Original Research

The Effect of Dietary Copper, Zinc and Their Combination on Somatic Cell Counts and Daily Milk Yield of Kankrej Cattle in Arid Zone of Rajasthan

Narender Kumar Poonia*[1], Arun Kumar Jhirwal[2], Subhash Chandra Goswami[2] and Garima Choudhary[3]

College of Veterinary and Animal Science, RAJUVAS, Bikaner, Rajasthan, INDIA

[1]Centre for Conservation of Animal Biodiversity, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, INDIA
[2]Department of Livestock Production Management
[3]Department of Animal Genetics and Breeding

***Corresponding author:** drnkpoonia07@gmail.com

Rec. Date:	Jul 16, 2018 17:53
Accept Date:	Oct 08, 2018 17:38
DOI	10.5455/ijlr.20180716055304

Abstract

A herd of Kankrej cows were grouped in 4 homogenous groups of 5 each as control, supplemented Copper, supplemented Zinc and supplemented with combination of copper and zinc. About 100 ml of milk samples at day 7th, 15th, 30th, 45th post-partum from individual animals of each milking was collected to analyse of somatic cell counts. The milk somatic cell counts was estimated lowest in supplemented combination followed by zinc, copper and control group of cows respectively. There was significant ($P \leq 0.01$) effect of treatment on somatic cell counts. The somatic cell counts were significantly ($P \leq 0.01$) higher in day 7th of lactation and decreased significantly ($P \leq 0.01$) on day 15th and the lower somatic cell count was observed on the day 45th. The daily milk yield was observed highest in combination group and the effect of treatment, days and the interaction due to the treatment x days were statistically significant ($P \leq 0.01$).

Key words: Copper, Milk Yield, Kankrej, Somatic Cell Counts, Zinc

How to cite: Poonia, N., Jhirwal, A., Goswami, S., & Choudhary, G. (2019). The Effect of Dietary Copper, Zinc and Their Combination on Somatic Cell Counts and Daily Milk Yield of Kankrej Cattle in Arid Zone of Rajasthan. International Journal of Livestock Research, 9(1), 350-359. doi: 10.5455/ijlr.20180716055304

Introduction

There are scientific evidence that the nutrient status of lactating dairy cows can have a direct bearing on production performance, reproduction performance and health status. Copper (Cu) and zinc (Zn) are important trace minerals in dairy cattle feeding, as both elements are widely distributed in the body. Zinc induces synthesis of metallothionein, a metal binding protein that may scavenge hydroxide radicals (Prasad *et al.*, 2004). In addition to an antioxidant role, Zn may affect immunity via its important role in cell replication and proliferation (Weiss and Spears, 2006). Zn is required for production of protective keratins in the hoof and teat. Therefore, it plays role in maintaining structural integrity and health of the hoof and udder (Tomlinson *et al.,* 2004). Proper Copper supplementation of the sire is needed for production of quality semen provision of adequate mineral and vitamin nutrition during the transition period may be used as a strategy to not only enhance the cow's immunity against disease but also maintain milk quality and production (Weiss and Wyatt, 2002; Cortinhas *et al*., 2010). Orr *et al*. (1990) found that as Zn status is reduced, along with stress (which can lead to a further depression in Zn status), a reduction in immune response and compromise of skin integrity the mammary glands natural defense system is weakened. These results in higher somatic cell counts and increased incidence of Mastitis. Jongbloed *et al*. (2001) reported that effects of Zn supply to dairy cows on somatic cell counts (SCC) are scarce and conflicting. 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, $P < 0.05$).

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 the quality and quantity of milk but also health and productive performance of an animal. Hence, present study was undertaken to estimate the somatic cell counts in milk and evaluate the impact of copper and zinc supplementation on milk yield of Kankrej cattle.

Material and Methods

The desired information for present study was obtained after conducting experiments in the cattle yard, collecting data from the records of Livestock Research Station (L.R.S), Kodamdesar of Rajasthan University of Veterinary and Animal Sciences, Bikaner - from May, 2017 to September, 2017.

Selection of Experimental Animals

Twenty pregnant Kankrej cows in their late gestation at 30 days before the expected date of calving were selected 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 expected to calve from June, 2017 to July, 2017 were selected. Based on their milk yield and parity, the animals within the same range of yield and at the same parity were blocked.

Determination of Practical Quantity of Copper and Zinc

Copper

15.2 mg per kg of DM (NRC, 2001)

Molecular weight of $CuSO_4 \cdot 5H_2O$ = 249.68

 DOI 10.5455/ijlr.20180716055304

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 $CuSO_4 \cdot 5H_2O$ (pure)

15.2 mg Cu → (249.68 x 15.2 mg)/ 63.546 $CuSO_4 \cdot 5H_2O$(pure)

→ 59.7 mg $CuSO_4 \cdot 5H_2O$ (pure)

→ 60.3 mg $CuSO_4 \cdot 5H_2O$ 99% purity by each kg of DMI

Based on the average requirement of DM/animal/day of 10 Kg, the daily amount of $CuSO_4 \cdot 5H_2O$ (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 $ZnSO_4 \cdot 7H_2O$ = 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 $ZnSO_4 \cdot 7H_2O$ (pure)

31 mg Zn → (287.54 x 31 mg)/ 65.38 $ZnSO_4 \cdot 7H_2O$ (pure)

→ 136.3 mg $ZnSO_4 \cdot 7H_2O$ (pure)

→ 137.6 mg $ZnSO_4 \cdot 7H_2O$ 99% pure by each kg of DMI

Based on the average requirement of DM/animal/day of 10 Kg, the daily amount of $ZnSO_4 \cdot 7H_2O$ (99% pure) to be supplemented was (137.6 mg x 10) = 1376 mg or 1.3 gm.

Table 1: Supplementation of micronutrients around peripartum period to Kankrej cows

Groups	No. of Animals	Treatment	Dose/Animal/day
Control	5	Nil	Control
Supplemented Copper	5	Copper	15.2mg/kg DMI
Supplemented Zinc	5	Zinc	31mg/kg DMI
Supplemented Combination	5	Combination of both	Cu - 15.2mg/kg DMI + Zn - 31mg/kg DMI

Time Period for Sample Collection

For analysis of milk parameters, samples were collected at the day 7th, 15th, 30th, 45th day post-partum.

DOI 10.5455/ijlr.20180716055304

Milk Yield

Milking was done twice a day and milk yield records were maintained throughout the experimental period (45 days after onset of lactation).

Milk Somatic Cell Counts

Milk quality in terms of somatic cell counts was determined up to 45 days of lactation. The somatic cell counts on milk sample were performed as described by Schalm *et al*. (1971). However, for staining of milk smear, Giemsa stain was used.

Preparation of Milk Smear

Before the preparation of milk smear, milk sample was mix thoroughly so as to obtain uniform distribution of cell. The sample was allowed to stand for two to five minutes to permit air bubbles to rise and foam to disappear. Grease free slide was placed on a level area over a template to outline 1 cm^2 area. 0.01 ml (10µl) of milk was withdrawn by micropipette and spread evenly on a glass slide in 1 cm^2 area. The smear was dried in air. Thereafter, a few drops of xylene were poured on glass slide over the milk smear and kept for 2 minutes to dissolve the fat globules of milk. Then smear was air dried and fixed with 99 per cent methanol for 2 minutes and washed with distilled water. After fixing smear, it was stained with Giemsa stain for 30 minutes. After staining, the smear was kept in phosphate buffer solution (pH 7.0) in coupling jar for 5 minutes and bloat dried.

Counting of Cell

Examination of milk smear was done at random under oil immersion.1 cm^2 area of smear was divided into four equal parts by dividing it at the right angle. Cells were counted in five fields from each divided area. Thus the cells were counted in total 20 fields. The average number of cells per sq cm area was calculated. For counting of cells per ml of milk the average number of cells per field was multiplied by microscopic factor.

Derivation of Common Microscopic Factor

Common microscopic factor was determined as per Prescott and Breed (1910) in following manner- Diameter of an oil immersion microscopic field of Nikon microscope used in study = 190µ or 0.019 cm.

Radius of an oil immersion microscopic factor = 0.0095 cm.

Area of oil immersion microscopic field = πr^2

$$= 22/7 \times (0.0095)^2 \ cm^2$$
$$= 3.1428 \times 0.0095 \times 0.0095 \ cm^2$$
$$= 0.0002833 \times 10^{-5} \ cm^2$$
$$= 28.33 \times 10^{-5} \ cm^2$$

 DOI 10.5455/ijlr.20180716055304

$$\text{No. of field in 1 cm2 area} = \frac{1\text{cm}}{\text{Area of an oil immersion}}$$

$$\text{Microscopic field} = \frac{1}{28.33 \times 10-5}$$

$$= 3529.82$$

Number of cell counted in 20 fields = X
Then total numbers of cells in 1 cm^2 area are -

$$= \frac{X}{20} \times 3529.82 \text{ in 0.01 ml of milk}$$

So number of cells in 1 ml of milk = $\frac{X \times 3529.82 \times 100}{20}$

$$\frac{X}{20} \times 352982(\textit{multiplication factor})$$

Here, $\frac{X}{20}$ = Average no. of cells in 20 field of 1 cm^2 area
Total number of cells in 1 ml of milk = Avg. no. of cells in 20 fields of 1 cm^2 area × 352982.

Statistical Analysis

Data were analyzed by general linear model analysis which includes 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. Statistical analysis was carried out by using SPSS software, version 20.0. The significance of mean difference was tested by Duncan's New Multiple Range Test (DMRT) as modified by Kramer (1957).

Results and Discussion

Daily Average Milk Yield

Individual daily milk yield of the experimental Kankrej cows were recorded up to 6th weeks post-partum from daily milk recording register. The Least Square means of daily milk production in the groups supplemented with copper, zinc and combination and the control group have been presented in Table 2 and shown in Fig. 1. The daily milk yield was observed highest in combination group followed by copper, zinc and control cows. The comparisons between days of lactation revealed that the milk yield was significantly ($P \leq 0.05$) higher from the 1st week to 6th week of lactation as shown in Table 2.

 DOI 10.5455/ijlr.20180716055304

Table 2: Mean (±SE) of milk yield (Kg/day) of control Kankrej cows and cows supplemented with copper, zinc and their combination

Groups	Days of Lactation						Overall Mean± SE
	1st week	2nd week	3rd week	4th week	5th week	6th week	
Control	3.66^{aA}± 0.13	4.20^{bA}± 0.1	5.02^{cA}± 0.15	5.23^{cA}± 0.13	5.93^{dA}± 0.2	6.45^{eA}± 0.19	5.08^{A}± 0.18
Supplemented Copper	4.16^{aB}± 0.29	5.15^{bC}± 0.21	5.408^{bA}± 0.19	6.11^{cB}± 0.2	7.248^{dB}± 0.13	8.194^{eB}± 0.13	6.04± 0.26
Supplemented Zinc	4.08^{aAB}± 0.13	4.64^{bB}± 0.09	5.32^{cA}± 0.07	5.53^{cA}± 0.06	6.21^{dA}± 0.03	6.67^{eA}± 0.11	5.41^{B}± 0.16
Supplemented combination	4.76^{aC}± 0.08	5.97^{bD}± 0.1	6.42^{cB}± 0.07	7.13^{dC}± 0.21	8.36^{eC}± 0.19	9.21^{fC}± 0.12	6.98^{D}± 0.28
Overall mean±SE	**4.16^{a}± 0.24**	**4.99^{b}± 0.32**	**5.54^{c}± 0.27**	**6.00^{d}± 0.36**	**6.94^{e}± 0.46**	**7.63^{f}± 0.53**	**5.88± 0.13**

The values bearing different superscripts (a, b, c …) differ significantly (P ≤0.05); the values bearing different superscripts (A, B, C…) differ significantly (P ≤ 0.05).

The analysis of variance of the milk yield revealed that the effect of treatment, weeks and the interaction of treatment x weeks were statistically significant (P≤0.01) as presented in Table 3.

Table 3: Analysis of variance of milk yield of control Kankrej cows and cows supplemented with copper, zinc and their combination

Source	DF	SS	Mean Sum of squares	F Ratio
Groups	3	62.477	20.826	175.565**
Weeks	5	161.141	32.228	271.68**
Groups x Weeks	15	9.174	0.612	5.15**
Residual	96	11.388	0.119	-

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

Similar positive effect on milk yield was reported by Scaletti *et al*. (2003) and Siciliano-Jones *et al*. (2008). They reported that micro minerals such as Cu, Zn and Mn were shown to have beneficial effect in improving udder health by reducing somatic cell counts and decreasing the incidence of mastitis in dairy cows. Kinal *et al*. (2005) also reported similar finding 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). Nocek *et al.* (2006) reported that first lactation cows had no differences in SCC when fed Zn, Mn, Cu and Co in complex or inorganic form at 75 or 100% of NRC above the basal diets, but a small significant milk production response was noted between the organic and the inorganic minerals fed cows. Cope *et al.* (2009) evaluated the effects of the level and form of dietary Zn on milk performance, and found that cows supplemented with organically chelated Zn at the recommended level had a higher milk yield (37.6 kg/day) than those fed inorganic Zn at the recommended level (35.2 kg/day), or organically chelated Zn at low level (35.2 kg/day), but there was no difference from those fed inorganic Zn at the low level (36.0 kg/day). Milk composition was unaffected by dietary treatment. Animal that received the low level of zinc had higher somatic cell counts.

 DOI 10.5455/ijlr.20180716055304

In this study, we observed that micronutrient supplementation decreased milk somatic cell counts of cows and thus these supplemented cows had lower incidence of clinical mastitis, which subsequently increased the milk production.

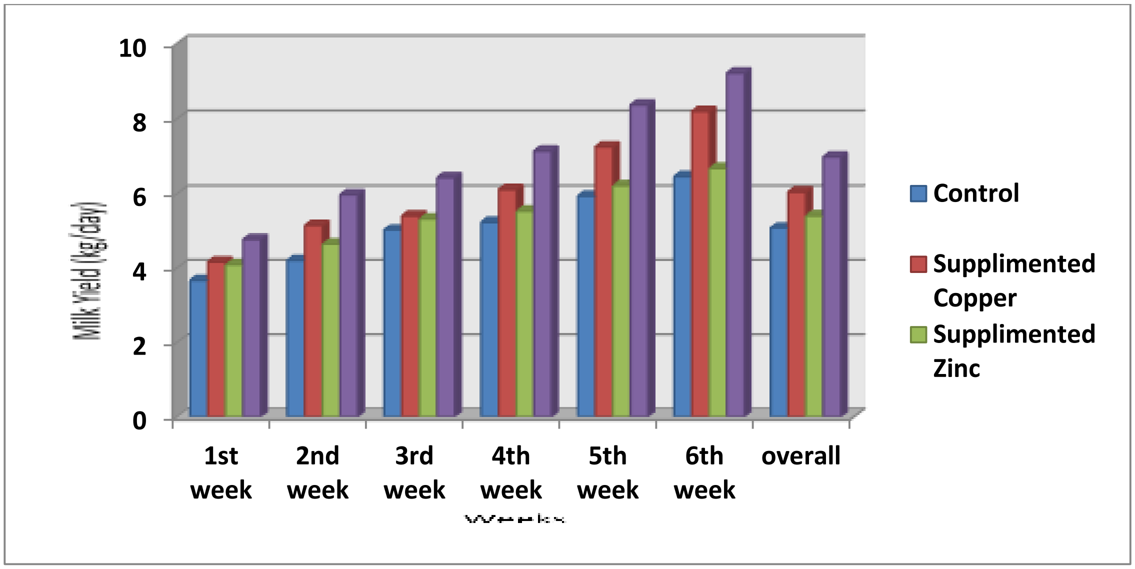

Fig. 1: Average daily milk yield (kg/day/cow) of control and supplemented groups with copper, zinc and their combination during different time period

Somatic Cell Counts (SCC)

The results of least square means of somatic cell counts (x10^5 cells /ml) in milk during different days of lactation up to the 45th day of control and cows supplemented with copper, zinc and combination have been presented in Table 4 and shown by Fig. 2. The lowest value of mean of milk somatic cell counts was estimated in supplemented combination group followed by zinc, copper and control group of cows. The somatic cell counts were significantly ($P \leq 0.05$) higher in day 7th of lactation of all experimental cows. These values decreased significantly ($P \leq 0.05$) on day 15th and the lower somatic cell counts were observed on the day 45th of lactation of all cows of four groups.

Table 4: Mean (±SE) of milk somatic cell counts (10^5)/ml 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	2.71± 0.16	2.67± 0.2	2.57± 0.08	2.53± 0.08	**2.62^{c}± 0.06**
Supplemented Copper	1.83± 0.13	1.76± 0.12	1.72± 0.16	1.68± 0.11	**1.75^{b}± 0.06**
Supplemented Zinc	1.61± 0.27	1.62± 0.1	1.58± 0.12	1.51± 0.08	**1.58^{ab}± 0.07**
Supplemented combination	1.44± 0.15	1.40± 0.12	1.44± 0.21	1.37± 0.06	**1.41^{a}± 0.06**
Overall mean± SE	**1.90± 0.14**	**1.86± 0.12**	**1.83± 0.12**	**1.77± 0.11**	**-**

The values bearing different superscripts (a, b, c ...) differ significantly ($P \leq 0.05$).

DOI 10.5455/ijlr.20180716055304

The interaction effect of group x days was found non- significant on somatic cell counts. The maximum somatic cell counts were estimated in control group. There was significant (P≤0.01) effect of treatment on somatic cell count as shown in Table 2(b) and also the somatic cell counts were significantly (P≤0.05) lower in combination group to the supplemented zinc, copper and control groups respectively increased as shown in Table 4.

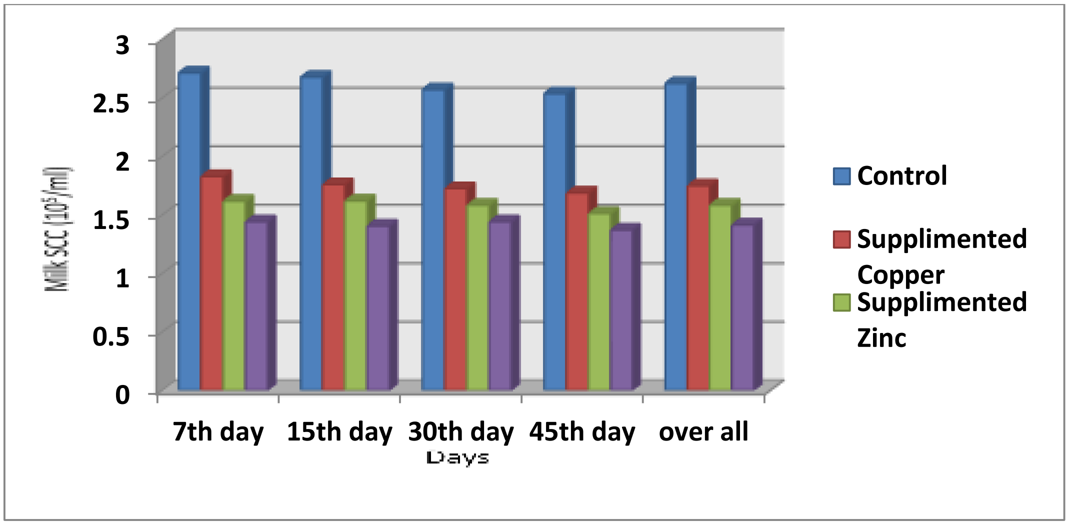

Fig. 2: Average milk somatic cell counts of control and supplemented groups with copper, zinc and their combination during different time period

Similar results were also reported by Popovic (2004) and he found that supplementation of organic Zn for 45 days pre-calving until 100 days post-calving lowered somatic cell count by (158,840/ml vs.193, 530/ml) day 10 of lactation in dairy cows. Results also resembles with the findings of Cortinhas *et al*. (2010). We also found that the supplementation of combination of micronutrients reduced the milk somatic cell counts significantly (P≤0.01) as compared to control group.

Table 5: Analysis of variance of milk somatic cell counts of control Kankrej cows and cows supplemented with copper, zinc and their combination

Source of Variation	DF	SS	Mean Sum of Squares	F Ratio
Groups	3	17.4172	5.80575	51.56**
Days	3	0.16648	0.05549	0.49
Groups x Days	9	0.04874	0.00541	0.04^{NS}
Residual	64	7.2052	0.11258	-

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

 DOI 10.5455/ijlr.20180716055304

Conclusion

Supplementation of micronutrients improved the udder health by reducing milk somatic cell counts. Of all the micronutrients maximum beneficial effect was seen when all the micronutrients (Copper + Zinc) are fed together to the peripartum cows. Supplementing dietary trace elements complex in concentrate is desirable as it can achieve a major economic return with increase in milking performance of grazing dairy cows.In our study the dietary treatment significantly affected the milk production and reduces somatic cell counts in milk as compare to control group cows. There is need of supplementing the trace elements during feeding of animals, where soil has no micronutrients in its composition.

References

1. Cope, C. M., Mackenzie, A. M., Wilde, D. and Sinclair, L. A. (2009). Effects of level and form of dietary zinc on dairy cow performance and health. Journal of dairy science, 92(5): 2128-2135.
2. Cortinhas, C. S., Botaro, B. G., Sucupira, M. C. A., Rennó, F. P. and Santos, M. V. D. (2010). Antioxidant enzymes and somatic cell count in dairy cows fed with organic source of zinc, copper and selenium. *Livestock Science*, 127(1): 84-87.
3. Gupta, R., Singh, K., Sharma, M. and Kumar, M. (2017). Effect of Mineral Mixture Feeding on the Productive and Reproductive Performance of Crossbred Cattle. *International Journal of Livestock Research*, 7(12): 231-236.
4. Jongbloed, A.W., Van den Top, A.M., Beynen, A.C., Van der Klis, J.D., Kemme, P.A., Valk, H. (2001). Consequences of newly proposed maximum contents of copper and zinc in diets for cattle pigs and poultry on animal performance and health. Report ID-Lelystad no. 2097.
5. Kinal, S., Korniewicz, A., Jamroz, D., Zieminski, R. and Slupczynska, M. (2005). Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. *J. Food Agric. Environ*, 3(1): 168-172.
6. Kramer, C.Y. (1957). Extension of multiple range tests to group correlation.
7. National Research Council (2001). Nutrient Requirements of Dairy Cattle. 7th rev. edition. Natl. Acad. Sci., Washington, DC.
8. Nocek, J. E., Socha, M. T. and Tomlinson, D. J. (2006). The effect of trace mineral fortification level and source on performance of dairy cattle. *Journal of Dairy Science*, 89(7): 2679-2693.
9. Orr, C. L., Hutcheson, D. P., Grainger, R. B., Cummins, J. M. and Mock, R. E. (1990). Serum copper, zinc, calcium and phosphorus concentrations of calves stressed by bovine respiratory disease and infectious bovine rhinotracheitis. *Journal of Animal Science*, 68(9): 2893-2900.
10. Popovic, Z. (2004). Performance and udder health status of dairy cows influenced by organically bound zinc and chromium. Ph.D. Thesis, University of Belgrade, Belgrade.
11. Prasad, A. S., Bao, B., Beck, Jr. F. W., Kucuk, O. and Sarkar, F. H. (2004). Antioxidant effect of zinc in humans. Free Radical Biology and Medicine. 37: 1182–1190.
12. Rehman, H.U., Khan, M.A., Kakar, M.A., Ahmad, M., Ahmad, S. and Ahmad, Z. (2017) Fundamentals of Zinc, Manganese and Copper in the Metabolism of the Rumen and Immune Function. *International Journal of Livestock Research*, 7(11): 55-76
13. Scaletti, R.W., Trammell D.S., Smith B A. and Harmon R.J. (2003). Role of Dietary Copper in Enhancing Resistance to Escherichia coli Mastitis. *Journal of Dairy Science*, 86:1240–1249.
14. Schalm, O.W., Carroll, E. J. and Jain, N. C. (1971). Bovine Mastitis. Lea and Febiger, Philadelphia.
15. Siciliano-Jones, J. L., Socha, M. T., Tomlinson, D. J. and DeFrain, J. M. (2008). Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. *Journal of Dairy Science*, 91(5): 1985-1995.

  DOI 10.5455/ijlr.20180716055304

16. Tomlinson, D. J., Mülling, C. H. and Fakler, T. M. (2004). Invited review: formation of keratins in the bovine claw: roles of hormones, minerals, and vitamins in functional claw integrity. *Journal of Dairy Science*, 87(4): 797-809.
17. Weiss, W. (2002). Relationship of mineral and vitamin supplementation with mastitis and milk quality. In annual meeting-national mastitis council incorporated (Vol. 41, pp. 37-44). National Mastitis Council; 1999.
18. Weiss, W. P. and Wyatt, D. J. (2002). Effects of feeding diets based on silage from corn hybrids that differed in concentration and in vitro digestibility of neutral detergent fiber to dairy cows. *Journal of Dairy Science*, 85(12): 3462-3469.
19. Weiss, W.P. and Spears, J.W. (2006). Vitamin and trace mineral effects on immune function of ruminants. In: Sejrsen, K., Hvelplund, T., Nielsen, M.O. (Eds.), Ruminant Physiology. Wageningen Academic Publishers, Utrecht, The Netherlands, pp. 473– 496.

  DOI 10.5455/ijlr.20180716055304