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Dynamics of Selenium Deficiency in Bovines in District Kasur, Punjab, Pakistan

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Authors' Contribution
KP conducted and planned the study. MSK, MI and AAA supervised the study. JAK, NA, RA, AM and SU wrote the manusript. YC helped in statistical analysis.

Key words
Risk factors, Selenium, Cattle, Buffaloes, Udder health.

ABSTRACT

The present study was designed to determine the serum selenium status in association of its risk factors in cattle and buffaloes of district Kasur, Punjab. Selenium status was evaluated by Atomic Absorptions Spectrophotometery (AAS) with respect to sex of animals, geographical area, age of animals, herd size, stage of animals, production level and concentrate feeding. Selenium deficiency was also evaluated as a risk factor of mastitis and repeat breeding. About 48.43% cattle and 72.39% buffaloes were found positive in selenium deficiency. Species (cattle and buffaloes) were significantly (χ^2=23.042, df=1, p<0.05) associated with selenium deficiency. The highest rates (90.62 %) of selenium deficient animals were observed in village Nathoki of tehsil Kasur. The risk factors such as Gender (χ^2=11.31, df=1, p<0.05), feed components (χ^2=4.47, df=1, p<0.05) and high or low milk production (χ^2=36.42, df=3, p<0.05) are significantly associated with selenium deficiency. Other risk factors like age of animals (χ^2=3.47, df=4, p>0.05) and herd size (χ^2=2.45, df=2, p>0.05) are not significantly associated with selenium deficiency. selenium status is association with udder and reproductive system health (P<0.05). Dairy animals at milk producing stage, high milk producers, and animals that are not supplemented with extra concentrate were found to be at higher risk. Males are more at risk than the females. Present study concludes that almost half of the animals surveyed were deficient in selenium. Milking animals with high production levels and older animals require selenium supplementation.

INTRODUCTION

Selenium is one of the essential micro minerals of the bodies of living organisms. It is required for various body functions such as growth, reproduction, immune system and protection of tissue integrity and coordination in various parts of the body. Biological functions associated with Se are to protect biological membranes from oxidative damage from free radicals. Deficiency of this element in the body results in tissue break down and degeneration (Papp *et al*., 2007). Selenium is one of the most important trace minerals for ruminants (Graham, 1991). Selenium deficiency negatively impacts the bovine agronomy, and is associated with many clinical and subclinical conditions like muscle necrosis, shoulder lameness, compromised calf health, reproductive abnormalities, reduced fertility, placental retention, metritis, mastitis, reduced performance, and pneumonia (Spears *et al*., 1986; Erskine *et al*., 1989; Gunning and Walters, 1994; Spears and Weiss, 2008; Ceballos-Marquez *et al*., 2010; Hefnawy and Tórtora-Pérez, 2012; Sordillo, 2013). Suppression of immune system and increase susceptibility to infections has been observed in selenium deficiency (Droke and Loerch, 1989; Swecker *et al*., 1989). The basic role of the selenium is the protection of tissues against hydrogen peroxide produced during a host of metabolic reactions (El-Demerdash,

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2004; Sordillo, 2016). Selenium supplementation in deficient calves improves animal growth (Castellan *et al.*, 1999; Salles *et al.*, 2014). Excess of selenium may cause selenosis, selenium toxicity, however selenium deficiency is far more common and has a much greater impact on bovine agronomy than selenosis (Zagrodzki *et al.*, 1998).

Selenium is important mineral for the immune system (Nair and Schwartz, 1990). Selenium supplementation is known to enhance both innate and acquired immune responses (Salman *et al.*, 2009) humoral or cell mediated (Petrie *et al.*, 1989) and active or passive (Rowntree *et al.*, 2004; Guyot *et al.*, 2007; Hefnawy and Tórtora-Pérez, 2012; Hall *et al.*, 2014). Selenium improves antibody titers in both serum and colostrum (Kamada *et al.*, 2007), as well as immune responses in newborn calves living under stressful conditions (Schrama *et al.*, 1993).

Incidence of metritis, ovarian cysts and retained placenta were found more in selenium deficient animals (Wilde, 2006; Spears and Weiss, 2008). Selenium supplementation improves conception rates in cattle and secondary follicle formation in goats (Kommisrud *et al.*, 2005; Wu *et al.*, 2011). Selenium deficiency impairs testosterone and spermatozoon synthesis, at both gross and microscopic levels (Rayman, 2012; Ahsan *et al.*, 2014). Antioxidant and phagocytic properties of milk are enhanced with selenium supplementation (Sordillo, 2013; Abuelo *et al.*, 2014). Rates of mammary infections are higher in selenium deficient animals (Finch and Turner, 1996; Ceballos-Marquez *et al.*, 2010).

Forage is most important source of nutrients for the animals and inadequate level of selenium has been associated to low levels in the soil (Ceballos-Marquez *et al.*, 2010). Selenium, after absorption in plants, replaces sulfur in cysteine and methionine changing these two to selenomethionine and selenosystine, which are absorbed by animals (Pereira *et al.*, 2012). Plants higher in these amino acids have higher levels of selenium in them. Selenium content of forage varies area to area and region to region (Campbell *et al.*, 1995). Higher amount of selenium is found in stems, leaves and seeds of plants. Soil texture, type, organic matter and humidity are factors influencing uptake and assimilation. Redox status, pH and microbial activity are also important factors (Mehdi *et al.*, 2013). Extensive and organic farming strategies tend to favour selenium deficiency (Schöne *et al.*, 2013). Selenium deficiency has been reported as widespread in the soil of the Sargodha (Ahmad *et al.*, 2009) and semi-arid areas of Pakistan (Khan *et al.*, 2005). Keeping in view the importance of selenium to livestock, this investigation was carried out evaluate the selenium status and its association with its risk factors in bovines of district Kasur, Punjab.

MATERIALS AND METHODS

Area and sampling

The blood samples (n=384, 192 each for cattle and buffaloes) were collected from the district Kasur, Punjab-Pakistan. Sample size was estimated through simple random sampling method as described by Thrushfield (2005). Blood samples were collected from jugular veins and tail veins of young and adult animals, respectively and serum was separated by centrifugation at 1500 rpm. A questionnaire was filled regarding the information of age, gender, parity, stage, and production level, occurrence of infectious, non-infectious, and reproductive diseases. California Mastitis Test (CMT) was used to grade udder health and one quarter positive for mastitis was considered positive case for analysis of data.

Determination of selenium

Serum selenium level of samples was estimated using an Atomic Absorption Spectrophotometer (AAS). Before use of AAS, the samples were processed by wet digestion method to separate selenium from bound to unbound form following Hseu (2004) with some modifications. Briefly, 1mL serum sample was digested with 10 mL of digestion mixture ($HClO_4$ and HNO_3 in 1:2 ratio) at 150°C for 30 min. After that, temperature of mixture was raised to 250°C until the color disappeared. After cooling the mixture, distilled water was added in it to adjust volume up to 10 mL. The resulting solution was analyzed using AAS (accompanied by standard controls). Normal level of the selenium in cattle and buffaloes is about 0.11-0.13 ppm (Maas et al., 1992). Animals with lower level of selenium than this were considered as selenium deficient.

Statistical analysis

A questionnaire was filled and all the data were collected and arranged in single Microsoft Excel 2016 sheet. The association of selenium deficiency with various risk factors was calculated by Pearson Chi-Square test using SPSS 20.0 and with 5% level of significance. Odd ratio was calculated to estimate the effect of selenium deficiency on reproductive and udder health.

RESULTS

The highest rates of selenium deficient animals were observed in village Nathoki (92.62 %) tehsil Kasur followed by Khudian (81.25 %) and Sarhali (78.12%), respectively (Table I). Selenium status of animal varies from area to area and there is association of selenium deficiency with tehsils (χ^2=38.047, df=2, p<0.05) and villages (χ^2=47.65.042, df=11, p<0.05). Results about the various risk factors associated with selenium deficiency is shown in Table II. Two species were studied, out of these, buffaloes (72.39%) were more at risk (χ^2=23.042, df=1, p<0.05) than the cattle (48.43%). Gender as a risk factor of selenium deficiency shows that Males (81.96%) are at more risk (χ^2=11.31, df=1, p<0.05) than females (56.69%). Animals were divided into four categories depending on the age and production status *i.e.* calf, heifer, milking and dry animals out of these stages milking animals (65.94 %) are at more risk (χ^2=27.88, df=4, p<0.05), followed by the calves (63.82%).

Table I.- Area-wise status of selenium in district Kasur, Punjab, Pakistan.

Tehsil / Villages	Total	Deficient		P value
		n	%	
Kasur				
Sarhali	32	25	78.12	P<0.05
Khara	32	24	75.00	
Khudian	32	26	81.25	
Nathoki	32	29	90.62	
Chunian				
Moujuki	32	20	62.50	
Jajjal	32	20	62.50	
Talwandi	32	19	59.37	
Kotha	32	17	53.12	
Pattoki				
Habibabad	32	17	53.12	
Dinanath	32	18	56.25	
Halla	32	12	37.50	
Sheikham	32	10	31.25	

The milk producing animal were divided into three categories depending on the milk production low milk producers (<5 L), moderate milk producers (5-10 L) and high milk producers (>10 L). Animals producing more than 10 L Milk are more at risk (χ^2=36.42, df=2, p<0.05) to selenium deficiency. The animals included in our study which were given any type concentrate, whether conventional (sunflower Meal, Canola meal and wheat bran etc.) or Commercial (pelleted or meshed cattle feed by various companies) were less at risk to selenium deficiency (χ^2=4.73, df=1, p<0.05). Herd size risk (χ^2=2.45, df=2, p>0.05) and Age of animals (χ^2=3.47, df=4, p>0.05) were observed to have no effect on selenium deficiency.

DISCUSSION

Selenium is important micromineral in the living beings and performs various vital functions in the animal body including the most important aspect of animal life *i.e.* protection of body from external invaders through immune system. Like other micro and macro minerals selenium could not be produced in the body. Though selenium could be supplemented as inorganic salt, its natural source for the animals is forage (López-Alonso, 2012). Forages take all minerals from the soil depending on its level in the soil and it is well known reality that the selenium levels in forage

Table II.- Risk factors of serum selenium status of bovine in district Kasur, Punjab.

Parameters	No. examined	Selenium deficient		P value
		n	%	
Herd size				
< 5	92	58	63.04	P>0.05
5 to 10	186	105	56.54	
10 to 15	106	69	65.09	
Species				
Buffaloes	139	192	72.39	P<0.05
Cattle	93	192	48.43	
Age of animals				
< 1	49	30	61.22	P>0.05
1 to 3	154	89	57.79	
3 to 6	93	54	58.06	
6 to 9	41	25	60.97	
>9	47	34	72.34	
Gender				
Female	321	182	56.69	P<0.05
Male	63	50	81.96	
Stage of animal				
Calf	47	30	63.82	P<0.05
Heifer	66	37	56.06	
Milking	138	91	65.94	
Dry	75	28	37.33	
Concentrate				
Yes	139	94	67.62	P<0.05
No	245	138	56.35	
Production				
Low	31	9	29.03	P<0.05
Moderate	46	25	54.34	
High	72	63	87.5	

The present study shows that selenium deficient animals are at risk of repeat breeding (OR= 11.08, P<0.05). Selenium status is also associated to the mastitis (OR= 6.19, p<0.05). Results are shown in Table III.

Table III.- Mastitis and repeat breeding in relation to selenium deficiency.

Disease	Selenium status	Total No.	Diseased animals		Odd ratio/ p value
			n	%	
Mastitis	Deficient	84	15	17.85	6.19/
	Normal	52	1	0.02	P < 0.05
Repeat breeding	Deficient	154	32	20.78	11.08/
	Normal	123	5	0.04	P<0.05

varies greatly from one area to the other (Pereira et al., 2012). One study was conducted in arid agricultural areas of Pakistan and it was concluded that plants in the study areas including Kasur were deficient in selenium (Khan *et al*., 2005). Lot of factors affect the selenium status of soil including soil texture, humidity, chemical composition, cultivation stress, soil pH and organic contents (Mehdi *et al*., 2013). And that varied from Soil in Kasur typically has high pH, Organic matter, lime, iron, zinc and manganese, all which tend to reduce selenium levels (Baig *et al*., 1990). Above account justifies the varying percentages of selenium in animals of various areas.

Males in our study were at higher risk to selenium deficiency which are in contrast to the Erasmus *et al*. (2000), who demonstrated that females are more deficient in selenium. no doubt female animals are kept for milk production by most of farmers and they need more selenium but out study calves were not given any type of extra concentrate and they were feed deficient so that could be associated to the cause of selenium deficiency.

In general, there was no association age with selenium status of the animal in our study. Though, highest selenium deficient animals were observed in the group having ages above 9 years but it is statistically in significant and this do not conform with previous researches who observed serum selenium level in blood was higher in older animals as compared to the younger animals (Stowe and Herdt, 1992).

Milking animals and weaned calves are more at risk of the pregnancy due to increased demand of selenium and feeding negligence. Previous studies showed that the late pregnancy is most mineral deficient stage because at that time animal needs higher amount of selenium (Meglia *et al*., 2004). In Norway heifers and dry cows were found to have low blood selenium content due to farmers administer Se supplemented feed during productive periods (Kommisrud *et al*., 2005). Same was the case with our study, farmer do not supplement concentrate in the dry animals and calves after weaning, Though, pregnant animals require more minerals as compared to other stages (Guyot *et al*., 2011).

Present study shows that selenium deficient group of animals has higher number of repeat breeding animals that have taken more than two services per conception. These results are in accordance with Roche (2006) and Guyot *et al*. (2009) who reported that selenium level influences reproductive levels and incidence of repeat breeding. Selenium deficiency has a strongly negative effect on bovine reproduction (Malbe *et al*., 1995; Allison and Laven, 2000; Hemingway, 2003; Bourne *et al*., 2008).

Higher number CMT positive animals was observed in the selenium deficient animals which means animals deficient in selenium are at higher risk of mastitis. Results of the present study are in accordance to Kommisrud *et al*. (2005) and Enjalbert *et al*. (1999) who concluded that selenium deficiency is important risk factor for mastitis. Supplementation with selenium reduced mastitic infection rates (Wilde, 2006). Atroshi *et al*. (1986) and Hogan *et al*. (1993) concluded that selenium deficiency contributes to higher rates of mastitis via reduction of glutathione peroxidase levels in tissue cells. Kruze *et al*. (2007) showed that selenium supplementation increases glutathione peroxidase activity which lower somatic cell count in *Staphylococcus aureus* infected cattle. Lower level of glutathione peroxide increases somatic cell count because it is an integral part of defense system (Mukherjee, 2008; Pilarczyk *et al*., 2012). Selenium is integral part of glutathione peroxide and reduction in selenium reduces antioxidant properties and increases inflammatory cells in mammary gland which increases the somatic cell count. Selenium supplementation reduces incidence of mastitis and somatic cell count in cattle (Barbano *et al*., 2006; Rabiee *et al*., 2010). Results are in contrast with Weiss *et al*. (1990) and Ndiweni *et al*. (1991) whose studies indicate that there is no relationship of selenium with udder health.

CONCLUSION

In Pakistan, the population of both cattle and buffaloes suffer high rates of selenium deficiencies; this negatively impacts productivity and animal health, as well as the domestic livestock agronomy, as a whole. Animals over 9 years of age, productive animals, high producers, concentrate deficient animals and male calves appear to be most at risk for developing problems associated with reduced selenium uptake and levels.

Statement of conflict of interest

Authors have declared no conflict of interest.

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