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PROTEIN FRACTIONS OF INTERCROPPED PEA AND OAT FOR RUMINANT NUTRITION

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Abstract: 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 ruminants. The first factor (A) is ratio of germinated seed in mixtures. The pea and oat were tested at two different mixture rates: A_1 – 50% pea + 50% oat and A_2 – 75% pea + 25% oat. The second factor (B) is 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 (DM)). 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.

Key words: protein fractions, pea-oat mixtures

Introduction

The goal of most dairy farmers is to use the available land to produce cheaper but high quality feed with adequate protein content for lactating cows in order to maximize milk yield. 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. 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*).

Annual legumes is mostly grown as a sole crop, but in some countries intercropping with cereals is a common practice (*Klimek-Kopyra et al.* 2014). Legume-cereal mixtures are important protein and carbohydrate sources for livestock. The most common application in practice is a mixture of field pea and oat, because of the high yield and quality of biomass (*Uzun and Asik, 2012*). This may lead to the better utilization of these mixtures as livestock feed.

The objective of the present study was to quantify the main CP fractions during the growing period of field pea and oat mixtures. Determination of protein fraction would improve balancing rations for animals, especially for dairy cows.

Materials and methods

Field pea and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac – Serbia (21º19′35″ E, 43º34′58″ N). Experiment was carried out in autumn in 2012, on October the 20th in a randomized block design with three replications. The first factor (A) is ratio of germinated seed in mixtures. The pea and oat were tested at two different mixture rates: A_1 – 50% pea + 50% oat and A_2 – 75% pea + 25% oat. The second factor (B) is a time of cutting of biomass with 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. Both mixtures were sown on plots of 20 m^2 with three replications for each time of cutting. Plant samples were taken from the surface of 1 m^2 for chemical analysis.

The CP of the samples was determined using Kjeldahl method. The NPN, NDICP, ADICP, SolP, TP (True protein) and IP (Insoluble protein) were determined by *Licitra et al.* (*1996*). The CP, NPN, SolCP, NDICP, ADICP, TP and IP were calculated as follows:

CP = Total N x 6.25

NPN = (Total CP – Residusl CP_{NPN})/CP x 1000

SolCP = (Total CP – Residual CP_{SolCP})/CP x 1000

ADICP = Residual CP_{ADICP}/CP x 1000

NDICP = Residual CP_{NDICP}/CP x 1000

TP = Residual CP_{NPN}/CP x 1000

IP = Residual CP_{SolCP}/CP x 1000

NPN_{SolCP} = NPN/SolCP x 1000

Where, CP is the crude protein, NPN - non-protein nitrogen (g kg^{-1} CP); SolCP, the soluble crude protein (g kg^{-1} CP); NDICP, the neutral detergent insoluble crude protein (g kg^{-1} CP); ADICP, the acid detergent insoluble crude protein (g kg^{-1} CP); TP – true protein (g kg^{-1} CP); IP – insoluble crude protein (g kg^{-1} CP) and NPN_{SolCP}, (g NPN kg^{-1} SolCP^{-1}).

The CNCPS (Cornell Net Carbohydrates and Protein System) crude protein fractions of the samples, PA, PB_1, PB_2, PB_3 and PC were calculated based on CP, NPN, SolCP, NDICP, ADICP contents of samples according to *Fox et al.* (*2004*).

PA = NPN

PB_1 = SolCP – NPN

PB_2 = CP – SolCP – NDICP

PB_3 = NDICP – ADICP

PB = 1000 – PA – PC

PC = ADICP

Where, PA refers to the non-protein nitrogen (g kg^{-1} CP); PB_1, the rapidly degraded crude protein (g kg^{-1} CP); PB_2, the intermediately degraded crude protein (g kg^{-1} CP); PB_3, the slowly degraded crude protein (g kg^{-1} CP) and PC, the bound crude protein (g kg^{-1} CP).

The data were processed by the two way ANOVA in a randomized block design. Effects were considered different based on significant ($p< 0.05$) F ratio. The significance of differences between arithmetic means was tested by Tukey test.

Results and discussion

The protein fractions of of field pea and oat mixture are shown in Table 1. The crude protein (CP) concentration decreased ($p< 0.05$) during the investigated period as the maturity advanced. The average level of CP in the mixture R_2 was for 20.59 g kg^{-1} DM higher than in mixture R_1 ($p< 0.05$). This was expected due to the higher quantity of pea in the mixture R_2. The CP content in both mixtures were significantly higher than *Kocer and Albayrak* (*2012*) reported for mixtures with similar pea : oat ratios as well as the CP values that reported *Omokanye* (*2014*) for field pea intercropping with oat and barley. The decreasing trend in CP content can be explained by increasing oat weight and reducing pea weight in mixtures with plant development from flowering to grain filling. Obtained results are consistent with results by *Uzun and Asik* (*2012*), who stated that, in the pea monoculture and in pea - barley mixtures, maturing plants decrease CP level.

In both mixtures and during maturation, except stage P_3, the soluble fraction PA was above 500 g kg^{-1} (Table 1). *Vahdani et al.* (*2014*) reported that the highest fraction of CP in DM of grass pea hay was PB_2 that is potentially degradable in rumen. These authors showed that content of PA, PB_1, PB_2, PB_3 and PC fractions were 107.8, 266.5, 511.6, 54.5 and 59.5 g kg^{-1} CP, respectively. Maturity of forage mixtures had contrasting effects on fraction PA and fraction PB_1. Fraction PA increased ($p< 0.05$) and fraction PB_1 decreased from the stage P_1 to the stage P_2, and then fraction PA decreased from stage P_2 to stage P_3, and fraction PB_1 increased. This contrasting pattern between the two soluble CP fractions can be explained by the process of accumulation and distribution of CP during the period of rapid growth. However, fraction PA is NPN, whereas fraction PB_1 is soluble true protein. As seed makes a large fraction of total forage, these results suggest that a higher proportion of soluble true protein is accumulated in the seed as compared with NPN or that some NPN of vegetative plant parts is redistributed as true protein of the seed.

Across oat : pea ratios in mixtures and harvesting stages, result showed that intermediately degraded fractions PB_2 accounted 310.23 gkg^{-1} CP in mixture R_1, and 330.76 gkg^{-1} CP ($p< 0.05$) in mixture R_2 (Table 1).

During growing season, fraction PB_2 decreased from the stage P_1 to the stage P_2, and after that from stage P_2 to P_3 this fraction increased ($p< 0.05$). A higher proportion of seeds can be the cause of this difference between stages of growth. These results illustrate the fact that maturity had a greater effect on CP concentration than on the proportion of the main CP fractions.

Table 1. Fraction of protein by Cornell Net Carbohydrate and Protein (CNCPS)

Factors		CP $g\ kg^{-1}$ DM	PA $g\ kg^{-1}$ CP	PB_1 $g\ kg^{-1}$ CP	PB_2 $g\ kg^{-1}$ CP	PB_3 $g\ kg^{-1}$ CP	PC $g\ kg^{-1}$ CP
R_1	P_1	176.82b	473.70e	79.72a	279.06c	82.87a	84.65d
	P_2	152.58d	549.85b	17.98d	276.02d	52.50c	103.65a
	P_3	136.00e	500.60d	11.97e	375.60a	15.23f	96.60b
R_2	P_1	192.58a	535.55c	9.78e	350.37b	37.65d	66.65f
	P_2	167.06c	559.70a	33.10c	267.05e	63.75b	76.40e
	P_3	166.80c	459.80f	51.20b	374.85a	20.65e	93.50c
$\overline{X}$ R_1		155.13b	508.05b	36.55a	310.23b	50.20a	94.97a
$\overline{X}$ R_2		175.72a	518.35a	31.37b	330.76a	40.67b	78.85b
$\overline{X}$ P_1		184.85a	504.63b	44.75a	314.74b	60.23a	75.65c
$\overline{X}$ P_2		160.03b	554.78a	25.54c	271.53c	58.12b	90.03b
$\overline{X}$ P_3		151.45c	480.20c	31.58b	375.22a	17.95c	95.05a

R_1- first mixture, 50% pea + 50% oat; R_2- second mixture, 75% pea + 25% oat; P_1- a cutting of the biomassat the start of flowering pea (10% of flowering); P_2- a cutting of biomass at forming the first pods on 2/3 plants of pea; P_3- cutting of biomass at forming gren seeds in 2/3 pods; CP – Crude Protein, PA - non-protein nitrogen; PB_1 - the rapidly degraded crude protein; PB_2 - the intermediately degraded crude protein; PB_3 - the slowly degraded crude protein; PC - the bound crude protein; Different letters denote significantly different means ($P< 0.05$)

Fraction PB_3 includes CP that is insoluble in NDF but soluble in ADF. Changes in NDF concentration of plant parts with maturity may largely explain the differences in proportions of fraction PB_3 (as percentages of total CP). Results of this investigation showed that this protein fraction was higher in mixture R_1 than in mixture R_2 ($p< 0.05$). It is assumed that higher levels of oat in the mixture R_1 contributed to these results. This is consistent with the results of the quality oat and pea that were presented (*Kocer and Albayrak, 2012*).
Unavailable fraction PC (ADICP) represent bound protein that is not degraded in the rumen and is not digested in the small intestine. This fraction increased with plant maturation from 75.65 to 95.05 gkg^{-1} of CP. Statistically significant difference was observed between stages of growth. Mixture R_1 had higher content of PC fraction than mixture R_2 ($p< 0.05$). In contrast to these results, in the biomass of grass pea (*Vahdani et al*. 2014) the largest fraction was PB_2.
One, anticipated advantage of feeding bi-crop forages of cereal and legume is an improvement in the efficiency of nutrient utilization due to the possible synchronous supply of readily fermentable energy and protein in the rumen. Choosing the most efficient combination of forage species, timing the harvest could increase CP quality for ruminant production. If protein degradation is rapid or non-protein nitrogen value is higher than the capacity of ruminal microbes to utilize released amino acids or ammonia, this could lead to inefficiencies in ruminal nutrition.

Conclusions

In conclusion, 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 levels of crude protein in dry matter were obtained in pea in flowering stage (184.85 g kg^{-1} DM). Knowledge of the structure of the protein fractions gives us the possibility of combining the proper balance of nutrients in the livestock diet. The high level of easily soluble protein and non-protein nitrogen compounds (over 50%) represent specific characteristics of the mixture. This allows easier mixing of pea-oat mixtures with other livestock feed which have different structure of protein fractions. Thus, mixing pea-oat biomass with other perennial legume (alfalfa, red clover) can give bulky part of a ration with high protein value and a balanced ratio and representation of all protein fractions.

Proteinske frakcije u združenom usevu ovsa i graška za ishranu preživara

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Rezime

Određivanje količine proteinskih frakcija u združenom usevu graška i ovsa može poslužiti za optimalno korišćenje ovih krmnih biljaka. Poznavanje proteinskih frakcija u krmi može poboljšati balansiranje obroka za preživare. Prvi factor u ovim istraživanjima jeste odnos klijavih semena u smešama. Odnosi klijavih semena graška i ovsa su bili: 120 semena graška i 120 semena ovsa (A_1 – 2 / 4 : 2 / 4) u prvoj smeši i 180 semena graška I 60 semena ovsa (A_2 – 3 / 4 : 1 / 4) u drugoj smeši. Drugi factor je bio košenje biomase u tri različite faze razvića: B_1 – početak cvetanja graška (10% procvetalih biljaka), B_2 – faza kada su 2 /3 biljaka formirale prve mahune i B_3 – faza kada su 2 /3 biljaka formirale zeleno seme u mahunama. Faza razvića i odnos graška i ovsa u smešama su značajno uticale na sadržaj proteinskih frakcija u biomasi. Najveći sadržaj sirovih proteina je ustanovljen u fazi cvetanja graška (184,85 g kg^{-1} SM). Visok nivo lakorastvorljivih i neproteinskih azotnih jedinjenja predstavljaju specifičnu karakteristiku ovih smeša. Sadržaj nedostupne proteinske frakcije, PC, se povećavao sa rastom I razvićem biljaka od 75,65 do 95,05 g kg^{-1} SM.

Ključne reči: proteinske frakcije, smeše graška i ovsa

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