INFLUENCE OF RUMINAL OR DUODENAL SOYBEAN OIL INFUSION ON INTAKE, RUMINAL FERMENTATION, SITE AND EXTENT OF DIGESTION, AND MICROBIAL PROTEIN SYNTHESIS IN BEEF HEIFERS CONSUMING GRASS HAY

L. J. Krysl, M. B. Judkins and V. R. Bohman

University of Nevada, Reno 89557-0104

ABSTRACT

Six heifers (two Hereford × Jersey, four Hereford × Longhorn; average BW 278 kg) cannulated at the rumen and duodenum and fed a grass hay (fescue/orchardgrass) diet were used in a replicated 3 × 3 Latin square. Treatments were either no infusion (C), 150 ml of duodenally infused soybean oil (DI), or 150 ml of ruminally infused soybean oil (RI)/heifer twice daily for a total daily infusion of 300 ml of soybean oil. Periods of the Latin square included 18 d for adaptation and 5 d for collection. Forage OM, ADF, NDF, and N intakes were not affected (P > .10) by soybean oil infusion. Ruminal (P = .11) and total tract (P < .10) OM digestibilities were decreased by RI compared with C or DI, but ADF and NDF digestibilities were not affected by treatment. Duodenal N (P < .05) and microbial N flows were increased (P < .10) for C and RI compared with DI. Microbial efficiency (g of N/kg of OM truly fermented) was improved (P < .10) by RI compared with DI but did not differ (P > .10) from C. Ruminal pH was lower (P < .05) with RI than with either C or DI. Ruminal NH3 N, total VFA, and acetate were not affected (P > .10) by treatment. Propionate (mol/100 mol) was greater (P < .05) with RI than with DI and C, but the proportion of butyrate did not differ among treatments. These data indicate minimal direct benefits for improving forage usage as a result of soybean oil infusion with a 100% grass diet; however, animals should realize benefits from additional dietary energy provided by infused lipid.

Key Words: Beef Cattle, Dietary Fat, Rumen Digestion, Microbial Proteins, Hay


Introduction

Ruminal fermentation and fiber digestion are often decreased by the addition of fats or oils to the diet (Czerkawski, 1973; Kowalczyk et al., 1977; Jenkins, 1987; Jenkins et al., 1989; Jenkins and Fotouhi, 1990); however, the degree of inhibition varies with amount and type (unsaturated vs saturated) of supplemental lipid. Considerable research with fat or oil additions to sheep diets has been conducted, but few reports (Erwin et al., 1956; Moore et al. 1986) are available concerning effects of fats or oils on beef cattle consuming high-roughage diets. Limited research indicates that 4 to 7% added fat or oil decreases intake and digestibility of high-roughage diets (Jenkins and Pahquist 1984; Moore et al. 1986). Supplementing low-quality roughage with energy supplements would be preferable to feeding protein meals (Adams, 1986). We examined the influence of ruminal or duodenal infusion of soybean oil on OM intake, ruminal fermentation, NDF digestion, and duodenal nutrient flows in beef heifers consuming grass hay.

Experimental Procedure

Sample Collection Periods. Six ruminally and duodenally (T-type) cannulated heifers (two Hereford × Jersey, four Hereford ×
Longhorn, 2 yr old, average BW 278 kg) were used in a replicated 3 x 3 Latin square to study effects of either no supplement (C), 150 ml of duodenally infused soybean oil2 (D1), or 150 ml of ruminally infused soybean oil (RI)/heifer twice daily (every day of study) for a total daily infusion of 300 ml of soybean oil. These infusions supplied 1.8 Mcal of ME (NRC, 1984) to the animals daily. Each period of the Latin square included 18-d adaptation and 5-d collection phases. Heifers were housed in 2-m x 2.5-m tie stalls (covered barn) with free access to water and trace mineralized salt.3 Surgical procedures were approved by the University Animal Care Committee, and animal care followed procedures outlined by the Consortium (1988).

Heifers were fed chopped grass hay free choice (130% of voluntary intake, adjusted daily). Hay was a mixture of fescue/orchardgrass (approximately 80%;20%; maximal particle length = 2.5 cm; DM = 92.1%; ash = 12.2%; NDF = 56.5%; ADF = 35.4%; ADL = 2.8%; N = 1.37%) fed twice daily (0700 and 1900). Daily DMI was measured directly by weighing hay offered each morning and evening and weighing and discarding orts before the next feeding. Heifers were supplemented (0700 and 1900) via ruminal or duodenal cannula with 150 ml of soybean oil. Ruminal infusion was accomplished by direct administration of soybean oil through a syringe, and duodenal infusion was accomplished with an i.v. drip line set to infuse approximately 3 ml/min.

On d 1 of each 5-d collection period, ruminal samples (250 ml) were collected via ruminal cannula at 0, 2, 4, 8, and 12 h after feeding; 0 h occurred just before the 0700 feeding and supplementation. Samples were analyzed immediately for pH using a combination electrode. All samples were strained through four layers of cheesecloth, acidified with 1 ml of 7.2 N H2SO4/100 ml of strained fluid, and frozen (−40°C).

Heifers were dosed via ruminal cannula with 20 g/d of chromic oxide, the digesta flow marker, in two equal proportions at 0700 and 1900, beginning 7 d before sampling and continuing throughout the sampling period. Duodenal samples were taken at 0, 4, 8, 12, 16, 20, 26, 30, 34, 38, 42, and 46 h into the collection period. Approximately 250 ml of digesta was obtained from the duodenum at each collection time, composited within heifer across days, and stored frozen (−40°C). Rectal grab samples were taken on d 1 through 5 during each collection phase at 0700 and 1900 feedings, composited within heifer, and frozen (−40°C).

On d 1 through 4 of the collection phase, approximately 3 g of grass hay ground in a Wiley mill to pass a 2-mm screen were placed in 9-cm x 16-cm nylon bags (pore size 27 x 47 µm). Bags were suspended in the rumen of each heifer for 72, 48, 36, 24, 12, 8, 4, and 2 h. One empty bag served as a blank for each time. After removal, bags were rinsed in cold tap water until effluent was clear, dried at 60°C for 24 h, and then dried at 100°C for 24 h.

On d 5 of the collection phase at 0900 (2 h after feeding) approximately 2 liters of ruminal fluid were removed from each heifer by straining ruminal contents through four layers of cheesecloth and were preserved with formaldehyde (25 ml of .9% [wt/vol] NaCl in 37% formaldehyde/100 ml of ruminal fluid). The mixture was centrifuged at 1,000 x g for 5 min to remove feed particles and protozoa. Bacteria were separated from the supernatant fluid by centrifugation for 20 min at 20,000 x g, washed with .9% (wt/vol) NaCl, recentrifuged (20 min at 20,000 x g), and rinsed with distilled water (Merchen and Satter, 1983).

Laboratory Analyses. Fecal, duodenal, and isolated bacterial samples were lyophilized4. Feed, orts, fecal and duodenal samples were ground in a Wiley mill to pass a 2-mm screen. All samples were analyzed for DM and ash (AOAC, 1984). Feed samples were analyzed nonsequentially for dietary fiber constituents (NDF, ADF, ADL; Goering and Van Soest, 1970) and for Kjeldahl N (AOAC, 1984). After drying, nylon bag residues were analyzed for NDF content to determine extent and rate of digestion. Chromium concentration in duodenal and fecal samples was determined by atomic absorption spectroscopy with a nitrous oxide/acetylene flame (Williams et al., 1962).

2Soybean oil composition percentage: myristic, .10; palmitic, .11.2; margaric, .10; stearic, 4.17; arachidonic, .26; oleic, 25.85; linoleic, 51.32; linolenic, 7.09; total saturated, 16%; total unsaturated, 84%.

3Diamond Trace Mineralized Salt, Diamond Crystal Salt Co., St. Clair, MI. Salt, 97 to 99; Zn, .85; Mn, .22; Fe, .21; Mg, .10; Cu, .30; I, .01; and Co, .006 (expressed as percentage).

4Model 600 SL, Virtus Freeze Drier, Virtus Corporation, Gardner, NY.
SOYBEAN OIL INFUSION OF GRASS-FED HEIFERS

Table 1. Influence of soybean oil infusion on ruminal pH, NH₃ N, and VFA concentrations in beef heifers consuming grass hay.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Ruminal infusion</th>
<th>Duodenal infusion</th>
<th>Stb</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.6d</td>
<td>6.4d</td>
<td>6.6e</td>
<td>.03</td>
</tr>
<tr>
<td>NH₃ N, mg/dl</td>
<td>5.3</td>
<td>6.2</td>
<td>6.9</td>
<td>.6</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>81.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>67.5</td>
<td>67.0</td>
<td>68.5</td>
<td>.8</td>
</tr>
<tr>
<td>Propionate</td>
<td>19.3c</td>
<td>22.2d</td>
<td>18.9c</td>
<td>.5</td>
</tr>
<tr>
<td>Butyrate</td>
<td>11.0</td>
<td>10.5</td>
<td>10.3</td>
<td>.2</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>.7c</td>
<td>.7c</td>
<td>.8d</td>
<td>.03</td>
</tr>
<tr>
<td>Valerate</td>
<td>.9c</td>
<td>1.1d</td>
<td>.8e</td>
<td>.04</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>.5c</td>
<td>.6e</td>
<td>.8d</td>
<td>.04</td>
</tr>
<tr>
<td>Acetate: propionate ratio</td>
<td>3.5d</td>
<td>3.0e</td>
<td>3.6d</td>
<td>.07</td>
</tr>
</tbody>
</table>

*aMeans averaged over sampling times of 0, 2, 4, 8, and 12 h after 0700 soybean oil infusion. bN = 30. c,dRow means that do not have common letters in their superscripts differ (P < .05).

Duodenal contents were analyzed for NH₃ N (AOAC, 1984), purines (Zinn and Owens, 1986), and NDF and ADF as described previously. Isolated, lyophilized bacterial cells also were analyzed for DM, ash, N, and purines. Feces were analyzed for N, NDF, and ADF as described previously.

Ruminal samples were thawed at room temperature and centrifuged at 10,000 × g for 10 min. Supernatant fluid was decanted and analyzed for NH₃ N by a phenol-hypochlorite procedure (Broderick and Kang, 1980). After addition of 2-ethylbutyric acid as an internal standard, fluid was recentrifuged for 10 min at 10,000 × g, and VFA concentrations were analyzed by gas chromatography (Goetsch and Galyean, 1983).

Calculations. Organic matter flow to each segment of the tract was calculated by dividing daily Cr dose by Cr concentration (OM basis) in duodenal or fecal samples. Individual dietary constituent flows were calculated by multiplying dietary constituent concentrations (OM basis) by OM flows. Ratio of nucleic acids to N in isolated microbial cells was used to calculate microbial flow to the small intestine. Passage of apparent feed N was determined by subtracting microbial N and NH₃ N from total N passage to the duodenum. Rate of NDF disappearance was calculated from the natural logarithmic regression of potentially digestible NDF (1 minus decimal fraction of NDF of the 72-h residue) remaining against time (Mertens and Loften, 1980).

Statistical Analysis. Intake, digesta flow, digestibility, and microbial protein data were analyzed as a replicated 3 × 3 Latin square with treatment, square, period, and heifer within square as effects in the model using the GLM procedure of SAS (1987). Time sequence data (ruminal pH, NH₃ N, and VFA) were analyzed as a split-plot within a 3 × 3 replicated Latin square with treatment as the main plot and sampling time in the subplot (Gill, 1986). The split-plot model included effects for heifer within square, treatment, square, heifer × period × treatment within square, time, time × treatment, time within heifer within square, time × square, and residual. Treatment effects were tested with heifer × period × treatment within square, and the time × treatment interaction was tested with residual error. Treatment means were separated by the lsd method (Snedecor and Cochran, 1980) protected by a preliminary F-test (P < .05).

Results and Discussion

Ruminal Fermentation. Time × treatment interactions were not detected (P > .10) for ruminal pH, NH₃ N, and VFA concentrations; therefore, treatment means were pooled across time (Table 1). Ruminal pH was lower (P < .05) for RI heifers than for either C or DI heifers. Values were slightly less than those reported as optimal for fiber digestion (Mertens, 1979) and cellulolytic bacterial growth.
TABLE 2. INFLUENCE OF SOYBEAN OIL INFUSION ON OM INTAKE (OMI) AND RUMINAL AND TOTAL TRACT OM, NDF, AND ADF DIGESTIBILITIES IN BEEF HEIFERS CONSUMING GRASS HAY

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Ruminal infusion</th>
<th>Duodenal infusion</th>
<th>SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OMI, g/d</td>
<td>6,813</td>
<td>6,394</td>
<td>5,808</td>
<td>353</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruminalb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent OM</td>
<td>40.4</td>
<td>34.1</td>
<td>45.2</td>
<td>3.2</td>
</tr>
<tr>
<td>True OMc</td>
<td>46.8</td>
<td>41.4</td>
<td>51.2</td>
<td>2.8</td>
</tr>
<tr>
<td>NDF</td>
<td>33.7</td>
<td>32.4</td>
<td>44.9</td>
<td>4.2</td>
</tr>
<tr>
<td>ADF</td>
<td>33.5</td>
<td>28.4</td>
<td>40.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Total tract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OM</td>
<td>54.3e</td>
<td>48.1d</td>
<td>59.2e</td>
<td>3.1</td>
</tr>
<tr>
<td>NDF</td>
<td>53.1</td>
<td>54.0</td>
<td>57.3</td>
<td>3.7</td>
</tr>
<tr>
<td>ADF</td>
<td>46.4</td>
<td>42.5</td>
<td>50.1</td>
<td>3.9</td>
</tr>
</tbody>
</table>

aN = 6.
bPercentage of intake.
cCorrected for microbial OM synthesis in the rumen.
dRow means that do not have common letters in their superscripts differ (P < .10).

(Ørskov, 1982) but were not decreased enough to have a deleterious effect on fiber digestion. Ruminal NH₃ N concentrations were not affected (P > .10; Table 1) by treatment; however, soybean oil-infused groups tended (P = .17) to have a greater ruminal NH₃ N concentration. This result disagrees with the findings of Jenkins and Fotouhi (1990) in which addition of either lecithin or corn oil to a concentrate/roughage diet decreased ruminal NH₃ N concentrations. Likewise, Kowalczyk et al. (1977) reported decreased ruminal NH₃ N with increasing level of tallow added to grass hay diets.

Neither total VFA concentration (mM) nor molar proportions of acetate and butyrate were altered (Table 1; P > .10) by soybean oil infusion. Proportion of propionate was greater (P < .05) for RI heifers than for C and DI heifers. Increased propionate coincides with VFA changes reported by others feeding tallow (Kowalczyk et al., 1977), linseed oil (Ikwuegbu and Sutton, 1982), yellow grease (Zinn, 1989), and corn oil and lecithin (Jenkins and Fotouhi, 1990). As a result, the acetate:propionate ratio was least (P < .05) for the RI group. Lowering this ratio is generally associated with decreased fiber digestion (Jenkins and Fotouhi, 1990). Ruminal valerate was greater (P < .05) in RI heifers than in C or DI heifers. Heifers receiving DI had greater (P < .05) concentrations of both isobutyrate and isovalerate than either C or RI heifers.

Intake and Digestion. Organic matter, NDF, ADF, and N intakes (g/d) did not differ (P > .10) among treatments. Ruminal and total tract NDF and ADF digestibilities were not affected (P > .10) by treatment (Table 2). True ruminal OM digestibility tended (P = .11) to be decreased in RI heifers compared with DI and C heifers (Table 2); total tract OM digestibility also was decreased (P < .10) by RI. Despite decreased ruminal digestibilities, Jenkins and Fotouhi (1990) found little effect on total tract digestibilities with corn oil or lecithin supplementation of sheep. Postruminal digestibilities, expressed as a percentage of intake (data not shown), were not influenced (P > .10) by soybean oil infusion.

In situ NDF disappearance of forage at different incubation times (data not shown) was not altered (P > .10) by infusion of soybean oil. Extent of NDF digestion after 72 h of ruminal incubation for infused heifers did not differ (P > .10) from that of controls (C = 72.4; DI = 70.2; RI = 69.6; SE = 1.8). Likewise, supplementation with soybean oil had no effect (P > .10) on rate (%/h) of NDF digestion (C = 4.0; DI = 3.6; RI = 3.9; SE = .3).

Nitrogen Flow and Micr0bial Protein Synthesis. Total N flow to the duodenum was greater (P < .10) for C and RI heifers than for DI heifers (Table 3). Possible explanations for this phenomenon include feedback of fat in duodenum to rumen via digestive hormones.
TABLE 3. INFLUENCE OF SOYBEAN OIL INFUSION ON DUODENAL NITROGEN FLOW AND MICROBIAL EFFICIENCY IN BEEF HEIFERS CONSUMING GRASS HAY

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Ruminal infusion</th>
<th>Duodenal infusion</th>
<th>SEa</th>
</tr>
</thead>
<tbody>
<tr>
<td>N intake, g/d</td>
<td>96</td>
<td>91</td>
<td>82</td>
<td>5</td>
</tr>
<tr>
<td>Duodenal flow, g/d</td>
<td>156.7d</td>
<td>147.2d</td>
<td>118.6c</td>
<td>11.2</td>
</tr>
<tr>
<td>Total N</td>
<td>99.5</td>
<td>91.6</td>
<td>75.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Microbial N</td>
<td>48.5d</td>
<td>47.7d</td>
<td>34.9e</td>
<td>4.6</td>
</tr>
<tr>
<td>Ammonia N</td>
<td>8.7d</td>
<td>7.9e</td>
<td>7.8e</td>
<td>2</td>
</tr>
<tr>
<td>Nonammonia, nonmicrobial N flow</td>
<td>15.2d</td>
<td>18.0f</td>
<td>11.7f</td>
<td>2.0</td>
</tr>
<tr>
<td>Total tract N</td>
<td>39.1</td>
<td>34.6</td>
<td>35.9</td>
<td>4.0</td>
</tr>
</tbody>
</table>

\[N = 6.\]

\[M\text{OEFP} = \text{efficiency of microbial growth (g of microbial N/kg of OM truly fermented in rumen).}\]

\[\text{Row means that do not have common letters in their superscripts differ (} P < .10).\]

More likely, however, this difference reflects lower forage intake by DI heifers. Likewise, duodenal microbial N flow was greater \(P < .10\) for C and RI than for DI. Total tract N digestibility was not altered \(P > .10\) by either RI or DI. Jenkins and Fotouhi (1990) reported that added fat had negative effects on both ruminal and total tract N digestibility. Feeding forages low in N is normally associated with more N reaching the duodenum than was ingested (Egan et al., 1975) because of N recycling to the rumen. Other researchers also have reported a greater N flow to the duodenum than was ingested with low-N diets (Funk et al., 1987; McCollum et al., 1987; Stokes et al., 1988; Krysl et al., 1989; Gunter et al., 1990). Ammonia N flow was greater \(P < .10\) for C heifers than for either DI or RI heifers. Nonammonia, nonmicrobial N (presumably feed N) flows were not different \(P > .10\) among treatments.

Microbial efficiency \(5\) (Table 3; g of N/kg of OM truly fermented) was not influenced \(P > .10\) by RI; however, DI reduced \(P < .10\) microbial efficiency. In contrast, Ikwuegbu and Sutton (1982) and Jenkins and Fotouhi (1990) reported improved microbial efficiency with ruminal supplementation of lecithin, corn oil, and linseed oil, respectively. Microbial efficiencies reported in the present study are similar to those reported for cattle grazing blue grama rangeland (1.13 to 1.86% N; Funk et al., 1987) and cattle fed either low-quality (.58 to .66% N) prairie hay (McCollum et al., 1987) or grass hay (1.36% N; Gunter et al., 1990) but are substantially greater than those reported by Stokes et al. (1988) in cows consuming low-quality (.77% N) prairie hay.

Implications

Ruminal infusion of soybean oil decreased ruminal and total tract organic matter digestibilities; however, fiber digestion was not altered despite a lower acetate:propionate ratio. Duodenal infusion of soybean oil did not alter any digestibility measurements. Duodenal infusion of soybean oil decreased N flow to the duodenum, which was most likely a result of a nonsignificant reduction in feed intake with this treatment. These data indicate that no direct benefits for improving forage usage were derived from soybean oil infusion of heifers fed a 100% grass hay diet; however, animal should realize benefits from additional dietary energy provided by the infusion.

Literature Cited


simultaneous determinations of ammonia and total amino acids in ruminal fluid and in vitro media. J. Dairy Sci. 33:64.


