The effects of continuous ruminal dosing with dioctyl sodium sulphosuccinate on ruminal and metabolic characteristics of lactating Holstein cows

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Rumen-cannulated Holstein cows were used to study the effect of intraruminal dosing of dioctyl sodium sulphosuccinate (DSS; 0·07 g/kg body weight per d) for 4 weeks. DSS was suspended in nylon bags to allow it to be released slowly into the rumen. Cows were offered a diet containing grass silage and concentrate (45:55, w/w). Intakes of control cows were regulated to those of DSS-dosed cows. Cows dosed with DSS had no rumen ciliate protozoa, lower rumen NH_3-N concentrations and acetate and butyrate proportions, higher propionate, isovalerate, and valerate proportions. *In vitro* fibre digestion by non-ciliate rumen fluid from DSS-dosed cows was apparently impaired. When cows were dosed with DSS, levels of neutral- and acid-detergent fibre in whole rumen contents were increased, rumen solids turnover rate was slower, and whole tract apparent digestibility of cellulose and diethyl ether extract was decreased. Dosing of DSS led to reduced concentrations of blood acetoacetate but elevated plasma glucose levels. Milk protein content was higher, however, lactose content was lower for DSS-dosed than control cows. Milk fat of DSS-dosed cows had a smaller proportion of short-chain fatty acids but a greater proportion of unsaturated fatty acids.

Rumen: Dioctyl sodium sulphosuccinate: Lactating cow

Rumen ciliates can account for half the rumen biomass and significantly contribute to the fermentative activity in the rumen (Jouany *et al.* 1988). Controlling the population size of rumen ciliates offers an opportunity for altering rumen fermentation and productivity of ruminant animals (Jouany *et al.* 1988). Pulse-dose of DSS into the rumen has been used often to study the effects on rumen fermentation without ciliates (see Williams, 1986). Some studies (Lovelock *et al.* 1982; Veira *et al.* 1983) have reported that ciliates reappeared in the rumen (refaunation) after defaunation even when defaunated animals were kept in isolation from normally faunated animals. The causes for spontaneous refaunation after a period of defaunation are not certain. The present study was conducted to evaluate the effect of continuous ruminal dosing but slow-release of DSS into the rumen to prevent refaunation and to determine effects on ruminal and metabolic responses to this application in lactating Holstein cows.

MATERIALS AND METHODS

Experimental animals and design

Four rumen-cannulated Holstein cows were used in a crossover design with three periods of 28 d each. Cows were paired into two groups as similar as possible with regard to their age and period of lactation (average 125 d postpartum), live body weight (BW) (average 642 kg), and milk production (average 35 kg/d) at the beginning of the experiment. Within

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Table 1. *Ingredient and chemical composition of the diet*

Component					
Ingredient composition (g/kg dry matter (DM))			Chemical composition		
			Grass silage	Concentrate	TMR*
Grass silage	450	Dry matter	311	908	487
Shelled maize, cracked	333	Organic matter	916	928	923
Soya-bean meal (480 g/kg)	146	Diethyl ether extract	51	23	36
Oats	22	Crude protein	159	210	187
Molasses, liquid	18	NDF	621	173	375
Limestone	11	ADF	362	39	184
Dicalcium phosphate	3·2	Cellulose	316	30	159
Salt	4·4	TNC	85	522	325
Sodium bicarbonate	7·1	Gross energy	18·0	16·7	17·3
Magox†	1·4				
Selenium premix‡	1·3				
Dynamate§	1·2				
Trace mineral premix‖	1·1				

TMR, total mixed ration; NDF, neutral detergent fibre; ADF, acid detergent fibre; TNC, total non-structural carbohydrate (1000 − ash − diethyl ether extract − crude protein (N × 6·25) − NDF).

* Values for TMR are calculated from grass silage and concentrate. Net energy for lactation for TMR, estimated from ADF percentage (Adams, 1980), is 12·2 MJ/kg DM.

† Magox contained (mg/g): Mg 545, Mn 0·1.

‡ Se premix contained (mg/g): Se 0·2.

§ Dynamate contained (mg/g): Mg 111, K 182, S 222.

‖ Trace mineral premix contained (mg/g): Ca 260, Mg 14, Cu 6, Zn 54, Fe 27.

each group cows were randomly assigned to either no DSS (control) or DSS dosing. Cows were housed in tie stalls with plywood as partitions to minimize physical contact.

Experimental diet and feeding

The diet consisted of grass silage and concentrate (45:55, w/w; dry matter (DM) basis) and was formulated to meet nutrient requirements of cows (National Research Council, 1978) based on milk production at the start of the trial. Table 1 lists the ingredients and chemical composition of the diet.

Since DSS can reduce animal appetite (Orpin, 1977) a potential negative effect of DSS dosing on feed intake could be realized. Therefore, to avoid DSS effects being confounded with the effect due to feed intake differences the diet was fed *ad lib.* to DSS-dosed cows in two equal portions at 07.30 and 16.30 hours. For control cows the diet was also provided in equal portions twice daily; however, the amount of feed offered was regulated daily to be similar to that of DSS-dosed cows (paired counterparts) on a BW basis. Access to water was provided continuously and water consumption was measured daily using in-line water meters.

Procedures, sample collection, and chemical analyses

Ruminal DSS dosing and refaunation. On two successive days at the beginning of each experimental period cows assigned to DSS dosing were dosed intraruminally with DSS at the daily rate of 0·16 g/kg BW to eliminate rumen ciliates completely. Starting on day 3 the amount of DSS was reduced to 0·07 g/kg BW per d. The daily amount of DSS was partitioned into two doses according to a 24 h feeding interval and was administered

at feeding time. DSS (Fisher Scientific Co., USA) was weighed into a nylon bag (205 × 125 mm) which was tied with a nylon cord and attached to the cap of the rumen cannula while suspended in the rumen. This allowed a slow release of DSS into the rumen and prevented DSS from bypassing the rumen before being completely dissolved therein. Samples of rumen contents from DSS-dosed cows were examined for ciliates microscopically every other day. At 2 d before the beginning of periods 2 and 3, cows dosed with DSS in the previous period and then assigned to the control treatment were refaunated by direct addition of 2 litres of fresh whole rumen contents, obtained from their paired counterparts (control cows), into the rumen through the cannula.

Chemical analysis of feedstuffs. After determination of DM (55°, 48 h), feed samples were ground (1 mm) and analysed for neutral-detergent fibre (NDF), acid-detergent fibre (ADF), cellulose, and sulphuric acid (720 ml/l)-detergent lignin (Goering & Van Soest, 1970). Hemicellulose content was determined by subtraction of ADF from NDF. Ash, DM, crude protein (N × 6·25; CP), and diethyl ether extract determinations were made according to the Association of Official Analytical Chemists (1980). Gross energy was determined using a Parr Adiabatic bomb calorimeter (Parr Instrument Company, Inc., Moline, IL).

Whole tract nutrient digestibility measurements. Between days 10 and 19 of each experimental period, apparent whole tract digestibility of dietary nutrient components was estimated by using Cr_2O_3 as an inert digestibility marker. Cr_2O_3 in gelatin capsules was administered intraruminally at the rate of 10 g/d for 10 d. The first 5 d period was to ensure equilibration of Cr_2O_3 throughout the digestive tract. The last 5 d period was for the collection of orts and faecal samples. Portions of orts and faecal samples were composited over the 5 d period by cows. Dried (55°, 48 h) orts and faecal samples were then ground (1 mm) and analysed for ash, CP, NDF, ADF, cellulose, diethyl ether extract, and gross energy. Analysis of Cr in faeces was as described by Williams *et al.* (1962).

Comparison of non-ciliate rumen fluid. Forage digestibility by non-ciliate rumen fluid isolated from control and DSS-dosed cows was compared *in vitro*. After an 11 or 13 d adaptation into each experimental period for control or DSS-dosed cows, rumen fluid was drawn via rumen cannula 4 h after the 07.30 hours feeding. This rumen fluid was strained through four layers of cheesecloth. Non-ciliate rumen fluid was isolated by a sedimentation method (John & Ulyatt, 1984). The one-stage *in vitro* rumen fermentation conditions were similar to those described by Goering & Van Soest (1970). Each fermentation vessel used for incubation (anaerobic, 39°, 24 h) contained 20 ml artificial saliva (McDougall, 1948), 5 ml non-ciliate rumen fluid, and 250 mg dried (55°, 48 h), ground (4 mm) grass silage or lucerne (*Medicago sativa*) hay (g/kg; CP 156, NDF 496, ADF 363) as substrate. At the end of the incubation the contents of fermentation vessels were measured for DM, NDF, and ADF (Goering & Van Soest, 1970).

Blood measurements. Blood samples were taken from the tail vein or artery 4 h after the 07.30 hours feeding on day 18. Blood was determined for packed cell volume and plasma was prepared by centrifugation (3000 ***g***, 20 min, 4°). Plasma was frozen until quantified for total lipid (Folch *et al.* 1957), triacylglycerol (Sigma Chemical Co., 1989), phospholipids (Stewart, 1980), non-esterified fatty acids (NEFA; Wako Chemicals USA Inc., 1989), glucose (Sigma Chemical Co., 1989), urea-N (Technicon Instruments Co. Ltd, 1977), and insulin (Cambridge Medical Technology Corp., 1990). Acetoacetate was determined on whole blood (Mellanby & Williamson, 1974).

Rumen digestion. Rumen DM digestibility of forage and concentrate was determined *in situ* using nylon bags (Varga & Hoover, 1983) during days 20 and 22 of each period. Bags (205 × 125 mm, pore size 47 μm) containing 8 g dried (55°) and ground (4 mm) forage or 20 g concentrate were placed into the rumen of each cow at various time-intervals between

1 and 72 h, before the removal of all bags at one time. After removal, bags were washed with tap water and dried (55°).

Rumen digesta kinetics. From days 24 to 28, rumen digesta turnover rates were determined. Co-EDTA was used for the measurement of rumen liquid turnover rate and volume (Uden *et al.* 1980). Yb, as $YbCl_3.xH_2O$, was used as the particulate turnover marker (Yang & Varga, 1989).

Composition of rumen contents. On day 28 whole rumen contents were sampled from each cow immediately before the 07.30 hours feeding (0 h) and at 3 and 6 h after the 07.30 hours feeding to determine the effect of feeding on the rumen. Equal amounts of whole rumen contents were obtained from the dorsal and ventral regions and composited. A portion of the composited whole rumen contents was frozen immediately. Another portion of whole rumen contents was strained through four layers of cheesecloth to yield rumen fluid for pH measurement using a glass electrode (Fisher Accumet Model no. 610A) and for volatile fatty acid (VFA) (Yang & Varga, 1989) and NH_3-N (Chaney & Marbach, 1962) analyses. Rumen fluid obtained from control cows was preserved for ciliate protozoa counting (Yang & Varga, 1989). Frozen whole rumen contents samples were subsequently lyophilized, ground (1 mm), and analysed for ash, NDF, ADF, and lipid.

Milk sampling and analyses. Daily milk production was recorded at milking (05.30 and 15.30 hours) during days 15–28. Milk samples for chemical analyses were collected from each milking and immediately analysed for fat and protein using a Foss 203B Milko-Scan (Foss Electric, Hillerod, Denmark). A portion of the milk sample was frozen for later analyses of milk fatty acids, total solids (100°, 3 h), and ash. Lactose concentration was determined by subtraction of milk fat, protein, and ash contents from total solids contents. Milk fat for fatty acid analysis was extracted from milk by the Mojonnier Bros. Co. (1925) method. Methyl esters of milk fat were prepared according to the procedure of Christopherson & Glass (1969) and subsequently analysed by gas–liquid chromatography (Model 3290; Perkin-Elmer Corp., Norwalk, CT, USA).

Statistical analysis

Values were analysed as a crossover design by SAS PROC GLM (SAS, 1985). The model (main plot) employed for analysis of variance consisted of cow (3 df), period (2 df), and treatment (1 df). Sampling time effect (2 df) and its interaction with treatment (2 df) were added to the model as sub-plot for rumen variables. The *in vitro* experiment had non-ciliate inoculum source (1 df), forage species (1 df), and period (2 df) as sources of variation in the model. Means reported are least squares means. Treatment effect was determined by an F ratio and, with only four animals used in the present study, $P < 0{\cdot}10$ was considered significant.

RESULTS

Feed and water intake

Although every effort was made to equalize the intakes of each control cow with its DSS-dosed pair, control cows tended to consume more feed DM (Table 2). However, differences in DM intake and DM intake as a proportion of BW were not statistically significant. There was also no difference in water consumption.

Rumen measurements

No ciliate protozoa were observed in rumen fluid of DSS-dosed cows. The density of the ciliate population ranged between 5 and 8×10^5/ml in rumen fluid of control cows. Entodiniomorphs consisted of more than 95% of the total ciliate protozoa population density; the remainder were holotrichs.

Table 2. *Daily feed dry matter (DM) and water intake for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows**

Treatment group...	C	D	SE (5 df)	Statistical significance of difference: $P <$
DM intake: kg/d	19·4	18·1	0·48	0·13
kg/d per kg BW	0·030	0·029	0·0005	0·12
Water intake (l/d)	96·6	92·9	2·93	0·45

BW, body weight.
* For details of procedures, see pp. 398–399.

Table 3. In vitro *(24 h incubation) rumen forage digestibility (coefficient) by non-ciliate rumen fluid from control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows*†

	Grass silage		Lucerne (*Medicago sativa*) hay		
Treatment group...	C	D	C	D	SE (7 df)
DM	0·373	0·329	0·513	0·510	0·0261*
NDF	0·294	0·279	0·343	0·323	0·0187*
ADF	0·371	0·303	0·266	0·225	0·0279*

DM, dry matter; NDF, neutral-detergent fibre; ADF, acid-detergent fibre. The effect of forage species was significant (1 df): $*P < 0·05$.
† For details of procedures, see p. 399.

In vitro digestibility of ADF tended ($P = 0·10$) to be depressed when grass silage and lucerne hay were incubated with non-ciliate rumen fluid from DSS-dosed cows compared with that from control cows (Table 3). Digestibilities of DM and NDF did not differ.

Rumen DM digestibility of grass silage and concentrate determined *in situ* is presented in Table 4. There was no difference between the two treatments in ruminal disappearance of grass silage DM through 12 h of incubation. More grass silage DM tended to disappear after 24 h of incubation for the control cows than for the DSS-dosed cows. These differences were significant ($P < 0·07$) at the 48 h incubation. For the concentrate portion of the diet, DSS dosing decreased ($P < 0·10$) rumen DM disappearance at the 4 h incubation.

Dosing of DSS did not alter rumen fluid dilution rate, volume, or outflow rate; however, rumen solids turnover rate was higher ($P < 0·07$) in control cows (Table 5). Retention time of solid digesta in the rumen was shorter ($P < 0·08$) for the control compared with the DSS-dosed cows.

Mean organic matter and lipid levels of whole rumen contents across sampling time did not differ between the two treatments (Table 6). Mean levels of NDF, ADF, and hemicellulose were lower ($P < 0·05$) for control cows compared with DSS-dosed cows. Decreases ($P < 0·05$) in NDF, ADF, and hemicellulose concentrations in the whole rumen contents were observed up to 6 h after feeding (values not shown).

Mean pH over sampling time was not affected by DSS dosing; mean NH_3-N concentrations were higher ($P < 0·05$) for control cows than for DSS-dosed cows (Table 6).

Table 4. *Rumen dry matter digestibility* in situ *for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows**

Incubation period (h)	Treatment group...	Grass silage C	Grass silage D	Grass silage SE (5 df)	Grass silage Statistical significance of difference: $P <$	Concentrate C	Concentrate D	Concentrate SE (5 df)	Concentrate Statistical significance of difference: $P <$
1		—	—	—	—	0·327	0·330	0·0030	0·49
2		0·249	0·242	0·0054	0·40	0·333	0·331	0·0048	0·81
4		0·253	0·255	0·0040	0·81	0·380	0·362	0·0060	0·10
6		0·276	0·283	0·0041	0·33	—	—	—	—
8		—	—	—	—	0·503	0·471	0·0223	0·37
12		0·376	0·384	0·0063	0·44	0·583	0·578	0·0187	0·86
24		0·529	0·507	0·0122	0·27	—	—	—	—
48		0·674	0·628	0·0137	0·07	—	—	—	—
72		0·752	0·697	0·0324	0·29	—	—	—	—

* For details of procedures, see p. 399.

Table 5. *Rumen digesta kinetics for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows**

Treatment group...	C	D	SE (5 df)	Statistical significance of difference: $P <$
Fluid volume (l)	62·5	56·1	6·22	0·53
Fluid dilution (/h)	0·160	0·155	0·108	0·77
Fluid outflow (l/h)	9·5	8·1	0·61	0·19
Solids turnover (/h)	0·046	0·042	0·001	0·07
Solids retention time (h)	21·7	24·0	0·67	0·08

* For details of procedures, see p. 400.

Concentrations of NH_3-N (176, 342 and 248 mg/l for control cows and 163, 202 and 157 mg/l for DSS-dosed cows at 07.30, 10.30 and 13.30 hours respectively) varied with time ($P < 0·05$) after the 07.30 hours feeding, with highest concentrations occurring at 3 h post feeding for both treatments. There was a treatment × sampling time interaction effect ($P < 0·05$) for NH_3-N. Concentrations of NH_3-N were higher for control cows than for DSS-dosed cows at 3 and 6 h post feeding; however, the concentrations did not differ between the two treatments just before feeding.

Mean total VFA concentrations were not affected by DSS dosing but there were marked changes in molar proportions of individual VFA (Table 6). Proportions of acetate and butyrate were higher ($P < 0·05$), but those of propionate, isovalerate, and valerate were lower ($P < 0·10$) for control cows compared with DSS-dosed cows. The mean acetate:propionate values (not shown) were reduced ($P < 0·10$) by DSS dosing.

Apparent whole tract nutrient digestibility

Cows digested less ($P < 0·10$) diethyl ether extract and cellulose in the whole digestive tract with DSS dosing (Table 7). There was a tendency for lower ($P = 0·10$) hemicellulose digestibilities for DSS-dosed cows than for control cows. Apparent digestibilities of DM,

Table 6. *Composition (g/kg dry matter) of whole rumen contents and rumen pH, NH_3-N (mg/l), total volatile fatty acid (VFA) concentration (mmol/l) and proportions of individual VFA (mmol/mol) for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows*

Treatment group...	C	D	SE (5 df)	Statistical significance of difference: $P <$
Whole rumen contents				
Organic matter	907	913	02·7	0·62
Lipid	64	64	01·0	0·85
NDF	465	529	05·6	0·03
ADF	281	316	02·5	0·05
Hemicellulose	185	213	03·6	0·05
Rumen fluid				
pH	6·15	6·22	0·080	0·38
NH_3-N	256	174	13·0	0·03
Total VFA	81·5	80·1	2·93	0·65
Acetate	664	624	2·5	0·05
Propionate	174	236	5·7	0·07
Isobutyrate	10·0	10·1	0·53	0·52
Butyrate	124	96·3	3·58	0·04
Isovalerate	15·7	18·2	0·68	0·07
Valerate	12·4	15·6	0·46	0·10

NDF, neutral-detergent fibre; ADF, acid-detergent fibre.

Table 7. *Faecal measurements and apparent whole tract nutrient digestibility for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows**

Treatment group...	C	D	SE (5 df)	Statistical significance of difference: $P <$
Faeces				
pH	6·44	6·31	0·051	0·16
DM (g/kg)	148	156	1·9	0·05
DM output (kg/d)	7·2	7·1	0·37	0·96
Digestibility				
DM	0·628	0·605	0·0154	0·38
Organic matter	0·637	0·614	0·0152	0·35
Diethyl ether extract	0·600	0·516	0·0237	0·07
Crude protein (N × 6·25)	0·615	0·635	0·0141	0·38
Cell solubles	0·672	0·682	0·0109	0·56
NDF	0·546	0·502	0·0172	0·15
ADF	0·480	0·367	0·0427	0·14
Hemicellulose	0·626	0·554	0·0246	0·11
Cellulose	0·595	0·489	0·0345	0·10
TNC	0·749	0·762	0·0095	0·39
Gross energy	0·600	0·571	0·0156	0·27

DM, dry matter; NDF, neutral-detergent fibre; ADF, acid-detergent fibre; TNC, total non-structural carbohydrate.

* For details of procedures, see p. 399.

Table 8. *Blood measurements for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows**

Treatment group...	C	D	SE (5 df)	Statistical significance of difference: $P <$
Whole blood				
Packed cell volume	0·306	0·324	0·0068	0·13
Acetoacetate (mg/l)	8·3	6·6	0·30	0·03
Plasma				
Glucose (mg/l)	639	748	36·4	0·02
Urea N (mg/l)	230	227	6·2	0·76
Total lipids (mg/l)	5135	5423	376	0·64
Triacylglycerols (mg/l)	446	424	8·4	0·16
Phospholipids (mg/l)	2998	2800	130	0·36
NEFA (μmol/l)	144·1	140·3	6·93	0·77
Insulin (mU/l)	14·5	16·0	0·62	0·12

NEFA, non-esterified free fatty acids.
* For details of procedures, see p. 399.

Table 9. *Milk yield and composition for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows**

Treatment group...	C	D	SE (5 df)	Statistical significance of difference: $P <$
Yield				
Milk (kg/d)	27·7	24·6	0·62	0·04
4% Fat-correct milk (kg/d)	23·6	20·8	0·90	0·10
Solids (g/d)	3220	2860	90	0·06
Fat (g/d)	840	730	50	0·21
Protein (g/d)	802	770	20	0·16
Ash (g/d)	180	160	6	0·05
Lactose (g/d)	1370	1170	40	0·03
Efficiency†	1·22	1·19	0·05	0·72
Composition (g/kg)				
Solids	117	116	1·6	0·77
Fat	30·9	30·3	1·4	0·81
Protein	30·0	31·3	0·3	0·04
Ash	6·6	6·3	0·2	0·41
Lactose	49·8	47·4	0·8	0·10

* For details of procedures, see p. 400.
† 4% Fat-corrected milk yield/dry matter intake.

OM, CP, total non-structural carbohydrate, cell solubles and energy were not significantly affected by DSS dosing. Faecal pH and daily output were similar between treatments while DM content of faeces was lower ($P < 0·05$) for control cows compared with DSS-dosed cows.

Blood metabolite measurements

Selected blood metabolite measurements are shown in Table 8. Blood packed cell volume values tended to be lower in control cows compared with DSS-dosed cows. Whole blood

Table 10. *Milk fatty acid composition (g/kg) for control (C) and dioctyl sodium sulphosuccinate-dosed (D) cows**

Fatty acid	Treatment group... C	D	SE (5 df)	Statistical significance of difference: $P <$
Short-chain				
4:0	56	47	2·4	0·06
6:0	26	17	2·3	0·06
8:0	15	10	0·8	0·03
10:0	35	29	2·9	0·23
11:0	4	3	0·3	0·03
12:0	41	37	2·0	0·22
13:0	3	2	0·6	0·36
Total	181	146	8·7	0·05
Medium-chain				
14:0	132	126	6·4	0·54
14:1	20	24	1·3	0·07
15:0	13	17	1·1	0·07
16:0	324	350	16·9	0·34
16:1	29	45	4·4	0·07
17:0	7	8	0·5	0·11
Total	525	571	23·3	0·24
Long-chain				
18:0	89	60	8·9	0·09
18:1	176	202	14·9	0·30
18:2	20	17	1·0	0·10
18:3	7	7	0·3	0·67
20:0	3	1	0·6	0·13
Total	294	287	23·2	0·84
Saturated	749	708	14·2	0·11
Unsaturated	252	296	14·2	0·10
Even-no.	974	974	2·8	0·95
Odd-no.	27	30	1·0	0·15

* For details of procedures, see p. 400.

acetoacetate concentrations ($P < 0·03$) were higher while plasma glucose ($P < 0·02$) levels were lower in control cows compared with DSS-dosed cows. Plasma urea-N, total lipid, triacylglycerol, phospholipids, NEFA, and insulin concentrations were not different.

Milk yield and composition

Daily yield of milk ($P < 0·04$) and 4% fat-corrected milk ($P < 0·10$) were higher in control cows compared with DSS-dosed cows (Table 9). All milk component yields were numerically higher in control cows with significant differences ($P < 0·10$) detected in yield of milk total solids, ash, and lactose. Milk yield efficiency and total solids, fat, and ash contents in milk were similar. Milk protein content was higher ($P < 0·04$) for DSS-dosed cows, whereas milk lactose content was greater ($P < 0·10$) for control cows than that for DSS-dosed cows.

Dosing of DSS had a significant effect ($P < 0·10$) on the proportions of many individual fatty acids of milk fat (Table 10). When pooling values by the chain length (C numbers) of fatty acids, milk fat of control cows contained a greater ($P < 0·05$) proportion of short-chain fatty acids and a smaller ($P < 0·10$) proportion of unsaturated fatty acids than that of DSS-dosed cows.

DISCUSSION

Animals

In the present study we observed that cows reduced DM intake by 50% for 2–4 d after receiving the initial dose of DSS. Thereafter, when cows were maintained on the reduced continuous ruminal slow-release dose of DSS, feed intakes increased to 90–95% of pretreatment intakes. During the course of the experiment DSS-dosed cows did not have any health problems.

Rumen metabolism

Previous studies (Orpin, 1977; Eadie & Shand, 1981; Nocek *et al.* 1988) showed that surfactants such as DSS can have a negative effect on bacterial populations in the rumen. This may be a contributing factor to the observed depression in digestibility of forages by non-ciliate rumen fluid, which contained mainly bacteria, isolated from DSS-dosed cows (Table 3). Another surfactant, Tween 80, has been used to dislodge rumen bacteria from particulate matter (Minoto & Suto, 1978). Because DSS has been shown to be associated with particulate matter rather than with the aqueous phase (Wright & Curtis, 1976; Orpin, 1977), continuous dosing of DSS in the present study might have reduced attachment of non-ciliate rumen micro-organisms to particulate matter and subsequently decreased substrate digestion in the rumen (Table 4). Reduced rumen fibre digestion in DSS-dosed cows is also suggested by the higher concentrations of NDF, ADF, and hemicellulose in whole rumen contents (Table 6) and a reduced rumen solids digesta turnover rate (Table 5). Rumen NH_3-N concentration was decreased by DSS dosing after feeding but not during the period immediately before feeding. The difference in postprandial changes in rumen ammonia might have been due to a decreased degradation of dietary protein, greater incorporation by bacteria into microbial protein or reduction in protozoal turnover of bacterial protein in DSS-dosed compared with control cows.

Apparent whole tract nutrient digestibility

The reduced whole tract cellulose digestibility in the present study during DSS dosing (Table 7) may be attributed to decreased cellulolytic activity of non-ciliate rumen fluid observed *in vitro* (Table 3). A trend for lower whole tract NDF, ADF, and hemicellulose digestibilities was also evident during dosing with DSS (Table 7). However, DM digestibility in DSS-dosed cows was not reduced to the extent observed for apparent fibre digestibility. This might have been a result of numerically higher CP, cell solubles, and total non-structural carbohydrate digestibilities for DSS-dosed cows. It appears that slight increases in the digestibility of some non-fibre components in the dietary DM might have compensated for the decreased fibre digestibility during DSS dosing. As a result, whole tract DM digestibility did not differ significantly between treatments.

Blood metabolites

Higher blood acetoacetate concentration in control cows (Table 8) could be a result of higher proportions of butyrate in the rumen (Table 6). Acetoacetate is a major intermediate during butyrate oxidation in the tissue (Kronfeld *et al.* 1968). Increased blood acetoacetate concentrations in control cows did not appear to be associated with body fat mobilization since plasma NEFA concentration (Table 8) was not different between treatments. Increased plasma glucose concentrations could be attributed to elevated rumen propionate levels for cows dosed with DSS (Table 6). In ruminants a significant proportion of plasma glucose is produced by hepatic glucogenesis from propionate (Wiltrout & Satter, 1972). Lower rumen NH_3 concentrations (Table 6) resulting from DSS dosing might have reduced

the absorption of NH_3 across the rumen wall. However, plasma urea-N concentrations (Table 8) were not different between control and DSS-dosed cows. Although DSS dosing decreased apparent whole tract lipid digestibility (Table 7), some plasma lipid metabolites did not change (Table 8).

Lactational responses

Lower milk yield (Table 9) in the present study for DSS-dosed cows compared with control cows was probably the result of slightly lower feed intake (Table 2), since milk yield efficiency did not differ (Table 9). Although feed intake was regulated in the present study, values for milk composition for control and DSS-dosed cows are typical for the Holstein breed (Jenness, 1985). Concentration of milk lactose, the main osmotically active constituent in milk that determines the volume of milk secreted (Larson, 1985), was lower for DSS-dosed cows. This coincides with the lower milk yield for DSS-dosed cows in comparison with control cows. Dosing of DSS did not influence milk fat content, although the proportion of rumen butyrate, acetate:propionate (Table 6), and whole tract lipid digestibility (Table 7) were decreased in DSS-dosed cows.

Most milk short-chain fatty acids are synthesized *de novo* in the mammary gland with β-hydroxybutyrate as a precursor (Moore & Christie, 1981). The increased proportions of short-chain fatty acids (Table 10) in milk fat of control cows could be an outcome of higher rumen butyrate (Table 6). Increased proportions of 15:0 and 17:0 odd-numbered-C fatty acids for DSS-dosed cows could be related to the increased propionate concentration in the rumen (Table 6). This suggests an increase in *de novo* synthesis of fatty acids containing odd-numbered C atoms from propionate in the mammary gland. Odd-numbered-C fatty acids can be synthesized in the mammary gland by addition of propionic acid to even-numbered C fatty acid precursors (Moore & Christie, 1981). Dosing of DSS decreased proportions of 14:1 and 16:1 fatty acids but increased proportions of 18:2 fatty acid. This implies that DSS might have affected rumen hydrogenation of mono- and polyunsaturated fatty acids through different mechanisms. But, overall, dosing of DSS resulted in milk fat with a smaller extent of saturation of fatty acids.

Conclusions

Continuous ruminal slow-release of DSS (0·07 g/kg body-weight per d) maintained rumens ciliate-free for 4 weeks in lactating Holstein cows and had a significant effect on rumen metabolism. The primary influence of DSS appeared to be a decrease in rumen fibre digestion. This influence was reflected in altered rumen fermentation patterns and digesta kinetics, apparent whole tract nutrient digestibilities, blood metabolites, and milk composition.

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