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ORIGINAL SCIENTIFIC PAPER

The effect of partial replacement of maize with dried whey on N bilance from a diet based on alfalfa haylage in wether sheep

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Abstract
The aim of this study was to determine the effect of partial replacement of ground maize grain (GM) with dried whey (DW), as an supplement to the alfalfa haylage (AH), on the nitrogen (N) bilance in wether sheep. Four feeding treatments, with the addition of 30 g kg^{-1} $M^{0,75}$ of concentrate, were investigated: only GM (treatment A) or GM replaced with DW in the quantity of 10% (treatment B), 20% (treatment C) or 30% (treatment D). According to results obratined a DW has a potential to replace a GM, as a supplement to AH of medium quality. It was concluded that the replacement of 10%, 20% and 30% has no negative affect on N intake, N excretion, nor the N balance in wether sheep. A decision of replacement and quantity of GM to be replaced by DW is dependent on the prodction economy and market prices of these feeds.

Key words: alfalfa haylage, maize grain, dried whey, N bilance

Introduction

The alfalfa haylage (AH) is rich in crude protein (CP) but is poor in energy (Miller et al. 2001). If the diet does not contain a sufficient amount of energy, significant losses of N occur with the excretion via urine and faeces from the organism (Miller et al. 2001). Previous studies have demonstrated that the energy supplements to diets based on haylage increases nitrogen utilization in wether sheep (Vranić et al., 2011; 2018). Dried whey (DW) is a by-product of the dairy industry. It is rich in lactose as an energy source (about 700 g kg^{-1} DM) (DeFrain et al., 2004) which is similar to corn (11.6 and 12.17 MJ kg^{-1} ME for ruminants, respectively) (Schingoethe and Beardsley, 1975). The final product of lactose degradation in rumen is butyric acid while starch propionic acid (Chamberlain et al., 1993), so the mechanism and rate of absorption of newly formed acids through the rumen wall is different and their impact on nutrient utilization (DeFrain et al., 2004). It has been reported that the total or partial replacement of maize with DW (50 or 100%) increases urine excretion, but does not affect the N bilance in dairy cows (Susmel et al., 1995). Furthermore, the addition of DW to forage increases the utilization of nitrogen components in diets (Calsamiglia et al., 2010), whereas the degradation and absorption of whey proteins (lactoglobulins) in the small intestine increases the retention of N (Susmel et al., 1995). The hypothesis of this study was that the replacement of maize with DW, as a supplement to alfalfa haylage, increases feed intake and N bilance in sheep. The aim of this study was to determine the effect of partial replacement of maize with DW on N intake and N bilance from a diet based on alfalfa haylage in wether sheep nutrition.

Materials and Methods

Alfalfa crop (*Medicago sativa* L.) was mown in the early flowering growth stage (around 30 % of the crop was in flowering growth stage), wilted during 24 h at 500 – 600 g (DM) kg^{-1} fresh crop, and baled in the roller bales wrapped with 6 layers of light blue plastic film. Alfalfa haylage (AH), for experimental purposes, was chopped to particle size 3 – 5 cm, compressed into plastic bags (around 10 kg bag^{-1}), and stored in the cold chamber at 4 °C until feeding.

The maize BC 566 were harvested after they reached physiological maturity, and stored in a dry chamber in 3 plastic bins, mechanically ground (GM) to the particle size of 3 mm and stored. DW was obtained by Dukat d.d., M. Čavić 9, 10 000 Zagreb, Croatia. The animals were fed twice a day (9 a.m. and 4 p.m.) with an equal amount of concentrate separated from the voluminous diet thus enabling the intake of the offered total daily meal. The total amount of concentrate intake was 30 g d^{-1} kg^{-1} $M^{0.75}$ (GM or GM + DW). Four wether sheep (Suffolk x Lake-Solčava sheep), aged about 2 years and body weight of 60 – 66 kg were selected. Four feeding treatments, with the addition of 30 g kg^{-1} $M^{0,75}$ of concentrate, were investigated: only GM (treatment A) or GM replaced with DW in the quantity of 10% (treatment B), 20% (treatment C), or 30% (treatment D).

Experimental design

A feeding trial was performed with 4 sheep and 4 feeding treatments over 4 periods using an incomplete change-over design. Each period consisted of 3 phases: acclimatization period, measurement period of *ad libitum* intake, and measurement period of N bilance parameters. During the acclimatization period, the animals were located in individual pens (1.5 x 2.2 m) followed by their translocation to individual crates (1.36 m x 1.53 m x 1.49 m) and a 2-days measurement of *ad libitum* intake. Afterwards, the N bilance measurement was performed during a period of 5 days. Each of the four experimental periods lasted 17 days.

The feed intake and produced amount of faeces and urine were recorded daily. Likewise, the samples of an offered feed, refused feed, faeces and urine were collected daily and stored at a temperature 4 °C. A 30 mL of 2M H_2SO_4 was added daily to each urine container to prevent the evaporation of N. By the end of each experimental phase, the samples collected were stored at -20 °C. The DM contents of samples were determined by oven drying to a constant weight at 60 °C in a fan-assisted oven (ELE International). The ash contents of the offered feed, refused feed and faeces were measured by igniting the samples in a microwave oven (Milestone PIYRO, Italy) at 550 °C for 3 h. The total N concentrations were determined by the Kjeldahl method (AOAC 1990, ID 954.01) using a Gerhardt nitrogen analyser. In addition, N concentration was expressed as crude protein (CP) (total N x 6.25) g kg^{-1} DM for the offered feed and refused feed. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using the procedure described by Van Soest et al. (1991) utilising Ankom Filter bag technology (USA). The pH of the silage was determined in a water extract containing 10 g of fresh silage and 100 ml of distilled water using a pH meter 315i (WTW). The efficiency of N was determined using the following equations: (i) N intake = N offered - N in food residues and (ii) N utilisation = N intake – N excreted (via faeces + urine)

Statistical analysis of the results were performed using SAS statistical software (SAS Institute, 1999); GLM, and MIXED procedure. The statistical significance was defined as $P < 0.05$.

Results and Discussion

The chemical composition of the feeds used in this experiment is presented in Table 1. The average CP content of alfalfa haylage was from 77 to 167.5 g kg^{-1} DM determined in the grass silages produced on family farms in the northwest part of Croatia (Vranić et al., 2005).

Table 1. The chemical composition of the alfalfa haylage, maize grain and dried whey used in the experiment (in g kg^{-1} DM unless stated otherwise).

Chemical parameter	Alfalfa haylage	Maize grain	Dried whey
DM	549.39	918.30	950
OM	913.31	979.60	NA
CP	122.45	84.26	90
Starch	103.96	584.16	NA
Lactose	NA	NA	750
NDF	534.56	219.99	NA
ADF	397.42	31.17	NA
pH	6.02	NA	NA
NH_3-N (g N kg^{-1} total N)	38.31	NA	NA
Butyric acid	5.47	NA	NA
Acetic acid	5.13	NA	NA
Lactic acid	41.02	NA	NA

DM - dry matter; OM - organic matter; NH_3-N - ammonium nitrogen; CP - crude protein; NA - not analyzed

The high pH value of AH and the low concentration of lactic acid (41.02 g kg^{-1} DM) are consistent with previous studies (Hopkins, 2000) where a high pH value and low acetic acid content of wilted haylage at a high DM content was observed. According to Chamberlain and Wilkinson (1996), anaerobic conditions in the silo are difficult to achieve when ensiling a feed with a high DM content. The N intake, excretion and N bilance are presented in Table 2.

Table 2. Intake, excretion, and N balance of the investigated feeding treatments (in g kg^{-1} DM)

Feeding treatment	N intake			N excreted		
	haylage	maize/whey	total	urine	faeces	N Bilance
A	18.1	7.8	25.0	9.1	10.4	6.5
B	19.3	7.9	27.3	9.5	10.8	6.9
C	19.5	8.0	27.4	10.1	11.2	6.1
D	19.3	8.3	27.6	9.7	10.8	7.1
Significancy	NS	NS	NS	NS	NS	NS
SEM	0.56	0.23	0.51	0.47	0.36	0.58
0 vs. add	NS	NS	NS	NS	NS	NS
lin	NS	NS	NS	NS	NS	NS
Q	NS	NS	NS	NS	NS	NS

N, nitrogen; A, B, C, D, feeding treatments, with the addition of 30 g kg^{-1} $M^{0,75}$ of concentrate, which consisted of only ground maize (treatment A) or ground maize supplemented with dried whey in the quantity of 10 % (treatment B); 20% (treatment C) or 30% (treatment D); SEM, standard error of the mean; Lin, the linear effect of the ground maize supplementation; Q, the quadratic effect of the ground maize supplementation; values in the same column did not differ significantly $P>0.05$

The effect of energy supplements to forage, on the nutritive value and nutrients utilization in ruminants, is variable and depends not only on the type and amount of carbohydrate feed (Chamberlain et al., 1993) but also on the type and nutritive value of the basic forage (Orr et al. , 2001; Vranic et al., 2018). Sugar supplementation, in a haylage-based ruminant diet, is a more efficient energy source for microbial protein synthesis which reduces the NH_3 content in the rumen and increases the utilization of N (compared to a starch supplementation) (Chamberlain et al., 1993) if the basic forage is of high nutritive value (Kim et al., 2000). Otherwise, if the basic forage has a low CP content (62 g kg^{-1} ST), energy

supplements can lead to a negative N balance (Kim et al., 2000). In this study, alfalfa haylage was of average nutritive value (CP content 120 g kg^{-1} DM) and the replacement of GM with DW did not have a negative effect on the investigated N balance parameters in wether sheep ($P>0.05$). However, a tendency of an increased N intake from a diet increased secretion of N from the organism, and the increased N balance with an increase of DW ratio in a diet, were observed (Table 2). Furthermore, a maximum of 30% of GM was replaced by DW in this study, while in previous studies 100% maize replacement by DW resulted in better utilization of N components of a meal (Calsamiglia et al., 2010). In the studies where a higher replacement of maize with DW (50 or 100%), as a concentrate in a diet based on a haylage, did not affect the balance and utilization of N, whereas an almost double increased urinary excretion of N (with complete replacement of maize with DW) due to the diuretic effect of whey was observed (Susmel et al., 1995). Moreover, the addition of DW to AH resulted in lower *in vivo* digestibility of CP as a consequence of the faster passage of feed through the digestive system (Vranić et al., 2016).

Conclusion

Dried whey has a potential to replace a corn grain, as a supplement to alfalfa haylage of medium quality. The replacement of 10%, 20% and 30% has no negative affect on N intake, N excretion, nor the N balance in wether sheep. A decision of replacement and quantity of GM to be replaced by DW is dependent on the prodction economy and market prices of these feeds.

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Utjecaj djelomične zamjene kukuruza sirutkom u prahu, kao dodatka sjenaži lucerne, na bilancu dušika u hranidbi kastriranih ovnova

Sažetak
Cilj istraživanja bio je utvrditi utjecaj djelomične zamjene mljevenog zrna kukuruza sirutkom u prahu, kao dodatka sjenaži lucerne (SL), na bilancu dušika (N) u hranidbi kastriranih ovnova. Istraživana su četiri hranidbena tretmana hranidbe SL uz dodatak 30 g koncentrata kg^{-1} $M^{0,75}$ koji se sastojao od samo mljevenog zrna kukuruza (tretman A) ili mljvenog zrna kukuruza zamijenjenog sa sirutkom u prahu 10 % (tretman B), 20 % (tretman C) ili 30 % (tretman (D). Zaključeno je da sirutka u prahu ima potencijal zamijene zrna kukuruza, kao dodatak sjenaži lucerne srednje kvalitete. Zamjena 10 %, 20 % ili 30 % nema negativnog utjecaja na konzumaciju N, izlučivanje N, niti na bilancu N kod kastriranih ovnova. Odluka o zamjeni i količini krmiva istraživanih krmiva u obroku obuhvaća područje ekonomike i tržišnih cijena ovih krmiva.

Ključne riječi: sjenaža lucerne, zrno kukuruza, sirutka u prahu, bilanca N