Original paper

EFFECT OF CONSECUTIVE CUT AND VEGETATION STAGE ON CNCPS PROTEIN FRACTIONS IN ALFALFA (*Medicago sativa* L.)

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Abstract

Crude protein (CP) of forages can be separated into fractions of differentiated abilities to provide available amino acids in the lower gut of ruminants. This knowledge is critical to develop feeding systems and to predict animal responses. The objective of this research was to asses whether CP concentrations and the relative proportion of CP fractions by CNCPS in alfalfa (*Medicago sativa* L.) *cv* K-28 were affected by different cuts and vegetation stages. Fraction B_2, which represents true protein of intermediate ruminal degradation rate, was the largest single fraction in all cuts except in the third cut. Soluble fraction A was less than 400 g kg^{-1} CP in all cuts except in the third cut, while the unavailable fraction C ranged from 56 g kg^{-1} CP in the first cut to 134.8 g kg^{-1} CP in the fourth cut. The remaining fraction B_3 (true protein of very low degradation rate) only represented less than 60 g kg^{-1} of total CP. Results showed that undegraded dietary protein represented a small proportion of total CP in alfalfa from the first to the fourth cut.

Key words: *alfalfa, CNCPS, cut, protein*

Introduction

Livestock enterprises are significant contributors to nonpoint sources of environmental N pollution because of their contributions to ammonia emissions and nitrate contamination of surface and ground water (NRC, 1993). Purchased feed, especially protein supplements, is a major source of imported nutrients and farm expenses on dairy farms (Klausner et al., 1998). Under these economic and environmental constraints, improving the efficiency of N utilization and thus reducing N excretion are very important to maintain the sustainability of dairy farms, and nutrition models became an effective farm management tool to accomplish these tasks (Dinn et al., 1998; Wattiaux and Karg, 2004).

Milk production will be reduced when protein supplied by the diet is below energy-allowable milk production, which is affected by protein degradation rates (Fox et al., 2004). Feed protein fractionation systems have been integrated into nutrition models to account for differences in protein availability and utilization. The in situ techniques and schemes based on solubility in buffers and detergent solutions have been adopted by the NRC (2001) and the

Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 2004) to measure protein fractions in feeds.

In the CNCPS the CP (Crude Protein) of feeds for ruminants is divided into three fractions: the non-protein nitrogen (NPN, PA), the true protein (PB) and the combined protein (PC) (Sniffen et al., 1992), of which the true protein (PB) is further divided into PB_1, PB_2 and PB_3. The PA and PB_1 are soluble in buffer and highly degradable, whereas PB_3 and PB_2 are combined with plant cell wall with different degradabilities in the rumen. Although the calculation of PA, PB_1, PB_2, PB_3 and PC is based on the chemical analysis of the CP, the soluble crude protein (SCP), the neutral detergent insoluble crude protein (NDICP) and the acid detergent insoluble crude protein (ADICP) of feeds, PA, PB_1, PB_2, PB_3 and PC have been closely related to the rumen degradation characteristics of feeds (Sniffen et al., 1992).

The objective of this study was to determine the effect of cutting alfalfa at different periods of vegetation on protein fractions determined as CNCPS.

Materials and methods

The experiment was designed as a factorial trial, by randomized block system in three replicates. Samples of alfalfa, *cv* K 28 was taken on May 4th at mid-bud stage in the first cut, on June 6th at early flowering in the second cut, on July 6th at mid-bloom in the third cut and on August 8th at full bloom in the fourth cut. Dry matter was determined by drying out samples at 65º C and grinding and sieving them to 1 mm particle size. The samples were dried in an oven at 105º C for 6 h for dry matter determination.

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 – Residual 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 crude protein fractions of the samples, PA, PB, PB_1, PB_2, PB_3 and PC were calculated based on CP, NPN, SolCP, NDICP, ADICP contents of samples according to Sniffen et al. (1992).

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).

Data were processed by the analysis of variance in a randomized block design. Effects were considered different based on significant (P< 0.01) F ratio.

Results and discussion

The analyses of variance (Table 1) revealed a statisticaly significant effects of the cut on crude protein content in dry matter of alfalfa. The cut was an important source of variability for all investigated protein fractions. Alfalfa had the highest content of crude protein at mid-bud stage in the first cut (199.1 g kg^{-1} DM) and the lowest content of CP at mid-bloom stage in the third cut. This is in agreement with Taylor and Quesenberry (1996) who reported that in early spring young plants of alfalfa have a large proportion of leaves, a high moisture content, protein and minerals.

The highest contents of NDICP and ADICP were in full-bloom stage in the fourth cut. Yari et al. (2012) concluded that alfalfa at the flowering stage had higher content of NDF, ADF and NDICP compared to alfalfa at early and late bud stage. The contents of IP were similar in the first and the second cut of alfalfa, but the lowest content was observed at the mid-bloom stage in the third cut. The values for buffer soluble CP in alfalfa were slightly lower than those reported by Sniffen et al. (1992) and Yari et al. (2012) for spring growth in alfalfa. Results in this investigation showed that all content of SolCP is represented by NPN.

Table 1. *Content of crude protein fractions in alfalfa, cv K 28, g kg^{-1} CP*

Cut	CP	NDICP	ADICP	IP	SolCP	TP	NPN	NPN_{SolCP}
I	199.1^{a}	104.0^{c}	55.9^{d}	653.6^{a}	346.3^{c}	588.1^{b}	411.8^{c}	1000.0^{a}
II	198.3^{ab}	120.6^{b}	90.7^{b}	656.8^{a}	343.5^{c}	675.4^{a}	324.5^{d}	942.8^{b}
III	182.2^{c}	77.5^{d}	70.6^{c}	496.8^{c}	503.1^{a}	471.1^{d}	528.9^{a}	1000.0^{a}
IV	198.0^{b}	194.6^{a}	134.8^{a}	629.1^{b}	370.8^{b}	553.3^{c}	454.9^{b}	1000.0^{a}

Different letters denote significantly different means (P< 0.01)

In models designed to asses utilization of dietary protein by ruminants, it is assumed that most of the soluble protein (PA and PB_1) is completely degraded in the rumen, and varying proportions of insoluble fractions (PB_2, PB_3 and PC) escape ruminal degradation depending on the interactive effects of digestion and passage (Sniffen et al., 1992). Because various

protein fractions differ in rate and extent of ruminal degradation, the proportions of these different protein fractions in alfalfa are believed to influence the amounts of ruminally degraded and escape protein consumed by animals (Elizalde et al., 1999). The results of these protein fractions by CNCPS are presented in Table 2.

Table 2. *Content of crude protein fractions in alfalfa, cv K 28 by CNCPS, g kg^{-1} CP*

Cut	PA	PB	PB_1	PB_2	PB_3	PC
I	346.3^{c}	597.7^{a}	0.0^{b}	549.5^{a}	48.1^{a}	55.9^{d}
II	323.8^{c}	585.7^{a}	19.3^{a}	536.1^{a}	29.9^{b}	90.7^{b}
III	503.1^{a}	426.3^{c}	0.0^{b}	419.4^{b}	7.0^{c}	70.4^{c}
IV	370.8^{b}	494.3^{b}	0.0^{b}	434.5^{b}	59.9^{a}	134.7^{a}

Different letters denote significantly different means ($P< 0.01$)

The results indicate that alfalfa cut at mid-bloom stage in third cut had the highest rapidly degradable NPN fraction-PA fraction of crude protein. The highest content of undegradable PC fraction, associated with the lignin and cell wall was observed at full-bloom stage in the fourth cut. The slowly degradable PB_3 fraction associated with the plant cell wall was the lowest at mid-bloom stage of alfalfa in the third cut. PB_2 fraction, which is intermediately degradable in the rumen was the highest protein fraction in all the cuts of alfalfa. Values for this fraction were similar in the first and the second cut, but higher than values for PB_2 fraction in the third and the fourth cut.

The protein fractions of alfalfa in this study differed from tabular values in NRC (2001). Fraction PA was higher, fraction PB ($PB = PB_1 + PB_2 + PB_3$) was lower and fraction PC was higher that the tabular value in NRC (2001), except for the value of PC fraction in the first cut. Sniffen et al. (1992) found that the fraction PB_2 was the largest CP fraction in alfalfa, with a mean value of 41% of the total CP, which is in agreement with our results in the third and the fourth cut. Elizalde et al. (1999) reported a PB_2 value of 51.6% of the total CP in alfalfa, which is in agreement with our results in the first and the second cut. The values for PB_3 fraction of alfalfa in this study were similar to the results obtained by Elizalde et al. (1999) who reported that this value amounted to 3% of CP in alfalfa. From a nutritive point of view, PC fraction appears to be essentially indigestible and the amount apparently digested is poorly used by ruminant animal (Sniffen et al., 1992). In this study, the proportion of fraction PC averaged approximately 9% of the total CP which is higher than the results obtained by Cherney et al. (1992) for alfalfa (1.8-4.6% CP).

Conclusion

Cutting alfalfa at different cuts and different vegetation stages had profound influence on the protein fractions as determined by CNCPS. The present data indicate that alfalfa from different cuts and different vegetation stages differ in proportions of protein fractions, which account for different rumen degradation characteristics.

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References

1. Cherney DJR., Volenec JJ and Cherney JH 1992. Protein solubility and degradation *in vitro* as influenced by buffer and maturity of alfalfa. Anim. Feed Sci. technol, 37, 9-20.
2. Dinn NE, Shelford JA and Fisher LJ 1998. Use of the Cornell Net Carbohydrate and Protein System and rumen-protected lysine and methionine to reduce nitrogen excretion from lactating dairy cows. Journal of Dairy Science, 81, 229-237.
3. Elizalde JC, Merchen NR and Faulkner DB 1999. Fractionation of fiber and crude protein in fresh forages during the spring growth. J. Anim. Sci., 77, 476-484.
4. Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE, Pell AN and Overton TR 2004. The Cornell Net carbohydrate and protein System model for evaluating herd nutrition and nutrient excretion. Animal Feed Science and Technology. 112, 29-78.
5. Klausner SD, Fox DG, Rasmussen CN, Pitt RE, Tylutki TP, Wright PE, Chase LE and Stone WC 1998. Improving dairy farm sustainability I: An approach to animal and cop nutrient management planning. J. prod. Agric, 11, 225-233.
6. Licitra G, Hernandez TM and Van Soest PJ 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science Technology, 57, 347-358.
7. NRC 1993. Soil and water quality: An agenda for agriculture. National Academic Press, Washington, DC.
8. NRC 2001. Nutrient Requirements for Dairy Cattle. National Academic Press, Washington, DC.
9. Sniffen CJ, O'Connor DJ, Van Soest PJ, Fox DG and Russell JB 1992. A net carbohydrate and protein system for evaluating cattle diets. II: Carbohydrate and protein availability. J. Anim. Sci., 70, 3562-3577.
10. Taylor NL and Quesenberry KH 1999. Red clover science. Kluwer, Academic Publishers.
11. Wattiaux MA and Karg KL 2004. Protein level for alfalfa and corn silage-based diets: II. Nitrogen balance and manure characteristics. Journal of Dairy Science. 87, 3492-3502.
12. Yari M, Valizadeh R, Naserian AA, Ghorbani GR, Moghaddam PR, Jonker A and Yu P 2012. Botanical traits, protein and carbohydrate fractions, ruminal degradablity and energy contents of alfalfa hay harvested at three stages of maturity and in the afternoon and morning. Anim. Feed Sci. Technol., 172, 162-170.