



## Effect of customized mineral supplement on blood biochemical profile, antioxidant indices and immunity in kids

ROJITA YENGKHOM<sup>1</sup>, A K VERMA<sup>2</sup>, NARAYAN DUTTA<sup>3</sup>, S E JADHAV<sup>4</sup> and A K PATTANAIK<sup>5</sup>

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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### ABSTRACT

The study was carried out to examine the effects of customized mineral supplement on blood-biochemical profile, antioxidant indices and immunity in kids. Kids (18) were randomly divided into 3 groups with 6 kids in each group in a CRD. The kids were randomly allocated into CON, CMM and CMS groups. In CON group, kids were offered a concentrate mixture without mineral mixture; however, kids in CMM and CMS groups were provided concentrate mixture having commercial and customized mineral supplement, respectively. The feeding trial lasted for 135 days. Haematocrit and serum glucose levels were significantly lower in CON as compared to CMS and CMM groups. The mean Hb was significantly higher in CMS relative to CON. Total protein was significantly higher in CMS group as compared to CMM and CON, while serum urea, albumin, globulin, A:G, serum enzymes (ALT and AST) did not differ significantly among treatments. The LPO levels were decreased in CMS group than CON. The CAT and GSH levels were comparable irrespective of treatments, however, SOD levels increased significantly in CMS followed by CMM and CON groups, respectively. The CMI response was significantly higher in CMS than CON and CMM groups. The mean antibody titer (log base<sub>2</sub>) against chicken RBC was significantly higher in CMS and CMM groups relative to CON. It may be concluded that the supplementation of customized mineral supplement considerably improved the haematological parameters, antioxidant indices and immune response in kids.

**Key words:** Antioxidant indices, Biochemical profile, Immunity, Kids, Metabolic profile, Mineral supplement

Minerals have a great impact on animal performance and its imbalance adversely affects growth, fertility, immunity and ultimately affecting production (Bhandari *et al.* 2016). Health and production of livestock is thus greatly influenced by optimal level of essential and trace mineral in the body (Mohanta and Garg 2014, Yengkhom *et al.* 2018). Supplementation of all the elements in diet may not always be desirable, because many of them such as Mg, S, K, I, Co, Fe and Mn are present in required concentrations in feeds and fodders of specific areas. At present, commercial mineral mixtures are prepared and marketed without considering the actual deficiency or excess of minerals in animals of a specific region. Keeping this background in view, the proposed research work was planned to assess the effects of customized mineral supplement on blood-biochemical profile, antioxidant indices and immune response in kids.

### MATERIALS AND METHODS

**Experiment, animals and diets:** The experiment was carried out at Animal Nutrition Research Shed of the

Institute. Eighteen kids of about 6 months old with mean BW of 10.70±0.7 kg were randomly divided into 3 groups with 6 kids in each group in a completely randomized block design. The kids were randomly allocated into 3 dietary treatments, viz. control (CON) and 2 treatment groups (CMM and CMS). The experimental kids in CON group were offered a concentrate mixture without mineral mixture; however, kids in CMM and CMS groups were given concentrate mixture having commercial mineral mixture and customized mineral supplement, respectively. All the kids were dewormed at the onset of the experiment and reared under uniform managerial conditions for a period of 135 days. All the kids were housed in well ventilated shed having provision for individual feeding.

The kids were provided daily a weighed amount of respective concentrate mixture and wheat straw in the morning at 10.00 AM to meet their nutrient requirements for maintenance and 50 g daily growth (Kearl 1982). Wheat straw was offered *ad lib.* after ensuring complete consumption of the concentrate mixture. Wheat straw refusals were weighted daily on the next morning to estimate wheat straw consumption per day and sampled at weekly intervals for subsequent analysis of dry matter (DM) to assess the average DM intake during the experimental period. A small amount of green oats (200 g) was given to

Present address: <sup>1</sup>PhD Scholar (rojityeng11@gmail.com), <sup>2</sup>Head (vermaak62@gmail.com), <sup>3,5</sup>Principal Scientist (dutta65@gmail.com, akpattanaik1@gmail.com), <sup>4</sup>Senior Scientist (sejadhav1@gmail.com).

all the experimental kids to satisfy the part of their respective nutrient requirements. The animals were provided with fresh and clean tap water free choice twice daily. The body weight of the individual kid was recorded at fortnightly intervals in the morning before feeding and watering for 135 d in order to assess the change in body weight.

**Blood collection and estimation of blood biochemical profile:** Blood samples (6 ml) were collected from all the experimental kids early in the morning before feeding at 0, 60 and 135 d, post-feeding, aseptically by jugular vein puncture. Out of 6 ml, 2 ml was added with EDTA for haematological parameters and the remaining was taken in a well cleaned sterilized centrifuge tubes to collect sera. Serum was separated and preserved at  $-20^{\circ}\text{C}$  for further analysis. The metabolic profile was estimated by using diagnostic kits. Haemoglobin (Hb) and hematocrit were estimated in whole blood immediately after blood collection by cyanomethaemoglobin method and Wintrobe's tube, respectively. Serum glucose concentration was determined colorimetrically. The serum protein and albumin content were measured as per Vatzidis (1977) and Doumas *et al.* (1971), respectively. Serum urea and serum glucose was estimated as per Hallett and Cook (1971). Activity of serum enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined as per standard methods described by Thefeld *et al.* (1974) whereas alkaline phosphatase (ALP) was determined as per Bretaudiere *et al.* (1977).

**Antioxidant indices and immune response:** On each collection, blood samples were collected aseptically from jugular vein of every kid in 3.0 ml wintrobe tube with acid citrate dextrose buffer @ 1.5 ml/10.0 ml of blood. The collected samples were processed to prepare haemolysate and kept at  $-20^{\circ}\text{C}$  and used for antioxidant assays. The superoxide dismutase (SOD) activity of RBC haemolysate samples was measured using nitro blue tetrazolium (NBT) as a substrate after suitable dilution according to the method of Marklund and Marklund (1974), with certain modifications as suggested by Minami and Yoshikawa (1979). Lipid peroxidation (LPO) was determined by estimating the concentration of malondialdehyde (MDA) in Hb. The reduced glutathione (GSH) and catalase (CAT) were estimated by Dithio-bis-2 nitrobenzoic acid (DTNB) method of Prins and Loos (1969) and Bergmeyer (1983), respectively. Non-protein thiol (NP-SH) group in the erythrocytes was estimated following the method of Sedlak and Lindsay (1968).

The cell mediated immune (CMI) response was assessed by delayed-type hypersensitivity (DTH) response to intra-dermal inoculation of phytohaemagglutinin-P (PHA-P) as described by Dey *et al.* (2015). Humoral immune response was assessed by challenging the animals with intravascular single dose of chicken red blood cells (CRBC, 20% suspension in phosphate-buffered saline; PBS) following the method of Dutta *et al.* (2012).

**Statistical analysis:** Data obtained were subjected to analysis of variance using SPSS software (v11.0) and

treatment means were ranked using Duncan's multiple range tests. Significant variance between treatments were measured at  $P < 0.05$ . Statistical analyses were carried as per the procedures of Snedecor and Cochran (1994).

## RESULTS AND DISCUSSION

**Chemical composition of feeds:** The chemical composition of concentrate mixtures CON, CMM and CMS offered to experimental kids was analogous with the values reported for goats in previous reports (Patra *et al.* 2006, Dey *et al.* 2015). All the supplements were isonitrogenous and isocaloric (Table 1). The chemical composition of green oats and wheat straw was also found to be in normal range of Indian feeds (Dey *et al.* 2015).

**Blood-biochemical parameters:** The haematocrit (%) was significantly ( $P < 0.05$ ) lower in CON group as compared to CMS and CMM groups. Mean Hb (g/dL) values were significantly ( $P < 0.05$ ) higher in CMS relative to CON, while CMM has an intermediate position between CMS and CON groups (Table 2). Serum glucose (mg/dL) was significantly ( $P < 0.01$ ) lower in CON as compared to CMM and CMS. Several previous reports (Satapathy *et al.* 2016, Kiomurasi *et al.* 2011, Godara *et al.* 2015, Khan *et al.* 2015) support the fact that supplementation of mineral mixture/area specific mineral mixture improved serum/plasma glucose levels in goats and buffaloes. This might be attributed to the effect of minerals either as the cofactors and/or activators of many enzymatic systems associated with the metabolism of nutrients and again Zn is known to alter molar proportion of VFA in the rumen with an increase in propionate (Arelovich *et al.* 2000) resulting in increased glucose level in the plasma (Aliarabi and Chhabra 2006). Total protein (g/dL) was significantly ( $P < 0.01$ ) higher in CMS group as compared to CMM and CON groups, while serum urea (mg/dL), albumin (g/dL), globulin (g/dL) and A:G ratio did not differ significantly ( $P > 0.05$ ) among the treatment groups. The present research findings are supported by earlier workers (Huert *et al.* 2002, Kinal *et al.* 2007), who reported no change in plasma/serum urea level after mineral supplementation. Satapathy *et al.* (2016) also observed no

Table 1. Chemical composition of feeds (% DM basis)

| Attribute | Concentrate supplements |       |       | Green oats | Wheat straw |
|-----------|-------------------------|-------|-------|------------|-------------|
|           | CON                     | CMM   | CMS   |            |             |
| OM        | 90.96                   | 91.03 | 91.27 | 91.57      | 90.15       |
| CP        | 22.32                   | 22.36 | 22.40 | 8.43       | 3.32        |
| EE        | 3.10                    | 3.22  | 3.15  | 2.49       | 1.62        |
| Total Ash | 8.80                    | 8.81  | 8.73  | 8.43       | 9.85        |
| NDF       | 43.89                   | 45.16 | 44.62 | 65.97      | 81.33       |
| ADF       | 25.18                   | 25.10 | 25.09 | 36.20      | 52.32       |
| Ca        | 0.88                    | 1.16  | 1.39  | 2.21       | 0.23        |
| P         | 0.32                    | 0.58  | 0.69  | 0.34       | 0.15        |

CON: Maize-30, Wheat bran-37, DSBM-30, Salt-1.0; CMM: Maize-30, Wheat bran-37, DSBM-30, CMM- 2.0, Salt-1.0; CMS: Maize-30, Wheat bran-37, DSBM-30, CMS - 2.0, Salt-1.0.

Table 2. Effect of various mineral supplements on metabolic profile in kids

| Treatment                    | Periods (d)              |                           |                          | Treatment mean±SE         | P value |       |       |
|------------------------------|--------------------------|---------------------------|--------------------------|---------------------------|---------|-------|-------|
|                              | 0                        | 60                        | 135                      |                           | G       | P     | G*P   |
| <i>Haematocrit (%)</i>       |                          |                           |                          |                           |         |       |       |
| CON                          | 33.80 <sup>a</sup>       | 35.60 <sup>b</sup>        | 37.17 <sup>bcd</sup>     | 35.60 <sup>A</sup> ±0.33  | 0.028   | 0.00  | 0.000 |
| CMM                          | 36.00 <sup>b</sup>       | 36.40 <sup>bc</sup>       | 38.00 <sup>cd</sup>      | 36.80 <sup>B</sup> ±0.33  |         |       |       |
| CMS                          | 35.40 <sup>ab</sup>      | 36.00 <sup>b</sup>        | 38.60 <sup>d</sup>       | 36.67 <sup>B</sup> ±0.33  |         |       |       |
| Period mean±SE               | 35.07 <sup>a</sup> ±0.33 | 36.00 <sup>a</sup> ±0.33  | 38.00 <sup>b</sup> ±0.33 |                           |         |       |       |
| <i>Haemoglobin (g/dL)</i>    |                          |                           |                          |                           |         |       |       |
| CON                          | 9.11 <sup>a</sup>        | 10.74 <sup>bcd</sup>      | 10.55 <sup>bc</sup>      | 10.13 <sup>A</sup> ±0.20  | 0.051   | 0.00  | 0.000 |
| CMM                          | 9.88 <sup>ab</sup>       | 11.11 <sup>cd</sup>       | 11.69 <sup>d</sup>       | 10.81 <sup>AB</sup> ±0.20 |         |       |       |
| CMS                          | 10.22 <sup>abc</sup>     | 11.03 <sup>bcd</sup>      | 11.73 <sup>d</sup>       | 11.03 <sup>B</sup> ±0.20  |         |       |       |
| Period mean±SE               | 9.74 <sup>a</sup> ±0.21  | 11.00 <sup>b</sup> ±0.21  | 11.32 <sup>c</sup> ±0.21 |                           |         |       |       |
| <i>Serum glucose (mg/dL)</i> |                          |                           |                          |                           |         |       |       |
| CON                          | 62.17 <sup>a</sup>       | 62.97 <sup>a</sup>        | 63.63 <sup>ab</sup>      | 62.91 <sup>A</sup> ±1.38  | 0.000   | 0.749 | 0.010 |
| CMM                          | 68.64 <sup>abc</sup>     | 69.45 <sup>abc</sup>      | 69.53 <sup>abc</sup>     | 69.20 <sup>B</sup> ±1.38  |         |       |       |
| CMS                          | 71.62 <sup>c</sup>       | 71.09 <sup>bc</sup>       | 72.97 <sup>c</sup>       | 71.41 <sup>B</sup> ±1.38  |         |       |       |
| Period mean±SE               | 67.74±1.38               | 67.36±1.38                | 68.70±1.38               |                           |         |       |       |
| <i>Total protein (g/dL)</i>  |                          |                           |                          |                           |         |       |       |
| CON                          | 6.49                     | 6.52                      | 6.72                     | 6.53 <sup>A</sup> ±0.01   |         |       |       |
| CMM                          | 6.53                     | 6.59                      | 6.65                     | 6.59 <sup>A</sup> ±0.01   |         |       |       |
| CMS                          | 6.86                     | 6.87                      | 7.08                     | 6.70 <sup>B</sup> ±0.01   |         |       |       |
| Period mean±SE               | 6.62±0.09                | 6.70±0.09                 | 6.77±0.09                |                           |         |       |       |
| <i>Albumin (g/dL)</i>        |                          |                           |                          |                           |         |       |       |
| CON                          | 3.66                     | 3.58                      | 3.63                     | 3.63±0.05                 |         |       |       |
| CMM                          | 3.63                     | 3.64                      | 3.65                     | 3.64±0.05                 |         |       |       |
| CMS                          | 6.66                     | 3.73                      | 3.70                     | 3.68±0.05                 |         |       |       |
| Period mean±SE               | 3.65±0.05                | 3.63±0.05                 | 3.67±0.05                |                           |         |       |       |
| <i>Globulin (g/dL)</i>       |                          |                           |                          |                           |         |       |       |
| CON                          | 2.84                     | 2.95                      | 2.92                     | 2.90 <sup>A</sup> ±0.13   |         |       |       |
| CMM                          | 2.91                     | 2.96                      | 3.00                     | 2.96 <sup>AB</sup> ±0.13  |         |       |       |
| CMS                          | 3.20                     | 3.30                      | 3.38                     | 3.29 <sup>B</sup> ±0.13   |         |       |       |
| Period mean±SE               | 2.98±0.13                | 3.07±0.13                 | 3.10±0.13                |                           |         |       |       |
| <i>A:G ratio</i>             |                          |                           |                          |                           |         |       |       |
| CON                          | 1.31                     | 1.24                      | 1.27                     | 1.27±0.06                 |         |       |       |
| CMM                          | 1.29                     | 1.27                      | 1.26                     | 1.27±0.06                 |         |       |       |
| CMS                          | 1.18                     | 1.14                      | 1.12                     | 1.15±0.06                 |         |       |       |
| Period mean±SE               | 1.26±0.06                | 1.22±0.06                 | 1.22±0.06                |                           |         |       |       |
| <i>Serum urea (mg/dL)</i>    |                          |                           |                          |                           |         |       |       |
| CON                          | 15.30 <sup>a</sup>       | 16.56 <sup>abc</sup>      | 17.09 <sup>bc</sup>      | 16.32±0.31                | 0.104   | 0.003 | 0.024 |
| CMM                          | 16.85 <sup>abc</sup>     | 16.73 <sup>abc</sup>      | 17.81 <sup>bc</sup>      | 17.13±0.31                |         |       |       |
| CMS                          | 16.08 <sup>ab</sup>      | 17.25 <sup>bc</sup>       | 18.14 <sup>c</sup>       | 17.16±0.31                |         |       |       |
| Period mean±SE               | 16.08 <sup>a</sup> ±0.31 | 16.85 <sup>ab</sup> ±0.31 | 17.68 <sup>b</sup> ±0.31 |                           |         |       |       |
| <i>ALP (IU/L)</i>            |                          |                           |                          |                           |         |       |       |
| CON                          | 142.47                   | 122.83                    | 123.97                   | 123.09±2.77               | 0.918   | 0.923 | 1.000 |
| CMM                          | 120.74                   | 121.72                    | 122.08                   | 122.17±2.77               |         |       |       |
| CMS                          | 121.08                   | 121.96                    | 122.94                   | 121.99±2.77               |         |       |       |
| Periodmean±SE                | 121.43±2.77              | 122.17±2.77               | 123.00±2.77              |                           |         |       |       |
| <i>AST (IU/L)</i>            |                          |                           |                          |                           |         |       |       |
| CON                          | 82.81                    | 82.94                     | 82.81                    | 82.85±4.34                | 0.996   | 0.999 | 1.000 |
| CMM                          | 82.69                    | 82.06                     | 82.81                    | 82.52±4.34                |         |       |       |
| CMS                          | 82.19                    | 82.31                     | 82.44                    | 82.31±4.34                |         |       |       |
| Period mean±SE               | 82.58±4.34               | 82.44±4.34                | 82.69±4.34               |                           |         |       |       |
| <i>ALT (IU/L)</i>            |                          |                           |                          |                           |         |       |       |
| CON                          | 19.59                    | 21.69                     | 22.18                    | 21.16 <sup>B</sup> ±0.99  | 0.040   | 0.190 | 0.260 |
| CMM                          | 16.06                    | 18.10                     | 19.02                    | 17.73 <sup>A</sup> ±0.99  |         |       |       |
| CMS                          | 17.00                    | 18.72                     | 18.88                    | 18.20 <sup>A</sup> ±0.99  |         |       |       |
| Period mean±SE               | 17.55±0.99               | 19.50±0.99                | 20.03±0.99               |                           |         |       |       |

abcd,AB Means with different superscripts within a row and column differ significantly.

significant changes in serum urea, albumin, globulin, A:G after supplementing area specific mineral mixture. Serum protein level depends on various factors including extent, duration and nature of hepatic disorders and the presence of the other pathological conditions (Kaneko 1997). No significant ( $P>0.05$ ) difference was observed in serum enzymes irrespective of treatment groups except ALT which is significantly higher ( $P<0.05$ ) in CON group as compared to CMM and CMS groups. Pandey *et al.* (2018) also reported no effect of organic form of minerals (Mn, Cu and Zn and Se) on AST level. ALP concentration was similar before and after the treatment. Godara *et al.* (2015) also support the fact that supplementation of commercial/area specific mineral mixture did not make any change in the AST and ALT levels in goats. Interestingly, normal levels of AST and ALT indicate no adverse on liver and muscle functions.

**Antioxidant indices:** Antioxidant enzymes, viz. GSH, CAT, SOD, LPO and NPSH levels play a significant role in body defense mechanism by scavenging reactive oxygen species (ROS). Antioxidants comprising of an array of endogenous and exogenous substances serve to stabilize these highly reactive free radicals, thereby remaining the structural and functional integrity of cells including important immune cells (Bisla *et al.* 2002, Han *et al.* 2004). The LPO is used as an indicator of oxidative stress in cells and tissues. The LPO levels were decreased ( $P<0.06$ ) in

CMS group as compared to CON, while CMM has the intermediate position. Similarly, LPO level was significantly ( $P<0.05$ ) reduced at III<sup>rd</sup> period as compared to I<sup>st</sup> period (Table 3). The present research findings are supported by previous report (Spears and Weiss 2008), that the essential trace minerals, viz. Zn, Cu, Mn and Se are involved in the antioxidant defense system.

The CAT and GSH levels were comparable ( $P>0.05$ ) irrespective of treatments, however, SOD levels increased significantly ( $P<0.05$ ) in CMS followed by CMM and CON groups, respectively. Correspondingly, SOD levels increased significantly ( $P<0.01$ ) from I<sup>st</sup> period onwards. Our results are in conformity with the finding of Jing *et al.* (2007), who fed rats on different Zn levels for 6 weeks and reported that rats fed on Zn deficient diet had reduced activities of copper-zinc superoxide dismutase (Cu-Zn SOD) and CAT; and increased malondialdehyde as well as hydrogen peroxide concentrations in liver. Similarly, Muhammad (2012) found that supplementation of antioxidant minerals to the albino rats increased the activities of CAT, Gpx, and SOD as compared with hypertensive control. Nevertheless, CAT activity in the present study was comparable among treatment groups. Prohaska and Brokate (2001) reported that rats fed a low copper diet had lower Cu, Zn-SOD activity in the liver, heart, and kidney. In addition, there was a significant reduction in Cu, Zn-SOD protein detected by Western

Table 3. Effect of mineral supplements on serum LPO, CAT, SOD and GSH activity in kids

| Treatments             | Period (d)               |                           |                          | Treatment<br>mean±SE     | Period (d) |       |       |
|------------------------|--------------------------|---------------------------|--------------------------|--------------------------|------------|-------|-------|
|                        | 0                        | 60                        | 135                      |                          | G          | P     | G*P   |
| <i>LPO (μmol/mgHb)</i> |                          |                           |                          |                          |            |       |       |
| CON                    | 3.34 <sup>d</sup>        | 2.83 <sup>abc</sup>       | 2.72 <sup>abc</sup>      | 2.97 <sup>B</sup> ±0.08  | 0.066      | 0.000 | 0.004 |
| CMM                    | 3.14                     | 2.84 <sup>abc</sup>       | 2.71 <sup>abc</sup>      | 2.90 <sup>AB</sup> ±0.08 |            |       |       |
| CMS                    | 2.94 <sup>bcd</sup>      | 2.69 <sup>ab</sup>        | 2.49 <sup>a</sup>        | 2.71 <sup>A</sup> ±0.08  |            |       |       |
| Period mean±SE         | 3.14 <sup>b</sup> ±0.08  | 2.79 <sup>ab</sup> ±0.08  | 2.64 <sup>a</sup> ±0.08  |                          |            |       |       |
| <i>CAT (U/mgHb)</i>    |                          |                           |                          |                          |            |       |       |
| CON                    | 77.08 <sup>b</sup>       | 81.03 <sup>b</sup>        | 64.74 <sup>a</sup>       | 74.28±1.97               | 0.321      | 0.018 | 0.031 |
| CMM                    | 78.88 <sup>b</sup>       | 73.39 <sup>ab</sup>       | 75.49 <sup>b</sup>       | 75.92±1.97               |            |       |       |
| CMS                    | 83.62 <sup>b</sup>       | 77.07 <sup>b</sup>        | 74.82 <sup>ab</sup>      | 78.51±1.97               |            |       |       |
| Period mean±SE         | 79.86 <sup>b</sup> ±1.97 | 77.16 <sup>ab</sup> ±1.97 | 71.69 <sup>a</sup> ±1.97 |                          |            |       |       |
| <i>SOD (U/mgHb)</i>    |                          |                           |                          |                          |            |       |       |
| CON                    | 52.45 <sup>a</sup>       | 60.27 <sup>ab</sup>       | 63.59 <sup>bc</sup>      | 58.77 <sup>A</sup> ±1.21 | 0.051      | 0.000 | 0.004 |
| CMM                    | 55.97 <sup>ab</sup>      | 59.45 <sup>ab</sup>       | 66.21 <sup>bc</sup>      | 60.54 <sup>B</sup> ±1.21 |            |       |       |
| CMS                    | 55.99 <sup>ab</sup>      | 61.07 <sup>ab</sup>       | 71.92 <sup>c</sup>       | 63.00 <sup>C</sup> ±1.21 |            |       |       |
| Period mean±SE         | 54.80 <sup>a</sup> ±1.81 | 60.26 <sup>b</sup> ±1.81  | 67.24 <sup>c</sup> ±1.81 |                          |            |       |       |
| <i>GSH (μmol/mgHb)</i> |                          |                           |                          |                          |            |       |       |
| CON                    | 21.96 <sup>b</sup>       | 18.29 <sup>a</sup>        | 17.09 <sup>a</sup>       | 19.11±0.54               | 0.234      | 0.000 | 0.007 |
| CMM                    | 19.64 <sup>ab</sup>      | 17.40 <sup>a</sup>        | 16.92 <sup>a</sup>       | 17.87±0.54               |            |       |       |
| CMS                    | 19.24 <sup>ab</sup>      | 17.91 <sup>a</sup>        | 16.60 <sup>a</sup>       | 16.87±0.54               |            |       |       |
| Period mean±SE         | 20.28 <sup>b</sup> ±0.54 | 17.87 <sup>a</sup> ±0.54  | 16.87 <sup>a</sup> ±0.54 |                          |            |       |       |
| <i>NSPH (μmol/ml)</i>  |                          |                           |                          |                          |            |       |       |
| CON                    | 0.84 <sup>a</sup>        | 0.85 <sup>ab</sup>        | 0.86 <sup>abc</sup>      | 0.85 <sup>A</sup> ±0.00  | 0.001      | 0.000 | 0.000 |
| CMM                    | 0.85 <sup>ab</sup>       | 0.87 <sup>bc</sup>        | 0.88 <sup>cd</sup>       | 0.87 <sup>B</sup> ±0.00  |            |       |       |
| CMS                    | 0.85 <sup>ab</sup>       | 0.88 <sup>c</sup>         | 0.90 <sup>d</sup>        | 0.88 <sup>B</sup> ±0.00  |            |       |       |
| Period mean±SE         | 0.85 <sup>b</sup> ±0.00  | 0.86 <sup>b</sup> ±0.00   | 0.88 <sup>c</sup> ±0.00  |                          |            |       |       |

abcd,AB Means with different superscripts within a row and column differ significantly.

Table 4. Effect of mineral supplements on DTH response (skin thickness in mm) of kids to PHA-p

| Treatment | Periods (h post inoculation) |                         |                         |                         |                         |                         | Treatment mean±SE       | P value |       |       |
|-----------|------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|---------|-------|-------|
|           | 0                            | 12                      | 24                      | 48                      | 72                      | 96                      |                         | G       | P     | G*P   |
| CON       | 2.32 <sup>a</sup>            | 3.90 <sup>efgh</sup>    | 3.69 <sup>def</sup>     | 3.42 <sup>cde</sup>     | 2.90 <sup>abc</sup>     | 2.57 <sup>ab</sup>      | 3.13 <sup>A</sup> ±0.09 | 0.001   | 0.000 | 0.000 |
| CMM       | 2.32 <sup>a</sup>            | 4.21 <sup>fgh</sup>     | 3.89 <sup>efgh</sup>    | 3.37 <sup>bcd</sup>     | 3.04 <sup>bcd</sup>     | 2.75 <sup>abc</sup>     | 3.26 <sup>A</sup> ±0.09 |         |       |       |
| CMS       | 2.49 <sup>ab</sup>           | 4.56 <sup>h</sup>       | 4.41 <sup>gh</sup>      | 3.81 <sup>efg</sup>     | 3.41 <sup>cde</sup>     | 3.17 <sup>bcd</sup>     | 3.64 <sup>B</sup> ±0.09 |         |       |       |
| Period    | 2.38 <sup>a</sup> ±0.13      | 4.22 <sup>d</sup> ±0.01 | 4.00 <sup>b</sup> ±0.13 | 3.54 <sup>c</sup> ±0.13 | 3.12 <sup>b</sup> ±0.13 | 2.83 <sup>b</sup> ±0.13 |                         |         |       |       |
| mean±SE   |                              |                         |                         |                         |                         |                         |                         |         |       |       |

abcdefgh,AB Means with different superscripts within a row and column differ significantly.

immunoblotting that was proportional ( $r=0.96$ ) to the reduction in Cu, Zn-SOD activity. The NPSH ( $\mu\text{mol/ml}$ ) level was significantly ( $P<0.05$ ) higher in CMS group as compared to CMM and CON. The increased NPSH levels in mineral supplemented groups as compared to control indicate better antioxidant status of kids. Thiol groups act as intracellular antioxidants by scavenging free radicals and through enzymatic reactions. The water soluble thiol group protects biological membrane (Moscio *et al.* 1994, Dey 2005, Dubey 2007).

**Immune response:** The kids under CON, CMM and CMS groups exhibited an increased in skin thickness following the intra dermal injection of PHA-p. The mean values for skin thickness were significantly ( $P<0.01$ ) higher in CMS as compared to CON and CMM groups (Table 4). The increased CMI response in CMS supplemented kids may be attributed to the higher bioavailability of essential trace minerals like Zn, Cu, I and Mn. The mean antibody titre (log base 2) against chicken RBC was significantly ( $P<0.01$ ) higher in CMS and CMM groups relative to CON. The mean antibody titre differed significantly ( $P<0.01$ ) among different periods and being significantly ( $P<0.01$ ) higher at 14<sup>th</sup> day as compared to comparable values between 7<sup>th</sup> and 21<sup>st</sup> day of post inoculation (Fig 1). The interaction between period and treatment was also significant ( $P<0.01$ ). Trace minerals Zn, Cu, Mn and Se are important for the immune system and their deficiency can cause immune dysfunction because minerals are essential to cell function, cell development, and metabolic reactions. Zn is an important cofactor for numerous enzymes involved in cell metabolism and Zn deficiency can result in a profoundly immune deficient state (Saker, 2006). Cu plays a role in the development and maintenance of the immune system and Cu status alerts several aspects of neutrophil, monocyte, and T cell function (Wintergerst *et al.* 2007). Cu deficiency exerts deleterious effects on cell-mediated as well as the humoral response (Lukasewycz *et al.* 1989) in mixed-lymphocyte reaction, which is an *in vitro* assay of T cell proliferation in a cell-mediated response, the proliferative response of lymphocytes from Cu-deficient mice was diminished and Cu deficiency led to an impairment of the *in vivo* antibody response in mice to sheep red blood cell antigens. Ceruloplasmin (Cp), the major Cu-containing protein in serum, reflects Cu status. In Cu-deficient cattle, serum Cp activity decreased along with reduced SOD and

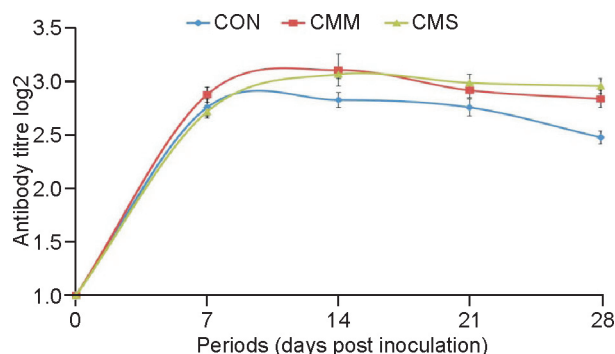


Fig. 1. Effect of mineral supplements on humoral immune response in kids.

cytochrome c oxidase activities in leukocyte, which may impair the cell immune function (Cerone *et al.* 2000). In the present study higher humoral immune response in CMS supplemented kids may be attributed to higher bioavailability of macro and micro minerals in CMS group as indicated by higher nutrient balances and serum levels of Ca, P, Zn, Cu and Mn in kids.

On the basis of above results, it may be concluded that the supplementation of customized mineral supplement @ 2% in the concentrate mixture, substantially improved the haematological parameters, serum glucose and total protein, antioxidant indices, cell mediated and humoral immune response in kids as compared to control.

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#### REFERENCES

- Aliarabi H and Chhabra A. 2006. Effect of inorganic and chelated zinc supplementation on the performance of crossbred calves. *Indian Journal of Animal Nutrition* **23**: 141–45.
- Arelovich H M, Owens F N, Horn G W and Vizcarra J A. 2000. Effect of supplemental zinc and manganese on ruminal fermentation, forage intake and digestion by cattle fed prairie hay and urea. *Journal of Animal Science* **78**: 2972–79.
- Bergmeyer H U. 1983. *Methods of Enzymatic Analysis*. 2<sup>nd</sup> edn. Verlag Chemie, Weinheim, pp 273–86.
- Bhandari B M, Goswami A, Garg M R and Samanta A. 2016. Study on minerals status of dairy cows and their

- supplementation through area specific mineral mixture in the state of Jharkhand. *Journal of Animal Science and Technology* **58**: 42.
- Bisla R S, Singh J and Krishnamurthy D. 2002. Assessment of oxidative stress in buffaloes suffering from diaphragmatic hernia. *Indian Journal of Veterinary Surgery* **23**: 77–80.
- Bretaudiere J P, Vassault A, Amsellem L, Pourci M L, Thieche-Phung H and Bailly M. 1977. Criteria for establishing a standardized method for determining alkaline phosphatase activity in human serum. *Clinical Chemistry* **23**: 2263–74.
- Cerone S I, Sansinanea A S, Streitenberg S A, Garcia M C and Auza N J. 2000. Cytochrome c-oxidase, Cu, Zn-superoxide dismutase and ceruloplasmin activities in copper-deficient bovines. *Biological Trace Element Research* **73**: 269–78.
- Dey A, Narayan Dutta, Pattanaik A K and Sharma K. 2015. Antioxidant status, metabolic profile and immune response of lambs supplemented with tannin rich *Ficus infectoria* leaf meal. *Japanese Journal of Veterinary Research* **63**: 15–24.
- Godara R S, Naskar S, Das B C, Godara A S, Ghosh M K, Mondal M and Bhat S A. 2015. Effect of area specific mineral supplementation on growth and reproductive performance of female black bengal goats. *Journal of Animal Research* **6**: 155.
- Doumas B T, Watson W A and Biggs H G. 1971. Albumin standards and the measurements of serum albumin with bromocresol green. *Clinica Chimica Acta* **31**: 87–96.
- Dubey M. 2007. 'Strategic use of tanniferous tree leaves on nutrient utilization, growth and GI parasites in goats.' MVSc Thesis, Deemed University, IVRI, Izatnagar, UP, India.
- Dutta N, Dubey M, Banerjee P S, Pattanaik A K, Sharma K, Kumar P and Narang A. 2012. Effect of supplementing tanniferous tree leaves mixture on immune response and GI nematodes in kids. *Livestock Research for Rural Development*. <http://www.lrrd.org/lrrd24/2/dutt24035.htm>
- Hallett C J and Cook J G H. 1971. Produced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. *Clinica Chimica Acta* **35**: 33–37.
- Han B, Yoon S, Su J, Han H R, Wang M, Qu W and Zhong D. 2004. Effects of selenium, copper and magnesium on antioxidant enzymes and lipid peroxidation in bovines fluorosis. *Asian Australasian Journal of Animal Sciences* **17**: 1695–99.
- Huert M, Kincaid R L, Cronrath J D, Busboom J, Johnson A B and Swenson C K. 2002. Interaction of dietary zinc and growth implants on weight gain, carcass traits and zinc in tissues of growing beef steers and heifers. *Animal Feed Science Technology* **95**: 15–32.
- Jain N, Tiwari S P and Singh P. 2005. Effect of urea molasses mineral granule on rumen fermentation pattern and blood biochemical constituents in goat kids fed sola grass based diet. *Veterinaeski Arhiv* **75**: 521–30.
- Jing M Y, Sun J Y and Weng X Y. 2007. Insights on zinc regulation of food intake and macronutrient selection. *Biological Trace Element Research* **115**: 197–94.
- Kaneko J J. 1997. Serum proteins and the dysproteinemias. *Clinical Biochemistry of Domestic Animals*. 5th edn. Academic Press, London. pp 117–38.
- Kearl L C. 1982. *Nutrient Requirements of Ruminants in Developing Countries*. International Feedstuffs Institute, Utah State University, Logan, UT, USA, pp 45–81.
- Khan H M, Mohanty T K, Bhakat M, Gupta A K, Tyagi A K and Mondal G. 2015. Effect of Vitamin E and mineral supplementation on biochemical profile and reproductive performance of buffaloes. *Buffalo Bulletin* **34**: 63–78.
- Kinal S, Korniewicz A, Slupczynska M, Bodarski R, Korniewicz D and Cermak B. 2007. Effect of the application of bioplexes of zinc, copper and manganese on milk quality and composition of milk and colostrum and some indices of the blood metabolic profile of cows. *Czech Journal of Animal Science* **52**: 423–29.
- Kioumars H, Yahaya Z S and Rahman A W. 2011. The effect of molasses/mineral feed blocks along with the use of medicated blocks on haematological and biochemical blood parameters in Boer goats. *Asian Journal of Animal and Veterinary Advances* **6**: 1683–91.
- Lukasewycz O A and Prohaska J R. 1989. Increased interleukin-1 (IL-1) production and decreased interleukin 2 (IL-2) production in copper deficient mice. *FASEB Journal* **3**: A665.
- Marklund S and Marklund G. 1974. Involvement of superoxide anion radical in autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**: 469–74.
- Minami M and Yoshikawa H. 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clinica Chimica Acta* **92**: 337–42.
- Mohanta R K and Garg A K. 2014. Organic trace minerals: immunity, health, production and reproduction in farm animals. *Indian Journal of Animal Nutrition* **31**: 203–12.
- Moscio A, Bufano G, Sevi A, Manchisi A, Martemucci G, Casamassima D, Djajanegara A and Sukmawati A. 1994. Effect of slaughtering age and dietary energy level on productive performance of fattening lambs. Proceedings of the 7<sup>th</sup> AAAP Animal Science Congress, Bali, Indonesia, pp. 75–76.
- Muhammad S A, Bilbis L S, Saidu Y and Adamu Y. 2012. Effect of antioxidant mineral elements supplementation in the treatment of hypertension in albino rats. *Oxidative Medicine and Cellular Longevity* **2012**: 1–8.
- Pandey V K, Gendley M K, Tiwari S P and Prusty S. 2018. Effect of area specific mineral mixture on serum biochemical parameters and milk yield in dairy cattle. *International Journal of Agriculture Sciences* **10**: 4965–68.
- Patra R C, Swarup D, Sharma M C and Naresh R. 2006. Trace mineral profile in blood and hair from cattle environmentally exposed to lead and cadmium around different industrial units. *Journal of Veterinary Medicine* **A53**: 511–17.
- Prins A and Loos G. 1969. *Biochemical Methods in Red Cell Genetics*. Academic Press, pp 127–29.
- Prohaska J R and Brokate B. 2001. Dietary copper deficiency alter protein levels of dopamine beta monooxygenase and tyrosine monooxygenase. *Experimental Biology and Medicine* **226**: 199–07.
- Saker K E. 2006. Nutrition and immune function. *Veterinary Clinics of North America: Small Animal Practice* **36**: 1199–24.
- Satapathy D, Mishra S K, Swain R K, Sethy K and Sahoo G R. 2016. Effect of supplementation of area specific mineral mixture on performance of crossbred cows with reproductive disorders in kakatpur block. *Indian Journal of Animal Nutrition* **33**: 279–84.
- Sedlak J and Lindsay R. 1968. Estimation of total protein bound and non-protein sulphydryl groups in tissue with Ellman's reagent. *Biophysica Acta* **25**: 92–105.
- Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. East West Press Pvt. Ltd., New Delhi, India.
- Spears J W and Weiss W P. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. *Veterinary Journal* **176**: 70–76.

- Thefeld W, Hoffmeister H, Busch E W, Koller P U and Vollmar J. 1974. Referenzwerte für die Bestimmungen der Transaminasen GOT und GPT sowie der alkalischen phosphatase im serum mit optimierten standardmethoden. *Deutsche. Medizinische. Wochenschrift* **99**: 343–51.
- Vatzidis H. 1977. An improved biuret reagent. *Clinical Chemistry* **23**: 908.
- Wintergerst E A, Maggini S and Hornig D H. 2007. Contribution of selected vitamins and trace elements to immune function. *Annals of Nutrition and Metabolism* **51**: 301–23.
- Yengkhom R, Verma A K, Dutta N, Jadhav S E and Pattanaik A K. 2018. Effect of a customized mineral supplement on nutrient metabolism, serum mineral profile and growth performance of kids. *Animal Nutrition and Feed Technology* **18**: 177–87.