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# Effect of fats and ionophores on in vitro fermentation of a high concentrate diet

## Abstract

Batch culture fermentations were used to determine the effects of fat type [none, animal tallow (AT), soybean oil soapstock (SOY), or yellow grease (YG)] and ionophore type [none, lasalocid (L), monensin + tylosin in a ratio of 2.5:1 (MT), or a 50:50 combination of Land MT (LMT)] on in vitro concentrations of lactate (LA) and volatile fatty acids (VFA). Fat-containing substrates had 4% fat on a dry basis. No significant interactions between fat and ionophore treatments were observed. Ionophore treatment resulted in a reduced pH, with the greatest reduction in the L treatment. Total VFA and LA increased with ionophore treatment and were highest with L treatment. All ionophore treatments decreased molar proportions of acetate and butyrate and increased propionate. Lasalocid produced a lower molar proportion of acetate and a higher molar proportion of propionate than did MT. Adding fat resulted in a reduction in total VFA and an increase in pH. The reduction in total VFA was less for SOY than AT or YG treatments. No significant differences in LA or VFA molar proportions were observed among fat treatments. Our results indicate that the rate of starch digestion may be slowed by fat, which may translate into a decreased incidence of ruminal acidosis.

## Keywords

Cattlemen's Day, 1990; Kansas Agricultural Experiment Station contribution; no. 90-361-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 592; Beef; Fats; Ionophores; Fermentation; Volatile fatty acids; Lactic acid

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**EFFECT OF FATS AND IONOPHORES  
ON IN VITRO FERMENTATION  
OF A HIGH CONCENTRATE DIET**

**E. M. Clary, R. T. Brandt, Jr.,  
and T. G. Nagaraja**

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**Summary**

Batch culture fermentations were used to determine the effects of fat type [none, animal tallow (AT), soybean oil soapstock (SOY), or yellow grease (YG)] and ionophore type [none, lasalocid (L), monensin + tylosin in a ratio of 2.5:1 (MT), or a 50:50 combination of L and MT (LMT)] on in vitro concentrations of lactate (LA) and volatile fatty acids (VFA). Fat-containing substrates had 4% fat on a dry basis. No significant interactions between fat and ionophore treatments were observed. Ionophore treatment resulted in a reduced pH, with the greatest reduction in the L treatment. Total VFA and LA increased with ionophore treatment and were highest with L treatment. All ionophore treatments decreased molar proportions of acetate and butyrate and increased propionate. Lasalocid produced a lower molar proportion of acetate and a higher molar proportion of propionate than did MT. Adding fat resulted in a reduction in total VFA and an increase in pH. The reduction in total VFA was less for SOY than AT or YG treatments. No significant differences in LA or VFA molar proportions were observed among fat treatments. Our results indicate that the rate of starch digestion may be slowed by fat, which may translate into a decreased incidence of ruminal acidosis.

(Key Words: Fats, Ionophores, Fermentation, Volatile Fatty Acids, Lactic Acid.)

**Introduction**

Supplemental fat and ionophores are commonly fed to finishing cattle. Fat at 2 to 5% of the diet dry matter controls dust, improves ration consistency, and aids feed processing. It also increases diet energy density and total energy intake. Ionophores improve nitrogen metabolism and the efficiency of energy metabolism in the rumen and also help control feedlot disorders such as lactic acidosis and bloat, probably because of shifts in the ruminal microbial flora. Previous research at Kansas State University suggests that the effects of feeding supplemental fat may be altered by the presence of an ionophore. This paper presents the results of an in vitro experiment designed to explore potential mechanisms for such interactions.

**Experimental Procedures**

Batch culture fermentations with substrates (Table 5.1) based on flaked grain were used in a 4 × 4 factorially arranged experiment (five replications). Main effects were supplemental fat type [none, animal tallow (AT), soybean oil soapstock (SOY), or yellow grease (YG)] and ionophore type [none, lasalocid (L), monensin + tylosin in a 2.5:1 ratio (MT), or a 50:50 combination of L and MT (LMT)]. Ionophores were dissolved in ethanol and added at 10 µg of total ionophore per ml of inoculum. To each tube was added 1 g of ground (Wiley mill, 2 mm sieve) substrate, 15 ml of McDougall's buffer, 15 ml of strained rumen fluid

**Table 5.1. Composition of Diets<sup>1</sup>**

Fat treatment	Dry rolled wheat	Dry rolled milo	Corn silage	Supplement	Beet molasses	Fat
No fat	40.4	40.4	10.0	5.2	4.0	0.0
Fat	40.3	40.3	10.0	5.4	0.0	4.0

<sup>1</sup>Dry matter basis.

obtained 12 to 14 hr post-feeding from a steer fed 80% alfalfa hay and 20% grain, and 100  $\mu$ l of ionophore preparation (control tubes received 100  $\mu$ l ethanol). Each tube was flushed with CO<sub>2</sub>, stoppered with a rubber stopper and a Bunsen valve, and incubated at 39 C for 12 hr. Fermentations were conducted in duplicate for each treatment combina-

tion. Final pH's were recorded, and aliquots from each tube were taken for subsequent VFA and LA analysis.

### Results and Discussion

Effects of fat treatment are listed in Table 5.2. Fat treatment significantly increased pH, which may simply reflect the changes in total VFA concentration. Animal tallow and YG reduced ( $P < .0001$ ) total VFA compared to the no fat control. Fat did not significantly affect VFA molar proportions or LA.

Proposed mechanisms for fat's action on rumen fermentation include physical coating of feed particles, direct toxic effects of the fats on rumen microbes, and decreased cation availability through soap formation. Fiber digestion is generally reduced by supplemental fat, resulting in a decreased acetate:propionate ratio, and this effect is usually greater with polyunsaturated than saturated fats. Thus, one might expect SOY to decrease the acetate:propionate ratio and reduce total VFA more than AT or YG. Such changes were not observed in this experiment, perhaps because the substrate contained so little fiber that change in fiber digestion would have minimal effect.

**Table 5.2. Effects of Fat Treatment on VFA Profile, pH, and Lactate Concentration**

Treatment	pH <sup>a</sup>	Lactate, mM	Total VFA <sup>a</sup> , mM	%Acetate	%Propionate	%Butyrate
No fat	5.64 <sup>b</sup>	.198	179.5 <sup>b</sup>	59.0	31.2	9.8
Animal tallow	5.77 <sup>c</sup>	.171	170.4 <sup>c</sup>	59.2	30.7	10.1
Soy oil soapstock	5.72 <sup>c</sup>	.185	175.4 <sup>b</sup>	59.2	30.8	10.0
Yellow grease	5.77 <sup>c</sup>	.169	168.6 <sup>c</sup>	59.0	30.7	10.3
SE	.023	.019	1.48	.20	.40	.26

<sup>a</sup>Fat effect ( $P < .001$ ).<sup>bc</sup>Means in a column with unlike superscripts differ ( $P < .05$ ).

Table 5.3 lists the effects of ionophore treatment on in vitro batch culture fermentations. Lasalocid resulted in a lower ( $P<.05$ ) pH than other ionophore treatments. The no-ionophore control treatment resulted in a higher pH than MT ( $P<.10$ ) and LMT ( $P<.05$ ). Changes in pH resulting from ionophore treatment were likely due to higher total VFA, which was significantly higher in all ionophore treatments vs the no-ionophore control. This is consistent with other in vitro work with ionophores at KSU and suggests that ionophores may alter fermentation endproducts via anti-protozoal activity. Lasalocid treatment resulted in a higher ( $P<.05$ ) total VFA than LMT, but did not differ significantly from MT. Lasalocid treatment resulted in higher ( $P<.0001$ ) LA than other ionophore treatments. No LA was detected in the control. Alterations in VFA profile associated with ionophore treatment included a reduction in the molar proportions of acetate and butyrate and an increase in the molar proportion of propionate. Lasalocid resulted in a lower molar proportion of acetate and higher molar proportion of propionate than the MT treatment ( $P<.05$ ). Molar proportions of acetate and propionate for LMT were intermediate to those for L and MT treatments, but did not differ significantly from either. The effects of ionophores on ruminal fermentation are thought to be the result of changes in rumen microbial populations. Bacteria sensitive to ionophores include those that produce LA, butyric acid, formic acid, and hydrogen, whereas bacteria producing succinate and propionate are relatively resistant to the effects of ionophores. Reductions in rumen formic acid and hydrogen concentrations result in reduced methane production, thereby increasing efficiency of energy metabolism in the rumen. No significant interactions between fat and ionophore treatments were observed.

**Table 5.3. Effects of Ionophore Treatment on VFA Profile, pH, and Lactate Concentration**

Treatment	pH <sup>a</sup>	Lactate <sup>a</sup> , mM	Total VFA <sup>a</sup> , mM	%Acetate <sup>a</sup>	%Propionate <sup>a</sup>	%Butyrate <sup>a</sup>
No ionophore	5.80 <sup>b</sup>	.00 <sup>b</sup>	168.8 <sup>b</sup>	61.6 <sup>b</sup>	25.6 <sup>b</sup>	12.8 <sup>b</sup>
Lasalocid	5.63 <sup>c</sup>	.37 <sup>c</sup>	177.3 <sup>c</sup>	57.9 <sup>c</sup>	33.2 <sup>c</sup>	8.9 <sup>c</sup>
Monensin/Tylosin	5.74 <sup>bd</sup>	.11 <sup>d</sup>	174.7 <sup>cd</sup>	58.6 <sup>d</sup>	32.0 <sup>d</sup>	9.4 <sup>c</sup>
Las/Mon/Tylosin	5.72 <sup>d</sup>	.24 <sup>e</sup>	173.1 <sup>d</sup>	58.3 <sup>cd</sup>	32.6 <sup>cd</sup>	9.1 <sup>c</sup>
SE	.023	.019	1.48	.20	.40	.26

<sup>a</sup>Ionophore effect ( $P<.001$ ).

<sup>bcd</sup>Means in a column with unlike superscripts differ ( $P<.05$ ).

The reduction in total VFA and increase in pH resulting from the more saturated fats suggests a potential alteration in the rate of starch digestion, which, in turn, may reduce the incidence of acidosis. Further research should be conducted to verify this observation.