

Wood molasses as a preservative for high moisture barley. 2. Ration digestibility and rumen fermentation in sheep

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Abstract. The effect of wood molasses ensiled barley on ration digestibility and nitrogen utilization (Exp. 1 and Exp. 2) and on rumen fermentation and degradation rate *in sacco* (Exp. 2) was investigated in two experiments. The ration contained 0.9 kg of DM. The proportion of hay was 30 % in Exp. 1 and 40 % in Exp. 2. In Exp. 1 the experimental diets were dried barley (DB) and barley ensiled with a level of 8 (WMB8) or 16 % (WMB16) wood molasses of barley DM. In Exp. 2 the diets were dried barley (DB), propionic acid-treated barley (PAB) and barleys ensiled with 0.3 % v/w % of AIV II solution (AIVB) or with wood molasses at a level of 12 % of barley DM (WMB12).

The ration digestibility decreased with increasing levels of wood molasses. The difference in DM and organic matter (OM) digestibility was significant ($P < 0.05$) between DB and WMB16. Wood molasses tended to decrease the apparent digestibility of crude protein and crude fibre and to increase nitrogen retention. The percentages of nitrogen retained of ingested were in Exp. 1 on DB, WMB8 and WMB16 diets 13.1, 17.8 and 15.8 % and in Exp. 2 on DB, PAB, AIVB and WMB12 diets 13.8, 13.2, 10.3 and 14.5 %, respectively.

After feeding, the concentration of total VFA in the rumen was higher when ensiled barleys were fed. The proportion of propionic acid in the total VFA was greater with DB and PAB than with AIVB and WMB12 diets ($P < 0.01$), and butyric acid was correspondingly lower ($P < 0.01$ and $P > 0.05$). The proportion of isovaleric and valeric acids was highest on AIVB diet. On DB and PAB diets the ammonia concentration in the rumen decreased after feeding, but on AIVB and WMB12 diets the highest value was reached 1.5 hours after feeding. The degradation rate of DM and CP as determined by nylon bag method was faster on AIVB and WMB12 than on DB and PAB diets.

Introduction

Various types of wood molasses have been fed to livestock, with a feeding value equal to cane molasses (TURNER 1964, AL-CHALABI et al. 1974, CRAWFORD et al. 1978). CHANG et al.

(1977) found no differences in diets containing 8 and 12 % of spent sulphite liquor and a control diet in beef cattle. HARTNELL and SATTER (1978) suggested that the polyphenolic fraction in wood molasses (Masonex) binds with certain proteins to decrease

their microbiological degradation and increase the amount of dietary nitrogen that escapes the rumen fermentation.

SALO (1978) and HUHTANEN (1984 a) reported wood molasses, a by-product from the wood processing industry, to be an efficient preservative for high moisture barley. The digestibility of barley ensiled with wood molasses was lower than dried barley in pigs (HUHTANEN 1984 a) and in the diet of pigs 10–20 % wood molasses decreased the ration digestibility (NÄSI 1984).

The objective of the present study was to investigate the effects of wood molasses ensiled barley on ration digestibility, nitrogen utilization and rumen fermentation in sheep and to evaluate the optimum level of wood molasses in ensiled barley as an energy source for ruminants. The wood molasses ensiled barleys were the same used in the previous experiment (HUHTANEN 1984 a).

Material and methods

The digestibility and rumen fermentation trials were performed with three (Exp. 1) and four (Exp. 2) Finnsheep rams in 3×3 and 4×4 Latin square arrangements. The average weight of the sheep was 48 kg in Exp. 1 and 55 kg in Exp. 2. The trials consisted of transition, standardization and collection periods lasting 5, 9 and 7 days each.

The sheep were kept in metabolism cages allowing a separate collection of faeces and urine. During the collection periods the sheep were provided with faeces-collecting harnesses.

In Exp. 1, diets comprised 300 g of hay and either 700 g of dried barley (DB) or an equal amount of DM of barley ensiled with wood molasses at a level of 8 (WMB8) or 16 % (WMB16) of barley DM; in Exp. 2 diets comprised 400 g of hay and 480 g DM of dried barley (DB), propionic acid-treated barley (PAB), barley ensiled with 0.3 % v/w of AIV II solution (AIVB) or barley ensiled with wood molasses at a level of 12 % barley DM (WMB12). In addition the animals re-

ceived a mineral mixture ad libitum in Exp. 1 and 20 g/d in Exp. 2. Water was given freely and its consumption was measured during the collection periods. Feeding took place twice daily. The sheep were weighed before and after the collection periods.

The faeces and urine were collected in the morning and representative samples were taken for analysis and stored frozen until analyzed. DM determinations were made at 103°C; the samples for analysis were dried in vacuum at 50°C for 2–3 days and milled through a 1-mm screen. The DM contents of ensiled barleys were corrected according to JARL and HELLEDAY (1948). The feed analyses were made according to standard procedures and VFA determinations by the method of HUIDA (1973).

During the two last days of collection periods in Exp. 2, rumen samples were taken through the fistula before and 1.5, 3, 4.5 and 6 hours after the morning feeding. pH-measurements were made immediately. The samples were centrifuged for 10 min at 2000 rpm. Ammonia N and VFA determinations were made on the supernatant by the methods of McCULLOUGH (1967) and HUIDA (1973). Samples for determination of rumen microbiota were taken 6 hours after feeding. Five ml of rumen content was transferred to a glass bottle containing 45 ml of 10 % formalin. The total number of protozoa was calculated according to WESTERLING (1970) and the number of bacteria using a counting chamber of dimensions 1×1 mm and 0.2 mm depth. Four preparations were made of each sample for count.

The DM and crude protein (CP) degradabilities of DB, PAB, AIVB and WMB12 were determined in Exp. 2 by the method of SETÄLÄ (1983). The experimental design was 4×4 Latin square and the incubation times were 2, 5, 9 and 24 hours. The degradability measurements were performed on the same barley fed in the diets.

The results were tested by analysis of variance and the differences between the means by the Tukey-test.

Results and discussion

Digestibility of rations

The chemical composition of the experimental feeds is presented in Table 1. Wood molasses used at levels of 12 and 16 % decreased the digestibility of the ration (Table 2). The differences in the DM and OM digestibilities of DB and WMB16 were significant ($P < 0.05$). Also, the CP and crude fibre digestibilities tended to decrease with increasing levels of wood molasses. Ammonium spent sulphite liquor (SALO and PUUMALA 1978) and spent sulphite liquor (CHANG et al. 1977), which contain more lignosulphonates than wood molasses have been found to

decrease the digestibility of forages and soyabean-alfalfa substrate *in vitro*. In contrast, relative to cane molasses wood molasses (Masonex) has been found to have positive effect on cellulose digestibility in steers (CRAWFORD et al. 1978). The wood molasses used in the present experiment contains, however, more lignosulphonates than Masonex.

The effects of the various preserving methods on the ration digestibility agree with the results of WEISSBACH and SCHADEREIT (1968), KORHONEN et al. (1973) and INGALLS et al. (1974). In contrast, CLARK and HARSBERGER (1972) obtained higher DM and OM digestibilities on high moisture ensiled corn diet than on dried corn diet. McKNIGHT et al.

Table 1. Chemical composition of experimental feeds

	Exp. 1				Exp. 2				
	Hay	DB	WMB8	WMB16	Hay	DB	PAB	AIVB	WMB12
Dry matter, %	84.5	87.5	57.2	56.0	80.9	87.0	83.0	55.0	54.9
In dry matter, %									
Ash	7.7	3.0	4.0	5.2	5.9	2.7	2.8	3.2	3.5
Crude protein	10.2	11.8	12.1	11.0	8.9	11.6	13.7	12.7	12.1
Ether extract	1.7	2.2	2.6	2.3	2.2	2.3	2.5	2.8	2.7
Crude fibre	35.8	6.0	5.5	5.3	33.5	5.2	5.6	5.7	5.5
NFE	44.6	77.0	75.8	76.2	49.6	78.3	75.5	75.6	76.2

Table 2. Digestibility coefficients of total ration

Exp. 1	DB		WMB8		WMB16			
	\bar{x}	s.d.	\bar{x}	s.d.	\bar{x}	s.d.		
Dry matter	71.5 ^a	3.1	70.7 ^{ab}	2.0	68.3 ^b	2.6		
Organic matter	73.5 ^a	3.0	72.8 ^{ab}	2.0	70.4 ^b	2.3		
Crude protein	68.1	2.3	67.8	2.1	63.1	2.3		
Ether extract	70.3	4.1	77.9	1.9	78.3	1.0		
Crude fibre	51.1	6.2	46.3	4.9	42.3	6.0		
NFE	79.6	2.5	79.1	1.3	77.3	1.7		
Exp. 2	DB		PAB		AIVB		WMB12	
	\bar{x}	s.d.	\bar{x}	s.d.	\bar{x}	s.d.	\bar{x}	s.d.
Dry matter	71.3	2.3	72.1	3.2	71.9	0.4	69.8	2.2
Organic matter	73.2	2.2	74.1	3.1	73.7	0.5	72.0	2.0
Crude protein	67.4	1.9	73.0	2.4	69.8	3.0	68.4	4.4
Ether extract	72.9	3.4	75.2	1.0	76.8	4.6	76.7	5.0
Crude fibre	49.5	2.9	50.5	6.5	51.7	1.5	48.1	3.8
NFE	80.0	2.1	80.2	2.8	80.0	0.6	78.5	1.9

Means with different letters were significantly different: a, b ($P < 0.05$)

(1973) reported greater ruminal digestion of DM, OM and starch for ensiled and acid-treated corn diets than for dried corn diet and this resulted in slightly better overall digestibility of energy and DM. Likewise GALYEAN et al. (1976) found better ruminal starch digestion on ensiled corn than on dried corn diet, but DM or OM digestion in the rumen were not affected by the preserving method. The explanation for the higher apparent CP digestibility of PAB in Exp. 2 could be the higher CP content of PAB.

Rumen fermentation

There were no significant differences in the average pH-values or total VFA concentrations in the rumen (Table 3). At 1.5 and 3 hours after feeding, rumen pH was lower and VFA concentration higher on the ensiled barley diets than on DB or PAB diets (Fig. 1). Similar effects of ensiled grain on fermentation rate in the rumen have reported by TORRY and PERRY (1974), PRIGGE et al. (1976) and GALYEAN et al. (1976) *in vivo* and DANLEY and VETTER (1974) and GALYEAN et al. (1976) *in vitro*.

The proportion of acetic acid was lower on the PAB diet than on the other diets. The difference was significant ($P < 0.05$) between PAB and AIVB. Molar per cent of propionic acid was higher ($P < 0.01$) on DP and PAB diets than on ensiled barley diets, and molar per cent of butyric acid lower ($P < 0.05$) on DB than on AIVB or WMB12 diets. Similar changes in VFA ratios when ensiled grain is used have been reported by PRATT and CONRAD (1970), INGALLS et al. (1974) and TONREY and PERRY (1974). GALYEAN et al. (1976), in contrast, reported a lower acetate-propionate ratio when ensiled corn was fed to steers instead of dried corn. The slightly lower acetate-propionate ratio on the PAB diet than on the DB diet agrees with the results of CLARK et al. (1973) and may be due to the prior treatment of barley with propionic acid.

The high proportion of butyric acid on ensiled barley diets may partly be explained by higher sugar content of ensiled barley than of dried barley. Increased levels of molasses in the diet have been found to promote butyrate production (KARALAZOS and SWAN

Table 3. Rumen pH, ammonia N, VFA and microbes on experimental diets. The values are averages of samples taken at different times.

	DB		PAB		AIVB		WMB12	
	\bar{x}	s.d.	\bar{x}	s.d.	\bar{x}	s.d.	\bar{x}	s.d.
pH	5.97	0.32	5.97	0.31	5.90	0.36	5.86	0.28
Ammonia N mmol/l	8.1 ^a	5.2	12.3 ^{ab}	2.6	11.1 ^{ab}	3.9	12.7 ^b	5.9
Total VFA mmol/l	81.2	10.0	79.7	6.5	82.5	13.9	85.3	11.3
Molar per cent of								
Acetic acid	59.4 ^{ab}	1.8	57.1 ^b	2.1	60.2 ^a	2.9	59.1 ^{ab}	4.6
Propionic acid	25.9 ^d	3.1	26.6 ^d	3.3	20.7 ^e	3.2	22.2 ^e	2.5
Butyric acid	11.0 ^e	3.0	13.0 ^{de}	1.8	14.1 ^d	2.0	14.7 ^d	2.4
Isovaleric acid	2.3 ^d	0.7	1.8 ^d	0.4	2.9 ^e	1.0	2.1 ^d	0.3
Valeric acid	1.3 ^d	0.3	1.5 ^{de}	0.2	2.0 ^f	0.7	1.7 ^{ef}	0.5
Caproic acid	0.0 ^a	0.0	0.0 ^a	0.0	0.1 ^{ab}	0.1	0.2 ^b	0.2
Ratio A:P	2.3 ^{ef}	0.3	2.2 ^f	0.4	3.0 ^d	0.5	2.7 ^{de}	0.5
Ratio A:B	5.9 ^d	2.1	4.5 ^{de}	0.7	4.4 ^{de}	0.8	4.2 ^e	1.1
Ratio P:B	2.6 ^a	1.0	2.1 ^{ab}	0.5	1.9 ^b	0.9	1.8 ^b	0.6
Bacteria $n \times 10^9$ ¹⁾	4.67 ^a		4.00 ^{ab}		2.84 ^{ab}		2.16 ^b	
Protozoa $n \times 10^4$ ¹⁾	87.4		115.2		114.9		90.3	

¹⁾ 6 hours after feeding

Means with different letters significantly different: a, b, c ($P < 0.05$), c, d, f ($P < 0.01$)

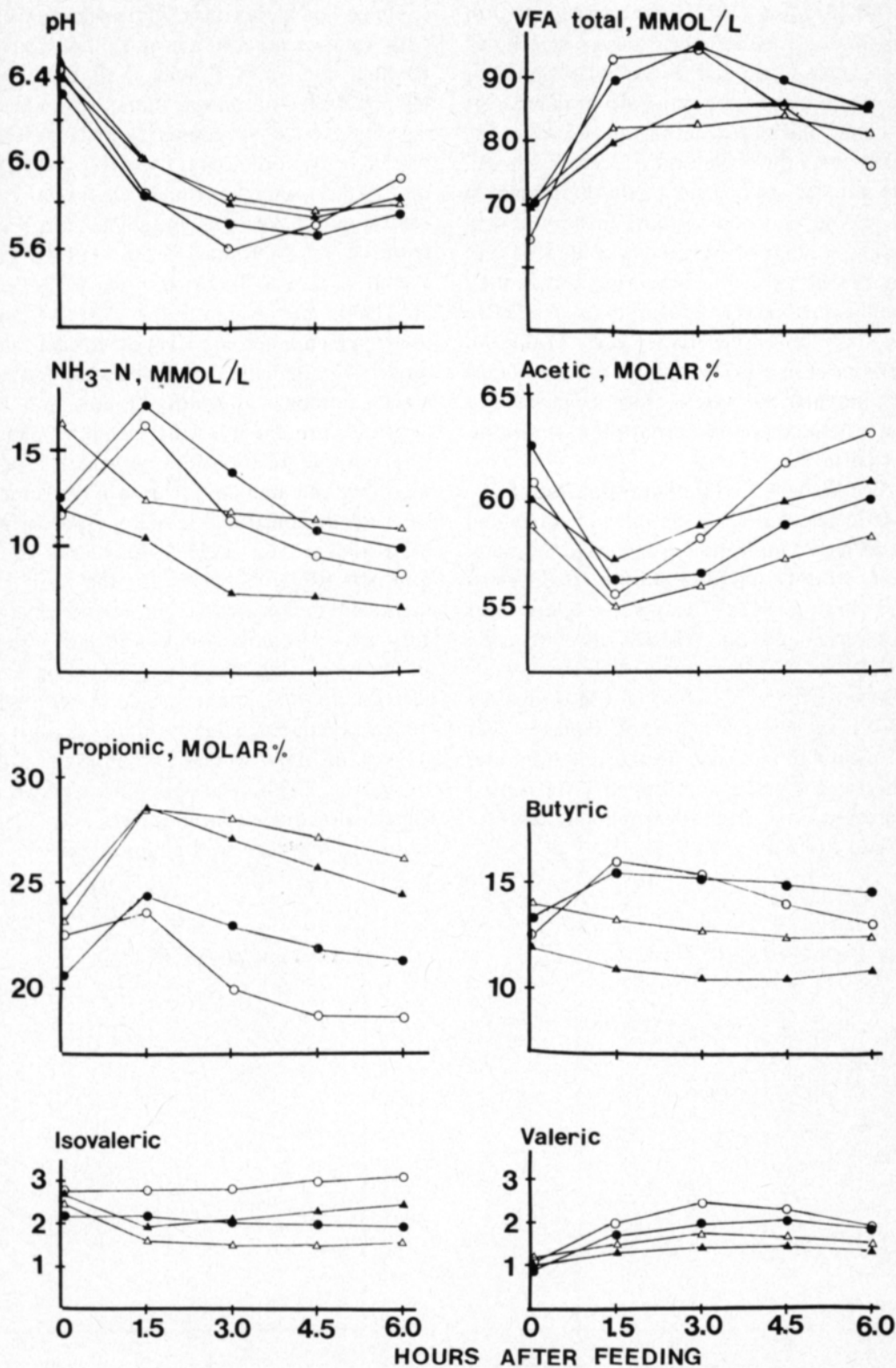


Fig. 1. Rumen pH, ammonia N and VFA of sheep fed with different barleys (\blacktriangle DB, \triangle PAB, \circ AIVB and \bullet WMB12)

1974). SYRJÄLÄ (1972) reported higher proportion of butyrate when sucrose was added to a grass silage diet instead of starch. But also a change in the ratio of the number of bacteria and protozoa has an effect on the propionate-butyrate ratio. Increases in ciliate number are proportionate to decrease in propionic acid and increase in butyric acid (EADIE et al. 1970, WHITELAW et al. 1972). In the present experiment the ratio between the number of bacteria and protozoa tended to be lower on ensiled barley diets (Table 3). ISHAQUE et al. (1971) reported that a change in fermentation pattern from propionate to butyrate increases the proportion of OM digested in the rumen.

The higher ($P < 0.01$) proportion of iso-valeric acid on AIVB diet may be attributed to decreased utilization or increased deamination by rumen micro-organisms (BRÜGGEMAN and GIESEKE 1967). The lower proportion of iso-valeric acid on WMB12 diet than on AIVB diet is in agreement with *in vitro* results reported by CHALUPA and MONTGOMERY (1979). At lower levels wood molasses was fermented to methane, acetate and butyrate, whereas increased supplement level caused increased production of propionate (CHALUPA and MONTGOMERY 1979).

On ensiled barley diets, rumen ammonia N concentration reached a peak 1.5 hours after feeding, but on DB and PAB diets the highest concentration was found before feeding. On average, ammonia N concentration was higher ($P < 0.05$) on WMB12 diet than on DB diet. A higher rumen ammonia concentration with ensiled grains has also been reported by McKNIGHT et al. (1973) and PRIGGE et al. (1978). In contrast, INGALLS et al. (1974) and PRIGGE et al. (1976) found lower rumen ammonia level on ensiled than on dried grain diets. One explanation for the higher ammonia level after feeding may be increased protein solubility during ensiling. The positive relationship between protein solubility and ammonia release in the rumen has been demonstrated in many experiments (ANNISON et al. 1956, DONALDSON and EDWARDS 1977). A reason for the different shapes of ammonia curves may be the change in rumen microbiota. It has been shown that an increase in the number of protozoa and decrease in the number bacteria increases rumen ammonia concentration (KLOPFENSTEIN et al. 1966 MALES and PURSER 1970, VEIRA et al. 1983). The correlations between rumen ammonia concentration and VFA ratios in the rumen were in good agreement

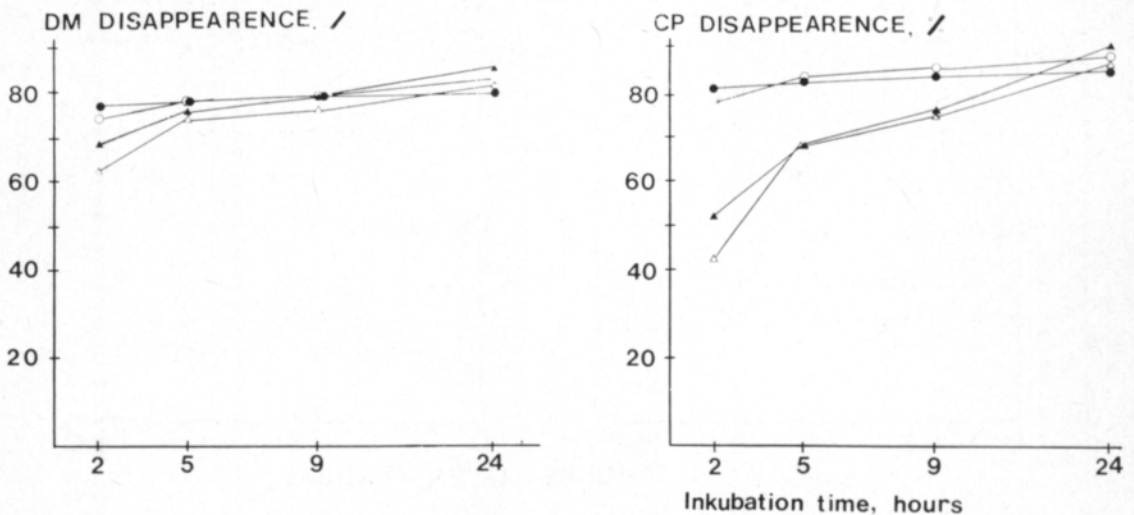


Fig. 2. DM and CP disappearance of different barleys in the rumen (▲ DB, △ PAB, ○ AIVB and ● WMB12)

with those observed by MALES and PURSER (1970).

Degradability of DM and CP

DM and CP degradation rates in the rumen were faster for ensiled barleys than for DB and PAB (Fig. 2) which is in agreement with the rumen fermentation studies and *in sacco* studies by GALYEAN et al. (1977) with ensiled corn. The difference in the degradation rates of DM and CP decreased with longer incubation times, and after an incubation time of 24 hours the DM degradability of DB was higher ($P < 0.05$) than to WMB12. Dried barley had a slower degradation rate than reported earlier by SETÄLÄ (1983) and the values were more similar to those reported by LINDBERG and VARVIKKO (1982).

Nitrogen utilization

In Exp. 1 the use of wood molasses as a preservative tended to increase nitrogen retention in sheep aged 8–10 months, compared with DB (Table 4). The excretion of nitrogen in urine was lower ($P < 0.05$) and in faeces higher ($P < 0.05$) on WMB16 than on DB diet. Blood urea N was not determined, but was lower ($P < 0.01$) in growing bulls fed on WMB12 diet than in bulls fed on AIVB diet (HUHTANEN 1984 b). A reason for the slightly better protein utilization on WMB diets is suggested by the finding that

xylose is a better energy source for microbial protein synthesis *in vitro* than sucrose or mannose (HENDERICKX and MARTIN 1963).

The increased protein solubility of the ensiled barleys had no adverse effect on protein utilization. After feeding of ensiled barley diets microbes had more rapidly fermenting organic matter available to use soluble nitrogen for protein synthesis. Generally, proteins of low solubility are used more efficiently than proteins of high solubility because more dietary protein reaches the duodenum intact and less cycles through the process of degradation to ammonia. However, PRIGGE et al. (1976) observed greater nitrogen retention with high moisture ensiled corn than with dried rolled corn. PRIGGE et al. (1978) reported abomasal nitrogen flow and efficiency of microbial synthesis to be greater on ensiled or acid-treated corn than on dried corn. The increased microbial synthesis was related to increased rumen turnover rate. Increased rumen dilution rate has been found to be associated with increased acetate and butyrate and decreased propionate in rumen VFA (THOMSON et al. 1975, OWENS and ISAACSON 1977). This effect was also found on the ensiled barley diets of the present study.

When dietary protein is of poor quality, utilization can be improved by rumen degradation and conversion to microbial protein (LITTLE et al. 1963, PRIGGE et al. 1976). Moreover, when the grain protein is protected against microbial degradation in the rumen, no positive effect on protein utiliza-

Table 4. Nitrogen balance and excretion of nitrogen in faeces and urine.

	Exp. 1			Exp. 2			
	DB	WMB8	WMB16	DB	PAB	AIVB	WMB12
N intake, g/d	15.7	16.3	15.0	13.5 ^a	15.4 ^b	14.8 ^{a,b}	13.7 ^a
N in faeces, g/d	5.0 ^a	5.3 ^{a,b}	5.5 ^b	4.4	4.2	4.5	4.3
N in urine, g/d	8.8 ^a	8.1 ^{a,b}	7.1 ^b	7.2	9.2	8.9	7.4
N balance, g/d	2.1	2.9	2.6	1.9	2.0	1.5	2.0
% of intake	13.1	17.8	15.8	13.8	13.2	10.3	14.5
% of absorbed	19.6	26.4	25.3	20.6	18.2	14.6	20.4

Means with different letters significantly different: a, b ($P < 0.05$)

tion is found (DAVIS and FAICHNEY 1973, THORNTON et al. 1977). In contrast to the results of HARTNELL and SATTER (1978), in the present experiment wood molasses did not slow the degradation of protein by rumen microbes. But it did decrease the breakdown of barley protein in the silo compared with AIV II solution (HUHTANEN 1984 a).

When wood molasses was used as a preservative for high moisture barley at a level of

8—12 % of barley DM, no significant effect on ration digestibility was found and it tended to improve protein utilization. Wood molasses at this level can therefore serve as an energy source as well as a preservative for grain.

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Puumelassi tuoreen ohran säilöntäaineena.

2. Vaikutus rehun sulavuuteen ja pötsifermentaatioon lampaalla

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Kahdessa kokeessa selvitettiin puumelassilla säilötyn ohran vaikutusta rehuannoksen sulavuuteen ja typen hyväksikäyttöön (koe 1 ja 2). Kokeessa 2 selvitettiin lisäksi vaikutusta pötsifermentaatioon ja rehun hajomismnopeuteen pötsissä. Koe-eläimet saivat kuiva-ainetta 0.9 kg/pv, josta heinän osuus oli kokeessa 1 30 % ja kokeessa 2 40 %. Koerehuina oli kokeessa 1 kuivattu (KO), sekä 8 (PMO8) ja 16 %:n puumelassilisäyksellä säilötty ohra (PMO16) sekä kokeessa 2 kuivattu (KO), propionihapolla jyväsäilötty (PrO), AIV II:lla (AIV2O) tai puumelassilla (12 % ohran ka:sta) säilötty ohra (PMO12).

Puumelassitason noustessa rehuannoksen sulavuus huononi. Ero oli merkitsevä ($P < 0.05$) KO:n ja PMO16:n kuiva-aineen ja orgaanisen aineen sulavuudessa. Puumelassilla säilötty ohra huononsi hieman raakavalkuaisen ja raakakuidun näennäistä sulavuutta ja lisäsi typen pidättymistä ($P > 0.05$). Pidättyneen typen

osuus typen saannista oli kokeessa 1 KO-, PMO8- ja PMO16-ruokinnalla 13.1, 17.8 ja 15.8 % sekä kokeessa 2 KO-, PrO-, AIV2O- ja PMO12- ruokinnalla 13.8, 13.2, 10.3 ja 14.5 % vastaavasti.

KO- ja PrO-ruokinnalla pötsinesteen pH laski ja VFA-pitoisuus nousi vähemmän kuin murskesäilötyillä viljoilla. Propionihapon osuus VFA:sta oli KO- ja PrO-ruokinnalla korkeampi ($P < 0.01$) ja voihiapon osuus KO-ruokinnalla alempi ($P < 0.01$) kuin AIV2O- ja PMO12-ruokinnalla. Murskesäilöttyä ohraa käytettäessä pitkäketjuisten rasvahappojen osuus VFA:sta lisääntyi. KO- ja PrO-ruokinnalla pötsinesteen ammoniakkipitoisuus alkoi laskea ruokinnan jälkeen, mutta AIV2O- ja PMO12-ruokinnalla ammoniakkihiippu saavutettiin 1.5 tuntia ruokinnan jälkeen. Murskesäilöntä lisäsi sekä kuiva-aineen että raakavalkuaisen hajomismnopeutta pötsissä nailonpussimenetelmällä määritettynä.