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RESEARCH ARTICLE

Impact of Organic Metabolites on the Occurrence of Dystocia in Nili-Ravi Buffaloes of Three Different Agro-ecological Zones of Punjab, Pakistan

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ABSTRACT

The present study was conducted to find out the role of organic metabolites on the occurrence of dystocia in Nili-Ravi buffaloes of three different agro-ecological zones of Punjab, Pakistan. Serum samples of healthy control (150) and dystocia affected (23) buffaloes were collected and subjected to biochemical analyses to determine metabolites (Urea, Total Proteins, Albumin, Globulins and Albumin-Globulin ratio) by using commercially available kits. The finding indicated significantly higher ($P < 0.05$) levels of serum urea in dystocia affected buffaloes from southern irrigated zone (259.3 ± 71.4 mg/dl) and arid zone (276.2 ± 70.7 mg/dl) while non-significant difference was observed in northern irrigated zone (106.8 ± 1.03 mg/dl), in comparison to the healthy control buffaloes of the same region (95.6 ± 4.54 mg/dl, 63.9 ± 4.68 mg/dl and 82.1 ± 4.37 mg/dl) respectively. While serum total proteins and albumin concentrations were significantly higher ($P < 0.05$) in healthy control buffaloes in comparison to dystocia affected buffaloes of northern irrigated zone while non-significant difference was observed in southern irrigated zone and arid zone buffaloes in comparison to the dystocia affected buffaloes of the southern irrigated and arid zones. Whereas the serum globulin concentration of dystocia affected buffaloes of northern irrigated zone was found to be significantly lower ($P < 0.05$) compared to healthy controls while no significant difference was found among the dystocia affected and healthy control buffaloes of southern irrigated and arid zone. Similarly, the albumin to globulin ratio was found to be significantly lower ($P < 0.05$) among healthy control as compared to dystocia affected buffaloes of northern irrigated zone while no significant difference was found among the dystocia affected and healthy control buffaloes of the southern irrigated and arid zone. The present study concludes that the deviations of serum metabolites could be useful to predict the reproductive health status of the Nili-Ravi Buffaloes of Punjab, Pakistan.

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INTRODUCTION

Dystocia is a major cause of lower productivity in the livestock industry, including foetal and maternal losses, consequent infertility, culling rate and treatment costs (Tenhagen et al., 2007; Mee, 2008). The direct causes of dystocia vary depending on the species. The most common cause of dystocia is an aberrant presentation, position, or posture (Vandeplasseche, 1987; Noakes et al.,

2019; McCue and Ferris, 2012). Feto-pelvic disproportion is common in bovines, especially Primiparous. Buffalo is one of the main sources of milk and meat production in Pakistan. Current buffalo population of Pakistan is touching the estimated figure of 42.4 million heads (Anonymous, 2020a) and contributing 1/6th to the world's population (Anonymous, 2020b).

Among famous breeds of Buffaloes of Pakistan, Nili-Ravi is of great importance considering its milk

production. It is also called Black Gold of Pakistan due to its excellent productive features and its milk is liked due to its beneficent characteristics (Warriach et al., 2012). To enhance the milk production high yielding animals, need to be propagated. During the transition period i.e. 3 weeks prior to parturition through 3 weeks after parturition the buffaloes are at high risk for the occurrence of most of production diseases. Fetal size increases greatly during last trimester of pregnancy, so in this period demand for energy, protein and other nutrients increases and can greatly affect the health of the animal and its production in the upcoming lactation (Sharma, 2010; Keshri et al., 2020). Excellent reproductive health is the only way to enhance the number of best producers of milk and meat.

As parturition problems like dystocia, retention of fetal membranes and uterine prolapse are responsible to prolong the uterine and cervical involution and ultimately resumption of post-partum ovarian activity which result in prolonged calving intervals (El-Wishy, 2007; Kour, 2020). Occurrence of dystocia was reported to be 20.7% in river buffaloes (Ali et al., 2009). It is therefore suggested that particular attention should be paid to animals that suffered from calving problems, especially dystocia, uterine prolapse and retention of fetal membranes for successive fertility (Anonymous, 2005). The biochemical components are crucial for maintaining the normal physiology. As a result, blood biochemical components are crucial for the reproductive systems, normal functioning. Many problems can occur if any of the blood components, such as glucose, urea, cholesterol, total protein (TP), and other metabolites, have altered. As a result, understanding the serum biochemical profile of dairy animals during various stages of health and disease is critical. Metabolic problems will be better understood through biochemical profiling. Fertility and cyclicity in farm animals have been linked to blood cholesterol, glucose, urea and TP levels (Qureshi, 1998). Adequate nutritional availability improves postpartum reproductive performance by raising the animals' energy levels. Repeat breeding has been linked to changes in cholesterol, glucose, protein, albumin, and globulin levels (Barson et al., 2019) in a few studies. However, information regarding biochemical profile of Nili Ravi buffaloes is scarce. Keeping in view, this study was conducted to determine the impact of organic metabolites alteration on dystocia in Nili Ravi Buffaloes from three different agro-ecological zones of Punjab, Pakistan.

MATERIALS AND METHODS

A sum of (n=173) buffaloes 150 Healthy Control and 23 dystocia affected from three different districts representing each of three agro-ecological zones of Punjab province, Pakistan, namely District Multan from Southern zone, District Sialkot from Northern zone and

District Chakwal from Arid zone were selected. The healthy (control) and dystocia affected buffaloes were homogeneous.

Sampling

A total of 20 ml blood from each buffalo was collected from jugular vein in a sterile plastic test tube with screw cap using a sterile 16-gauge needle. The test tubes containing blood were placed in slanting position for one hour to let the serum ooze out. The serum was then aspirated carefully with a pipette, placed in glass vials, labelled and stored at -20°C for further analysis.

Urea estimation

The serum urea concentration was measured using a commercially available kit and an enzymatic UV test (Dia Sys GmbH-Germany). On blank, standard, and sample tubes, one ml of working solution (mono-reagent) was poured. The standard was urea concentration of 50 mg/dl, and 10 µl of standard or sample was added to the corresponding tubes, mixed, and incubated at 37°C for 30-40 seconds. The standard and sample concentrations were determined.

Serum proteins

Total protein was determined by colorimetric method using commercially available kit (Wiener Lab-Argentina) as per manufacturer's instruction. Briefly, distilled water (50 µl) was taken in tubes marked as blank, 50 µl of standard serum was added to tubes marked as standard and 50 µl of serum sample (the total protein concentration to be determined) was added to tube marked as sample. Then 3.5 ml of EDTA/Cu reagent was added to all the tubes mentioned above. Mixed with rod and incubated at 37°C for 15 minutes and then the spectrophotometer was standardized running blank and standard serum and the concentration in the serum samples were determined.

Albumin was determined by colorimetric method using commercially available kit (Wiener Lab- Argentina) following manufacturer's instruction; whereas, globulin concentration was determined by the given formula:

Globulin (g/dl) = Total Protein (g/dl) – Albumin (g/dl)

Albumin-Globulin ratio was determined by using the

formula: $A/G \text{ Ratio} = \frac{\text{Albumin}}{\text{Globulin}}$

Data analysis

The data thus collected was subjected to statistical analysis using one-way ANOVA and Duncan's Multiple Range test for determining significant differences at 5% level of significance using SPSS software.

RESULTS

Urea

The mean (\pm SE) serum urea level was always higher in dystocia affected buffaloes than control group (healthy control buffaloes) (Table 1). Buffaloes in Arid and Northern zones had the highest and the lowest

Table 1: Mean (\pm SE) serum urea levels (mg/dl) in affected and healthy buffaloes maintained in three agro ecological zones of Punjab, Pakistan

Calving Disorder	Southern Irrigated Zone	Northern Irrigated Zone	Arid Zone
Dystocia	259.33 \pm 71.42 ^A	106.75 \pm 1.03 ^{AB}	276.22 \pm 70.67 ^A
Control	95.56 \pm 4.54 ^{aC}	82.12 \pm 4.37 ^{aB}	63.86 \pm 4.68 ^{bC}

The values with different superscripts in the same row (small letters) and in the same column (capital letters) differ significantly ($P < 0.05$).

Table 2: Mean (\pm SE) serum total protein levels (g/dl) in affected and healthy buffaloes maintained in three agro ecological zones of Punjab, Pakistan

Parameter	Agro-ecological Zone		
	Southern Irrigated Zone	Northern Irrigated Zone	Arid Zone
Protein Concentration (g/dl; Mean \pm SE)			
Dystocia	6.70 \pm 0.45 ^{Ab}	5.33 \pm 0.13 ^{Cb}	6.14 \pm 0.44 ^{ABb}
Control	6.10 \pm 0.05 ^{Ab}	7.03 \pm 0.07 ^{Aa}	5.98 \pm 0.07 ^{Bb}
Serum albumin levels (g/dl; Mean \pm SE)			
Dystocia	3.60 \pm 0.10 ^A	2.83 \pm 0.15 ^B	2.94 \pm 0.21 ^{AB}
Control	3.47 \pm 0.04 ^a	3.34 \pm 0.04 ^{aA}	2.49 \pm 0.09 ^{bB}
Serum globulin levels (g/dl; Mean \pm SE)			
Dystocia	3.10 \pm 0.40 ^A	2.50 \pm 0.13 ^B	3.20 \pm 0.33 ^{AB}
Control	2.64 \pm 0.05 ^{bAB}	3.69 \pm 0.06 ^{aA}	3.49 \pm 0.12 ^{aA}
Serum albumin-globulin (A/G) ratio (Mean \pm SE)			
Dystocia	1.20 \pm 0.16 ^{AB}	1.14 \pm 0.10 ^A	0.99 \pm 0.1 ^{AB}
Control	1.35 \pm 0.04 ^{aB}	0.92 \pm 0.02 ^{bB}	0.82 \pm 0.07 ^{bB}

For each parameter, the values with different superscripts in the same row (small letters) and in the same column (capital letters) differ significantly ($P < 0.05$).

concentrations of urea among affected buffaloes, respectively. The control group of Arid and Southern zones possessed the lowest and highest urea levels. The differences among groups and zones were significant ($P < 0.05$).

Total proteins

The buffaloes with dystocia had the greatest mean (SE) serum total protein levels in the Southern irrigated zone and the lowest in the Northern irrigated zone. Although the total proteins of dystocia affected and healthy control buffaloes were similar, there were significant differences ($P < 0.05$) between groups and zones. The northern irrigated zone's control group had the greatest total protein concentration (Table 2).

Albumin

The mean (\pm SE) serum albumin levels of the buffaloes suffering from dystocia, and their healthy controls for each zone were compared (Table 2) among themselves and among three agro-ecological zones. In the southern irrigated zone, no significant difference ($P > 0.05$) was recorded in the mean serum albumin levels between the animals suffering from dystocia, compared with the controls. In the northern irrigated zone, no difference was recorded in the mean serum albumin levels of affected buffaloes within themselves but control buffaloes had significantly higher ($P < 0.05$) mean serum

albumin levels compared with those suffering from dystocia. In the arid zone, no difference was recorded when comparison was made between healthy control buffaloes and those affected with dystocia. The comparison amongst the agro-ecological zones revealed no significant difference in the mean serum albumin levels of buffaloes suffering from dystocia in all the three zones.

In healthy control buffaloes, a significantly lower ($P < 0.05$) mean serum albumin level was found in the arid zone compared with those in the northern irrigated zone and the southern irrigated zone and the later two did not differ between themselves. When the total affected and total controls were compared, no difference ($P > 0.05$) was recorded in the mean serum albumin levels.

Globulin

The mean (\pm SE) serum globulin levels of the buffaloes suffering from dystocia were highest in Arid zone and lowest in Northern Irrigated zone (Table 2). In control group highest and lowest levels were in Northern and Southern Irrigated zones. Healthy buffaloes had significantly higher level of globulins in Northern Irrigated and Arid zones ($P < 0.05$) than in dystocia affected buffaloes of the respective zones.

Albumin-globulin ratio (A/G)

The mean (\pm SE) serum albumin-globulin ratio of the buffaloes suffering from dystocia were higher than respective controls in northern irrigated and arid zone while lower in southern irrigated zone. The highest and lowest ratios were found in Southern and Northern irrigated zones, respectively (Table 2) for both dystocia affected and control buffaloes.

DISCUSSION

This study revealed the impact of organic metabolites on the occurrence of dystocia in Nili –Ravi buffaloes of three different agro-ecological zones of Punjab, Pakistan. Dystocia and associated inflammatory responses could cause sufficient losses in buffaloes in terms of production and economics. The levels of metabolites could be used as indicators of such conditions. Concentration of metabolites like total proteins, albumins, globulins and urea are important predictors of reproductive problems. Regular and routine examination and biochemical tests could provide early clues to the problems. Hypoproteinemia is also associated with liver malfunction and negative nitrogen balance because of reduced protein intake. Increased urea concentration in dystocia affected buffaloes compared with normal parturient buffaloes were recorded during the present study, similar findings have been recorded previously in cattle and buffaloes (Jeengar et al., 2015; Chaudhary et al., 2020). The increased urea levels in dystocia affected buffaloes,

could be due to dehydration, stress and reduced blood flow to kidneys, or kidney damage due to toxins from the dead fetuses (Jeengar et al., 2015). Also in the subcontinent, generally the feeding practice is not based upon requirements of the dairy animals. A same scale feeding is normally practiced exposing the low yielding animals to adverse effects of overfeeding. Increased intake of crude protein varied between seasons and was positively correlated with serum urea levels (Qureshi, 2009). Increased urea levels are indicative of faulty or poor prognosis. Some scientists found similar results. When dystocia case was presented, a substantial increase in plasma urea was recorded, which indicated poor prognosis as suggested by Mahmoud et al., (2020). Decrease or increase plasma proteins and albumin concentration can be a character of reproductive problem. The findings of current study are in line with the study of (Kour, 2020) as a decrease in the total plasma proteins and albumin level was consistently observed in dystocia affected buffaloes.

The results given by Molefe and Mwanza (2019) were also in line with present findings. total protein, albumin, globulin, AG ratio, were investigated by Permuhah and Rajkhowa (2013) and matching results were given by them. Results of other researchers about the serum total proteins in animals with retained placenta were found to be within the reference range of cattle (Radostits et al., 2000; Zaman et al., 2006; Regmi and Pande, 2018). Albumin globulin ratio was altered. Globulin was higher than the albumin fraction, which indicates there was increase in the immunoglobulin level as the animal suffered from bacterial infections. The variations of total proteins, albumin, globulin, and albumin-globulin ratios could be due to metabolic problems like imbalance of essential macro and micro minerals which are of utmost significance for normal physiological functioning of the animal. This finding was also in agreement with observations of Plaza et al., (2019). The findings of this study revealed that the serum metabolites levels are a key factor affecting the reproductive health of the animal so the monitoring of concentration of these metabolites can help us in reducing the reproductive and calving disorders as well as also help in enhancing productivity of the animals which will elevate the socio economic status of the farmer and boost the national economy.

Conclusion

It is concluded that metabolite levels were variable among buffaloes suffering from dystocia. The strict monitoring of buffaloes prior to parturition can be helpful in reducing dystocia and other peri-partum problems. Metabolite levels are indicative of many problems in bovines and their detection can be predictor of prior detection of reproductive problems.

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Authors' contributions

All authors contributed equally in writing this manuscript.

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