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Longitudinal study on evaluation of serum micromineral and hormone interrelation in cattle

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Abstract
To evaluate serum micromineral and hormone interaction in cattle, thirty crossbred cows from Holstein Friesian herd were selected and divided into three groups *viz.* dry, lactating and pregnant. Each group was having ten animals (n=10). Serum microminerals like copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) were estimated by atomic absorption spectrophotometry and serum hormones like oestrogen (E_2), progesterone (P_4), trioiodothyronine (T_3) and tetraiodothyronine (T_4) by radio immuno assay (RIA). Serum microminerals and hormone levels were in deficient range. Positive and significant ($p<0.05$) correlation was found between Zn, Mn and estrogen; Cu, Fe, Zn, Mn and progesterone. Positive and significant ($p<0.05$) correlation was found between Cu, Fe, Zn and T_3 and T_4. Significant ($p<0.05$) improvement in Cu, Fe, Zn and progesterone was noticed 30 days post mineral supplementation in pregnant cows and oestrogen in dry cows. Microminerals are involved in hormone synthesis and production and hence deficiency of microminerals causes hormonal imbalance thus affecting health and production.

Keywords: cow, micromineral, steroid, thyroid, hormone

Introduction

Microminerals are important for functioning of numerous metalloenzymes [1] and metalloproteins [2] These enzymes and proteins are involved in a no. of biological processes [3] including hormone synthesis [4]. Hence deficiency of microminerals causes imbalance of hormones affecting health [5] and production [6]. Keeping this in view, the present study was undertaken to study micromineral-hormone interaction and alleviation of through supplementation.

Materials and Methods

Thirty cows from crossbred Holstein Friesian herd in Kashmir valley were selected and divided into three groups *viz.* dry, lactating and pregnant group. Each group was having ten animals (n=10). Blood samples (10ml) were collected by jugular veinpuncture into vials without anticoagulant and serum was harvested for estimating microminerals and hormones. Serum Cu, Fe, Zn and Mn concentration was estimated by atomic absorption spectrophotometry (Model No. AAS 4141, ECIL, India) after digesting the serum samples by the procedure of [7]. Standards for spectrophotometery were procured from Fluka (Sigma-Aldrich).

Serum estrogen (E_2), progesterone (P_4), triiodothyronin (T_3) and tetraiodothyronin (T_4) was determined by radioimmunoassay (RIA) technique using gamma scintillation counter (I^{125} calibrated), at Nuclear Research Laboratory, Division of Physiology & Climatology, IVRI, Izatnagar. Estradiol, T_3 and T_4 radioimmuno assay kits were obtained from Immunotech, France whereas progesterone radioimmunoassay kit was obtained from Bhabha Atomic Research Centre (BARC), Mumbai, India.

All animals were reared under similar managental conditions. Each animal was supplemented with 25g of area specific mineral supplement (Table 6) daily for 30 days. Re-evaluation for serum microminerals and hormones was done 30 days post supplementation.

Data collected from this study were analyzed for correlation, mean, standard error and analysis of variance (ANOVA) as per the method described by [8].

Results

Mean±SE of serum microminerals is given in Table 1. Serum micromineral levels were on deficient side of normal range. Significantly ($p<0.05$) lower Cu and Fe levels were recorded in pregnant cows than dry and lactating cows. Zn and Mn showed nonsignificant difference between the different groups. Mean±SE of serum hormones is given in Table 2. Lower serum

Hormone levels were recorded in all groups. Significantly ($p<0.05$) lower levels of oestrogen were reported in lactating cows than dry and pregnant cows. Progesterone and T_4 levels showed significant ($p<0.05$) difference between pregnant cows and dry and lactating cows. Nonsignificant ($p<0.05$) difference was noticed for T_3 levels between different groups.
Correlation coefficient between serum micromineral levels and serum hormone levels in three groups of cows is given in Table 3. In all groups positive and significant ($p<0.05$) correlation was found between Zn, Mn and oestrogen and between Cu, Fe, Zn, Mn and progesterone whereas positive but nonsignificant ($p<0.05$) correlation was found between Cu, Fe and oestrogen. In all groups positive and significant ($p<0.05$) correlation was found between Cu, Fe, Zn and T_3 and T_4 whereas positive but nonsignificant ($p<0.05$) correlation was found between Mn and T_3 and T_4.
Significant ($p<0.05$) increase in serum Cu, Zn and Fe was found in pregnant cows post 30 day supplementation whereas Mn showed non-significant ($p<0.05$) increase (Table 3). In dry and lactating groups non-significant ($p<0.05$) increase was noticed in different micro minerals after supplementation. Significant ($p<0.05$) increase in serum progesterone and T_4 in pregnant cows and oestrogen in dry cows was noticed 30 days after supplementation.

Table 1: Mean±SE of serum microminerals (ppm) in different groups of cows.

Goup	Cu	Fe	Zn	Mn
Dry	0.82±0.39 ^{a}	1.21±0.58 ^{a}	0.89±0.35	0.18±0.09
Lactating	0.64±0.25 ^{a}	1.10±0.45 ^{a}	0.81±0.28	0.18±0.06
Pregnant	0.59±0.21 ^{b}	0.92±0.33 ^{b}	0.77±0.25	0.17±0.07

Values with different superscript differ significantly ($P<0.05$).

Table 2: Mean±SE of serum hormones in different groups of cows.

Goup	Estrogen (pg/ml)	progesterone (ng/ml)	triiodothyronin (nmol/L)	tetraiodothyronin (nmol/L)
Dry	17.66±0.83 ^{a}	0.68±0.056 ^{a}	1.15±0.05	31.25±1.14 ^{a}
Lactating	11.54±0.55 ^{b}	0.76±0.060 ^{a}	1.00±0.03	29.75±0.87 ^{a}
Pregnant	18.21±0.94 ^{a}	3.01±0.121 ^{b}	0.98±0.03	21.89±1.67 ^{b}

Values with different superscript differ significantly ($P<0.05$).

Table 3: Correlation between micro mineral and hormones.

Group	Micromineral	Estrogen	progesterone	triiodothyronin	tetraiodothyronin
Dry	Cu	0.46	0.65	0.78	0.82
	Fe	0.48	0.66	0.67	0.72
	Zn	0.74	0.81	0.64	0.61
	Mn	0.85	0.80	0.20	0.10
Lactating	Cu	0.50	0.72	0.61	0.58
	Fe	0.56	0.51	0.57	0.54
	Zn	0.79	0.74	0.82	0.73
	Mn	0.68	0.71	0.20	0.40
Pregnant	Cu	0.52	0.69	0.69	0.63
	Fe	0.59	0.63	0.81	0.78
	Zn	0.62	0.64	0.72	0.70
	Mn	0.72	0.68	0.30	0.10

Table 4: Mean±SE of serum microminerals (ppm) in different groups of cows 30 days post supplementation.

Goup	Cu	Fe	Zn	Mn
Dry	0.86±0.47 ^{a}	1.52±0.63 ^{a}	0.98±0.42 ^{a}	0.20±0.10
Lactating	0.72±0.36 ^{a}	1.44±0.54 ^{a}	0.93±0.37 ^{a}	0.18±0.04
Pregnant	0.85±0.44 ^{b}	1.25±0.23 ^{b}	0.89±0.31 ^{b}	0.19±0.06

Values with different superscript differ significantly ($P<0.05$).

Table 5: Mean±SE of serum hormones in different groups of cows 30 days post supplementation.

Goup	Estrogen (pg/ml)	progesterone (ng/ml)	triiodothyronin (nmol/L)	tetraiodothyronin (nmol/L)
Dry	19.75±0.88 ^{a}	0.82±0.036 ^{a}	1.32±0.08	35.43±2.04 ^{a}
Lactating	12.63±0.62 ^{b}	0.94±0.048 ^{a}	1.20±0.05	31.22±1.92 ^{a}
Pregnant	18.98±0.84 ^{ab}	4.11±0.160^b	1.13±0.04	24.87±1.10 ^{b}

Values with different superscript differ significantly ($P<0.05$).

Table 6: Composition of area specific mineral supplement.

Ingredients	Area specific mineral supplement
Dicalcium phosphate (%)	0.43
Calcium carbonate (%)	0.21
Magnesium sulphate (%)	0.12
Magnesium oxide (%)	0.3
Sodium chloride (%)	0.16
Potassium iodide (%)	0.5
Copper sulphate (mg/kg)	18.75
Ferrous sulphate (mg/kg)	11.25
Cobalt chloride (mg/kg)	0.11
Manganese sulphate (mg/kg)	23.75
Zinc sulphate (mg/kg)	31.25
Vitamin A	$20x10^6$ IU
Vitamin D_3	$10x10^6$ IU
Vitamin E	30ng

Discussion

Cu levels ranged from deficient to slightly normal level when compared to normal and critical serum Cu levels which are 0.57–0.96 and 0.19–0.57 μg/ml, respectively [9]. Lower serum Cu levels in pregnant cows may be due to the fact that pregnancy is Cu dependent process as demands of Cu increase during gestation [10]. Slightly deficient to normal serum Fe levels were found in cows when the normal and critical serum Fe level is 1–2 and 0.5–1 μg/ml, respectively [9]. This may be due to the fact that forages contain adequate Fe [11]. Deficient levels in pregnant cows may be due to the additional demands of gestation. Deficient serum Zn levels were found in cows when compared to normal and critical serum Zn levels for cattle which are 0.8–1.2 and 0.4–0.6 μg/ml, respectively [9] Lower Zn levels in pregnant cows may be due to depletion of Zn reserves and fall in plasma Zn concentration during pregnancy [12]. Mn values ranged from deficient to slightly normal level which is 0.18-0.19 ppm as per [13]. Deficient levels in pregnant cows are attributed to excess demands of gestation. Mn levels decrease during pregnancy [13].
The concentration of both steroid and thyroid hormones was lower than normal levels [14]. This may be attributed to the micromineral deficiency [15] as micromineral deficiency affects both steroid [16] and thyroid [17] hormone production. Correlation between microminerals and steroid and thyroid hormones is due to influence of microminerals on ovarian and thyroid activity of ruminants affecting synthesis and production of these hormones [16, 17]. Positive and significant correlation between Cu and progesterone can be due to role of Cu in regulating progesterone production by luteal cells via involvement of superoxide dismutase [18]. Positive and nonsignificant correlation between Cu and oestrogen may be due to its indirect role in steroidogenic enzymes cytochrome P450 17α-hydroxylase and cytochrome P450 side-chain cleavage and lysyl oxidase [19]. Positive and significant correlation between Zn, oestrogen and progesterone may be

due to involvement of Zn in the reorganization of ovarian follicle, source of progesterone through the involvement of metalloproteinase-2 (MMP-2), member of Zn endopeptidase family [20]. Zn is also involved in regulating progesterone production by luteal cells *via* involvement of superoxide dismutase [18]. Positive and significant correlation between Mn and oestrogen, and progesterone may be due to the fact that Mn acts as a cofactor in the synthesis of cholesterol, a precursor of steroids, including estrogen and progesterone [21]. Positive and significant correlation between Fe and progesterone can be attributed to its role in ovarian activity [22]. Positive correlation was also reported by [23] between serum progesterone level and Cu-Zn in cows.

Positive and significant correlation between Cu, Fe, Zn and thyroid hormones can be due to their role in synthesis or conversion of thyroid hormones [17]. Cu deficiency impairs secretion of trysonise hydroxylase and dopamine beta-enzyme systems which are both Cu containing, in hypothalamic neurons. This causes inhibition of synthesis of thyroid hormone releasing factor. The Cu containing peroxidase enzyme of the thyroid gland impairs thyroid hormone secretion [24]. Fe deficiency lower thyroid peroxidase (TPO) activity and thereby interfere with iodine metabolism in the thyroid [17]. The T_3 receptor is thought to require Zn to adopt its biologically active conformation. Some of the effects of Zn deficiency, therefore, may be due to loss of zinc from the T_3 receptor and impairment of T_3 action [5]. Increase in serum microminerals post supplementation is related to properly balanced micromineral content in the supplied mineral supplement. These results are in corroboration with Gressley (2009) and Noaman *et al.* (2011). Improvement in serum hormone levels post supplementation may be due the diverse roles of microminerals in steroid [18, 19 and 21] and thyroid [18 and 19] hormone synthesis and production.

Conclusion

Microminerals being essential for hormonal systems, their deficiency can affect different physiological and metabolic processes. Hence micromineral supplementation can maintain normal health and production.

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