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PROTEIN QUALITY OF LEGUME-CEREAL MIXTURES IN RUMINANTS' NUTRITION

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ABSTRACT

Legumes are the most important crops within sustainable agriculture. In animal nutrition they represent an important source of proteins. The aim of this study was to focus legumes and legume-cereal mixtures as a suitable source of protein in ruminants' nutrition. The influence of climatic conditions on the nutritional value of legumes and legume-cereal mixtures in two years of growing were compared. Quality of feed protein was studied according to the Cornell Net Carbohydrate and Protein System (CNCPS) chemical fractionation method. The highest value of fraction A was determined in 2015 in a monoculture – as high as 39.9 % in peas with leafs, in 2017 it was highest in the mixture of peas with leafs and wheat (40.7 %). During the second harvest of 2015, the amount of soluble crude protein (A) decreased in the monoculture of leafy peas by 11 % down to 28.6 %. Important for feed quality evaluation are the values of fractions B2 and B3. These are proteins that are slowly soluble in the rumen, which increases their by-pass into the intestine as well as their digestibility. Fraction B2 content exceeded 40 % in all samples from 2015. The highest fraction B2 content 52.6 % was determined in a mixture of faba bean and oats in the first harvest. The lowest value was determined in 2017 in peas with leafs-wheat mixture in both harvests (30.6 % and 35.6 %).

Key words: legumes; legume-cereal mixtures; CNCPS

INTRODUCTION

The large diversity of species allows to grow legumes in areas with significantly different natural conditions. They have valuable agronomic characteristics. They have a positive influence on soil fertility and therefore play an important role in the crop rotation system. They are an excellent fore-crop especially for cereals; they have a phytosanitary effect. Their main physiological and biochemical advantage is their ability to synthetize large amount of protein.

Mixtures of spring cereals with legumes are considered good agricultural practice in many European countries, especially in organic and low-input farming system (Knudsen *et al*., 2004). Cultivation of mixtures contributes to the complementary use of habitat resources and compensatory growth of individual plant species, causing an increased productivity and greater stability of yield (Niggli *et al*., 2008; Doré *et al*., 2011). Mixtures have a positive effect on the soil fertility, enriching it with nitrogen through a symbiosis of legumes with nodule bacteria and in organic matter due to the huge amount of crop residue left behind (Song *et al*., 2007; Iqbal *et al*., 2018).

The cultivation of mixtures of legumes and cereals offers a number of potential agronomic benefits.

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Coming from two different plant families, legumes and cereals complement each other in the capture of resources. Cereal crops growing in the vicinity of legumes benefit from nitrogen assimilated by legume root nodule bacteria. Increasing the supply of nitrogen by applying fertilizer caused in a substantial reduction of fixation of atmospheric nitrogen by legume crops. Mixtures are particularly relevant to the exploitation of poorer soils which are unsuitable for the production of either component grown as a sole crop. Yielding of the mixtures is highly dependent on the species and proportion of component. The share of legumes in the seed mixture in terms of seed number is recommended to range from 30 to 50 %. Total seed yield of mixtures decreases with increasing share of legume seeds in sowing. Increasing the dose of nitrogen for the cultivation of mixtures usually leads to increasein the yield of cereal component, but reduces the proportion of legume seeds in the crop. Increasing the share of legume seeds at sowing increases the protein concentration, digestibility and improves the protein value of the feed made of the mixtures. Legume-cereal mixtures are a good forecrop for cereals. They reduce the negative effects associated with sowing of cereals one after another. Mixtures enrich the soil with organic matter and nutrients, but the value of their post-crop area depends on the choice of components, their share in the stand, the level of yields and soil conditions. Cultivation of cereals after legume-cereal mixtures is characterized by higher yield stability. The benefits of mixed sowings of legumes with cereals are associated with a significant reduction of weed infestation, especially in organic farming. (Staniak *et al*., 2014, Huňady and Seidenglanz, 2016).

From the complex aspect of growing, ensilaging and feeding, it is advantageous to grow legumes not only as monocultures, but in combination with cereals as cereal-legume mixtures. These mixtures play the same role in the sowing system as legumes alone (improving crop, low demand for Nitrogen fertilization). Their undeniable advantage is easier ensilaging, which is based on high content of saccharides soluble in water which are essential for lactic acid bacteria and therefore for successful fermentation process in the silage (Loučka *et al*., 2013).

Plants cultivated in mixed sowings have stronger immunity against unfavourable course of weather than in pure sowings (Ceglarek *et al*., 2002; Rudnicki, 1999).

Peas is a sustainable source of plant-based protein in livestock nutrition. It proved beneficial to sow alfalfa to the peas as a cover. This has been due to the new varieties of tendrils peas, which have leafs replaced by tendrils. Those do not cast as much shadow, they are resistant to lodging and do not repress the growth of alfalfa (Loučka *et al*., 2013). Although peas is a variable, undemanding and widely utilizable source of protein and energy, it is still underutilized.

The objective of this study was to show the possibilities for use of legume and legume-cereal mixtures' as a suitable source of protein in ruminant nutrition. Their excellent advantage is that these protein feeds can be produced by the agricultural enterprises themselves, which does not create a pressure for the primary producers to buy expensive alternative feeds.

The aim of this study was to compare the influence of climatic conditions on the nutritional value of legume-cereal mixtures in two years of growing (2015 and 2017).

MATERIAL AND METHODS

In order to compare the quality of proteins of legume-cereal mixtures, we used different types of mixtures harvested in two harvests per year and in two years (2015 and 2017) [2015 – first harvest 8 July (n = 6) and second harvest 14 July (n = 6); 2017 – first harvest 14 July (n = 6) and second 20 July (n = 6)]. Samples were grown on experimental plots in Rapotin in the district Šumperk in the Czech Republic.

- Peas with leafs 100 % monoculture (variety: PROTECTA)
- Peas with leafs 70 % + wheat 30 % (variety: PROTECTA + ZUZANA)
- Leafless peas 100 % monoculture (variety: ESO)
- Leafless peas 70 % + wheat 30 % (variety: ESO + ZUZANA)
- Faba bean 100 % monoculture (variety: MERLIN)
- Faba bean 70 % + oats 30 % (variety: MERLIN + KOROK)

Dry samples were milled on hammer mill with a 1 mm screen for chemical and solubility analysis. In the samples, dry matter content and the content of crude protein was determined in accordance with the directive of the Commission (ES) no. 152/2009 from 27th January 2009, which defines the methods of sample collection and analysis for the purposes of official feed quality control.

N fractions were determined: N soluble in phosphate-borate buffer, non-protein N (NPN) as the ratio of total N and precipitated true protein with sodium tungstate, N bound to NDF and N bound to ADF according to Licitra *et al*. (1996). Based on the above mentioned N fractions, we calculated fractions A, B_1, B_2, B_3 and C, from which rumen degradability of crude protein is predicted.

The RDP value was calculated according to NRC (2001) as:

$$RDP = A + B_1 [kdB_1 / (kdB_1 + kp)] + B_2 [kdB_2 / (kdB_2 + kp)] + B_3 [kdB_3 / (kdB_3 + kp)]$$

Where fraction A (NPN) is the percentage of CP that is instantaneously solubilized at time zero, estimated as soluble in borate-phosphate buffer but not precipitated with the protein denaturant TCA; B_1 = Fraction B_1 is the percentage of total CP soluble in borate phosphate buffer and precipitated with TCA; B_2 = Fraction B_2 is the remaining CP and is calculated as total CP minus the sum of fractions A, B_1, B_3, and C; B_3 = Fraction B_3 is calculated as the difference between the portions of total CP recovered in NDF (i.e., NDIN) and ADF (i.e., fraction C); kdB_1, kdB_2, kdB_3 = rates of degradation of fraction B_1, B_2 and B_3 in the rumen; kp = rate of passage from the rumen. Degradation rate and passage rate of individual N fractions were available from CNCPS version 5.0 (2003).

RESULTS AND DISCUSSION

The average temperature and precipitation in the monitored years were quite different. This is illustrated by Figures 1 and 2.

Crude protein content and the nitrogen fractions in the examined samples are presented in Table 1. From the collected data, we can see the differences in crude protein content as well as in nitrogen fractions between the harvest as well as years.

The largest difference in crude protein content was between the monocultures in both years and in the first harvest it was higher in all the studied samples. We confirmed that there are large differences in the nutritive contents between types of feed but also within a given variety, which are influenced by agrotechnology as well as climatic conditions of growing (Chrenková *et al*., 2004).

Pozdíšek et al., 2018 when comparing the content of crude protein in legume-cereal (pea + barley) mixture silages, determined a decrease of crude protein concentration between the first (145.4 g.kg^{-1} DM) and second (135.64 g.kg^{-1} DM) harvest. In pea + wheat mixtures, the concentration of crude protein remained unchanged between harvests (144.6 and 144.8 g.kg^{-1} DM). In the balance experiment on heifers, coefficient of CP digestibility in pea + barley mixtures was determined to be 63 % in the first and 61.38 % in the second harvest. In wheat mixtures, higher digestibility coefficient of crude protein was determined: 66.59 % (first harvest) and 65.32 % (second harvest).

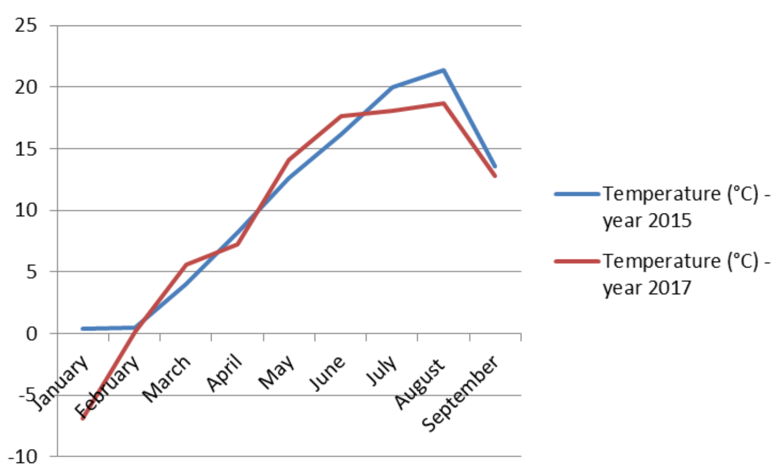

Figure 1. Average monthly temperatures in the year 2015 and 2017

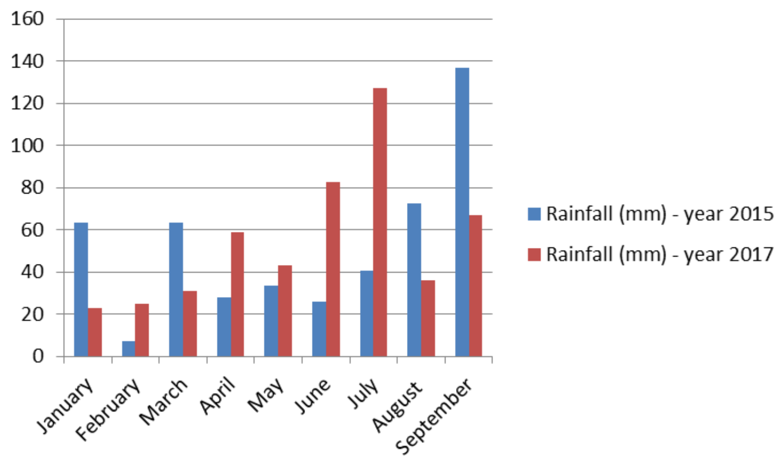

Figure 2. Average monthly rainfall in 2015 and 2017

Fraction A (crude protein very well soluble in rumen) in peas-based feeds (monoculture as well as mixtures) was higher in the first harvest in both years. It was highest in the leafy peas monoculture (39.9 %) in 2015, in 2017 it was highest in the leafy peas + wheat mixtures (40.7 %).

Fraction B_1 (rate of degradation in rumen is high) was lower in the first harvest of 2015 than in the second, while in 2017 it was reverse with the exception of peas + wheat mixtures. Total soluble crude protein content is determined by summing fractions A and B_1, which are quickly degraded in the rumen and utilized primarily by the rumen microorganisms. In the first harvest of 2017, we determined the highest content of soluble crude protein in the leafy peas monoculture (51.5 %) and the lowest in the first harvest of 2015 in the mixture of faba bean and oats (28.6 %).

Important for feed quality evaluation are the contents of B_2 and B_3 fractions. These are crude protein fractions slowly soluble in rumen, which increases their by-pass into the intestine, and also their digestibility. Fraction B_2 content was higher than 40 % in all samples in year 2015. The highest fraction B_2 content was determined in the mixture of faba bean and oats in the first harvest (52.6 %). The lowest content was determined in 2017 in leafy peas and wheat mixture in both harvests (30.6 % and 35.6 %).

Fraction C is very variable and changes based on certain technology of feed treatment (thermic treatment, incorrect ensilaging, etc.). This fraction is non-degradable in rumen and indigestible. These are crude proteins bound to acido-detergent fibre (cellulose, lignin, etc.). The fraction C content decreased with increased ripeness in both harvests in both years.

From the studied samples in 2015, the highest content of utilizable crude protein (fractions B_2 and B_3) was determined in the mixture of 70 % faba bean and 30 % oats in both harvests and in 2017 in the faba bean monoculture in the first harvest.

Our results confirmed the influence of climatic conditions on crude protein content and the values of CNCPS in the studied feed samples (Table 1).

Płaza *et al.* (2008) found in their experiments that the conditions of vegetation period significantly modified the yield of seeds and efficiency of true protein from the seeds of field pea and spring triticale mixtures. The highest yield of seeds was obtained from mixtures of field pea and spring triticale of component content 60 + 40 %, and the highest efficiency of true protein was obtained from field pea seeds cultivated in pure sowing. The mixtures of field pea with spring triticale provided more true protein from seeds than spring triticale cultivated in pure sowing.

The quantification of the main crude protein (CP) fractions during the growing period of pea and oat mixtures may be used to optimize the forage management. The determination of protein fraction could improve balancing rations for

Table 1. Fractions of crude protein according to CNCPS in years 2015 (n = 12) and 2017 (n = 12)

		% CP						
1st harvest (8.7.2015)	CP ($g \cdot kg^{-1}$ DM)	A	B_1	B_2	B_3	C	Insoluble CP	Soluble CP
Peas with leafs 100 %	161.7	39.9	7.1	40.9	2.1	10.1	53.1	46.9
Peas with leafs 70 % + Wheat 30 %	111.8	26.9	10.7	43.0	7.0	12.5	62.4	37.6
Leafless peas 100 %	139.9	31.4	13.4	40.2	4.6	10.5	55.3	44.7
Leafless peas 70 % + Wheat 30 %	108.1	26.5	12.1	42.7	6.4	12.4	61.5	38.5
Faba bean 100 %	206.1	33.0	9.9	44.3	5.1	7.7	57.1	42.9
Faba bean 70 % + oats 30 %	104.7	20.4	8.2	52.6	6.3	12.5	71.4	28.6
2nd harvest (14.7.2015)								
Peas with leafs 100 %	137.8	28.6	17.7	41.6	3.8	8.3	53.9	46.3
Peas with leafs 70 % + Wheat 30 %	113.5	22.6	15.1	43.1	8.6	10.7	62.3	37.7
Leafless peas 100 %	113.8	18.8	21.0	45.5	4.4	10.3	60.2	39.8
Leafless peas 70 % + Wheat 30 %	94.9	23.9	14.4	43.5	3.4	14.8	61.7	38.3
Faba bean 100 %	181.1	33.1	10.7	44.1	4.4	7.8	56.2	43.8
Faba bean 70 % + oats 30 %	106.0	23.0	10.7	45.9	10.0	10.4	66.4	33.7
		% CP						
1st harvest (14.7.2017)	CP ($g \cdot kg^{-1}$ DM)	A	B_1	B_2	B_3	C	Insoluble CP	Soluble CP
Peas with leafs 100 %	207.9	37.6	13.9	30.7	5.9	11.9	48.5	51.5
Peas with leafs 70 % + Wheat 30 %	197.2	40.7	10.5	30.6	5.9	12.4	48.8	51.2
Leafless peas 100 %	187.9	32.9	12.4	33.9	7.5	13.3	54.7	45.3
Leafless peas 70 % + Wheat 30 %	177.8	35.8	10.1	37.4	3.8	12.9	54.1	45.9
Faba bean 100 %	228.2	18.9	11.7	51.1	7.2	11.1	69.4	30.6
Faba bean 70 % + oats 30 %	186.4	35.8	10.9	31.7	8.7	13.0	53.3	46.7
2nd harvest (20.7.2017)								
Peas with leafs 100 %	184.3	35.7	10.5	37.4	6.8	9.6	53.8	46.2
Peas with leafs 70 % + Wheat 30 %	182.8	35.0	11.6	35.6	7.3	10.6	53.5	46.5
Leafless peas 100 %	168.0	31.8	7.2	43.1	6.9	11.0	61.0	39.0
Leafless peas 70 % + Wheat 30 %	167.9	30.7	11.1	40.3	7.1	10.8	58.2	41.8
Faba bean 100 %	217.3	34.0	9.0	42.6	4.9	9.5	57.0	43.0
Faba bean 70 % + oats 30 %	172.0	31.6	9.9	40.3	7.9	10.3	58.5	41.5

A – fraction of non-protein Nitrogen – highly soluble in rumen, B_1 – fraction of crude protein soluble in buffer and precipitated with TCA – the rate of degradation in rumen is high, B_2 – fraction of crude protein non-soluble in buffer, but soluble in neutral and acidic agents – rate of degradation in rumen is lower, B_3 – fraction of crude protein soluble in acidodetergent agent, rate of degradation is lower, C – fraction of crude protein, which are not soluble even in acidodetergent agent.

ruminants (Marković *et al.*, 2017). Marković *et al.*, 2017 in their work tested: The pea and oat at two different mixture rates: A_1 – 50 % pea + 50 % oat and A_2 – 75 % pea + 25 % oat and different a cutting time in three stages of growth: B_1 – a cutting of biomass at the start of flowering pea (10 % of flowering), B_2 – a cutting of biomass at forming the first pods on 2/3 plants of pea, and B_3 – cutting of biomass at forming green seeds in 2/3 pods. Stage of growth and pea-oat ratio in mixtures are significantly related to the change in the quality and chemical composition of biomass. The highest level of crude protein was obtained in pea at flowering stage (184.85 $g.kg^{-1}$) dry matter. The high level of easily soluble protein and non-protein nitrogen compounds (over 50 %) represent specific characteristics of the mixture. Unavailable fraction PC increased with plant maturation from 75.65 to 95.05 $g.kg^{-1}$ of CP.

CONCLUSION

Not only the legumes represent a quality feed, but their growing and feeding is also one of the options to cover a portion of the economically demanding protein requirements in animal nutrition using domestic feed sources. An undeniable advantage of feeding green legumes is that they increase the content of digestible crude protein in the mixtures. Legumes can be utilized in a variety of ways, which is confirmed also by our results.

Legume-cereal mixtures represent an excellent fore-crop in the crop rotation system, have a positive influence on soil quality, are more resistant against diseases and pests, repress weed and enrich soil with Nitrogen. They are a suitable source of crude protein for livestock.

The quality of legumes and the studied mixtures changes not only with the vegetation period but also under the influence of climatic conditions.

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REFERENCES

Ceglarek, F., Buraczyńska, D. & Brodowski, H. (2002). Porównanie plonowania i składu chemicznego mieszanek strączkowo-zbożowych. *Pam. Puławski*, 130(1), 81–89.

Doré, T., Makowski, D., Malézieux, E., Munier-Jolain, N., Tchamitchian, M. & Tittonell, P. (2011). Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge. *European Journal of Agronomy*, 34(4), 197–210.

Huňady, I. & Seidenglanz, M. (2016). Legume-cereal intercropping in organic farming. Proceedings of the 7th Scientific Conference with International Participation: *Growing technologies and their importance for practice*, 1. 12. 2016, Piešťany, NPPC–VÚRV Piešťany, p. 17–21. ISBN 978-80-89417-72-8.

Chrenková, M., Čerešňáková, Z. & Gálová, Z. (2004). Kvalita rastlinnych bilkovin ve vzťahu k vyžive. In: Proteiny 2004. Brno: MZLU v Brně, p. 57–62. ISBN 80-7157-779-0.

Iqbal, M. A., Iqbal, A., Maqbool, Z., Ahmad, Z., Ali, E., Siddiqui, M. H. & Ali, S. (2018). Revamping soil quality and correlation studies for yield and yield attributes in sorghum-legumes intercropping systems. *Bioscience Journal*, 34, 1165–1176. http://dx.doi.org/10.14393/BJ-v34n3a2018-36561

Knudsen, M. T., Hauggard-Nielsen, H. & Jensen, E. S. (2004). Cereal-grain legume intercrops in organic farming – Danish survey. In: *European Agriculture in global context*: Proceedings of VIII ESA Congress, 11-15 July 2004, Copenhagen, Denmark.

Licitra, G., Hernandez, T. M. & Van Soest, P. J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, 57, 347–358.

Loučka, R., Homolka, P. & Jančík, F. (2013). Cereals and legumes for silage, 334-342. Chapter from the book: Třináctý et al. (2013). *Feed assessment for dairy cows*, Pohořelice, 2013, p. 590. ISBN 978-80-260-2514-6.

Marković, J., Blagojević, M., Kostić, I., Vasić, T., Anđelković, S., Petrović, M. & Đokić, D. (2017). Protein Fractions of Intercropped Pea and Oat for Ruminant Nutrition. *Biotechnology in Animal Husbandry*, 33(2), p. 243–249.

Niggli, U., Slab, A., Schmid, O., Halberg, N. & Schlüter, M. (2008). Vision for an Organic Food and Farming Research Agenda to 2025. Report IFOAM EU Group and FiBL 2008.

NRC. (2001). Nutrient Requirements of Dairy Cattle, Seventh Revised Edition. National Academy Press, Washington, D.C.

Płaza, A., Ceglarek, F., Buraczyńska, D. & Gąsiorowska, B. (2008). The yielding of field pea and spring triticale mixture in climatic conditions of Poland. In: *Conference Proceedings 13th International Conference – Forage Conservation*, 3 – 5 September 2008, Research Institute for Animal Production Nitra, SARC RIAP Nitra, PDF Print unlimited company, Poprad, 190 p. ISBN 978-80-888-72-78-8

Pozdíšek, J., Huňady, I., Látal, O. & Dufek, A. (2018). Determination of the parameters of nutritional value and nitrogen retention in silage of the selected type of legume-cereal intercrop harvested in two maturity stages. *Výzkum v chovu skotu / Cattle Research*, LX, 3(221), 9–14.

Rudnicki, F. (1999). Środowiskowe uwarunkowania uprawy mieszanek w świetle literatury. Mat. konf. nauk. nt. "*Przyrodnicze i produkcyjne aspekty uprawy roślin w mieszankach*". Poznań 2-3 grudnia 1999, pp. 28–39.

Song, Y. N., Zhang, F. S., Marschner, P., Fan, F. L., Gao, H. M., Bao, X. G. & Li, L. 2007. Effect of intercropping on crop yield and chemical and microbiological properties in rhizosphere of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and faba bean (*Vicia faba* L.). *Biology and Fertility of Soils*, 43, 565–574.

Staniak, M., Księżak, J. & Bojarszczuk, J. (2014). Mixtures of Legumes with Cereals as a Source of Feed for Animals. http://dx.doi.org/10.5772/58358

The Directive of the Commission (ES) no. 152/2009 from 27th January 2009, which defines the methods of sample collection and analysis for the purposes of official feed quality control.