzymes), one cannot overlook the activity of rye's enzymatic inhibitors.

In this work we strove to determine:

1) the digestibility of rye and triticale protein after cooking of rye and triticale grain or bran prior to and following extraction with acetone (alkylresorcinols extraction) and TI inactivation;

2) coefficients of digestibility of casein protein after additions of TI isolated from rye and triticale to experimental rat diets;

3) the effect of TI isolated from rye in the diet on growth and diet utilization by chicken.

MATERIALS

Experiments were performed with Dańkowskie Złote rye and two triticale cultivars — Lasko and Grado — as well as with rye and wheat (mixed grain) bran obtained from a mill. The following thermal treatments were used to inactivate proteolytic enzymes inhibitors:

Toasting — rye grain was ground, its humidity adjusted to 20%, 3 cm layers placed on trays, covered with aluminum, foil, and heated for 30 min at 120°C (procedure a); cooking — whole rye grain was placed in a saucepan with an equal volume of water and cooked covered for 30 (procedure b), 60 (c) or 120 min (d) after bringing the water to boil; as in (d) but with three times the amount of water which was poured out after cooking (e); autoclaving rye or wheat bran for 1 h at 120°C (f); extracting rye and wheat bran with acetone (thrice) in order to

denature inhibitors protein and at the same time remove alkylresorcinols (g).

All rye grain or bran samples were dried in an air-flow drier at 25-30°C. Rye and triticale trypsin inhibitors were isolated at the Institute of Biotechnology, Wrocław University, by Tłuścik's method (patent pending).

METHODS

Protein was determined by Kjeldahl's method using a semi-automatic Kjeltec analyzer manufactured by Tecator. TI activity was determined by the Rackis method (chicken trypsin, BAPA substrate), and alkylresorcinols (AR) content according to Mejbaum et al. [12]. True digestibility (TD) and biological value (BV) of protein from rye and triticale, rye and wheat bran (both untreated and following thermal treatment or AR extraction) were determined in balance experiments with male rats according to the method described in detail by Eggum [3].

In order to determine the concentration of cereal TI which may reduce protein digestibility, the inhibitor, isolated from grain of one rye and two triticale cultivars, was added to standard casein diet. Chicken growth was analyzed in an experiment described by Boros [2], using four-day Astra B chicks kept singly in plastic cages to enable diet consumption determination; there were eight repetitions of each experiment.

The composition of the experimental rat diet was as follows: rye or triticale grain, rye and wheat bran and casein were the only sources of protein amounting to 9.4% of air-dry mass of diets. Casein diet consisted of standard casein (11.6%) with a 1% addition of methionine. The diets were isocalorific, and their energetic value was increased by a 6% addition of soybean oil; also added were vitamins (1%), mineral salts (3%) and proteinless wheat starch which made up the volume. The diets for chicken had 20.9% of protein, and consisted of: 40% wheat, 10% powdered egg, 8% casein, 5% gluten, 4% mineral salts, 1% vitamins; also added were increasing doses of trypsin inhibitor (o.75, 1.5 and 3.9 TUI (trypsin unit inhibitor) g diet.

RESULTS

Neither toasting nor cooking Dańskowskie Złote rye of initial inhibitor activity of 1.44 TUI/g for 30-120 min (with and without pouring out the water) resulted in improved digestibility (TD) of rye protein, despite the fact that TI activity dropped to about 0.3 TUI/g. Likewise in the case of triticale grain, a drop of TI activity from 1.29 to about 0.3 TUI/g failed to improve the coefficient of protein digestibility (Table 1). The TD coefficients of rye and triticale grain remained unchanged at 76-77 and 84-85% respectively.

Doses equivalent to single and double inhibitor activities in Lasko and Grado triticale cultivars did not lead to statistically significant changes in casein protein digestibility coefficients (Table 2), although a slight decrease of digestiblitity was

observed. In the case of TI isolated from Dańkowskie Złote rye grain, the does equivalent to the natural level in grain (1.44 TUI/g diet) did not significantly reduce TD, but a dose exceeding about twice the TI activity in grain (2.64 TUI of rye/g diet) reduced casein protein digestibility from 90.7 to 86.4% the difference being significant at $P = 0.05$.

Table 1. Effect of grain heating on digestibility by rats of rye and triticale protein

	Grain	Trypsin inhibitor activity (TUI/g)	Coefficient of protein time digestibility (TD)
1.	Dańkowskie Złote rye		
	untreated	1.44	77.9 ± 11.1 a
	cooked for 30 min in water	0.30	77.4 ± 3.8 a
	cooked for 60 min in water (1:1 seed-to-water ratio)	0.27	76.8 ± 3.6 a
	cooked for 120 min in water (1:3 seed-to-water ratio, water poured off)	0.27	76.1 ± 2.5 a
	toasted at 120°C for 30 min	0.92	76.9 ± 3.5 a
2.	Triticale		
	untreated	1.29	84.7 ± 1.6 b
	cooked for 60 min in water (1:1 seed-to-water ratio)	0.29	85.6 ± 2.7 b

Significance of differences marked with different letters on the basis of statistical analysis and division into homogeneous groups according to Duncan's test ($P \leq 0.05$)

Table 2. Effect of trypsin inhibitor (TI) isolated from triticale and rye grain on digestibility (TD) of casein protein by rats

Diet*	TI isolate (TUI/g diet)	Coefficient of protein time digestibility TD	S.D.
casein	0	91.5	2.8 a
TI 1 x Lasko	1.29	90.0	2.5 a
TI 2 x Grado	1.20	89.6	2.0 a
TI 1 x Dańkowskie Złote	1.44	90.7	2.7 a
TI 1.83 x Dańkowskie Złote	2.64	86.4	2.9 b

* TI dose equal to (1 x) or higher (up to 2 x) than in parent cereal Division into homogeneous gronps performed with Duncan's test ($P \leq 0.05$)

The isolated trypsin inhibitor added to chicken diet did not affect the growth of animals or feed consumption (Table 3).

Rye and wheat bran, relatively rich in protein (14-16%) and boasting an advantageous aminoacid composition, exhibit low protein digestibility coefficients. Rye and wheat bran contained 1.0% and 2.216 TUI/g and also 4.2 and

2.1 g AR/kg, respectively. At the same time they are a rich source of cellulose, both soluble and insoluble in water. In order to determine the degrees in which TI and cellulose are responsible for reduced digestiblility of protein, we determined TD coefficients in rats. The diet of the animals included rye and wheat bran, either untreated, or autoclaved (TI elimination) or acetone-extracted (AR elimination, TI denaturation), and autoclaved. The cellulose content was not affected by any of these treatments. The protein digestibility coefficients (Table 4) did not improve, and indeed they decreased significantly following autoclaving. Neither TI elimination nor AR extraction improved protein digestibility.

Table 3. Effect of trypsin inhibitor isolated from rye on growth and feed utilization in experiments with chicke

Diet	Chicken weight increments* (g/10 days)	Feed intake weight increment*
control	78.4 ± 12.6	1.62 ± 0.10
control + 0.75 TUI/g dict	75.9 ± 10.9	1.68 ± 0.10
control + 1.5 TUI/g diet	69.1 ± 5.6	1.68 ± 0.07
control + 3.0 TUI/g dict	78.6 ± 9.5	1.61 ± 0.14

* Differences statistically insignificant

Table 4. Effect of trypsin inhibitor (TI) and alkylresorcinols (AR) from rye and wheat bran on true digestibility (TD) of protcin by rats

	TI activity/g and AR content	Coefficient of protein digestibility S.D.
untreated rye bran	I TUI/g 4.2 mg AR/g	71.0 ± 1.5 b
autoclaved rye bran	0 TUI/g 4.2 mg AR/g	63.5 ± 1.9 c
acetone-extracted (-AR) and autoclaved rye bran	0 TUI/g 0.8 mg AR/g	65.0 ± 2.5 c
wheat bran	0.216 TUI/g 2.1 mg AR/g	80.3 ± 1.8 a
acetone-extracted and autoclaved wheat bran	0 TUI/g 0.35 mg AR/g	72.8 ± 3.7 b

Division into homogenous groups performed with Duncan's test ($P \leq 0.05$)

DISCUSSION

Our experiments with rye and triticale grain containing more trypsin inhibitors than wheat grain demonstrated that protein TD is not improved by prolonged (30-120 min) cooking in water. Similar results were obtained by

Sosulski et al. [17] who fed untreated or autoclaved wheat to mice: after TI activity elimination there was no improvement of protein digestibility, body growth or feed utilization. These authors also found that protein digestibility failed to improve after AR extraction, and these results confirm our findings regarding digestibility of rye and wheat bran proteins deprived of AR (by extraction with acetone). TI isolated from triticale grain and added to the casein diet in proportions corresponding to their content in Grado and Lasko triticale did not produce statistically significant reductions of digestibility coefficients of casein protein.

Pedersen et al. [13] obtained similar results, that is to say they did not observe reduced digestibility of powdered milk after addition to it of pepsin-resistant TI isolated from the Riso barley mutant characterized by a TI level four times that in farm-grown barleys. However, a dose of TI isolated from Dańkowskie Złote rye about twice exceeding the inhibitor level in this grain reduced casein protein digestibility from 91 to 86% ($P = 0.05$). This fact shows that TI measurement must be taken into account when selecting rye and triticale (which inherits rye TI) having improved nutritional characteristics.

In our experiments, additions of TI isolated from rye to chicken diet (0.75, 1.5 and 3.0 TUI/g) did not inhibit growth or affect diet utilization by chicken, which suggests that these animals are less susceptible to TI than laboratory rats or mice. Sosulski at al. [17] found that nutrition indices did not improve when chicken were fed with autoclaved rye grain in which TI activity was eliminated.

CONCLUSIONS

1. Trypsin inhibitor in the medium concentration found in rye grain does not reduce protein digestibility coefficients. TI activity ought to be considered when evaluating rye and triticale cultivars, since forms with upwards of 2 TUI/g grain are not desirable.

2. Alkylresorcinols of rye or wheat grain do not reduce the digestiblity of protein in rye grain and bran, even at the high concentrations found in bran.

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STUDIA NAD ANTYODŻYWCZYMI SUBSTANCJAMI ŻYTA. IV. WPŁYW INHIBITORÓW TRYPSYNY ZIARNA ŻYTA, PSZENICY I PSZENŻYTA NA STRAWNOŚĆ BIAŁKA

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Streszczenie

Stwierdzono doświadczalnie na szczurach laboratoryjnych brak poprawy współczynników strawności białka (TD) ziarna żyta i pszenżyta poddanych inaktywacji inhibitorów enzymów proteolitycznych przez gotowanie w wodzie oraz w strawności białka otrąb żytnich i pszennych ekstrahowanych acetonem (denaturacja TI usunięcia alkilorezorcyn) oraz poddanych autoklawowaniu (1 h — inaktywacja TI). Dodatek izolowanego inhibitora trypsyny (TI) z pszenżyta Lasco i Grado, w ilości 1-1,29 TUI/g diety kazeinowej (tj. w koncentracji występującej w surowym ziarnie) spowodował nieistotny statystycznie spadek współczynników strawności białka. W przypadku izolatu TI z ziarna żyta istotny spadek TD nastąpił po podaniu 2,64 TUI/g diety, co odpowiada ok. 2-krotnemu stężeniu inhibitora w porównaniu z wartościami średnimi dla żyta. Podobnie dodanie izolatu inhibitorów trypsyny w ilości 0,75, 1,5 i 3,0 TUI/g diety nie obniżyło przyrostów u kurcząt, ani też nie spowodowało zmian w wykorzystaniu diety na przyrost masy tuszek w ciągu 10-dniowego okresu doświadczenia.