Original Article

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DETECTION OF SUBCLINICAL KETOSIS IN DAIRY BUFFALO HERDS OF TEHSIL JHANG, PUNJAB, PAKISTAN

Amar Nasir[1,*], Arbab Sikandar[2], Abdul Shakoor[1], Muhammad Kashif[1], Rana Waqar Tabish[1], Asghar Hussain[3], Adnan Hassan Tahir[1], Mazhar Abbas[1] and Muhammad Umer Iqbal[1]

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ABSTRACT

Bovine ketosis is a major metabolic disorder emerging as a result of negative energy balance in the post calving period and subsequent lactation of lactating dairy animals. The ketone bodies (beta-hydroxybutyrate, acetoacetate and acetone etc.) are accumulated that can be detected in milk, blood, and urine samples. The present study was accomplished to sort out the frequency of bovine ketosis in dairy buffalo herds during the first 2 months of lactation in buffalo to detect bovine ketosis. For this purpose, blood, and urine samples (n=50) were randomly collected from private dairy farms (n=5) having buffalos as a pilot study employing commercial test strips i.e., Free Style Optium β-ketone test strips (Abbot Healthcare, Pvt. Ltd. UK) for blood and JusChek (Muenster, Germany) for urine analysis. Resultantly, as a whole prevalence of bovine ketosis found using blood sample was 18% and via urine was 12% that indicates high prevalence of subclinical ketosis during 2nd month of lactation. Animals in the age group of 5 to 7 years were found to be significantly more susceptible ($P<0.05$) with an incidence of 37.5%. The results revealed that a significant subclinical ketosis exists in the dairy buffaloes, which causes decreased production associated with significant economic loss to the farmers. It was concluded that a moderately high incidence of sub clinical bovine ketosis predominates in the study area which may be curtailed by early diagnostic and remedial approaches using field tests to save the economics of the farmers.

Keywords: *Bubalus bubalis*, buffaloes, prevalence, sub-clinical ketosis, dairy buffaloes, urine analysis

INTRODUCTION

Among metabolic disorders, ketosis is a compound ailment of milking dairy animals. Various biochemical (Kinoshita *et al*., 2010) and hormonal disturbance accompanied by number of other risk factors are responsible for this (Ghanem and El-Deeb, 2010; Liu *et al*., 2010). Usually, this is considered a prime disorder or occasionally this could be linked with concurrent disease situation including secondary ketosis that appeared through some systemic maladies. By abrupt decrease in milk

[1]Department of Clinical Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan, *E-mail: amar.nasir@uvas.edu.pk

[2]Department of Basic Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan

[3]Department of Animal Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan

production, selective inappetance, hypoglycemia and elevated ketone bodies in body fluids such as urine, milk and blood, the disease can be illustrated. In modern dairying, the disorder is incriminated to mismanagement, ignoring nutritional demands for physiological processes of the animal, undernourishment, higher energy requirements and lack of exercise during postpartum stage. Decline in milk yield, body condition deteriorating pattern of this ailment coupled with escalated risk of allied abnormalities including displacement of abomasum and diminish reproductive output are the main source of economic losses. In the absence of clinical signs, subclinical ketosis (SCK) is exhibited through elevated level of circulating ketone bodies. Prevalence of SCK is 7 to 41% (Geishauser *et al*., 1998; Enjalbert *et al*., 2001). The ailment needs to be precisely diagnosed at the subclinical stage to avoid gigantic economic losses to the stake holders. Treatment should be initiated as soon as possible to overcome the economic losses. The present study was conducted to evaluate the status of (SCK) among the indigenous buffalo herds of Tehsil Jhang, through farm side testing for timely identification of bovine ketosis. It was speculated that (SCK) may be the chief contributor in milk production decline among buffaloes pressing towards the diagnosis of this issue at early stage.

MATERIALS AND METHODS

The current study was undertaken at privately owned buffalo dairy herds (n=5) with varying group of animals i.e., 7 to 25 having combined population of both cattle and buffalo, located in various areas of tehsil Jhang, Punjab, Pakistan. Differing ages lactating buffalos in the first two months of postpartum were selected for survey. Nili-Ravi and local bred lactating buffaloes (n=50) were haphazardly selected to check the influence of SCK. Blood samples (1 ml) and urine samples (50 ml) were taken in sterile 1 ml disposable syringe and falcon test tubes, respectively. Ethical manners were kept in mind while collecting samples to avoid undue stress to animals. Ear veins were used for collection of blood sample. The perineal area was cleaned, and vulva lips were massage gently before collection of urine samples. A performa was designed to rack up Information associated with age, breed, management of farm, post calving period, feeding procedure, quantity of milk production, milking type either hand milking or machine milking and incidence of abnormal behavior or nervous expression, sweetish odor from oral cavity and use of feed additives at the farm.

Blood specimens were tested through Free Style Optium β-ketone test strips (Abbot Healthcare Pvt. Ltd. UK) while the urine samples were analyzed through the commercial strips for ketone bodies detection (JusChek, Muenster Germany) for the detection of ketone bodies (beta-hydroxybutyrate, acetoacetate and acetone). For this purpose, a single blood drop was instantly placed on strip the well with the strip placed inside the glucometer previously. The results were shown by the glucometer within 10 seconds refereeing SCK or clinical ketosis. The cut off value of SCK was 1 to 1.4 Mmol/L as reported in an earlier study (Basbug *et al*., 2014). To detect the ketone bodies holding the urine specimen, the strips for this purpose were fully submerged in the test tube containing the urine. Subsequently, strips were taken out of the sample tube after 40 seconds and kept horizontally to avoid the unspecified mingle of the chemical substances. The change of strip

color was compared with the bands color given on the external labeling of the box carrying tests strips. The outcomes were noted as per the shown color strength varying from dark pink to purple for positive tests.

The Pearson Correlation test (SPSS version, 18) was used to analyze the data pertaining to age, breed, milk production, post-calving period and dairy farms whereas the effect of the predisposing factors were compared with the occurrence of bubaline ketosis.

RESULTS AND DISCUSSIONS

The outcome of the field testing revealed that out of 50 buffaloes included in the study, 9 were detected sub-clinically positive through blood testing while 6 were positive by urine strip testing displaying an overall incidence of 18% and 12%, respectively. Based on the age, the animals in the >5 to 7 years. Age group were found to be highly affected with an incidence of 37.5% and 25% through blood and urine, respectively. It followed the occurrence among the age group of >7 years. with incidences of 16.12% and 12.90% through blood and urine samples testing, respectively. The least occurrence in the current study was recorded in 3 to 5 years. age group of buffaloes (Table 1).

Farm based bovine ketosis occurrence was 0 to 25% through blood ketone bodies testing and 0 to 16.66% through urine specimen testing. Among the dairy properties Allah Ditta dairy farm presented the highest occurrence carrying 3 ketosis positive buffaloes out of 12 tested animals (Table 2).

On the basis of post-calving duration, the highest occurrence was recorded in the buffaloes during 2nd month (19.3%) compared with the 1st month (15.7%) post calving. The second month post-calving is the period of initiation of peak lactation phase acting as a risk factor for compelling the poorly fed animals to negative energy balance. It results in hypoglycemia mainly mediated by reduced intake of concentrate ration and quality feed (Table 3).

Pertaining to the effect of age, breed, milk production, post-calving period and farm on Ketosis positive (Urine) revealed (Table 4) that age ($P<0.01$) and milk production are significantly ($P<0.05$) correlated with the instigation of Ketosis, although the factors such as breed, post-calving duration and dairy farm property had non-significant ($P>0/05$) towards the incidence of Ketosis.

Correlation coefficient mean and standard deviation for age, breed, farm, and post-calving milk production for Ketone and urine analysis are presented in Table 5.

The statistical analysis of the data showed that the Correlation coefficient (with a value of 0.381) between post-calving milk production and Ketosis positivity (detected through Blood) was significantly different ($P<0.01$). Likewise, the correlation (0.592) between milk production and Ketosis positivity (Blood) as well as milk production and Ketosis occurrence (urine) with a value of 0.545, were also significantly different ($P<0.01$).

The finding of highest ($P<0.05$) incidence of ketosis among >5-7 years age group animals in the present investigation resembles to the outcome of a similar research (Kumar *et al.* 2015) conducted in Murrah buffaloes of the Hisar district (Haryana), India. Their findings showed that the age group 5 years or so was the mean of animals affected with bovine ketosis. It suggested a high association of lactation peak during dam's productive life with the incidence of bovine ketosis. The occurrence

Table 1. Overall and age wise incidence of bovine ketosis among dairy buffalo herds from tehsil Jhang.

Age of the animal	No. of animals tested(n)	Ketosis +ve (Blood)	Incidence %	Ketosis +ve (Urine)	Incidence %
3-5 yrs.	11	1	9.09*	0	0
>5-7 yrs.	8	3	37.5*	2	25
>7 yrs.	31	5	16.12*	4	12.90
Overall	50	09	18	06	12

*Age was a significant (P<0.05) risk factor in the incidence of bubaline ketosis (Pearson Association test).

Table 2. Farm wise incidence of bovine ketosis in Jhang city.

Name of property /farm	No. of animals tested (n)	Ketosis +ve (Blood)	Incidence %	Ketosis +ve (Urine)	Incidence %
Allah Ditta farm	12	3	25	2	16.66
Aman Ullah Sial dairy farm	7	1	14.28	1	14.28
Shakil dairy farm	11	2	18.18	1	9.09
Nawab Maqbool dairy farm	6	0	0	0	0
Chenab dairy farm	14	3	21.42	2	14.28

Table 3. Incidence of bovine ketosis between dairy buffalo herds from tehsil Jhang based on post-calving period.

Post-calving period	Buffalos tested (n)	Sub-clinically + ive	Incidence %
1st month post-calving	19	3	15.7
2nd month post-calving	31	6	19.3

*Post-calving period was a non-significant (P<0.05) risk factor in the incidence of bubaline ketosis (Pearson Association test).

Table 4. Analysis of variance between age of the animal, breed, milk production, Post-calving period and farm on subclinical Ketosis tested through urinalysis.

SOV	df	Mean Square	F. value	Sig.
Age	2	0.455	11.375	0.019**
Breed	2	0.117	2.932	0.119
Milk Prod	11	0.221	5.288	0.036*
Post-calving	22	0.034	0.855	0.641
Farm	4	0.051	1.273	0.365
Error	7	0.040		
Total	50			

Table 5. Correlation coefficient, mean and standard deviation for age, breed, farm, Post-calving and milk production for blood and urine Ketone analysis.

Parameters	No. of Obs.	Mean Values	Std. Deviation	Correlation coefficient	
				Ketosis +ve (Blood)	Ketosis +ve (Urine)
Age	50	2.40	0.833	0.199	0.119
Breed	50	2.50	0.647	-0.068	0.000
Farm	50	2.94	1.463	-0.156	0.438
Post-calving	50	38.18	14.831	0.381**	0.016
Milk Prod	50	9.34	3.305	0.592**	0.545**
Ketosis +ve (Blood)	50	2.12	0.328		
Ketosis +ve (Urine)	50	0.732	0.2781		

**(P<0.01)

among various age categories explored in the current investigation was also inline and significantly different (P<0.05). Similarly, they described the highest occurrence of bovine ketosis during the initial couple of months of milking (91.6%) compared with the later months being in harmony with the findings (19.3%) of our study. The buffaloes with high mean milk production manifested a highest occurrence (P<0.05) of this metabolic malady versus other buffaloes counterparts of lower mean production worth. Alike findings were reported from another research activity carried out on the Dutch cows (Vanholder *et al*., 2015) reporting increase milk production as one of the chiefs threaten factors linked with subclinical and clinical form of bovine ketosis.

The occurrence of bovine ketosis at different farms in the current study ranged 0 to 25% on the basis of blood testing and 0 to 16.66% on the basis of urine testing presenting statistically significantly (P<0.05) different tests performance. The incidence was highest (19.3%) in 2nd month post calving, phase of peak lactation phase making the animal more vulnerable to go into negative energy balance largely owing to hypoglycemia modulated by lowered availability of concentrates and balanced feed. Feed intake generally reaches to optimal at 8 to 10 weeks after calving (Das *et al*., 2008; LeBlanc 2010; Asrat *et al*., 2013). While the peak lactation reaches at about 4 to 6 weeks. This lag phase is the critical period perpetuating the mobilization of adipose tissue and subsequent synthesis of ketone bodies ensuing bovine ketosis.

The milk yield range of 2 to 22 liters of the animals in the present study be also a plausible factor triggering the production of ketones in blood and urine. This coincides with the findings of an earlier study (Kumar *et al*., 2015) stating the association of milk yield average of 14.42 liters with ketosis. Although, the acetone odor in breath previously recorded (Sharma and Rakesh, 2001) was not detected in our investigation which might be due to the finding of predominant subclinical form of the disease.

CONCLUSION

It was concluded that based on findings of the present study regarding the lack of organized farming practices in the study area, an utmost need exists for the routine systemized testing of the lactating animals at the earlier phase of lactation cycle through using farm side tests (strip testing and glucometers). It is highly anticipated to save the economics of the farmer and state as well.

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