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Effect of a partial replacement of ground corn grain with dried whey on the quality of the ration based on different forage species in wether sheep

Utjecaj djelomične zamjene mljevenog zrna kukuruza sirutkom u prahu na kvalitetu obroka baziranog na različitim vrstama voluminozne krme u hranidbi kastriranih ovnova

Marina VRANIĆ[1], Krešimir BOŠNJAK[1] (✉), Božica LUKŠIĆ[1], Ivana ČAČIĆ[1], Nataša PINTIĆ PUKEC[2], Ivica VRANIĆ[2], Krešimir KRAPINEC[3], Ranko GANTNER[4], Kristina STARČEVIĆ[5], Tomislav MAŠEK[5], Andreja BABIĆ[2]

[1] University of Zagreb Faculty of Agriculture, Department of Field Crops, Forage and Grassland, Svetošimunska cesta 25, 10000 Zagreb, Croatia

[2] Croatian Agency for Agriculture and Food, Poljana Križevačka 185, 48260 Križevci;

[3] University of Zagreb, Faculty of Forestry and Wood Technology, Department of Forestry, Svetošimunska cesta 23, 10000 Zagreb, Croatia

[4] Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Department of plant production and biotechnology, 31000 Osijek, Croatia

[5] University of Zagreb, Faculty of Veterinarian Medicine, Department of Animal Nutrition and Dietetics, Heinzelova 55, 10000 Zagreb, Croatia

✉ Corresponding author: kbosnjak@agr.hr

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ABSTRACT

An *in vivo* study was conducted into the effects of partial replacement of dried ground corn grain with dried whey – as a supplement to alfalfa haylage (AH) or Italian ryegrass haylage (IRH) - on *ad libitum* intake, *in vivo* digestibility and N retention in wether sheep. The chemical composition of AH and IRH was roughly the same, except for the crude protein (CP) content which was much higher in AH compared with IRH. Sheep fed the AH-based diet had higher feed intake. Higher CP digestibility and higher N retention, as well as higher levels of excreted N, were shown compared IRH-based diet. The IRH-based diet increased the digestibility of chemical parameters studied, except CP and starch. The partial replacement of corn grain with dried whey reduced the intake of acid detergent fibre (ADF), improved the starch digestibility in AH and IRH-based diets, and increased the overall N intake in wethers fed the AH-based diet. In practical conditions, replacing 10% of corn grain with dried whey in a ration based on higher quality forage is a way to increase N intake in sheep. In conclusion, the partial replacement of corn grain with dried whey in a forage-based diet with higher CP content improves the nitrogen efficiency in the diet of wether sheep.

Keywords: whey in the diet of sheep, alfalfa haylage, Italian ryegrass haylage, intake, digestibility, N retention

SAŽETAK

Provedeno je *in vivo* istraživanje utjecaja djelomične zamjene suhog mljevenog zrna kukuruza sirutkom u prahu kao dodatka sjenaži lucerne (SL) ili sjenaži talijanskog ljulja (STL) na *ad libitum* konzumaciju, *in vivo* probavljivost i bilancu N u hranidbi kastriranih ovnova. Kemijski sastav SL i STL je bio otprilike isti, osim sadržaja sirovih proteina (SP) koji je bio veći kod SL u usporedbi s STL. Hranidba bazirana na SL imala je veću konzumaciju, veću probavljivost SP i veću bilancu N, kao i više izlučenog N u usporedbi s hranidbom baziranom na STL. Hranidba bazirana na STL je imala veću probavljivost istraživanih kemijskih parametara, osim SP i škroba. Djelomična zamjena zrna kukuruza sirutkom u prahu smanjila je konzumaciju kiselih detergent vlakana (KDV), povećala probavljivost škroba u hranidbi baziranoj na SL i STL i povećala ukupnu konzumaciju N u hranidbi baziranoj na SL. U praktičnim uvjetima zamjena 10% zrna kukuruza sirutkom u prahu u obroku na bazi kvalitetnije krme dovoljna je za povećanje konzumacije N. Zaključno, djelomična zamjena zrna kukuruza sirutkom u prahu u krmnoj osnovi s većim sadržajem SP povećava energetsku učinkovitost obroka u smislu konzumacije N i metabolizma.

Ključne riječi: energetski dodatak, sjenaža lucerne, sjenaža talijanskog ljulja, konzumacija, probavljivost, bilanca N

INTRODUCTION

Forage nutritive value, especially crude protein and digestibility, are associated with the passage rate of the forage through the gastrointestinal tract. Digestion rates of the forage carbohydrate fractions are much slower than the corresponding protein fractions which leads to nutrient asynchrony for rumen microorganisms (Hersom, 2007). Improvement of digestibility and intake are the two major factors for increasing the nutritive value of forage for ruminants. A diet with higher content in CP and lower content in fibre may result in a higher feed intake but might cause lower digestibility due to faster passage of ration through the digestive tract. One way of improving forage intake and nutrient utilization is to increase microbial activity in the rumen by supplementing the diet with feeds high in rumen-degradable organic matter and thereby increasing microbial protein synthesis and SCFA production.

Dried corn grain is the most frequently used high-energy supplement to forage in the diet of ruminants. It is rich in starch (Heldt et al., 1999) of 55 to 70% in the rumen (Huntington 1997), and about 95% degradable in the whole digestive tract (Rahimi et al., 2020).

Dried whey is a by-product in milk production rich in lactose (around 700 g/kg DM) as an energy source (DeFrain et al., 2004).

Compared with starch, however, lactose will ferment faster and lead to a quicker drop in the rumen's pH value (Susmel et al., 1995), but will not depress it if supplemented up to 44% DM intake (Oba, 2011). The end product of lactose degradation in the rumen is butyric acid, while that of starch is propionic acid (Chamberlain et al., 1993), which is why the mechanism and rate of absorption of the newly emerging acids by the rumen's walls are different, and so is their effect on nutrient intake from feed (DeFrain et al., 2004).

Dried whey fed to sheep as a supplement to alfalfa haylage (AH) in 5 and 10% ratio of DM will increase the *ad libitum* intake and reduce *in vivo* digestibility of DM, organic matter (OM) and CP (Vranić et al., 2016). It has been found that better dietary results are achieved through the combined effects of whey and corn, as compared to whey or corn grain alone, as an energy source additional to the main forage feed (Susmel et al., 1995).

Partial or full replacement of corn with whey (50 or 100% respectively) will increase the output of urine (that will almost double if corn is fully replaced by whey), but will not affect N retention in dairy cows (Susmel et al., 1995). Supplementing forage with whey will increase the intake of nitrogen in feed (Calsamiglia et al., 2010), since the protein in whey (lactoglobulin) is highly efficient in feed and will mostly increase N retention as it is broken down in the small intestine (Susmel et al., 1995) if the main forage is sufficiently rich in CP (Oba, 2011).

For the foregoing reasons, high-energy supplements to forage will have a variable effect on the nutritional value and energy efficiency of diet for ruminants. It depends on the type and quantity of supplement (Chamberlain et al., 1993) as well as the main forage (Orr et al., 2001; Vranic et al., 2007; Salcedo et al., 2010; Vranic et al., 2013; Vranic et al., 2018) that can have a higher impact on intake and digestibility than the high-energy supplement itself (Waldo, 1986). The aim of our study is to establish the effects of partial replacement of corn grain with dried whey on *ad libitum* feed intake, *in vivo* digestibility and N retention, based on two different types of forage fed to wether sheep.

The present study hypothesized that (i) replacing corn grain with dried whey would increase the intake, digestibility and N retention of feed based on alfalfa haylage (AH) or on Italian ryegrass haylage (IRH), and that (ii) an AH-based diet would have higher intake, digestibility and N retention compared to IRH-based diet.

MATERIALS AND METHODS

Experimental feed

Alfalfa haylage (AH) (*Medicago sativa* L.) was produced at the early flowering stage (cca 30% of the flowering crop) while Italian ryegrass (*Lolium multiflorum* L.) haylage (IRH) at R2-R3 stage (the mid of tasseling) (Moore et al., 1991). Both crops were grown in continental Croatia under favorable agro ecological conditions. It was the third vegetation year of alfalfa and the first of Italian ryegrass utilization.

Both crops were mown and allowed to wilt for 24 hours before being harvested at 500-600 g DM/kg fresh forage with a round baler. No additives were used for haylage conservation. The bales were wrapped in four to six layers of 500-mm wide light blue plastic film. The weather at harvest was warm and sunny with an average daily temperature of 27 °C which varies from 20.5 °C to 30.6 °C. The wrapped bales were stored for 2.5 months in the covered space of the Grassland Research Center of the University of Zagreb, Faculty of Agriculture i.e. until the beginning of the study. Maize hybrid FAO 500 was sown on the 5th of May 2018 at a row distance of 0.7m at the Experimental station Maksimir of the Faculty of Agriculture Zagreb. The soil was previously cultivated (30-32 cm plowing), seedbed prepared and fertilized (150 kg N/ha, 120 kg P_2O_5/ha, 120 kg K_2O/ha). The corn crop was harvested after it reached the phase of physiological maturity recognized with the emergence of the black layer based on the grain (Daynard and Duncan, 1969). After artificially drying at the temperature from 35 to 46 °C the dry corn was stored at room temperature unground in 3 plastic bins (50 L each) until required for experimental purposes. Dried whey (pasteurized sweet whey defense) in 2 packages, each of 25 kg, for the research needs was provided by the dairy industry Dukat d.d., Zagreb, Croatia.

Just prior to the start of the experiment the dry corn was ground using a hammer mill with a 3-mm screen, compressed into 5 plastic bags (approximately 30 L each) and stored at room temperature similar to dried whey.

Before the start of the experiment, the AH and IRH were chopped separately to approximately 3-5 cm using a commercial chopper. The chopped material was compressed into plastic bags (approximately 10 kg/bag) and stored in the cold chamber where the temperature was held at 4 °C. The amount of concentrate for daily feeding was measured separately into plastic bags and fed in two equal rations at 0:9 a.m. and 0:4 p.m. with no refusals remaining. No additional supplementary feeds were provided.

Dietary treatments

The concentrate (corn or corn partly replaced by whey) was fed at the quantity of 30 g/kg $M^{0.75}$ wether sheep/d. The experiment consisted of 8 feeding treatments for crossbreed wethers (Suffolk x Lake-Solčava sheep): (i) AH supplemented with corn (AH-0W); (ii) AH supplemented with 90% corn and 10% whey (AH-10W); (iii) AH supplemented with 80% corn and 20% whey (AH-20W); (iv) AH supplemented with 70% corn and 30% whey (AH-30W); (v) IRH supplemented with corn (IRH-0W), (vi) IRH supplemented with 90% corn and 10% whey (IRH-10W); (vii) IRH supplemented with 80% corn and 20% whey (IRH-20W); (viii) IRH supplemented with 70% corn and 30% whey (IRH-30W).

Animal management and the experimental design

Eight 1.5-year-old crossbreed wethers were selected based on BW (mean BW 60 kg, sd. 2.0 kg) and condition scores. All animals were treated for internal parasites before the experiment. The sheep were subjected to artificial lighting from 08:00 a.m. to 08:00 p.m. daily.

A feeding trial with the eight wethers and eight feeding treatments was performed indoors. Each sheep was randomly allocated to the treatment sequences in an incomplete changeover design with four periods.

A 10-day acclimatization period was followed by a 7-day measurement period (a 2-day *ad libitum* intake period was followed by 5 days of *ad libitum* intake, digestibility and N retention measurements), in which the offered feed, the refused feed, faeces and urine were measured. The experiment took place in September 2018 and lasted for 68 days. The animals were housed in individual pens (1.5 x 2.2 m) during the acclimatization period and in individual cages (1.36 m x 1.53 m x 1.49 m) during the measurement period. The cages were equipped with individual drinkers that were cleaned daily and the animals were provided *ad libitum* fresh water throughout the experiment. Animals were fed twice a day (09:00 a.m. and 04:00 p.m.) in equal amounts. They were fed AH and IRH *ad libitum* during the 2 days, while over the following 5-day period the rations were designed to ensure a refusal margin of 10–15% AH and IRH each day.

During the measurement period, the fresh weights and DM contents of the corn, whey, AH and IRH offered and the AH and IRH refused were recorded daily. Samples of offered corn and dried whey were taken at the beginning of each experimental period in the quantity of about 1,5 kg and stored separately at the ambient temperature until analyzed. Haylage samples were taken daily (about 10 dkg of AH and IRH) at weighing the daily amount of forage and stored at a temperature of -20 °C until the end of the experiment when they were separately composited before chemical analyses. The refused feed was composited per animal and stored at a temperature of -20 °C before chemical analyses.

During the measurement period, the animals had attached faecal bags. The daily excretion of faeces was measured. Daily samples of faeces (10% of the excreted faeces) from individual animals were stored frozen at -20 °C until the completion of the collection period. Composited faecal output from each animal was weighed and sampled prior to subsequent analyses. Urine was collected into plastic containers (40 cm x 20 cm x 30 cm) to which it was delivered by an inclined metal base set under the cages' performed floor. The daily output of urine from each animal was preserved by acidification (100 ml of 2 mol/l sulphuric acid was added to achieve a pH value of 2-3), and its volume was measured. Daily samples of urine (10% of the excreted urine) from individual animals were then composited over the measurement 5-day period and stored at a temperature of -20 °C until analyzed. The sheep were weighed on the 10th and 17th day of each period, and the mean weight was used to calculate the daily corn and whey supplements for each animal.

Digestibility was calculated using the equations (Rymer, 2000): DMD = (DM intake - faecal DM excreted)/ DM intake; OMD = (OM intake – faecal OM excreted/OM intake); D-value = (OM intake – faecal OM excreted/ DM intake; starch digestibility = (starch intake – faecal starch excreted)/starch intake; NDF digestibility = (NDF intake – faecal NDF excreted)/NDF intake; ADF digestibility = (ADF intake - faecal ADF excreted)/ADF intake.

The conducted experiment followed the Council Directive issued by the European Economic Community (EEC) (1986) on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. Also, animal husbandry and sampling were approved by the Scientific Research Ethic Committee University of Zagreb Faculty of Agriculture (Ethic Reference No: 380-71-01-06-1).

Chemical analyses

The DM contents of offered feed, refused feed and faeces were determined by oven drying to a constant weight at a temperature of 60 °C in a fan-assisted oven (ELE International) (Bedfordshire, United Kingdom). The ash contents of 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 of offered feed, refused feed, faeces and urine were determined by the Kjeldahl method (Freitas et al., 2013) using a Gerhardt nitrogen analyzer. In addition, N concentration was expressed as crude protein (CP) (total N x 6.25) g/kg DM for offered feed, refused feed and faeces.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analyzed using the previously described procedure (Van Soest et al., 1991) using the

Ankom Filter bags technology (USA) with an Ancom fiber bags. 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). Short-chain fatty acids (SCFA) (and lactose were determined by high-performance liquid gas chromatography (Fussell and Mccalley, 1987). Lactic acid content was determined enzymatically on an Express Auto biochemical analyzer in water extract using the juice expressed from the silage. The starch content of feed offered, feed refused and faeces were determined by an enzymatic method (Norris and Rooney, 1970).

Statistical analyses

Results were analyzed using mixed model procedures (SAS, 1999). The model applied: $Y_{ij}=\mu+T_i+P_j+e_{ij}$ where Y is the overall model, μ is the grand mean, T is the treatment, P is the period, e is the experimental error, i is the number of treatments, and j is the number of periods.

The experiment was set up as an incomplete change-over design with four periods, eight feeding treatments and eight animals. Each sheep received four feeding treatments over the experimental period. All model components were considered fixed while the experimental unit was animal within each period.

The orthogonal contrasts as well as linear and quadratic effects of the level of whey inclusion in AH and IRH well as were made using the CONTRAST statement of SAS.

RESULTS

The chemical composition of forage used in the study is presented in Table 1. The AH and IRH used in the study (Table 1) had a high DM content that is usual when ensiling forage into bales wrapped with plastic (Vranic et al., 2018). Corn grain had a high starch content and dried whey had a high lactose content (Table 1).

Table 1. Composition of forage and energy supplements (g/kg DM unless stated otherwise) fed to wether sheep

Chemical parameter	AH	IRH	Corn grain	Dried whey
DM (g/kg fresh sample)	549.39	472.44	918.30	950
OM	913.31	909.98	979.60	NA
CP	142.45	81.32	84.26	90
Starch	103.96	88.44	584.16	NA
Lactose	NA	NA	NA	750
NDF	524.56	548.98	219.99	NA
ADF	367.42	375.46	31.17	NA
pH	5.84	5.81	NA	NA
NH_3-N (g N/kg total N)	38.31	70.14	NA	NA
Butyric acid	5.47	4.33	NA	NA
Acetic acid	5.13	15.38	NA	NA
Lactic acid	41.02	16.85	NA	NA

AH, alfalfa haylage; IRH, Italian ryegrass haylage; DM, dry matter; OM, organic matter; NH_3-N, ammonium nitrogen; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; NA, not analyzed.

Table 2 presents the effect of partial replacement of dietary corn with dried whey on daily dry feed intake of wether sheep fed AH or IRH.

Replacing corn with whey increased the intake of CP ($P < 0.01$) and reduced the intake of NDF ($P < 0.001$) and starch ($P < 0.001$) in both types of haylage.

The replacement of corn with whey led to a linear increase in the intake of CP ($P < 0.05$) and a linear decrease in the intake of ADF ($P < 0.05$) and starch ($P < 0.001$) in the AH diet. The replacement of corn with whey resulted in a linear decrease in the intake of NDF ($P < 0.001$), ADF ($P < 0.001$) and starch ($P < 0.001$) in the IRH diet.

Effects of partial replacement of dietary corn grain with dried whey on apparent digestibility for wether sheep fed AH or IRH is presented in Table 3.

A higher level of digestibility of CP ($P < 0.001$) and starch ($P < 0.001$) was recorded in wethers fed the AH-based diet compared to the IRH-based diet. However, the IRH-based diet had a higher digestibility of DM ($P < 0.001$), OM ($P < 0.01$), D-value ($P < 0.001$), NDF ($P < 0.001$) and ADF ($P < 0.001$) compared with AH-based dietary treatments.

Table 2. Effects of partial replacement of dietary corn with whey on daily dry feed intake (g/kg $M^{0.75}$) of wether sheep fed alfalfa or Italian ryegrass haylage

Treatment	DM	OM	CP	NDF	ADF	starch
AH-0W	70.1^{ab}	65.9^{ab}	7.6^{a}	31.7^{a}	28.3^{a}	22.9^{a}
AH-10W	72.6^{a}	68.1^{a}	7.9^{a}	31.5^{a}	27.6^{ab}	21.9^{ab}
AH-20W	74.0^{a}	69.2^{a}	8.0^{a}	31.4^{a}	27.0^{ab}	20.7^{c}
AH-30W	73.4^{a}	68.5^{a}	8.2^{a}	29.9^{ab}	28.8^{bc}	18.9^{d}
IRH-0W	66.8^{b}	62.8^{b}	5.5^{b}	31.9^{a}	26.7^{ab}	21.8^{b}
IRH-10W	65.5^{b}	61.6^{b}	5.4^{b}	29.0^{b}	24.0^{dc}	20.3^{c}
IRH-20W	66.8^{b}	62.6^{b}	5.5^{b}	28.9^{b}	23.4^{d}	19.2^{d}
IRH-30W	65.9^{b}	61.9^{b}	5.5^{b}	27.7^{b}	22.2^{d}	17.3^{e}
Significance	**	**	**	*	**	*
SEM	1.54	1.42	0.17	0.82	0.55	0.38
AH vs. IRH	***	***	***	**	***	***
no whey vs. whey	NS	NS	**	***	NS	***
Lin in AH	NS	NS	*	NS	**	***
Quad in AH	NS	NS	NS	NS	NS	NS
Lin in IRH	NS	NS	NS	***	***	***
Quad in IRH	NS	NS	NS	NS	NS	NS

$^{a-e}$, Means values within columns for each item with clarification of the significant difference in the forms of superscripts (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). NS, non-significant ($P > 0.05$); SEM, standard error of the mean; Lin, linear; Quad, quadratic; Treatments, AH-0W, alfalfa haylage supplemented with corn; AH-10W, alfalfa haylage supplemented with 90% corn and 10% whey; AH-20W, alfalfa haylage supplemented with 80% corn and 20% whey; AH-30W, alfalfa haylage supplemented with 70% corn and 30% whey; IRH-0W, Italian ryegrass haylage supplemented with corn; IRH-10W, Italian ryegrass haylage supplemented with 90% corn and 10% whey; IRH-20W, Italian ryegrass haylage supplemented with 80% corn and 20% whey; IRH-30W, Italian ryegrass haylage supplemented with 70% corn and 30% whey; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; AH, alfalfa haylage; IRH, Italian ryegrass haylage.

Table 3. Effects of partial replacement of dietary corn grain with whey on apparent digestibility (g/kg DM) of wether sheep fed alfalfa or Italian ryegrass haylage

Treatment	DM	OM	D-value	CP	NDF	ADF	starch
AH-0W	662[b]	686[bc]	645[ab]	596[a]	584[cd]	639[bc]	491[a]
AH-10W	678[ab]	704[abc]	660[ab]	602[a]	566[cd]	627[bc]	474[ab]
AH-20W	660[b]	684[bc]	640[ab]	591[a]	535[d]	580[c]	443[c]
AH-30W	662[b]	682[c]	637[b]	608[a]	581[cd]	619[bc]	401[d]
IRH-0W	700[a]	715[a]	673[a]	529[bc]	660[a]	722[a]	466[b]
IRH-10W	697[a]	712[ab]	669[ab]	521[bc]	644[ab]	706[a]	436[c]
IRH-20W	694[a]	711[ab]	666[ab]	489[c]	605[bc]	664[ab]	414[d]
IRH-30W	701[a]	718[a]	674[a]	539[b]	639[ab]	674[ab]	368[e]
Significance	*	*	**	*	*	**	*
SEM	10.78	9.95	9.33	15.26	18.37	16.78	7.7
AH vs. IRH	***	**	***	***	***	***	***
no whey vs. whey	NS	NS	NS	NS	NS	*	***
Lin in AH	NS	NS	NS	NS	NS	NS	***
Quad in AH	NS	NS	NS	NS	NS	NS	NS
Lin in IRH	NS	NS	NS	NS	NS	*	***
Quad in IRH	NS	NS	NS	NS	NS	NS	NS

[a-e], Means values within columns for each item with clarification of the significant difference in the forms of superscripts (*, $P < 0.05$; **$P < 0.01$; ***, $P < 0.001$). NS, non-significant ($P > 0.05$). SEM, standard error of the mean; Lin, linear; Quad, quadratic; Treatments, AH-0W, alfalfa haylage supplemented with corn; AH-10W, alfalfa haylage supplemented with 90% corn and 10% whey; AH-20W, alfalfa haylage supplemented with 80% corn and 20% whey; AH-30W, alfalfa haylage supplemented with 70% corn and 30% whey; IRH-0W, Italian ryegrass haylage supplemented with corn; IRH-10W, Italian ryegrass haylage supplemented with 90% corn and 10% whey; IRH-20W, Italian ryegrass haylage supplemented with 80% corn and 20% whey; IRH-30W, Italian ryegrass haylage supplemented with 70% corn and 30% whey; DM, dry matter; OM, organic matter; D-value, the digestibility of the organic matter in the dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; AH, alfalfa haylage; IR, Italian ryegrass haylage.

The replacement of corn with whey resulted in a linear decrease of the digestibility of starch and ADF ($P < 0.05$).

Effects of partial replacement of dietary corn with dried whey on nitrogen retention of wether sheep fed AH or IRH is presented in Table 4.

The AH-based feed had higher N intake ($P < 0.001$), higher N excretion in faeces ($P < 0.001$) and urine ($P < 0.001$), and higher N retention ($P < 0.01$) compared with the IRH-based feed. Replacing corn with whey did not affect the N intake, N excretion or N retention in either type of haylage.

The replacement of corn with whey in AH-based feed led to an increase in the overall intake of N ($P < 0.05$).

Table 4. Effects of partial replacement of dietary corn with dried whey on nitrogen retention (g/kg DM) of wether sheep fed alfalfa or Italian ryegrass haylage

Treatment	N intake			N excreted		N retention	Fecal N/ N intake	Urin N / N intake
	haylage	concentrate	total	urin	feces			
AH-0W	18.1	7.8	26.0^{b}	9.1^{a}	10.4^{ab}	6.5^{abc}	0.41^{c}	0.35^{a}
AH-10W	19.3	7.9	27.3^{a}	9.5^{a}	10.8^{a}	6.9^{ab}	0.39^{c}	0.35^{a}
AH-20W	19.5	8.0	27.4^{a}	10.1^{a}	11.2^{a}	6.1^{abc}	0.41^{c}	0.37^{a}
AH-30W	19.3	8.3	27.6^{a}	9.7^{a}	10.8^{a}	7.1^{a}	0.39^{c}	0.35^{a}
IRH-0W	11.2	7.8	19.0^{c}	4.0^{b}	8.9^{cd}	6.1^{abc}	0.48^{ab}	0.21^{b}
IRH-10W	10.7	7.9	18.5^{c}	4.4^{b}	8.9^{cd}	5.2^{c}	0.48^{ab}	0.25^{b}
IRH-20W	11.0	8.0	19.0^{c}	4.4^{b}	9.7^{bc}	4.9^{c}	0.52^{a}	0.23^{b}
IRH-30W	10.5	8.3	18.8^{c}	4.8^{b}	8.66^{d}	5.37^{bc}	0.46^{b}	0.26^{b}
Significance	**	NS	*	**	*	*	*	*
SEM	0.56	0.23	0.51	0.47	0.36	0.58	0.02	0.03
AH vs. IRH	***	NS	***	***	***	**	***	***
no whey vs. whey	NS	NS	NS	NS	NS	NS	NS	NS
Lin in AH	NS	NS	*	NS	NS	NS	NS	NS
Quad in AH	NS	NS	NS	NS	NS	NS	NS	NS
Lin in IRH	NS	NS	NS	NS	NS	NS	NS	NS
Quad in IRH	NS	NS	NS	NS	NS	NS	NS	NS

$^{a-e}$, Means values within columns for each item with clarification of the significant difference in the forms of superscripts (*, P < 0.05; **, P < 0.01; ***, P < 0.001). NS, non significant (P > 0.05). SEM, standard error of mean; Lin, linear; Quad, quadratic; AH, alfalfa haylage; IR, Italian ryegrass haylage; Treatments, AH-0W, alfalfa haylage supplemented with corn; AH-10W, alfalfa haylage supplemented with 90% corn and 10% whey; AH-20W, alfalfa haylage supplemented with 80% corn and 20% whey; AH-30W, alfalfa haylage supplemented with 70% corn and 30% whey; IRH-0W, Italian ryegrass haylage supplemented with corn; IRH-10W, Italian ryegrass haylage supplemented with 90% corn and 10% whey; IRH-20W, Italian ryegrass haylage supplemented with 80% corn and 20% whey; IRH-30W, Italian ryegrass haylage supplemented with 70% corn and 30% whey; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre.

DISCUSSION

Chemical Composition

Forages ensiled at higher DM content (>300 g/kg fresh forage) have reduced risk of clostridial fermentation (Kung et al., 2010), but restrict in-silo fermentation. This results in high pH values and aerobically unstable fermented forage (Vranic et al., 2011). As the DM content in the haylage used for ensiling increases, the pH value of the fermented forage increases. The pH value of IRH can range from pH 3.7 of plant mass in direct ensiling (DM content 159 g/kg fresh sample), to pH 4.1 and 4.9 in 1-day and 2-day wilted crop before ensiling (DM content 336 and 469 g/kg fresh forage, respectively) (McDonald, 1982). This is consistent with the results on DM content and high pH values of the haylage used in the study (Table 1). AH had lower CP content and higher NDF content (Table 1) compared with the typical CP and NDF content in high-quality AH (Kung et al., 2010). Similarly, IRH had lower CP content and higher NDF content (Table 1) compared with the typical CP and NDF content in high-quality IRH (> 190 g CP/kg DM and <550 g NDF/kg DM) (McCormick et al., 1998). However, the haylage used in the study had CP content higher than 60 g/kg DM that can provide sufficient N for rumen microbes (Kim et al.,

1999) and prevent a negative N balance caused by protein malnutrition (Lloyd et al., 2012). The high values of starch content found in corn grain and lactose in dried whey (Table 1) are typical for the high-energy feed used (Heldt et al., 1999; Susmel et al., 1995). The present study found lower CP content in corn grain (84.26 g/kg DM) and dried whey (90 g/kg DM) compared to an earlier study (109 and 122 g/kg DM respectively) (Susmel et al., 1995).

Effect on feed intake

The feed intake and animal performance largely depend on the chemical composition of feed, primarily on the CP, NDF, ADF and energy content (Waldo, 1986).

A diet with higher content of CP and lower content in fibre is of higher digestibility and higher feed intake (Vranic et al., 2009). However, the digestibility of nutrients in higher animal intake can be lower due to the accelerated passage of food through the digestive tract (DeFrain et al., 2004). For optimal uptake of nutrients from feed, the energy content and protein content in the rumen should be aligned, because higher accessibility of carbohydrates over protein can lead to energy loss in rumen microorganisms, whereas higher CP content can lead to N loss through urine and faeces (Russell, 1998). An unbalanced diet can therefore lead to energy and nitrogen loss in an organism, lower synthesis of microbial protein and lower uptake by the animal (NRC, 2001). The intake of DM is proportional to the DM content and quality of feed (Bruinenberg et al., 2002). The AH dietary treatments under this study had a higher intake of all the studied dietary chemical elements than the IRH treatments, which is consistent with earlier studies that found that supplementing carbohydrate-rich forage to feed higher in CP content had a positive effect on feed intake, compared to feed with lower CP content (De Visser et al., 1998; Heldt et al., 1999; Huntington, 1997; Vranic et al., 2008). Furthermore, a strong negative correlation had previously been found between the NDF content in feed and DM intake (DMI) (Hayirli et al., 2003), as well as a higher rumen filling effect of NDF grasses compared with NDF legumes (Dewhurst et al., 2010). This is consistent with the present study where the AH as legume had lower NDF content (Table 1) and consequently, the AH-based feed had higher DMI compared with IRH feed. Moreover, the positive correlation between the DM and DMI content of feed established earlier (Steen and Kilpatrick, 2000) is consistent with the results of the present study where a higher DMI value was found in AH-based feed that contained more DM (549.39 g/kg fresh specimen) compared with IRH (472.44 g DM/kg of fresh sample) (Table 1).

Animals prefer feed that is high in energy and sugar (Murphy et al., 1997), although low quantities of sugar (1.5% of sucrose in DM of feed) will not affect feed intake (Nombekela and Murphy, 1995). In the present study, the share of lactose in feed amounted to 0; 4.5; 9 and 13% in DM feed based on AH or IRH. In earlier studies, the replacement of corn with whey up to 12% (De Seram et al., 2019) or up to 15.7% had no effect (DeFrain et al., 2006) or increased (DeFrain et al., 2004) the DMI in the feed for dairy cows, as did the replacement of corn with lactose of up to 10.6% of DM feed (Susmel et al., 1995) or with sucrose (Broderick and Radloff, 2004; Penner and Oba, 2009). In the present study, the replacement of corn with whey in up to 30% of concentrate fed, and up to 12% of DMI, increased the intake of DM, OM and CP ($P < 0.05$) in AH-based feed and resulted in a linear reduction in the intake of ADF ($P < 0.05$) and starch ($P < 0.001$) in AH and IRH, and a linear reduction in NDF intake in IRH-based feed.

Earlier studies in general did not find any negative effects of sugar supplements on feed intake, nor should any negative effects be expected in a diet with reasonable sugar levels (Oba, 2011). This is consistent with the present study where replacing starch with lactose led to an increased intake of all the studied chemical elements of AH-based feed, as compared with IRH feed where no negative effect was found of starch replacement with lactose in the chemical parameters studied.

In an earlier study, the DM intake had a tendency to rise when up to 14% of sugar was added to feed (DeFrain et al., 2004). As it pertains to AH dietary treatments, the finding is partly consistent with the present study.

However, the results of earlier studies where corn was replaced by sugar in the feed for ruminants are not consistent. It was found that replacing corn with lactose in up to 13% of DM in feed did not affect the pH value of the rumen (DeFrain et al., 2004), but that replacing 6% of starch with sucrose led to a tendency to increase (Penner and Oba, 2009), whereas replacing 9% of starch with sugar resulted in an increased pH value in the rumen (Martel et al., 2011). Manipulating the type and share of forage and carbohydrate-rich forage in the feed will have an impact on the growth and development of microbiota and fermentation in the rumen. A more intensive fermentation will reduce the pH value of the rumen, as well as the activity of cellulolytic ruminal bacteria that break down the fibre (Pu et al., 2020). In the present study, the replacement of starch with lactose has led to a linear reduction in the digestibility of NDF in AH-based feed, as well as that of NDF and ADF in IRH-based feed, which indicates that replacing starch with lactose has led to a drop in the pH value of the rumen and lower activity of cellulolytic ruminal bacteria.

Effect on digestibility

Compared with IRH-based feed, a higher intake of AH-based feed resulted in lower digestibility of the studied chemical elements of feed, except in CP. This is partly consistent with earlier studies that found that a synchronized supplement of sugar and nitrogen had a positive effect on the production of microbial protein (Kim et al., 1999), especially if sugar was added at a point when protein degradation in the rumen was at its maximum (Trevaskis et al., 2001). In line with the results of the present study, previous studies did not find any positive effects from a synchronized presence of CP and sugar in the rumen on the digestibility of a forage-based diet with high CP content. However, it was established that the feed was more efficient based on its digestibility and milk production when sugar was added to forage with low content in rumen degradable protein (RDP) and when starch was added to forage high in RDP content (Hall et al., 2010). The level of replacement of starch with lactose can have a significant effect on the digestibility of feed. It was found that replacing 50% of corn with whey had no impact on CP digestibility but that replacing 100% of corn with whey increased CP digestibility compared with a corn-only supplement (Susmel et al., 1995).

The higher digestibility of DM, OM, NDF and higher D-value of a low intake of forage-based dietary treatments can be accounted for by the longer presence of forage high in fiber and low in CP in the digestive tract of ruminants. The intake of low-quality forage will be limited by the rumen's physical fill (Allen, 2000), and the intake of high-quality forage by the energy consumed (Vranic et al., 2018). The high-quality forage with a high DMI will linger in the digestive tract of ruminants for one to two days and that of low quality and low DMI for more than three days (Sayan et al., 2010). The earlier studies with a supplement of 5 or 10% of dried whey to AH/kg DMI found that there was higher *ad libitum* digestibility of DMI and OM and lower *in vivo* digestibility of DM (P < 0.01), OM (P < 0.05) and CP (P < 0.001), as a result of a faster passage of feed through the digestive tract (Vranić et al., 2016).

Replacing corn with whey in the present study did not have any impact on NDF digestibility but NDF and ADF digestibility of the IRH-based diet was higher compared with AH, which is consistent with earlier studies where adding sugar worth 0.3% of body mass increased the digestibility of fiber in low-quality forage (Heldt et al., 1999).

In line with these results, high-energy supplements to high-quality forage may lead to a negative associative effect of two types of forage on fiber digestibility (Pordomingo et al., 1991). Higher fiber digestibility in a diet based on high-quality forage may be achieved by adding low-quality forage that will ensure a longer presence of feed in the digestive tract (Matejovsky and Sanson, 1995).

Effect on N retention

Higher N retention is an indication of improved microbial N use in the rumen (Moss et al., 1992). A synchronized fermentation of carbohydrates and protein degradation in the rumen can theoretically increase the production of microbial protein (Calsamiglia et al., 2010). Lactose ferments faster and might be more efficient in feeding than starch if basic forage is reached in nutrients (Susmel et al., 1995). Adding lactose to the main diet will help release a higher amount of energy and enhance fermentation, while leading to a lower content of NH_3 in the rumen and protozoa that negatively affect the synthesis of microbial protein (Chamberlain et al., 1993), especially in a highly nutrient forage-based diet (Kim et al., 2000). A higher quantity of microbial N (Chamberlain et al., 1993) is also the result of the absorption of butyric acid following lactose degradation, which stops short-chain fatty acids (SCFA) from accumulating (DeFrain et al., 2004, 2006).

On average compared with IRH dietary treatments, the AH-based dietary treatments had about a 75% higher intake of N from haylage, 43% higher total intake of N (AH + concentrate), as much as 118% higher N output through urine, and 19% through faeces. Similarly, N retention in AH dietary treatments was 23% higher compared with IRH dietary treatments, which is consistent with the earlier study (Susmel et al., 1995).

Replacing barley starch with lactose led to a reduction in the content of NH_3 in the rumen of ruminants but did not affect N retention (De Seram et al., 2019). Since N retention is dependent on the intake of DM and N (Adesogan et al., 2002), the present study found that N retention was higher in a forage diet with a high CP content and higher DM intake, when compared with a forage diet with a low CP content and low DM intake.

The explanation for this lies in the higher CP content in AH where the available energy results in a more efficient use of N, while in forage diets with low CP content, the available energy is left partly unspent since the rumen's microbes do not need it to generate their protein (Russell, 1998).

The protein in whey (lactoglobulin) is highly efficient in feeding and its degradation in the small intestine (Susmel et al., 1995) can partly account for the increase in N retention in AH diets where starch is replaced by lactose. However, replacing starch with lactose in IRH diets clearly leads to a drop in N retention.

An increased synthesis of microbial protein in the rumen will lead to a more efficient use of N and a lower output of N through urine (De Seram et al., 2019). This study found that there was more efficient use (higher retention) of N, as well as a higher output of N from the organism in urine and faeces, in AH diets rather than IRH. However, replacing starch with lactose did not have an impact on N retention across individual feeding treatments based on AH or IRH, although the AH-based treatments had higher N retention than IRH-based treatments. A positive combined effect on N retention in AH-based diets, unlike IRH, may be the result of higher DM and N intake in AH-based feed, as mentioned above (Adesogan et al., 2002).

CONCLUSION

From the practical point of view, it can be concluded that the replacement of corn with whey in the wether sheep diet: (i) reduces the intake and digestibility of fibre and digestibility of starch regardless of the quality of the basic forage used and (ii) improves N utilization of the ration based on higher quality and higher protein forage.

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