Reduction in the content of antinutritional substances in pea seeds (*Pisum sativum* L.) by different treatments

R. Dvořák[1], A. Pechová[1], L. Pavlata[1], J. Filípek[1], J. Dostálová[2], Z. Réblová[2], B. Klejdus[3], K. Kovaříčík[4], J. Poul[5]

[1]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[2]Institute of Chemical Technology, Prague, Czech Republic
[3]Mendel University of Agriculture and Forestry, Brno, Czech Republic
[4]Veterinary Research Institute, Brno, Czech Republic
[5]Agricultural and Commercial Cooperative Society Žichlínek, Žichlínek, Czech Republic

ABSTRACT: The goal of the trial was to reduce the content of antinutritional substances in pea (*Pisum sativum* L.) seeds in order to enhance its use in livestock nutrition. A variety of field pea (*Pisum sativum* L.) with a high content of antinutritional substances and favourable production traits (Gotik) was chosen. Native and heat-treated pea seeds were used to collect representative samples (n = 6) for analytical purposes. The technology (V-0 technology, Czech patent No. 285745) was further modified by adjusting the reactor temperature, the duration of exposure to that temperature, and the duration of ageing of the material treated in this way (V-I and V-II technologies). The methodology of treatment is based on exposing pea seeds to vapour, organic acids and selected oxides. The monitored parameters included antinutritional substances. As far as the antinutritional substances were concerned, the content of trypsin inhibitors in native pea seeds (P) was around 15.4 ± 0.5 TIU. After treatment with technologies V-0, V-I, and V-II its activity dropped by 83.8, 80.5 and 83.8%, respectively. The pre-treatment titre of lectins (P) was 717 ± 376. It dropped by 70.3, 35.7 and 73.2% after treatment with technologies V-0, V-I and V-II, respectively. The content of tannins measured by the amount of gallic acid in native pea seeds was 49.1 ± 2.7 mg per kg. It dropped by 41.4, 32.0 and 46.2% after the application of the above-mentioned technologies. The content of indigestible oligosaccharides causing flatulence was less affected by the treatments. The pre-treatment content of raffinose was 9.5 ± 0.5 g/kg. The drop associated with the treatment was 9.5, 6.3 and 10.5%, respectively. The pre-treatment content of stachyose was 21.4 ± 0.8 g/kg and after treatment with technologies V-0 and V-II it dropped by 7.0% and by 16.4%, respectively. The application of technology V-I did not result in a drop in the content of stachyose. The content of verbascose in native pea seeds was 16.1 g/kg and the treatment with technologies V-0; V-I and V-II resulted in a drop by 7.5, 5.6 and 20.5%, respectively. As for the detected phenolic acids, with the exception of caffeic acid, not a drop, but an increase in their content was recorded. Isoflavone oestrogens such as daidzein and genistein also recorded a small increase in their content. The results of the trial lead us to conclude that the above-described methods of pea seed treatment, especially the V-II variant, proved to be useful and can be recommended for practical use.

Keywords: pea (*Pisum sativum* L.*)* seeds; trypsin inhibitors; lectins; tannins; raffinose; stachyose; verbascose; daidzein; genistein

Legumes rank among the traditional commodities produced in the Czech Republic. Like all other legumes, pea seeds provide an important source of proteins, fibre, potassium, B vitamins and carbohydrate complexes (Savage and Deo, 1989; Corbett et al., 1995; Alonso et al., 1998). Compared with soybeans and oilseed rape, pea seeds contain less protein (24% vs. 49.9% and 40.6%). The content

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of undegraded protein amounts to around 22% while the respective levels for soybeans and oilseed rape are 35% and 28%. The content of fibre in pea seeds and soybeans is similar: 6.9% and 7.0% while oilseed rape has a higher fibre content, around 13.2%. Thanks to the high content of high-quality starch (54%; McLean et al., 1974), pea seeds can serve as a very good source of energy as well as protein for milk cows at a lower production level or in the declining stage of lactation period when the amount of milk collected per day is not higher than 25 kg (Khorasani et al., 1992). Pea seeds have a relatively favourable lysine content, but the content of methionine and tryptophan has to be regarded as insufficient. As for mineral substances, potassium and phosphorus dominate while the calcium content is relatively low. The microelements represented in pea seeds include especially iron, manganese, copper, cobalt, and zinc (Savage and Deo, 1989; Sommer et al., 1994). Pea seeds are highly digestible, but contain some antinutritional substances. According to their importance, these are: trypsin inhibitors, lectins (phytohaemagglutinins), indigestible oligosaccharides causing flatulence, gallic acid, and other phenolic acids and substances with phytoestrogenic effects (Kalač and Míka, 1997).

Protease inhibitors are substances of proteinic or polypeptidic nature forming relatively stable complexes with proteolytic enzymes, but without enzymatic activity of their own. They inhibit the growth of animals by inhibiting protein degradation and thus reducing protein digestibility. Their inhibitory effect on proteases causes secondary hypertrophy and hyperplasia of the pancreas. Young and monogastric animals are the most vulnerable (Gatel, 1994; Kalač and Míka, 1997).

Lectins are substances of proteinic nature differing from all other plant proteins especially in their ability to bind to polysaccharides of the epithelium of the digestive system. Fortunately, as lectins in pea seeds do not bind to the epithelium of the digestive system very firmly, mainly the young are at risk. The general negative effects of lectins include growth retardation to stoppage and damage to the epithelium of the digestive system, especially the intestine, leading to its hypertrophy. Other effects include hypertrophy of the pancreas, hepatic hypertrophy, and premature thymic atrophy in the young. Last but not least, lectins have a negative effect on intestinal microflora and inhibit the enteric enzyme activity (Oboh et al., 1998).

Saccharides such as raffinose, stachyose and verbascose are significantly represented in leguminous seeds. It is mainly stachyose and verbascose that are regarded as the key oligosaccharides in pea seeds (Reddy et al., 1980; Alonso et al., 2001; Vidal-Valverde et al., 2003). The above-mentioned sugars have no reducing effect and contain α-galactosidoglucosic and α-galactosidogalactosic bonds. The intake of greater amounts of legumes leads to gas production in the digestive system.

Phenolic substances are a very numerous, and also very heterogeneous group of substances contained in plants. Tannins coagulate proteins and are characterized by an astringent taste (Kalač and Míka, 1997). It is relatively difficult to assess tannins as in spite of ranking among antinutritional substances, they also have a positive effect in ruminants, preventing ruminal tympany (Vrzgula et al., 1990). The ruminal microflora can adapt itself to different contents of tannins in various kinds of fodder (Reed, 1995). Phenolic acids decrease cell digestibility, some of them being toxic to the ruminal microflora and interfering with some enzymes (Kalač and Míka, 1997). Especially ferulic, *p*-coumaric and hydroxycinnamic acids have an inhibitory effect. By contrast, syringic acid and *p*-hydroxybenzoic acid enhance the growth of some ruminal bacteria. Some of them affect the taste and smell of the seeds and/or mediate a sense of bitterness, acidity and astringency (Kalač and Míka, 1997). Phenolic substances also include isoflavones, substances improving the natural resistance of plants to diseases and insects, and phenolic substances contained in chloroplasts have an oestrogenic effect. The oestrogenic activity of isoflavones is relatively low. Phytoestrogens may impair the secretion of animal oestrogens, thus inhibiting ovulation, transport of ova in the oviduct, and may provoke false heat, false pregnancy, and degenerative changes in the reproductive system. The above-mentioned mechanisms affect the metabolic functions (Kalač and Míka, 1997).

Intensive breeding efforts have helped to reduce the content and range of antinutritional substances. The issue should, however, be viewed in its full context as antinutritional factors may also have a number of positive roles in plants. Most often these include a positive effect contributing to the protection of different parts of the plant body from insects and microorganisms as well as higher animal consumers. They also help to protect the cytosol from endogenous proteases and are utilized as reserve

proteins (Norton, 1991). According to some theories and hypotheses plants can transform primary metabolites into antinutritional substances (tannins) in stress situations. It has also been recognised that varieties with a higher content of antinutritional substances produce higher yields, possibly due to the existence of mechanisms of higher resistance of these varieties to diseases and animal predators. It may be assumed in this context that the cultivation of varieties free of antinutritional factors but vulnerable to insects would not be an optimum solution to the problem. Combining two approaches consisting in partly reducing the content of antinutritional substances by cultivation while using some adequate and economic technologies of legume treatment aimed at significantly reducing or removing antinutritional substances prior to their use in human and animal nutrition appears as a better way of handling the problem.

The goal of the trial was to verify an industrially applicable treatment of seeds of *Pisum sativum* L. designed by us to reduce the content of antinutritional substances.

MATERIAL AND METHODS

A variety of field pea (*Pisum sativum* L.) with a high content of antinutritional substances and favourable production traits (Gotik) was chosen. This source material (P) was then treated with three technological variants (V-0; V-I; V-II).

The technology referred to as V-0 applied the results of Project RP 7187 of the National Agency for Agricultural Research, leading to the formulation of a technology of treatment of pressed oilseed rape used to reduce the content of antinutritional substances (glucosinolate content was reduced by more than 90%). This technology is protected by Czech patent No. 285745. The above-described technological modification is based on exposing coarsely extracted pea seeds to moist heat, organic acids and selected oxides in a reactor. The V-0 technology was further modified by adjusting the reactor temperature, the duration of exposure to that temperature and the duration of ageing of the material treated in this way. The V-I technology was defined by an exposure to the temperature of 80 to 90°C in the reactor for 30 minutes, the duration of ageing being 40 minutes. The V-II technology was characterized by an exposure to the temperature of 80–90°C in the reactor for as long as 50 minutes, the ageing interval being 60 minutes. Native pea seeds of the variety Gotik before treatment are referred to by the symbol (P).

The above-described technological modifications were developed on the basis of preliminary laboratory trials. Pilot trials conducted *in vitro* conditions provided a basis for application of the above-described technological modifications on a technological equipment of the cooperating institution Agricultural and Commercial Cooperative Society Žichlínek, which owns a reactor with water and vapour supply, temperature regulation and a stirring unit.

Pisum sativum L. samples were analysed for the most important antinutritional substances. Representative samples (n = 6) of native *Pisum sativum* L. prior to treatment (P) and subjected to the three types of treatment (V-0; V-I; V-II) were collected as materials for these analyses.

Trypsin inhibitors were determined using a methodology developed in Agritec Ltd. laboratories by the two-cycle method, the sample dilutions being 3× and 5× in triplets in microtitration plates at 37°C. Absorbance was taken at 410 nm using an ELISA reader. The calculations were made in TIU, one TIU being defined as the drop in absorbance A_{410} within 10 minutes by 0.01 per mg of determined mass.

The haemagglutination activity of lectins present in pea seeds was determined using our own modification of the method described by Grant et al. (1983). The haemagglutination activity is expressed directly by the lectin titre at which a microscopic evidence for haemagglutination becomes available.

Indigestible oligosaccharides causing flatulence (raffinose, stachyose, and verbascose) as well as saccharose were determined within one analysis (in sample extracts prepared by extraction with 80% boiling ethanol for 1 hour) by HPLC with refractometric detection using analyte separation to a stationary stage with combined amino groups. The method has commonly been used for determination of the different mono- and oligosaccharides in foodstuffs and similar samples (Scott, 1992). The method was slightly modified to suit the material analysed and saccharide determination and validated at the Department of Chemistry and Food Analysis of the Institute of Chemical Technology, Prague (Zátopková et al., 2001). The correctness of the results provided by this method was verified as the yield of extraction of the added known

amounts of saccharides. The yield was not statistically significantly lower than 100%. Repeatability of this method including the effect of extraction was determined. This repeatability expressed as a relative standard deviation ranged between 1% and 3%. Detection limits for saccharides were as follows: 0.1 g/kg for saccharose, 0.2 g/kg for raffinose, 0.3 g/kg for stachyose and 0.4 g/kg for verbascose. Determination limits are three times higher.

Tannins, as measured by the content of gallic acid, were determined using the method developed by Kahkonen et al. (1999). Phenolic acids were determined using liquid chromatography on an HP 1100 (Hewlett Packard) liquid chromatograph. Extraction was performed using a fex IKA Werke extraction device.

As for substances with oestrogenic effects, daidzein and genistein were determined. Both substances were determined by HPLC on an HP 1100 (Hewlett Packard) liquid chromatograph. Extraction was performed using a fex IKA Werke extraction device. The used HPLC-grade methanol (> 99.9%; v/v) was supplied by Merck (Darmstadt, Germany). Isoflavones, phenolic acids and all other reagents of ACS purity were purchased from Sigma Aldrich (St. Louis, USA). The stock standard solutions of isoflavones and phenolic acids (c = 1 mg per ml) were prepared in aqueous methanol (7:3, v/v) and stored in darkness at 4°C. The working standard solutions (0–300 μg/ml) were prepared daily by diluting stock solutions in aqueous methanol. All solutions were filtered through 0.45 μm teflon membrane filters (MetaChem, Torrance, USA) prior to HPLC separations.

An HP 1100 liquid chromatographic system (Hewlett Packard, Waldbronn, Germany) was equipped with a vacuum degasser (G1322A), binary pump (G1312A), auto-sampler (G1313A), column thermostat (G1316A) and UV-VIS diode array detector (model G1315A) working at 254 nm. The isoflavones and phenolic acids were separated on an Atlantis dC_{18} reversed-phase fast chromatographic column (20 mm × 4.6 mm, 3 μm particle size, Waters Corp., Ireland).

Extraction procedures

A homogenised sample (100 ± 0.5 mg) was used for stirred extraction using a computer-controlled commercially available fex Ika Werke 50 device (IKA-Werke GmbH and Co., Staufen, KG, Germany) related to Soxhlet apparatus. A two-step temperature program (first step: cooling/heating block temperature of 160°C for 30 min, cooling/heating block 30°C for 5 min; second step: 160°C for 30 min, cooling 30°C for 5 min) was applied to isolate 2M HCl (v/v) aqueous solutions (50 ml). The obtained extract was treated as described above (Klejdus et al., 1999; Klejdus and Kubáň, 2000).

The resulting antinutritional factor contents were statistically processed using the F-test to evaluate the variance of the individual sets and, depending on the result, paired Student's test for equal/unequal variance sets. The statistical evaluation was performed using MS-Excel® (Microsoft Corp., Inc.) software.

RESULTS AND DISCUSSION

The results specifying the trypsin inhibitor content in untreated and treated pea seeds are presented in Table 1. The table shows that the treatment had a very positive effect, namely a drop in the trypsin inhibitor. The content of this undesirable antinutritional factor was reduced by 84%. All three treatment variants provided very positive results and the differences compared with the original mass were statistically significant ($P < 0.001$). The technological modifications based on the exposure to temperature and chemical substances (organic acids and oxides) in the reactor seem to be the main factors that caused the drop in inhibitory activity. Our results may be compared with those of Alonso et al. (1998), who tested some other treatments based on maceration, dehulling and seed sprouting and who did not observe a drop in the antitrypsin activity. Similar positive results of pea seed heat treatment were recorded by Habiba (2002). As for the trypsin inhibitor content, all three treatment variants may be regarded as very appropriate. The best results were yielded by variants V-0 and V-II.

The changes in lectin content determined on the basis of their haemagglutination activity are presented in Table 1. The highest haemagglutination activity titre was recorded in untreated pea seeds (717 ± 376). The results also show a relatively high degree of variability. The treatment resulted in a significant drop in the haemagglutination activity titre. The titre dropped to 213 ± 60 in variant V-0 and to 192 ± 64 in variant V-II. The drop in the titre was less significant in variant V-I. The difference in the titre between untreated pea seeds and

Table 1. Contents of trypsin inhibitors, gallic acid and titres of lectins (average ± standard deviation) in native (P) and treated (V-0; V-I; V-II) field pea samples

		P	V-0	V-I	V-II
Gallic acid (mg/kg)		49.1 ± 2.7	28.8 ± 1.5	33.4 ± 1.7	26.4 ± 1.4
	V-0	***			
Comparison between groups	V-I	***	**		
	V-II	***	*	***	
Trypsin inhibitors (TIU)		15.4 ± 0.5	2.5 ± 0.2	3.0 ± 0.3	2.5 ± 0.4
	V-0	***			
Comparison between groups	V-I	***	*		
	V-II	***			
Lectins (titre)		717 ± 376	213 ± 60	461 ± 102	192 ± 64
	V-0				
Comparison between groups	V-I		**		
	V-II	*		**	

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

treatment variant V-II reached the level of statistical significance ($P < 0.05$). The results are in line with published data as an exposure to temperatures around 100°C and legume cooking are held to diminish the lectin content (Kalač and Míka, 1997; Habiba, 2002). The methods such as seed dehulling, maceration or seed sprouting for 24, 48 or 72 hours did not have any effects in terms of the lectin content reduction (Alonso et al., 1998). We may conclude that the technological treatment had a positive effect consisting in a reduction in lectin content, the greatest drop in haemagglutination activity being achieved in V-II and V-0 variants.

The determined contents of indigestible oligosaccharides causing flatulence (raffinose, stachyose, verbascose) are presented in Table 2. The raffinose content of untreated pea seeds was 9.5 ± 0.5 g/kg. The most significant drop after treatment was

Table 2. Contents of indigestible oligosaccharides causing flatulence (mean ± SD) in native (P) and treated (V-0; V-I; V-II) field pea samples

		P	V-0	V-I	V-II
Saccharose (g/kg)		24.8 ± 0.7	26.6 ± 1.4	29.7 ± 1.1	27.6 ± 1.3
	V-0	*			
Comparison between groups	V-I	***	**		
	V-II	**		*	
Raffinose (g/kg)		9.5 ± 0.5	8.6 ± 0.4	8.9 ± 0.4	8.5 ± 0.3
	V-0	**			
Comparison between groups	V-I				
	V-II	**		*	
Stachyose (g/kg)		21.4 ± 0.8	19.9 ± 0.5	22.5 ± 0.4	17.9 ± 0.5
	V-0	**			
Comparison between groups	V-I	*	***		
	V-II	***	***	***	
Verbascose (g/kg)		16.1 ± 0.8	14.9 ± 0.9	15.2 ± 1.0	12.8 ± 0.6
	V-0	*			
Comparison between groups	V-I				
	V-II	***	**	**	

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

observed in variant V-II: the drop by 10.5% is a relatively small one although it reached the level of statistical significance. Similar results may be documented for variants V-0 and V-I.

As for stachyose, the greatest drop – by 16.4% – was also observed after V-II treatment. Although the drops after all types of treatment compared with untreated pea seeds reached the level of statistical significance, we do not consider the result as entirely satisfactory with regard to our objectives. The content of verbascose in untreated pea seeds was 16.1 ± 0.8 g/kg. In this case, the greatest drop in the content of this oligosaccharide – by 20.5% – was also observed after V-II treatment. The difference between native and treated pea seeds was highly statistically significant ($P < 0.001$). The drop in verbascose was, in fact, the highest drop that occurred. Although not optimum, the result may be regarded as encouraging. It is also to be said that stachyose and verbascose are the most abundant oligosaccha-

Table 3. Contents of phenolic acids (average ± standard deviation) in native (P) and treated (V-0; V-I; V-II) field pea samples

		P	V-0	V-I	V-II
Protocatechuic acid (mg/kg)		25.9 ± 6.7	36.5 ± 8.2	42.9 ± 12.1	36.4 ± 14.6
	V-0	*			
Comparison between groups	V-I	*			
	V-II				
p-hydroxybenzoic acid (mg/kg)		15.3 ± 5.8	22.8 ± 7.5	22.2 ± 8.1	19.3 ± 5.9
	V-0				
Comparison between groups	V-I				
	V-II				
Vanillic acid (mg/kg)		15.5 ± 8.5	29.3 ± 13.5	30.6 ± 14.3	23.7 ± 11.2
	V-0				
Comparison between groups	V-I				
	V-II				
Caffeic acid (mg/kg)		5.5 ± 2.4	3.6 ± 3.0	3.5 ± 3.4	2.2 ± 2.0
	V-0				
Comparison between groups	V-I				
	V-II				
Syringic acid (mg/kg)		0.0 ± 0.0	8.8 ± 5.9	13.0 ± 7.9	6.3 ± 2.2
	V-0	**			
Comparison between groups	V-I	**			
	V-II	***			
p-coumaric acid (mg/kg)		1.2 ± 2.0	3.6 ± 2.9	6.0 ± 4.5	2.7 ± 2.2
	V-0				
Comparison between groups	V-I				
	V-II				
Ferulic acid (mg/kg)		12.4 ± 2.9	18.4 ± 5.9	19.0 ± 7.6	14.6 ± 4.6
	V-0				
Comparison between groups	V-I				
	V-II				
Sinapic acid (mg/kg)		10.7 ± 6.1	12.2 ± 4.8	17.3 ± 7.0	13.4 ± 5.2
	V-0				
Comparison between groups	V-I				
	V-II				

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

rides in pea seeds as reported by Reddy et al. (1980), Alonso et al. (2001) and Vidal-Valverde et al. (2003). Stachyose and verbascose were also represented at the highest amounts according to our results. On the other hand, it should be recognized that flatulent oligosaccharides do not rank among toxic substances. They are substances rather diminishing the quality of the environment and animal welfare and/or the attending staff welfare. The saccharose content increased after all types of treatment, by 20% in V-I, and we consider such an increase as a positive result due to the higher availability of cell saccharose enabling an increase in available energy for the rumen microflora upon including treated pea seeds in cereal mixtures for ruminants. The increase in saccharose content seems to be due to degradation of the trisaccharide raffinose during the treatment of pea seeds.

The contents of tannins, as measured by the content of gallic acid, are presented in Table 1. The content of tannins decreased after treatment, by 46.2% in the V-II technological variant. The other treatment variants also yielded positive results, and the difference between the contents of native and treated pea seeds was highly statistically significance in all cases ($P < 0.001$). The drop by 46% may be regarded as satisfactory as tannins also have a positive effect in ruminants, especially an anti-foam effect, thanks to which they prevent ruminal tympany, and/or may enhance the content of undegradable protein in the rumen. A drop in the content of tannins in pea seeds by 20% was recorded by Alonso et al. (1998), who applied dehulling, maceration and sprouting. Conventional cooking for 40 minutes reduced the content of tannins by 6% only (Habiba, 2002). The technological modifications proposed by us yielded considerably better results.

The contents of phenolic acids are presented in Table 3. The results concerning phenolic acids we arrived at were interesting and rather surprising at the first glance. The content of all phenolic acids except caffeic acid increased after all treatments, by as much as 100% for vanillic acid. Syringic acid, which had not been identified in the native samples at all, was determined after treatment. Our explanation of these results is that the treatment made the above-mentioned acids available; they are otherwise contained in cells and cell organelles and the analytical determination does not lead to their full extraction from native pea seeds. The treatment increases the utilization of phenolic acids by disturbance of cell walls and cell organelles. It is difficult to interpret the results as these substances can be found in other legumes or seeds of a wide range of plants and crops including soybeans, while these feeds are regarded as superb in terms of animal nutrition. Available literature presents no data on the content of these acids in pea seeds or other legumes. We therefore regard our results as not of ultimate significance as far as the utilization of *Pisum sativum* L. in the nutrition of ruminants or other farm animals is concerned.

The results regarding the contents of isoflavone oestrogens, daidzein and genistein, are presented in Table 4. It is noteworthy that the lowest content of these substances was determined in untreated native pea seeds. The daidzein content was 2.2 mg/kg and genistein was not identified in native pea seeds at all. The content of these substances increased several times after treatment. The highest levels were associated with the V-I treatment. The content of daidzein and genistein increased to 55.3 ± 31.8 and 22.5 ± 18.4 mg/kg, respectively. The increase in the content of these phytoestrogens in treated mate-

Table 4. Content of isoflavone oestrogens (average ± standard deviation) in native (P) and treated (V-0; V-I; V-II) field pea samples

		P	V-0	V-I	V-II
Daidzein (mg/kg)		2.2 ± 4.9	22.5 ± 7.8	55.3 ± 31.8	29.3 ± 13.5
	V-0	**			
Comparison between groups	V-I	*			
	V-II	**		–	
Genistein (mg/kg)		0.0 ± 0.0	8.8 ± 5.3	22.5 ± 18.4	6.8 ± 3.6
	V-0	**			
Comparison between groups	V-I	*			
	V-II	**			

*$P \leq 0.05$; **$P \leq 0.01$;

rial was probably due to the release of these substances from stronger bonds as a consequence of treatments. Similar situations may occur e.g. during ensilaging when oestrogen levels in silage material also increase, oestrogens being released by the enzymatic action (Kalač and Míka, 1997). In literature, pea seeds are not held to be a legume associated with a risk of negative effects of phytoestrogenic substances. Despite the content increase, we are speaking of milligrams here, these amounts having hardly any effect on the reproduction function. The real effect of these substances or groups of substances will have to be verified in animal experiments. The increased content after treatment is, however, not to be regarded as important as these substances may be assumed to be released from pea seeds during fermentation in the rumen or by enzymatic processes in the intestine anyway and therefore the treatment as such did not increase their content, just made them more readily available and triggered off the process before its natural onset during fermentation in the rumen or digestion in the intestine.

The results of the trial lead us to claim that the above-described methods of pea treatment, especially the V-II variant, proved practical and can be recommended for practical use.

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Corresponding Author

Prof. MVDr. Rudolf Dvořák, DrSc., Clinic of Diseases of Ruminants, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1-3, 612 42 Brno, Czech Republic
Tel. +420 541 562 403, fax +420 541 562 413, e-mail: dvorakr@vfu.cz