The Effect of Copper, Zinc, and Manganese Amino Acid Chelates on Dairy Cow Reproduction on Eight Farms: A Field Trial

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ABSTRACT

A field trial involving 182 multiparous dairy cows from 8 different farms was designed to compare the effects of Cu, Zn, and Mn amino acid chelates (AAC) against Cu, Zn, and Mn inorganic metal salts (IM) on reproductive performance. Each farm had equal numbers of cows in the AAC group and the IM group. Cows in each group commenced receiving 50 g daily of an isomineral formulation containing AAC or IM following parturition and continuing for the first 120 days of lactation. Mean services per conception was less in the AAC group compared with the IM group (1.50 versus 1.90) ($P < 0.001$). Confirmed pregnancies following first services was 36% greater the AAC group compared with the IM group ($P < 0.05$). In the IM group, 87% of the cows ultimately became pregnant compared with 96% in the AAC group ($P < 0.05$). Based on the retail costs of the 2 mineral supplements, the economic return due to improved reproductive performances was approximately 10:1 in favor of the amino acid chelates.

INTRODUCTION

The effects of metal amino acid chelates (AAC) on reproductive performance in cattle have been studied by several investigators.[1-4]

Manspeaker et al[1] divided 40 first calf Holstein heifers from the University of Maryland dairy herd into 2 groups. They demonstrated that animals receiving a formulation containing Mg, Fe, Mn, Cu, and Zn as AAC exhibited improved fertility ($P < 0.05$) compared with herdmates receiving the same minerals as inorganic metal salts (IM). The AAC group had 75% more mature ovarian follicles with vaginal secretions indicating estrus ($P < 0.05$), 80% less uterine bacterial infections ($P < 0.05$), 83% less periglandular fibrosis ($P < 0.05$), and 45% less early embryonic mortality ($P < 0.05$). Mean first conception occurred 45 days earlier in the AAC group (AAC = 90 days versus IM = 135 days; $P < 0.05$).[1]

Kropp[2] sequestered 40 first calf heifers from the Oklahoma State University herd and put them on the same diet, which included AAC, for 1 year. Following first parturition, the animals were divided into 2 groups (18 AAC and 19 IM). Both groups received identical feed and for the first 75 days of lactation, a mineral supplement containing Mg, Cu, Mn, and Zn as either AAC or IM plus K as an amino acid complex, or IM. Each animal received Synchromate B (Norgestomet) as an injectable and as an implant. Following removal of the implant, 78% of the heifers in the AAC group exhibited estrus compared with 42% in the IM group ($P < 0.05$). Of those exhibiting estrus, 69% in the AAC group conceived on first service compared with 25% in the IM group ($P < 0.05$). The AAC group conceived 19 days earlier than the IM group (86 days versus 105 days).[2]

In a third study, Bonomi et al[3] administered a mineral supplement containing Cu, Fe, Zn, Mn, and Co as AAC or IM to multiparous cows selected from the University of Parma dairy herd. They reported that services per conception were 26% lower in cows receiving AAC (AAC = 1.7 versus IM = 2.15), mean first insemination occurred 16.8 days sooner (AAC = 64.3 days versus IM = 81.1 days), and confirmed conception was achieved 35 days sooner (AAC = 100.65 days versus IM = 130.50 days) in cows receiving AAC during lactation.[3]

In a fourth study, Ashmead et al[4] reported that 46 registered Holstein first calf heifer herdmates receiving the same feed were divided into 2 groups. Following calving, both groups were provided an isomineral supplement formula at milking that contained either AAC or IM sources of Cu, Mn, Zn, and Mg plus K as an amino acid complex, or IM. Mean rebreeding time for cows in the AAC group was 89 days compared with 130.5 days for cows in the IM group ($P < 0.01$). Mean services per conception were 1.50 (AAC) compared with 2.58 (IM) ($P < 0.01$). Uterine horn size at the time of confirmed second pregnancy was 6.2% smaller in the AAC group; this is important because the size of the uterine horn has been related to conception. Early embryonic mortality was less in the AAC group than in the IM group (5% versus 21%, respectively; $P < 0.01$).[4]

All of the above research was conducted between herdmates under controlled conditions. None of the studies considered the effects of AAC on multiparous cows from different farms with different handlers, etc. which would simulate commercial conditions. Consequently, a study was designed to determine if similar reproductive results could be obtained in dairy cows under naturally occurring farm conditions.

MATERIALS AND METHODS

Eight dairy farms from the Brittany area of France were selected for inclusion in this study. Each farm contained between 45 and 65 lactating cows of similar breed (Prim Holstein) with mean milk production ranging between 8,000 and 9,000 kg of milk/year/cow. While not identical, each dairy barn in the study had similar construction and milking equipment. All producers were purchasing a similar commercial feed supplement from the same company. All animals were on a similar forage program.

Animals involved in this study were assigned to either an IM or an AAC group at the time of parturition. The first cow to calve on a farm after study commencement was assigned to the IM group. The second cow to calve was assigned to the AAC group. As a cow entered the study, it was identified as part of either the AAC or IM group by a tag secured around its neck by a chain. The tag also indicated the date the animal entered the study and the completion date for providing the supplemental minerals described below. All cows with obvious pathologies (mastitis, foot problems, ketosis, displaced abomasums, etc) were disqualified from the study.

Each farm had an equal number of cows in the AAC group and the IM group, but not all farms had an equal number of animals participating in the study. The total number of cows involved in the study was 182 (91 AAC and 91 IM). All cows entering the study completed it although some cows from both groups that could not be rebred were ultimately removed from their herds. Their removal did not affect statistical analysis since they were included in the study as non-conceiving cows.

Each group contained cows of all ages and parturition numbers that ranged from first calf heifers to eighth calving (Table 1). An analysis of variance (ANOVA) based on parity and milk production determined that there was no significant difference between the 2 groups of cows ($P = 0.673$).

Table 1. Assignment of Cows to Groups by Parity (lactation number).

Lactation Number/Parity	AAC Group	IM Group
1	25	23
2	28	23
3	18	24
4	12	12
5	5	7
6	1	1
7	1	1
8	1	—

A commercial feed supplement (Zootech, Yffiniac, France) fed at the rate of 250 g/head/day was provided to all cows at milking. It consisted of a soya-based protein concentrate, wheat, and barley plus a vitamin and trace mineral concentrate. The trace mineral concentrate provided 1200 mg Zn, 950 mg Mn, and 150 mg Cu daily, all as IM. Each producer also fed maize silage and grass hay that were grown on the farms of the individual producers. All cows were allowed access to fresh pasture grass. Following calving, each cow in the study received an additional experimental lactation mineral supplement for the first 120 days of the study period that provided an additional 300 mg Zn, 240 mg Mn, and 150 mg Cu daily as either AAC (Albion, Clearfield, Utah) or IM. Both experimental mineral lactation supplements (AAC or IM) were pelleted and then packaged in bags containing instructions as to which supplement was to be fed to which animals and how much supplement to administer. Each cow was hand-fed 50 g of its assigned experimental lactation mineral supplement plus 250 g of the regular commercial feed supplement (described above) daily at milking.

Experimental lactation mineral supplementation commenced at calving and continued for the first 120 days of lactation. Not all of the cows calved at the same time. Consequently, the study lasted approximately 11 months (commencing in August and ending the following June). In this study, spring/summer months were designated as April to September and fall/winter months were October to March.

The reproductive parameters recorded by the producers were mean days to first and last estrus, mean days to first, second, and last services, number of services per conception, and pregnancy. Pregnancy was confirmed by palpation by a veterinarian but recorded by the producer. For statistical analysis, all animals requiring 3 or more services were grouped together. Each producer also reported any observed health problems. The 100-day standardized milk, 100-day standardized fat, and 100-day standardized protein were calculated for both groups. Seasonal effects on some of the above parameters were also studied to determine if either source of minerals ameliorated those effects.

For statistical analysis, all 8 farms were considered to be part of the same population. Data were subjected to analysis of variance using Student's *t*-test. To further test for significance, certain data were rearranged and Pearson Chi-Square analysis applied. A P value of <0.05 was considered significant. A P value of <0.01 was considered to be highly significant. A P value between 0.05 and 0.10 was considered to be trending towards significance.

RESULTS

Supplementing with different sources of an isomineral formula during lactation resulted in several significant differences between groups; AAC supplementation had a positive effect on reproductive performance in cows compared with cows receiving the IM supplement (Table 2).

Table 2. Effect of Mineral Sources on Services per Conception and Pregnancy.

		Mean Services Per Conception	First Service Cows Pregnant		Second Service Cows Pregnant		Third + Services Cows Pregnant		Total Pregnancies		Total Non-pregnancies	
			n	%	n	%	n	%	n	%	n	%
Total Services per Conception and Pregnancies	IM	1.90^{a}	42^{a}	53^{a}	23^{a}	29^{a}	14	18	79^{a}	87^{a}	12^{a}	13^{a}
	AAC	1.50^{b}	57^{b}	66^{b}	15^{b}	17^{b}	15	17	87^{b}	96^{b}	4^{b}	4^{b}
Spring/Summer Services per Conception and Pregnancies	IM	1.93^{a}	22^{a}	52	12^{a}	52^{a}	10	71^{a}	44	48	—	—
	AAC	1.52^{b}	29^{b}	51	9^{b}	60^{b}	8	53^{b}	46	52	—	—
Fall/Winter Services per Conception and Pregnancies	IM	1.86^{a}	20^{a}	48	11^{a}	48^{a}	4	29^{a}	36	39^{a}	—	—
	AAC	1.48^{b}	28^{b}	49	5^{b}	33^{b}	7	47^{b}	40	44^{b}	—	—

$^{a,b}P < 0.05$.

The mean number of days from calving to first reported estrus (when first service occurred) was significantly different between the 2 groups (AAC = 78 days versus IM = 72 days; $P < 0.05$). For those cows that did not conceive on first and second services, the mean number of days from calving to last estrus (3 or more services) was the same for both groups (AAC = 96 days versus IM = 99 days). The difference in the number of days from calving to first estrus seemed to be greater in the spring/summer than in the fall/winter for both groups, but the differences between seasons were not significant.

Mean service per conception in the IM group was 1.90 services compared with 1.50 services in the AAC group ($P < 0.001$). Mean services per conception for both the spring/summer and fall/winter periods were significantly less for the AAC group compared with the IM group ($P < 0.05$). Season did not affect services per conception in the AAC group but trended towards having a significant effect in the IM group ($P < 0.10$).

In total, 36% more cows in the AAC group conceived following first service compared with the cows in the IM group ($P < 0.05$). In the spring/summer period, 32% more cows in the AAC group conceived following first service compared with cows in the IM group ($P < 0.05$). In the fall/winter period, 40% more cows in the AAC group conceived following first service compared with the IM group ($P < 0.05$).

In the IM group, 29% of the group ultimately became pregnant following 2 services, whereas the AAC group had 17% of its total group becoming pregnant following 2 services ($P < 0.05$). The number of cows becoming pregnant following second service was also significantly different between groups ($P < 0.05$). Fewer cows from the AAC group became pregnant following second service compared with the IM group due to a higher number of animals in the AAC group becoming pregnant following first service. As noted with first services, there were fewer pregnancies in both groups in the fall/winter period compared to the spring/summer period. Seasonal effects were significant in both groups ($P < 0.05$).

There was no significant difference between groups requiring 3 or more services. Season, however, appeared to affect conception rates in both groups when 3 or more services were required ($P < 0.05$).

Twelve cows failed to conceive in the IM group, and 4 cows failed to conceive in the AAC group. This difference was significant ($P < 0.05$). When these non-conceiving cows were removed from both groups and services per conception were compared in only those cows that became pregnant, the difference between groups remained significant ($P < 0.05$) with 96% of the AAC group becoming pregnant compared with 87% in the IM group.

While the AAC group had less reported health problems associated with reproduction, the difference in the number of observed problems was not significant between groups.

The 100-day standardized milk production (AAC = 38.9 kg/day versus IM = 38.5 kg/day) and fat production (AAC = 3.216% versus IM = 3.109%) were the same for both groups. The percent of milk protein between groups (AAC = 4.102% versus IM = 4.050%) trended towards significance ($P < 0.10$).

DISCUSSION

This study confirmed that metal AAC have a positive effect on bovine reproduction under commercial field conditions. Many reproductive improvements can accrue by changing nutrition or addressing bovine health or by giving attention to the overall management practices of a producer. This study only looked at one aspect, that of changing mineral nutrition sources. When dairy cows received AAC minerals with higher bioavailability than standard IM,[4,6,7] reproductive performance tended to improve as demonstrated by this and other studies.[1,3,4]

Bonomi et al[3] indicated that mean first estrus occurred in the University of Parma dairy herd 16.8 days sooner when AAC were supplemented compared with cows receiving IM; Kropp[2] reported the difference as being 19 days. This seems to suggest that, contrary to the reported data in this field trial, the number of days from calving to first estrus may be less in cows receiving AAC supplementation. More research is needed to verify this.

It is clear that when AAC are supplemented, they result in a reduction in services per conception. The IM group required an average of 1.90 services whereas the AAC group required an average of 1.50 services for conception to occur ($P < 0.001$). The AAC group's mean services per conception are consistent with published data[3,4] whereas the IM group's mean services per conception are better than what has been published. The 21% reduction in services to achieve conception in this field trial is less than reported by Ashmead et al[4] in their double-blind, split herd study. In this field trial, the smaller difference between the groups reflects the better-than-average services per conception in the IM group. Identification of the precise reasons for the better-than-expected services per conception in the IM group is unknown but warrants further study.

In this field trial, 57 cows in the AAC group became pregnant following first service compared with 42 cows in the IM group. That 36% increase in pregnancies was significant ($P < 0.05$). This study did not result in as great of a difference between groups as reported in the controlled study at

Oklahoma State University.[2] Investigators in that study reported that when estrus was synchronized, 71% of the AAC group and 25% of the IM group conceived following first service.[2] There was no attempt to synchronize estrus in this field trial, so absolute comparisons are not justified. Nevertheless, both studies underscore the fact that supplementing AAC during lactation results in better conception rates.

Following first service, 36% more cows from the AAC group in the field trial became pregnant ($P < 0.05$). Inclusion of the second service with the first service resulted in a total of 11% more cows in the AAC group becoming pregnant than in the IM group ($P < 0.05$). When cows requiring 3 or more services are added, (first, second, and third or more services), a total of 10% more cows in the AAC group became pregnant ($P < 0.05$).

No attempt was made in this field trial to ascertain the reasons for the improved conception rates observed in the AAC group. Manspeaker et al[1] reported that when a cow becomes pregnant but then exhibits subsequent estrus, it is an indication of early embryonic mortality. Early embryonic mortality can be a result of inadequate uterine tissue mineral concentrations, particularly Mn and Zn. It has been reported that these tissue deficiencies can be rectified by feeding AAC.[5] Early embryonic mortality can also result from swollen uterine horns that have not returned to normal following a previous parturition at the time of breeding.[4] It has been reported that supplementing dairy feed with Mn, Zn, and Cu AAC following parturition results in a more rapid return of the uterine tissue to normal size.[1,4,5] Finally, it has been reported that lactating cows fed a mineral supplement containing AAC exhibit significantly less periglandular fibrosis ($P < 0.05$) than do lactating cows receiving the same minerals as IM.[1] In the past, conception rates have been directly related to the incidence and severity of periglandular fibrosis.[1]

Each of the above observations suggests a possible reason for the improved conception rate observed in the AAC group in this field trial. Since neither pathological nor histopathological studies were a part of this field trial design, one can only speculate on the causal differences in conception rates. It was noted that there were less health problems in the group receiving the AAC, but no attempt was made to identify the exact natures of these pathologies. Furthermore, the reported problems were a result of subjective observations by the producers rather than well-defined clinical diagnosis. Perhaps a future study should include the taking of uterine biopsies and conducting clinical evaluations to determine the reasons for the observed differences observed in this field trial.

While there was an attempt to measure the effects of seasons on reproductive performance, no well-defined patterns emerged. There were 5 months included in the spring/summer period and 6 in the fall/winter period. In theory, more activity should have occurred in the fall/winter period because an extra month was included. The season did not seem to affect services per conception in the AAC group. There was a weak trend suggesting the need for more services per conception in the IM group in the spring/summer period compared to the fall/winter period ($P < 0.10$). Perhaps a future investigation should focus on this aspect of the study. If AAC can ameliorate the effects of seasons, it would prove interesting to elucidate the reason(s).

The fact that 13% of the cows from the IM group and 4% of the cows from the AAC group failed to conceive after 3 or more services ($P < 0.05$) suggests that AAC may effect a difference in the reproductive condition of cows receiving them. The only measured variable between the groups was the source of the isomineral supplement for the first 120 days of lactation. All other variables tended to "wash out" since animals in both groups came from all 8 farms. While no clinical examination was conducted to ascertain the cause of infertility in either group, it is clear that in some as yet undefined way, the AAC significantly reduced infertility and improved reproductive performance ($P < 0.05$). Since the cost of culling is high, it behooves the producer to extend the useful life of his herd by whatever means possible. Supplementing AAC may be able to extend that useful herd life. This should be the subject of another study.

In a double-blind, controlled study using 50 registered Holstein first calf heifers, it was reported that milk production was 5.2% higher, fat production 5.32% higher, and milk protein 3.9% higher in the group receiving AAC.[3] In this field trial, there was a 3.9% difference between groups on the 100-day standardized milk production, a 1.2% increase in 100-day standardized fat production, and a 4.4% increase in 100-day standardized protein production. While in the double-blind study all of the differences between the AAC and IM group were significant, only the increase in the 100-day standardized protein production trended towards significance in the field trial ($P < 0.10$).

Milk, fat, and protein production are a function of diet and health. None of these factors were controlled in the field trial to the extent they had been in the double-blind study. Since there was a trend in the field trial towards significance (protein) and since the difference between groups was somewhat comparable in the 2 studies, this argues for further field studies comparing milk production in larger populations. The larger population may result in fewer outliers thus allowing significance, if it exists, to be calculated.

CONCLUSION

This study was conducted under normal dairy cow conditions on 8 different farms managed by 8 different dairy producers. In spite of the large number of naturally occurring variables from farm to farm that would not have existed had a split herd on a single dairy farm been used, there were still several significant observations. When amino acid chelates of Cu, Zn, and Mn were fed to dairy cows during the first 120 days of lactation, services per conception were significantly reduced in those animals receiving the AAC. Dairy cows receiving the AAC supplement had almost 36% more confirmed pregnancies following first service than did the cows receiving IM. Furthermore, the 10% greater number of total confirmed pregnancies in the AAC group of these multiparous herds was significantly higher, ultimately resulted in a lower culling rate. When these benefits were evaluated by subtracting the retail cost of the minerals from either source, there was an economic return of approximately 10:1 in favor of supplementing Cu, Zn, and Mn AAC over the IM salts during the first 120 days of lactation following parturition.

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