Variability of antinutritive compounds in flaxseed flours

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Abstract

The use of flaxseed flours in the diets of poultry and broilers may be limited by the presence of antinutritive compounds. The content of cyanogenic glycosides, phytic acid, condensed tannins and trypsin inhibitors was evaluated in seven varieties of *Linum usitatissimum*. Phytic acid, condensed tannins and trypsin inhibitors showed significant differences among varieties. Only the concentration of cyanogenic glycosides and phytic acid in the flour deserves attention, while the content of condensed tannins and trypsin inhibitors are to acceptable levels. Since the flax meal is an important source of omega-3 for poultry and broilers, the cyanogenic glycoside and phytic acid contents in linseed has to be reduced to increase the ration to be included in the diet.

Introduction

Flaxseed (*Linum usitatissimum* L.) is one of the world's oldest cultivated crops. The crop is prized for fibre and oil but, recently, a renewed interest in using flaxseed in animal ration is grown.[1,2] Flaxseed may be processed by mechanical expellers or solvent extraction and the residual linseed meal is available as an animal feed ingredient.

Linseed is a rich source of protein and energy.[2] However, it does contain some antinutritional components that need to be considered when feeding this product. Flax seed contains cyanogenic glycosides (linustatin, neolinustatin and linamarin) which, when degraded by β-glucosidase, may release hydrogen cyanide.[1] This compounds is a powerful respiratory inhibitor if absorbed in sufficient quantities.

Phytic acid (inositol exaphosphate) is the major phosphorus storage compound in plant seed. The chelation ability towards many mineral elements (Ca, Mg, Zn, Fe) and the interaction with proteins and digestive enzymes may render them insoluble and biologically unavailable.[3,4] Condensed tannins (flavan-3-ol based biopolymers) are antinutrient compounds which can precipitate proteins, inhibit digestive enzyme and decrease the utilization of vitamins and minerals. They tend to complexing proteins and enzymes, thus rendering the meal protein indigestible directly linking them or indirectly interfering with the action of digestive enzymes such as trypsin and chymotrypsin.[5] Moreover, tannins can create complex with vitamin B12, thus leading to a decrease of its absorption.[6] Trypsin inhibitors in seeds are likely protector molecules against attack by predators. Trypsin inhibitors can have a major impact on nutritional value as they impair protein digestion.[7] The resulting effect is the inhibition of animal growth. In the present study, the content of antinutritive compounds was evaluated in seven flax varieties in order to better understand the limit of application of linseed as feed ingredient.

Materials and Methods

Plant material and flour preparation

Seeds from seven flax varieties were obtained from Semfor (Italy). The varieties were the following: Festival, Kaolin, Linoal, Merlin, Natural, Solal and Valoal. Defatting of flaxseed samples was carried out by extracting the samples with hexane (1:10, w/v, twice).

Extraction and assay of cyanogenic glycosides

Cyanogenic glycosides were extracted from defatted flour with 80% ethanol at 70°C twice. The samples were evaporated to dryness and then resuspended in water: n-butanol (50:50, v/v). After 1 h of agitation, the samples were centrifuged and the lower water layer recovered. Cyanogenic glycosides were assayed on the water extracts by the Spectroquant Cyanide-Test (Merck, Germany) according to the manufacturer's protocol.

Extraction and separation of phytic acid

Phytic acid was isolated from defatted flour using a modified method by de Boland *et al.*[8] Phytic acid was acid extracted with 0.4N HCl plus 0.7M Na_2S0_4 and then precipitated by 15 mM $FeCl_3$ in 0.2N HCl. The phosphorus content of the precipitate was determined, after acid digestion with sulfuric acid, colorimetrically according to Chen *et al*.[9]

Extraction and separation of condensed tannins

The determination of condensed tannins was carried out as described by Butler *et al*.[10] Tannins were extracted from defatted flour with 70% acetone, the samples evaporated to dryness and then resuspended in methanol. Condensed tannins were determined by the vanillin method (absorbance at 500 nm) using catechin as a standard.

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Extraction and assay of trypsin inhibitors

Trypsin inhibitors was extracted from defatted flour with 2 mM glycine buffer pH 11 containing 2 mM NaCl, 10 mM urea and 25 mM EDTA. Trypsin inhibitor activity was measured using N-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate. A solution containing 200 μL of extracted sample and 200 μL (40 μg/mL) trypsin were pre-incubated at 37°C for 3 min. Then 500 μL (0.4 mg/mL) of BAPNA (pre-warmed to 37°C) was added to start reaction. After incubation at 37°C for 10 min, 100 μL 30% (v/v) acetic acid was added to terminate reaction and then subjected to centrifugation. Activity of trypsin was measured by the absorbance at 410 nm due to p-nitroaniline released. One unit of trypsin inhibitor was defined as 0.01 decreases in absorbance at 410 nm under assay conditions compared with the control (without inhibitor).

Statistical analysis

All statistical analysis were performed by SPSS version 16.0 software. Analysis of Variance (ANOVA) was applied to establish significant differences ($P<0.01$) between flax genotypes in the levels of antinutritive compounds. Mean separation was performed using Duncan's test and referring to $P\leq0.05$ probability level. Pearson's correlations between antinutritive compounds were also calculated.

Results

In Table 1 is shown ANOVA for phytic acid, cyanogenic glycosides, condensed tannins and trypsin inhibitor for seven varieties of flax. ANOVA showed highly significant genotypic variation (P<0.01) for all the antinutritive compounds with the exception of cyanogenic glycosides.

In Table 2 are shown the levels of antinutritive compounds for seven flax varieties. As can be seen, the mean content of cyanogenic glycosides was 0.64 g Kg^{-1} dry matter (DM) (Table 2). Values of cyanogenic glycosides in excess of 100 ppm are considered a danger to health.[11,12] Thus, the mean level of 640 ppm here observed suggests that flax flour in animal rations cannot exceed 15% in DM.

Phytic acid ranged from 33.4 to 40.7 g Kg^{-1} DM, with the variety Natural exhibiting the highest level of phytic acid. Significant differences between genotypes were put in evidence by the Duncan's range test, with the group marked with the letter c (Festival, Kaolin, Linoal and Valoal) showing contents below the general mean. The phytic acid content in flax results higher than the content of phytic acid in soybean,[13] which flour is the main ingredient used in animal diet.

The variation of the condensed tannin content in different flax varieties was quite high. In Table 2, the contents varied from 0.43 to 0.82 g Kg^{-1} of defatted flour. These levels are no toxic in animal diets since they are definitely below 1% DM.[14]

Also the variation in trypsin inhibitor activity in different flax varieties was quite high. In Table 2, the activity varied from 12.5 to 24.6 unit mg^{-1} of defatted flour. Varieties Festival, Natural and Valoal (marked with letter e by the Duncan's range test) exhibited trypsin inhibitor activities below the general mean. These activities resulted lower than those observed in some cereals or legumes.[5,15]

In Table 3 is shown the correlation matrix calculated for the different antinutritional compounds. In this table, Pearson correlation coefficients are given as a measure of linearity between two class of compounds. From Table 3, it becomes clear that trypsin inhibitors are highly inversely correlated (P<0.0.1) with condensed tannins and correlated (P<0.0.5) with cyanogenic glycosides.

Discussion

A renewed interest of flax oil for biodiesel, make the remaining meal available for use as animal feed.[16] Linseed can serve as a useful source of nutrients for many class of livestock and could satisfactory replace the protein equivalent of soybean meal in the diet. However, high levels of flax flour caused reduction in gain and feed efficiency in broilers and poults.[17,18] This adverse effect was speculated to be due to the toxicity of cyanogenic glycosides. Instead, ruminants are more tolerant of these compounds and linseed may be added at 12-14% (DM basis) to their diets.[2] Linseed is commonly used even in feeding of swine and horses.[1,2] In this work, we confirm that attention have to be put on the content of these substances for poultry and broiler rations. Data here presented would suggest to diluite at least seven times linseed in animal feed, although boiling or autoclaving of seeds can partly remove cyanogenic glycosides.[19] Necessarily having to dilute the contents of cyanogenic glycosides in flour is also simultaneously lowered phytic acid to acceptable levels. Low cyanogenic glycoside levels would also lead to a low activity of trypsin inhibitors (Table 3). A variety like Festival, being low in antinutritive compounds (phytic acid and trypsin inhibitors), would be a good starting material for breeding (Table 2). Festival variety reproduced in field confirmed to have less cyanogenic glycosides and trypsin inhibitors than other varieties (data not shown). Moreover, must be taken into account that cyanogenic glycoside content is significantly influenced by growth location and season.[20] Thus, growth conditions have to be also depth investigated since they may alter the cyanogenic glycoside content.

Flax is one of the most concentrated sources of omega-3 unsaturated fatty acid available in natural feedstuffs for poultry.[21] However, the maximum recomemnded inclusion of linseed in broiler diets is 3%.[1,17] To enhance the commercial value of linseed meal for broilers and poultry and to avoid expensive processes (heat treatments) is therefore advisable to decrease the content of cyanogenic glycosides.

Table 1. Mean square and F value from Analysis of Variance (ANOVA) for antinutritive compounds in flours of seven varieties of *Linum usitatissimum*.

Chemical compound	d.f.	Mean square	F value
Cyanogenic glycosides	6	0.017	7.49
Phytic acid	6	20.68	18.50*
Condensed tannins	6	0.053	10.31*
Trypsin inhibitors	6	56.46	62.74*

d.f., degrees of freedom. *Significant at P≤ 0.01.

Table 2. Antinutritive compounds in flours of seven varieties of *Linum usitatissimum*.

Variety	Cyanogenic glycosides*	Phytic acid*	Condensed tannins*	Trypsin inhibitors**
Festival	0.63	33.4 (c)	0.82 (a)	14.0 (cde)
Kaolin	0.57	34.2 (c)	0.63 (b)	15.7 (c)
Linoal	0.59	34.1 (c)	0.46 (cd)	20.4 (b)
Merlin	0.60	36.8 (b)	0.69 (b)	15.0 (cd)
Natural	0.59	40.7 (a)	0.56 (bcd)	12.5 (e)
Solal	0.77	36.5 (b)	0.43 (d)	24.6 (a)
Valoal	0.72	34.0 (c)	0.58 (bc)	13.8 (de)
Mean±SE	0.64±0.02	35.6±0.6	0.60±0.03	16.6±0.9

*Data expressed as g Kg^{-1}; **data expressed as unit mg^{-1}. Means with different letters in parentheses within the same row differ significantly by Duncan's range test (P≤0.05).

Table 3. Pearson correlation coefficients (r) among antinutritive compounds in seven varieties of *Linum usitatissimum*.

	Cyanogenic glycosides	Phytic acid	Condensed tannins	Trypsin inhibitors
Cyanogenic glycosides	1			
Phytic acid	-0.013	1		
Condensed tannins	-0.215	-0.199	1	
Trypsin inhibitors	0.456*	-0.120	-0.609**	1

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

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