EFFECTS OF SLAFRAMINE ON RUMINANT DIGESTIVE FUNCTION:
LIQUID TURNOVER RATE AND FERMENTATION PATTERNS
IN SHEEP AND CATTLE

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ABSTRACT

Two trials were initiated to determine if slaframine (SF) can be used to alter fluid digesta flow
and fermentation patterns in the rumen. In trial 1, a preliminary experiment, four Dorset X
Barbados Black-belly ruminal-cannulated wethers (avg weight 41.6 ± 8.7 kg) given ad libitum
access to a pelleted concentrate/hay diet were injected intramuscularly with 0, 12, 24 or 48 μg
SF/kg body weight (BW) in a 4 × 4 Latin-square design. Ruminal fluid dilution rate was de-
termined using a single intraruminal infusion of polyethylene glycol (7 g), followed by seven
hourly ruminal fluid samples. The administration of 48 μg SF/kg BW increased (P<.10) ruminal
volume and outflow by 27 and 25%, respectively, compared with controls. In trial 2, two Hereford
and two Angus ruminal cannulated steers (avg weight 568 ± 93 kg) were injected with 0, 6, 12
or 24 μg SF/kg BW at 8-h intervals over a 24-h period in a 4 × 4 Latin-square design. Steers were
fed a concentrate diet at twice maintenance in 24 equal portions daily. Ruminal fluid dilution was
measured using a single intraruminal infusion of cobalt-ethylenediamine tetraacetic acid (20 g)
administered 9 h after the initial SF injection. Ruminal fluid was collected each hour during 8 to
24 h after the initial SF injection and analyzed for pH, osmolality and volatile fatty acids (VFA).
For the 24 μg SF/kg BW treatment, ruminal fluid dilution rate (P<.16) and outflow (P<.04) were
26% greater, ruminal pH was .33 units higher (P<.05) and ruminal propionate concentration and
molar proportion (mol/100 mol total VFA) were 36 and 26% lower (P<.05) than the control
treatment. Saliva was estimated to contribute 50% more (P<.05) liquid to the ruminal fluid phase
for the 24 μg/kg BW treatment than the control treatment. These studies demonstrate the poten-
tial for altering the physiological processes controlling ruminal environment and its dependent
microbial fermentation using an exogenously administered sialagogue.

(Key Words: Parasympathomimetics, Salivary Secretions, Rumen Fluid, Dilution, Rumen Fer-
mentation.)

Introduction

Ruminal microbial fermentation is main-
tained within a tightly regulated environment
controlled by the host animal. Ruminal liquid
dilution rate exerts a major influence
upon the type and extent of fermentation
and is largely a function of both salivation
(Poutianen, 1968; Bartley, 1976) and ruminal
motility (Wyburn, 1980; Sissons et al., 1984).
All of these processes are highly related to
the physical form and nutrient density of
the feed consumed (Bull et al., 1979). Greater
rates of salivation, ruminal motility and liquid
flow have been associated with the ingestion
and rumination of coarse fibrous feeds, whereas
chronic reductions in these characteristics
result during the feeding of restricted roughage

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diets; this may lead to metabolic disorders and suboptimal feed utilization (Svendson, 1975; Dougherty, 1976; Kromann, 1976; Kronfeld, 1976). Supplementing with mineral salt buffers is sometimes effective at minimizing the negative effects of restricted roughage diets upon animal performance (Emerick, 1976; Rogers et al., 1985). Another approach, yet to be tested, is to stimulate digestive processes at the physiological control point by pharmacological administration of neurotransmitter-like substances.

In relatively short-term experiments, slaframine (SF), a parasympathomimetic secretagogue, has been found to stimulate salivary secretion without reducing feed consumption of cattle fed hourly at twice their maintenance requirement for digestible energy (Froetschel et al., 1986b). It may be possible to alter ruminal function by stimulating salivation or other digestive processes with SF or related pharmacological agents. Furthermore, the use of SF to experimentally manipulate digestive characteristics such as salivation would result in a better understanding of physiological control of ruminal digesta flow and its influence upon the nutrition of the animal. The objective of the present trials was to determine the effects of various levels of SF upon ruminal liquid digesta flow and fermentation in sheep and cattle fed concentrate diets.

Materials and Methods

**Trial 1.** The effects of SF/kg body weight (BW) on the flow of ruminal liquid digesta were investigated using four ruminal-cannulated Dorset x Barbados Black-belly wethers (avg BW 43.6 ± 8.7 kg) in a 4 × 4 Latin-square design. Wethers were given ad libitum access to a commercial pelleted diet and a mixture of long-stemmed alfalfa and grass hay. At weekly intervals, wethers were moved to individual metabolism crates, denied access to feed and water, SF was administered and ruminal liquid dilution rate was estimated.

Slaframine was prepared from cultures of Rhizoctonia leguminicola and administered with saline intramuscularly in the region of the biceps femoris as stated previously (Froetschel et al., 1986b). Ruminal liquid dilution was estimated from the dilution of polyethylene glycol (PEG) in the rumen. Immediately following SF injection, 7 g of PEG in 100 ml of water were infused into the rumen. Ruminal fluid samples were aspirated under low vacuum pressure from several sites at hourly intervals for the next 7 h and stored at 0°C until analyzed. Ruminal fluid was filtered through a 1-mm mesh during sampling. Concentration of PEG in clarified ruminal fluid was determined according to Bauman et al. (1971). Ruminal fluid volume and dilution rate were calculated using the slope and intercept of the best-fit line generated with a non-linear iterative procedure (SAS, 1982), regressing the exponential decay of PEG concentration against time after dosing. Ruminal fluid outflow was estimated as a product of ruminal volume and fluid dilution rate (Poutianen, 1968). Overall statistical differences were determined among means of ruminal fluid measurements by analysis of variance using the General Linear Model procedure of the Statistical Analysis System (SAS, 1982). Differences between each treatment and the control least-squares mean were assessed using non-orthogonal single-degree-of-freedom contrasts when significant F-values (P<.05) due to treatment were observed.

**Trial 2.** Two Angus and two Hereford steers averaging 568 ± 93 kg were used to study the effects of repeated administration of SF on ruminal measurements in cattle. Steers were allowed free access to water and fed a concentrate diet (table 1) in 24 equal portions daily, at hourly intervals (Croom et al., 1982), at twice their net energy requirement for maintenance, along with 1 kg of chopped switchgrass hay daily. The energy content of the experimental diet was estimated to contain 2.07 Mcal of net energy for maintenance per kilogram of dry matter. Steers were allowed to adjust to this diet 2 wk before experimentation. Once per week steers were injected intramuscularly with either saline (control), 6, 12 or 24 μg SF/kg BW in a 4 × 4 Latin-square design at 8-h intervals during a 24-h experimental period as in a previous experiment (Froetschel et al.,

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9 Anderson-8702, Maumee, OH.
10 Carbowax 3350, Fisher Scientific, Pittsburgh, PA.
TABLE 1. SELECTED COMPONENTS OF EXPERIMENTAL DIET a

<table>
<thead>
<tr>
<th>Dietary ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>76</td>
</tr>
<tr>
<td>Cottonseed hulls</td>
<td>15</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>8.5</td>
</tr>
<tr>
<td>Trace mineral salt b</td>
<td>.5</td>
</tr>
</tbody>
</table>

 a Feed composition expressed on a dry matter basis.

 b Trace mineralized salt contained a minimum of: NaCl, 96.5%; Zn, .75%; Fe, .35%; Mn, .25%; Cu, .05%; Co, .01%; I, .007%.

1986b). Feeding and access to water were maintained throughout experimental periods. Steers were initially dosed with SF at 2400 and again at 0800 and 1600 on the following day of an experimental period. At 0900, steers were dosed intraruminally with 300 ml of a 67 g/liter solution of cobalt-ethylene-diamine tetraacetic acid (Co-EDTA) as a water soluble marker (Uden et al., 1980). Hourly samples of ruminal fluid were collected as in trial 1 during the last 16 h of each experimental period. The cobalt concentrations of the Co-EDTA solution infused and the ruminal fluid samples collected after infusion were determined with atomic absorption spectrophotometry. Ruminal fluid dilution rate, volume and outflow were calculated with the same regression procedures used to describe PEG dilution in sheep (trial 1). Salivary flow rate was estimated according to Poutianen (1968) by subtracting ruminal outflow from water intake, the latter measured with a flow meter in line to each cattle waterer. Along with digesta marker analysis, ruminal fluid sampled hourly during the last 16 h of the experimental periods was analyzed for volatile fatty acids (VFA), pH and osmolality. At 2400, ruminal digesta was removed from steers and subsequently weighed, mixed and sampled for dry matter analysis and replaced. A 6-d separation was allowed between each treatment period.

Statistical analyses in trial 2 were conducted in a manner similar to that described in trial 1 with the exception of VFA, pH and osmolality data. These data were analyzed as a Latin-square split-plot in time using the General Linear Model procedure of the Statistical Analysis System (SAS, 1982). Animal, period and treatment were independent variables and the animal x period x treatment nested interaction was used as the error term to test these main effects. The sub-plot consisted of a time x treatment interaction term. Differences between each treatment and the control least-square mean were assessed using non-orthogonal single-degree-of-freedom contrasts when significant F-values (P<.05) for treatment were observed.

Results and Discussion

The effects of SF upon ruminal fluid measurements in wethers as estimated from the dilution of intraruminal PEG are depicted in figure 1 and table 2. Even though the ruminal fluid dilution rate in wethers was not affected by SF treatment, both volume and outflow tended to increase, with the 48-μg SF treatment 27% and 25% greater than control measurements, respectively (P<.10). Differences in ruminal outflow reflect changes in ruminal volume because outflow is a product of both ruminal volume and fractional dilution rates. Increased salivation due to a single dose of SF may not be sufficient to cause an immediate change in ruminal fluid dilution. The observed increases in ruminal volume (table 2) may preface long-term changes in ruminal fluid dilution rate.

Changes in ruminal fluid dilution, attributable to SF, as measured by Co-EDTA dilution, were more marked in trial 2 (figure 2 and table 3). Although not significant, the ruminal fluid dilution rate of the 24-μg SF treatment was 26% greater than that of the control treatment. Ruminal fluid volume was relatively consistent across SF treatments, but ruminal fluid outflow for the 24-μg SF treatment was 26% greater than that of the controls (P<.05). In contrast to trial 1, changes in ruminal fluid outflow in cattle were primarily a consequence of increased ruminal dilution rate. Differences in the magnitude of response between trial 1 and trial 2 are probably not attributable to different levels of SF administration (mg/kg BW) because these levels were equal when expressed on a metabolic body size basis. Instead, the duration of SF administration to steers may have allowed more
2.5 mr- /~uZfl.Cl of primary ruminal contractions found with laframine/kg BW occurred in spite of the decreased frequency of primary ruminal contractions found with this level of SF administration (Froetschel et al., 1986a).

Differences attributable to the effects of SF upon ruminal liquid volumes and contents in steers measured directly after completing each experimental period (2400) were in agreement with measurements estimated from Co-EDTA dilution (tables 3 and 4). Total ruminal contents tended to be greater (P<.12) for the 12- and 24-μg SF treatments than for the control treatment (table 4). A trend for increased ruminal content weight (P<.12) with the 24-μg SF treatment may have resulted from tendencies for both ruminal dry matter and water to be greater.

Salivary contributions to ruminal fluid dilution were estimated from differences between ruminal fluid outflow and water intake (table 3), assuming negligible net ruminal transepithelial flux (Poutianen, 1968; von Engelhardt, 1970). Based upon these estimates, ruminal salivary influx for the 24-μg SF treatment was calculated to be 46% greater than for the control treatment (P<.05). No significant differences were noted with lower levels of SF administration. Previous research with esophageal cannulated steers have shown 12 μg SF/kg BW to be effective in stimulating salivatory flow (Froetschel et al., 1986b), 30 to 50% above controls. The difference between estimates of saliva entering the rumen from dilution rate of Co-EDTA and direct measurements of salivation from esophageal fistulated steers, measured previously (Froetschel et al., 1986b), may reflect the inadequacies of saliva flow estimation by the water difference method (Woodford et al., 1984) and suggests that

time for the ruminal environment to adjust to the effects of SF. Increased ruminal dilution and outflow observed in both sheep and cattle is probably due to a greater influx of saliva (Froestchel et al., 1986b), and occurred in spite of the decreased frequency of primary ruminal contractions found with this level of SF administration (Froetschel et al., 1986a).

TABLE 2. THE EFFECTS OF VARIOUS LEVELS OF SLAFRAMINE (SF) ADMINISTRATION UPON RUMINAL LIQUID MEASUREMENTS IN WETERS

<table>
<thead>
<tr>
<th>Measurement</th>
<th>SF, μg/kg body wt</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>12</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Dilution rate, %/h</td>
<td>17.4</td>
<td>17.8</td>
<td>17.3</td>
<td>16.4</td>
</tr>
<tr>
<td>Ruminal volume, liters</td>
<td>3.25</td>
<td>3.07</td>
<td>3.11</td>
<td>4.13</td>
</tr>
<tr>
<td>Ruminal outflow, liters/h</td>
<td>.56</td>
<td>.58</td>
<td>.50</td>
<td>.70</td>
</tr>
</tbody>
</table>

a Values are least-squares means calculated from four observations.

b Standard error of the mean.
Table 3. Effects of Various Levels of Slaframine (SF) Administration Upon Ruminal Liquid Measurements in Steers

<table>
<thead>
<tr>
<th>Measurement</th>
<th>SF, µg/kg body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Dilution rate, %/h</td>
<td>7.7</td>
</tr>
<tr>
<td>Ruminal volume, liters</td>
<td>49.8</td>
</tr>
<tr>
<td>Ruminal outflow, liters/h</td>
<td>3.8</td>
</tr>
<tr>
<td>Water intake, liters/16 h</td>
<td>32.5</td>
</tr>
<tr>
<td>Saliva, liters/h</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Values are least-squares means calculated from four observations.

Standard error of the mean.

Single-degree-of-freedom comparison different from control (P<.05) with upper level of significance for F-test across means at P<.05.

Estimated from the difference between ruminal outflow and water intake.

Figure 2. The exponential decay of ruminal cobalt-ethylenediamine tetraacetic acid (Co-EDTA) concentration plotted against time from steers treated with 0, 6, 12 or 24 µg slaframine (SF)/kg body weight (BW). Each data point is a mean of four steers.
TABLE 4. EFFECTS OF SLAFRAMINE ADMINISTRATION UPON RUMINAL DIGESTA OF STEERS a

<table>
<thead>
<tr>
<th>Measurement</th>
<th>SF, µg/kg body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Contents, kg</td>
<td>55.0</td>
</tr>
<tr>
<td>Dry matter, %</td>
<td>15.6</td>
</tr>
<tr>
<td>Ruminal water, kg</td>
<td>46.4</td>
</tr>
<tr>
<td>Ruminal dry matter, kg</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Values are least-squares means calculated from four observations. Standard error of the mean.

A substantial portion (40 to 70%) of saliva may be entering the reticulum and passing directly through the nearby reticul-omasal orifice and not equilibrating with the ruminal fluid phase. The contribution of saliva to the ruminal fluid phase has been demonstrated to decrease when the proportion of dietary concentrates increase (Poutianen, 1968). Differences in physical form and hydration capacity between roughages and concentrates are likely responsible for this phenomena.

In the present study, SF increases ruminal liquid dilution at dosages that decreased ruminal motility in previous studies (Froetschel, 1986a). In both wethers and steers, increases in ruminal outflow were observed with SF.

TABLE 5. EFFECTS OF SLAFRAMINE FROM RUMINAL pH, OSMOLALITY AND VOLATILE FATTY ACIDS (VFA) IN STEERS a

<table>
<thead>
<tr>
<th>Measurement</th>
<th>SF, µg/kg body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>6.26</td>
</tr>
<tr>
<td>Osmolality, milliosmoles/kg</td>
<td>268</td>
</tr>
<tr>
<td>VFA, mM</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>57.4</td>
</tr>
<tr>
<td>Propionate</td>
<td>21.3</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>1.6</td>
</tr>
<tr>
<td>Butyrate</td>
<td>13.1</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>3.2</td>
</tr>
<tr>
<td>Valerate</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>98.0</td>
</tr>
<tr>
<td>VFA, mol/100 mol</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>57.9</td>
</tr>
<tr>
<td>Propionate</td>
<td>22.4</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>1.6</td>
</tr>
<tr>
<td>Butyrate</td>
<td>13.5</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>3.2</td>
</tr>
<tr>
<td>Valerate</td>
<td>1.4</td>
</tr>
<tr>
<td>A b d ratio</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Least-squares means calculated from 64 observations. Standard error of the mean. Single-degree-of-freedom comparison different from control (P<.05) with upper level of significance for F-test across treatment means at P<.05. Ratio of molar proportions of acetate to propionate.
levels that have been found to decrease frequency of primary ruminal contractions (Froetschel et al., 1986a). It is possible that decreased ruminal motility increases the proportion of ruminal liquid contributed by saliva and may enhance dilution rate. In addition, SF may cause relaxation of the reticulo-omasal orifice, which is under vagal control (Ruckebusch, 1983).

Slaframine administration resulted in changes in both ruminal pH and the pattern of VFA (table 5). Ruminal pH for the 12- and 24-μg SF treatments was .07 and .33 units greater than the control treatment, respectively (P<.05). The concentration of total ruminal VFA tended to be (P<.15) lower for the 24-μg SF treatment, and is consistent with the changes in ruminal pH. Additionally, greater ruminal buffering capacity as a result of increased saliva entering the rumen may contribute partly to changes observed in ruminal pH. Slaframine administration did not affect ruminal osmolality (table 5).

Of the individual ruminal VFA, propionate was most affected by SF administration. The ruminal concentration of propionate for the 6- and 24-μg SF treatments was 16 and 36% less than for the control treatment; whereas, its concentration for the 12-μg SF treatment was 19.7% greater (P<.05). Accordingly, the molar proportions (mol/100 mol total VFA) of propionate for the 6- and 24-μg SF treatments were 19.6 and 25.8% less than the control, while for the 12-μg SF treatment the molar proportion of propionate was 17% greater (P<.04). A negative correlation between liquid dilution rate and the molar proportion of propionic acid in the rumen is well documented (Hodgson et al., 1975; Thomson et al., 1975; Owens and Isaacs, 1977). As a result of the relative changes in both molar proportions of acetate and propionate, the acetate to propionate ratio for the 24-μg SF treatment tended to increase; although the trend was not significant. Isovalerate concentration was increased 28 to 75% in the ruminal fluid for the 6- and 24-μg SF treatments and its molar proportion increased 53 to 66% (P<.05).

In the present trial, the changes in VFA patterns associated with SF administration reflect unstable modifications in bacterial populations or their metabolism as a result of alterations in the ruminal environment. The changes observed in fermentation patterns in this experiment are likely due to the intense selection pressures exerted upon the ruminal microflora by the changes in ruminal environment due to SF treatment. Longer-term investigations with SF are needed to describe changes in fermentation patterns representing a stable microbial population.

This study is the first to demonstrate the capability of pharmacologically controlling the ruminal environment and its dependent microbial fermentation by administration of a neurotransmitter-like substance. In this and previous studies, SF administration resulted in changes in salivation, ruminal motility and liquid outflow without affecting feed intake. As a result of these changes, ruminal fermentation patterns in steers were altered within a 24-h period, even though nutritional inputs were constant. This research indicates that SF and related compounds may be valuable tools in assessing the relative importance of saliva and motility in maintaining the ruminal environment. Of special interest is the potential use of these substances to alter ruminal fermentation in such a manner as to influence beneficiary feed utilization, growth and lactation.

Literature Cited


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