Ann. Anim. Sci., Vol. 15, No. 1 (2015) 143–154 *DOI: 10.2478/aoas-2014-0080*

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EFFECT OF VARIETY AND PLANT PROTECTION METHOD ON CHEMICAL COMPOSITION AND *IN VITRO* DIGESTIBILITY OF FABA BEAN (*VICIA FABA*) SEEDS*

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Abstract

The aim of the work was to determine the effect of faba bean varieties and plant protection method on their yield of seeds and protein, chemical composition, *in vitro* true digestibility (IVTD) and nutritive value for ruminant feeding. The study was carried out on four cultivars of faba beans grown in similar environmental conditions in a two-factorial field experiment. The variation between cultivars, modified by the plant protection method, in chemical composition and IVTD of seeds may suggest that there would be a need to estimate its real nutritive value used for diet formulation instead of one common value for faba bean species read from feed tables. It was shown that low-tannin varieties are characterized by slightly higher CP and lower NDF content and significantly (P<0.01) higher IVTD digestibility compared to traditional or terminal inflorescence varieties. Regardless of type of faba been variety, the highest seed and protein yields were found when insecticides were applied, with minor effect on chemical composition and the nutritive value of seeds.

Key words: chemical composition, faba bean, nutritive value, ruminants, seeds

In the early nineties, a cheap soybean meal and meat meal became easily available on the European feed market. However, the bovine spongiform encephalopathy (BSE) epidemic contributed to the prohibition of the use of animal origin feeds, while soybean meal became more expensive and in most cases is genetically modified (Vindis et al., 2007). This has forced the farmers in animal nutrition to use alternative sources of vegetable protein on a larger scale. Among the agricultural plants accumulating the greatest amount of protein are legumes (Chrenková et al., 2011), which can be successfully cultivated in conventional or organic farming systems (Micek et al., 2012), because a characteristic feature of legumes is symbiosis with bacteria (*Rhizobium leguminozarum*) binding atmospheric nitrogen and for this reason plants do not require intensive nitrogen fertilization (El Fiel et al., 2002). Moreover, faba bean, an

*Work financed from statutory activity, project no. DS-3217/KZiDZ/2014.

important representative of legumes, helps to reduce phytosanitary development of root rot and gangrene, and improves the physical and chemical properties of soil. In addition, shading the soil for a long growing season promotes the development of desirable soil microorganisms.

The main reason for growing faba bean are the seeds, whose yield in favourable conditions may achieve 4 tons per ha. This feed can be successfully used in nutrition of adult farm animals as the component of diet, mixtures or protein concentrates. The presence of antinutritional compounds in faba bean seeds limits their usefulness in rations for growing animals. However, Strzetelski et al. (1996) showed that in starter mixtures for young calves the soybean-extracted protein meal could be completely replaced by a meal from faba bean seeds (Nadwiślański cultivar), without any negative effect on body weight gains and feed utilization. Also, Bidwell-Porębska and Piotrowski (1991) showed that a 10% share of faba bean seeds in mixtures for calves does not cause any danger of health complications and provides the satisfactory average daily gain.

The main component of the faba bean seed storage proteins are globulins. Their share in the total protein ranges from 55 to 70%. Compared with soybean meal, faba bean protein contains similar quantities of lysine, but less methionine and cystine. Undesirable weather conditions during maturation and seed filling may significantly affect the protein content in seeds, which in turn may be correlated with the type of faba bean varieties (Crépon et al., 2010).

Faba bean seeds contain antinutritional substances such as tannins, lectins, glycosides, phytates, oligosaccharides, and inhibitors of enzymes (trypsin, chymotrypsin, α-amylase). In animal nutrition, the most undesirable are glycosides and tannins. Glycosides are mainly located in the embryo and their content ranges from 0.58 to 1.04% of dry matter (DM), depending on the genotype. In turn, tannin content in the whole seed of traditional varieties is at the level of 2 to 5 $mg \cdot g^{-1}$ but mere epidermis of the seed may contain 6.75 $mg \cdot g^{-1}$ of these substances (Minakowski et al., 1996). Tannins are resistant to heat and dry heating – they neither cause changes in chemical structure nor reduce content and activity. In normal conditions tannins may form low digestible tannin–protein complexes, which reduce the susceptibility of proteins to degradation in the rumen and decrease nutrient digestibility in the whole gastrointestinal tract of ruminants (Frutos et al., 2004).

Pests significantly impact the yield of faba bean seeds. It was observed that the use of chemical plant protection effectively reduces the incidence of insect pests and increases seed yield (Ropek and Kulig, 2002). However, nowadays organic production, which is becoming increasingly popular, aims at minimizing the use of chemical pesticides and fertilizers. Unfortunately, the most important preventive measures in such a farming method are less effective and rely on strengthening the natural enemies of pests and reduction of the vulnerability of crops to diseases and pests. Therefore, in organic farming, losses caused by pests and fungi alike should be expected. Ropek and Kulig (2001) comparing the health status of plants protected chemically and unprotected from the fungi and pests, have shown that significantly more damaged plants were on the untreated plots. In view of the increasing scale of organic production, which withdraws any chemicals from use, there is little in-

formation about the effect of absence of such substances on the yield, digestibility and nutritional value of seeds for ruminants related to different varieties of faba bean.

The aim of the present study was to determine the effect of faba bean varieties as well as kind of plant protection method on chemical composition, *in vitro* true digestibility and nutritive value of seeds for ruminants.

Material and methods

The field experiment was carried out at Experimental Station in Prusy near Kraków using randomized block design with four replications. The study was performed on representative samples of faba bean seeds from 4 cultivars (Nadwiślański, Optimal, Olga and Albus) grown in similar environmental conditions in a two-factorial field experiment (Table 1). The first experimental factor was a type of cultivar (traditional, with terminal inflorescence or low-tannin) and the second the kind of chemical plant protection (no chemical protection, insecticides, fungicides or insecticides and fungicides applied simultaneously). Average tannin content determined by Kuhla and Emmeier (1981) method was 0.472 for Nadwiślański, 0.507 for Optimal, 0.033 for Olga and 0.050 $mg \cdot g^{-1}$ DM for Albus cultivar. In total 64 plots (n=64) were used, each size of 10 m^2. Agronomic treatments and fertilization of plants were in accordance with the generally applicable recommendations.

Table 1. Experimental design

Experimental factor	
Type of variety (cultivar)	Plant protection method*
Traditional (Nadwiślański)	K
	I
	F
	IF
With terminal inflorescence (Optimal)	K
	I
	F
	IF
Low-tannin (Olga)	K
	I
	F
	IF
Low-tannin (Albus)	K
	I
	F
	IF

* K – control – no chemical protection; I – insecticides;
F – fungicides; IF – insecticides and fungicides.

Plant protection

During the experiment 4 different plant protection methods were used: i/ K – control group without any chemical treatment of plants; ii/ I – insecticides – pest control chemicals using Karate 025 EC, Syngenta Crop Protection UK at a dose of 0.15 $l \cdot ha^{-1}$; treatments were carried out 3 times with 4 weeks interval; iii/ F – fungicides – chemical control of fungal diseases using first Sumilex 500 SC, Sumitomo Chemical Company Japan at a dose of 1.5 $l \cdot ha^{-1}$ and then (10 weeks later) Bravo 500 SC, Syngenta Crop Protection UK at a dose of 3 $l \cdot ha^{-1}$; iv/ IF – insecticides and fungicides, the simultaneous control of pests and fungal diseases using chemicals in identical doses and schedule as above.

Chemical analysis

The chemical composition (DM – dry matter, Ash – crude ash, CP – crude protein, TP – true protein, NPN – non protein nitrogen, CF – crude fibre, EE – ether extract and NFE – N-free extractives) was determined using standard methods (AOAC, 2007). Additionally, the content of NDF and ADF was assessed according to the Goering and Van Soest method (1970). Both heat-stable amylase and sodium sulfite were used to obtain NDF with minimum contamination by either starch or protein.

***In vitro* digestibility studies**

In vitro true digestibility (IVTD; Ankom Technology, 2014) of seeds was estimated in Daisy^{II} Incubator (Ankom Co, Fairport, NY) based on the Van Soest et al. (1966) procedure. Dried and ground (1.5 mm) samples of faba bean were placed in the filter bags (F57) made from polyester/polyethylene extruded filaments (50 × 55 mm exterior size). The bags (4 jars × 4 replications) were then incubated in the incubation jar in buffering ruminal fluid for 24 h. According to Ankom recommendation 0.25 g of sample per bag were weighed. Rumen fluid was collected by rumen cannula from two dry Holstein-Friesian cows (LW 660±20 kg) fed with 5 $kg \cdot d^{-1}$ of meadow hay and 3.3 $kg \cdot d^{-1}$ of concentrate mixture with mineral supplementation. The rumen fluid was collected directly before assessments. After incubation, the jars were drained and the bags rinsed thoroughly with cold tap water. The bags with residues were boiled for 75 min. in neutral detergent solution (Ankom^{220} apparatus). After the solution was removed, 2000 ml of hot (90–100°C) H_2O and 4.0 ml of alpha-amylase were used in the first and second rinses of bags. The bags were then dried and weighed.

Nutritive value

The nutritive value of faba bean seeds for ruminants was calculated in French system units (INRA, 2007), using PrevAlim v.3.0 software. Calculations were based on own chemical composition of samples, standard coefficients of ruminal degradability and intestinal digestibility of protein and total tract digestibility of organic matter (INRA, 2007). Energy content was expressed as milk feeding units (UFL) and meat feeding units (UFV), which are the net energy of lactation or growth content relative to that of the reference barley. The protein content of seeds was expressed

as protein truly digested in the small intestine (PDI), where rumen available energy (PDIE) or rumen available nitrogen (PDIN) were limiting for microbial growth in the rumen.

Statistical analysis

To assess the effect of type of faba bean variety and plant protection method the data was subjected to two-way analysis of variance using GLM procedure of SAS (1999–2000). The following model was used:

$$Y_{ijk}=\mu + \tau_j + \lambda_k + (\tau\lambda)_{jk} + \varepsilon_{ijk}$$

where:

Y_{ij} – the random variable that represents the response from the j^{th} observation of the i^{th} treatment,

μ – the overall mean,

τ_j – the effect of the j^{th} treatment on the response,

λ_k – the effect of the k^{th} treatment on the response,

$(\tau\lambda)_{jk}$ – is the interaction effect for the combination of the j^{th} and the k^{th} category,

ε_{ij} – independent random variables.

Means were separated using Student-Newman-Keuls (SNK) multiple range test. Effects between experimental groups were considered statistically significant at $P\leq0.05$. In the Tables, the results are presented as mean values with pooled standard errors.

Results

Yields of faba bean seeds and protein as related to main experimental factors are presented in Table 2. Irrespective of plant protection method the average yield of seeds per ha was 3.40 $t \cdot ha^{-1}$. The highest seed and protein yield (3.71 and 0.94 $t \cdot ha^{-1}$ respectively) has been found for traditional variety type (Nadwiślański), while the lowest was for cultivar Olga (low-tannin variety). Regardless of the variety, the plants protected by insecticides had 159% higher seed yield compared to the control group (non protected; $P<0.05$). In turn, plants protected by fungicides produced 55% more seeds than control ($P>0.05$). The use of both substances together (insecticides and fungicides) resulted in slightly lower yield of seeds than in the case when only insecticides were applied ($P>0.05$).

A similar relationship was observed for protein yield, as a consequence of the seed yield. The highest result was obtained for insecticides treated and the lowest for non-protected plants ($P<0.05$). It is noteworthy that the tested varieties reacted differently to the applied chemicals (Figures 1 and 2).

Table 2. Seed and crude protein yield (t·ha^{-1}) of faba bean (effect of main factors)

Effect		Yield	
		seed	crude protein
Type of variety	Traditional	3.71	0.94
	With terminal inflorescence	3.17	0.80
	Low-tannin	3.31	0.86
Plant protection method*	K	1.77 b	0.47 b
	I	4.58 a	1.15 a
	F	2.95 b	0.73 b
	IF	4.42 a	1.12 a
Interaction (P-value)		0.82	0.77
SEM		0.32	0.08

a, b – values in columns with different letters differ significantly (P≤0.05); SEM – standard error of the mean.

* as – described in Table 1.

Chemical composition of seeds (Table 3) was significantly (P<0.05) influenced by both type of cultivar and plant protection method. Lack of plant protection in group K caused an increase in ash content (P<0.05). The highest ash content was also observed in seeds of low-tannin variety. CP content was the highest in K and F groups, and the lowest in group I (P<0.05). In turn, irrespective of protection method, low-tannin cultivars were characterized by the highest CP and EE contents (P>0.05) and the lowest of NFE (P<0.05). The traditional variety (Nadwiślanski) and that with terminal inflorescence (Optimal) had same average CP content. On the other hand, regardless of the type of variety the highest content of CP was found in seeds from control group (not protected) and protected by fungicides (P<0.05).

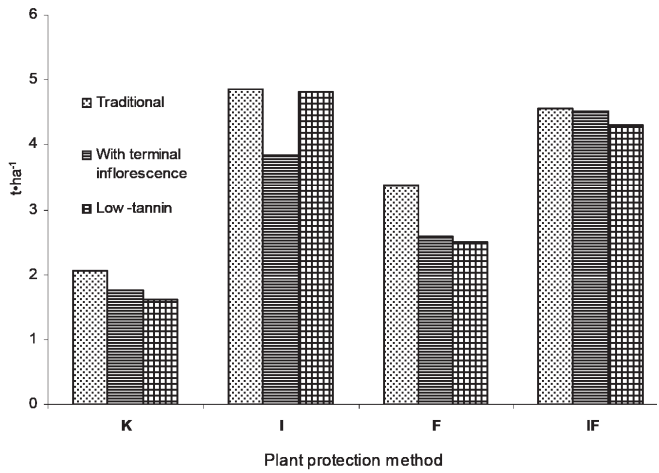

Figure 1. Seed yield (t·ha^{-1}) of different types of faba bean varieties depending on the plant protection method

The average CF value in the seeds of all types of varieties was about 79 $g \cdot kg^{-1}$ but significant differences between means were observed ($P<0.05$). Additionally, faba bean seeds did not differ in NDF content. However, regardless of plant protection method, the NDF content was the lowest in seeds of low-tannin varieties and the highest in seeds of traditional variety ($P>0.05$). Similarly, there were no differences in ADF content between experimental varieties ($P>0.05$).

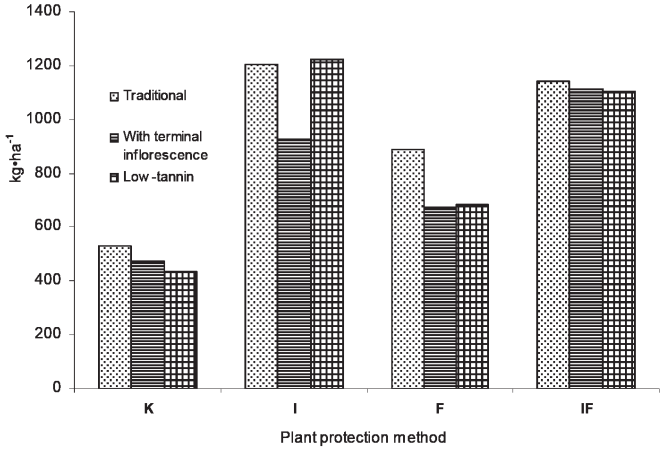

Figure 2. Protein yield of different types of faba bean varieties depending on the plant protection method

Regardless of the plant protection method the highest digestibility IVTD ($P<0.05$) was observed in seeds of low-tannin varieties (Table 3), which was accompanied by the lowest NDF and the highest (numerically) protein content. Generally, a positive correlation ($P>0.05$) was found between crude protein content in seeds and IVTD and negative correlation between NFE content and IVTD ($P>0.05$). There was no effect of plant protection method on IVTD of seeds. Regardless of the type of seed variety, IVTD ranged from 92 to 93%.

A statistically significant ($P<0.05$) effect of both type of variety and plant protection method of faba bean seeds on nutritive value for ruminants expressed in French system units (INRA, 2007) was found (Table 4). Particularly noteworthy is an effect of the type of variety on net energy content (UFL, UFV) but not on the protein value of seeds (Table 4). Low-tannin variety had the highest content of UFL (1.26) and UFV (1.28). These samples were also characterized by high IVTD digestibility and protein content in the seeds (Table 3).

The lowest content of net energy (UFL as well as UFV) was found in the traditional-type seeds because of high NDF and ADF contents and low digestibility. On the other hand, there was a significant effect ($P<0.05$) of plant protection method on protein value of seeds (PDI), mostly correlated with crude protein content in seeds (Tables 3 and 4). Higher PDI values were observed in material from the plants not treated with insecticides (group K and F), where protein concentration was higher in comparison to group I and IF.

Table 3. Chemical composition (g·kg^{-1} DM) and *in vitro* true digestibility (IVTD, %) of faba bean seeds

Type of variety (cultivar)		Plant protection method*	Ash	CP	TP	NPN	CF	EE	NFE	NDF	ADF	IVTD
Traditional (Nadwiślański) *n*=16		K	40	305	255	50	87	14	555	178	151	89.5
		I	34	292	247	46	87	14	573	186	136	88.8
		F	36	309	264	45	77	11	566	150	138	91.7
		IF	37	295	249	46	82	12	574	140	125	92.1
With terminal inflorescence (Optimal) *n*=16		K	39	315	264	51	61	15	569	155	90	93.3
		I	33	285	245	40	70	11	600	139	104	91.8
		F	35	308	254	54	74	11	572	190	128	91.8
		IF	33	290	243	48	73	11	592	144	134	89.2
Low-tannin (Olga) *n*=16		K	44	326	265	61	78	13	539	149	124	93.6
		I	37	304	261	44	84	14	561	176	122	94.5
		F	40	327	265	63	87	14	532	161	131	94.6
		IF	38	303	255	49	81	15	563	145	136	93.2
Low-tannin (Albus) *n*=16		K	45	312	252	60	89	13	541	161	143	94.3
		I	38	294	245	50	92	13	563	164	137	94.2
		F	40	316	257	59	79	12	553	128	113	92.9
		IF	38	300	249	52	83	12	567	140	127	93.4
Effect	Type of variety	Traditional	37 b	300	254	47 b	83 a	13	567 b	164	138	90.5 b
		With terminal inflorescence	35 c	300	252	48 b	70 b	12	583 a	157	114	91.5 b
		Low-tannin	40 a	310	256	55 a	84 a	13	552 c	153	129	93.8 a
	Plant protection method	K	42 a	315 a	259	56 a	79	14	551 b	161	127	92.7
		I	36 d	294 b	250	45 b	83	13	574 a	166	125	92.3
		F	38 b	315 a	260	55 a	79	12	556 ab	157	128	92.8
		IF	37 c	297 ab	249	49 ab	80	13	574 a	142	131	92.0
Interaction (P-value)			0.11	0.90	0.87	0.22	0.57	0.45	0.82	0.18	0.22	0.05
SEM			0.86	3.07	1.92	1.67	2.00	0.36	4.45	4.56	3.77	0.47

a, b, c – values in columns with different letters differ significantly (P≤0.05), SEM – standard error of the mean.
* see: Table 1

Table 4. Nutritional value for ruminants (INRA 2007 units) of faba bean seeds (in kg of DM)

Type of variety (cultivar)		Plant protection method	UFL	UFV	PDIN	PDIE	PDIA
					($g \cdot kg^{-1}$ DM)		
Traditional		K	1.19	1.19	195	113	54
(Nadwiślański)		I	1.19	1.18	186	111	52
n=16		F	1.23	1.24	197	116	55
		IF	1.23	1.24	188	114	52
With terminal inflorescence		K	1.26	1.27	201	117	56
(Optimal)		I	1.23	1.24	182	112	51
n=16		F	1.23	1.24	197	116	55
		IF	1.19	1.19	185	111	52
Low-tannin		K	1.25	1.27	207	116	55
(Olga)		I	1.28	1.30	193	114	51
n=16		F	1.28	1.31	208	117	55
		IF	1.26	1.27	192	113	51
Low-tannin		K	1.26	1.28	198	114	52
(Albus)		I	1.27	1.29	187	112	49
n=16		F	1.25	1.26	201	115	53
		IF	1.25	1.27	191	113	50
Effect	Type of variety	Traditional	1.21 b	1.21 b	192	114	53
		With terminal inflorescence	1.23 b	1.24 b	191	114	54
		Low-tannin	1.26 a	1.28 a	197	114	52
	Plant protection method	K	1.24	1.25	200 a	115 ab	54 b
		I	1.24	1.25	187 b	112 b	51 a
		F	1.25	1.26	201 a	116 a	55 b
		IF	1.23	1.24	189 b	113 b	51 a
Interaction (P-value)			0.08	0.14	0.91	0.34	0.96
SEM			0.01	0.01	1.92	0.50	0.52

a, b – values in columns with different letters differ significantly ($P \leq 0.05$). SEM – standard error of the mean.

Discussion

Large differences in seed yield were found both between studied varieties (effect of genotype) as well as between untreated and chemically treated plants. The plants protected by insecticides had higher seed yield, which may suggest an important influence of insects on this factor. Also, plants protected by fungicides produced more (numerically) seeds than the control. However, it still remains unclear why the use of both substances together (insecticides and fungicides) resulted in lower (numerically) yield of seeds than when only insecticides were applied. If the effect of fungicides and insecticides used simultaneously was similar to the case when insecticides were applied alone, this probably means that the plantations were significantly infected by insects and not by the fungus.

Positive effects of chemical plant protection on faba bean seed yield have been also demonstrated by Nadolnik et al. (2001). They proved that the use of fungicides

alone might significantly increase seed yield by up to 27.5% compared to the control group. Also Ropek and Kulig (2002) showed that the chemical control of pests and fungi had a significant effect on faba bean plant health and thereby increased the yield and a thousand seed weight. In their experiment, chemically protected cultivars (Kodam and Titus) produced an average seed yield of 5.0 and 4.0 $t \cdot ha^{-1}$ respectively, whereas in the control groups (not protected), both varieties gave an average yield of 3.4 $t \cdot ha^{-1}$.

In the present study chemical protection of plant increased an average yield of seeds from 1.77 to 3.98 $t \cdot ha^{-1}$, regardless of the variety and method. Also chemical composition of seeds was influenced by both the type of cultivar and plant protection method. For example, a lack of protection of plants caused an increase in ash concentration. High ash content was also observed in seeds of low-tannin variety, although Jasińska and Kotecki (1998) did not indicate a clear influence of genetic factors on the ash level. In turn low-tannin cultivars were characterized by the highest CP content and, irrespective of the type of variety, the highest content of this nutrient was observed in seeds from unprotected plants.

Frejnagel et al. (1997) studied the differences in chemical composition and nutritive value of seeds from low- and high-tannin faba bean varieties and found similar results. CP content in the seeds of Nadwiślański cultivar (high-tannin), examined in their experiment, did not differ from the results obtained in the present study, while slightly lower results were observed by Pisulewska et al. (1998). According to Jasińska and Kotecki (1998), CP content in faba bean seeds was determined more by genetic factors (type of varieties) than by the weather or soil condition. Low-tannin varieties seeds examined in the present study contained more crude protein (on average about 68 $g \cdot kg^{-1}$ DM) compared with the low-tannin Caspar cultivar studied by Frejnagel et al. (1997). The CP content in the seeds with terminal inflorescence variety did not differ significantly from the results obtained with the same cultivar by Jasińska and Kotecki (1998).

Ramos-Morales et al. (2010) studying the N and energy utilization of diets with different legume seeds (lupins, faba beans, bitter vetch and vetch) in lactating goats concluded that the partition of the N balance between milk production and deposit within the body varied depending on the diet. Although this N available to the metabolism was the highest for the faba bean diet, the quantity of this directed to milk was the lowest. However, the authors suggest that faba bean seems to be the better choice, considering the N available to the metabolism and its partition between milk production and deposit within the body. In turn Tufatelli et al. (2012) summarizing their research concluded that feeding faba bean seeds in a lactation diet for cows supports lactation performance similar to cows fed traditional soybean meal (SBM) based diet, and that the results may elicit great interest for countries where soybean utilization is adversely influenced by high supply costs.

N-free extractives is a component which is present in the greatest amount in faba bean seeds. Results of Jasińska and Kotecki (1998) confirmed the theory that the NFE content is positively correlated with the mass of a thousand seeds. Average CF content in the seeds of varieties used in the current study was much lower than the CF content in the seeds of the varieties studied by Pisulewska et al. (1998). In their

study CF content in seeds was proportional to the seed yield (the higher the yield, the greater the CF content). According to Jasińska and Kotecki (1998) the genetic factor has little effect on accumulation of crude fiber in the seeds and the content of this component is strongly correlated with the weather factor.

Relatively clear was the effect of type of faba bean variety on net energy content in seeds which might be related with their chemical composition, especially CP, NDF and ADF contents, and IVTD digestibility. An effect of plant protection method on protein value of seeds (PDI) was also shown. Because higher PDI values were observed in seeds from the plants not treated with insecticides, where crude protein concentration was higher, it can be assumed that a lack of plant chemical protection results in an increased incidence of pests in the seeds. It was confirmed by the observation of seeds with much more frequent holes made by insects. The results of these tests allow us to hypothesize that uncontrolled development of insects in seeds, even during vegetation of plants, leads to reduction in seed yield per hectare which at the same time increases protein concentration. This is probably due to the decreased content of other nutrients in seeds or changes in their structure. This hypothesis could not be verified or discussed because of the lack of available scientific papers on this topic.

Conclusions

Type of variety as well as the plant protection method has a significant influence on the chemical composition, *in vitro* digestibility and nutritive value of faba bean seeds. Irrespective of a variety, application of chemical plant protection, especially insecticides, significantly increases seeds and protein yield per ha with no significant differences in the net energy content. The variation between cultivars, modified by plant protection method, in chemical composition and IVTD of seeds may suggest that there would be a need to estimate its real nutritive value used for diet formulation instead of one common value for faba bean species read from feed tables.

Important variation in nutritive value of faba bean seeds between varieties may suggest a need to include in the feeding tables for ruminants the data on type of variety instead of one common value for faba bean species.

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Received: 28 II 2014
Accepted: 6 X 2014