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Short Communication

Incidence of Subclinical Ketosis and Evaluation of Associated Risk Factors in Early Lactating Dairy Buffaloes

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ABSTRACT

Ketosis is a metabolic disorder of dairy animals that appear mostly in the early lactation period of animals. Current study was aimed to estimate occurrence of ketosis and associated risk factors in dairy buffaloes. In this regard, milk and urine samples were individually collected from 96 buffaloes and analysed by Ross Modification of Rothera's test and Abbott Precision Xtra™ meter to assess the ketone bodies levels. We observed a total of eight out of 96 (8.33%) buffaloes positive for ketosis. The peak prevalence (13.3%) of ketosis was detected during the third week of lactation. Animals with body condition score >4 (15%), buffaloes yielding 18 liter milk in a day (33.3%), buffaloes in 3rd parity (11.1%), and 8-9 years old buffaloes (11.1%) were identified to be at higher risk of ketosis. A significant co-relation was observed between ketosis and milk yield, body condition score and puerperal metritis. Collectively, the animals with high milk yield, in third week of lactation, carrying >4 body condition score, in third parity and aged buffaloes were more prone to ketosis. These results highlight the occurrence of ketosis in buffaloes and offers an action plan to mitigate the risk of ketosis which may result in better production in livestock.

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MZA, KA, MAA and MHT designed the study. Sampling was done by MG, AA, and AH. Experiments were carried out by MZA, KA, MHT, and MUN. MZA wrote the original draft. MAA and AIA statistically analyzed, reviewed, and edited the manuscript.

Key words
Ketosis, Dairy buffaloes, Risk factors

Owing to high population of cattle (~51.5 million) and buffalo (~42.4 million), Pakistan stands among the highest milk producers (Pakistan Economic Survey, 2020). Pakistan livestock section attributes approximately 57,890 thousand tons milk per annum with an average of 1.46 L/animal/day. Compared to the number of livestock animals and agricultural land, theses production marks are considered low and substandard according to international standards. This suboptimum milk production is not only due to the poor genetic potential but also caused by a variety of infectious and metabolic diseases. Among various metabolic diseases, ketosis is a common metabolic disease that is characterized by the imbalance metabolism of carbohydrate and volatile fatty acids (VFAs). During early lactation periods, animals produce more milk than glucose/glycogen are fed, and this led to negative energy balance that causes mobilization of body fat reserves in order to meet the body requirements. The mobilization of fat leads to production of fatty acids at high rates which are responsible for ketone bodies formation. The high level of ketone bodies formation leads to development of ketosis (Radostits *et al*., 2007).

The salient features of this disease are ketonemia, ketonuria, hypoglycemia and low level of hepatic glycogen (Tehrani-Sharif *et al*., 2012). Clinical ketosis typically appears within the first six to eight weeks after calving and results in anorexia, licking, blindness, hard dry faeces, rapid loss of condition and decreased milk production (Youssef *et al*., 2010). Subclinical ketosis (SCK) are accompanied by decreased milk yield and lower milk protein and lactose contents (Vanholder *et al*., 2015). Ketosis prone animals to delayed oestrus, low conception rate, increased calving intervals, cystic ovarian disease and mastitis (Radostits *et al*., 2007).

There are number of risk factors that can lead to

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ketosis such as dystocia, retention of foetal membrane, high milk production capacities, puerperal metritis and milk fever (Vanholder *et al*., 2015). While the condition is common, limited information is available in dairy buffaloes, which comprise about 60% of the total lactating herds of Punjab province of Pakistan. Therefore, the present study was conducted to assess the occurrence and prevalence of ketosis in buffaloes. Additionally, we aimed to evaluate associated risk factors which can alter the ketosis in lactating buffaloes.

Materials and methods

This study encompassed different dairy farms in the vicinity of district Faisalabad. Blood, milk and urine samples were collected from a total of 96 buffaloes from dairy farms. The study was approved by Faculty Board and Graduate Studies for all kinds of ethical norms of research notification vide letter No. CE 841/15/08/15. Milk and urine samples from all quarters of buffaloes were collected in separate sterile screw-caped bottles. Both samples were analysed for ketosis by Ross Modification of Rothera's Test (Farooq, 2014). Blood was taken from jugular vein and analyzed by ketometer (Abbott Precision Xtra™) to detect the level of ketone bodies (Iwersen *et al*., 2009) according to the manufacturer's instructions. A cut-off point (≥1.2mmol/L) was used to differentiate between positive and negative samples (Iwersen *et al*., 2009). Prevalence was estimated at 95% confidence interval (CI). Chi square test was used to analyze the data collected (Schork and Remington, 2010) to perform test of association between variables and results were significant at $p<0.05$.

Results and discussion

Analysis of the ketosis, determined by the ketometer, demonstrated a positivity rate of 8.33% (8/96) in total collected and analyzed samples. Further analysis of the disease occurrence across the lactation period indicated a high incidence rate during the third (13.3%) and fourth week (11.1%) post-partum. The buffaloes carrying body condition score 4 (15%) and >4 (20%) were found to have higher ketosis. The body condition scoring was performed as described previously (Radostits *et al*., 2007). The ketosis was found to be higher in 8-9 (11.1%) and 10-11 (10.0%) years of age. Likewise, animals with 18 and 16 liters of milk yield were found more ketoic with 33.3% and 10.0%, respectively. Regarding parity number, buffaloes during 3rd and 4th parity carried 11.1% and 10.5% ketosis, respectively. The association of postpartum duration was found to be statistically non-significant (χ^2=1.520, p-value= 0.971). There was a significant ($p<0.05$) co-relation with ketosis in milk yield and body condition score while non-significant ($p>0.05$) relationship was found between age, postpartum duration, and parity (Table I). Significant ($p<0.05$) relation was found with puerperal metritis while non-significant ($p>0.05$) relationship was identified between mastitis, dystocia and retained placenta (Table II). The current findings corelated with various studies where ketosis prevalence was ranged from 0% to 33.9% in the first two months of lactation (Dohoo and Martin, 1984; Andersson and Emanuelson, 1985; Nielen *et al*., 1994; Duffield *et al*., 1997). First eight weeks of postpartum was considered suitable to check the prevalence of ketosis. It was recorded high during the third (13.3%) and fourth week (11.1%) of lactation. Findings of this study are in line with the previous studies showing that the peak prevalence of subclinical ketosis occurs in the first 2-4 weeks of lactation (Duffield *et al*., 1997, 1998) which is probably due to high milk production in these weeks.

The association of body condition score (BCS) was statistically significant (χ^2= 8.104, p-value = 0.044). Buffaloes having BCS 3.5-4 and >4 were more ketoic with a prevalence of 15% and 20%, respectively. It has previously been reported that BCS at calving higher than 3.25 or 3.5 on a 5-point scale was associated with a higher risk of development of ketosis (Gillund *et al*., 2001; Busato *et al*., 2002). This may be due to animals having high BCS at pre-calving carry lower dry matter intake (DMI). Such animals eat less at calving than thin cows which may lead to high concentrations of non-esterified fatty acids (NEFA) in plasma and high negative energy balance postpartum leading to the development of ketosis (Vanholder *et al*., 2015). The high milk production causes intensive negative energy balance, increased hepatic ketogenesis and fat mobilization which cumulatively result in ketosis (Duffield, 2000; Detilleux *et al*., 1994). The buffaloes in 3rd (11.1%) and 4th (10.5%) parity were showed higher ketosis. The association of parity was found statistically non-significant (χ^2=1.317, p-value=0.971). The study findings are in line with already published reports. The chances of ketosis increase with the increase in parity. This is attributed to high milk production in this period that leads to high prevalence of ketosis (Vanholder *et al*., 2015). In this study, the peak prevalence of ketosis was recorded in 8-9 (11.1%) and 10-13 (10%) years of age. It was observed that association of age was statistically non-significant (χ^2=0.938, p-value=0.800). In a couple of studies, it has been documented that the likelihood of ketosis increases with the increase in age (Vanholder *et al*., 2015). Among eight ketosis positive buffaloes, three were mastitis positive. In our current study, the association of mastitis was found statistically non-significant (χ^2=0.108, p-value=0.742). The buffaloes with mastitis have 1.28 times more chances of ketosis as compared to mastitis negative animals. Previously, it has been reported that mastitis increases the chances of ketosis in high

producing animals. This discrepancy could be attributed to the impairment of udder defence mechanisms during hyperketonemia (Suriyasathaporn *et al*., 2000).

It was observed that animals with dystocia have 1.50 times higher risk of ketosis in the recent lactation as has been reported by Anna and Vertenten (2014). Notably, no significant increased ketosis was noticed in dairy animals having dystocia and twins, however, only two buffaloes carried retained placenta among eight ketosis positives animals. Collectively, in this study, no non-significant association between ketosis and retained placenta was noticed as previously described by Vanholder *et al*. (2015). However, there was 1.296 times more chances of ketosis in buffaloes having retention of placenta, whereas metritis positive animals carried 4.680 times higher chances of ketosis compared to metritis negative animals. It has been reported in previous studies that milking animals in early lactation with subclinical ketosis had an increased risk of metritis (Dohoo and Martin, 1984). However, most studies including ours, proposed that ketosis is an outcome rather than a cause of the metritis.

Table I. Risk factors of ketosis in dairy buffaloes.

Category	Variable	Positive	Prevalence (95% CI)	OR (95% CI)	χ^2-value and p-value
BCS	>4.0	4	20 (8.07-41.6)	12.51 (0.67-232.83)	$\chi^2 = 8.104$ $p = 0.044$
	4.0	3	15 (5.24-36.04)	1.33 (0.27-6.51)	
	3.0-3.5	1	3.6 (0.63-17.71)	5.60 (0.61-51.76)	
	<3.0	0	0.0 (0-12.06)	-	
Age	3-5 yr	1	5.0 (0.89-23.61)	-	$\chi^2 = 0.800$ $p = 0.938$
	6-7 yr	1	5.6 (0.9-25.76)	2.00 (0.18-22.55)	
	8-9 yr	2	11.1 (3.1-32.8)	2.22 (0.20-25.06)	
	10-11 yr	2	10.0 (2.8-30.1)	1.11 (0.15-8.30)	
	12-13 yr	2	10.0 (2.8-30.1)	1.11 (0.15-8.30)	
Postpartum duration (weeks)	3rd	2	13.3 (3.73-37.88)	3.39 (0.17-68.06)	$\chi^2 = 1.520$ $p = 0.982$
	4th	1	11.1 (1.99-43.5)	1.20 (0.11-13.59)	
	6th	1	9.1 (1.62-37.73)	1.47 (0.13-16.61)	
	8th	1	8.3 (1.49-35.38)	1.60 (0.14-18.12)	
	7th	1	7.7 (1.37-33.31)	1.73 (0.15-19.63)	
	5th	1	7.7 (1.37-33.31)	1.73 (0.15-19.63)	
	2nd	1	7.7 (1.37-33.31)	1.73 (0.15-19.63)	
	1st	0	0 (0-27.75)	-	
Parity	3rd	2	11.1 (3.1-32.8)	3.11 (0.15-62.53)	$\chi^2 = 1.317$ $p = 0.971$
	4th	2	10.5 (2.94-31.4)	1.06 (0.14-7.90)	
	2nd	1	9.1 (1.62-37.73)	1.22 (0.11-13.78)	
	5th	1	8.3 (1.49-35.38)	1.33 (0.12-15.03)	
	7th	1	8.3 (1.49-35.38)	1.33 (0.12-15.03)	
	6th	1	7.7 (1.37-33.31)	1.44 (0.13-16.29)	
	1st	0	0.0 (0-25.88)	-	
Milk yield (Liters)	18	4	33.3 (13.81-60.93)	13.32 (0.71-248.73)	$\chi^2 = 12.484$ $p = 0.014$
	16	2	10.0 (2.8-30.1)	3.33 (0.55-20.04)	
	14	1	4.3 (0.77-20.99)	7.67 (0.81-72.41)	
	12	1	4.3 (0.77-20.99)	7.67 (0.81-72.41)	
	Upto 11	0	0.0 (0-17.59)	-	
Dystocia	Yes	3	3.125 (1.07-8.8)	1.50 (0.25-9.09)	$\chi^2 = 1.724$ p= 0.189
	No	93	96.88 (91.22-98.93)		
Mastitis	Yes	3	3.125 (1.07-8.8)	1.50 (0.25-9.09)	$\chi^2 = 0.108$ p= 0.742
	No	93	96.88 (91.22-98.93)		
Metritis	Yes	3	3.125 (1.07-8.8)	1.50 (0.25-9.09)	$\chi^2 = 4.279$ p= 0.039
	No	93	96.88 (91.22-98.93)		
Retained placenta	Yes	2	2.08 (0.92-11.36)	-	$\chi^2 = 0.092$ p= 0.762
	No	94	97.92 (92.72-99.43)		

$p<0.05$ indicate significant association.

Table II. Analysis of the different reproductive diseases and conditions as risk factors of ketosis in dairy buffaloes.

Category	Variable	Pos./ tested	Prev. (95% CI)	Odds ratio (95% CI)	χ^2-value p-value
Dystocia	Yes	3/93	3.125 (1.07-8.8)	1.50 (0.25-9.09)	$\chi^2 = 1.724$ p= 0.189
Mastitis	Yes	3/93	3.125 (1.07-8.8)	1.50 (0.25-9.09)	$\chi^2 = 0.108$ p= 0.742
Metritis	Yes	3/93	3.125 (1.07-8.8)	1.50 (0.25-9.09)	$\chi^2 = 4.279$ p= 0.039
Retained placenta	Yes	2/94	2.08 (0.92-11.36)	-	$\chi^2 = 0.092$ p= 0.762

$p<0.05$ indicate significant association.

Conclusion

Taken together, the presents study found a higher prevalence of ketosis in buffalos. It was noticed that animals having high milk yield, being in the third week of lactation, having 3.5-4 body condition score, and in the third parity are more prone to ketosis compared to normal and healthy animals.

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Ethical statement

The study completely adhered to animal ethics and welfare as outlined in the method section of the article.

Statement of conflict of interest

The authors have declared no conflict of interest.

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