Tarım Bilimleri Dergisi
Tar. Bil. Der.
Dergi web sayfası:
www.agri.ankara.edu.tr/dergi

Journal of Agricultural Sciences
Journal homepage:
www.agri.ankara.edu.tr/journal

Determination of Green Forage and Silage Protein Degradability of Some Pea (*Pisum sativum* L.) + Oat (*Avena sativa* L.) Mixtures Grown in Serbia

Milomir BLAGOJEVIĆ[a], Nenad ĐORĐEVIĆ[b], Bora DINIĆ[a], Tanja VASIĆ[a], Jasmina MILENKOVIĆ[a], Mirjana PETROVIĆ[a], Jordan MARKOVIĆ[a]

[a]*Institute for Forage Crops, Kruševac, 37251 Globoder, SERBIA*

[b]*University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun-Belgrade, SERBIA*

ARTICLE INFO
Research Article
Corresponding Author: Jordan MARKOVIĆ, E-mail: jordan.markovic@ikbks.com, Tel: +381 (37) 442 583
Received: 24 December 2015, Received in Revised Form: 29 March 2016, Accepted: 29 March 2016

ABSTRACT

This research was conducted to determine the effect of seed rates in mixtures of pea + oat on the green forage and silage protein fractions evaluated by Cornell net carbohydrate and protein system (CNCPS). Experiment was established in autumn of 2012, on October the 20th and plant samples were taken in spring 2013 at forming the first pods on 2/3 plants of pea at Institute for forage crops, Kruševac, Republic of Serbia, using five different mixture rates of pea and oat crops (100% pea + 0% oat; 0% pea + 100% oat; 25% pea + 75% oat; 50% pea + 50% oat and 75% pea + 25% oat). After harvesting pea:oat mixtures were treated with bacterial inoculant and ensiled in anaerobic jars for 45 days. Green forages and silage samples were analyzed for DM (dry matter), CP (crude protein), primary protein fractions-TP (true protein), NPN (non protein nitrogen), IP (insoluble protein), SolP (soluble protein), NDICP (neutral detergent insoluble crude protein) and ADICP (acid detergent insoluble crude protein) and protein fractions by CNCPS. An analysis of variance found statistically significant differences among mixture rates for all variables, except IP and SolP. Silage from monoculture pea had the highest NPN (696.2 g kg^{-1} CP) and SolP (713.8 g kg^{-1} CP), followed by the mixture of pea with oat 75:25 (662.5 and 653.4 g kg^{-1} CP, respectively). Analyzing the CNCPS protein fractions of pea:oat silages it was found that silage from pea monoculture contained the highest PA fraction (non protein nitrogen, immediately degraded in the rumen) and that PA fraction increased with increasing pea ratios in silages, which was a direct reflection of their high NPN and SolP in green forages and in silages. Because of those facts 25:50 and 50:50 pea:oat mixtures silages could be recommended for ruminant feeding. The investigated bacterial inoculant can increase the TP content, as well as PB_1 (true protein rapidly degraded in the rumen) and PB_3 (slowly degraded True Protein in the rumen, because it is associated with the cell wall) fractions.

Keywords: Pea:oat mixture; Protein fractions; Degradability

Sırbistan Koşullarında Yetiştirilen Kimi Bezelye (*Pisum sativum* L.) + Yulaf (*Avena sativa* L.) Yeşil Ot ve Silaj Protein Parçalanabilirliğinin Belirlenmesi

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Jordan MARKOVIĆ, E-posta: jordan.markovic@ikbks.com, Tel: +381 (37) 442 583
Geliş Tarihi: 24 Aralık 2015, Düzeltmelerin Gelişi: 29 Mart 2016, Kabul: 29 Mart 2016

ÖZET

Bu çalışma bezelye + yulaf karışımlarında tohumluk oranının yeşil ot ve Cornell net karbohidrat ve protein sistemi (CNCPS) ile belirlenen silaj protein fraksiyonlarına etkisini belirlemek üzere yürütülmüştür. Kruševac, Sırbistan Cumhuriyeti'nde 5 farklı bezelye ve yulaf karışımı (% 100 bezelye + % 0 yulaf; % 0 bezelye + % 100 yulaf; % 25 bezelye + % 75 yulaf; % 50 bezelye + % 50 yulaf ve % 75 bezelye + % 25 yulaf) ile yürütülen deneme, 2012 sonbaharında 20 Ekim'de tesis edilmiş ve 2013 yılında bezelye bitkilerinin 2/3'ünde kapsül oluşumu döneminde bitki örnekleri alınmıştır. Hasat sonrası bezelye:yulaf karışımları bakteri ile aşılandıktan sonra havasız koşullarda kavanozlarda 45 gün süreyle silolanmıştır. Yeşil ot ve silaj örneklerinde DM (kuru madde), ham protein (CP), temel protein fraksiyonları (TP) olarak gerçek protein (TP), protein olmayan azot (NPN), çözünemez protein (IP), çözünebilir protein (SolP), nötral deterjanda çözünemez ham protein (NDICP), asit deterjanda çözünemez ham protein (ADICP) ve CNCPS ile belirlenen protein fraksiyonları belirlenmiştir. Sonuçlar; IP ve SolP hariç tüm özelliklere farklı karışımların etkisinin istatistiki olarak önemli olduğunu göstermiştir. En yüksek NPN (696.2 g kg^{-1} CP) ve SolP (713.8 g kg^{-1} CP) değerleri tek başına bezelyeden elde edilmiş ve bunu 75:25 bezelye yulaf karışımı izlemiştir (sırasıyla 662.5 ve 653.4 g kg^{-1} CP) izlemiştir. Bezelye:yulaf silajlarının CNCPS ile belirlenen protein fraksiyonları tek başına bezelye silajının en yüksek PA (rumende çabuk parçalanan protein olmayan azot) değerine sahip olduğunu ve PA değerinin karışımlarda artan bezelye oranına bağlı olarak arttığını, bunun da esasen yeşil ot ve silajlardaki NPN ve SolP değerlerinin yüksek olmasının bir yansıması olduğunu göstermiştir. Bu sonuçlara göre ruminant beslenmesinde 25:50 ve 50:50 bezelye: yulaf karışımlarının önerilmesinin faydalı olabileceği tespit edilmiştir. Kullanılan bakteri aşısının PB_1 (rumende çabuk parçalanan gerçek protein) ve PB_3 (rumende çabuk parçalanan gerçek protein) fraksiyonları yanında TP içeriğini de artırdığı belirlenmiştir.

Anahtar Kelimeler: Bezelye:yulaf karışımı; Protein fraksiyonu; Parçalanabilirlik

1. Introduction

Field pea (*Pisum sativum* L.) is an annual plant which is grown in many parts of the world. It is highly valued for its high crude protein content. Cultivation of field pea is also beneficial to improve soil fertility by the root-nodule bacteria (*Rhizobia*) that are able to introduce atmospheric nitrogen into soil (Kwabiah 2004). Although it is mainly used for seed production in Serbia, the whole plant can be processed into silage. Oat (*Avena sativa* L.) is often used as forage (hay). However, it also has some less desirable characteristics such as low protein content (6.45-7.84%) compared to other cereals used for other forms of livestock feeding systems such as grazing and silage (Omokanye 2014). Combining annual crop species for improved forage productivity should clearly have nutritional and financial benefits in the overall livestock production (Kwabiah 2004).

Modern scientific models of solubility analysis of the proteins and carbohydrates, as well as digestibility of organic matter, enabled the detailed analysis of the quality of various animal feeds. Information about the extent and rate of degradation, also give us information about their passage and absorption in the digestive tract. This allows adequate characterization of animal feedstuffs and their variability, depending on the manner and time of use. Such an approach to the analysis of nutrients allows balancing the rations in according to the need of each group of animals. The Cornell net carbohydrate and protein system (CNCPS) allows predicting the extent and rate of degradation of carbohydrates and protein in digestive tract, the amount of degradable and undegradable protein as well as microbial protein supply (Lanzas et al 2007; 2008).

The proteins are one of the most expensive components in animal feeds, with a large impact on the cost of production. In addition to the level of protein in a ration, the model of protein use is of great importance for a proper diet. Feeding excess CP can result in unnecessary feed expenses with no return in milk or milk protein yield. Furthermore, the majority of excess dietary N is excreted in the urine, which is the most environmentally labile form (Higgs et al 2012).

The nutritional quality of CP in forages is determined by its rate and extent of degradation in the rumen, and this can be enhanced by increasing true protein that is resistant to microbial degradation in the rumen. Choosing the most efficient combination of forage species, timing the harvest and silage additives or bacterial inoculants could increase CP quality for ruminant production (Tremblay et al 2003; Guo et al 2008). Bacterial inoculant seemed to produce more lactic acid and caused a rapid drop in pH in the silage. Plant proteases are more active between pH 6 and 7 than at lower pH (Heron et al 1989). Several findings indicate that microbial inoculants generally do not affect total N content, but their effect on the composition of silage N is more variable (McAllister et al 1995; Mandebvu et al 1999; Moshtaghi Nia & Wittenberg 1999).

Knowledge of potential rumen degradability of feed fractions is key to assess their nutritive values and extent of utilization in ruminants. The CNCPS accounts for the effects of variation due to feed protein fractions, their relative ruminal degradation rates and ultimately their rate of passage through the digestive tract. Thus, the present study was undertaken to evaluate certain pea:oat green forage mixtures and silages as per CNCPS model and to assess the acceptability of this model in the preparation of balanced rations for dairy animals. In terms of improving the protein fraction distribution, the main aim of bacterial inoculant addition is to obtain low concentration of protein fraction A in the silage.

2. Material and Methods

Field pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac-Serbia (21º 19′ 35″ E, 43º 34′ 58″ N). The study area was situated at altitude of 166 m above sea level in Central Serbia. Soil type was with humus content of approximately 3.5% and a pH in H_2O 6.87; pH in 1 N KCl 5.85; nitrogen content of 0.176%; AL-soluble P_2O_5 and K_2O 3.6 and 28.6 mg 100 g^{-1}, respectively. The mean annual temperature and the total precipitation from 40-years for the region and in research period are reported in Table 1.

The experiment was designed with three replication according to a randomized complete block. Experiment was established in autumn in 2012, on October the 20th. The pea and oat were tested at five different mixture rates: A1) 100% pea + 0% oat; A2) 0% pea + 100% oat; A3) 25% pea + 75% oat; A4) 50% pea + 50% oat and A5) 75% pea + 25% oat. All mixtures were sown on plots of 20 m^2. One level of fertilizer was applied, 300 kg ha^{-1} NPK (15:15:15) before the seeding. Plant samples were taken in spring 2013, at forming the first pods on 2/3 plants of pea.

The pea:oat mixtures were ensiled in the experimental containers holding 130 dm^3, with three replications. After compaction, silomass was covered with plastic wrap, and covered with a layer of sand thickness of about 10 cm as the main load. Bacterial inoculant *BioStabil Plus* which contained homo-fermentative lactic acid bacteria (*Enterococus faecium* and *Bacillus plantarum*) and hetero-

Table 1- Climatic dates from 40-years for the region and in research period

Year	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*Total/Mean*
Mean monthly temperature (ºC)													
1971-2011	0.6	2.4	7.2	12.6	16.8	20.4	22.2	22.3	18.6	11.2	7.8	3.2	12.1
2012	0.6	-4.1	8.3	12.9	16.2	22.5	25.4	24.1	20.5	14.6	9.6	0.9	12.6
2013	2.6	4.1	6.5	13.4	18.3	20.1	21.9	23.9	16.3	13.1	9.0	1.2	12.5
Total monthly precipitation (mm)													
1971-2011	37.7	63.4	49.4	44.0	55.9	90.8	62.0	34.8	27.8	71.9	34.4	72.1	644.2
2012	118	62.1	20	69.1	150	29.3	33.3	0.0	15.2	55.4	12.2	88.6	653.2
2013	43.5	76.5	78.4	54.5	102	44.0	6.0	14.4	53.5	48.9	58.2	18.0	612.6

fermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of 5×10^{10} CFU per gram was added, and ensiled in anaerobic jars for 45 days.

Green forage and silage samples were assayed for DM (dry matter) by oven drying at 60 ºC for 48 h. Neutral (NDICP) and acid (ADICP) detergent insoluble CP (crude protein) was determined on the samples obtained from NDF (neutral detergent fiber) and ADF (acid detergent fiber) residues. The Kjeldahl method according to AOAC (1990) was used to determine CP content of all samples. CP of NPN (non protein nitrogen) origin was estimated as the difference between total CP and CP of true protein (TP) origin precipitated with 10% trichloracetic acid solution. Similarly, SolP (soluble protein) was calculated as the difference between total CP and buffer insoluble CP (IP) estimated with borate phosphate buffer (pH 6.7-6.8) and freshly prepared (1 g 10 mL^{-1}) sodium azide solution, according to the method of Licitra et al (1996).

Protein fractionation as percentage of total CP was made by the CNCPS according to the method of Fox et al (2004). According to CNCPS, CP is partitioned into 3 fractions. Briefly, the PA fraction is NPN, the PB fraction is a degradable protein, and the PC fraction is an undegradable and unavailable protein. The PB fraction is further divided into 3 subfractions according to solubility and rate of ruminal degradation. The degradation rates in the rumen of borate phosphate buffer soluble PB_1, neutral detergent soluble PB_2 and acid detergent soluble PB_3 fractions are rapid, intermediate and slow, respectively.

The experimental data were analyzed by a factorial analysis of variance for green forage samples in a completely randomized design using a model that accounted for the main effects of pea:oat mixtures, and by a two-way analysis of variance for silage samples using a model that accounted for the main effects of pea:oat mixtures and addition of inoculant. Effects were considered significant at P<0.05 level. The significance of differences between arithmetic means was tested by LSD test (STATISTICA 6, Stat. Soft. 2006).

3. Results and Discussion

The pea:oat primary protein fractions and protein fractions by CNCPS are presented in Table 2. The effects of the mixture rates were significant for the DM content. With regard to the mixture rates, DM content decreased with the decreasing rate of oat (from 75 to 50%) in the mixture. With further decreasing rate of oat (from 50 to 25%) in the mixture DM content increased, although the highest DM content (280 g kg^{-1}) was obtained from the 100% oat plots. Furthermore, oat physically supported the pea plants in such mixtures and provided most of the DM production (Uzun & Asik 2012). Similarly effect of the mixture rates on DM content in silages were obtained (Table 3).

Table 2- Primary protein fractions and protein fractions by CNCPS of pea:oat mixture

Protein fractions	*Pea:oat mixture*				
	A1	*A2*	*A3*	*A4*	*A5*
DM, g kg^{-1}	248.3^{d}	280.0^{a}	271.6^{b}	263.0^{c}	270.3^{b}
CP, g kg^{-1} DM	190.3^{a}	114.5^{e}	126.8^{d}	152.8^{c}	167.3^{b}
NDICP, g kg^{-1} CP	155.7^{ns}	146.2^{ns}	145.4^{ns}	156.1^{ns}	140.1^{ns}
ADICP, g kg^{-1} CP	79.9^{c}	96.7^{ab}	86.8^{bc}	103.6^{a}	76.3^{c}
IP, g kg^{-1} CP	447.3^{ns}	438.6^{ns}	444.1^{ns}	435.4^{ns}	407.2^{ns}
SolP, g kg^{-1} CP	552.7^{ns}	561.3^{ns}	555.8^{ns}	564.5^{ns}	592.8^{ns}
TP, g kg^{-1} CP	417.0^{b}	442.9^{ab}	444.3^{ab}	449.0^{a}	440.3^{ab}
NPN, g kg^{-1} CP	582.9^{a}	557.1^{ab}	555.6^{ab}	550.9^{b}	559.7^{ab}
PA, g kg^{-1} CP	563.0^{ab}	552.1^{ab}	565.5^{ab}	551.0^{b}	579.7^{a}
PB_1, g kg^{-1} CP	6.8^{ab}	9.3^{ab}	1.2^{b}	14.1^{a}	13.1^{ab}
PB_2, g kg^{-1} CP	276.7^{ab}	292.4^{a}	288.7^{ab}	279.3^{ab}	267.0^{b}
PB_3, g kg^{-1} CP	73.5^{a}	49.5^{c}	58.6^{bc}	52.5^{c}	63.7^{bc}
PC, g kg^{-1} CP	79.9^{c}	96.7^{ab}	86.8^{bc}	103.6^{a}	76.3^{c}

A1, 100% pea + 0% oat; A2, 0% pea + 100% oat; A3, 25% pea + 75% oat; A4, 50% pea + 50% oat; A5, 75% pea + 25% oat; DM, dry matter; CP, crude protein; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; IP, insoluble protein; SolP, soluble protein; TP, true protein; NPN, non protein nitrogen; PA, non-protein nitrogen, immediately degraded in the rumen; PB_1, soluble true protein, rapidly degraded in the rumen; PB_2, buffer insoluble protein minus protein insoluble in neutral detergent, some fraction PB_2 is fermented in the rumen and some escapes to the lower gut; PB_3, true protein insoluble in neutral detergent but soluble in acid detergent, slowly degraded in the rumen because it is associated with the cell wall; PC, protein that is insoluble in the acid detergent, unavailable or bound protein; different letters in a row denote significant differences between means (P<0.05); ns, not significant

As seen in Table 2, the highest CP ratio (190.3 g kg^{-1} DM) was observed in the 100% pea plots, and the lowest CP ratio was obtained from 100% oat (114.5 g kg^{-1} DM). Among the mixtures, the highest CP ratio (167.3 g kg^{-1} DM) was found in the 75:25 pea:oat mixture (Table 2). As Uzun & Asik (2012) indicated, CP content increased as the percentage of pea in mixtures increased, because pea had higher CP content. The CP content in all mixtures was significantly higher than Kocer & Albayrak (2012) reported for mixtures with similar pea:oat ratios, as well as the CP values that reported Omakanye (2014) for field pea intercropping with oat and barley. Kocer & Albayrak (2012) also reported that CP content increased in pea:oat mixtures with increasing pea ratio in the mixture.

In all silages, the CP content increased as pea proportion in mixtures increased. Monoculture pea had the highest CP content (227.0 g kg^{-1} DM), followed by the mixture of pea with oat 75:25 (192.0 g kg^{-1} DM). In contrast, oat monoculture silage had the lowest CP content. The addition of inoculant slightly decreased ($P<0.05$) the CP content of silages (Table 3).

Proteins of forage legumes, particularly peas, are subject to rapid and extensive degradation in the rumen. Improving the efficiency of nutrient use requires accurate predictions of how various feed fractions behave as they flow through the digestive tract. The CNCPS is a mathematical model designed to evaluate the nutrient requirements and supply of cattle over a wide range of environmental, dietary, management and production situations (Van Amburgh et al 2007). The solubility and degradability of protein as well as the representation of individual fractions in pea:oat mixtures depends on the pea:oat ratio in the mixtures (Table 2).

Statistical analysis showed that the pea:oat ratios in investigated mixtures did not significantly affected the NDICP, IP and SolP content. The pea:oat ratios in the mixtures significantly influenced the amount of ADICP ($P<0.05$). Significantly greater ADICP content was recorded in the 50:50 pea:oat mixture, whereas the highest NPN content was observed in the 100% pea forage treatment (Table 2). Recent report in the present study area (Omokanye 2014) showed that legumes are good source of protein and can be used to compensate cereal protein shortage in livestock feeds and cereal-field pea intercrop appeared to improve forage nutritive value. This author indicated that the content of CP, IP, SolP and ADICP was 9.85, 5.72, 4.12 and 0.84%, respectively in DM of oat, whereas contents of these protein fractions were 14.4, 7.45, 6.90 and 1.16% respectively in DM of field pea. Das et al (2015) regarding NDICP and ADICP reported values of 20.9 and 5.7% CP, respectively in DM of oat.

Primary protein fractions (NDICP, ADICP, TP, NPN, IP and SolP) of pea:oat silages are presented in Table 3. The effects of the mixture rates showed significant differences ($P<0.05$) for the primary protein fractions. Silage from monoculture pea had the highest NPN (696.2 g kg^{-1} CP) and SolP (713.8 g kg^{-1} CP), followed by the mixture of pea with oat 75:25 (662.5 and 653.4 g kg^{-1} CP, respectively). The highest content of IP and ADICP was observed in silage from oat monoculture (496.6 and 179.9 g kg^{-1} CP, respectively), followed by the mixture of 25:75 pea:oat (491.0 and 167.2 g kg^{-1} CP, respectively). Pea silage and mixtures with increasing rates of pea contained higher values of SolP indicating their high protein availability in the rumen. ADICP content represents the fraction of feed protein which is neither available to microbes nor to the animal in ruminants. ADICP content was higher in silages than in green forages. Bacterial inoculant decreased NPN and SolP content in silages, but increased TP, IP, NDICP and ADICP content ($P<0.05$).

In all five treatments of the investigation, the soluble fraction PA averaged above 50% of total CP (Table 2). Despite the results obtained in this investigation, Vahdani et al (2014) reported that the highest CP fraction of pea was PB_2 that is potentially degradable in the rumen. Results from this investigation indicate that the highest PA fraction was recorded in 75:25 pea:oat mixture. Concentration of PB_1 protein fraction was the lowest, from 1.2 g kg^{-1} CP in 25:75 pea:oat mixture to 14.2 g kg^{-1} CP in 50:50 pea:oat mixture. Across pea:oat ratios in mixtures, results showed that intermediately degraded fraction PB_2 accounted from 292.4 g kg^{-1} CP in 0:100 pea:oat mixture to

Table 3- Primary protein fractions of pea:oat bi-crop silages

Protein fractions		*Pea:oat mixture*					
		A1	*A2*	*A3*	*A4*	*A5*	$\overline{X}_B$
DM, g kg^{-1}	B1	271.0^{ns}	274.3^{ns}	269.3^{ns}	272.0^{ns}	277.0^{ns}	272.7^{ns}
	B2	270.3^{ns}	279.0^{ns}	276.6^{ns}	273.6^{ns}	274.6^{ns}	274.8^{ns}
	$\overline{X}_A$	270.6^{b}	276.6^{a}	273.0^{ab}	272.8^{ab}	275.8^{ab}	
CP, g kg^{-1} DM	B1	230.1^{a}	96.3^{fg}	113.0^{e}	159.3^{c}	195.3^{b}	158.8^{a}
	B2	223.9^{a}	87.5^{g}	102.1^{f}	147.1^{d}	188.6^{b}	149.8^{b}
	$\overline{X}_A$	227.0^{a}	91.8^{e}	107.5^{d}	153.2^{c}	192.0^{b}	
NDICP, g kg^{-1} CP	B1	85.3^{e}	168.7^{c}	169.9^{c}	118.1^{d}	91.3^{e}	126.6^{b}
	B2	90.0^{e}	234.9^{b}	252.3^{a}	178.8^{c}	111.5^{d}	173.5^{a}
	$\overline{X}_A$	87.7^{e}	201.7^{b}	211.1^{a}	148.4^{c}	101.4^{d}	
ADICP, g kg^{-1} CP	B1	74.7^{d}	160.2^{b}	143.7^{b}	101.1^{c}	80.1^{cd}	111.9^{b}
	B2	77.8^{cd}	199.6^{a}	190.7^{a}	159.4^{b}	97.1^{cd}	144.9^{a}
	$\overline{X}_A$	76.2^{c}	179.9^{a}	167.2^{a}	130.2^{b}	88.6^{c}	
IP, g kg^{-1} CP	B1	276.5^{d}	343.3^{c}	354.9^{c}	461.1^{b}	513.0^{a}	386.7^{b}
	B2	295.8^{d}	349.9^{c}	453.0^{b}	480.1^{b}	529.1^{a}	424.6^{a}
	$\overline{X}_A$	286.2^{d}	496.6^{a}	491.0^{a}	408.0^{b}	346.6^{c}	
SolP, g kg^{-1} CP	B1	704.1^{a}	519.9^{c}	547.0^{c}	645.1^{b}	650.1^{b}	613.2^{a}
	B2	723.4^{a}	486.9^{d}	470.8^{d}	538.9^{c}	656.7^{b}	575.3^{b}
	$\overline{X}_A$	713.8^{a}	503.4^{d}	508.9^{d}	592.0^{c}	653.4^{b}	
TP, g kg^{-1} CP	B1	312.9^{g}	559.9^{b}	518.7^{d}	376.6^{e}	336.7^{f}	420.9^{b}
	B2	294.7^{h}	552.4^{bc}	634.4^{a}	548.2^{c}	338.4^{f}	473.6^{a}
	$\overline{X}_A$	303.8^{e}	556.1^{b}	576.6^{a}	462.4^{c}	337.5^{d}	
NPN, g kg^{-1} CP	B1	687.1^{b}	440.0^{g}	481.3^{e}	623.4^{d}	663.3^{c}	579.0^{a}
	B2	705.3^{a}	447.6^{fg}	365.6^{h}	451.7^{f}	661.6^{c}	526.4^{b}
	$\overline{X}_A$	696.2^{a}	443.8^{d}	423.4^{e}	537.5^{c}	662.5^{b}	

A_1, 100% pea + 0% oat; A_2, 0% pea + 100% oat; A_3, 25% pea + 75% oat; A_4, 50% pea + 50% oat; A_5, 75% pea + 25% oat; B1, control treatment without bacterial inoculant; B2, treatment with bacterial inoculant; DM, dry matter; CP, crude protein; NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein; IP, insoluble protein; SolP, soluble protein; TP, true protein; NPN, non protein nitrogen; different letters in a row denote significant differences between means ($P<0.05$) ; ns, not significant

267.0 g kg^{-1} CP in 75:25 pea:oat mixture. Fraction PB_3 includes CP that in insoluble in NDF but soluble in ADF. Changes in NDF concentration of plant parts may largely explain the differences in proportions of fraction PB_3. Results of this investigation showed that this protein fraction was the highest in 100:0 pea:oat mixture. Unavailable fraction PC represent bound protein that is not degraded in the rumen and is not digested in the small intestine. Mixtures 100:0 and 75:25 pea:oat had significantly lower concentration of PC fraction in comparison with other investigated mixtures. In contrast to these results, in the biomass of grass pea (Vahdani et al 2014) the largest fraction was PB_2, while Das et al (2015) showed significantly higher values for PB_3 and PC fractions for different grass species.

Analyzes of CNCPS protein fractions in pea:oat silages (Table 4) showed that silage from pea monoculture contained the highest PA fraction and that PA fraction increased with increasing pea ratios in silages, which was direct reflection of their high NPN and SolP in green forages and in silages. Other protein fractions were higher in oat silages and their content

Table 4- Protein fractions by CNCPS of pea:oat bi-crop silages

Protein fractions		*Pea:oat mixture*					
		A1	*A2*	*A3*	*A4*	*A5*	$\overline{X}_B$
PA, g kg^{-1} CP	B1	687.1^b	440.0^f	481.3^e	623.4^d	647.2^c	575.8^a
	B2	705.2^a	447.6^f	365.6^g	451.7^f	648.2^c	523.7^b
	$\overline{X}_A$	696.2^a	443.8^d	423.4^e	537.5^c	647.7^b	
PB_1, g kg^{-1} CP	B1	17.0^e	79.8^{bc}	65.7^c	21.7^{de}	2.8^e	37.4^b
	B2	18.2^e	39.3^d	105.3^a	87.1^{ab}	8.5^e	51.6^a
	$\overline{X}_A$	17.6^c	59.6^b	85.5^a	54.4^b	5.6^c	
PB_2, g kg^{-1} CP	B1	210.5^{cd}	311.4^a	283.0^b	236.8^c	258.5^{bc}	260.0^{ns}
	B2	186.5^d	278.2^b	276.8^b	282.4^b	231.7^c	251.1^{ns}
	$\overline{X}_A$	198.5^c	294.8^a	279.9^a	259.6^b	245.1^b	
PB_3, g kg^{-1} CP	B1	10.6^d	8.5^d	26.2^{bc}	17.0^c	11.2^d	14.7^b
	B2	12.2^d	35.2^b	101.5^a	19.4^c	14.5^c	36.5^a
	$\overline{X}_A$	11.4^c	21.8^b	63.9^a	18.1^{bc}	12.8^{bc}	
PC, g kg^{-1} CP	B1	74.7^e	160.2^b	143.8^c	101.1^d	80.1^e	111.9^b
	B2	77.8^e	199.6^a	150.7^{bc}	159.4^b	97.1^d	136.9^a
	$\overline{X}_A$	76.2^e	179.9^a	147.2^b	130.2^c	88.6^d	

A1, 100% pea + 0% oat; A2, 0% pea + 100% oat; A3, 25% pea + 75% oat; A4, 50% pea + 50% oat; A5, 75% pea + 25% oat; B1, control treatment without bacterial inoculant; B2, treatment with bacterial inoculant; PA, non-protein nitrogen, immediately degraded in the rumen; PB_1, soluble true protein, rapidly degraded in the rumen; PB_2, buffer insoluble protein minus protein insoluble in neutral detergent, some fraction PB_2 is fermented in the rumen and some escapes to the lower gut; PB_3, true protein insoluble in neutral detergent but soluble in acid detergent, slowly degraded in the rumen because it is associated with the cell wall; PC, protein that is insoluble in the acid detergent, unavailable or bound protein; different letters in a row denote significant differences between means ($P<0.05$) ; ns, not significant

decreased with increasing pea ratio in mixtures. PC fraction in silages was higher than in green forages.

Bacterial inoculant decreased ($P<0.05$) PA fraction of pea:oat silages, but increased PB_1, PB_3 and PC protein fractions in ensiled pea:oat mixtures. Bacterial inoculants reduced the breakdown of protein with reduced NPN values in ensiled 25:75 and 50:50 pea:oat mixtures from 481.3 to 365.6 g kg^{-1} CP and from 623.4 to 451.7 g kg^{-1} CP, respectively. Differences in PB_2 fraction content with and without inoculant were not significant ($P>0.05$). The reduced proteolysis with the bacterial inoculants resulted in an increase in PB_1 and PB_3 fractions for all ensiled pea:oat mixtures, which is in consistent with results by Keleş et al (2014). The increase in PB_1 fraction and decrease in PA fraction with the addition of bacterial inoculant suggests that inoculants can increase the true protein content, considering silages have more NPN than dried forages (Edmunds et al 2012), this could pose a nutritional advantage for ruminant nutrition.

4. Conclusions

Data obtained from this investigation show that pea and oat mixtures can be planted successfully for forage and hay production. Moreover, pea and oat mixtures can be successfully ensiled and obtained high quality silages. According to the results, cultivation of a 25% pea and 75% oat mixture for higher PB_2 fraction is recommended. Increasing the oat ratio in the mixture could increase TP and IP content. Higher oat ratio in mixtures decreased PA fraction which is rapidly degraded in the rumen, but increased PC fraction which is unavailable. Because of those facts 25:50 and 50:50 pea:oat mixture silages could be recommended for ruminant feeding. Bacterial inoculant evaluated can improve protein quality of ensiled pea:oat mixtures and increase the TP content, as well as PB_1 and PB_3 fractions and decrease NPN content and PA fraction of protein.

Acknowledgements

The authors thank the Ministry of Education, Science and Technological Development of Republic of Serbia who funded this research as part of the project TR-31057.

References

AOAC (1990). Official method 984.13 Crude protein in animal feed, forage, grain and oil seeds. Official methods of analysis of AOAC International, 15th ed., NY

Das K l, Kundu S S, Kuwar D & Datt C (2015). Fractionation of carbohydrate and protein content of some forage feeds of ruminants for nutritive evaluation. *Veterinary World* **8**: 197-202

Edmunds B, Südekum H H, Spiekers H & Schwarz F J (2012). Estimating ruminal crude protein degradation of forages using in situ and in vitro techniques. *Animal Feed Science and Technology* **175**: 95-105

Fox D G, Tedeschi L O, Tylutki T P, Russell J B, Van Amburgh M E, Chase L E, Pell A N & Overton T R (2004). The Cornell net carbohydrate and protein system model for evaluating herd nutrition and nutrient excretion. *Animal Feed Science and Technology* **112**(1-4): 29-78

Guo X S, Ding W R, Han J G & Zhou H (2008). Characterization of protein fractions and aminoacids in ensiled alfalfa treated with different chemical additives. *Animal Feed Science and Technology* **142**: 89-98

Higgs R J, Chase L E & Van Amburgh M E (2012). Case study: Application and evaluation of the Cornell net carbohydrate and protein system as a tool to improve nitrogen utilization in commercial dairy herds. *The Professional Animal Scientist* **28**: 370-378

Keleş G, Coşkun B, İnal F, Selçuk Alataş M & Ateş S (2014). Conservation characteristics and protein fractions of cereal silages ensiled with additives at the booting and dough stages of maturity. *Turkish Journal of Veterinary and Animal Sciences* **38**: 285-294

Kocer A & Albayrak S (2012). Determination of forage yield and quality of pea (*Pisum sativum* L.) mixtures with oat and barley. *Turkish Journal of Field Crops* **17**(1): 96-99

Kwabiah A B (2004). Biological efficiency and economic benefits of pea-barley and pea-oat intercrops. *Journal of Sustainable Agriculture* **25**(1): 117-128

Lanzas C, Tedeschi L O, Seo S & Fox D G (2007). Evaluation of protein fractionation systems used in formulating rations for dairy cattle. *Journal of Dairy Science* **90**(50): 7-21

Lanzas C, Broderick G A & Fox D G (2008). Improved feed protein fractionation schemes for formulating rations with the Cornell Net Carbohydrate and Protein System. *Journal of Dairy Science* **91**: 4881-4891

Licitra G, Hernandez T & Van Soest P (1996). Standardization of procedures for nitrogen fractioning in ruminal feeds. *Animal Feed Science and Technology* **57**: 347-358

Mandebvu P, West J W, Froetschel M A, Hatfield R D, Gates R N & Hill G M (1999). Effect of enzyme or microbial treatment of bermudagrass forage before ensiling on cell wall composition and products of silage fermentation and *in situ* digestion kinetics. *Animal Feed Science and Technology* **77**: 317-329

McAllister T A, Selinger L B, McMahan L R, Bae H D, Lysyk T J, Oasting S J & Cheng K J (1995). Intake, digestibility and aerobic stability of barley silage inoculated with mixtures of *Lactobacillus plantarum* and *Enterococcus faecium*. *Canadian Journal of Animal Sciences* **75**: 425-432

Moshtaghi Nia S A & Wittenberg K M (1999). Use of forage inoculants with or without enzymes to improve preservation and quality of whole crop barley forage ensiled as large bales. *Canadian Journal of Animal Sciences* **79**: 525-532

Omokanye T A (2014). On-farm testing of strip intercropping of annual crops for forage yield and quality. *International Journal of Agronomy and Agricultural Research* **4**(4): 65-76

Tremblay G F, Michaud R & Belanger G (2003). Protein fractions and ruminal undegradable proteins in alfalfa. *Canadian Journal of Plant Science* **83**: 555-559

Uzun A & Asik F F (2012). The effect of mixture rates and cutting stages on some yield and quality characters of pea (*Pisum sativum* L.) + oat (*Avena sativa* L.) mixture. *Turkish Journal of Field Crops* **17**(1): 62-66

Vahdani N, Moravej H, Rezayazdi K & Dehghan-Banadaki M (2014). Evaluation of nutritive value of grass pea hay in sheep nutrition and its palatability as compared with alfalfa. *Journal of Agricultural Science and Technology* **16**: 537-550

Van Amburgh M E, Recktenwald E B, Ross D A, Overton T R & Chase L E (2007). Achieving better nitrogen efficiency in lactating dairy cattle: Updating field usable tools to improve nitrogen efficiency. In: *Proceedings of 18th Cornell Nutrition Conference*, 30-31 January, Syracuse, NY, pp. 25-38