Effects of Metal Amino Acid Chelates on Milk Production, Reproduction, and Body Condition in Holstein First Calf Heifers

H. Dewayne Ashmead, PhD
Stephen D. Ashmead, PhD
Royce A. Samford, PhD

Albion Advanced Nutrition
101 North Main Street
Clearfield, Utah 84015

KEY WORDS: first calf heifers, metal amino acid chelates, inorganic minerals, milk production, milk fat, milk protein, open days, services per conception, body condition scores

ABSTRACT

First calf heifers are still growing through their first lactation and second gestation, and consequently have higher mineral requirements than mature cows. This study demonstrates that first calf heifers produce more milk and milk components with fewer open days (days from parturition to estrus) and better breeding efficiency, along with higher body condition scores, when supplemented with metal amino acid chelates (AACs) than herdmates supplemented with inorganic minerals (IOMs). Two identical groups of Holstein first calf heifers (24 and 22 heifers, respectively) were given supplements containing copper, manganese, zinc, magnesium, and potassium as either IOMs or as metal AACs. Supplementation commenced upon calving and continued for 180 days. The AAC group produced 9.3% more total milk fat ($P <. 01$) than the IOM group due to greater milk production. Milk protein was 3.01% in the IOM group and 3.06% in the AAC group, with the total protein production 8.1% greater in the AAC group ($P < .05$). Mean number of open days was 130.5 days (IOM) and 89.0 days (AAC) ($P < .01$). The IOM group required 2.58 services per conception compared with 1.50 in the AAC group ($P < .01$). Beginning with parturition and continuing through 11 months of lactation, the AAC group had higher body condition scores ($P < .01$). It was concluded that AACs were more bioavailable than IOMs. Heifers supplemented with AACs demonstrated greater mineral utilization from this source than from IOMs. The improved mineral nutrition from the chelate supplement enhanced milk production, reproduction, and body condition compared with herdmates given IOMs.

INTRODUCTION

Modern dairy production encourages breeding Holstein replacement heifers at about 407 days of age or 352 kg of weight.[1] Though sexually mature, these animals are not full-grown. During first pregnancy, average daily weight gain is about 0.87 kg/day, due in part to the growing fetus; the rest of the weight gain is dedicated to the heifer's own growth.[1,2] An average first calf heifer's postpartum weight should be 77% to 83% that of a mature cow, and she should continue to gain between 0.136 kg/day and 0.148 kg/day during her first lactation.[1,3] Because of a first calf heifer's unique nutritional needs, her nutrient requirements are greater than those of mature cows.[3]

In addition to vitamin, protein, and energy requirements, the first calf heifer also needs certain trace elements including zinc, copper, and manganese, plus the microelements magnesium and potassium for lactation and reproduction concurrent with growth and maintenance of body tissues.[4-18] During periods of dietary mineral insufficiencies, essential minerals will be cannibalized from body tissues to support milk production, which ultimately affects the quality and quantity of milk as well as reproduction.[19-22]

Using x-ray dispersion microanalysis, Manspeaker and Robl assayed tissue biopsied from the medial surfaces of the left and right uterine horns and the body of the uterus as close to the cervix as possible in fertile and infertile cows.[20] They reported that uterine tissue of infertile cows was manganese deficient ($P < .05$) with a trend (not significant) toward zinc deficiency ($P < .10$). Conception occurred only in uterine horns with manganese sufficiency, thus establishing a direct relationship between the concentration of manganese in uterine tissue and fertility. Quantitatively, there was also less copper, magnesium, and potassium in the uterine tissue of infertile cows.[20]

In a follow-up study, Manspeaker et al[23] demonstrated that supplemental metal amino acid chelates (AACs) were more bioavailable than inorganic metal salts (IOMs) and resulted in improved reproductive performance. Thirty days before expected parturition they supplemented the feed of first calf heifers with AACs or IOMs and continued supplementation into lactation until each of the 40 animals in the study became pregnant with a second calf. Heifers receiving the AAC supplement conceived 48 days earlier than heifers receiving the IOM supplement (90 days versus 138 days) ($P < .05$) and experienced 45% less early embryonic mortality (EEM) ($P < .05$).[23]

Other researchers have also reported that AAC supplementation enhanced reproductive performance, but their studies have tended to focus on multiparous cows.[24-26]

This research considers the unique mineral needs of the first calf heifer and examines the effects of different sources of minerals on lactation, reproduction, and overall body condition in these animals.

MATERIALS AND METHODS

A double-blind study was conducted on a commercial dairy farm containing a stanchion barn capable of being divided into 2 color-coded blocks (green and red). Fifty registered Holstein first calf heifers in various stages of gestation were assigned to 1 of 2 groups: 25 in the IOM group and 25 in the AAC group, blocked by age, weight, and expected parturition date. An analysis of variance (ANOVA) indicated no significant differences between these groups ($P > .05$) at the start of the study. All animals calved on their expected calving dates except for 1 heifer in the IOM group, which calved 25 days past her expected calving date. Each animal was identified by a tag on a chain around the neck indicating the assigned group and matching the block color in the barn. Three heifers in the AAC group and 1 in the IOM group were subsequently sold by the producer before calving, leaving a total of 46 heifers to complete the study (24 IOM, 22 AAC).

Based on data developed by Manspeaker and Robl[20] relating to mineral insufficiencies in the uterine tissues of infertile cattle, two isomineral, isonitrogenous grain-based pelleted supplements containing either IOM or AAC were formulated. Using Association of American Feed Control Officials (AAFCO) recognized mineral sources with assigned international feed ingredient numbers (IFNs), the IOM mineral sources were copper sulfate (IFN 6-01-717), manganese oxide (IFN 6-03-054), zinc oxide (IFN 6-05-553), magnesium oxide (IFN 6-02-756), and potassi-

Table 1. Mineral supplementations fed per day as a top dressing to first calf heifers during the first 180 days of their first lactation

Mineral	0–90 days lactation (mg/head/d)	91–180 days lactation (mg/head/d)
Copper	181	127
Manganese	363	255
Zinc	727	511
Magnesium	355	248
Potassium	355	248

um chloride (IFN 6-03-755). The AAC mineral sources were copper (IFN 6-20-983), manganese (IFN 6-20-988), zinc (IFN 6-20-987), and magnesium (IFN 6-20-987); potassium was given as an amino acid complex (IFN 6-32-059) (Albion Advanced Nutrition). Prior to pelleting, mineral quantities in each supplement were verified by inductively coupled plasma mass spectrometry (HP 4500 ICP-MS, Agilent Technologies) using Environmental Protection Agency methods.[28,29] Any required adjustments in mineral content were made based on the analytical results. Crude protein content was confirmed by Dumas combustion analysis (LECO CNS-2000, LECO Manufacturing), and any needed adjustments to protein content were made.[29] Following pelleting, but before feeding, supplements were reassayed to confirm the amount of each test mineral and the protein content.

The pelleted supplements were packaged in identical 24.8-kg bags and labeled with either green or red feed tags that corresponded to the color of the blocks in the milking barn. The experimental design called for the quantity of supplemental minerals to be reduced in the second trimester of lactation (91 to 180 days), followed by discontinuance of supplemental minerals after 180 days of lactation. Consequently, the feed tags also identified in which trimester the supplements were to be fed.

At parturition, each first calf heifer was fed 454 g daily of the pelleted mineral supplement assigned to the animal's group as a top dressing on the feed at milking time. All of the supplement provided to each animal was consumed during the milking period. The heifers in the green group received IOMs, while the heifers in the red group received AACs. Table 1 summarizes the quantities of minerals fed daily from Day 1 to Day 90 and from Day 91 to Day 180 of lactation. In the second trimester of lactation, the supplement contained 70% of the quantity of minerals provided daily in the first 90 days. At the conclusion of 180 days of lactation, the experimental mineral supplements were discontinued and all heifers were fed similarly throughout the remainder of their lactation.

Other than feeding the pelleted supplements containing different mineral sources for the first 180 days of lactation, all animals were handled similarly. All heifers received the same daily ration of food that was routinely supplied. Water was provided ad libitum.

Statistical analysis

The data generated by this study were grouped into 3 categories: lactation, reproduction, and body condition. Lactation data included the quantity of milk, milk fat (butterfat), milk protein, and somatic cell count. Raw data were obtained from Wisconsin Dairy Herd Improvement Association (DHIA) records. These data were analyzed by using two-way analysis of variance test (ANOVA) with treatment and time as the main effects on body condition scores using Systat version 10 (Systat Software, Inc.). Student *t*-tests were conducted on quantity of milk, milk fat, milk protein, and somatic cell count.

Reproduction data included the number of open days, services per conception, rate of EEM, uterine horn size, and number of cases of cystitis and metritis. The same veterinarian was used throughout the study. He examined each freshened heifer within 2 weeks of calving with follow-up examinations conducted every 2 weeks until confirmation of second pregnancy.

Table 2. Milk and milk component production data in first calf heifers given supplements of either inorganic metals (IOMs) or amino acid chelates (AACs) in a 305-day milking period

Study groups	Mean milk production, 305 days (kg)	Mean production/d for 305 days (kg)	Mean milk fat (%)	Mean total milk fat (kg)	Mean milk protein (%)	Mean total milk protein (kg)
IOM group	10,047 ± 1456.36	32.94	3.57 ± 0.48	355.9 ± 46.11[a]	3.01 ± 0.21	302.1 ± 40.76[c]
AAC group	10,568 ± 911.76	34.65	3.76 ± 0.35	389.1 ± 47.02[b]	3.06 ± 0.17	326.4 ± 29.89[d]
% difference IOM/AAC	5.2%	5.2%	5.32%	9.3%	3.3%	8.1%

[ab]Significant difference at $P < .01$.
[cd]Significant difference at $P < .05$.

The effect of each mineral source on the number of open days from calving to estrus was tested by the Student *t*-test. Conception data were analyzed using logistic regression analysis in SAS statistical software (SAS Institute). Differences in the incidences of cystitis for the two groups were tested using a Chi-square test and logistic regression.

Throughout the study, each animal's body condition score was determined monthly by the same trained individual[30,31] and differences analyzed by both Student *t*-test (data for each month) and ANOVA to determine the effects of mineral source on body condition (all data).

In all statistical tests, an alpha level of 0.05 was set for determining statistical significance. A *P*-value of less than 0.01 was considered highly significant.

All individuals involved in handling the animals or in generating or analyzing data were kept blind as to which source of minerals (IOMs or AACs) was fed to which heifer.

RESULTS

Milk Production

There appeared to be a relationship between milk production and mineral source. In a 305-day milking period, the IOM group's mean total milk production was 10,047 kg compared with 10,568 kg in the AAC group.

On a percentage basis, the IOM group's milk contained 3.57% ± 0.48% fat. The AAC group's milk contained 3.76% ± 0.35% fat. On a weight basis, the IOM group's mean total production of milk fat was 355.9 kg compared with 389.1 kg for the AAC group. The latter difference was highly significant ($P < .01$).

Milk in the IOM group contained 3.01% ± 0.21% protein while the AAC group's milk contained 3.06% ± 0.17% protein. The IOM group's mean milk protein production was 302.1 kg compared with the AAC group's mean production of 326.4 kg, an 8% increase ($P < .05$). Milk production data are summarized in Table 2.

The monthly somatic cell counts in the AAC group were lower than in the IOM group, but the differences were not significant.

Reproductive Performance

The month of lactation from commencement of lactation until the heifer became pregnant for the second time had no effect on reproductive performance. Reproduction was, however, affected by the source of supplemental minerals. The mean number of days from parturition to estrus in the IOM group was 130.5 days. In the AAC group the mean number of days from parturition to estrus was 89.0 days. This 31.8% reduction in number of open days was highly significant ($P < .01$).

The IOM group required a mean of 2.58 services per conception compared with 1.50 services per conception in the AAC group, a 41.9% reduction ($P < .01$).

Following confirmed conception in the second pregnancies, the IOM group had 5 cases of EEM. One heifer in the AAC group experienced EEM.

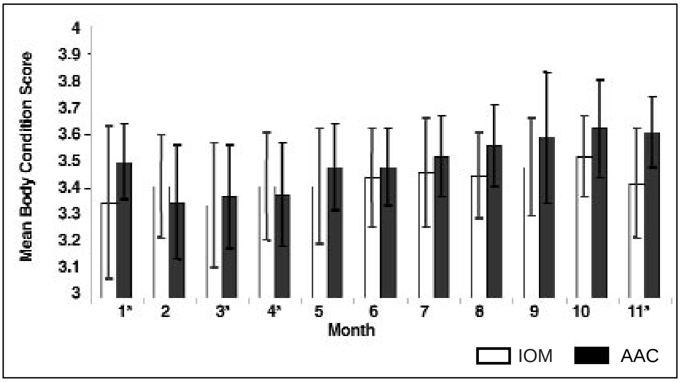

Figure 1. Mean monthly body condition scores in first calf heifers receiving either inorganic mineral (IOM) or amino acid chelate (AAC) supplementation. Mean 11-month difference between groups is highly significant (*P* < .01).

[a]*Significant difference at P < .05.*
[*]*Significant difference at P < .05.*

Heifers in the AAC group had 27% fewer cases of cystitis than did heifers in the IOM group (11 IOM versus 8 AAC). There appeared to be a relationship between the incidences of cystitis and services per conception, but it was not significant (*P* < .09). There also appeared to be a relationship between the incidence of cystitis and uterine horn size, but, again, it was not significant (*P* = .06). At 3 to 7 weeks after calving, mean uterine horn size for the IOM group was 33.7 mm compared with 31.6 mm for the AAC group. Four heifers in the AAC group had larger-than-normal uterine horns on both sides compared with 6 in the IOM group. The size of the uterine horn was inversely related to the incidence of metritis and was highly significant (*P* < .01).

Body Condition

Beginning with parturition and continuing through 11 months of lactation, the monthly body condition scores were recorded for each heifer (Figure 1). Applying the Student *t*-test, the mean monthly body condition scores in months 1, 4, 8, and 11 were significantly different (*P* < .05). When the data were analyzed by 2-way ANOVA with factors of treatment and month, the month of lactation had no effect on these differences, while the source of supplemental minerals had a highly significant effect on the monthly body condition scores of the heifers throughout the entire study period (*P* < .01). Mean 11-month body condition scores of heifers receiving the AAC-containing supplements were significantly higher than heifers receiving the IOM-containing supplement (*P* < .05).

DISCUSSION

Because a first calf heifer is still growing during her first pregnancy and lactation, she requires additional nutrients above those needed by a mature cow producing a comparable amount of milk. If the need for additional nutrient is not addressed during initial pregnancy and lactation, the heifer's nutritional status and body condition can potentially be compromised more dramatically than that of a mature cow. Reduced milk production, lowered milk quality, poor reproduction, or a combination of the three can result.

Sprengel's Law of the Minimum

Using growth as an example, Sprengel's Law of the Minimum states that improving growth by increasing one growth factor (ie, protein, carbohydrate, or lipid) will have no effect on overall growth rates if another growth factor (ie, mineral) is limited or deficient.[32] Optimal mineral nutrition is necessary for efficient use of vitamins, protein, lipids, and carbohydrates for most favorable growth and/or development in a pregnant or lactating heifer. Optimum mineral nutrition does not appear to be as likely when IOMs are supplemented; metal AACs appear to be a more bioavailable source of minerals.[22,25,26,28] When the limiting growth factor is alleviated, through increased absorption of minerals from the AAC source, it results in improved growth (body condition) and/or production and reproduction. This study seemed to demonstrate the application of Sprengel's Law.

Sprengel's Law can also be applied to milk production. Total milk production was increased, although not significantly, as a result of supplementing AACs for the first 180 days of lactation. National DHIA records report that average milk production for first calf Holstein heifers is 27.3 kg/day.[32] Both groups in this study generated more milk [32.7 kg (IOM); 34.6 kg (AAC)] than national DHIA averages for first lactation heifers. They also produced higher percentages of milk components such as fat and protein than national DHIA averages.[32] These results suggest that better-than-average nutrition was being provided to all the heifers used in this study, but data also indicate that the source of supplemented minerals affected both total milk production and milk component production.

The exact metabolic pathway(s) of AACs in increasing milk production or milk components remains to be elucidated. Determining whether the observed improvements are a direct result of the enhanced uptake of minerals from the AAC source or a consequence of increased amounts of minerals interacting with other nutrients essential for milk and milk component production requires further study. It is clear, however, that supplementing dairy feed with AACs tended to increase both total milk production and production of milk components in the first calf heifers more than was possible with IOM supplementation. Other studies have also confirmed this.[24, 26]

Reproduction

The number of open days in the IOM group (130.5 days) was about the same as the DHIA national average (139 days) for first calf heifers.[32] Herdmates in the AAC group had significantly ($P < .01$) fewer open days (89 days) than the IOM group, a difference of 41 days. The AAC group became pregnant 50 days sooner than the first calf heifers used to compute the DHIA national average.

Mineral supplementation from either the AACs or IOMs was provided to all of the heifers in the study for the first 180 days of lactation. All animals in the AAC group had displayed estrus by 113 days (89 ± 24 days), which meant estrus occurred in all the animals in the AAC group before the end of their supplementation period. In the IOM group, estrus occurred in some animals as late as 206 days post-calving and fell outside the supplement period by as much as 26 days. Based on other studies it is postulated that the reduction in the number of open days observed in the AAC group was due to an increased concentration of supplemented minerals in their uterine tissue.[20] This suggests that if the onset of estrus requires, in part, optimal concentrations of minerals in the tissues of the reproductive organs, then the IOM heifers were unable to absorb enough essential minerals from the IOM sources during the supplemental period to achieve the required concentrations of these essential minerals in their uterine tissue, whereas the AAC group did achieve this. More research could clarify this finding.

The source of minerals also affected conception rates. The heifers receiving the supplement containing AAC required 42% fewer services per conception than the IOM group ($P < .01$). The fact that both supplements were formulated with the same mineral concentrations indicates that the source of the minerals was the mitigating factor affecting the difference in services per conception. Manspeaker and Robl[20] and Manspeaker et al[23] demonstrated that better conception rates occurred in first calf heifers receiving AACs because that source of minerals increased the concentration of specific minerals in their uterine tissue. The AAC group in this study received mineral supplementation throughout the time that estrus, rebreeding, and conception occurred. Part of the IOM group's initial estrus and conception occurred after mineral supplementation had terminated. The delay in estrus and the greater number of services per conception in the IOM group may have been a result of lower uterine tissue levels of specific minerals resulting from lower absorption of the IOM source of minerals.

The cessation of mineral supplementation at 180 days of lactation may have induced a reduction in tissue mineral levels that further exacerbated the compromised reproductive performance in the IOM group.

Body condition scores were also affected by the source of supplemental minerals. Most of the significant differences between the two groups occurred in the latter stages of lactation at the time the heifers in the IOM group were still exhibiting estrus and those in the AAC group were pregnant.

Previous research has reported that the level of specific minerals in uterine tissue will also affect uterine horn size following parturition. The size of the uterine horn is also related to the incidences of cystitis and metritis, which in turn affect conception.[19,20,23]

When measured at the time of confirmed second pregnancy, the mean size of the uterine horns in the AAC group was 6.2% smaller than those in the IOM group, with 33% more heifers in the IOM group having larger uterine horns on both sides than in the AAC group. The results of this study suggest that the source of the supplemental minerals affected uterine horn size, which in turn affected reproduction.[19,20,23] The exact mechanism of how more bioavailable minerals influence the size of the uterine horns is unknown at present and should be the subject of a future study.

There was an indication of a positive correlation between the size of the uterine horn and the incidence of metritis. This suggests another area for additional study.

Investigators have previously reported that EEM was significantly reduced when AAC supplements were provided ($P < .05$).[20, 23] In the current study, 21% of the heifers in the IOM group aborted one or more times. Only one animal in the AAC group experienced EEM. While the data regarding the influence of AACs on EEM seem to confirm the findings of earlier studies, additional research is indicated to elucidate exactly why this happens.

Body Condition

Body condition scores of the two groups of heifers (Figure 1) link the different parts of the study. If first calf heifers have high milk output and superior reproduction at the expense of maintaining good body condition, then the future performance of the animals is more likely to be poor. Adequate body condition must be maintained for future productivity. The AAC group had higher milk production, greater milk component production, less cystitis and metritis, and improved reproductive performance, which all appear to be related to enhanced availability of the supplemental minerals from the AAC source of minerals. In addition, the 11-month mean body condition scores of the AAC group were significantly higher than those in the IOM group ($P < .05$). Whether the significantly improved body condition scores are directly related to increased uptake of minerals from the AAC supplement or the synergistic effect of increased absorption of AAC with other nutrients necessary for the growth and maintenance of body tissues (Sprengel's Law of the Minimum) requires further study.

When inorganic mineral sources were substituted for the AAC minerals in the supplements, first calf heifers, from the same genetic stock, who were housed together and received the same dairy feed except for the source of supplemented minerals, were not capable of equivalent performance. Regardless of how or why this occurred, this study demonstrates that when AACs are given as supplements for the first 180 days of lactation, performance as expressed by milk production (either amount or components), reproduction, and body condition improved, and those improvements had to be related to the source of the minerals because that was the only variable in the test.

The study design called for mineral supplementation for only 180 days of the first lactation period. The heifers continued to grow, lactate, and in some of the IOM group animals, commence second estrus and conception after discontinuing mineral supplementation. What effect, if any, continuous supplementation would have had on the performance of these heifers in either group is unknown. This suggests the need for a future study in which the supplemental minerals are fed continuously.

Finally, this study employed specific minerals in certain concentrations that were based on previous research observations. It is unknown what, if any, differences might have occurred if other minerals had been supplemented or if different concentrations of minerals were supplemented (eg, dose-response). Additional studies can be designed to answer these questions.

CONCLUSION

This study demonstrated that supplementing minerals in the AAC form rather than in the IOM form resulted in more milk production, and significantly more total milk fat and total milk protein in first calf heifers. Both the number of open days from first calving to the subsequent estrus and the number of services per conception were significantly reduced when first calf heifers received supplemental minerals as AACs. Uterine horn size was smaller in the heifers supplemented with AACs and those same animals exhibited decreases in cystitis, metritis, and a reduction in EEM. In spite of the higher relative mineral demands necessary to support increased milk production and improved reproduction, the body condition scores of the first calf heifers receiving metal AACs were higher than herdmates fed equivalent amounts of the same minerals in the IOM form. This suggests that the heifers in the AAC group were obtaining more usable mineral nutrition from the supplements containing AACs than were the heifers that received supplements containing IOMs.

REFERENCES

1. VanAmburgh ME, Fox DG, Galton DM, Bauman DE, Chase LE. Evaluation of National Research Council and Cornell net carbohydrate and protein systems for predicting requirements of Holstein heifers. *J Dairy Sci.* 1998;81:509–526.
2. Hoffman PC. Optimum body size of Holstein replacement heifers. *J Anim Sci.* 1997;75:836–845.
3. Schutz LH. Relationship of rearing rate of dairy heifers to mature performance. *J Dairy Sci.* 1969;52:1321–1329.
4. Zin Z, Waterman DF, Neinken RW, Harmon RJ. Copper status and requirement during the dry period and early lactation in multiparous Holstein cows. *J Dairy Sci.* 1993;76:2711, 2716.
5. Walravens PA, Hambidge KM. Growth of infants fed a zinc supplemented formula. *Am J Clin Nutr.* 1976;29:1114–1121.
6. Kirchgessner M, M Weigand. Optimal zinc requirements of lactating dairy cows based on various dose-response relationships [in German]. *Arch Tierenahr.* 1982;32:569–578.
7. Bentley OG, Phillips PH. The effect of low manganese rations upon the dairy cows. *J Dairy Sci.* 1951;34:396–403.
8. Howes AD, Dyer IA. Diet and supplemental mineral effects on manganese metabolism in newborn calves. *J Anim Sci.* 1971;32:141–145.
9. Askew EW, Benson JD, Thomas JW, Emery RS. Metabolism of fatty acids by mammary glands of cows fed normal, restricted roughage on magnesium oxide supplemented rations. *J Dairy Sci.* 1971;4:854–859.
10. Ramberg CT Jr. Kinetic analysis of calcium metabolism in the cow. *Fed Proc.* 1974;33:183–187.
11. Dennis RJ, Hemken RW. Potassium requirements of dairy cows in early and mid-lactation. *J Dairy Sci.* 1978;61:757–761.
12. Mills CF. Trace element nutrition of the dairy herd. *J Royal Assoc Br Dairy Farmers.* 1964;68:1–10.
13. Suttle NF, Angus KW. Effects of experimental copper deficiency on the skeleton of the calf. *J Comp Path.* 1978;88:137–148.
14. Miller WJ. Zinc nutrition of cattle: A review. *J Dairy Sci.* 1970;53:1123–1135.
15. National Research Council. *Nutrient Requirements of Dairy Cattle*. Washington DC: National Academy Press; 2001:139.
16. Rook JAF, Storry JE. Magnesium in the nutrition of farm animals. *Nutr Abstr Rev.* 1962;32:1055.
17. National Research Council. *Nutrient Requirements of Dairy Cattle.* Washington DC:National Academy Press; 2001:109.
18. Weil HJ, Haverland LH, Cassard DW. *Potassium requirement of dairy calves*. *J Dairy Sci.* 1988;71:1868–1872.
19. Manspeaker JE, Robl MG, Edwards GH, Douglass LW. Chelated minerals: Their role in bovine fertility. *Vet Med.* 1987;82:951–956.
20. Manspeaker JE, Robl MG. The Use of Amino Acid Chelates in Bovine Fertility and Embryonic Viability. *Proceedings of the American Association of Bovine Practitioners,* Des Moines, IA, 1984.

21. Betteride K, Miller RB. Embryonic Losses from Conception Today. *Proceedings of the American Association of Bovine Practitioners,* Buffalo, NY, 1985.

22. Ayalon N. A review of embryonic mortality in cattle. *J Reprod Fert.* 1978;54:483–493.

23. Manspeaker JE, Robl MG, Edwards GH. *Prevention of early embryonic mortality in bovine fed metalosates.* Proceedings of the IVth International Symposium of Veterinary Laboratory Diagnosticians, Amsterdam, 1986.

24. Bonomi A, Quarantelli A, Sabbioni A, Superchi P. The chelated trace elements in the feeding of dairy cows. *Progresso Vet.* 1986;41:673–682.

25. Kropp JR. *Reproductive Performance of First Calf Heifers Supplemented with Amino Acid Chelated Minerals.* Animal Science Research Report MP-129. Stillwater: Oklahoma State University; 1990:35–43.

26. Ashmead HD, Samford R. Effects of metal amino acid chelates or inorganic minerals on three successive lactations in dairy cows. *Int J Appl Res Vet Med*. 2004;2:181–188.

27. United States Environmental Protection Agency. *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods.* Final Update III, Method 3050B, US Environmental Protection Agency SW–846; 1997.

28. United States Environmental Protection Agency. *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods*. Final Update III, Method 6010B, US Environmental Protection Agency SW–846; 1997.

29. Sweeney RA. Generic combustion method for determination of crude protein in feeds: Collaborative study. *J Assoc Off Analytic Chem.* 1989;72:770–774.

30. Heinrichs AJ, Ishler VA. *Body Condition Scoring as a Tool for Dairy Herd Management.* College of Agriculture Corporate Extension Circular 363. State College, Penn: Pennsylvania State University; 1989.

31. Wattiax MA. *Body Condition Scores.* Babcock Institute for International Dairy Research and Development. Madison, Wisc:University of Wisconsin, Madison; 1995: 45–48.

32. Dairy Herd Improvement Association. Computing Services Inc. Characteristics of high milk Holstein herds. *Agribusiness Dairyman*. 1996;(November):24–25.