Acta vet. scand. 1980, *21*, 1—10.

From the State Veterinary Research Station for Small Ruminants, Høyland, Sandnes, Norway.

LISTERIOSIS IN SHEEP

LISTERIA MONOCYTOGENES IN SHEEP FED HAY OR GRASS SILAGE DURING PREGNANCY. IMMUNOLOGICAL STATE, WHITE BLOOD CELLS, TOTAL SERUM PROTEIN AND SERUM IRON *

By

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GRØNSTØL, H.: *Listeriosis in sheep. Listeria monocytogenes in sheep fed hay or grass silage during pregnancy. Immunological state, white blood cells, total serum protein and serum iron.* Acta vet. scand. 1980, *21*, 1—10. — A comparison was made between a hay fed group, consisting of 23 ewes, and a grass silage fed group of 22 ewes, all pregnant. Excretion of Listeria monocytogenes (Lm) in the faeces and milk, antibody titres in sera and whey and delayed hypersensitivity against Lm, and several blood components were determined. The animals had previously been exposed to Lm, and Lm was isolated from the faeces from several animals when the experiment started.

No significant difference in number of excretors between the 2 groups was found during the experimental period. The haemagglutination titres in both sera and whey were low and on the same level in both groups. The titres were higher in animals with 1 foetus than in animals with more than 1 foetus.

In the first part of the experimental period the silage group had a reduced number of lymphocytes, lower total serum protein values and higher serum iron values, compared with the hay group.

The silage group also had a stronger delayed hypersensitivity reaction against Lm than the hay group, and in the silage group the reaction was significantly stronger in ewes with 3 or more foetuses than in ewes with 1 foetus.

In conclusion, the combined effect of some of the changes found in animals fed grass silage may leave them more susceptible to infections.

Listeria monocytogenes; grass silage; hay; sheep; immunology; blood components.

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* This work was supported by grants from the Norwegian Agricultural Research Council.

Outbreaks of clinical listeriosis seem to be associated with grass silage feeding (*Pálsson* 1963, *Dijkstra* 1965, *Grønstøl* 1979 b). Little is known, however, about the significance of such feeding in the pathogenesis of listeriosis, but Listeria monocytogenes (Lm) has been isolated from grass silage samples (*Dijkstra, Grønstøl* 1979 c). Whether the grass silage feeding in addition may have a predisposing effect is not clear, although previous experiments at this Research Station indicated that sheep fed grass silage have a reduced degree of nutritional immunity (*Kochan* 1973) compared with sheep fed hay (*Øverås* 1974).

The present experiment was performed to compare grass silage fed sheep with hay fed sheep with regard to excretion of Lm, possible antibody titres and delayed hypersensitivity against Lm. In addition, the effect of the diet on some blood components was determined.

MATERIALS AND METHODS

Animals

The animals belonged to the experimental flock at this Research Station. Feeding and management of the flock have been described elsewhere (*Grønstøl* 1979a).

When the indoor season started, on 16th December, 2 groups of ewes were selected for the experiment. Each group consisted of 24 animals, 2—8 years old, of the Dala and Rygja breeds. They were kept on slatted floors until lambing, when they were transferred to individual pens. One ewe which died (silage group) and 2 which were barren (1 from each group) were excluded from the material.

The 2 groups were fed either grass silage or hay ad libitum. In addition, each animal was given a daily ration of 0.3 kg concentrates with a crude protein content of 12.5 %.

During the winter the animals appeared to be healthy and in good condition. They were shorn on 6th March and lambed in April and early May.

Samples

Samples and sampling dates are recorded in Table 1. Grass silage, faeces and milk were examined bacteriologically as described previously (*Grønstøl* 1979a). Whey and sera were examined serologically by an indirect haemagglutination method

Table 1. Sampling dates and number of samples in an experiment with hay fed and silage fed ewes.

Date	Faeces	Silage	Blood	Milk
Dec. 17	45	—	—	—
Dec. 30	—	4	—	—
Jan. 27	45	—	45	—
Feb. 23	45	—	45	—
Mar. 23	45	—	45	—
At lambing	45	—	45	45

(*Grønstøl* 1979a). Blood samples were examined for content of total serum protein, serum iron, plasma glucose, whole blood haemoglobin, packed cell volume and number of red cells and leucocytes.

Differential leucocyte counts were performed. The methods are given by *Øverås* (1969, 1974) and *Waldeland* (1977).

Skin test

On 8th April the animals were tested intradermally as described by *Grønstøl* (1979a).

RESULTS

Bacteriological examination

Lm was not isolated from any of the silage samples. The isolation of Lm from the faeces and milk is recorded in Table 2. At the start of the experiment, 8 of the 22 silage fed ewes and 4 of the 23 hay fed ewes excreted Lm in the faeces, and similar results were found on the second sampling date. Later,

Table 2. Isolations of Listeria monocytogenes (Lm) from the faeces and milk of 23 hay fed ewes and 22 grass silage fed ewes on the various sampling dates.

Date	Hay	%	Silage	%	Sample
Dec. 17	4	17	8	36	Faeces
Jan. 27	3	13	8	36	—
Feb. 23	0	0	2	3	—
Mar. 23	0	0	3	14	—
At lambing	2	9	3	14	—
At lambing	3	13	3	14	Milk

a lower excretion rate was found, but the number of positive samples was slightly higher in the silage group than in the hay group.

Serological examination

The reciprocal geometrical mean titres (GMT) for the hay group ranged from 13 to 29, and for the silage group from 13 to 36. In both groups GMT was higher for ewes with 1 foetus than for the remainder (Table 3). GMT in whey from the 2 groups were 12 and 13, respectively.

Table 3. Reciprocal geometrical mean titres (GMT) in sera and whey in the 2 groups according to number of foetuses.

Number of foetuses	Group*	Sample	Jan. 27	Feb. 23	Mar. 23	At lambing
≥ 3	H	sera	28	20	14	14
		whey	—	—	—	14
2	H	sera	13	26	11	13
		whey	—	—	—	18
1	H	sera	36	45	23	28
		whey	—	—	—	23
≥ 3	S	sera	13	32	20	20
		whey	—	—	—	25
2	S	sera	14	26	12	15
		whey	—	—	—	12
1	S	sera	28	71	28	64
		whey	—	—	—	12

* H = Hay fed. S = Silage fed.

Blood components

Fig. 1 shows that the number of leucocytes in the silage group was lower than in the hay group on the first ($P < 0.001$, Students t-test) and second sampling date ($P < 0.05$). This was mainly due to a reduction in number of lymphocytes. The silage group also had significantly lower total serum protein values on the first ($P < 0.001$) and second sampling date ($P < 0.01$), and the values remained lower in this group throughout the experimental period (Fig. 2).

Fig. 3 illustrates that serum iron was higher in the silage group than in the hay group. The difference was statistically

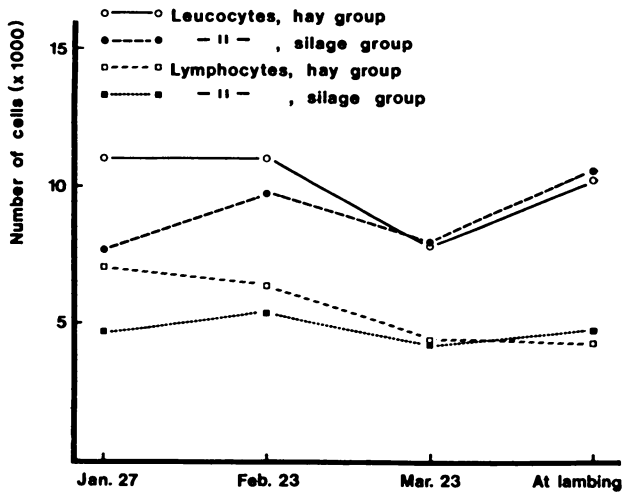

Figure 1. Average numbers of leucocytes and lymphocytes in 23 hay fed and 22 grass silage fed ewes.

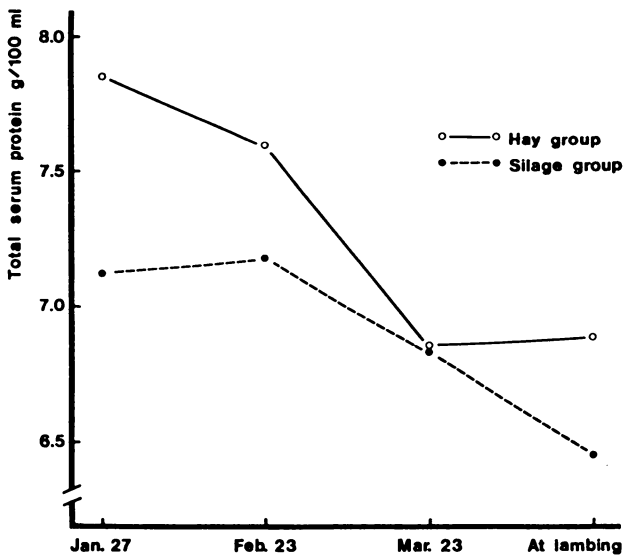

Figure 2. Average total serum protein values in 23 hay fed and 22 grass silage fed ewes.

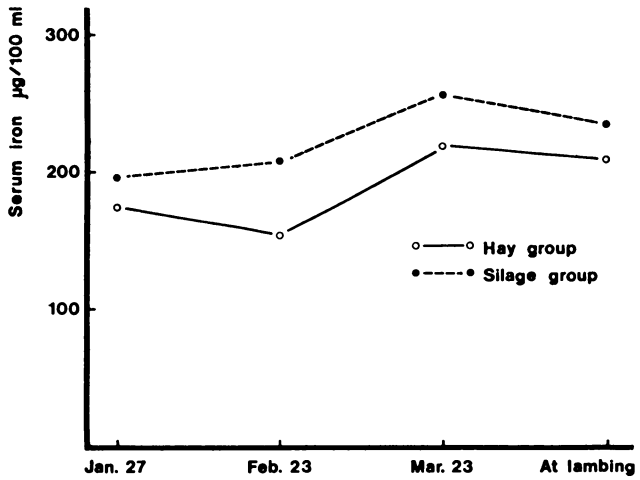

Figure 3. Average serum iron values in 23 hay fed and 22 grass silage fed ewes.

significant on the first 3 sampling dates ($P < 0.05$, $P < 0.001$ and $P < 0.002$, respectively). For the remaining blood components no statistically significant differences could be found.

Skin test

Results from the skin test are recorded in Table 4. The greatest increase was found in ewes fed silage. The difference between the 2 groups was statistically significant ($P < 0.001$). In the silage group the increase was greatest for ewes with ≥ 3 foetuses and smallest for ewes with 1 foetus. The difference between these categories was significant ($P < 0.05$). For the hay fed ewes no significant differences between groups according to number of foetuses were found.

Table 4. Average increase in thickness (mm) in the skin test.

Number of animals	Group*	Number of foetuses	Increase
23	H	1—4	1.8
22	S	1—4	3.3
3	S	3	3.8
13	S	2	3.4
6	S	1	2.6

* H = Hay fed. S = Silage fed.

DISCUSSION

An excretion pattern of Lm in healthy sheep fed grass silage of good quality during the indoor season, has been described previously (*Grønstøl* 1979a). None of the ewes excreted Lm until lambing, when 64 % of the ewes were found to excrete Lm in the faeces, and 41 % in the milk.

Another pattern was found in a flock with listeric encephalitis (*Grønstøl* 1979b). Lm was isolated from the faeces of about 25 % of the ewes in the first part of the indoor season, and 10—30 % had positive faecal samples during the gestation period. The rise in number of excretors towards lambing as seen in the healthy flock, was not found, and Lm was isolated from the milk from 15 % of the ewes.

In the present experiment the excretion seemed to follow the latter pattern, with the highest number of excretors during the first 6 weeks and a low number during the rest of the experimental period. Such a pattern may be the consequence of a clinical or subclinical listeric infection in the recent past.

Hay samples were not examined for the presence of Lm as previous investigations had shown that Lm rarely could be isolated from such samples (*Grønstøl*, unpublished). Lm was not isolated from any of the grass silage samples. Considering the difference in number of excretors when the experiment started, no difference in excretion rate was found between the groups during the experimental period.

Both groups had low antibody titres on the same level.

The leucocyte counts in the silage fed ewes were significantly below those recorded in the hay group on the 2 first sampling dates. This was mainly due to a reduced number of lymphocytes. Although the counts were within the normal range, the reduction may impair the immunity. *Vanel* (1974) postulated that serum from sheep fed maize silage contained a substance which was toxic for lymphocytes, but he was unable to identify this factor.

Glucocorticoids have a lytic effect on lymphocytes. The effect of grass silage feeding on the level of glucocorticoids in sheep has not been studied, but in cattle *Buruiana & Susan* (1973) found an increase in glucocorticoids in blood, urine and milk after feeding with maize silage. The values remained high as long as this feeding regime lasted, and returned to normal when the maize silage feeding was terminated.

Hjelle (1969) found that sheep fed grass silage and concentrates had acid urine, while sheep fed hay and concentrates had alcaline urine. This may be interpreted as a moderate metabolic acidosis in the silage fed sheep. The effect of a metabolic acidosis on cell mediated immunity is not known, but *Lachmann & Fürll* (1977) found that animals in a state of acidosis had a reduced ability to develop antibodies after exposure to an antigen. In the present experiment the animals were probably not exposed to sufficiently strong antigen concentrations (Lm) to mount an antibody response during the experimental period, and hence, no such effect was seen.

This experiment also confirmed previous findings that the titres were associated with the number of foetuses (*Grønstøl* 1979a, b).

Total serum protein was significantly lower in the silage group than in the hay group on the first 2 sampling dates. *Lachmann & Fürll* found that metabolic acidosis led to a reduction in γ-globulins. In the present work electrophoresis of the sera was not performed, and consequently nothing can be said about which protein fractions that were reduced. *Øverås* (1974), on the other hand, found no significant differences in total protein values in serum between silage fed and hay fed ewes.

The elevated serum iron content in the silage group is in accordance with *Øverås* (1974) and may be of importance in mechanisms of infections (*Sword* 1966, *Kochan* 1973).

The skin test showed a significantly stronger delayed hypersensitivity reaction in the silage group than in the hay group, as also reported earlier (*Grønstøl* 1979a). In the silage group a stronger reaction was found in ewes with ≥ 3 foetuses than in ewes with 1 foetus, while a corresponding difference was not recorded in the hay group. This pattern is opposite to that seen for GMT where ewes with 1 foetus had the highest values. If grass silage feeding has a suppressive effect on humoral immunity, the humoral branch of the immune system has a reduced ability to deal with antigens. The stimulation of the cell mediated branch may then be stronger, and this effect is expressed through an increased delayed hypersensitivity reaction.

The strong circumstantial evidence for an association between silage feeding and listeriosis in sheep (*Pálsson* 1963, *Dijkstra* 1965) points towards effects of the silage other than just as a vehicle for Lm. The reduction in number of lymphocytes,

in total serum protein values and the increase in serum iron values indicate a suppression of the immune system.

Nygaard & Nedkvitne (1977) recorded significantly lower body temperatures in grass silage fed ewes than in hay fed ewes. The greatest differences were found during the coldest periods. The effect of this temperature reduction on the resistance against infection is not known, and a more comprehensive study of the effect of grass silage feeding in sheep should be made.

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SAMMENDRAG

Listeriose hos sau. Gransking av utskiljing av Listeria monocytogenes, immunologiske tilhøve og visse komponentar i blodet. Ei samanlikning mellom dyr fôra med silofôr og dyr fôra med høy.

Drektige sauer blei delte i 2 grupper. Den eine, på 23 dyr, blei fôra med høy og kraftfôr, den andre, på 22 dyr, med silofôr og kraftfôr. Talet på dyr som skilde ut Listeria monocytogenes (Lm) i avføring og mjølk, blei undersøkt, antistoff mot Lm blei titrert og seinka hypersensitivitet mot Lm blei målt. Dessutan blei fleire komponentar i blodet bestemt. Listeriabakterier fanst i dyra sitt miljø, og fleire dyr skilde ut Lm i avføringen då granskinga starta.

Det blei ikkje funne noko tydeleg skilnad mellom tala på utskiljarar i dei to gruppene. Begge gruppene hadde låge antistofftiter mot Lm i sera og mjølk. Dyr med eitt foster hadde høgare titer enn dyr med fleire foster.

I fyrste delen av granskingsperioden hadde silogruppa lågare lymfocytt-tal, lågare innhald i serum av totalprotein og høgare innhald av jern, enn høygruppa.

Silogruppa hadde sterkare seinka hypersensitivitetsreaksjonar mot Lm enn høygruppa, og innan silogruppa var desse reaksjonane sterkare hos dyr med $\geq$ 3 foster enn hos dyr med 1 foster.

Det ser ut som dei endringane silofôringa fører med seg, kan setja ned motstandskrafta mot infeksjonar.

(Received April 4, 1979).

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