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Journal of Veterinary Practice and Health

Prevalence of subclinical ketosis in Chilika buffaloes and associated hematobiochemical and urinary changes

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Received: 01, 07, 2025; Accepted: 20, 09, 2025; Published: 18, 11, 2025

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Abstract

The present study was aimed at assessing the prevalence of subclinical ketosis and the associated risk factors in 526 lactating Chilika buffaloes, maintained in 29 herds, belonging to marginal or small farmers in 16 villages, in three Chilika Lake-adjointing districts, viz. Puri, Khordha, and Ganjam, in the state of Odisha, India. Rother's test on urine samples from 526 lactating Chilika buffaloes diagnosed subclinical ketosis in 41 animals (7.79%). The blood, milk, and urine sampling were done from 20 lactating buffaloes, consisting of 10 apparently healthy buffaloes, and another ten with subclinical ketosis. The serum samples were analyzed for biochemical parameters, namely triglyceride, cholesterol, protein, albumin, globulin, activities of liver-specific enzymes, and milk fat% and SNF%. The urine samples from these buffaloes were tested using multi-diagnostic urinalysis strip. The highest prevalence of subclinical ketosis was observed in 4th parity (15.38%), followed by 3rd parity (11.20%), and in buffaloes yielding ≥ 3 kg of milk/ day (17.07%). The buffaloes with subclinical ketosis had severe hypoglycaemia (38.5 ± 0.598 mg/dL vs. 66.721 ± 1.923 mg/dL). There was an increase in the mean serum triglycerides (mg/dl) level, activity of Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT). The mean level of serum calcium (4.55 ± 0.01 vs. 9.88 ± 0.05), magnesium (1.35 ± 0.01 vs. 3.9 ± 0.31), and phosphorus (2.35 ± 0.01 vs. 5.08 ± 0.01) was significantly lower in buffaloes with subclinical ketosis. The subclinical ketosis was more common during peak production in Chilika buffaloes, associated with hepatic dysfunction.

Keywords: Chilika buffaloes, Subclinical ketosis, Prevalence, Hematobiochemical tests, Ross test, Rother's test

1. Introduction

Subclinical ketosis, produced by negative energy balance, is an important production disease that arises due to the imbalance in carbohydrate and fat metabolism in dairy animals. It is characterized by higher concentrations of ketone

bodies, namely acetoacetate, β -hydroxy butyrate, and acetone, in the body tissue and fluids with concurrent decreases of blood glucose levels and milk production [1, 2]. Dairy cows are challenged with a shortage of energy supply to the mammary gland during early lactation. Such a situation

Research Article

prevails due to gradually decreasing the feed intake, with an increasing level of milk synthesis [3]. The available adipose tissue fat becomes the main source of energy due to the negative energy balance. The changeover from carbohydrate to fat metabolism enhances production of ketones in the blood, called hyperketonaemia, resulting in clinical or subclinical ketosis with impaired milk production [4]. Ketosis develops into a disease state when production and absorption of ketone bodies exceed their use by the ruminants as an energy source, resulting in raised blood ketones, free or non-esterified fatty acid (NEFA), and decreased levels of blood glucose [5]. It may appear as a primary disease or as a consequence of other pathological conditions in dairy animals. The latter is termed secondary ketosis, which occurs in animals, mainly during winter and spring months. The negative energy balance leading to hypoglycaemia and ketonemia is the primary biochemical alterations, as maintenance of adequate blood glucose concentration is critical in high-yielding cows during the first few weeks of parturition [6]. A significant increase in energy requirements during the late pregnancy and early lactation subjects the dairy cows to a negative energy balance [7]. The metabolic adjustment to a negative energy balance (NEB) necessitates the mobilization of other metabolic fuels, and its failure may take place in tissues such as adipose tissue, liver, and others [4]. The intensified oxidation of non-esterified fatty acids (NEFA) in the liver may increase the production of reactive oxygen species and the development of oxidative stress [8].

Considerable economic losses occur due to a decrease in milk yield, cost of the treatment, failure of the recovered lactating animals to return to the normal milk production potential, decreased market value of the animal because of severe wasting, and occasionally on account of death and disposal of the animal. Ketosis mostly occurs in its secondary form in India, due to unhygienic farm conditions, and the pure primary ketosis is comparatively less [9].

Chilika buffalo (water buffalo) is a unique buffalo breed that has been named after the place of origin, Chilika Lake in Odisha. These animals spend the whole night grazing on weeds that grow in the Chilika water. The buffaloes usually return the next morning to the master's house. This breed has an excellent competence in converting the saline biomass of the lake into the most precious milk and dung. The buffaloes are milked after their return to the owner's

house, and they move in and around the house in daytime. As the night comes, those who return to the Chilika Lake, thus called "the night queen of Chilika". Some buffaloes follow a daytime grazing routine [10]. The population of Chilika buffaloes is estimated to be around 30,000.

Ketosis continues as a major disease in buffaloes that affects productivity. However, there are very limited studies on the clinical and therapeutic aspects of bubaline ketosis [11, 12, 13]. The clinical ketosis is associated with serum biochemical derangement and higher oxidative stress indices, as compared to normal buffaloes or those with subclinical ketosis [14]. The primary subclinical ketosis in Murrah buffaloes is most commonly recorded within the first two months after parturition in the third lactation, with characteristic clinical signs without variation in vital parameters. Still, it seriously hampers production [15]. The seasonal effects on milk yield and blood metabolites have been recently confirmed in dairy cows reared in high ambient temperature [16]. The current study was designed to determine the prevalence of subclinical ketosis, its risk factors, and associated clinicopathological alterations in Chilika buffaloes, maintained with typical management and feeding practices.

2. Materials and methods

2.1. Study location

The present study was performed on 526 Chilika buffaloes reared in 29 herds, owned by small or marginal farmers in 16 villages in three Chilika Lake-adjointing districts, Puri, Khordha and Ganjam, in Odisha, India, Chilika Lake is the Asia's largest inland brackish water lagoon, situated along the eastern coastline in between Latitude 19° 28'N to 19° 54' N and Longitude E 85° 06' to E 85°15' of Indian peninsula, spreading over 1,100 square km of wet land area, and has a catchment area of more than 3,500 sq. km. It is listed as a tentative UNESCO World Heritage site for its rich aquatic flora and fauna.

2.2. Owners' consent and experimental design

This clinical study was carried out by registered veterinary physicians, observing all guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). No invasive or painful procedures were performed in the study. The owner's consent was obtained to conduct the buffalo/ cow side test on their milch Chilika buffaloes. A total number of 526 lactating Chilika

Research Article

buffaloes were screened by Ross test in milk and Rothera's test in urine during the period from December 2019 to June 2020. Briefly, early morning milk samples, collected for buffalo-side test in sterile dry containers, were used for the Ross test for the detection of acetone. Five ml of milk (5ml) was saturated with granules of ammonium sulphate. A few drops of freshly-prepared sodium nitroprusside solution were added and mixed by tilting the test tube several times. Sodium hydroxide flake was added to the solution. Purple colour (like potassium permanganate) indicated the presence of ketone bodies [17]. The early morning urine samples, collected in sterile containers, were subjected to Rothera's test for the detection of acetone. Five ml of urine sample was collected from each Chilika buffalo in a clean and dry glass test tube saturated with granules of ammonium sulphate. A few drops of freshly prepared sodium nitroprusside solution were added and mixed by tilting the test tube several times. Then, concentrated ammonium hydroxide solution (1ml) was carefully layered over it. A purple colour (like potassium permanganate) ring at the interface established the presence of ketone body [17].

Screened 526 Chilika buffaloes were further grouped as per parity (1st/ 2nd / 3rd /4th / 5th /6th or above), stage of lactation (Early, Mid, Late mid or Late lactation) based on the period after parturition (0 to 60 days, 61-120 days, 121 to 180 days or above 180 days, respectively) and amount of milk yield/ day (up to 1 lt, 1-2 lt, 2-3 lt or above 3 lt) to establish the prevalence of sub clinical ketosis with respect to parity, stages of lactation and milk yield. The data on milk yield were collected through a predesigned questionnaire.

2.3. Collection and processing of blood sample

As a buffalo side test, a glucometer (On Call Plus blood glucose meter kit, Right Med Bio System, Chennai) was used for quantitative estimation of blood glucose concentration (mg/dl). A drop of blood was placed on a disposable test strip, which the glucometer read within seconds.

About 8 ml blood samples from each of the ten randomly selected subclinically ketotic and ten apparently healthy lactating Chilika buffaloes were collected by jugular vein puncture with the help of a sterilized 1.5-inch, 18-gauge needle after application of rectified spirit on the collection site. Approximately 4 ml of blood was collected in a clot activator vial (Accuvet plus) for the separation of serum for biochemical analysis. The biochemical profile, like blood glucose, serum

triglycerides, cholesterol, total protein, albumin, globulin, serum minerals such as calcium, magnesium, phosphorus, and liver-specific serum enzymes, namely, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), was estimated in all samples. The blood glucose was estimated by the GOD/ POD method, using a reagent kit, Crest Biosystem, Goa [18]. The serum triglyceride was estimated by the GPO/ PAP method, using the Crest Biosystem reagent kit [19]. The serum total protein and albumin were estimated by the Biuret and Bromocresol Green (BCG) dye methods, respectively [19, 20]. The activity of serum alanine aminotransferase (ALT) was estimated by the IFCC method, using the Crest Biosystem reagent kit [21]. The activity of serum aspartate aminotransferase (AST) was estimated by the IFCC method using the Crest Biosystem reagent kit [22]. The activity of serum Alkaline Phosphatase (ALP) was estimated by the pNPP kinetic method using the Crest Biosystem reagent kit [19]. Glucometer (On Call Plus blood glucose meter kit from Right Med Bio System, Chennai) was used for quantitative estimation of blood glucose concentration as a buffalo side test. A drop of blood was placed on a disposable test strip, which was read by the glucometer within seconds. The serum calcium, magnesium, and inorganic phosphorus were estimated by using Crest Biosystem reagent kits [19].

2.4. Analysis of milk and urine samples

The fresh milk was kept in a milk can/ vessel for 2 hours, and then filtered to separate the foreign particles. The milk was stirred well for 5 minutes in a circular motion using a mixing spoon with a long handle, so that the lower layers are reached and mixed properly for analysis. Stirring of milk in cans was done 5 to 8 times, with slow circular movements from the surface to the bottom and reverse. Clean, dry, glass/ metal/ plastic sample containers were used. The milk sample was poured several times out of the vessel into another and back forth, at least 3 times, just for uniform distribution of fat before testing. The fats get stuck to the walls of the container when stored for a long time. In such cases, milk was slowly heated to 35-40 °C, simultaneously shaken slowly, and then poured several times, for proper mixing of cream, and cooled. Samples for testing were used only once, and were not returned to the main vessel but discarded [23]. The Gerber method was used for a routine screening test for milk fat (IS:

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1223, 2001). The milk sample was added with sulphuric acid in a special Gerber tube/ butyrometer in order to dissolve protein and to release fat, and iso-amyl alcohol (IS: 360, 1964) was added for separation of fat (IS: 1224 Part 1, 1977). The lactometer was used to measure milk specific gravity. Richmond's formula was used to calculate the total solid not fat (SNF) and total solid of milk based on fat % and specific gravity. The milk was prewarmed to bring the fat to a liquid state and to ensure variation-free specific gravity reading (IS: 9385, 1980). Indiz milk stirrer and milk analyser were used for the estimation of fat % and SNF % in milk. The urine samples from these buffaloes were tested using multi-diagnostic urinalysis strip.

2.5. Statistical analysis

The data were analysed to express in mean $\pm$ standard error. One-way and repeated measure ANOVA and post-hoc analysis were conducted by Duncan multiple range test using SPSS-22 computer package.

3. Results

3.1. Prevalence of Subclinical ketosis in lactating Chilika buffaloes

Out of 526 lactating Chilika buffaloes screened, 41 animals were diagnosed positive for subclinical ketosis by Rother's test on urine samples, representing 7.79% of total animals examined (Table 1). However, Ross test on any of the milk samples from all 526 lactating Chilika buffaloes did not reveal positive reaction. Table 2 shows the prevalence of subclinical ketosis in Chilika buffaloes with respect to risk factors such as parity, lactation stage, and daily milk yield. Out of 157 Chilika buffaloes in early lactation (within 60 days postpartum), 26 had a positive reaction in Rother's test, representing the highest percentage of prevalence of 16.56% among all four lactation stages. There were 185 Chilika buffaloes in the mid lactation stage (within 61-120 days of lactation), and 12 out of those were positive, indicating a prevalence rate of 6.49% in mid lactation. This was followed by a prevalence rate of 2.03% (n=3) among 148 Chilika buffaloes in the late mid-lactation stage. All the 36 lactating Chilika buffaloes in the late lactation stage (180 days after parturition) were negative for both the Rother's test on urine, and Ross test on milk samples.

Sorting lactating Chilika buffaloes as per their parity (1st to 6th) revealed the highest prevalence of subclinical ketosis in the 4th

parity (15.38%), followed by 11.20% in the 3rd parity (Table 2). In the present study, out of 56 Chilika buffaloes in 1st lactation, only one was positive (1.79%) in Rother's test. Three Chilika buffaloes (n=3) were positive for subclinical ketosis (4.62%) out of 65 in 2nd lactation. However, only 6 Chilika buffaloes were positive out of the 67 lactating Chilika buffaloes (8.96%) in the 5th lactation. Only one buffalo out of 109 animals in 6th or above lactation (0.92%) was positive for subclinical ketosis.

A total of 132 Chilika buffaloes with the milk yield of less $\leq$ 1 kg milk/ day, were screened in this study. None of those buffaloes (n=0) were positive for subclinical ketosis (Table 2). Out of 125 Chilika buffaloes yielding 1-2 kg of milk/ day, only 6 animals (4.80%) were positive for subclinical ketosis. Chilika buffaloes with a daily milk yield of 2-3 kg (n=187) had a prevalence of 11.23% (21/187x100) for subclinical ketosis. The highest prevalence percentage of subclinical ketosis (17.07%; 14/ 82x100) was recorded among the Chilika buffaloes yielding $\geq$ 3 kg of milk/ day.

3.2. Clinical signs

Table 3 shows the frequency distribution of clinical signs in subclinical ketosis in forty-one (n=41) lactating Chilika buffaloes. The most common signs of subclinical ketosis in those buffaloes were reduced feed and fodder intake, a drop in daily milk yield, debility, incoordination in gait, and sweetish breath. The fall in the daily milk yield was observed in all 41 (100 %) Chilika buffaloes. The frequency of this sign was followed by that of reduced appetite (0.59; n=24), debility (0.46; n=19), incoordination in gait (0.097; n= 4/41), Chilika buffaloes, sweet smelling breath (0.024; n=1), and signs of central nervous system involvement (0.0; n=0).

3.3. Urinalysis using dip strips

Table 4 shows the urinary changes in subclinical ketosis in Chilika buffaloes. 41 out of 526 urine samples from the lactating Chilika buffaloes were positive for ketone bodies in Rother's test, showing (++) in sixteen and (+) in twenty-five samples. The urine samples from all the ketone bodies-positive buffaloes were negative for the presence of glucose, blood and bilirubin. This dipstick for multi-diagnostic urinalysis strip reaction test also revealed positive values for leucocytes, nitrates, urobilinogen, and protein. The mean pH of the urine from buffaloes with subclinical ketosis was 7.88 ± 0.073 , while the mean specific gravity was 1.02 ± 0.002 .

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Table 1. Prevalence of subclinical ketosis in lactating Chilka buffaloes by Rothera's test in urine and Ross test in milk samples.

Diagnostic test	No. of lactating Chilka buffalo screened	Positive for subclinical ketosis	Prevalence rate	Colour index*
Rothera's test	526	41	7.79%	±, +, or ++
Ross test in milk	526	nil	nil	-

No colour index - -ve; Very slightly/ faintly purple - ±; Slightly purple - +; Moderately purple - ++; Purple - +++; Deep purple - +++++.

Table 2. Risk analysis of subclinical ketosis in lactating Chilika buffaloes.

Risk factors	Group	No. of ketotic buffaloes screened	Frequency of Positive animals	Prevalence percentage
Lactation stage	Early (0-60 days)	157	26	16.56%
	Mid (61-120 days)	185	12	6.49%
	Late Mid (121-180 days)	148	3	2.03%
	Late (Above 180 days)	36	0	0.00%
Parity / Lactation number	1 st lactation	56	1	1.79%
	2 nd lactation	65	3	4.62%
	3 rd lactation	125	14	11.20%
	4 th lactation	104	16	15.38%
	5 th lactation	67	6	8.96%
	6 th lactation and above	109	1	0.92%
Range of milk yield (Kg/day)	Up to 1	132	0	0
	1-2	125	6	4.80%
	2-3	187	21	11.23%
	3 and above	82	14	17.07%
Irrespective of criterion		526	41	7.79%

Parity – calving number, the number of times a buffalo has given birth to a calf, regardless of whether the calf is born alive or stillborn; Lactation stage refers to period after calving.

Table 3. Frequency distribution of clinical signs in subclinical ketosis in lactating Chilika buffaloes.

Clinical findings	Number of buffaloes exhibiting signs	% of the total buffaloes (n= 41) with confirmed subclinical ketosis showing a particular clinical sign
Drop in daily milk yield	41	100
Reduced feed/ fodder intake	24	59
Debility	19	46
In-coordinated gait	4	10
Sweet smelling breath	1	2
Excitatory nervous signs	0	0

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Table 4. Sl. No. Parameters Test reaction in urine from subclinical ketotic buffaloes.

Sl. No.	Parameters	Test reaction in urine from subclinical ketotic buffaloes			
		++	+	-	Total
1	Leucocytes	3	2	36	41
2	Nitrate	0	6	35	41
3	Urobilinogen	38 (3.5)	17 (3)	-	41
4	Protein		3	38	41
5	pH				7.88±0.073
6	Presence of blood			41	41
7	Specific gravity				1.02±0.002
8	Ketone bodies	16	25	0	41
9	Bilirubin			41	41
10	Glucose			41	41

3.4. Quantitative biochemical parameters

The changes in blood or serum biochemical and milk parameters are presented in Figure 1 and 2, respectively. The mean blood glucose concentration of healthy control buffaloes was 66.721 ± 1.923 mg/dL, which was significantly higher than that of buffaloes with subclinical ketosis 38.5 ± 0.598 mg/dL, suggesting severe hypoglycaemia. There was an increase in the mean serum triglycerides (mg/dl) level, activity of Aspartate Aminotransferase, Alanine Aminotransferase, and Alkaline Phosphatase (ALP) (U/l). The serum mineral analysis revealed significant hypocalcemia, hypomagnesemia, and hypophosphatemia in lactating Chilika buffaloes diagnosed for subclinical ketosis (Figure 1). The urine samples from all 41 Chilika buffaloes, diagnosed positive for subclinical ketosis, were positive for glucose by the multi-diagnostic urinalysis strip reaction. Figure 2 shows daily milk yield, milk fat %, and the ratio of Milk Fat/ SNF. The mean level of these parameters in subclinical ketosis cases was 75.51, 137.17, 53.10, and 250.91% of the respective mean level in non-ketotic animals. There was a around one-fourth decrease in milk production and around a 50% decrease in SNF%.

4. Discussion

Subclinical ketosis, termed “the silent killer”, is one of the most important production diseases in dairy buffaloes that

adversely affects the quality and quantity of milk yield, in an unnoticed manner, as it does not show overt clinical signs, noticeable by the owners for the diagnosis. The Chilika buffaloes were let loose into the lake to graze on the natural grown flora and fauna. They were brought back in the evening for milking without provision of concentrate supplements and medications, thus predisposing dairy animals to imbalances in carbohydrate and fat metabolism during the transition period and milk production. The subclinical ketosis remains in a hidden form. It brings huge economic losses through changing the quantity and quality of milk for quite a long time, impacting the rural household economy of the poor dairy farmers. The present study is the first report on the prevalence of subclinical ketosis and the associated risk factors in these buffaloes. These dairy animals are unique in nature due to their habitat, feeding habits, milk quality, and other specific characteristics. They have acquired the ability to go into the brackish water and feed on submerged aquatic flora and fauna vegetation that grows in the lake, by adapting to the local harsh environment [24]. These buffaloes are reared completely in natural conditions without any supplementation of concentrates, minerals, and vitamins, even during their early lactation, for which they suffer from negative energy balance leading to subclinical to clinical ketosis. The result of the present study on Chilika buffaloes documents the prevalence of ketosis with respect to age, parity status, and stage of

Research Article

lactation, which indicates a higher prevalence of ketosis during the peak lactation phase.

Although there are several reports of bubaline ketosis in India but there are no reports on this unique buffalo breed [11, 16, 25-28]. The geopolitical difference based on region and country plays a vital role in dairy husbandry for ketosis prevalence. The differences in prevalence estimation may also be attributed to differences in study design and methodology. The deviations may be attributed to factors like geographical and environmental changes; animal breed, gestational stage, lactation capacity and status, and local community feeding customs and management practices. The present investigation was performed specifically on a landless farming community rearing Chilika buffaloes, in the native local tract, particularly in areas around the Chilika Lake. The farmers still follow age-old practices of their forefathers for their management, including housing and feeding. The lactating buffaloes thrive in this unique natural habitat by grazing on submerged flora and fauna. The diet of the night queens was devoid of the universal rural cattle feeds like paddy straw, broken pulses, oil cake, wheat/ rice bran, common salt, and mineral mixtures. The Chilika buffaloes are maintained without sheds under the sky or in an open shed with a thatched roof. Occasionally, some farmers were using plastic sheets on bamboo sticks to protect their livestock from rains [10]. The buffaloes thrived in very poor sanitary conditions, in knee-deep mud under very hostile natural environments.

The occurrence of subclinical ketosis largely depends on the amount of milk produced per day as well as the drainage of energy through milk. The feed intake and body energy reserves constitute the principal input of energy required for milk production. There is a steady increase in energy demand on a daily basis for the increased milk production during the peak lactation period. The negative energy balance results from the higher energy output required for milk production as compared to the energy received from the feed intake. However, this is considered a usual metabolic condition in high-yielding buffaloes. Therefore, lactating Chilika buffaloes with a higher daily milk yield, coupled with any stress, like changes in environmental conditions, suffer from anorexia, making them susceptible to subclinical ketosis. In this study, the highest occurrence was recorded in Chilika buffaloes, yielding more than 3kg of milk/day.

The highest prevalence of subclinical ketosis in Chilika buffaloes was recorded during the early lactation (0-60 days following calving) (n=26; 16.56%), followed by mid lactation (n=12; 6.49%) Chilika buffaloes, late mid lactation (n=3; 2.03%). No cases were recorded in late lactation (n=0). The peak milk production takes place during the first month following calving, thus increasing the energy demand at a steady rate during this period. The energy intake during the early lactation remains inadequate to meet the energy demand for the milk output, resulting in the higher prevalence of subclinical ketosis from day 0 to 60 of lactation. Environmental stress and reduced feed intake predispose these animals to subclinical ketosis.

The parity number influenced the prevalence of subclinical ketosis in lactating Chilika buffaloes. Buffaloes in the 4th parity were most susceptible (15.38%), followed by those in 3rd parity (11.20%), 5th parity (8.96%), and 2nd parity (4.62%). Out of 56 Chilika buffaloes in 1st lactation in the present study, only one buffalo was found positive, constituting only 1.79% of buffaloes of this group. This may be attributed to higher body reserves, efficient physiological adaptability to the adverse changes in young lactating Chilika bubaline mothers. The diverse physiological processes gradually weaken with the advancement in age. Therefore, the incidence of ketosis in Chilika buffaloes gradually increased with peak production capacity up to the 4th parity; thereafter, the decline begun.

The clinical signs observed in the present study, such as decreased feed and fodder intake, debility, drop in daily milk yield, uncoordinated gait, sweat-smelling breath, and excitatory nervous signs, were reported earlier in dairy cattle and are attributed to hypoglycaemia and hyper acetonemia [9, 29-33]. The decreased serum total protein and albumin level in subclinical ketosis in Chilika buffaloes might be attributed to hepatic insufficiency as seen in fatty liver syndrome in cattle [14, 28, 29, 34, 35]. The utilization of adipose tissue as the source of energy and subsequent production of acetyl CoA leads to a state of ketosis, as utilization of acetyl CoA in the TCA cycle is not optimal, resulting in accumulation and formation of ketone bodies and free fatty acids (FFA) [37-41]. The mean serum triglyceride concentration was significantly higher than the apparently healthy controls (154.4±0.81 mg/dl). The breakdown of body fat releases non-esterified fatty acids, which are oxidized to acetyl CoA or re-esterified

Research Article

to triglycerides in the liver [14]. The hormonal imbalances (insulin: glucagon ratio) also contribute to increased lipolysis and subsequent increase in plasma NEFA levels [41, 42].

The mean serum total protein concentration in buffaloes with subclinical ketosis was significantly lower than the apparently healthy controls (8.43 ± 0.02 g/dL), as seen in bovine ketosis [37, 43]. The reduction in total protein in this study might be attributed to hepatic dysfunction, reducing the synthesis of protein [43]. Besides, the protein catabolism for gluconeogenesis might be another reason for the decline in total protein levels. The energy-deficient animals with subclinical ketosis utilize the mobile pool of body protein as an alternative energy source for the synthesis of milk protein and lactose [5, 15, 44]. However, the present observations are contrary to the earlier findings reported in high-yielding ketotic cows [13, 14, 45].

The serum albumin and globulin concentrations in ketotic buffaloes were significantly lower (67.85% and 90.04%) as compared to apparently healthy controls. Significantly decreased blood glucose and increased AST, GGT, and urea concentrations have been reported in Anatolian Black cows

with primary ketosis [45]. Increased serum BHBA, decreased glucose and Ca concentrations, with statistically similar concentrations of vitamin C and P have been reported in clinically ketotic cows [46]. The reduction in serum albumin and globulin is a suggestive indicator for hepatic dysfunction/injury [47]. In energy-lacking animals with ketosis, the body's protein pool provides energy for the synthesis of milk lactose. The gluconeogenesis from the protein metabolism results in low serum albumin levels [15, 44]. The high protein intake exacerbates the energy deficit because of energy losses resulting from its metabolism and excretion [48]. The activity of serum ALT, AST, and ALP was significantly higher than that of control animals, which may be attributed to impaired liver function in buffaloes with clinical ketosis [2, 15, 44]. A significant hypocalcaemia was recorded earlier in primary ketosis in buffaloes [2, 44].

The lowered serum calcium in such cases can be attributed to a general state of sub-clinical hypocalcaemia, which is a normal consequence of the high metabolic demand of this mineral for higher milk yield. The reduced feed intake also results in secondary hypocalcaemia [41].

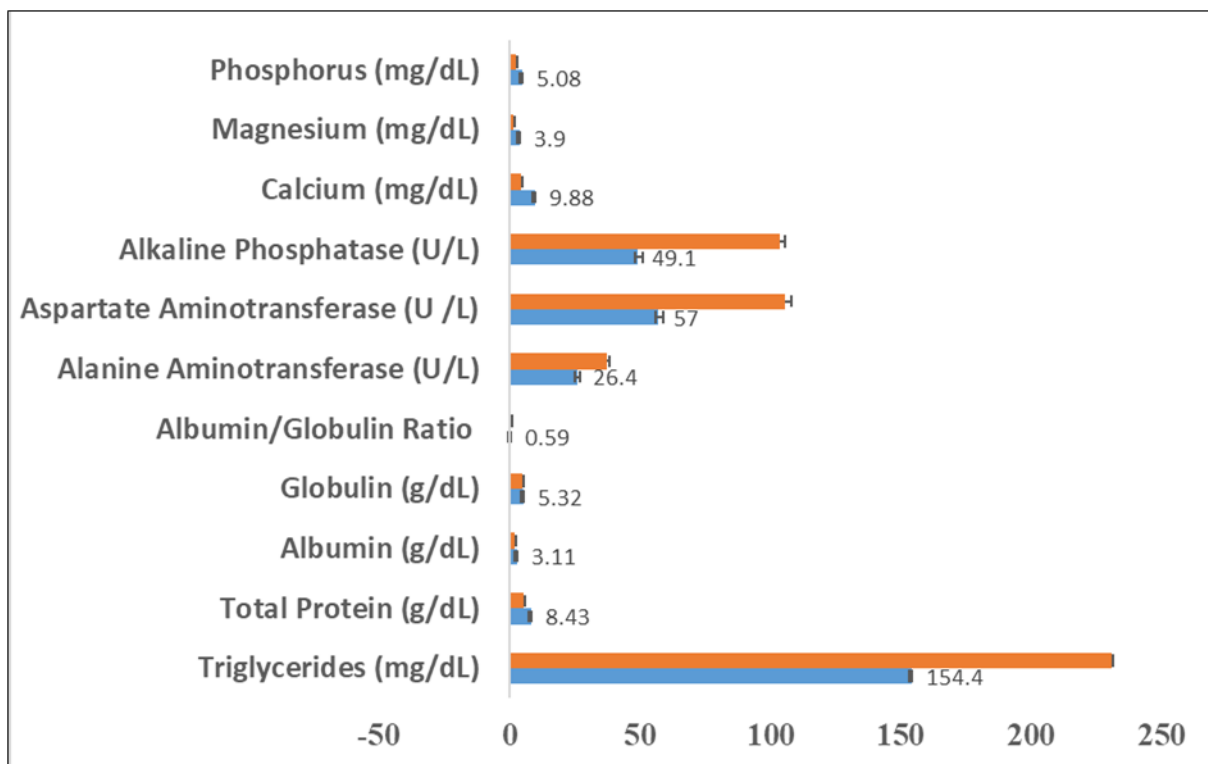


Figure 1. Changes in serum biochemical parameters in lactating Chilika buffaloes with subclinical ketosis.

Research Article

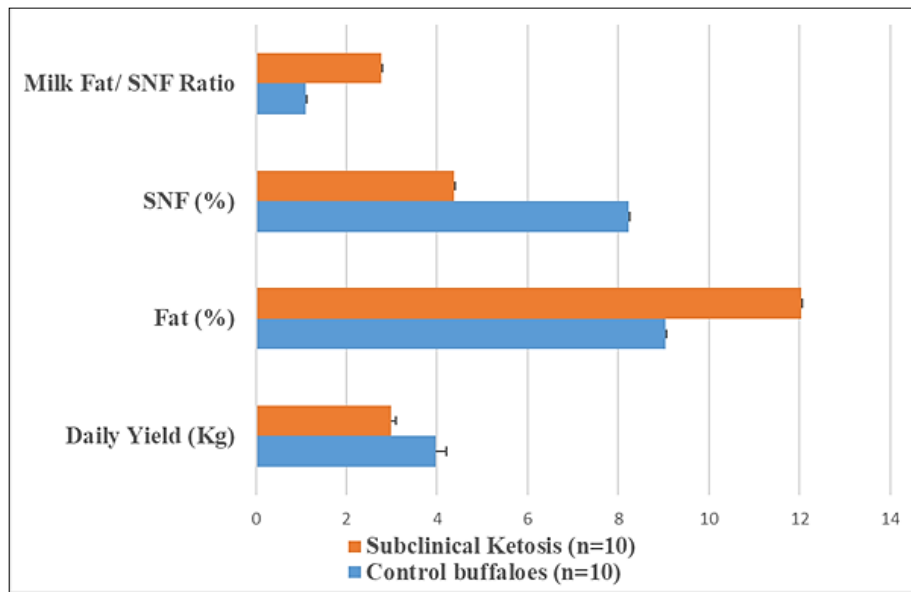


Figure 2. Changes in milk parameters in lactating buffaloes with subclinical ketosis.

The milk fat % in subclinical ketosis in buffaloes was significantly higher than the apparently healthy controls. The negative energy balance during the early lactation causes mobilization of body fat to fulfil energy requirements [48]. A chunk of mobilised fatty acids in subclinical ketosis in Chilika buffaloes is converted straight away into milk fat, thereby increasing milk fat% [49, 50]. The mean milk solid not fat (%) in subclinical ketosis in the buffaloes was significantly lower than the apparently healthy controls (8.232 ± 0.018 %). The milk protein % falls to some extent in ketotic animals due to a decrease in energy supply diminution [45]. This is in contrast to the milk fat percentage increase, a resultant of the bovine's fat mobilization. The increased level of milk solid not fat percent might be due to the alleviating hypoglycaemic condition and restoration of various enzyme activities. The daily milk yield (Kg) was significantly ($P \leq 0.05$) lower than the apparently healthy controls. The increase in blood ketone bodies and decreased availability of lactogenic precursor to the mammary gland in ketosis result in a lesser milk synthesis [51, 52]. However, the decrease in milk production was not proportional to the reduction in energy status due to excessive hormonal stimuli [5]. Urinary changes include a strong positive test reaction for ketone bodies in subclinically affected ketotic buffaloes, which could be attributed to acetonemia [51].

5. Conclusion

Subclinical ketosis accompanied by hepatic insufficiency prevailed in 7.79% of lactating Chilika buffaloes, and was more common during early (0-60 days) lactation in 4th parity (15.38 %) and with milk production of ≥ 3 lts / day (17.07%).

Funding

The research work did not have any specific funds from agencies in the public, commercial, or not-for-profit sectors. The work was accomplished by routine institute support.

Conflict of interest

No potential conflict of interest was reported by the author(s)

Author contributions

1. Ashish Dora, (dorashish@gmail.com), conducted the experiments, analysed data
2. S. K. Senapati, (drsenapati.ovc@gamil.com), supervised the experiments
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5. R. C Patra, (rccpatra@gmail.com), designed the experiments, and arranged resources

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Data availability

Data will be available upon request. The data generated in this study were only used to write the manuscript.

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Research Article

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