



Mineral profile of abandoned cows suffering from anestrus

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One of the reasons of abandoning a cow may be prolonged period of sexual quiescence or anestrus which is considered as one of the most prevalent reproductive disorders in dairy cows. Anestrus cows have small, flaccid uteri and small, inactive ovaries with no palpable corpus luteum or follicle (Hoedemaker 2008). Numerous underlying factors for anestrus have been described such as severe negative energy balance postpartum, high milk yield, age, breed, season and stress (Roche 2006, Tewari *et al.* 2014).

Physiological concentrations of the elements must always be maintained to assure the proper cellular functions of the animal. There is a general consensus that low production and suboptimal reproductive efficiency of our livestock is due to inadequate nutrition, particularly deficiency of minerals. The ovarian activity of ruminants is influenced by mineral deficiency (Ansotegui *et al.* 1999). Minerals may have a beneficial role to play in the resumption of follicular growth and fertility in dairy cows. It is well known that deficiency of vitamins and minerals induce or predispose animals to repeat breeding and anestrus (Boland 2003, Kilic *et al.* 2007).

Failure to conceive and give birth to a young one can result in economic losses, however, it is easy for the farmer in marginal farming system to abandon a cow not conceiving and rear a new one. Therefore, the present study envisages the mineral profile of abandoned cows having anestrus before and after nutritional supplementation.

Cows kept in various *Goshalas* in different districts of Himachal Pradesh were selected. History of the abandoned cows was taken from the care takers of the *Goshalas* which included age, parity, cycle length, feeding status, previous fertility records, date of last calving, post-partum estrus, service dates, natural service or artificial insemination etc. Cows were examined thoroughly by rectal examination method and overall 83 abandoned cows were diagnosed to be affected with anestrus. Initially, deworming of all the cows was done using bolus Fenbendazole at dose rate of 7.5 mg/kg body weight (BW) per os (p.o.) and/or Ivermectin at dose rate of 0.2 mg/kg BW sub-cutaneous (s/c). Deltamethrin spray at dose rate of 2 ml/L of water was used

in some cows with heavy ecto-parasitic infestation.

Cows were also provided concentrate ration and mineral mixture @ 50 g p.o. daily. Additionally, these cows were administered toldimphos sodium @ 5–10 ml i/m and calcium levulinate @ 15–20 ml i/m. Tablets CoFeCu were also given to each cow @ 2 tablets p.o. daily for 20 days. Vitamin supplementation was provided by injecting vitamin A, D₃, E @ of 5 ml i/m or Vitamin A, D₃, E and H @ of 5 ml i/m.

Blood samples were collected from each cow suffering from true anestrus, before treatment, through jugular venipuncture in two separate centrifuge tubes for separation of plasma. Sampling was repeated in 83 cows when those were examined to know the post-treatment recovery. For plasma, samples were harvested by centrifugation of tubes at 3,000 rpm for 10 min. Thereafter, samples were transferred to 2 ml eppendorf tubes (in duplicate) and stored at –20°C pending analysis.

The macro minerals, i.e. calcium (Ca), magnesium (Mg) and micro minerals, viz. copper (Cu), zinc (Zn), cobalt (Co) and manganese (Mn) were estimated from plasma using atomic absorption spectrophotometer (Perkin Elmer Analyst 400). The wavelengths used for estimation of Ca, Mg, Cu, Zn, Co and Mn were 422.7 nm, 285.2 nm, 324.8 nm, 213.9 nm, 240.7 nm and 279.5 nm, respectively. Plasma sodium (Na) and potassium (K) were estimated by Colorimetric method in semiautomatic biochemistry analyzer Photometer 5010 (ROBERT RIELE GmbH and Co KG, Berlin, Germany) using standard kits (Avecon Healthcare Pvt. Ltd., Himachal Pradesh, India). The inorganic Phosphorus (P), was estimated from plasma in semiautomatic biochemistry analyzer Photometer 5010 (ROBERT RIELE GmbH and Co. KG, Berlin, Germany) by using standard kits (Agappe Diagnostics Ltd., India) at wavelength of 340 nm for inorganic phosphorus. The obtained data was statistically analyzed using Student t-test for parametric data with SAS (Statistical Analysis Software), SAS® 9.2 TS Level version 2M2 for windows.

There was no significant difference ($P>0.05$) in pre- and post-treatment concentrations of all the minerals except inorganic phosphorus and copper in the recovered cows (Table 1). Non-significant ($P>0.05$) increase in calcium level was recorded following treatment in recovered as well

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as non-recovered cows. The Ca: P ratio, alteration may affect ovarian function and can result in prolongation of first estrus and ovulation, delayed uterine involution, increased incidence of dystocia, retention of placenta and prolapse of uterus (Kumar 2003). However, low calcium level in blood is also associated with anestrus whereas excess of calcium can affect the reproductive status of animal by impairing absorption of phosphorus, manganese, zinc, copper and other elements from gastrointestinal tract. There was significant increase ($P < 0.05$) in pre and post treatment inorganic phosphorus concentrations in recovered anestrus animals (Table 1). The mineral most frequently associated with infertility in cattle is phosphorus. Classical manifestations of phosphorus deficiency in reproduction involve alterations of estrus. The utilization of all nutrients such as proteins, carbohydrates and fat was dependent upon phosphorus in the form of ATP. A phosphorus deficiency induces anestrus, lowered conception rate, irregular estrus, decreased ovarian activity, increased incidence of cystic follicles and generally depressed fertility (Ceylan *et al.* 2008; Kumar 2010). Therefore, dairy cows should always be provided with adequate amounts of phosphorus to minimize health and fertility problems.

Irrespective of recovery, significantly higher copper concentration ($P < 0.05$) was recorded in treated anestrus cows. The biological role of copper is exerted through a number of copper containing proteins including ceruloplasmin and superoxide dismutase. Copper besides

acting as a metalloenzyme also plays an important role in FSH, LH and estrogen activity and could serve as a causative factor for anestrus condition (Mahajan 1999, Bindari *et al.* 2013). Ceylan *et al.* (2008) also reported low levels of serum copper in anestrus animals than in control animals. An increase in retained placenta, inactive ovaries, delayed estrus and infertility has been attributed to a copper deficiency in dairy cattle (Arthur *et al.* 2011).

Magnesium usually does not have direct impact on the reproductive status of animals, since in body it remains in almost antagonistic relation with calcium and any disturbance in Ca-P-Mg homeostasis can impart some influence on reproduction (Kumar 2003, Bindari *et al.* 2013).

Zinc deficiency has been associated for impaired reproductive performance, decreased fertility and abnormal estrus in cows and decreased cell mediated immunity (Sharma *et al.* 2005). Zinc has a critical role in repair and maintenance of uterine lining following parturition and early return to normal reproductive function and estrus (Greene *et al.* 1998).

No major change in sodium and potassium profile was seen following treatment in anestrus cows. Sodium and potassium are indirectly related to reproduction in animals as the deficiency of sodium can affect the normal reproductive physiology by preventing the utilization of protein and energy whereas deficiency of potassium is well known to cause muscular weakness and thereby affect the

Table 1. Pre- and post-treatment blood serum concentration of some minerals (Mean±SE) in recovered/not-recovered abandoned cows treated for anestrus in different *Goshalas* of Himachal Pradesh

Blood plasma profile	True anestrus (N=83)							
	Recovered (n=35)				Not-recovered (n=48)			
	Pre-treatment	Post-treatment	t-value	P	Pre-treatment	Post-treatment	t-value	P
<i>Mineral</i>								
Calcium (mg/dl)	8.44±0.29 (6.11–9.93)	9.16±0.25 (8.05–10.61)	1.826 NS	0.079	8.31±0.38 (4.25–10.32)	8.76±0.29 (6.99–10.10)	0.898 NS	0.377
Inorganic phosphorus (mg/dl)	5.07±0.17 (3.95–6.28)	5.91±0.36 (3.99–8.85)	2.175 NS	0.038	5.01±0.22 (3.48–6.62)	5.59±0.30 (3.86–7.48)	1.565 NS	0.1281
Magnesium (mg/dl)	1.98±0.10 (0.87–2.40)	1.95±0.09 (1.43–2.53)	0.278 NS	0.782	1.95±0.12 (0.87–2.77)	1.98±0.11 (1.21–2.77)	0.179 NS	0.858
Copper (ppm)	0.41±0.05 (0.13–0.75)	0.56±0.04 (0.19–0.90)	2.250 NS	0.032	0.44±0.05 (0.10–0.80)	0.62±0.05 (0.15–0.89)	2.583 NS	0.015
Zinc (ppm)	1.32±0.12 (0.50–2.04)	1.36±0.17 (0.51–3.22)	0.191 NS	0.849	1.32±0.09 (0.94–1.98)	1.39±0.17 (0.63–3.46)	0.253 NS	0.802
Cobalt (ppb)	0.04±0.03 (0.01–0.07)	0.01±0.01 (0.01–0.02)	0.487 NS	0.844	0.05±0.00 (0.05–0.05)	0.02±0.00 (0.02–0.02)	–	–
Sodium (mmole/L)	143.80±3.57 (105.10–159.20)	144.50±3.86 (100.40–169.90)	0.132 NS	0.896	150.58±3.06 (128.80–169.00)	141.15±5.10 (111.90–179.00)	1.527 NS	0.138
Potassium (mmole/L)	3.86±0.21 (1.81–5.08)	3.41±0.11 (2.38–3.90)	1.835 NS	0.078	3.86±0.12 (3.12–4.74)	3.48±0.15 (2.61–4.99)	2.004 NS	0.053
Chloride (mEq/L)	87.87±1.06 (82.33–94.88)	86.63±1.05 (79.97–95.59)	0.829 NS	0.415	85.97±0.99 (78.95–95.37)	87.04±0.93 (77.25–95.05)	0.784 NS	0.438

NS, Non-significant.

musculature of female genital tract causing impairment in the normal reproductive process (Kumar 2003, Bindari *et al.* 2013).

Nutritional deficiency in cows results in depletion of minerals and deranged enzymatic activity affecting the normal reproductive behaviour. Efficient production in cows requires the supply of nutrients in appropriate amount and form that is most biologically useful. As most of the cows are affected by poor management, therefore, farmers need extensive educative trainings on feeding and management to avoid their abandoning.

SUMMARY

The present investigation was carried out with an objective of studying the mineral profile in abandoned cows affected by anestrus (N=83). Overall 83 abandoned cows kept in 18 *Goshalas* were diagnosed to be affected with anestrus. The macro minerals, i.e. calcium (Ca), magnesium (Mg) and microminerals, viz. copper (Cu), zinc (Zn), cobalt (Co) and manganese (Mn) were estimated from plasma. Nutritional supplementation led to a successful recovery in 42.17% cows. Mineral profile suggested that inorganic phosphorus and copper concentration increased significantly in recovered anestrus cows. All other minerals also witnessed a non-significant increase in recovered as well as non-recovered cows after treatment. In conclusion, anestrus could be successfully treated to an extent following nutritional supplementation.

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