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Original Paper

Estimation of Ruminal Digestibility of Nutrient and Intestinal Digestibility of Un-Degradable Proteins at Different Feedstuffs

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The work evaluates the content of nutrients, rumen digestibility of NDF, degradability of CP, and intestinal digestibility of rumen un-degradable proteins in protein forages and concentrated feeds. The analysis of nutrient content confirmed along with the increase of plant maturity expressed by the increase of NDF, a significant decrease of NFC and CP in the regression dependence R^2 = 0.7649 and R^2 = 0.7116, respectively. The *in situ* method after 30 hours of incubation confirmed the higher NDF digestibility (P <0.001) of young roughages. The degradability of CP in alfalfa and grass silages was on average 78.7 ±6.1% or 63.7 ±9.7%. Higher protein degradability was confirmed in early-stage forages compared to mature forages for alfalfa (P <0.05) and grass (P <0.01) silages. Intestinal digestibility of un-degradable proteins determined by the modified three-step method was on average 64.1 ±2.5% in alfalfa silages and 51.0 ±5.6% in grass silages, with a statistically significant decrease (P <0.05) in mature forages. The obtained values of rumen degradability of crude protein confirmed a significant difference (P <0.001) between concentrates ranging from 20.3 to 76.0% (mean 58.1 ±3.0%) according to heat treatment. Intestinal digestibility of un-degraded protein varied from 54.5 ±1.4% in raw soybean to 95.2 ±1.0% in corn gluten feed. This study showed that the digestibility factor of crude protein and intestinal digestibility methods for protein feeds can be used in models to optimize the protein nutrition of dairy cows.

Keywords: digestibility, protein, fiber, forage, concentrate

1 Introduction

Protein balance in feed rations of high-producing dairy cows is important for increasing milk production and reducing the impact on the environment through the way (Powell et al., 2014) optimization of the amount of metabolizable proteins (Schwab 2010). Protein nutrition, both in terms of the amount of CP, the proportion and rate of rumen degradation, but also the amount of un-degradable proteins significantly affects the production, health, and reproductive parameters of the herd (Robinson et al., 2006). An important criterion for evaluating the quality of feed and ration is the analysis and ratio of rumen degradable proteins (RDP) and rumen un-degradable proteins (RUP), as well as intestinal digestibility of un-degradable proteins.

The trend of increasing the quantity and efficiency of milk production and the creation of production health is associated with increasing the utilization of nutrients by maximizing the intake of quality forages and decreasing the proportion of concentrated feed (Cauty, Perreau 2003). Forages are an important source of nutrients for the synthesis of dairy cow's milk, as well as stabilizing components of feeding for optimal rumen function.

Growth stage, cutting order, leaf-to-stem ratio, moisture at harvest, and processing method are the most important causes of forage quality variation (Mauriès 2003, Veronesi et al., 2010). Increasing the plant´s maturity increases the proportion of neutral detergent fiber (NDF) in the cell walls and reduces the amount and utilization of nutrients from the cell contents. The result of the increasing proportion of fiber can be:

- a decrease in the content of digestible nutrients and energy of animal feed;

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- a slowing down of rumen emptying, and a decrease in dry matter intake in animals.

Indicator of the nutritional value of forage is the content of neutral detergent fiber (NDF) and its digestibility in relation to the stage of maturity at harvest with the change of morphological composition of plant tissues (Sanderson al., 2004).

In protein forages, the concentration of RUP is influenced by the growth stage of harvest, preservation method, genotype, and cultivars (Guines et al., 2000; Tremblay et al., 2000). The increase in NDF content and decrease in protein with increasing maturity of forages is conditioned by a change in the structure of the cell walls, but also due to a decreasing leaf-to-stem ratio. The most important are changes in the amount and content of nutrients during the alfalfa growth phase, which are manifested by increasing the NDF content by 4.5–5.5 $g.kg^{-1}$ DM and decreasing CP 2.5–3.5 $g.kg^{-1}$ DM daily (Ball et al., 2001; Mitrík 2010)

Immature alfalfa harvested at the beginning of the growing season has a high protein content, which is quickly degraded to ammonia in the rumen and their utilization is less efficient (Undersander et al., 2011). The high proportion of protein and low concentration of fibre when using immature alfalfa as the main feed source limits the dietary properties of the ration in meeting the nutritional requirements for protein, energy, and structural fiber. Alfalfa fibre contains a high proportion of lignin compared to grasses, which results in lower digestibility of NDF in mature alfalfa. The potential digestibility of NDF in grass is 55 to 70%, but the NDF digestibility in alfalfa is 40–60% due to its high lignin content (Ball et al., 2001).

The aim of the work was to analyse and evaluate the influence of the composition, preservation and treatment of protein forages and concentrates on rumen degradability (CP and NDF) and intestinal digestibility of RUP.

2 Material and methods

2.1 Feedstuffs

The different protein feedstuffs routinely used in Slovakia were examined: a., forages (alfalfa and grass silages, alfalfa hay) and b., protein concentrates – raw soybean, soybean meal, Soypass (soybean meal treated with xylose), corn gluten meal, corn gluten feed, rapeseed meal.

The quality of farm-scale alfalfa silages (n = 14), grass silages (n = 12) and alfalfa hay (n = 7) from East part of Slovakia was determined on the Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy in Košice.

2.2 Chemical analyses of feed.

Feed samples were analysed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and ether extract (EE) according to conventional methods (Committee regulation ES No.152/2009 of 27.1. 2009). Dry matter was determined by weight upon drying the sample at 105 °C under prescribed conditions. The CP content was determined according to the Kjeldahl methods (N × 6.25) using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Sweden). Fat (as etheric extract) was determined by the device Det-gras (JP SELECTA, Spain). Neutral and acid detergent fiber were determined by the device Dosi-Fibre Analyzer (JP SELECTA, Spain).

2.3 Animals and in situ rumen incubation

Parameters of rumen degradation of crude protein (CP) and NDF were determined by using protocol Orskov and McDonald (1979), where 5 g of sample of each feed was weighed into bags (R1020 ANKOM technology, Macedon, NY) and heat-sealed. Samples of protein concentrates were incubated in duplicate in the rumen of each cow for 12 h, and protein forages of 16 h for protein and 24 h for NDF. Animals were fed *ad libitum* lactation diet (CP 15.0%, neutral detergent fibre 36.8%, and acid detergent fibre 25.3% of dry matter) composed of 53% forage and 47% of concentrate. After ruminal degradation, bags were rinsed with cold water to remove particulate matter.

2.4 The intestinal digestibility of RUP

The intestinal digestibility of RUP of selected feeds was performed by a modified three-step method (MTSP) (Gargallo et al., 2006). Approximately 1.0 g of the pooled rumen-exposed residue after 12 resp. 16 h rumen incubation was weighed into nylon bags (R 510 ANKOM Technology), heat-sealed and placed in a DaisyII incubator (ANKOM, Fairport, NY). Samples were incubated in 2 l of pre-warmed 0.1 N HCl solution adjusted to pH 1.9 and containing 1 $g.l^{-1}$ of pepsin (P-7000, Sigma, St. Louis). They were rotated constantly at 39° C for 1 h. After pepsin digestion, samples were rinsed in cold tap water until the runoff was clear before they were incubated in 2 l of pre-warmed pancreatin (Sigma P-7545, St. Louis) solution (0.5 M KH_2PO_4 buffer standardized at pH 7.8 and containing 50 ppm of thymol and 3 $g.l^{-1}$ of pancreatin) and rotated constantly at 39 °C for 24 h. After incubation, samples were rinsed in cold tap water until the runoff was clear and oven-dried at 55° C for 48 h.

The achieved results were processed by mathematical and statistical methods using the statistical program GraphPad Prism9. We evaluated the CP degradation results in the rumen, their intestinal digestibility and

their differences for each feed as variables using the Tukey-HSD test at the significant levels of $P \leq 0.01$ and $P \leq 0.05$. Each parameter was presented as its mean (x), and standard deviation (SD).

2.5 Ethical statement

All procedures concerning the animals were performed in compliance with the national guidelines for animal care.

3 Results and discussion

3.1 Nutritional composition of protein forages depending on the vegetation phase of harvesting

The content of nutrients (NDF, CP, and NFC) in alfalfa and grass silages is mainly influenced by the vegetation phase of ensiling matter during harvesting. The analysed content of nutrients in protein forages was divided into groups according to the vegetation phase during harvesting and evaluated according to NDF content and the method of preservation is summarized in Table 1.

In group I (immature alfalfa and grass silages) with an average NDF content of 36.9 ±1.51% respectively 44.0 ± 8.1%, a significantly higher proportion of the main nutrients (CP and NFC) in the cellular content of the feed was confirmed compared to group II (mature alfalfa and grass silage) with an NDF content of 47.8 ±1.32% resp. 55.93 ±53.8%. With the increase in the concentration of NDF in the analysed alfalfa and grass silages, the content of CP and NFC decreased, which was confirmed by the regression dependence with the coefficient of determination for NFC $R^2 = 0.7649$ and for CP $R^2 = 0.7116$ (Figure 1).

In the summary evaluation of alfalfa and grass silages, each increase in NDF content by 1.0% reduces NFC content by 5.7 g.kg^{-1} DM and CP content by 4.3 g.kg^{-1} DM. In the evaluation of alfalfa silages alone, an increase in NDF content by 1.0% confirms a higher decrease in CP of 5.0 g.kg^{-1} DM, while in the case of grass silages was confirmed a higher decrease in NFC content by 8.6 g.kg^{-1} DM.

In the case of alfalfa, Mitrík (2010) confirmed an increase in NDF content by 4.4 g.kg^{-1} DM and a decrease in CP content by 2.95 g.kg^{-1} DM in the daily dynamics. According to this dependence, the analysed difference in the content of NDF 109 g.kg^{-1} and CP 52 g.kg^{-1} DM between the groups in our monitoring corresponds to a shift in the time of collection of silage crops by 18–25 days.

The NDF content analysed represents the cell walls of the forages that form a complex lignin matrix, a small amount of protein, and various polysaccharides, especially cellulose, hemicelluloses, and pectin. The structure and composition of the cell wall vary depending on the type of plants and the type of plant tissues. The NDF content in the feed increases with the maturation of plants and depends on the growth environment including temperature, light intensity, water availability (Jung et al., 2012). In young (immature) plants, the cell walls of the stem and also the leaves are formed by the primary wall, with a high proportion of cellulose. As the vegetation maturity increased, the plants' stems increase the proportion of the secondary cell wall and the formation of the lignin-cellulose complex. As the forages mature, the leaf to the stem ratio decreases. During flowering, grass stems faster accumulate the cell wall material, while alfalfa is a more even increase in leaves and stems. The proportion of the cell wall of the leaves with the increasing maturity of alfalfa is dramatically not increasing. Immature alfalfa contains a smaller proportion of cell wall than grass and

Table 1 Nutritional composition of forages (g.kg^{-1} DM) used in these evaluated analyses

	DM	CP	NDF	ADF	EE	NFC	Ash
Alfalfa silages I. NDF <400 g.kg^{-1}	375.6 ±25.3	227.3* ±14.0	368.8** ±15.1	306.8* ±16.7	32.1* ±0.3	263.6* ± 11.0	108.1* ±6.0
Alfalfa silages II. NDF >450 g.kg^{-1}	446.6 ±73.1	174.6* ±4.5	477.7** ±13.2	411.5* ±18.3	24.1* ±2.4	235.4* ±21.7	88.1* ±1.9
Grass silages I. NDF <450 g.kg^{-1}	268.0 ±37.0	149.6* ±4.2	440.2* ±8.1	280.8** ±13.7	37.7 ±3.1	267.4** ± 21.4	105.1 ±16.1
Grass silages II. NDF >500 g.kg^{-1}	296.7 ±21.9	132.1* ±6.6	559.3* ±53.8	355.5** ±17.7	36.8 ±2.6	157.7** ±31.8	114.0 ±26.4
Alfalfa hay I. NDF <500 g.kg^{-1}	943.0 ±24.7	173.0 ±10.3	475.5 ±40.3	402.5 ±64.3	26.0 ±4.2	235.6 ±34.3	89.9 ±0.1
Alfalfa hay II. NDF >500 g.kg^{-1}	942.4 ±20.1	159.4 ±19.2	534.5 ±17.7	417.5 ±0.7	13.0 ±1.4	204.8 ±19.6	88.5 ±23.3

* P <0.05; ** P <0.01

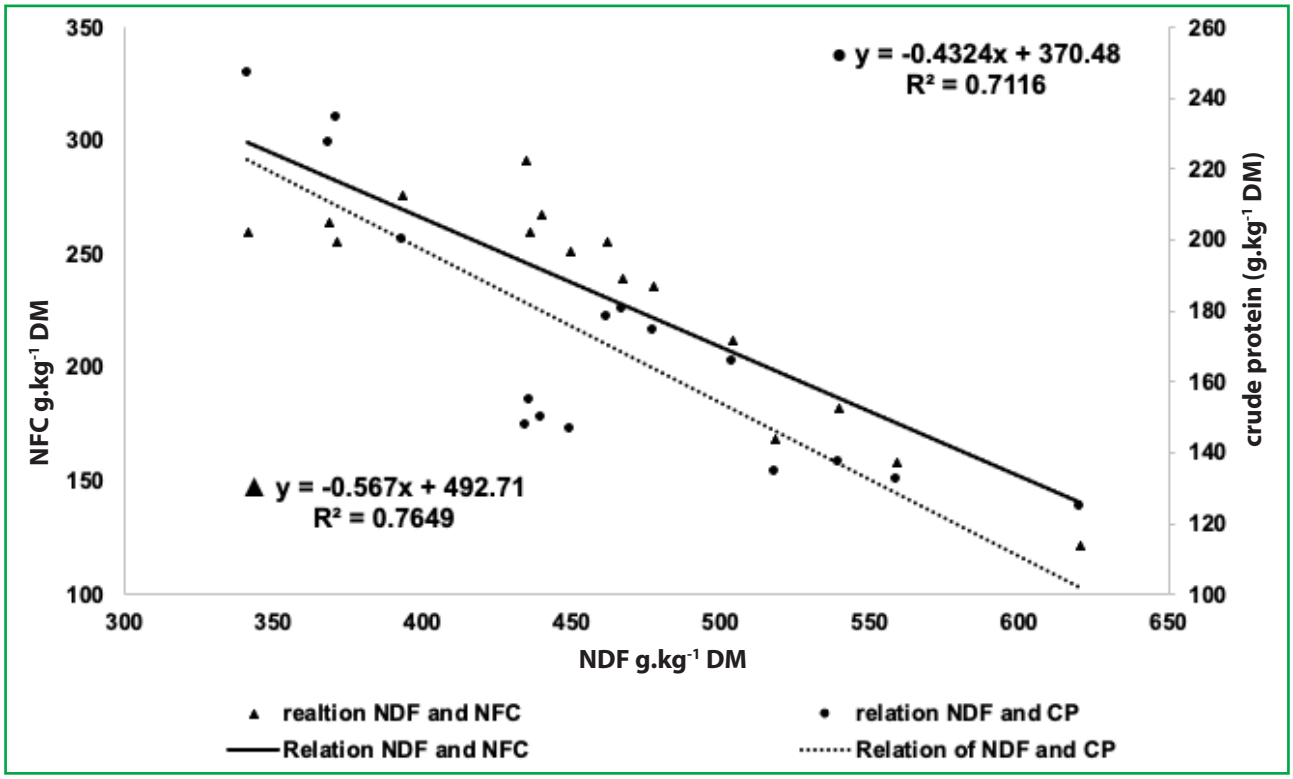

Figure 1 Dynamics of the analysed components of the cell wall (NDF) and cell content (NFC and CP) in protein forages

the proportion of the pectin is higher in the cell wall material (Jung and Engels 2002). Higher NDF content is represented in the leaves and stems of grass compared to the alfalfa. 25% NDF content in the leaves and 40 to 55% of the NDF content in the stems were observed in alfalfa, while the leaves and stems of the grass contained 50 resp. 70% NDF content at the same vegetation phase (Yu et al., 2003). The leaves have a stable and higher protein content than stems, which in the growth phase develops at the expense of leaves, and their cell walls increase in fiber content and the proportion of lignin as they mature (Veronesi et al., 2010).

3.1.1 Rumen degradability and intestinal digestibility of nutrients in forages

The results of analyses of tested protein silage in the experiment, evaluation of degradability of CP and digestibility of NDF in the rumen, and intestinal digestibility of un-degradable proteins, after statistical processing are summarized in Table 2. The amount and ruminal degradability of NDF and CP in forages is influenced by the maturity stage, growth conditions and method of preservation.

The rumen digestibility of NDF, analysed by the *in-situ* method after 30 hours of incubation in the rumen, was significantly higher (P <0.001) in younger protein forages an average of 52.1 ±2.0% with a tendency for a higher level of fermentation of silages compared to alfalfa hay. In alfalfa silage, NDF digestibility in the rumen was analysed on average 47.9 ±5.3%. At an early stage of maturity with a lower NDF content, the digestibility in the rumen reached an average of 52.4% compared to 43.5% NDF digestibility (P <0.01) in later stages of maturity with a higher NDF content and lignification of the plant stems. In grass silage, NDF digestibility was on average (46.5 ±6.0%) significantly higher (P <0.01) in younger immature grasses of 51.7 ±2.0% compared to 41.3 ±1.8% in vegetative mature forages.

The statistical evaluation of the correlation between NDF content of tested silages and NDF digestibility and degradability of the CP in the rumen is given in figure 2.

The dynamics of the NDF content in the evaluated protein forages in relation to the rumen digestibility of NDF confirms a regression dependence at the level of R^2 = 0.6458 and in relation to the rumen degradability of CP at the level of R^2 = 0.8633. The correlation dependence of the evaluated indicators confirmed that each increase in NDF content by 1% in alfalfa and grass silages reduces the rumen digestibility of NDF by 0.8% and the rumen degradability of CP by 1.3%.

Between groups of alfalfa and grass silage according to vegetation maturity, there was a difference in rumen digestibility of NDF on average 9 resp.10%. Yu et al. (2003) confirmed a lower decline in the rumen digestibility of

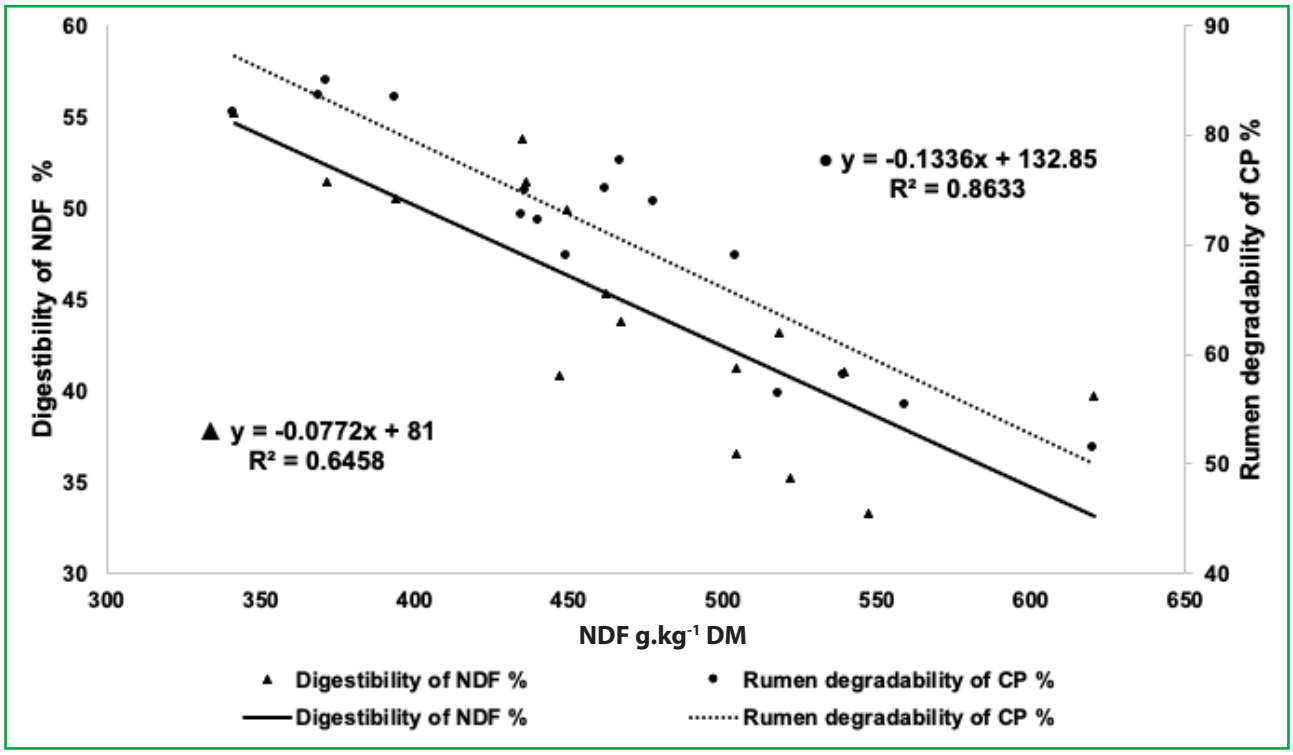

Figure 2 Rumen digestibility of NDF and degradability of CP in relation to the NDF content in forages

NDF between samples of alfalfa hybrid at the level 6.6% point, which represents 0.94% per day. This decline in digestibility of NDF in alfalfa was from 43.4% to 36.8% and expressed a delay of 7 days. In grasses, lignin binds to the fraction of hemicellulose through network bonds that lead to slower digestibility of the cell wall, regardless of lignin concentration (Grabber 2005). In alfalfa is assumed that lignin is cross-linked with cell wall polysaccharides, but the chemical bond has not been identified (Jung et al., 2004).

Rumen degradability of CP in evaluated protein silages (Table 2) was on average 71.2 ±11.0%. Higher degradability of CP was in groups of younger forages (77.8 ±6.5%) with significantly lower (P <0.05) degradability of CP (64.6 ±10.8%) in mature forages. The degradability of CP separately in alfalfa and grass silages was on average 78.7 ±6.1% resp. 63.7 ±9.7%. Statistically significant differences in the rumen degradability of CP were confirmed among the analysed samples of alfalfa silages of group I in the early stage of maturity compared

Table 2 Rumen degradability of CP and NDF and intestinal digestibility of non-degradable proteins according to NDV content in the forages

	Fermentability NDF% 30 h	*Degradability of CP% 16 h	Intestinal digestibility of RUP%	Feed intake potential * kg DM	NEL MJ.kg^{-1} DM
Alfalfa silages I. NDF <400 g.kg^{-1}	52.4 ±3.7	83.5 ±1.5	64.1 ±3.4	21.2 ±1.5	6.0 ±0.1
Alfalfa silages II. NDF >450 g.kg^{-1}	43.4 ±3.4	73.9 ±4.5	64.2 ±1.5	16.4 ±0.8	5.2 ±0.2
Grass silages I. NDF > 450 g.kg^{-1}	51.7 ±2.0	72.2 ±3.0	58.1 ±7.2	17.7 ±0.3	5.8 ±0.3
Grass silages II. NDF > 450 g.kg^{-1}	41.3 ±1.8	55.3 ±3.4	43.9 ±4.0	14.0 ±1.3	5.2 ±0.4
Alfalfa hay I. NDF <500 g.kg^{-1}	45.1 ±3.6	60.2 ±1.8	74.1 ±2. 1	16.5 ±1.3	5.2 ±0.3
Alfalfa hay II. NDF >500 g.kg^{-1}	39.8 ±1.6	49.0 ±5.9	67.1 ±0.8	14.6 ±0.5	4.8 ±0.5

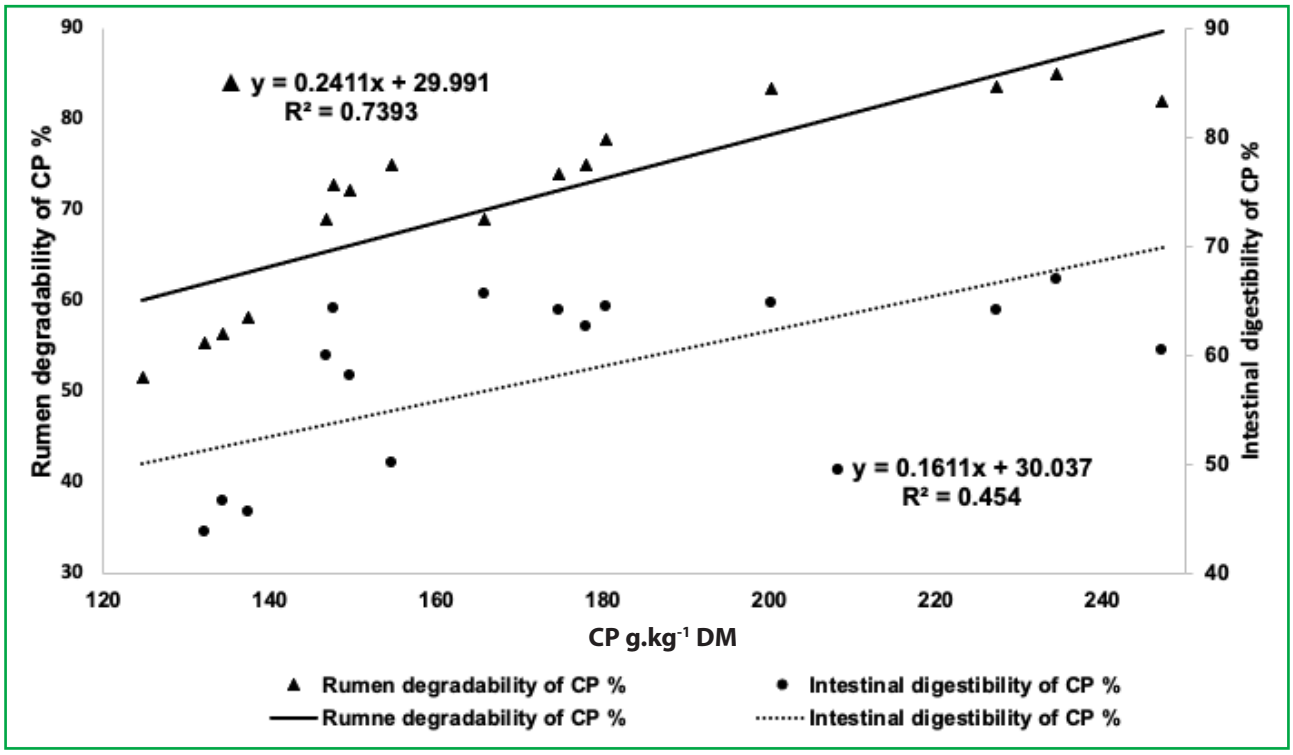

Figure 3 CP content in protein feeds in relation to rumen degradability and intestinal digestibility of proteins

to silage of group II (P <0.05) and among groups of grass silages P <0.01.

The dynamics of the content of CP in the evaluated protein forages (graph 3) in relation to the analysis of rumen degradability of CP and intestinal digestibility of RUP confirm a regression dependence with the coefficient of determination for rumen degradability of CP at the level (R^2 = 0.7393) and intestinal digestibility of RUP (R^2 = 0.454). Increasing the CP content by 1% increases the degradability of CP by 2.4% and the intestinal digestibility of RUP by 1.6% in the evaluated alfalfa and grass silages.

Intestinal digestibility of rumen un-degradable proteins (RUP) determined by the modified three-step *in vitro* method in the DaisyII incubator for all analysed samples (Table 2) reached an average level of 64.1 ±2.3%. Intestinal digestibility of RUP in group I of alfalfa silages with lower NDF content was on average 64.1 ±3.4% without significant differences compared to group II with an average value of 64.2 ±1.5%. The statistical difference (P <0.05) in intestinal digestibility of RUP was confirmed in grass silage groups with an average value of 58.1 ±7.2% or 43.9 ±4.0%. Less information is available in studying the intestinal digestibility of RUP of forages compared to studies on rumen protein degradability. The intestinal digestibility of the RUP is estimated at 80% as a fixed value, however in fact these values in forages significantly vary (Prestløkken and Rise, 2003). NRC (2001) uses the value of intestinal digestibility from 50 to 100%. The determined intestinal digestibility of RUP by the modified three-step method in our tested alfalfa silages was comparable to the study by Wang et al. (2015), ranging from 59.5 to 67.4%. In our grass silages, the analysed intestinal digestibility of RUP was on average higher than the average value of 39.3% reported by Wang et al. (2015). In a parallel analysis of the intestinal digestibility of RUP in forages (alfalfa and grass silage), a higher level of digestibility was confirmed by the mobile nylon bag method than by the modified three-step method (Wang et al., 2015). Intestinal digestibility, like rumen degradability in forage, is affected by the harvesting time of the forage. The influence of different nutritional values and content of nutrients (NDF, CP, EE) on the level of intestinal digestibility was confirmed by Wang et al. (2015) by regression dependence with the coefficient of determination R^2 = 0.8668 (P <0.01).

3.2 Nutritional composition of concentrated feeds and utilization of nutrients depending on the type and method of treatment

Chemical analysis of nutrients and calculated nutritional value of tested concentrates in the experiment is summarized in Table 3. The CP content of selected feeds varied from 205.7 to 748.4 g.kg^{-1} of DM among feedstuffs. As expected, there was a large range in EE content among all feedstuffs (19.2–214.3 g.kg^{-1} of DM).

Table 3 Nutritional composition (g.kg^{-1} DM) of protein concentrated feeds

	CP	CF	NFC	EE	NDF	Ash
Raw Soybean	319.4 ±14.1	73.5 ±13.1	396.4 ±29.3	214.3 ±13	163.5 ±32	59.1 ±4.0
Soybean meal	520.0 ±18.4	49.9 ±6.3	381.8 ±23.1	27.6 ±0.8	94.4 ±17.4	76.2 ±5.0
Soy-pass	501.3 ±14.6	51.1 ±12.1	276.4 ±16.3	38.6 ±0.6	105.1 ±14.2	78.6 ±4.1
Rapeseed meal	376.2 ±13.3	133.4 ±13.1	171.2 ±10.4	57.2 ±8.0	318.5 ±37.0	76.9 ±4.3
Corn gluten meal	748.4 ±29.0	9.1 ±4.1	169.8 ±7.8	19.2 ±1.2	41.2 ±2.5	21.1 ±0.8
Corn gluten feed	205.7 ±15.2	81.3 ±1.1	292.0 ±12.8	36.1 ±0.9	390.6 ±4.3	75.6 ±0.5

Results of rumen degradability of proteins (RDP) confirmed the effect of the type of feed and the way of its treatment (Table 4). Heat treatment of soybean-based feed significantly affected the degradability of CP in the rumen with the analysed value of 76.0% for raw soybean compared to 46.5% for Soypass. Stern et al. (2006) analysed the proportion of rumen-degradable proteins depending on the thermal treatment of soybean feed from 31.7 to 76.8%. The lowest rumen degradability of proteins was confirmed with the commercial product Soypass, with the same type of feed (extracted soybean meal treated with xylose) Borucki Castro et al. (2007) report rumen degradability at the level of 34.9%. A close level of rumen degradability for soybean extracted meal was found by Mjoun et al. (2010) at the level of 67.7%, as well as 70.2% Borucki Castro et al. (2007). A higher level of rumen degradability (77%) for soybean extracted meal is described by Falahatizow et al. (2015).

Rapeseed meal showed a mean degradability of CP equal to 60.5. Rapeseed meals reached a mean degradability of CP equal to 60.5%. By-products such as corn gluten feed with a proportion of CP 200–300 g.kg^{-1} of DM confirmed higher rumen degradability of 74.8% to compared corn gluten meal with the highest resistance to microbial degradability at the level of 20.3%. In our experiment, the value of rumen degradability for rapeseed at the level of 60.5% with the analysed ADIN content of 6.5% of CP is consistent with the findings of Maxin et al. (2013) and Falahatizow et al. (2015) at the level of 59.3 or 56.0% with an analysed ADIN content of 7.7% CP.

The intestinal digestibility of RUP (Table 4) was estimated by the modified method described by Gargallo et al. (2006). The mean intestinal digestibility of RUP was 78.4 ±13.0%. Rumen degradability of CP, as well as intestinal digestibility of RUP in analysed protein feeds, was significantly variable (P <0.01). The analysed intestinal digestibility of RUP in concentrates was on average 78.4% with fluctuations in the range of 54.5–95.2% for individual feeds, which is comparable to the values of intestinal digestibility of 80%, with also the same tendency of variability in ranging from 59.2–95.0% (NRC 2001). In the case of soybean feeds, we confirmed the intestinal digestibility of RUP in the range of 54.5 to 90.1%, which are comparable values as reported by Stern et al. (2006) for heat-treated soy proteins without thermal degradation ranging from 57.7% to 83.8%. From the available data, the highest intestinal digestibility RUP of soybean meal on average 97.7 ±0.75% for extracted soy meal and extruded soy was confirmed by Mjoun et al. (2010). This intestinal digestibility was significantly higher than the average intestinal digestibility of RUP (72.1 ±16.3%) in different soybean treatments than in our observations. (From) Of the observed soybean feeds, we found the lowest intestinal digestibility of 54.5% for untreated soybeans, while for heat-treated soybean feeds, the intestinal digestibility reached an average level of 77.9%, which is comparable to the data of Borucki Castro et al. (2007) with an average intestinal protein digestibility of 79.5% for the same soybean feeds.

The lower intestinal digestibility of RUP in raw soybean due can explain by the continuity of the effect of anti-nutritional substances (tannins, trypsin inhibitors, lectins, and saponins) in leguminous feeds (Shimelis and Rakshit 2005), which are partially disturbed in the rumen, but their fractions can affect intestinal digestibility. The intestinal digestibility of RUP given in literary sources is also significantly different according to the used diagnostic methods for determining digestibility.

The effects of heat treatment on rumen degradability of proteins and intestinal digestibility of rumen un-degradable proteins, which we confirmed in this study, are consistent with the findings of literature sources for protein feeds (Lund et al., 2008; Solanas et al., 2008). Ruminal degradability, as well as intestinal digestibility of RUP, is most affected by different combinations of temperature and treatment time of protein feeds (Stern et al., 2006). Other factors that affect the rumen degradability of proteins are fat content, feed structure, chemical structure, proportion and digestibility of fiber, as well as the type and relative representation of carbohydrates, which limit the degradation and rate of passage (Doreau et al., 2009).

Table 4 Rumen degradability and intestinal digestibility of CP in protein concentrates

	CP g.kg^{-1} DM	ADIN % of CP	Degradability of CP% 12 h	Intestinal digestibility of RUP%	NEL MJ.kg^{-1} DM
Raw Soybean	319.4 ±14.1	4.1	76.0 ±8.8	54.5 ±1.4	8.9
Soybean meal	520.0 ±18.4	3.6	71.0 ±1.8	90.1 ±0.6	8.68
Soy-pass	501.3 ±14.6	5.6	46.5 ±1.7	62.8 ±0.1	8.06
Rapeseed meal	376.2 ±13.3	6.5	60.5 ±3.1	81.3 ±1.4	7.91
Corn gluten meal	748.4 ±29.0	2.1	20.3 ±0.5	84.7 ±7.3	9.04
Corn gluten feed	205.7 ±15.2	2.2	74.8 ±0.1	95.2 ±1.9	8.54

Table 5 Proportion of NL usable (g.kg^{-1} DM) at the level of rumen and intestinal transformation from available protein feeds for dairy cows

	CP g.kg^{-1} DM	Usable in the rumen	Usable in the intestine	Excluded as unused
Alfalfa silages I. NDF <400 g.kg^{-1}	227.3 ±14.0	189.7 ±19.6	24.0 ±2.6	13.6 ±3.5
Alfalfa silages II. NDF >450 g.kg^{-1}	174.6 ±4.5	129.2 ±13.5	29.2 ±4.1	16.2 ±1.8
Grass silages I. NDF <450 g.kg^{-1}	149.6 ±4.2	108.1 ±7.3	24.2 ±4.2	17.3 ±2.6
Grass silages II. NDF >450 g.kg^{-1}	132.1 ±6.6	73.3 ±8.1	25.8 ±1.8	33.1 ±3.1
Alfalfa hay I. NDF <500 g.kg^{-1}	173.0 ±10.3	104.1	46.2	22.7
Alfalfa hay II. NDF >500 g.kg^{-1}	159.4 ±19.2	78.1	60.2	21.1
Raw Soybean	319.4 ±14.1	242.7	41.8	34.9
Soybean meal	520.0 ±18.4	369.2	135.9	14.9
Soy-pass	501.3 ±14.6	233.1	168.4	99.8
Rapeseed meal	376.2 ±13.3	227.6	120.8	27.8
Corn gluten meal	748.4 ±29.0	152.0	505.5	91.3
Corn gluten feed	205.7 ±15.2	153.9	39.3	2.5

Chemically bound CP to acid-detergent fiber expressed as acid-detergent insoluble nitrogen (ADIN) with a range from 2.1 to 6.5% of CP were analysed in the tested feeds as an indicator of the degree of heat treatment (Maillard reaction). During the heat treatment, in addition to denaturation, the thermal overheat is applied, analytically confirmed by the increased share of ADIN, which was at the level of 5.6% of CP in Soypass, while Borucki Castro et al. (2007) analysed 7.6% of CP, which can be related to a 1.8–11.6% lower proportion of ruminal protein degradation.

Currently, a heat treatment system is being applied industrially in order to increase the proportion of RUP in commercially produced concentrates. Thermal treatment of feeds is a common method that can be performed in a number of ways, including moist heat treatment with a positive relationship between steam pressure and temperature (Van der Poel et al., 2005).

According to the nature of proteins in feed for ruminants, they can be used at the level of ruminal digestion to support the synthesis of microbial proteins, as well as at the level of intestinal digestion, depending on the intestinal digestibility of ruminally un-degradable proteins. The estimated balance of usability in the evaluated feeds is summarized in Table 5. Of the evaluated forages, the higher utilization of proteins for the support of microbial protein synthesis was found in the rumen degradation of alfalfa silages, as well as alfalfa and grass silages in immature ones. Statistically significant differences were not confirmed for intestinal digestibility. From the point of view of the preservation method, the higher utilization of proteins at the level of intestinal digestion was confirmed in the evaluation of alfalfa hay. The utilization of proteins of concentrated feeds at the level of rumen and intestinal digestion was significantly influenced by the method of heat treatment.

4 Conclusions

The analysed of the nutrient content of forages determinates feed quality and confirms the importance of the correct choice of plants maturity at the time of harvest. Feed quality is determinate taking into account the amount and ratio of nutrients, their rumen

and intestinal utilization, but also the potential of feed intake according to the NDF content and the rate of passage through the digestive tract. The analysis of the quality and biological value of protein concentrates by determining the rumen degradability of proteins and the intestinal digestibility of RUP confirmed the influence – of the time dependence of incubation, as well as the type and method of treatment of the feed.

According to the chemical structure, natural binding to other nutrients and the method of treatment, the proteins of forages and concentrates not only have different rumen degradability and intestinal digestibility, but also different amino acid composition of individual protein fractions. The obtained results in the most frequently used feeds are applicable for the development of control systems and optimization of protein nutrition of dairy cows.

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