Lactation Performance and Fatty Acid Composition of Milk from Holstein Cows Fed 0 to 5% Oleamide

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ABSTRACT

Diets containing 0 to 5% oleamide were fed to Holstein cows to determine linear or nonlinear responses to the fat supplement on lactation performance and milk fatty acid composition. Six rations containing concentrate, corn silage, and 0, 1, 2, 3, 4, or 5% (dry matter basis) added oleamide were fed to six multiparous cows in a 6 × 6 Latin square for 2-wk periods. As the oleamide concentration in the ration increased from 0 to 5%, dry matter intake declined, fiber and dry matter digestibilities remained constant, and digestibilities of protein and fatty acids increased. Milk yield declined as dietary oleamide increased, although yield was not depressed numerically until oleamide exceeded 2% of the diet dry matter. The C18:1 concentration doubled in milk as oleamide in the diet increased from 0 to 5%. Ratios of C18:1 to C16:0 in milk fat were 0.56, 0.83, 1.34, 1.53, and 1.73 for the diets supplemented with 0, 1, 2, 3, 4, and 5% oleamide, respectively. No amide was detected in milk samples taken from cows fed the 5% oleamide diet. Results show that intake of diets containing 2 to 3% oleamide substantially increased the milk C18:1:C16:0 ratio without greatly affecting milk yield or causing detectable amounts of amide in milk. (Key words: oleamide, milk fatty acids, milk composition, digestibility)

INTRODUCTION

The conversion of oleic acid to a fatty acyl amide significantly reduces its hydrogenation by mixed ruminal microbes. Reeves et al. (18) reported that the disappearance rate of cis-C18:1 from ruminal cultures was 0.064/h for substrates with 4.5% added oleic acid but only 0.025/h for substrates containing 4.5% added oleamide. When oleamide was fed to sheep (18) and dairy cows (12), fecal excretion of C18:1 was not elevated, indicating normal postruminal digestion of the amide supplement. Schmid et al. (20) reported that fatty acids were released from fatty acyl amides fed to rats by the action of intestinal amidases.

Because oleamide resisted biohydrogenation but was still digested in the intestines, its intake had a substantial effect on altering milk fatty acid profile of lactating dairy cows. Intake of oleamide by lactating cows (12) resulted in a 37% increase in milk C18:1 compared with an equal quantity of high oleic canola oil (48.2 vs 35.1% of the total milk fatty acids, respectively); the oleamide increased milk C18:1 107.9% compared with the control diet (23.2% of the total fatty acids).

Oleamide also reduced feed intake of sheep (18) and dairy cows (12). Milk yield in the latter study was not statistically reduced by dietary oleamide, although milk yield was numerically lower for cows fed oleamide or canola oil than for cows fed the control diet.

Because the previous study (12) only examined the effects of oleamide at 3.5% inclusion in dairy rations, this study was conducted to determine the effects of 0 to 5% of the amide added to the ration. Rations containing 0 to 5% oleamide were fed to cows to determine linear or nonlinear responses to the amide on intake, lactation performance, and milk fatty acid composition.

MATERIALS AND METHODS

Diets

The TMR consisted of concentrate and corn silage containing 0, 1, 2, 3, 4, or 5% added oleamide (DM basis). The oleamide supplement contained 89.7% amide and 87.6% total fatty acids (12). Fatty acid composition of oleamide was 3.09% C14:0, 7.82% C16:0, 2.74% C18:0, 68.3% cis-C18:1, 7.75% trans-C18:1, 5.02% C18:2, and trace amounts (<1%) of C12:0, C14:0, C18:3, and C20:0 (12). Oleamide replaced both corn silage and corn grain to minimize decreases in nonstructural carbohydrate concentration as added fat increased (Table 1). The ratio of corn silage to corn was held constant (1.33:1) as oleamide increased in the diet. As oleamide concentration increased, CP was...
TABLE 1. Composition of total mixed rations.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>0% of DM</th>
<th>1% of DM</th>
<th>2% of DM</th>
<th>3% of DM</th>
<th>4% of DM</th>
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<tr>
<td>Dietary oleamide, %</td>
<td></td>
<td></td>
<td></td>
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<td>% of DM</td>
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</table>

- **Ingredients**
  - Corn silage: 45.4 44.1 43.9 41.9 40.7 39.6
  - Corn: 34.1 33.1 32.2 31.4 30.6 29.7
  - Soybean meal: 18.0 18.4 18.6 18.9 19.2 19.4
  - Cottonseed hulls: 0.0 0.82 1.60 2.22 2.92 3.61
  - Oleamide: 0.0 1.0 2.0 3.0 4.0 5.0
  - Limestone: 1.28 1.43 1.44 1.44 1.44 1.44
  - Dynamate¹: 0.3 0.3 0.3 0.3 0.3 0.3
  - Trace mineral salt²: 0.5 0.5 0.5 0.5 0.5 0.5
  - Zinc methionine³: 0.25 0.25 0.25 0.25 0.25 0.25
  - Vitamin premix⁴: 0.25 0.25 0.25 0.25 0.25 0.25

- **Composition, % of DM**
  - CP: 16.7 16.8 17.4 17.2 17.8 17.4
  - NDF: 28.1 27.6 28.1 28.5 27.5 29.3
  - Fatty acids: 2.24 3.15 4.04 4.82 5.66 7.00
  - Amide: 0.00 0.98 2.03 3.43 4.09 4.93
  - NE₇.5, Mcal/kg: 1.76 1.80 1.83 1.87 1.91 1.95

¹Dynamate® (Eastern Minerals Inc., Henderson, NC). Composition: not less than 18% K, 11% Mg, and 22% S.
²Morton Salt Division (Chicago, IL); specified to contain: 93 to 98% NaCl, not less than 0.35% Zn, 0.28% Mn, 0.175% Fe, 0.035% Cu, 0.007% I, and 0.007% Co.
³Zinpro Corporation (Chaska, MN).
⁴Supplied 220 IU of vitamin A and 440 IU of vitamin D₃/g.
⁵Estimated from NRC (16).

Cows were orally dosed with 10 g of Cr₂O₃ twice daily (1100 and 1900 h) during the last 10 d of each period. The Cr₂O₃ concentration in fecal samples was used to determine fecal DM excretion for calculation of total tract digestibilities. Eight grab fecal samples were taken by rectal palpation from each cow in each period on d 10 (0800 and 2300 h), d 11 (1100, 1700, and 0200 h), and d 12 (1400, 2000, and 0500 h). Feces were composited across times for each cow and immediately frozen.

**Sample Analysis**

Samples of TMR, orts, and feces were composited across periods, dried at 55°C, and ground in a centrifugal mill through a 1-mm sieve. Ground samples were analyzed for DM (100°C), Kjeldahl N (2), NDF (24), and amide (12). Feces were analyzed for Cr by the method of Fenton and Fenton (10).

Fatty acids in ground samples were determined according to the procedure of Sukhija and Palmquist (22), except that ground samples were methylated in acetyl chloride and methanol (1:10, vol/vol) at a higher temperature (80°C) and for a longer period of incubation (15 h) to ensure complete hydrolysis of the amide bond. Analysis of the reaction mixture by TLC verified that all amide had disappeared under...
Thies (14). Milk samples from cows fed the 0% amide diets were freeze-dried (Deerfield, IL) as described by Jenkins and Thies (14). Milk samples from cows fed the 0% amide diet were freeze-dried and analyzed for amide content by HPLC with an evaporative light-scattering mass detector (Varex MK III, Alltech Associates, Deerfield, IL) as described by Jenkins and Thies (14). Milk samples from cows fed the 0% amide diet were spiked with known quantities of oleamide to determine the percentage of amide recovery by the procedure just described. Known amounts of oleamide dissolved in the extraction mixture were introduced into the freeze-dried control milk (10 ml) during extraction. Spiked samples were analyzed for amide content by the same HPLC procedure. The milk of one cow fed the 5% amide diet was unavailable for analysis. Spiked samples were analyzed for amide content by the same HPLC procedure. The milk of one cow fed the 5% amide diet was unavailable for analysis due to insufficient sample size.

**Statistics**

Data were analyzed as a 6 × 6 Latin square by ANOVA using the general linear models procedure of SAS (19). The model included effects caused by diet, period, and cow. The diet sums of squares were divided into linear, quadratic, cubic, quartic, and quintic components and were tested against the error mean square. Significance of polynomial components was declared at P < 0.05. Data on milk yield, milk composition, and feed intake that are reported in the tables were averaged over the last 4-d of each period.

**RESULTS AND DISCUSSION**

Differences in CP and NDF contents were small among the six TMR (Table 1). Diets averaged 17.2% CP and 28.2% NDF (DM basis) compared with the recommended (16) minimum concentrations of 17% CP and 25% NDF for cows averaging 40 kg of milk/d.

Actual amide concentrations in the ration DM (0, 0.98, 2.03, 3.43, 4.09, and 4.93% of DM) were similar to the calculated concentrations of 0, 1, 2, 3, 4, and 5% (Table 1). The addition of oleamide increased total fatty acid concentration over the diet without oleamide by 0.91, 1.80, 2.58, 3.42, and 4.76 percentage units for the 1, 2, 3, 4, and 5% amide diets, respectively.

Daily DM, CP, and NDF intakes by the cows declined linearly (P < 0.05) as the percentage of oleamide in the ration increased from 0 to 5% (Table 2). Despite the drop in DMI caused by oleamide, total fatty acid intake still increased (P < 0.05) linearly as amide in the diet increased. Amide consumed also increased (P < 0.05) but in a quadratic fashion.

Oleamide reduced DMI in a previous study (12) when fed to lactating cows at 3.5% of the ration DM. Intake depressions in this present study were related proportionately to the percentage of oleamide added to the diet. As little as 1% oleamide in the ration DM was enough to depress DMI 1.2 kg/d. On average, DMI was reduced 1.6 kg/d for each percentage unit increase in ration oleamide.

The reduced DMI associated with increased oleamide in the diet could be attributed to intake regulation by chemostatic mechanisms that maintain constant digestible energy intake. A specific oleamide effect cannot be discerned from an energy effect in this study because the two were confounded. However, in the previous study (12), increased energy was eliminated as the cause of the DMI depression because oleamide reduced DMI more than did an isoenergetic diet containing canola oil.

An alternative explanation is that oleamide reduced DMI by resisting ruminal biohydrogenation and increasing the postruminal flow of unsaturated fatty acids. Bremmer et al. (4) reported decreased DMI associated with increasing amounts of unsaturated fatty acids infused abomasally into lactating cows. Abomasal infusion of fat sources that were mostly saturated had no effect on DMI. The Bremmer et al. study (4) did not distinguish with certainty whether the number of double bonds was a factor influencing the degree of intake depression. However, their results suggest that intake was reduced by fat sources that were high in either C 18:1 or C 18:2. Intake depression by high duodenal unsaturated fatty acids has been attributed to their ability to stimulate cholecystokinin release (26) or reduce gut motility (9).

Apparent digestibilities of DM and NDF were not affected by amide concentration in the ration DM. However, an increase in oleamide concentration in the ration increased (P < 0.05) protein and fatty acid digestibilities.

The digestibility of CP increased linearly from 69.8 to 75.4% as oleamide increased from 0 to 5% of the ration DM. A substantial increase in CP digestibility was reported previously when dairy cows were fed added oleamide (12). Amide N may be more digestible than N from other feed ingredients if the amide bond is cleaved postruminally, as suggested by Jenkins (12), resulting in release of ammonia.

Fat added to dairy rations in other studies has had variable effects on protein digestibility. Digestibility...
TABLE 2. Nutrient intakes and total tract digestibilities.

<table>
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<tr>
<th>Dietary oleamide, %</th>
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<th>Sig¹</th>
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</tr>
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<tr>
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Dietary oleamide, % kg/d

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<td>24.6</td>
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<td>4.13</td>
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<td>3.22</td>
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<td>NDF</td>
<td>7.21</td>
<td>6.78</td>
<td>6.21</td>
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<td>0.77</td>
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<td>0.44</td>
<td>0.69</td>
<td>0.75</td>
<td>0.86</td>
<td>0.034</td>
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Dietary oleamide, %

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<td>71.0</td>
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<td>74.6</td>
<td>74.6</td>
<td>75.4</td>
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<tr>
<td>NDF</td>
<td>42.1</td>
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<td>40.4</td>
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<td>85.6</td>
<td>86.1</td>
<td>90.3</td>
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<tr>
<td>Amide</td>
<td></td>
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<td>98.8</td>
<td>99.1</td>
<td>98.1</td>
<td>98.9</td>
</tr>
</tbody>
</table>

¹Orthogonal polynomials with linear (L), quadratic (Q), or cubic (C) components were significant (P < 0.05).

Milk and FCM, similar to DMI, declined (P < 0.05) as concentration of oleamide in the diet DM increased (Table 3). Milk yield was not numerically depressed until oleamide exceeded 2% of the diet DM. However, statistically, milk yield declined linearly as dietary oleamide increased, although there was a tendency (P < 0.10) for a cubic response. When milk yields for each percentage of oleamide were tested by pairwise t-tests against milk yield for the 0 diet, only the 4 and 5% oleamide diets were lower (P < 0.05).

Lactation efficiency, expressed as kilograms of milk per kilogram of DMI, increased (P < 0.05) up to 2% oleamide in the ration and then leveled off. Efficiency, expressed as kilogram of FCM per kilogram of DMI, increased linearly as concentration of oleamide in the diet increased.

Milk fat percentage declined (P < 0.05) as oleamide concentration in the diet DM increased from 0 to 5%. Fat percentage was not affected by dietary oleamide in the study by Jenkins (12). The addition of
formaldehyde-protected canola seeds to the diet of lactating cows increased milk fat percentage (1) from 3.37 to 3.83%. Depressions in milk fat percentage that accompany the dietary intake of fat are often associated with disruption of ruminal fermentation. Digestion in the rumen was not determined in this study, although DM and NDF digestibilities in the total tract were not affected by oleamide (Table 2). Reduced intake was not likely the reason for the milk fat depression caused by oleamide. A decrease in DMI while the concentrate:forage ratio is maintained constant has been shown to increase milk fat concentration (23).

Milk protein concentration declined linearly ($P < 0.05$) as oleamide concentration in the diet increased. The 0.2-unit decline in milk protein concentration (3.03 to 2.82%) from the 0 to 5% oleamide diets was within the 0.1 to 0.3 range normally reported with fat supplements (7).

Yields of milk fat and protein also declined ($P < 0.05$) as oleamide concentration in the diet increased. The decline in milk fat yield was quadratic ($P < 0.05$), and the greatest drop in fat yield occurred with 2% added oleamide. Milk protein yield, however, declined linearly ($P < 0.05$) from 1.28 to 1.03 kg/d as oleamide increased from 0 to 5% of the diet DM.

The saturated fatty acids in milk from C₆ to C₁₆ declined ($P < 0.05$) as oleamide concentration in the diet increased from 0 to 5% (Table 4). All of the C₄ to C₁₄ fatty acids and half of the C₁₆ fatty acids found in milk are derived from mammary de novo synthesis, which is inhibited by increased blood lipids that originate from dietary fat supplements (11). The C₁₈₂ concentration in milk fat also declined ($P < 0.05$) as dietary oleamide increased, although C₁₈₂ concentrations only varied between 2 and 3% of total milk fatty acids.

Fatty acids that increased in milk fat as oleamide concentration in the diet increased were C₁₆₁, C₁₈₀, and C₁₈₁. The C₁₈₁ concentration doubled as oleamide in the diet increased from 0% to 5%. Previously, oleamide at 3.5% of the diet DM also increased C₁₈₁ in milk fat from 23.2 to 48.2%; an equal quantity of canola oil increased milk C₁₈₁ to 35.1% of the milk fatty acids (12). Increases in milk C₁₈₁ from dietary oleamide is consistent with reports that rates of ruminal biohydrogenation were lower for oleamide than for oleic acid (18).

The oleamide supplement was effective in increasing the ratio of C₁₈₁ to C₁₆₀, a change that is consistent with current medical advice to improve public health and consumer acceptability of milk and dairy products (11, 15). Ratios of C₁₈₁ to C₁₆₀ in milk fat were 0.56, 0.83, 1.34, 1.53, and 1.73 for the diets supplemented with 0, 1, 2, 3, 4, and 5% oleamide, respectively. Oleamide progressively increased milk C₁₈₁ at the expense of C₁₆₀ through all concentrations in the ration DM.

Milk samples taken when the cows were fed the 5% oleamide diet were analyzed for amide concentration by HPLC. To verify the HPLC procedure, 15 milk samples from cows fed the 0% oleamide diet were spiked with oleamide ranging from 1.28 to 9.65 mg/10 ml (Figure 1). Recoveries averaged 102.3 ± 8.4%, and retention times averaged 2.53 ± 0.04 min, demonstrating acceptable accuracy and repeatability of the procedure. No peaks with a retention time near 2.53 min were detected in any of the milk samples taken from cows fed the highest percentage (5%) of oleamide (Figure 2). It was assumed that the 5% olea-

### TABLE 4. Fatty acid composition of milk from Holstein cows fed TMR supplemented with increasing concentrations of oleamide.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>SEM</th>
<th>Sig¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₄₀</td>
<td>1.64</td>
<td>1.73</td>
<td>1.65</td>
<td>1.63</td>
<td>1.63</td>
<td>1.55</td>
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<tr>
<td>C₆₀</td>
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<td>1.64</td>
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<tr>
<td>C₈₀</td>
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<td>C₁₀₀</td>
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</tr>
<tr>
<td>C₁₂₀</td>
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<td>4.06</td>
<td>3.08</td>
<td>2.73</td>
<td>2.16</td>
<td>1.94</td>
<td>0.14</td>
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<tr>
<td>C₁₄₀</td>
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<td>13.26</td>
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<td>C₁₄₁</td>
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¹Orthogonal polynomials with linear (L), quadratic (Q), or cubic (C) components were significant ($P < 0.05$).
Figure 1. The HPLC chromatograms of milk samples from cows fed a diet with no amide but spiked with a) 9.65, b) 4.78, c) 2.79, or d) 1.28 mg of oleamide/10 ml of milk. Oleamide peaks are shown with an arrow.

Figure 2. The HPLC chromatograms of milk samples from cows fed the diets containing 5% added oleamide. The sample taken from the cow in period 2 was not analyzed because of an insufficient sample size. Although oleamide peaks were not detected, arrows show their expected location.
mide treatment would yield the highest concentration of milk amide; cows fed the 5% oleamide diet had the highest amide intake (860 g/d) but the lowest milk yield (35.8 kg/d). Amide possibly was present in milk but was below the detection limit. If all 860 g of amide consumed by the cows from the 5% oleamide diet was transferred to milk, which averaged 35.8 kg/d, the amide concentration in milk would equal 247 mg/10 ml. Even as little as 1% transfer of amide yields a concentration of 2.47 mg/10 ml in milk, a value nearly double the lowest concentration (1.28 mg/10 mL) tested in spiked milk. The 1.28 mg of added oleamide/10 ml of milk was easily recognized by HPLC and had a high recovery (102.7%). Therefore, transfer of dietary oleamide to milk either was nonexistent or extremely low to avoid detection by the HPLC assay. These results are consistent with previous reports that fatty acyl amides are not absorbed intact. Cleavage of the amide bond in fatty acyl amides was proposed to occur by action of intestinal amidases (20) or proteolytic enzymes in pancreatic fluid (5).

CONCLUSIONS
Results from this investigation support previous reports that oleamide fed to lactating dairy cows substantially increases monounsaturated fatty acid concentration in milk primarily at the expense of palmitic acid. Changes in milk fatty acid composition were not accompanied by the appearance of amide in milk. Oleamide between 0 and 5% of the diet DM linearly reduced DMI, but milk yield was not numerically reduced until diet amide concentration exceeded 2%. Therefore, dairy cows fed diets containing 2 to 3% oleamide substantially increased the milk C18:1/C16:0 ratio without greatly affecting milk yield.

ACKNOWLEDGMENTS
Appreciation is extended to Meredith Harmon and Jennifer Clarke for their assistance with animal care and feeding and to Evanne Thies for assistance with analysis of samples. The donation of oleamide and partial financial support provided by Church & Dwight, Inc. (Princeton, NJ) are gratefully acknowledged.

REFERENCES