Original Research

Estimation of Neutrophils and Total Leucocyte Count in Blood and Neutrophils Count in Colostrum after Supplementation of Copper and Zinc to Kankrej Cows

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Abstract

The objective of the study was to estimate neutrophil and TLC in blood and neutrophil in colostrums after feeding micronutrients formulation to kankrej cows. The experimental treatments included four groups of animals as control, supplemented copper, supplemented zinc and supplemented with combination of copper and zinc. Maximum increase in blood neutrophils percentage, TLC and neutrophils percentage in colostrums was found in control cows as compared to the supplemented cows. Blood neutrophils percentage, TLC and neutrophils percentage in colostrums differed significantly (P≤0.01) between groups as well as between different days of peripartum period, day of calving and post-partum days. The interaction effect of group x days on cows of various group was observed non-significant for these traits.

Key words: Copper, Kankrej, Neutrophil in Blood, Neutrophil Count in Colostrum, Total Leucocyte Count, Zinc

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Introduction

The incidence of diseases and disorders can have a negative impact on reproductive and productive performance. Minerals and vitamin supplementation are necessary to maintain healthy immune system of an animal. The important trace elements are copper, cobalt, iron, iodine, zinc, manganese and selenium. Griffiths *et al*. (2007) reported that mineral matters like selenium, zinc, copper, cobalt and iodine play an important role in synthesis of proteins, forming of connective tissue, vitamin metabolism, and improvement of immune function. These minerals have an effect on health, lactation, fertility, and immune functions. Gengelback *et al*. (1997) found that low copper status has resulted in decrease humoral and cell-mediated immunity in cattle and decrease neutrophil bactericidal capability in steers. NRC (2001) reported that Zinc is a component of thymosin, a hormone produced by thymic cells that regulates cell-mediated immunity. In addition to the antioxidant role in combination with copper, zinc may contribute to the reduction of somatic cell counts due to its role in keratin formation.

Copper is important for adequate growth, reproduction and immunity. Unlike zinc, copper is stored tenaciously in the liver, and levels build up rapidly when animals are fed high levels of copper. Copper oxide should be avoided as a copper source because of its poor bioavailability, which will affect the level of copper required in supplements. High levels of molybdenum, sulfur, iron or zinc in the diet interfere with normal copper absorption and metabolism. Niederman *et al*. (1994) observed a reduced phagocytic activity in neutrophils from gestating beef cattle supplemented with copper and iron sulfate, assuming an unknown interaction between both. In addition, these animals were not copper-deficient according to liver levels of copper. They reported functions of copper in the immune system through energy production, neutrophil production and activity, antioxidant enzyme production, development of antibodies and lymphocyte replication. Stable *et al*. (1993) found the importance of copper for maintaining the functions of the immune system has been demonstrated in several studies. Viral and bacterial challenges have been shown to increase serum ceruloplasmin and plasma copper in copper-repleted cattle indicating a major protective role for copper in infectious diseases.

Recently, Van Knegsel *et al*. (2008) showed in a review the importance of a sufficient copper supply. Copper is involved in the development and maintenance of the immune system, and the copper status alters several aspects of neutrophil, monocyte, and T-cell function. However, the effect of copper on the immune response seems to be limited. This is mainly due to the fact that copper homeostasis is maintained over a wide range of intakes. Zinc is an essential mineral that is required by all cells in animals. Zinc plays a role in numerous enzymatic reactions. Deficiencies of zinc are associated with reduced growth, diminished reproductive performance, poor immune function due to reduce activity of neutrophils and NK cells and poor offspring viability, as well as skin lesions in severe cases. Rehman *et al*. (2017) reported that copper is necessary for the function of the superoxide dismutase and in the elimination of toxic by-products of metabolic pathways. The elimination of these toxic by-products allows metabolism to perform effectively, uninhibited by damaging oxygen free radicals. Galyean *et al*. (1999) and Jongbloed *et al*. (2001) have reported the effect of dietary zinc level on beef cattle health and immunity, especially under conditions of stress such as transportation. Previous reviews reported effects of the addition of copper and zinc to dietary levels above the current physiological requirements on milk yield, milk composition, health parameters and

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immunity of cattle. 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. Gupta *et al*. (2017) conducted a study on crossbred cow to find out the effect of mineral mixture feeding on the production and reproductive performance. It was observed that avg. daily milk yield, peak yield and total 180 days milk production were found significantly (P ≤ 0.05) higher by 13.4%, 16.17% and 13.07 % in mineral mixture fed group than control group, however milk composition parameter were differed non-significantly between treatment and control group.

Therefore, present study was undertaken to estimate the neutrophil count and total leucocyte count in blood and colosturm neutrophil count of Kankrej cattle.

Material and Methods

The experiment was conducted in the Kankrej cattle yard at Livestock Research Station (L.R.S), Kodamdesar of Rajasthan University of Veterinary and Animal Sciences, Bikaner.

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).

Table 1: Supplemented dose of micronutrients in different groups of experimental animals around peripartum period

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

Sample Collection

To estimate blood parameters blood samples were collected at 30th, 15th days of pre-partum, at the day of calving and 15th, 30th, 45th days of postpartum. For analysis of colostrums neutrophils, samples were collected at the day of calving, 3rd and 5th day post-partum.

Total Leukocyte Count (TLC) in Blood

a) Blood samples were collected from all cows on scheduled days. After collection TLC was done on haemocytometer.

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b) First the counting chamber under the microscope was examined and confined the lines clearly.
c) Then blood sample was drawn to the 0.5 mark in WBC diluting pipette.
d) Excess blood was removed from the tip of pipette and keeping pipette in vertical position, gently suck the WBC diluting fluid to 11 marks.
e) Mix the blood with diluting fluid by rolling the pipette between the palms.
f) 2-3 drops of fluid was discarded from the end and the haemocytometer was charged.
g) After that counting was carried under low power objective microscope in four larger corner squares. First the upper left square of 16 small squares, then the upper right, the lower right and last the lower left was counted. Those leucocytes which were half in and half out of the upper and the left hand line were included in the counting but not those halves in and half out of the lower and right hand lines.

Calculation

V = D X Dp X SA= 1/20 X1/10 X4 = 1/50 cu.mm
V = Volume of blood
D =Dilution of the blood sample (1: 20)
Dp = Depth of the counting chamber 1/10 mm
SA =Surface area counted (Area counted 4 sq. mm.)
C =NX1/V per cu.mm.
C =NX1/1/50
C =NX50
C = Cell count per cu.mm.
N = Number of cells actually counted in the chamber (sum of four squares)
V = Volume of the blood (from above formula)

Differential Leukocyte Count (Neutrophils) in Blood

a) Fix the blood smear in absolute methyl alcohol for 3-5 minutes and allow evaporating.
b) Place the fixed and dried smear in the diluted stain (1 part Giemsa stain to 9 parts neutral distilled water) in the jar for 30 minutes.
c) Wash the slide in neutral distilled water and air dry.
d) Examine under an oil immersion
e) Classify the entire leukocyte which is seen. Count 100 cells to determine the % of the leucocytes (neutrophils).

Neutrophis in Colostrums

About 100 ml of well mixed colostrum sample of morning milking were collected on days 0 (day of calving), 3^{th} and 5^{th} from each animal in a milk sample bottle and stored at low temperature till further analysis of neutrophils count were carried out.

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

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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).

Result and Discussion

Total Leukocyte Count

The least square means of total leukocyte count in blood in cows supplemented with copper, zinc and combination of both and the control cows during pre-partum, at day of calving and post calving period have been presented in Table 1 and Fig. 1. The result revealed that there is gradually increase in leukocyte count in all supplemented and control cows from 30th day's pre partum period to the day of calving and remain highest on the day of calving but there is gradually decrease number of leukocytes after calving and post calving period. Leukocyte count reaches the normal level by the 45 day post-partum.

Table 1: Mean (±SE) of total leukocyte count ($x10^3$)/mm^3 in blood of control Kankrej cows and cows supplemented with copper, zinc and their combination

Groups	Pre-partum Days		Calving Day	Post-partum Days			Overall Mean
	30th	15th	0 day	15th	30th	45th	
Control group	7.64±0.41	8.41±0.36	9.21±0.23	8.96±0.25	8.73±0.27	8.52±0.17	**8.58^B ±0.35**
Supplemented Copper group	8.12±0.30	8.64±0.39	9.28±0.17	8.68±0.27	8.32±0.26	8.17±0.27	**8.53^B±0.31**
Supplemented Zinc group	8.09±0.53	8.53±0.44	9.14±0.27	8.78±0.15	8.45±0.17	8.21±0.16	**8.53^B ±0.33**
Supplemented combination group	7.87±0.46	8.13±0.35	8.71±0.37	8.11±0.27	7.78±0.21	7.46±0.24	**8.01^A±0.35**
Overall mean± SE	**7.93^a**±0.4	**8.43^{bc}**±0.3	**9.09^d±0.27**	**8.63^c ±0.26**	**8.32^{abc}**±0.26	**8.09^b±0.26**	**8.41±0.35**

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).

Total leukocyte count of blood differed significantly (P≤0.01) between groups as well as between different days of peripartum period, day of calving and post parturient period. There was no significant effect due to the interaction of group x days on cows of various group as presented in Table 2.

Table 2: Analysis of variance of total leukocyte count in blood 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	6.558	2.186	4.485**
Days	5	16.971	3.394	6.963**
Groups x Days	15	3.28	0.219	0.449^{NS}
Residual	96	46.793	0.487	-

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

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The increase in the blood total leukocyte count around the day of calving may be due to a combination of increase number of blood neutrophils and various stressors. At the day of parturition, there may be a high risk of infections due to suppressed immune system of periparturient cows.

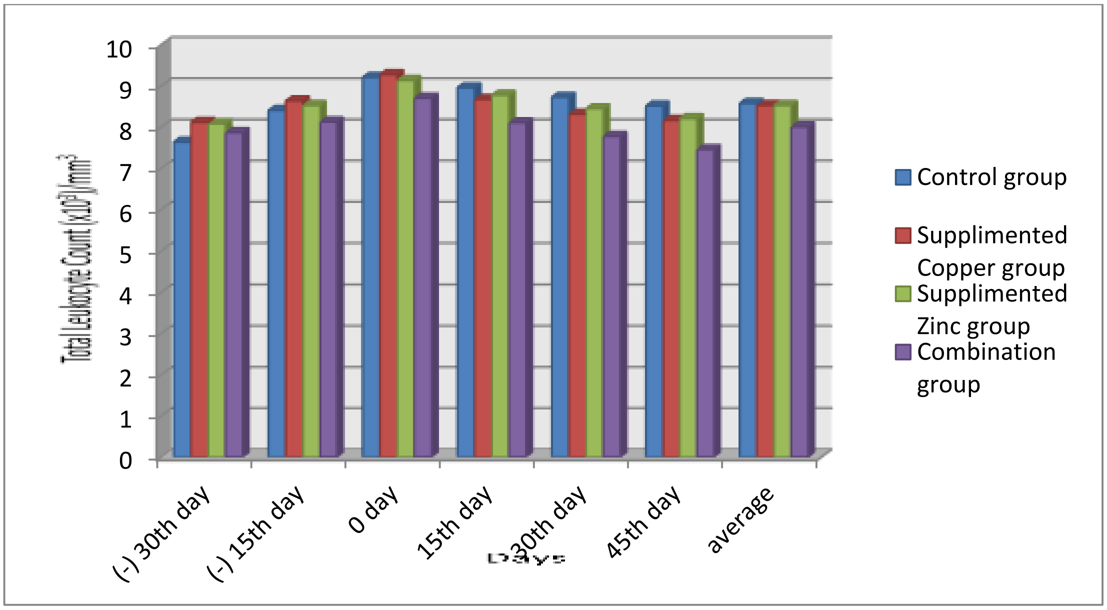

Fig. 1: Average total leukocyte counts in blood of control and supplemented groups with copper, zinc and their combination during different time period

Blood Neutrophil

The least square means of total neutrophils percentage in blood of cows supplemented with copper, zinc and combination of both and the control cows during pre-partum, at day of calving and post calving period have been presented in Table 3 and Fig. 2.

Table 3: Mean (±SE) of blood neutrophils percentage of control Kankrej cows and cows supplemented with copper, zinc and their combination

Groups	Pre-partum Days		Calving Day	Post-partum Days			Overall mean
	30^{th}	15^{th}	0 day	15^{th}	30^{th}	45^{th}	
Control group	29.30± 0.82	32.19± 0.82	33.90±0.8	29.86±0.87	27.66±0.86	25.89±0.88	**29.80^{B}±0.58**
Supplemented Copper group	21.61±0.8	22.70±0.94	24.52±1.06	23.49±1.08	22.76±0.94	21.89±0.63	**22.83^{A}±0.38**
Supplemented Zinc group	22.63±1	23.75±1.03	25.73±1.2	23.70±1.35	22.47±1.4	22.10±0.98	**23.40^{A}±0.49**
Supplemented combination group	21.40±1.01	22.37±1.09	25.87±1.36	23.33±1.43	21.89±1.1	20.68±1.2	**22.59^{A}±0.54**
Overall mean± SE	23.73^{ab}±1.71	25.25^{b}±2.06	27.50^{c}±2	25.10^{b}±1.68	23.70^{ab}±1.46	22.64^{a}±1.25	**24.65±1.82**

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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).

There was significant (P ≤ 0.05) difference found in copper, zinc and combination group with the control group but no significant difference among supplemented copper, zinc and combination group as presented Table 3. In all supplemented and control cow's blood neutrophils percentage gradually increased from 30 days prepartum to the day of calving and then again gradually decreased and reach the normal level by the 45 day post-partum. Blood neutrophils percentage remains highest on the day of calving in all the supplemented and control cows. Maximum increase in blood neutrophil percentage was found in control cows and minimum increase was observed in combination supplemented cows.

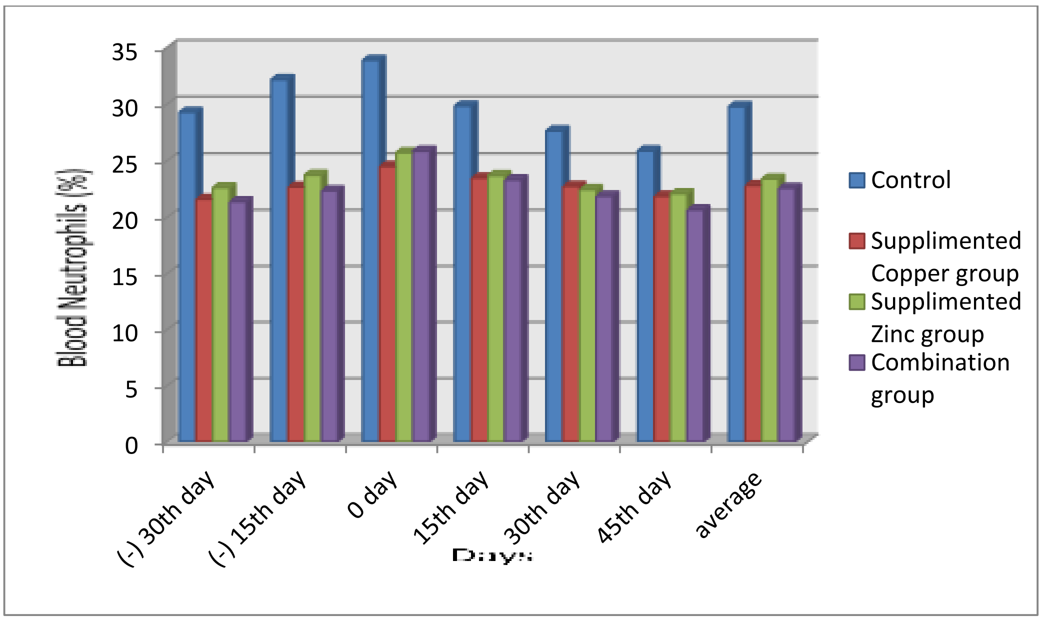

Fig. 2: Average neutrophils percentage in blood of control and supplemented groups with copper, zinc and their combination during different time period

The result revealed that blood neutrophils percentage differed significantly (P≤0.01) between groups as well as between different days of peripartum period, day of calving and post-partum days. The analysis of variance table showed non-significant interaction effect due to the group x days as shown in Table 4.

Table 4: Analysis of variance of blood neutrophils 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
Groups	3	1070.068	356.689	64.253**
Days	5	289.731	57.946	10.438**
Groups x Days	15	80.165	5.344	0.963 ^{NS}
Residual	96	532.93	5.551	-

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

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Results resembles with the previous findings of Gengelback *et al*. (1997) who found that low copper status has resulted in decreased humoral and cell-mediated immunity in cattle.

Neutrophils Percentage in Colostrums

Neutrophils percentage in colostrums of control group and supplemented cows with copper, zinc and combination of both at different days of lactation has been presented in Table 5. The highest value of mean of neutrophils percentage in colostrums was estimated in control as 26.80 percent followed by the 23.96 percent in supplemented zinc group, 24.27 percent in supplemented copper group and 22.46 percent in combination group, respectively. Result of this study revealed that the neutrophils percentage were significantly higher (P≤0.05) at day-0 (at day of calving) colostrums of all the cows of four groups. These values decreased significantly (P≤0.05) on day-3 and day-5 respectively. It was also noticed that significant difference was observed between day 3rd and day 5th in combination group as shown in Table 5.

Table 5: Mean (±SE) of neutrophils percentage in colostrums of control Kankrej cows and cows supplemented with copper, zinc and their combination

Groups	Days of Lactation			Overall Mean± SE
	0 day	**3rd day**	**5th day**	
Control group	28.81±0.42	26.83±0.43	24.75±0.43	**26.80^{c}±0.51**
Supplemented Copper group	25.83±0.71	24.37±0.44	21.67±0.44	**23.96^{b}±0.54**
Supplemented Zinc group	26.37±0.33	24.20±0.47	22.24±0.47	**24.27^{b}±0.49**
Supplemented combination group	24.77±0.76	22.85±0.58	19.76±0.58	**22.46^{a}±0.64**
Overall mean± SE	**26.44^{c}±0.43**	**24.56^{b}±0.39**	**22.10^{a}±0.46**	-

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

Table 6: Analysis of variance of colostrums neutrophils 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	145.996	48.6655	35.2417	2.84*	4.31**
Days	2	189.594	94.797	68.6483	3.23*	5.18**
Groups x Days	6	2.8374	0.47291	0.34246	2.34^{NS}	3.29^{NS}
Residual	48	66.2835	1.3809	-	-	-

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

An appraisal of analysis of variance table revealed that neutrophil percentage differed significantly (P≤0.01) between groups as well as between different days of lactation of cows. The interaction effect of group x days was found to be non-significant on neutrophils in colostrums in different days of lactation of cows. Similar results were reported by Droke *et al*. (1993) who reported that zinc deficiency and copper deficiency has been shown to have an important impact on immunity.

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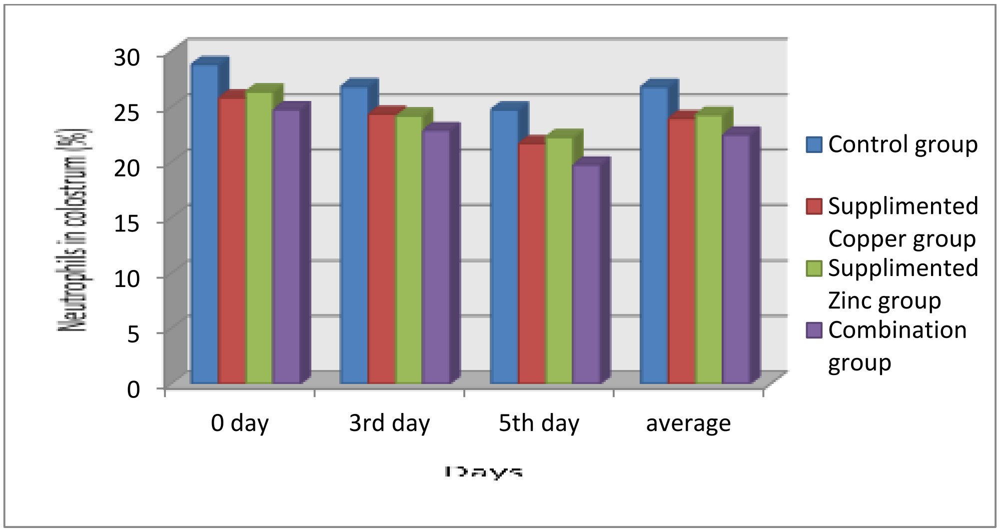

Fig. 3: Average colostrums neutrophils percentages of control and supplemented groups with copper, zinc and their combination during different time period

Conclusion

From the above study it can be concluded that supplementation of micronutrients such as copper and zinc improved the udder health by reducing percent neutrophils of milk. Of all the micronutrients maximum beneficial effect was seen when the micronutrients (Copper + Zinc) are fed in combination to the peripartum cows. Micronutrient supplementation improves immunity of an animal by reducing number of TLC in blood. So micronutrients also affect milk production because health of animal and udder are related with immune status.

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