Implication on Milk Protein and Milk Lactose by Supplementing Copper and Zinc to Kankrej Cattle in Pre and Postpartum Stage

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ABSTRACT

Introduction

Minerals are important for all physiological processes in animals including reproduction, production and immune system. There was scientific evidence that the nutrient status of lactating dairy cows can have a direct can have a bearing on production performance, reproduction performance and health status. Trace minerals and vitamins which influence udder health, milk production and milk quality include Selenium (Se), vitamin E, Copper, Zinc and vitamin A (Rourke, 2009). Animal breeders want to improve milk quality as well as milk production. Copper and Zinc or both supplements in the ration of the animals not only improves health and productive performance of an animal but also quality parameters of milk.

Copper (Cu) and Zinc (Zn) are important trace minerals in dairy cattle feeding, as both elements are widely distributed in the body. Cope et al., (2009) reported that the micronutrient Zn is an essential component of the dairy cow’s diet for maintaining health and performance.

Dietary nutrients not only affect productive and reproductive performance but also milk composition traits like milk protein and milk lactose. This study was conducted to observe effect of copper and zinc feeding on milk protein and milk lactose when these are fed in pre and post partum stage to kankrej cows. Twenty Kankrej pregnant cows (five in each group) at 30 days before the expected date of calving were used to present study and grouped into four groups (five in each group) as control, copper supplemented, zinc supplemented and supplemented with both elements. Milk lactose and protein percentage were observed highest when both nutrient were fed together. The protein content was significantly (P≤0.01) higher on the day 7th of lactation and there was a decrease up to the day 45th. The milk lactose content (%) was significantly (P≤0.05) lower at day 45th of lactation. There was no significant effect of interaction due to the group x days.

Keywords
Kankrej, Copper, Zinc, Milk protein, Milk lactose

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Kincaid and Cronrath (1992) found that Zn primarily binds to casein and to a small extent, to citrate in cow milk. Almost 90% of Zn binds to casein in mature milk in contrast to just 60% in the colostrums.

Scaletti and Harmon (2012) shown that organic supplementation of micro minerals has been associated with improvements in milk yield, clinical udder score and Escherichia coli counts in milk compared with inorganic and unsupplemented dairy cows following an E. coli challenge. Cope et al., (2009) observed that milk composition of cows was unaffected by dietary treatment but animals that received the low level of Zn had higher somatic cell counts in milk. Weiss and Wyatt (2002) reported that Zn and Cu supplementation has been associated with higher antioxidant capacity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) respectively, resulting in reduced somatic cell counts in dairy cows.

NRC (2001) reported that trace minerals and vitamins are essential in the cow’s diet to support overall health and longevity. A dairy cow’s requirements for vitamins and minerals are affected by a variety of factors such as pregnancy, age and production level or for the heifer, rate of growth. Kinal et al., (2005) reported that replacing 30% of the inorganic Cu, Zn and Manganese (Mn) for 6 weeks pre-calving until 305 days of lactation in dairy cows resulted in a 6.5% increase in milk yield (22.35 vs. 21.20 kg/day, P < 0.05). Kincaid and Cronrath (1992) found that Zn primarily binds to casein and to a small extent, to citrate in cow milk. Almost 90% of Zn binds to casein in mature milk in contrast to just 60% in the colostrums.

Uchida et al., (2001) reported that feeding a combination of Zn amino acid (AA), Mn AA and Cu AA complexes and Co glucoheptonate to early lactation Holstein cows had no effect on milk production, milk fat and protein content and linear somatic cell counts. Cortinhas et al., (2010) reported that provision of adequate mineral and vitamin nutrition during the transition period of dairy cattle may be used as a strategy to not only enhance the cow’s immunity against disease but also maintain milk quality and production.

Gilbert et al., (2011) reported that supplementation of micronutrients to peripartum Sahiwal cows not only increased the milk yield but also significantly (P<0.01) increased the fat and protein percentage. Maximum beneficial effect was seen in the combination group which showed a significant (P<0.01) increase in the fat, protein and lactose percentage as compared to the control groups.

Joksimović Todorović et al., (2016) reported that trace elements such as Copper, Zinc are indispensable nutrients for preserving major physiological functions and improving overall health state. Insufficient quantity of these microelements leads to impairment of the immune response due to metabolic and oxidative stress in dairy cows, which affect milk production also. Animal breeders want to improve not only milk production but also milk composition because milk composition has economic importance in dairy industry. The livestock sector alone contributes nearly about 25.6 per cent of current prices of total value of output in Agriculture, Fishing and Forestry sector. The overall contribution of the livestock sector in total GDP of India is nearly 4.11 per cent at current prices during 2012-13. India is the world largest producer of milk accounting for more than 18.5 per cent of world’s total milk production and it also has the world’s largest dairy herd. The milk production has increased from 146.3 million tonnes in 2014-15 to 155.5 in 2015-16 registering a growth of 6.27 per cent. Currently, the Indian dairy market is growing
Materials and Methods

Selection of experimental animals: Twenty pregnant Kankrej cows in their late gestation at 30 days before the expected date of calving were selected Cattle Yard of Livestock Research Station (L.R.S), Kodamdesar of Rajasthan University of Veterinary and Animal Sciences, Bikaner and divided into 4 groups of five each. Group-I without any supplementation was considered as a control. The experimental cows were supplemented individually with Copper (Group-II), Zinc (Group-III) and combination of Copper and Zinc (Group-IV).

Animals with previous milk yield records more than 1500-1800 Kg/305 days of lactation were selected. Based on their milk yield and parity, the animals within the same range of yield and at the same parity were blocked and randomly allocated to one of the four different treatments.

Determination of quantity of Copper and zinc which was offered to animals during feeding

Zinc, Copper and their combination feeding was conducted 30 days before the calving of selected animals and continued after the calving till 45 days.

Copper

15.2 mg per kg of DM (NRC, 2001)
• Molecular weight of CuSO4. 5H2O = 249.68
• Atomic weight of Cu = 63.546
• Purity of the product = 99%

The quantity of Copper sulfate to supplement daily was calculated as follow:

• 15.2 mg Cu  →  15.2 mg Cu by each kg of DMI
• 63.546 mg Cu  →  249.68 mg CuSO4. 5H2O (pure)
• 15.2 mg Cu  →  (249.68 x 15.2 mg)/ 63.546 CuSO4.
• 5H2O (pure)  →  59.7 mg
• CuSO4.5H2O (pure)  →  60.3 mg CuSO4.5H2O 99% purity by each kg of DMI

Based on the average requirement of DM/animal/day of 10 Kg, the daily amount of CuSO4.5H2O (99% pure) to be supplemented was (60.3 mg x 10) = 603 mg or 0.6 gm
Zinc

31 mg per kg of DM (NRC, 2001)
- Molecular weight of ZnSO4. 7H2O = 287.54
- Atomic weight of Zn = 65.38
- Purity of the product = 99%

Similarly, the quantity of Zinc sulfate to supplement daily was calculated as follow:

- 31 mg Zinc → 31 mg Zn by each kg of DMI
- 65.38 mg Zn → 287.54 mg ZnSO4. 7H2O (pure)
- 31 mg Zn → (287.54 x 31 mg)/ 65.38 ZnSO4. 7H2O (pure)
- → 136.3 mg ZnSO4.7H2O (pure)
- → 137.6 mg ZnSO4.7H2O 99% pure by each kg of DMI

Based on the average requirement of DM/animal/day of 10 Kg, the daily amount of ZnSO4.7H2O (99% pure) to be supplemented was (137.6 mg x 10) = 1376 mg or 1.3 gm

Collection of samples

For analysis of milk parameters, samples were collected at the day 7th, 15th, 30th, 45th day postpartum. About 100 ml of milk samples from individual animals of each milking were collected in a properly cleaned milk sample bottle and these samples were kept for two hours after collection and then stirred well for minimum 5 minutes by vertical and circular slow movements. The samples were brought to the laboratory immediately after collection and placed in refrigerator till use.

Preparation of sample for analysis

The milk samples were pre warmed at 39-40°C before analysis. Then samples were poured slowly from one vessel to another vessel by slightly tilting the vessel and using the side walls of the vessel to avoid formation of foams for the equal distribution of fat within the sample.

Analysis of the sample

After preparation of milk samples milk composition parameters like milk protein and milk lactose were estimated by automatic milk analyzer at Urmul dairy Bikaner.

Accuracy of milk analyzer

Accuracy of milk analyzer was checked by comparing the analyzed data of some measuring parameters with the corresponding reference method of analysis (Kjeldhal for milk protein etc.). If there was discrepancy then correction and calibration had done.

Statistical analysis

The data collected during the present investigation were subjected to statistical analysis by adopting appropriate methods of analysis of variance as described by Snedecor and Cochran (1994). Wherever, the variance ratio (F-values), were found significant at 5% and 1% level of significance.

The significance of mean difference was tested by Duncan’s New Multiple Range Test (DMRT) as modified by Kramer (1957).

Data were analyzed by general linear model analyses which include the effect of treatment (supplementation of Copper and Zinc), effect of days as well as interaction effect between treatment and days for various parameters studied.
Results and Discussion

Milk protein percentage

The least square means of milk protein content in control group and cows supplemented with Copper, Zinc and combination of both during the early lactation have been presented in Table 1(a).

The overall mean values for protein percent in milk during the experimental period were 3.52 in control cows and 3.88, 4.04 and 4.37 respectively for the cows supplemented with Copper, Zinc and their combination. The results revealed that the protein content (%) was statistically higher in combination followed by Zinc, Copper and control group. The group wise multiple comparisons between days showed that the protein content was significantly (P≤0.05) higher on the day 7th of lactation and there was a decrease up to the day 45. There was no significant difference in milk protein percent between supplemented Copper and Zinc group as presented in Table 1(a).

Analysis of variance of milk protein percentage in control and supplemented cows with Copper, Zinc and combination of both groups up to 45 days fortnightly interval of lactation is presented in Table 1(b).

Protein percentage of milk differed significantly (P≤0.01) between groups as well as between different days of lactation period. There was no significant effect due to the interaction of group x days on cows of various group as shown in Table 1(b).

Table 1a Mean (±SE) of fortnightly milk protein percentage of control Kankrej cows and cows supplemented with Copper, Zinc and their combination

| Groups                | Days of lactation | Overall mean± SE |
|-----------------------|-------------------|------------------|
|                       | 7th   | 15th  | 30th  | 45th  |                     |
| Control               | 3.78±0.19 | 3.62±0.07 | 3.42±0.06 | 3.26±0.08 | 3.52±0.07         |
| Supplemented Copper   | 4.13±0.26 | 3.94±0.16 | 3.74±0.21 | 3.68±0.14 | 3.88±0.10         |
| Supplemented Zinc     | 4.32±0.21 | 4.11±0.25 | 3.87±0.19 | 3.85±0.24 | 4.04±0.11         |
| Supplemented combination | 4.52±0.18 | 4.42±0.16 | 4.34±0.17 | 4.21±0.18 | 4.37±0.08         |
| Overall mean± SE      | 4.19b±0.11 | 4.02a±0.10 | 3.84a±0.10 | 3.75a±0.11 | -                  |

Note – The values bearing different superscripts (a, b, c …) differ significantly (P≤0.05).
**Table 1b** Analysis of variance of milk protein percentage of control Kankrej cows and cows supplemented with Copper, Zinc and their combination

| Source of variation | DF  | SS    | Mean Sum of squares | F Ratio | F(5%)S/N | F(1%)S/N |
|---------------------|-----|-------|---------------------|---------|----------|----------|
| Groups              | 3   | 7.568 | 2.522               | 14.46   | 2.76*    | 4.13**   |
| Days                | 3   | 2.286 | 0.762               | 4.36    | 2.76*    | 4.13**   |
| Groups x Day        | 9   | 0.134 | 0.014               | 0.08    | 2.1 NS   | 2.82 NS  |
| Residual            | 64  | 11.16 | 0.174               | -       | -        | -        |

Asterisks indicate level of significance (**P≤0.01, *P≤0.05)

**Table 2a** Mean (±SE) of fortnightly milk lactose percentage of control Kankrej cows and cows supplemented with Copper, Zinc and their combination

| Groups                        | Days of lactation | Overall mean | | | |
|-------------------------------|-------------------|--------------|---|---|---|
|                               | 7th               | 15th         | 30th | 45th | |
| Control                       | 4.53±0.07         | 4.47±0.11    | 4.30±0.07 | 4.16±0.02 | 4.36±0.05 |
| Supplemented Copper           | 4.98±0.14         | 4.78±0.16    | 4.56±0.19 | 4.42±0.14 | 4.68±0.08 |
| Supplemented Zinc             | 5.14±0.08         | 4.95±0.14    | 4.70±0.14 | 4.55±0.13 | 4.84±0.08 |
| Supplemented combination      | 5.52±0.03         | 5.42±0.03    | 5.31±0.08 | 5.13±0.10 | 5.34±0.04 |
| Overall mean± SE              | 5.04±0.09         | 4.91±0.09    | 4.72±0.10 | 4.56±0.09 | -        |

Note – The values bearing different superscripts (a, b, c …) differ significantly (P≤0.05).

**Table 2b** Analysis of variance of milk lactose percentage of control Kankrej cows and cows supplemented with Copper, Zinc and combination of both during the early lactation period under the study have been presented in the Table 2(a).

In conclusion, these data suggest that Zn and Cu supplementation to dietary levels above recommendations for cows may have a positive effect on milk performance and milk protein. Similar results were reported by Uchida et al., (2001) and Kincaid and Cronrath (1992).

**Milk lactose percentage**

The milk lactose percentage least square mean results for the control and cows supplemented with Copper, Zinc and combination of both during the early lactation period under the study have been presented in the Table 2(a). The overall average values for lactose percent in milk were 4.36 in control cows and 4.68, 4.84 and 5.34 respectively for the cows supplemented with Copper, Zinc and their combination. The highest value of mean of lactose percentage was reported in supplemented combination group followed by Zinc, Copper and Control group respectively.
The comparisons between days showed that the milk lactose content (%) was significantly (P≤0.05) lower on day 45\textsuperscript{th} of lactation in all experimental cows. There was no significant difference in milk lactose percentages between supplemented Copper and Zinc group as presented in Table 2(a).

Analysis of variance of milk lactose percentage in control and supplemented with Copper, Zinc and combination of both cows up to 45 days fortnightly of lactation is presented in Table 2(b).

The comparisons between treatments revealed that there was significant (P<0.01) difference between all the supplemented cows and control group. As compare among treatments there was also significant (P≤0.01) effect of supplementation of Copper, Zinc and combination group. Thus effects of treatment and days were statistically significant (P≤0.01) but the interaction due to the effect group x days was found non- significant as shown in Table 2(b).

It can be concluded that the effect of Cu, Zn and their combination is favorable on milk lactose parameter of selected supplemented cows. Similar results were reported by Uchida et al., (2001), Weiss and Wyatt (2002) and Cortinhas et al., (2010).

From the above study it can be concluded that:

Supplementation of micronutrients improved the udder health and milk production.

Micronutrient supplementation also increased the other milk parameters such as milk lactose and milk protein.

Micronutrients supplementation provided to pregnant dam has a significant effect on milk composition.

Micronutrient supplementation also improves immunity of an animal.

Of all the micronutrients maximum beneficial effect was seen when all the micronutrients (Copper + Zinc) are fed together to the peripartum cows.

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