Effects of Metal Amino Acid Chelates or Inorganic Minerals on Three Successive Lactations in Dairy Cows

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ABSTRACT
The residual or long-term effects of organic minerals on consecutive lactations has not been studied to date. This study compared the effects of copper, magnesium, zinc, and manganese as amino acid chelates (AACs) or as inorganic minerals (IOMs) on milk and milk component production through three lactation periods in Holstein cows. A high-production herd was divided into two groups. One group (n = 64) received AACs for the first 180 days of lactation. The second group (n = 65) received equivalent amounts of metals as oxides of magnesium, zinc, manganese, and copper sulfate for the first 180 days of lactation. The AAC group had significantly higher (P<.005) total milk production (kilograms of milk) and increasingly more milk in each successive lactation period compared with the IOM group (P<.005). The AAC group also produced significantly more milk fat by weight when milk was corrected to 3.5% fat (P<.05). Milk protein was consistently higher in the AAC group. Feeding AACs favorably affected genetic potential for milk production, with significant increases in predicted transmitting ability (P = .08). Body condition scores were also affected, with differences becoming significant by the third lactation (P<.01). Feeding dairy cows metal AACs improved dairy milk and milk component production, with the largest improvements occurring in later lactation periods.

INTRODUCTION
Bovine milk production is partially dependent on the cow’s mineral nutrition. When a cow is deprived of optimum amounts of essential minerals necessary for milk production, the system will initially cannibalize its own body stores. Once those mineral reserves are depleted, milk production will decline more rapidly than would be predicted in a normal milking cycle in which adequate mineral nutrition is provided.

Although organic minerals [complexes, proteinates, and amino acid chelates (AACs)] are reported to be more bioavailable than minerals from inorganic sources, very little has been published relating to their influence on milk production. Campbell et al reported that a supplement consisting of copper lysinate together with manganese and zinc methionate failed to increase milk yield or alter the milk compo-
nents in either Holstein or Jersey cows. Smith et al. reported that feeding supplemental zinc methionate to Holstein cows whose feed already contained four times the National Research Council’s zinc recommendation increased production of 3.5% fat-corrected milk ($P < .05$).

In another study, 354 dairy cows were divided into two groups. One group was fed zinc, manganese, and copper proteinates. The other group received an equivalent amount of the same minerals as inorganic salts (IOMs). Both supplements were fed for 120 days. Investigators reported that daily milk yield was lower from the proteinate group [49.5 lb (22.3 kg)] than from the IOM group [50.4 lb (22.7 kg)]. During the first 90 days of the study, the proteinate group’s daily milk production also declined more rapidly than did the group receiving IOM. In the last 30 days of the study, however, this trend reversed and the IOM group’s milk production declined more rapidly. The differences in decline were significant ($P < .01$).

Bonomi et al. reported that a supplement consisting of copper, iron, zinc, manganese, and calcium as AACs increased milk production in Fresian cows compared with controls, with greater divergence between groups occurring in the latter months of lactation. There were also significant ($P < .05$) increases in milk fat and protein production within 1 month following commencement of supplementation.

In a small trial at Bombay Veterinary College, milk production in crossbred (Gir x Holstein x Jersey) cows supplemented with a mixture of copper, cobalt, zinc, manganese, and magnesium AAC was measured against control cows for 90 days. Total milk production increased 15.5% ($P < .01$) and milk fat increased from 3.67% to 4.16% ($P < .01$). The percentage of milk protein was the same for both groups ($P < .10$).

Each of the above studies focused on changes in milk and milk component production during a single lactation. They provided no data on the residual or long-term effect of organic minerals on consecutive lactations. Consequently, a double-blind study extending through three lactations that compared both the immediate and long-term effects of AAC and IOM supplements on milk and milk component production was designed.

**MATERIALS AND METHODS**

**Test Animals and Facilities**

One hundred thirty-five registered Holstein cows from a midwestern US commercial dairy farm were initially included in the study, 66 in the IOM group and 69 in the AAC group. Using Wisconsin Dairy Herd Improvement Association (DHIA) records, the animals were matched as evenly as possible, based on milk production and number of lactations. Each cow was assigned to one of two groups and was identified by a tag on a chain around the neck. Six cows were culled (1 IOM and 5 AAC) when they failed to reconceive following the first lactation period, leaving 129 cows to complete the study (65 IOM, 64 AAC). This particular herd had a rolling herd average of 10,682 kg of milk per year at the start of the study.

The facility contained a stanchion barn with an 84-head capacity. The barn was divided into two blocks, with each block color coded for treatment group: one for the IOM group and the other for the AAC group. As each cow freshened and entered the milking herd, it was stalled in a stanchion within the assigned block, which had been vacated by a cow that had recently dried off. Each cow was milked three times daily and trained to go to the same stanchion for every milking.

**Supplements**

Two isomineral, isonitrogenous supplements were prepared and pelleted. All minerals used in the supplements were approved Association of American Feed Control Officials (AAFCO) sources with assigned International Feed Number (IFN). The mineral sources in the IM supplement were copper sulfate (IFN 6–01–717), manganese oxide (IFN 6–03–054), zinc oxide (IFN 6–05–553), magnesium oxide (IFN...
The copper (IFN 6–20–983), manganese (IFN 6–20–986), zinc (IFN 6–20–987), and magnesium (IFN 6–20–985) AACSs as well as the potassium amino acid complex (IFN 6–32–059) fed to the AAC group were supplied by Albion Advanced Nutrition. Each time the supplements were produced and prior to pelleting, mineral composition was verified by inductively coupled plasma mass spectrometry (ICP-MS; Hewlett-Packard 4500, Agilent Technologies). Required mineral adjustments (if any) were made prior to pelleting. Following pelleting and prior to feeding, both supplements were reassayed by ICP-MS to validate the quantitative presence of each test mineral. The amount of mineral per kilogram of supplemental pellets fed in the first 90 days (first trimester) of lactation was 1600 mg zinc, 780 mg magnesium, 400 mg copper, 780 mg manganese, and 780 mg potassium. The pellets fed in the second 90 days (second trimester) of lactation contained the following minerals per kilogram of pellets: 1120 mg zinc, 546 mg magnesium, 280 mg copper, 546 mg manganese, and 546 mg potassium. The protein content of both pelleted supplements was adjusted to be the same in all of the pellets and was between 18.5% and 18.6% as confirmed by Dumas combustion analysis (LECO CNS-2000, LECO Corporation). The pelleted supplements were packaged in identical bags and labels except for the color of the feed tags. The feed tag color corresponded to the assigned color of the block in the barn to help ensure that each animal received the correct mineral supplement. Since the level of minerals in the supplements changed from the first to the second trimester of lactation, the bags also identified whether the supplements were to be fed during the first trimester or second trimester.

The assigned supplement was provided to each cow starting as it freshened. As a top dressing during milking, 454 g of the supplement was hand-fed daily to each cow. This supplement was divided into three equal parts and one third administered at each milking period during the day. The supplement containing the higher level of minerals was fed for the first 90 days of lactation (first trimester). During the second 90 days of lactation (second trimester), 1 kilogram of supplement, which contained 70% of the quantity of minerals fed in the first trimester, was fed to each cow daily. Table 1 summarizes the amount of supplemental mineral fed to each cow per day during the first two trimesters of lactation.

At the conclusion of the second trimester of lactation, the supplemental pellets were discontinued in both groups, and all cows were fed similarly throughout the remainder of the lactation and dry cow periods.

When a cow freshened for the second and third lactations, the same nutritional treatment as the prior lactation and the above supplement program was repeated for the first 180 days of that new lactation period. The total study period was 40 months to allow each cow to complete her third lactation period.

Other than feeding the pelleted supplements containing different sources of minerals for the first 180 days of lactation, all animals were managed similarly. All cows, regardless of mineral treatment, received the same feeds normally fed by the producer throughout the lactation and dry phases. Water was provided ad libitum. Routine health care was the same for each animal.

**Data Analysis**

Lactation records for each cow were generated by the Wisconsin DHIA. The data were analyzed statistically using Systat Version 10 (Systat Software, Inc.). Analysis
of variance (ANOVA) was performed and, because all of the cows in the herd were registered, it was also possible to use the genetic indices predicted transmitting ability (PTA) and estimated relative producing ability (ERP A) as covariants to conduct an analysis of covariance (ANCOVA) on the effect of each source of minerals on genetic potential. \(^{18}\) Body condition was scored for each cow monthly by the same trained individual. \(^{19,20}\) All people involved in handling the cattle and generating and analyzing the data were kept blind as to the sources of minerals in the treatments.

**RESULTS**

There was a clear interaction between the lactation number and the source of the supplemental minerals \((P = .027)\). With each successive lactation period, the difference in total milk production from cows receiving AACs became greater compared with cows receiving IOM. As shown in Table 2, mean daily milk production per cow in the first lactation was 32.5 kg for the IOM group and 32.64 kg for the AAC group/head/day. In the second lactation period, the IOM group produced an average of 35.14 kg milk/head/day compared with 36.09 kg in the AAC group. In the third lactation, average daily milk production per head per day was 36.09 kg in the IOM group and 40.23 kg in the AAC group, an 11.5% difference \((P < .005)\).

When total milk production for the three lactations was combined, the difference between the two groups was significant \((P < .005)\). Average daily milk production increased 10.9% from the first lactation to the third lactation in the IOM group and 23.3% in the AAC group.

When milk production from the two groups was adjusted to 3.5% fat, the IOM group produced an average of 35.38 kg of milk per head per day compared with 33.85 kg in the AAC group during the first lactation (-4.32%). In the second lactation, average daily corrected milk production was 37.35 kg in the IOM group and 37.95 kg in the AAC group (+1.61%). Average daily corrected milk production in the third lactation was 36.50 kg in the IOM group and 41.38 kg in the AAC group (+13.35%). The total difference between the two groups was significant \((P < .05)\).

There were significant relationships between the genetic potential to produce milk and the source of supplemental minerals. The AAC group produced more milk than genetically similar cows in the IOM group. Consequently, the PTA of the cows in the AAC group was 109.09 kg more milk than the cows in the IOM group \((P = .08)\). PTA is a measure of the expected ability of an animal to produce milk and milk components based on the genetic potential transmitted to the offspring, and suggests that

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**Table 2.** Mean Milk Volume, Milk Fat, and Milk Protein by Lactation for Dairy Cows Given Inorganic Minerals or Metal Amino Acid Chelates as Nutritional Supplements

<table>
<thead>
<tr>
<th>Treatment/Lactation</th>
<th>Milk Production (kg/day)</th>
<th>Milk Fat</th>
<th>Milk Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Corrected to</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5% Fat</td>
<td>kg/day</td>
</tr>
<tr>
<td>Inorganic minerals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32.5 ± 0.3</td>
<td>35.1</td>
<td>3.78 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>35.1 ± 0.4</td>
<td>37.3</td>
<td>3.72 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>36.0 ± 0.3^a</td>
<td>36.4</td>
<td>3.54 ± 0.03</td>
</tr>
<tr>
<td>Metal amino acid chelates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32.6 ± 0.3</td>
<td>33.8</td>
<td>3.63 ± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>36.0 ± 0.3</td>
<td>37.9</td>
<td>3.68 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>40.1 ± 0.3^b</td>
<td>41.3</td>
<td>3.60 ± 0.03</td>
</tr>
</tbody>
</table>

Values for the two treatments within variables and lactation having different superscripts are significantly different \(^{a,b}P < .005; ^{c,d}P < .05\).
AACs improved the genetic potential to produce milk by approximately 0.36 kg more milk per day. After receiving the AAC supplement, cows in the AAC group also had an ERPA of 431.36 kg more milk compared with cows in the IOM group \((P = .16)\). This equates to approximately 1.41 kg more milk per day. ERPA estimates the cow’s genetic tendencies expressed in PTA but also measures the permanent environmental effects (i.e., the source of mineral supplementation on milk production, which is expressed as a deviation from herdmates).

As a percentage of total milk volume, the source of the minerals had no significant effect on fat production (Table 2). The average fat content in the first lactation was 3.78% (IOM) and 3.63% (AAC); the second lactation was 3.72% (IOM) and 3.68% (AAC); and the third lactation was 3.54% (IOM) and 3.60% (AAC).

The AAC group produced significantly more milk per lactation than the IOM group. Consequently, on a weight basis, each cow in the IOM group produced an average 1.23 kg of fat per day in the first lactation, 1.21 kg per day in the second lactation, and 1.28 kg per day in the third lactation. Each cow in the AAC group produced an average of 1.19 kg per day in the first lactation, 1.33 kg per day in the second lactation, and 1.45 kg per day in the third lactation. While the average weight differences in fat production per day between the cows in the IOM group and AAC group remained insignificant in the first lactation, the average differences between the kilograms of fat each animal produced per day in the two groups in both the second and third lactations were each significant \((P<.05)\).

Based on milk volume, there were no significant differences in the percentage of milk protein in any lactation period (Table 2) between the groups. The milk from the IOM group had 3.09%, 3.11%, and 3.01% protein in lactations 1, 2, and 3, respectively. The milk from the AAC group contained 3.08%, 3.09%, and 2.97% protein in lactations 1, 2, and 3, respectively. The trend toward increased amounts of protein in the milk from the second lactation followed by a decrease in the third lactation was similar for both groups.

When the quantity of milk protein was measured by weight, the AAC group’s milk contained more protein (Table 2). The IOM group produced an average of 1.00 kg protein per head per day in lactation 1, 1.01 kg per day in lactation 2, and 1.09 kg per day in lactation 3. The AAC group produced an average of 1.24 kg per head per day in lactation 1, 1.11 kg per day in lactation 2, and 1.20 kg per day in lactation 3. The AAC group produced 23.5% more protein per day in lactation 1, 9.9% more per day in lactation 2, and 10.0% more in lactation 3 than did the IOM group \((P<.05)\).

Evaluation of DHIA records indicates no difference in somatic cell count in either group in any lactation period. (Data not shown.)

A trend was observed in cows that received the AACs to achieve higher body condition scores sooner than the animals receiving IOM, and to maintain better body condition scores for longer periods of time. With each successive lactation period, the differences between the body condition scores for the two groups became greater and were significant by the third lactation \((P<.01)\). Figure 1 shows the differences between the two groups at the commencement of the third lactation period and continuing for one year.

**DISCUSSION**

These data show that metal AACs have a significant effect on the quantity of dairy cow milk and its components produced over three successive lactations compared with inorganic minerals. When cows received AACs, there was a significant increase in the total amount of milk produced in each lactation as well as the total amount of milk produced overall. The source of mineral supplementation had an effect on milk production. Intake of more bioavailable sources of minerals results in increased milk production.

With each successive lactation the differences in body condition and milk production
between cows receiving AACs and IOMs became greater. This divergence might indicate that when the amino acid chelated form of minerals is fed, there is more rapid mineral absorption and replenishment of the minerals into body stores for future use than is possible with IOMs. Although their studies were not performed in cattle, Pineda et al demonstrated that when therapeutic doses of iron were ingested daily as either ferrous sulfate or iron AACs for 30 days, both sources of iron were able to elevate hemoglobin levels, but only the AAC form of iron was able to replenish depleted ferritin levels in that short time frame.21,22 The same effect is possibly being observed in the current study based on the higher body condition scores of the AAC group. The difference between the two groups became significant by the third lactation (P<.01). All of the cows were deprived of supplemental minerals for approximately half of each lactation period, which may have necessitated mineral cannibalizing if insufficient mineral nutrition were obtained from the feed alone. While no tests were conducted to validate this supposition, it is possible that cannibalization occurred in the IOM group because in each successive lactation period, the AAC group’s body condition continued to improve at a more rapid rate than did the IOM group. Because the body condition scores of the two groups were similar at the beginning of this study, the subsequent higher scores in the AAC group suggest that as the study progressed, cows in the AAC group ultimately developed greater mineral reserves that were drawn upon for use in improved body condition and increased milk production. The difference in mineral reserves can only be attributed to the difference in mineral sources.

Body condition scores became significant (P<.01) during the 12 months following the start of the third lactation. As each group commenced the third lactation, the overall body condition score of the AAC group was slightly higher than that of the IOM group, possibly suggesting larger initial mineral reserves in the bodies of the AAC group, presumably as a consequence of supplementing AAC in the first two lactations. Even though supplementation of minerals commenced at the beginning of month 1, body condition scores of both groups dipped in the second and third months, suggesting the mineral reserves were probably being depleted more rapidly than they could be replenished from either the feed or feed supplement due to milk production generally being the greatest in the first trimester of lactation. In spite of this, the AAC group’s body condition score remained superior to that of the IOM group, suggesting greater mineral absorption from the chelate source and less consequential cannibalizing during that supplementation period. At 6 months, when mineral supplementation was discontinued, the AAC group continued to manifest improved body condition scores throughout the remainder of the lactation period and into the dry cow phase, suggesting that greater mineral reserves or a more efficient metabolism resulting from the increased activity of the AACs had been established during the supplementation period compared with IOMs. These inherent differences were sufficient to
support not only significantly improved body condition scores but also significantly more milk in the AAC group. The IOM group’s body condition scores did not improve as rapidly as the AAC group, suggesting that the mineral reserves in this group were being depleted at a higher rate or more completely than the AAC group. It appears that the cows that received the IOMs were unable to establish sufficient mineral reserves necessary to support the higher milk production observed in the AAC group even though they were potentially genetically capable of about the same production. Consequently, the IOM group entered each successive lactation period with a lower body condition score than did the AAC group. This may be due to lower bioavailability of IOMs compared with AACs. Mineral bioavailability and metabolism trials are needed to clarify this.

This study suggests a second area of research related to body conditions scores. It is not known whether the improved body condition reflects the direct or indirect role(s) of the minerals. Investigators have reported that when AACs are supplemented, metabolism of both carbohydrates and amino acids from maize, barley, wheat, corn silage, or alfalfa hay-based diets was significantly increased \((P<.05)\).\(^{23}\) Metabolizable energy from these same diets was also significantly increased \((P<.05)\).\(^{23,24}\) Additional research is required to ascertain the precise role of the AACs in improving body condition scores and how this relates to improved milk and milk component production.

The current study focused on the effect of supplemental minerals from two sources, which were fed during the first 180 days of lactation, when milk production was greatest, for three successive lactations. Different results might have been obtained had the two mineral supplements been fed continuously throughout the lactation period and perhaps even the dry periods. Future research could elucidate this.

This study suggested that with each successive lactation, greater amounts of fat were being produced in the milk from cows receiving AACs. As a percentage, there was no significant difference in milk fat between the IOM and AAC groups, but the AAC group produced significantly more milk \((P<.005)\). When the amount of milk in the two groups was equalized to 3.5% fat, the differences between the two groups became significant \((P<.05)\) in each lactation, with the differences between the groups becoming greater with each successive lactation. When milk production for the two groups was equalized, the AAC group produced 8.2% less (lactation 1), and 0.3% (lactation 2), and 15.5% (lactation 3) more fat than did the IOM group. Studies should be designed to more closely examine the effect of AACs on fat production in milk.

When the percentage of protein in the milk was examined, there was no significant difference between the two groups because of the greater volume of milk being produced by the AAC group. Nevertheless, the AAC group produced more kilograms of protein. When the quantity of milk was equalized, as described above, the AAC group was clearly producing at least 10% more protein per lactation than the IOM group, suggesting that AAC had an effect on milk protein production. On the basis of weight, the AAC group produced more protein in each lactation \((P<.05)\). This potential effect of AAC on protein production deserves further investigation.

**CONCLUSION**

When high-producing cows were fed a supplement containing copper, manganese, zinc, and magnesium amino acid chelated minerals for the first 180 days of lactation for three successive lactations, by the third lactation there was a significant increase in milk production compared with equivalent cows fed the same minerals from inorganic sources. The larger volume of milk from the AAC group resulted in significant increases of milk fat and protein (by weight) and suggested a possible trend favoring a greater percentage of fat and protein in milk from cows receiving the AACs. Milk and milk component production differences between the IOM...
REFERENCES


