SERUM BIOCHEMICAL PROFILE OF CROSSBRED COWS DURING HEALTHY AND INFECTIOUS UTERINE CONDITIONS

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ABSTRACT

Serum biochemical profile was studied of six healthy (Control) and fourteen crossbred cows with uterine infection. The infected cows were divided in two equal groups (n=7) for assessing the immunomodulatory effect of oyster glycogen and autologus plasma. The mean concentration of serum glucose, calcium, inorganic phosphorus and cholesterol was recorded as 51.85±4.02, 10.94±0.62, 5.85±0.19 and 157.10±14.46 mg/dl in healthy crossbred cows (Group I), whereas, in cows with uterine infection the values were recorded as 35.84±2.03, 8.82±0.33, 5.79±0.38 and 119.84±9.19 mg/dl in Group II (oyster glycogen) and 33.97±2.67, 9.25±0.65, 5.72±0.24 and 116.17±8.49 mg/dl in Group III (autologus plasma) cows, respectively. Before treatment the concentration of serum glucose, calcium and cholesterol in uterine infected cows was significantly lower as compared to healthy cows. No significant difference was observed in the values of serum inorganic phosphorus between healthy and infected cows. However, after intrauterine infusion of oyster glycogen and autologus plasma in infected cows the values of serum glucose, calcium, and cholesterol improved and no significant difference was observed between healthy and infected cows. It may be concluded that cows with uterine infection are sub-clinically hypoglycemic and have lower concentration of serum calcium. The increase in the risk of uterine diseases in early lactation may be attributed to the inadequate energy level and lower concentration of calcium which is crucial in maintaining the local uterine and systemic immune response towards the pathogens. Oyster glycogen and autologous plasma were quite effective in restoration of reduced glucose and calcium to normal level and enhancement of uterine defence mechanism.

Key words: Biochemical profile, crossbred cows, infectious uterine conditions

Introduction

The uterine tract is prone for bacterial infection during process of parturition as the anatomical barriers are removed and uterine tract remain open for few days. Both pathogenic and non-pathogenic bacteria can enter the uterus at the time of parturition and multiply rapidly (Usmani *et al.*, 2001). Two postpartum clinical conditions commonly occur in dairy cows after calving are metritis and endometritis. Metritis is characterised by an enlarged uterus containing a watery red-brown fluid to viscous off white purulent uterine discharge which often has a fetid odour, whereas, endometritis is an inflammation of inner uterine wall and characterised by presence of a purulent or mucopurulent discharge (Sheldon *et al.*, 2006, Dubuc *et al.*, 2010). The annual incidence of uterine infection in postpartum dairy cattle ranges from 10-50 per cent (Lewis, 1997). These abnormal uterine conditions increase the interval from calving to onset of oestrus as well as calving to conception by delaying the resumption of ovarian cycle (Sheldon *et al.,* 2009).

The bacterial contamination of the postpartum uterus is a common finding which by itself does not disturb the anatomical and histological restoration of tubular genital tract. The improper balance between uterine infection and the intrauterine antimicrobial self-defence mechanisms, however, often results in complications, such as puerperal metritis, clinical endometritis, pyometra and subclinical endometritis (Foldi *et al.,* 2006). The reduction of immune function during the peripartum period has been reported (Hammon *et al.,* 2006; Silvestre *et al.,* 2011), and this is suggested as one of the major reasons for the high incidence of bacterial diseases such as mastitis and metritis in early-lactation cows. Furthermore, the existence of metabolic conditions such as clinical hypocalcemia and exacerbated negative energy balance have been shown to suppress immune function (Hammon *et al.,* 2006; Kimura *et al.,* 2006; Galvao *et al.,* 2010).

The investigation of blood constituents in early postpartum period is drawing an attention since it is reported that the postpartum cows with uterine infection had low concentrations of serum glucose, calcium and cholesterol (Magnus and Lali, 2009; Ghawi and Hamedawi 2016, Sarkar *et al.,* 2016). The blood metabolites, general body condition, peri and postpartum health and resumption of postpartum ovarian cyclicity in dairy cows are closely related. Therefore, the present study was undertaken to estimate and compare the concentrations of blood constituent's viz. serum glucose, calcium, inorganic phosphorus and cholesterol in the crossbred cows during healthy and infectious uterine conditions and to determine their association with uterine diseases. Furthermore, these serum constituents were evaluated after intrauterine infusion of oyster glycogen and autologus plasma in infected cows which were used to enhance the uterine defence mechanism.

Materials and Methods

Total 20 crossbred cows with history of recent calving were selected for the study. Out of these twenty animals, fourteen cows showing clinical signs of uterine infection such as watery red-brown fluid to viscous off white purulent uterine discharge from the vulva were considered as unhealthy and divided into two equal groups (n=7). Remaining six cows without any clinical sign of uterine infection were considered as healthy and kept as control (Group I).

The infected cows were subjected to the immunomodulatory treatment of oyster glycogen and autologus plasma. Group II cows (n=7) were treated with intrauterine infusion of 500 mg oyster glycogen and Group III cows (n=7) with intrauterine infusion of 50 ml autologus plasma.

Oyster glycogen 500 mg (Himedia Laboratories Pvt. Ltd.) was thoroughly mixed with 40 ml normal saline solution and

administered intrauterine in the infected cows of Group II. Approximately 100 ml blood was collected from jugular vein of the experimental animals of Group III and immediately transferred it to the centrifuge tubes containing anticoagulant heparin. The blood was centrifuged @ 2700 RPM for 15-20 minutes. The supernatant plasma was separated and administered into the uterine lumen of the infected cows.

Ten ml blood was collected from the jugular vein of the experimental animals in sterile tubes and serum was harvested. The second blood sample was collected on day 10 post treatment. The cows with healthy uterus also bled during similar intervals. The serum biochemical estimation was carried out for glucose, calcium, inorganic phosphorus and cholesterol using semiautomatic biochemical analyser employing the commercially available reagent kits.

The data was analysed by the methods described by Snedecor and Cochran (1994).

Results and Discussion

The mean concentration of serum glucose, calcium, inorganic phosphorus and cholesterol was recorded as 51.85±4.02, 10.94±0.62, 5.85±0.19 and 157.10±14.46 mg/dl in healthy crossbred cows (Group I) and in cows with uterine infection the values were recorded as 35.84±2.03, 8.82±0.33, 5.79±0.38 and 119.84±9.19 mg/dl (Group II) and 33.97±2.67, 9.25±0.65, 5.72±0.24 and 116.17±8.49 mg/dl (Group III), respectively before treatment (Table 1). The serum glucose concentration in cows with uterine infection was below the reference value (45-75 mg/dl) and found significantly lower ($P<0.01$) as compared to healthy cows. Similarly, serum calcium in infected cows (Group II and III) was also below the reference value (9.7 to 12.4 mg/dl) and found significantly lower ($P<0.05$) when compared with the values recorded in healthy cows. The serum cholesterol recorded in cows with uterine infection was within normal range (80-180 mg/dl), however, the values found to be significantly lower ($P<0.05$) when compared with healthy cows. No significant difference was recorded in the concentration of inorganic phosphorus between healthy and infected cows and the values found to be within normal range (5.6 to 6.5 mg/dl).

The present findings are in agreement with Magnus and Lali (2009) who recorded serum glucose and calcium concentration as 22.30±2.18 mg/dl and 7.0±0.43 mg/dl, respectively in metritic cows which is much lower than the normal range. Similarly, Ghawi and Hamedawi (2016) recorded significantly less concentrations of serum glucose (28.4±3.27 vs 56.4±4.58 mg/dl) and calcium (6.8±0.52 vs 10.6±0.34 mg/dl) in puerperal metritic cows as compared to healthy cows which corroborates the present findings. Sarkar *et al.* (2016) also reported significantly lower ($P<0.01$) level of serum glucose in cows with uterine infection than healthy cows (38.51±0.81 vs 56.9±1.47 mg/dl). Jeon *et al.* (2016) studied the uterine microbiota and immune parameters in dairy cows with metritis during early postpartum period and reported lower serum total calcium as compared to healthy cows. Furthermore, it is reported that hypocalcemia is associated with the development of metritis. Similarly, Martinez *et al.* (2012) observed that cows that developed metritis had lower ($P<0.001$) concentration of Ca in serum starting on the day after calving until 12 DIM when compared with cows that did not develop metritis. During present study the concentration of inorganic phosphorus recorded in all the groups before treatment did not differ significantly amongst the groups. This observation corroborates the finding of Patil (2010) who reported no significant difference in the serum concentration of inorganic phosphorus between metritic and healthy buffaloes. Furthermore, Said *et al.* (1964) stated that no appreciable variations in serum inorganic phosphorus could be attributed to any of the uterine pathological conditions.

Sarkar *et al.* (2016) reported significantly ($P<0.01$) lower level of serum cholesterol in cows with uterine infection than healthy cows (81.34±2.44 vs 114.89±4.40 mg/dl) which corroborates the present finding wherein the serum cholesterol concentration in the cows with uterine infection was found significantly lower ($P<0.05$) than the values recorded in healthy cows. Kaczmarowski *et al.* (2006) stated that sick cows had cholesterol level similar to the lower limit of physiological norm; however, significant difference was not reported between infected and healthy cows.

Blood glucose level gives an indication of carbohydrate status of an animal and is very important to normalize the hormonal concentration and thus regulating the reproductive cycles (King, 1971). In present study the significant lower level of serum glucose indicated that the crossbred cows with uterine infection had low blood energy level. Kaczmarowski *et al.* (2006) reported positive correlation between concentrations of glucose and cholesterol in puerperal metritic cows. In another study undertaken by Divya and Ram Singh (2013) significant increase in the plasma cholesterol in the rats is reported after induction of diabetic condition. These reports demonstrates that the increase or decrease in the level of serum cholesterol is associated with the concentration of serum glucose. In present study serum glucose and serum cholesterol levels in infected cows were found to be significantly lower than healthy cows. The deficiency of glucose might have resulted in reduction of serum cholesterol. The synthesis of both cholesterol and fatty acids is greatly influenced by the amount of glucose being oxidized within the cell; however, the mechanism by which glucose produces these effects has remained obscure. Energy status in late gestation and early lactation has been linked to immune function in transition cows (Hammon *et al.,* 2006). In fact, cows that developed metritis and later subclinical endometritis had increased concentrations of NEFA and BHBA early postpartum and had neutrophils with less intracellular glycogen (Galvao *et al.,* 2010).

In present study the concentration of serum calcium in uterine infected cows was recorded in lower quantity as compared to healthy cows. It is reported that cows deficient in Ca have a greater risk of development of uterine infection than normocalcaemic cows. Hypocalcaemic cows have increased plasma concentrations of cortisol (Horst and Jorgensen, 1982), a reduced proportion of neutrophils with phagocytic activity (Ducusin *et al.,* 2003; Martinez *et al.,* 2012), and reduced concentrations of cytosolic Ca^{2+} in mononuclear cells (Kimura *et al.,* 2006). This reduction in measures of immune cell function has linked hypocalcaemia with periparturient diseases such as retained placenta (Melendez *et al.,* 2004) and mastitis (Curtis *et al.,* 1983). Ribeiro *et al.* (2011) stated that although the consequences of sub-clinical hypocalcaemia have not been fully established, early-lactation cows with low serum Ca in

Table 1: Mean±SE serum glucose, calcium, inorganic phosphorus and cholesterol (mg/dl) in crossbred cows of different groups

Collection of sample	Healthy cows	Cows with uterine infection		C. D.
	Group I (n=6)	Group II (n=7) Oyster glycogen	Group III (n=7) Autologus plasma	
Serum glucose(mg/dl)				
Before treatment	51.85±4.02^{bA}	35.84±2.03^{aA}	33.97±2.67^{aA}	11.64 (0.01%)
After treatment	50.57±2.67^{aA}	48.57±4.19^{aB}	46.28±3.09^{aB}	--
T value for interval 2.18 (0.05%)				
Serum calcium (mg/dl)				
Before treatment	10.94±0.62^{b}	8.82±0.33^{a}	9.25±0.65^{a}	1.60 (0.05%)
After treatment	10.98±0.43	10.17±0.42	9.86±0.58	
Serum inorganic phosphorus (mg/dl)				
Before treatment	5.85±0.19	5.79±0.38	5.72±0.24	--
After treatment	5.91±0.33	5.84±0.19	5.89±0.48	--
Serum cholesterol (mg/dl)				
Before treatment	157.10±14.46^{b}	119.84±9.19^{a}	116.17±8.49^{a}	31.00 (0.05%)
After treatment	151.02±10.07	141.14±6.95	139.37±11.36	--

Values with different superscripts (small letters) indicates significant differences between groups
Values with different superscripts (capital letters) indicates significant differences within group

the first seven DIM had an increased incidence of subclinical ketosis.

Serum biochemical values of cows after treatment are presented in Table 1. After treatment with oyster glycogen and autologus plasma the serum glucose in the experimental animals increased significantly (P<0.05) from its pre-treatment level and found comparable with the values recorded in healthy cows at similar interval. Similarly, serum calcium and cholesterol in cows with uterine infection also increased from their pre-treatment level and did not differ significantly from the respective values of healthy group estimated at similar interval. No significant difference was observed in the mean values of serum inorganic phosphorus estimated during pre and post-treatment period in all the groups.

Singh *et al*. (2003), Prasad *et al*. (2009), Sarma *et al*. (2010), Biswal *et al.* (2014) and Krishnan *et al.* (2015) studied the immunomodulatory effect of oyster glycogen in endometritic cows and reported the enhancement of uterine defence mechanism on the basis of increase in the total protein concentration, total leucocyte count, percent PMNs and the concentration of total immunoglobulins after intrauterine infusion of oyster glycogen. It is further reported that the bacterial infection was significantly reduced and conception rate was improved after intrauterine infusion of oyster glycogen. Similarly, Asbury *et al*. (1984) reported that addition of a small amount of serum to uterine secretions can increase the opsonising capacity and enhance the phagocytic ability of PMNs significantly. Venugopal (1995), Methai (1999), Sarma *et al.* (2012) recorded good recovery in endometritic cows after intrauterine infusion of autologous plasma. The present findings are in agreement with these reports since the uterine infected cows in the present study after treatment with oyster glycogen and autologous plasma did not show clinical signs of uterine infection and recorded non-significant differences in the concentration of serum glucose, calcium and cholesterol when compared with respective values estimated in healthy cows.

It may be concluded that the cows with uterine infection are sub-clinically hypoglycaemic and have lower level of serum calcium. The increase in the risk of uterine diseases in early lactation may be attributed to the inadequate energy levels and lower concentration of calcium which is crucial in maintaining the local uterine and systemic immune response towards the pathogenic bacteria. Oyster glycogen and autologous plasma were quite effective in restoring the reduced glucose and calcium to normal level and enhancement of uterine defence mechanism.

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