DIAGNOSTIC STUDIES ON CLINICAL KETOSIS IN DAIRY ANIMALS

Thesis

Submitted to Guru Angad Dev Veterinary and Animal Sciences University in partial fulfilment of the requirements for the degree of

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By

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CERTIFICATE – I

This is to certify that the thesis entitled, **"DIAGNOSTIC STUDIES ON CLINICAL KETOSIS IN DAIRY ANIMALS"** submitted for the degree of **M.V.Sc.,** in the subject of **Veterinary Medicine** (Minor subject: **Veterinary Public Health and Epidemiology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Sameer Sachdeva (L-2019-V-59-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

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ABSTRACT

The present study was aimed to evaluate the various diagnostic parameters for the diagnosis of different types of ketosis and their therapeutic management. In first experiment of study, approximately 200 animals were enrolled out of which 36 animals were positive, of these 22 were buffaloes and 14 were cattle. Majority of cases were of type II ketosis and only four cases were of type I ketosis. The animals were diagnosed using different diagnostic tests of blood, milk and urine. Hyperglycaemia and hypokalaemia were the consistent findings in the type II ketosis. Significant increase in the blood glucose, blood BHBA and milk BHBA concentration was recorded as well as significant decrease in the blood potassium concentration. Non-significant difference was recorded in the vitals and haematological parameters except hypomotile rumen in diseased animals as compared to healthy controls. Invariably there was increase in the urine ketone in all the diseased animals. In second part of study, 32 animals (20 buffaloes and 12 cattle) were enrolled for the study. Animals were given potassium chloride (16gm/100kg/day) and niacin (12gm/day). Good response of treatment was observed in 62.5 percent of animals. Twenty out of 32 animals responded to treatment and seven animals collapsed before the completion of appropriate treatment protocol of three to five days. Among the haematological and biochemical parameters, the concentration of blood glucose, potassium blood BHBA as well as milk BHBA were in physiological range after the treatment. Increase in the milk yield and performance of animals was observed after the treatment.

Keywords: Ketosis, BHBA, Dairy Animals, Potassium chloride, Niacin, Ketone Bodies

Signature of Major Advisor **Signature of the student**

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LIST OF ABBREVIATIONS

%	:	Percentage
<	:	Less than
>	:	Greater than
µmol/L	:	micro mole per litre
BHBA	:	Beta Hydroxy Butyrate
^{o}F	:	Degree Fahrenheit
bpm	:	beat per minute
bpm	:	breath per minute
d	:	day
e.g	:	For example
mmol/L	:	milli mole per litre
et al	:	and Co-workers
Fig.	:	Figure
i.e.	:	that is
NEFA	:	Non-Esterified Fatty Acid
mEq/L	:	milli Equivalent per litre
g/dl	:	gram per decilitre
mg/dl	:	milligram per decilitre
S.D	:	Standard Deviation
S.E	:	Standard Error
USG	:	Ultrasonography

CHAPTER I

INTRODUCTION

Ketosis is a complex multifactorial energy disorder that occurs in high producing dairy animals (cattle and buffaloes). Ketone bodies are normally absent or in trace amounts in body fluids. Ketosis is characterised by presence of elevated ketones in the blood (ketonemia), urine (ketonuria), and milk (ketolactia). It commonly affects lactating dairy cattle between one and six weeks after parturition and in early lactation period.

The transition period, which lasts from three weeks prepartum to three weeks after parturition, is marked by changes in metabolic state of animals. Throughout this period homeorhetic control of metabolic activities is important in order to fulfil needs of parturition and milk processing (lactogenesis) (Bauman & Currie, 1980). To support lactation, high demands for energy, fat and protein are associated with a significant decrease in dry matter intake at the time of calving leading to negative energy balance in lactation (Baird, 1982). Ketosis occurs when energy requirement of lactating animals exceeds the intake, resulting into breakdown of body stores and formation of ketones (Herdt, 2000). The accumulation of ketone bodies (acetone, aceto-acetate and β-hydroxy butyric acid) in the blood will result in the loss of appetite (either anorexia or inappetence) and worsening the problem. Many animals break down body stores to promote lactation, but severe catabolism can contribute to pathologic levels of ketone bodies. According to one earlier study, about 90% of cases of ketosis were identified within the first two months of calving and prevalence peaked in the 2^{nd} or 3^{rd} month following calving (Geishauser et al., 2000).

There is a significant increase in milk production during the period of early postpartum and intake of energy may not be sufficient to keep up with increase output, which results in negative energy balance in dairy animals and animals metabolise fat or adipose tissue in order to fulfil the energy requirements. Animals in negative energy balance (NEB) utilise more body fat resources and generate glycerol for energy, resulting in increased NEFA levels (DeVries & Veerkamp, 2000)

Ketone bodies are formed when non-esterified fatty acids (NEFA) are released during fat catabolism and are not fully oxidised. Lactose is required for milk synthesis; hence glucose demand is significantly increased during early lactation (Herdt & Emery, 1992). The ruminant's insufficient capacity to consume glucose from the diet immediately exacerbates the situation. As a result, ruminants depend on gluconeogenesis, especially during the early stages of lactation (Herdt & Emery, 1992). Neither NEFA nor ketone bodies may be used to produce glucose. However, in early lactation, each of these substances are essential for glucose control (Ingvartsen, 2006).

Acetone, acetoacetate and beta-hydroxybutyrate (BHBA) are the three main ketone bodies found in animals. Many tissues use ketone bodies as a fuel source, freeing up glucose for more important functions including milk production (Ingvartsen, 2006).

The presence of ketone bodies is not a pathological finding in ruminants. Butyrate (volatile fatty acid) which is a natural by-product of ruminant digestion, is converted into ketones by the rumen epithelium (Hird & Symons, 1961). Butyrate levels can be elevated in poorly preserved silages, resulting in higher blood ketone levels due to improved rumen absorption or lower feed consumption due to palatability (Ingvartsen, 2006).

Ketosis is categorized as primary or secondary depending on the onset of symptoms and the presence of other diseases in the animal. Primary ketosis or starvation ketosis occurs when the energy demands for milk production are not met due to inadequate nutrition or poor feed quality energy source. On the other hand, ketosis caused by a phase of anorexia due to a concurrent illness is called secondary ketosis (Baird, 1982).

Ketosis is also categorised into type I and type II. Type I ketosis is described in animal with low insulin and blood glucose levels at the time of hyperketonaemia and occurs due to high demand of energy for milk processing during peak lactation. It is not accompanied by any disease or fat accumulation in the liver and often seen in herds that feed a component ration (Holtenius & Holtenius, 1996).

Type II ketosis occurs between 5-21 days in milk and is described as animal with high insulin and transient or permanent high blood glucose levels along with fatty infiltration of liver due to inadequate activation of gluconeogenesis and ketogenesis. Overfeeding during dry period acts as a risk factor for developing type II ketosis and is sometime associated with some of insulin resistance and obesity in lactating animals.

Ketosis is also classified as subclinical and clinical. Clinical ketosis is described as presence of ketones in milk, urine and blood, as well as other noticeable symptoms like inappetence, sudden loss of body condition and depressed milk yield. Subclinical ketosis (SCK) is characterised as a rise in blood, urine or milk ketone bodies above a threshold level and in the absence of apparent clinical symptoms. Ketosis has been linked to increased risk of developing other illness such as DA, metritis and decreased milk yield (Duffield et al., 2009).

Detecting an individual animal experiencing clinical symptoms of disease has become difficult in large groups of loose-housed animals. Based on blood BHBA concentrations, attempts have been made to define ketosis as clinical or subclinical (McArt et al., 2011). The intensity of clinical signs appears to be determined by the ability of each animal to metabolise and tolerate the ketone bodies (Herdt, 2000). Rather than trying to discriminate between clinical and subclinical ketosis, the disorder may be better defined as hyperketonaemia.

The incidence of clinical ketosis in first month of lactation range from 2 to 15 percent, depending on the different diagnostic methods and screening frequency, although a 40 percent incidence of subclinical ketosis is common if animals are evaluated weekly during the same period (Duffield, 2000). There is also some evidence that animals with higher blood ketone body concentrations are associated with a higher chance of unfavourable consequences such as illness and culling of animals (McArt et al., 2012b).

Ketosis may be diagnosed by detecting high levels of ketone bodies in blood, urine and milk examination of diseased animals. However, the quantity of ketones varies in different body fluids. Ketone bodies have four time more concentration in urine than in blood (Schultz, 1971). The levels of acetoacetate in milk are almost half than that of blood and the concentration of BHBA in milk is one-eighth than that of blood (Anderson, 1984). The detection of BHBA value in blood is gold standard test for detection of ketone bodies because of its more stability than other ketones (Duffield, 2000; Herdt, 2000). In various earlier studies, the blood BHBA concentration that characterises hyper-ketonemia ranged from 1.0 mmol/L to 1.4

mmol/L (Kelly, 1977; Nielen et al., 1994; Geishauser et al., 1997; LeBlanc et al., 2005; Duffield et al., 2009; McArt et al., 2012b). During first week of lactation in animals with BHBA concentration in blood more than 1.2mmol/l were more likely to have increase risk of abomasal displacement and decreased production of milk. Although BHBA concentration of more than 1.8mmol/l caused significantly decrease in milk production in all the animals (Duffield et al., 2009).

Since 1950s, number of cow-side tests have been developed for promote identification of ketotic animals. Despite being the gold standard test, laboratory detection of ketone bodies in blood is more expensive, unpleasant and time-consuming, making individual animal diagnosis and therapy unfeasible. However, some other cow-sides tests were readily available for easy access and diagnosis of ketosis in dairy animals. The specificity and sensitivity of several of these tests were low (Geishauser et al., 1998; Carrier et al., 2004). Ketostix (for diagnose of urine ketone bodies), Porta BHBA strip (for diagnose of BHBA in milk), Precision Xtra ketone meter or Free Style optimum neo ketone meter (for diagnosis of BHBA in blood) were some cow-side tests for routine diagnosis of ketosis (Geishauser et al., 1998; Geishauser et al., 2000; Carrier et al., 2004; Denis-Robichaud et al., 2011). Out of these, Ketostix had more accuracy with 90 percent sensitivity and 86 percent specificity (when read after 5 seconds of dipping in urine) (Carrier et al., 2004). However, the difficulty of collecting urine from all animals restricted the use of extremely accurate cow-side ketostix test.

A relatively new hand-held ketone meter was developed as a cow-side diagnostic test to determine the ketone concentration in blood. In this assay, NAD^+ is reduced to NADH after BHBA is oxidised to acetoacetate. The current generated, when NADH is re-oxidized, was measured and was proportional to the concentration of BHBA present in the sample (Iwersen et al., 2009).

For routine monitoring of disease in a farm, milk ketone test is preferred due to easy collection and requires no training to perform the test. Keto-Test and Porta-BHB strip tests of milk works on same principle: a strip is dipped into the sample and the semi-quantitative result is read one to two minute later using a chart (0, 100, 200, 500, 1000 µmol/l as negative, trace and abnormal respectively) (Carrier et al., 2004; Denis-Robichaud et al., 2011). Using a cut value of 100 µmol/L on the strip, ketosis was defined as blood BHBA > 1.4 mmol/L and the Keto-Test strip had a sensitivity of 73 percent and a specificity of 96 percent (Carrier et al., 2004). As compared to keto-test, porta BHB milk strip test showed more sensitivity (92%) and specificity (78%) with cut-point of 100umol/L (Denis-Robichaud et al., 2011). Results achieved via porta BHB test strip were utilised using precision Xtra ketometer results (Denis-Robichaud et al., 2011). However, precision Xtra was accurate, less time consuming and highly sensitive test for detection of blood BHBA ketone bodies, but it was not always 100 percent (Konkol et al., 2010).

Different forms of ketosis require different treatment. In some studies, treatment of the diseased animal with parenteral dextrose therapy was found to be more effective than treatment them with other different combination of drugs like sodium propionate, niacin and jaggery (Venkateshwarlu & Choudhuri, 2000). Other studies reported that propylene glycol and niacin was more effective in case of sub clinical ketosis in animals (Nigam, 2016). The above studies were in context of type I ketosis. However, there were some studies which reported poor prognosis of type II ketosis and stated that treatment had no or very poor impact on the disease outcome and no effect on the production of animals. As in type II ketosis, animals lose its gluconeogenic capacity which was the main cause of failure of treatment in affected animals (Oetzel, 2007).

Very few or sparse studies have been reported on diagnosis and management of type II ketosis. So, the present study aimed on various aspect, of type II ketosis regarding the management in animals. On the basis of above facts, the current research investigated the following objectives:

- To evaluate various parameters for diagnosis of different types of ketosis in dairy animals.
- To study therapeutic management of ketosis Type II in dairy animals

CHAPTER II

REVIEW OF LITERATURE

Production diseases are a major problem for dairy farmers all over the world. Ketosis is a metabolic condition that affects a lot of high-yielders animals. Ketosis, also known as acetonemia, is a condition in which blood levels of "ketone bodies" (acetone, acetoacetate, and beta hydroxybutyric acid, all of which are referred to as ketones) rise to the point that they spill into urine and milk.

The text has been divided into sections for easier comprehension:

2.1 Epidemiology and occurrence studies on ketosis in bovines.

2.2 Studies on clinical symptoms

2.3 Diagnostic studies of ketosis

2.4 Treatment studies on bovine ketosis

2.1 Epidemiology and occurrence studies on ketosis in bovines.

Duffield (1997) observed the prevalence of ketosis among 1333 dairy cattle at various phases of lactation and at different parity. Reported the prevalence was higher in early lactation than mid lactation, late lactation. The prevalence was 14.1, 5.3, 3.2 and 1.6 per-cent at early lactation, mid lactation, late lactation and dry cows respectively among suspected cases.

Oetzel (2007) studied on the risk factor and prevalence of ketosis type 1 and type 2 in diseased animals. the study showed animals at DMI (0-20) at highest risk for type 2 ketosis and animals at DMI (21-42) were at highest risk for type 1 ketosis. on the basis of lactation, generally type 2 ketosis occur at 1st lactation and type 1 at 2nd, 3rd lactation.

Radostits et al. (2007) concluded that bovine ketosis happens more often in the Ist month of lactation, less frequently in the 2nd month and rarely in late pregnancy.

Tehrani-Sharif (2011) conducted a study on 190 dairy cattle in Iran and observe the prevalence of sub clinical ketosis in the dairy animals. Concluded that prevalence of sub clinical ketosis was 18.42 percent and diagnosed on the basis of serum β-hydroxy butyric acid estimation and serum glucose concentration. The glucose concentration in sub clinical cases was lower than the normal/healthy animals and serum BHBA concentration was higher than normal animals.

Cooper (2014) reported that risk for developing type I ketosis was high after 3-8 week of calving and usually due to underfeeding of dry matter during transition period. Also reported that risk of developing type II ketosis was more after 1-2 week of calving and usually due to overfeeding during the transition period.

Purohit et al. (2014) conducted a study on 729 buffaloes presented in the clinic at Bikaner in past 10 years. Out of which 286 cases were of post-partum metabolic disorder. They reported that highest case presented were of PPH followed by ketosis and milk fever. The prevalence rate of ketosis in past 10-year study was 16.59 percent.

Biswal et al. (2016) studied the prevalence of disease ketosis in Orisha state and reported the prevalence of ketosis was 36.7%. out of which prevalence of clinical ketosis was 27.2 and rest 9.5% was subclinical cases. He was also found that ketosis was high in age group of 8 year or at 4-5 parity of animal. Also observed that prevalence was greater at first lactation (56.7%) at 4-6 week after parturition.

Kumar et al. (2015) conducted a study on 145 buffaloes to check the incidence of ketosis in animals. Out of which 24 buffaloes were positive for ketosis and incidence was 16.55 percent among the clinical cases. The disease was diagnosed with the help of dipstick test and rothera’s test in urine and reported no significant difference was observed in temperature, respiration and heart rate of animals as compared to healthy animals.

Vanholder et al. (2015) reported that in comparison to heifers, the risk of developing subclinical ketosis in older cows was higher in second parity and whereas the risk of developing clinical ketosis in older cows was higher in cows with parity ≥ 3.

Kumar et al. (2016) studied 350 recent parturated cattle for ketosis with the history of decrease in milk yield and anorexia using rothera test and urine dipstick test for the diagnosis of clinical and subclinical cases among them. Reported the prevalence of ketosis is 11.42% (40 out of 350) among the suspected animals.

Sharma & Kumar (2016) reported 294 parturated cows and found the prevalence of ketosis was 10-20%. Ketosis was most common between 1-2 months after calving, at 8-9 years of age, and during the fourth lactation.

2.2 Studies on clinical symptoms

Lean et al. (1991) reported a study on the clinical signs of ketosis in lactating animals. He concluded that clinical signs like drop in milk yield, selective feeding (prefer to concentrate over roughages), anorexia, weight loss or loss of body condition, CNS involvement and partial or capricious anorexia were the major clinical symptoms in all animas that were diagnosed with ketosis.

Venkateshwarlu et al. (1993) conducted a study of 313 lactating buffaloes for the diagnosis of ketosis using Rothera's test and out of 313 buffaloes 46 (14.69%) were positive for the disease. He also observed that the vital/general parameters of animal and concluded that there was no change in these parameters like temperature, pulse rate, motility, and respiration rate. There was no significant difference in these animals as compared to control animals.

Mir & Malik (2003) conducted a study on the diseased animals and reported that clinical signs like selective feeding, decreased milk yield, fruity odour, depression and weight loss or wasting of animals were useful as diagnostic purpose of disease because these signs were found/reported in more than 50 percent of diseased animals.

Drackley & Dann (2005) reported a study on dairy animals and concluded that decrease in dry matter intake before parturition lead to an increase chance of subclinical ketosis and also concluded that more demand of glucose by mammary gland and inappropriate propionate production at early parturition could be a cause of ketosis after parturition.

Dar et al. (2014) reported a study on dairy cattle (64) that were presented with the sign of ketosis with age group of 4-13 years old and concluded that all of the ketotic cattle had almost normal temperature, normal respiration rate and elevated pulse/heart rate. The range of temperature, respiration rate and pulse rate were 101-103^{o}F, 30-40/min and 60-80/min respectively.

Kumar et al. (2015) reported a study on the buffaloes that were diagnosed with ketosis. Concluded that the diseased animals were exhibited clinical signs like selective feeding (feeding only concentrate), gradual reduction in milk yield, fruity odour from urine, fruity odour from breath and from milk also. He also concluded that other vital parameter or general parameters like heart rate, rumen motility, rectal temperature, pulse rate and respiration rate were in normal range like a non-disease animal.

Bali et al. (2016) in a study of 18 clinical cases of ketosis in buffaloes, reported a wide range of symptom occurrences with a precipitous drop in milk yield occurring in 100% of the clinical cases. He also reported the wide variation in the symptom of ketosis. The most common symptom noted were selective feeding (79.14%), wasting (49.91%), anorexia (29.37%), CNS involvement (6%), fruity odour (19.37%), mucoid dry faeces (12.90%) and depression (36.67%).

Vaja et al. (2016) studied a case of a seven-year-old Gir cattle had a sign of ketosis and found the range of all vital parameters and concluded that the animal had a normal body temperature, normal respiration rate and elevated pulse rate.

Constable et al. (2017) reported that many animals may be in a negative energy balance and ketone in urine, but they were not present clinical symptoms that farmers may recognise.

2.3 Diagnostic studies of ketosis

Schultz & Myers (1959) conducted a study on thirty cattle for the diagnosis of ketosis using milk test at regular interval (four weeks after parturition). Found that fifty percent of animals respond negative to milk test and 40 percent animals respond very low amount of ketone bodies in the milk and rest ten percent of animals showed the highest amount of ketone bodies in milk. He also concluded that as compared to blood ketone bodies, milk ketone was present in little less amount in milk (approximately half of blood ketone bodies).

Kronfeld (1972) concluded that nitroprussde test (rothera's test) detect only aceto-acetate and acetone ketone bodies but did not estimate the concentration of β-hydroxy butyric acid ketone body. He also reported that test was highest sensitivity toward acetone than aceto-acetate ketone body and had no effect on BHBA ketone body.

Brockman (1979) found that insulin concentration was less in early lactation than any other stage of lactation, with highest concentration of blood ketone bodies and low glucose concentration. also, concluded that during clinical ketosis low insulin concentration and high G/I ratio along with the higher blood ketone bodies promotes higher removal of liver fatty acid and promote ketogenesis.

Kalita et al. (1987) conducted a study on the cause and diagnosis of ketosis (acetonemia) after blood examination in high producing jersey cattle, between 2nd and 3rd lactation after parturition and found the decrease in neutrophils count (neutropenia), increase in number of lymphocyte (lymphocytosis) and eosinophilia.

Seker (1989) recorded a study on the ketotic cattle and studied the differentiate leukocyte count in diseased animals. He reported that thirty percent of animals were having eosinophilia, twenty seven percent of animals having lymphocytosis and thirteen percent animals were having lymphopenia.

Nielen et al. (1994) reported a study on 185 dairy cattle for diagnosis of disease using the cow-sides tests of milk and urine. He concluded that the specificity and sensitivity of milk cow-side test was more than the urine tests. Reported the sensitivity and specificity of milk was 90 and 96 percent respectively and of urine was 100 and less than 67 percent respectively while comparing blood BHBA concentration at >1.4mmol/L. also concluded that test for milk was preferred due to easy access and better results than urine samples.

Rajora & Pachauri (1994) conducted a study on haematological parameters in cattle both before and after parturition. They concluded that in haemoglobin concentration and packed cell volume concentration there was no significant difference either in before parturition and after parturition and this concentration was slightly less in animals before parturition.

Akhtar & Anjum (1997) conducted a study on fifteen diseased buffaloes (Nilli Ravi) and examine the blood picture in diseased animals as compared to healthy animals. he reported that there was significant difference in the haemoglobin concentration, packed cell volume concentration and total leukocyte count in diseased animals as compared to healthy buffaloes. Also, in diseased buffaloes there was significant increase in the neutrophil count and significant decrease in lymphocyte count.

Jorritsma et al. (1998) conducted a study of 190 dairy cattle for diagnosis of ketosis using milk test (ketolac) at two different cut point values 100umol/L and 200umol/L. out of which 26 animals were positive for disease. The blood BHBA value used for comparison was 1200umol/L. he concluded that test used at 100umol/L had highest sensitivity and specificity in milk and give more positive result than other. Also, this test be used for routine purpose at dairy farms.

Geishauser et al. (2000) reported that milk sample testing is more sensitive than urine as well as blood for detection of ketone bodies in sub clinical ketosis. Several cow-side tests were used to detect ketone bodies. Out of which ketolac BHB strip Test had more sensitivity (92 percent) and specificity than other used (PINK milk ketone test, keto-check, ketostix) when used at 50umol/L BHBA of milk. Also, this test was more sensitive than nitroprusside test (rothera's test) to determine the concentration of aceto-acetate in milk.

Carrier et al. (2004) used three cow-side tests of urine and milk for the diagnosis of ketosis in diseased cattle. The tests used were ketostix (urine), ketocheck powder (milk) and keto test strip (milk). The first two test detect the concentration of acetoacetate in urine and milk respectively and keto test strip test used to estimate the concentration of BHBA in milk. The sensitivity and specificity of ketocheck powder was 41 percent and 99 percent respectively. Also, the sensitivity and specificity of ketostix was 78 percent and 96 percent respectively and for keto test strip test was 73 percent and 96 percent when used at cut point of 100umol/L and 27 percent and 99 percent respectively when used at cut point of 200umol/L. the author concluded that ketostix and keto test strip test was most useful test for determine the ketone bodies in urine and milk and also a useful method for the detection of sub clinical ketosis in the diseased animals.

Nielsen et al. (2005) estimate the measurement of BHBA in milk and other than this, used biological risk factor for determination of ketosis in dairy animals. He concludes that risk factor like disease recording, fat percentage at calving, milk yield and parturition status also help in diagnose and monitoring of the disease. Although these factor does not reflect the BHBA value in milk.

Oetzel (2007) conducted a study on the diagnosis of different type of ketosis on the basis of different diagnostic tests. Blood, milk and urine test were performed to diagnose. He concluded that most accurate (gold standard) test for diagnosis of disease was estimation or detection of b-hydroxy butyric acid (BHBA) ketone body in blood. The cut point value used foe estimation of blood BHBA concentration was

14.8 mg/dl. Several cow-side tests of milk and urine was also performed but their sensitivity and specificity were less than blood BHBA and several false positive and false negative results after performing cow-side test of milk and urine.

Teli & Ali (2007) conducted study on twenty-four ketotic buffaloes and reported the decreased glucose (37.68 ± 0.84 mg/dl) and insulin (15.82 ± 0.35 µu/ ml) level in ketotic animals and increase in level of both glucose and insulin level after treatment. He also recorded the average decrease in milk production (36.70%) of animals during the disease and increase (25.30%) after treatment.

Upadhyay et al. (2007) conducted a case study on the nervous form of ketosis and reported that animal affected with nervous ketosis shows lower blood glucose (24mg/dl) and positive for urine rothera test. However, vital parameters were normal except pulse rate (92/min) and hypomotile rumen was observed.

Iwersen et al. (2009) reported a study to evaluate the electronic cow-side test for the diagnosis of ketosis and to compare the efficacy of hand-held BHBA estimation with serum BHBA estimation photometrically. The cow-side test used was blood BHBA using the hand-held Precision xtra ketone meter. For these 196 samples were used for detection of blood BHBA. Out of which seventeen samples BHBA was >1200umol/L and ten sample BHBA concentration was >1400umol/L. He found the positive correlation between these two-detection method for estimation of BHBA concentration. the specificity and sensitivity of precision xtra was 100 percent at BHBA concentration >1400umol/L and concluded that precision xtra was used as estimation of blood BHBA for the detection of subclinical ketosis in animals.

Elitok et al. (2010) conducted a study on 24 ketotic cattle with primary ketosis and ten healthy cattle to compare the haematological and biochemical parameters between diseased and healthy cattle. He concluded that there was no significant difference of haematological parameter in diseased cattle as compared to healthy cattle. Also, he concluded that there as significant difference in the AST and GGT concentration in diseased group as compared to control animals.

Sahinduran et al. (2010) conducted a study on the 21 ketotic cattle to examine the change in blood picture of diseased animals before and after treatment. He reported that there was no significant difference was observed in the erythrocyte and leukocyte count in diseased animals when compared to healthy animals and also, no significance difference was observed in animals before treatment and after treatment

Ospina et al. (2013) used the non-esterified fatty acid (NEFA) pre partum or postpartum and blood BHBA for the diagnosis of ketosis in animals. The cut point value of NEFA used for prepartum was 0.3 mEq/L and post-partum was 0.7 mEq/L or higher and for blood BHBA cut point used was 1mmol/L or higher. The sensitivity and specificity of blood BHBA when used at cut point of 1.2mmol/L was 88 percent and 96 percent. Also, the sensitivity and specificity were 96 percent and 97 percent when used at 1.4 mmol/L.

Suthar et al. (2013) conducted a study on HF cattle for the prevalence of subclinical ketosis with first two months after parturition. He found high prevalence of sub clinical ketosis within two to fifteen days after parturition diagnosed via estimation of blood BHBA. The authors concluded that during this period there was increase chance of presence of other illness like lameness, clinical mastitis, metritis, abomasal displacement and clinical ketosis.

White (2015) used cow-side tests of milk, urine and blood for the diagnosis of ketosis in animals. he found that blood cow-side test had highest sensitivity and sensitivity. Tests used: ketostix (urine), ketocheck powder (milk), ketotest (milk) and precision xtra (blood). The sensitivity of these test was 78 percent, 41 percent, 27-59 percent and 91 percent respectively and specificity was 96percent, 99 percent, 76-99 percent and 94 percent respectively.

Chuang et al. (2016) conducted a study on 84 cattle to evaluate the different biochemical parameters in type 1 and type 2 ketosis. The authors found that the concentration of blood glucose, blood ketone body (b-hydroxy butyric acid) and serum NEFA was increased in type 2 ketosis as compared to type 1 ketosis. He concluded that type 2 ketosis was mostly occurred at 1-2 weeks postpartum and type 1 mostly at 3-6 weeks after parturition and also concluded that type 2 ketosis was occurred not because of insulin resistance but also due to some oxidative stress and metabolism of energy.

Safdar (2015) studied on the estimation of ketone bodies (acetone and aceto-acetate) in milk using the nitroprusside test (rothera's test) which give purple colour after reacting with the sample. Although it was simple test but was poorly sensitive (<40 percent) in milk but highly specific (90 perent).

Bali et al. (2016) reported a study on 18 ketotic buffaloes and observed the changes in biochemical profile in ketotic animals as compared to healthy animals. He further concluded that ketotic animals exhibit clinical sign like drop in milk yield, fruity odour, selective feeding and significant decrease in the blood glucose level, total protein, calcium and magnesium level ($p<0.05$). Also, significant increase in the blood BHBA level in affected animals as compared to healthy ones.

Jezek et al. (2017) conducted a study on ninety-four cattle for the diagnosis of ketosis using milk BHBA estimation and to check the correlation between blood and milk BHBA. The author found that BHBA in blood was about 1.14mmol/L while in the milk the concentration of BHBA was tenfold lower (0.117mmol/L) than the blood BHBA. He concluded there was positive correlation between the milk BHBA and blood BHBA ($p<0.05$). The cut point value used for estimation of ketone bodies in milk was 0.08mmol/L and sensitivity and specificity of milk BHBA test was 94 percent and 76 percent respectively. He also concluded that dipstick test was useful method for the diagnosis of ketosis (either clinical or sub clinical) in high yielding cattle.

Macmillan et al. (2017) conducted a study on 11 cattle farm and blood sample was collected to evaluate the efficacy of cow-side test (free style precision neo ketone and glucose meter) for detection of BHBA and glucose using device and compare with the laboratory findings of same parameter. The authors concluded that device was accurate for estimation of beta-hydroxy butyrate but was not accurate for estimation for blood glucose concentration.

Marczuk et al. (2018) conducted a study to rule out the role of amino acid in the diagnosis of ketosis in cattle. For this he used fifteen diseased cattle and fifteen healthy cattle. The authors found the significant increase in the ketogenic as well as glucogenic amino acid in the ketotic animals than healthy animals. There was significantly increase in the glutamine, tyrosine, glutamic acid and isoleucine in ketotic cattle. Also, significant decrease in the asparagine, histidine, leucine, alanine, proline and serine in ketotic animals as compared to healthy animals.

Cuiyu et al. (2019) reported a study to evaluate the relation between insulin resistance and ketosis type 2 in sixteen cattle (eight cattle with disease and eight healthy cattle). For this a glucose tolerance test was performed and animals were divided into three different groups (abnormal glucose tolerance, normal glucose tolerance and healthy group) and check for BHB concentration, insulin resistance index, blood glucose and liver function test (AST). The values of insulin resistance index, AST, blood glucose and BHBA was higher in abnormal glucose tolerance group. Thus, he concluded that due to insulin resistance there is higher chances of occurrence of type 2 ketosis.

Savita et al. (2020) reported on the diagnosis of ketosis in buffaloes using the blood and urine examination. The blood sample was assessed for the estimation of blood glucose concentration and urine sample was assessed for detection of ketone bodies using the rothera’s test. He found that blood glucose was less than normal range and animals was highly positive (+++) for rothera’s test in urine.

Giannuzzi et al. (2021) conducted a study to check the efficacy of ultrasonography of liver and comparison this with hematochemical parameters for the diagnosis of hepatic lipidosis and ketosis in dairy cattle. The authors reported that USG is potential tool for the diagnosis because of lack of liver biopsy method. They recorded a negative correlation of ultrasonography measurement of triacylglycerol and hematochemical parameters except than globulin concentration. Also, negative correlation of measurement of portal vein area and hematobiochemical parametrs.

2.4 Treatment studies on bovine ketosis

Dufva et al. (1983) conducted a study on 20 dairy cattle to estimate the effect of niacin supplement to the milk yield of animals and in disease like ketosis. The animals were divided into two groups with ten animals each. In group 1 animals receive 12gm of niacin per day two weeks before parturition and four weeks postpartum and other group animals taken as control. He concluded, animals supplemented with niacin had low BHBA and low NEFA concentration and increase in milk yield than control animals. He also conducted another experiment in which fourty animals were selected and divided into four groups with ten animals each. One group is taken as control and other group receive three, six and 12gm of niacin per day for ten weeks. He found that in group four animals those supplemented with 12gm of niacin per day had more milk yield and milk fat than other groups.

Herdt & Emery (1992) conducted a study on the effect on niacin supplement in ketotic animals. He concluded that daily supplementation of 12gm niacin per day to animals help in improving the condition of animals and health of animals improves within seven days and also helps in reducing the ketone level in the blood. He also reported that supplementation of niacin in large doses does not decrease the level of ketone bodies and also not used in treatment of fatty liver conditions as they will hinder the liver functions and reduce the secretion of lipoproteins in fatty liver cases.

Foster (1988) reported a study on niacin supplementation to ketotic animals and concluded that daily supplementation of niacin helps in improving the condition of animals. also, reported that after first dose decrease in the ketone bodies and improvement in the feed intake of an animals. He also found that on the second and third day, relapse of disease occurred, and blood ketone bodies increase, and blood glucose also increase but regular supplementation of niacin improvement of condition and decrease in the ketone bodies in blood. The recommended dose given to diseased animals was 12gm per day for better response.

Drackley et al. (2001) reported a study on dairy cattle supplemented with niacin with daily dosage of 12 gm and fat. The authors concluded that daily feeding of 12 gm of niacin (nicotinic acid) increases the milk production, milk fat, lactose and decrease in concentration of non-esterified fatty acid and BHBA. Also, increase the blood glucose concentration and decrease the fat mobilisation.

Zialitis et al. (2007) reported a study on 70 animals selected form five different herds and out of which twenty animals were positive for ketosis. the animals were divided into two different groups ten animals each. Ten animals were treated with oral supplementation of propylene glycol (250 ml) for ten days and other ten animals were provided oral supplement of niacin (12 gm) daily for seven days. The blood glucose, BHBA concentration, calcium, lactose level and milk fat percentage were regularly checked. The study concluded that animals treared with this supplement show increase milk production, reduction in ketone bodies (b-hydroxy butyric acid), decreased milk fat and decrease in glucose level.

McArt et al. (2011) conducted a study on 741 cattle (372 treated and 369 control) to check the efficacy of propylene glycol for treatment of sub clinical ketosis. The cows were diagnosed using the blood BHBA concentration. The sub clinical ketosis was having BHBA concentration between 1.2-2.9 mmol/l. the drug was administered 300 ml per orally for three days. After three days blood BHBA concentration was assessed and found that animals treated with propylene glycol having the negative ketone bodies in blood or having BHBA value less than 1.2mmol/L and also there was increase in the milk yield of animals those treated with propylene glycol.

Tufani et al. (2011) conducted a study on 40 animals of different age groups for the treatment of ketosis and divided into group (n=4). The animals of all the group received 25% dextrose (one litre) once in day on first day followed by twice a day on 2^{nd} and 3^{rd} day. The animals of group 2, 3 and 4 were given vitamin B supplement (10ml). In addition to this, animals were in group 3 was given nandrolone (50mg) and dexamethasone to group 4 animals. reported the highest recovery in group 4 animals followed by third, second and first group of animals.

Wittek et al. (2019) conducted a study on 30 dairy cattle using three different potassium preparation for the treatment of hypokalemia. One group receive potassium bolus, other group receive potassium propionate gel and last group received potassium chloride solution. The authors concluded that all preparation were equally effective for the treatment and increase the level of potassium with twelve hour of administration.

Kushwah et al. (2020) conducted a study on the treatment of type II ketosis in dairy animals and reported that infusion of long-acting insulin @200-300 IU subcutaneous per day along with glucose precursor had better effect on animals as insulin inhibits fat mobilization from fatty tissue as well as ketogenesis, while promoting intracellular glucose absorption and liver gluconeogenesis. He reported other treatment like choline, niacin and steroids (Trenbolone acetate) also beneficial in the treatment of type II ketosis. Niacin inhibits lipolysis by reducing HSL phosphorylation.

Elmeligy et al. (2021) conducted a study on twenty dairy cattle to evaluate the effect of treatment and role of insulin and abomasal function after treatment.

They concluded the significant increase in the milk yield of animal, income of farmer and benefit analysis after treatment. also, significant increase in the insulin level, insulin sensitivity index and improvement in function of abomasal after treatment.

Mammi et al. (2021) conducted a study to check the efficacy of monensin for the prevention of ketosis during transition period. They concluded that monensin was helpful in the reduction in the concentration of BHBA, increase propionate production, increase liver function and also helps in the prevention of ketosis in cows.

CHAPTER III

MATERIALS AND METHODS

The study was undertaken to report common metabolic disease (ketosis) in cattle and buffaloes that were presented at large animal clinics. This objective of the study was to differentiate the various forms of ketosis on the basis of clinical signs, laboratory findings and detection of ketones employing different cow-side tests and their line of treatment in different type of ketosis.

Efforts were made to establish the diagnosis of various forms of ketosis in clinical cases using complete history and physical examination along with various diagnostic tests viz., complete blood count, serum biochemistry {potassium and glucose, AST and GGT (as and when required)} urine examination (physical examination and Rothera's test), milk examination (milk BHBA, Rothera test), blood BHBA examination and ultrasonography of liver (as and when required) and evaluate the line of treatment for various form of ketosis (mainly type II ketosis).

3.1. Location and place of work

The study was performed on 36 animals presented at referral animal hospital, (Medicine unit), College of Veterinary Science, Guru Angad Dev Veterinary and Animal Science University, Ludhiana Punjab during the period of past one year from August 2020 to July 2021. Twenty healthy animals (cattle and buffaloes) were taken as control from an organised dairy farm.

3.2. To evaluate the various parameters for diagnosis of different types of ketosis in dairy animals

The study was conducted on animals (preferably from 1^{st} week to 8^{th} week after calving and of different parity and of different age) with the symptoms of disease presented in University Referral Large Animal hospital, Ludhiana, for the treatment. Complete physical examination and different diagnostic tests were done in dairy animals for diagnosis of the disease

3.2.1. Signalment and history

Complete history taking was performed to rule out other diseases in animals presented to clinics. The data like case number, name and address of the owners and date and time of case presentation were recorded. Other details such as breed and age were recorded. A complete history regarding the present and past history about the other illness or previous episode of same or different disease, drop in milk yield, defecation, parturition status, any history of weight loss and any nervous disorder like change in mentation, and other signs observed by owner, any treatment if given and its response, vaccination and deworming history, feed and water intake along with the feeding history and lactation details and any other abnormalities observed by the owner were also recorded.

3.2.2. Physical and clinical examination

Vital/general parameters like rectal temperature, heart rate, respiratory rate, rumen motility and the color of the conjunctiva were recorded as follow:

3.2.2.1. Rectal temperature

The rectal temperature was recorded using the clinical thermometer (^{0}F). The thermometer was placed into rectum and ensured that it remained in touch with rectal mucosa for at least 1 minutes, after reducing the mercury up to the neck level.

3.2.2.2. Respiration rate

The rate was recorded by observing the movement of rib and sternum or using palm of hand paced near the nostrils for minimum 30 seconds and then converted into minute by multiply it by two. The respiration rate was recorded in breath/min**.**

3.2.2.3. Heart rate

The heart rate was recorded from the 3rd to 5th intercostal space on left side of the animal by stethoscope. It was also recorded for minimum 30 seconds and then converted into minute by multiplying with two. The heart rate was recorded in beat/min.

3.2.2.4. Rumen motility

The motility was recorded from the left paralumbar fossa by gentle palpation of hand over it. It was observed by the pushing of hand away from the body of animal and recorded for minimum 3 minutes.

3.2.3. Collection of samples

For the diagnostic and therapeutic study blood, urine and milk samples were collected from the diseased animals on the day of presentation.

3.2.3.1 Blood collection

For collection of blood sample, firstly the animals were restrained properly. Approximately five ml of blood was collected aseptically from the either jugular vein or tail vein, using a dry sterile syringe and was immediately transferred to both vacutainers, one containing Ethylene-Diamine Tetra-Acetic acid (EDTA) as an anticoagulant and other vial containing no anticoagulant.

For estimation of hematological parameters like Hb, TLC and DLC, blood was collected in vial containing EDTA as an anticoagulant and for estimation of biochemical parameters, blood was collected without anticoagulant in a clot activating vial and kept in a slanting position for some time to facilitate the separation of the serum and then centrifuged (2500 rpm for three minutes). The serum samples collected were labeled and preserved at -4°C (if necessary) for further estimation of biochemical parameters.

3.2.3.1.1. Hematology:

The parameters (Hb, PCV, TLC) were determined using MYTHIC 18 Hematology system (Siemens Healthcare diagnostics Inc. Deerfield, IL, USA). The differential leucocyte count (DLC) was assessed under microscope using blood smear stained by Giemsa/Leishman stain. The blood smear was examined to find out any abnormalities in the various blood cells.

3.2.3.1.2. Biochemical analysis:

Serum concentration of potassium and glucose, AST and GGT were determined (as and when required) using the commercial kits available.

3.2.3.1.3. Quantitative estimation of blood glucose

A glucometer (free style optimum neo-H, Abbott) was used to determine the concentration of blood glucose (mg/dl). A drop of blood was inserted in test strip and recorded after 5 seconds. Other method used to determine the concentration of blood glucose was with an automated clinical bio-chemistry analyzer by using standard kits (Virto's-Ortho-Clinical Diagnostics, Mumbai).

3.2.3.1.4. Quantitative estimation of blood potassium

To determine the concentration of blood potassium, an automated clinical bio-chemistry analyzer with the help of standard kits available (Virto's-Ortho-Clinical Diagnostics, Mumbai) was used.

3.2.3.1.5. Quantitative estimation of blood BHBA

To determine the blood BHBA (mmol/l) ketone bodies, Free Style optimum H glucose ketone meter (Abbott, U.K.) was used. For this a small drop of blood was inserted in ketone test strip in the meter that kept on recorder for 10 seconds, after which the value was recorded.

Fig. 1: Ketone meter along with strip used for estimation of blood BHBA

3.2.3.2. Milk collection

For collection of milk sample, first udder was cleaned and then wiped with clean cloth or cotton wetted with 70 percent alcohol. First few drops of milk were discarded for the sample collection and then approximately five to ten ml of milk was collected in sterile container.

3.2.3.2.1. Ketone bodies

For detection of ketone bodies in the milk, Rothera's test was performed using Rothera's reagent**.** The principle of Rothera's test was to determine the concentration of acetone and aceto-acetate ketone bodies. The formation of ring at the junction indicated the severity of disease.

It was done by adding the Rothera's reagent (sodium nitroprusside plus ammonium sulphate) in 5 ml of milk and then gently mixing the sample. After mixing, ammonium hydroxide was added drop wise through the side wall to form a ring at junction. The change in colour at junction determined the concentration of ketone bodies in milk.

Rothera's reaction	**Result**
No change in the colour	-
Slight Levander	+
Deep Levander	++
Beet red or purple	+++
Deep purple	++++

3.2.3.2.2. Detection of milk BHBA

BHB test strip is dipstick test for detection the concentration of beta hydroxy butyrate in milk. Beta hydroxy butyrate in milk pass through reagent of test strip and converted to acetoacetate with the help of enzyme beta hydroxy butyrate dehydrogenase. In this process NADH is produced from NAD and reduced nitro tetrazolium blue (NTB) to formazan (purple colour).

Procedure:

- ✓ Remove dipstick from the light sealed container, then immersed in the milk sample
- ✓ After 2 minutes read the pad and compare with the colour chart provided.

(a) Dipstick used for estimation **(b) Colour chart for the comparison of pad**

Fig. 2: Dipstick used for the estimation of milk BHBA

Interpretation:

BHBA concentration (μmol/L)	**Interpretation**
0 (0mg/dl)	negative/normal
100 (1mg/dl)	doubtful
200 (2mg/dl)	positive (+)

500 (5mg/dl)	high positive (++)
1000 (10mg/dl)	highly positive (+++)

3.2.3.3. Urine sample

Approximately ten ml of free voided urine was collected in a sterile container for the detection of ketone bodies via Rothera's test and urine biochemical parameters.

3.2.3.3.1. Urine-analysis parameters

For the detection of biochemical parameter of urine, multi-diagnostic strips/ dipsticks were used. The following parameters are recorded via dipsticks,

- Ketones
- Glucose
- Specific gravity
- Protein
- Bilirubin
- pH
- Urobilinogen

A urine strip could have 10 different chemical pads or electrodes, which react with sample when it is immersed in the urine sample. After dipping the test strip was removed from the sample and read after 1-2 minute of dipping. The change in the color reflected the concentration of different parameters. The colour change was detected with the help of meter provided along with the strips.

3.2.3.3.2. Rothera's test

Rothera's test was performed in urine to detect the ketone bodies in the urine sample. This test detects the acetone and aceto-acetate ketone bodies. About 5 ml of urine sample is required for the test.

Urine sample + Rothera's reagent ⟶ gently mix the sample

(Na nitroprusside + ammonium sulphate)

After mixing add, drop wise ammonium hydroxide through side walls, then observe the colour change at junction.

Rothera's reaction	**Result**
No change in the colour	-
Slight Lavender	+

Deep Lavender	++
Beet red or purple	+++
Deep purple	++++

3.2.4. Ultrasonography of liver

The ultrasonography was done with a portable ultrasound scanning machine (Sonosite M-Turbo) with a 10-5 MHz curvilinear transducer (L38, Sonosite, serial number: 03RQ5K). The changes in liver like fat deposition, liver congestion and the change in size and shape of caudal vena cava were recorded to access any liver abnormality and any change in liver morphology due to disease.

3.3. Therapeutic management

After making a diagnosis, the disease was differentiated into different type (either type I or type II). Thus, the animal suffering from ketosis were treated as per schedule described below (Table 1).

On the basis of history, clinical symptoms, presence of ketone bodies in blood, urine and milk sample and basis of concentration of blood glucose and potassium estimation, 36 animals were positive for the disease, out of which 22 were buffaloes and 14 were cattle. Out of 36 positive cases 4 cases were of type 1 ketosis and rest of cases were of type II ketosis. Twenty animals (10 buffaloes and 10 cattle) at an organised farm were taken as healthy animals for the comparison purpose.

Table 1: Treatment protocol allotted for various types of ketosis

Group	Treatment provided	Dose	Route	Duration
T1	Dextrose (50%) + dexamethasone + DNS (5%)	500ml+ 10 ml+ 3L	IV +IM +IV	3-5 days
T2	Potassium chloride Niacin	16gm/100kg/day 12gm/day	P.O.	3-5 days

3.3.1. Study on treatment efficacy

The efficacy of treatment was recorded using either diagnostic tests or telephonically (diminished clinical signs or improvement in animal health). The efficacy was recorded via detection of ketone bodies using the above-mentioned tests after the treatment protocol.

3.4. Statistical analysis

The data was statistically analysed with the SPSS software version 27 using independent student T test. The analysis was done to know about the efficacy of treatment provided and various level of significance ($p<0.05$, $p<0.01$).

CHAPTER IV

RESULTS AND DISCUSSION

Among all the metabolic or production diseases, ketosis is one of the most common disease. It causes substantial economic loss to the livestock industry because of high morbidity and sharp decline in milk yield. In prospective of economics, it is important to diagnose the disease at an early stage of lactation to prevent the loss and for early treatment. For diagnosis the studies about the disease and its relationship with blood parameters like blood glucose, blood potassium, BHBA, urine and milk examination and clinical signs are major considerations.

The present study was done to diagnose the various types of ketosis using various diagnostic tools and their treatment in dairy animals (cattle and buffaloes).

4.1 To evaluate the various parameters for diagnosis of different types of Ketosis in dairy animals

From epidemiological point of view, the study was performed on animals that were presented at the clinics with the sign of ketosis or animals that were suspected of ketosis. The study was performed on 198 lactating animals (cattle and buffaloes) suspected of ketosis on basis of history and presenting clinical signs. The suspected animals were screened with the various diagnostic tests and using dipstick tests. Thirty-six animals were positive for ketosis and an overall occurrence of ketosis was 18.18 percent (Table 2).

Out of 36 animals, 14 (38.89 percent) cases were of cattle and 22 (61.11 percent) were buffaloes. The results of the study correlate well with the findings of various research workers, Kumar et al. (2015) found approximately similar occurrence (16.5%) of ketosis in lactating buffaloes. Biswal et al. (2016) reported that overall occurrence of ketosis was 36.7% (1014/2760), out of which 27.2 percent cases were of clinical ketosis and 9.5% cases were of subclinical disease. Nazeer et al. (2019) conducted a study on prevalence of ketosis in cattle and reported comparatively less occurrence of disease (11.42%) in and around Bikaner.

Table 2: Overall occurrence/prevalence of ketosis in dairy animals (cattle and buffaloes)

Animals suspected	Confirmed	Prevalence (%)
198	36	18.18

Out of 36 positive cases, majority of cases were of type II ketosis and least number of cases were of type 1 ketosis. The prevalence of type II ketosis in present study was 88.89 percent (32/36 cases) and type 1 ketosis was 11.11 percent (4/36 cases).

The variation in management practices plays an important role in disease diagnosis and disease prevalence. Variations in prevalence might also be explained by differences in study design and method of diagnosis. Various factors like climate change, management practice, calving interval, parturition status and feeding habits can cause a wide variation in the prevalence of disease in different regions.

4.1.1. Parity wise occurrence of ketosis in dairy animals

The Parity wise occurrence of ketosis in dairy animals was observed in different groups (1^{st} to 6^{th}). Among the positive cases in the present study, maximum cases were recorded in 2^{nd} parity (37.5%), followed by animals in 3^{rd} parity (31.25%), and the lowest cases were recorded in 5^{th} and 6^{th} parity (3.12%) animals. The parity wise occurrence of ketosis was presented in Table 3 and Fig. 3.

In cattle, the highest number of cases were recorded in parity 1^{st} to 2^{nd} (58.33%), followed by parity 3^{rd} to 4^{th} (38.33%), and the lowest were recorded in parity 5^{th} to 6^{th} (8.33%) (Table 4).

In case of buffaloes, the highest no. of cases was recorded in parity 1^{st} to 2^{nd} (55%), followed by animals that were in parity 3^{rd} to 4^{th} (40%), and lowest were recorded in parity 5^{th} to 6^{th} (5%) (Table 5).

Table 3: Parity wise occurrence of ketosis in dairy animals

Parity	Animals positive	Percentage (%)
1^{st}	3	9.37
2^{nd}	12	37.5
3^{rd}	10	31.25
4^{th}	5	15.63
5^{th}	1	3.12
6^{th}	1	3.12

Table 4: Parity wise occurrence of ketosis in cattle

Parity	Animals positive	Percentage (%)
1^{st} - 2^{nd}	6	50
3^{rd} - 4^{th}	5	41.67
5^{th} – 6^{th}	1	8.33

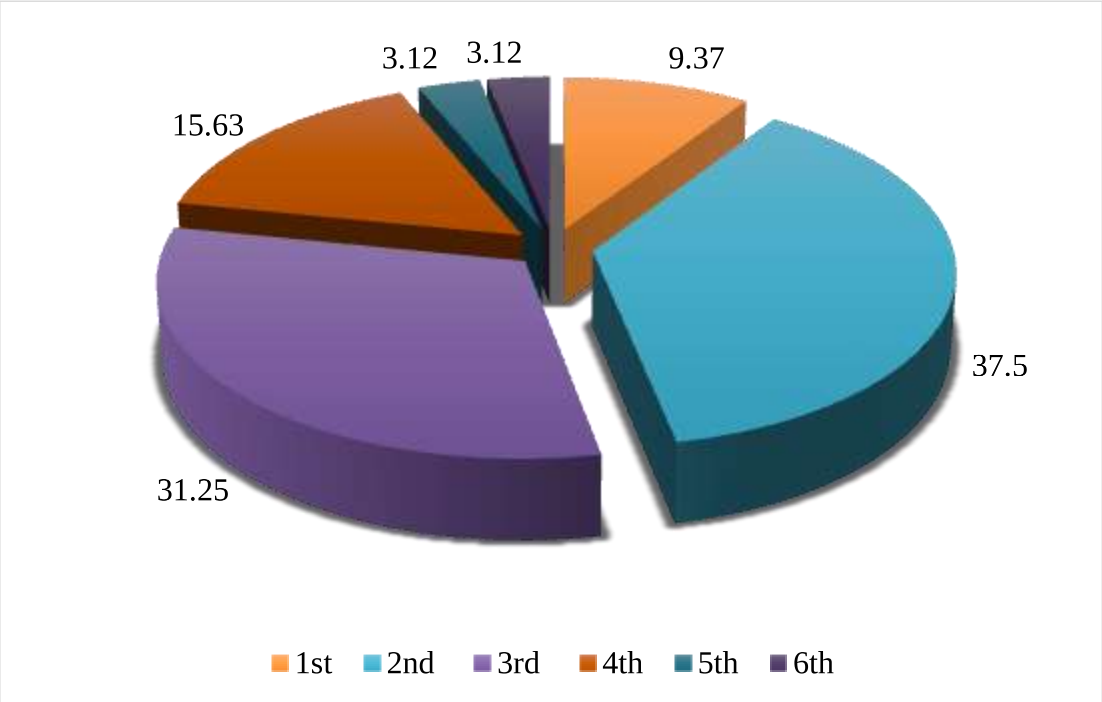


Fig. 3: Parity wise occurrence of ketosis in dairy animals

Table 5: Parity wise occurrence of ketosis in lactating buffaloes

Parity	Animals positive	Percentage (%)
1^{st} - 2^{nd}	9	45
3^{rd} - 4^{th}	10	50
5^{th} – 6^{th}	1	5

The results of this study correlate well with studies done by other research workers (Rautmare & Anantwar, 1993; Biswal et al., 2016). However, Rautmare & Anantwar (1993) reported the study in buffaloes and concluded that highest number of cases were in animals in 8-9 year of age group and 4^{th} to 5^{th} parity. Biswal et al. (2016) found the highest number of cases in buffaloes aged 7 years (4^{th} parity). Nazeer et al. (2019) reported the high occurrence of disease in the age group of 8-9 years and in 3^{rd} to 4^{th} (25%) parity.

Animals achieve their peak lifetime production during 2^{nd} to 3^{rd} parturition and thus high demand of glucose for milk production.

4.1.2. Lactation stage wise occurrence of ketosis in lactating buffaloes

Lactation wise occurrence of ketosis was presented in different groups: first lactation (0-1 month), second (1-2 month), third (2-3 month), and fourth (3-4 month).

The majority of overall cases were reported in first and second lactation followed by third lactation (Table 6).

In cattle, the majority of cases were recorded in first lactation (50%), followed by second lactation (33.33%), and lowest was recorded in third lactation (16.67%) (Table 8).

In case of buffalo, majority of cases were reported in second lactation (50%), followed by first lactation (40%), and least number of cases were reported in third (10%) (Table 7).

It was concluded that majority of cases were recorded in early lactation that is in first and second month of lactation and correlate well with similar studies and their findings. Oetzel (2007) found the prevalence in both type I and type II ketosis and out of which Type II was reported in first lactation and Type I were reported during second and third lactation. Nazifi et al. (2008) reported that majority or maximum number of cases of ketosis were recorded in early lactation (1-2 month after parturition). Nazeer et al. (2019) found that prevalence of ketosis was 11.42%, out of which maximum number of cases were reported at early lactation or at first and second month of lactation.

Table 6: Lactation wise occurrence of ketosis in dairy animals

Lactation	Positive animal	Percentage
First (0-1m)	16	50
Second (1-2m)	12	37.50
Third (2-3m)	4	12.5
Fourth (3-4 m)	0	0

Table 7: Lactation wise occurrence of ketosis in lactating buffaloes

Lactation	Positive animal	Percentage
First (0-1)	10	50
Second (1-2m)	8	40
Third (2-3m)	2	10
Fourth (3-4 m)	0	0

Table 8: Lactation wise occurrence of ketosis in cattle

Lactation	Positive animal	Percentage
First (0-1)	6	50
Second (1-2m)	4	33.33
Third (2-3m)	2	16.67
Fourth (3-4 m)	0	0

4.1.3. Clinical symptoms and parameters

4.1.3.1. Clinical symptoms

In the present study, varying degrees of clinical signs were observed in diseased animals and majority of the signs were similar in all (Table 9 and Fig. 4). The signs observed were anorexia, selective feeding, loss of body weight, fruity odour from nostrils and from urine, drop in milk yield, nervous signs like iron chewing, star gazing posture and depression.

Among all the clinical signs, the drop in milk yield (100%) was the most common clinical sign followed by loss of body weight (62.5%), depression (56.25%), complete anorexia (46.87%), selective feeding (37.5%) and the least common sign observed was fruity odour from nostril (25%) and from urine (9.37%) as well as nervous signs (6.25%). There was difference in clinical signs in both type 1 and type II ketosis.

Table 9: Percentage of clinical signs observed in dairy animals (cattle and buffaloes)

Signs	No. of animals	Percentage
Drop in milk yield	32	100
Complete anorexia	15	46.87
Selective feeding	12	37.5
Depression	18	56.25
Loss of body weight	20	62.5
Nervous involvement	2	6.25
Fruity odour from nostrils	8	25
Fruity odour from urine	3	9.37

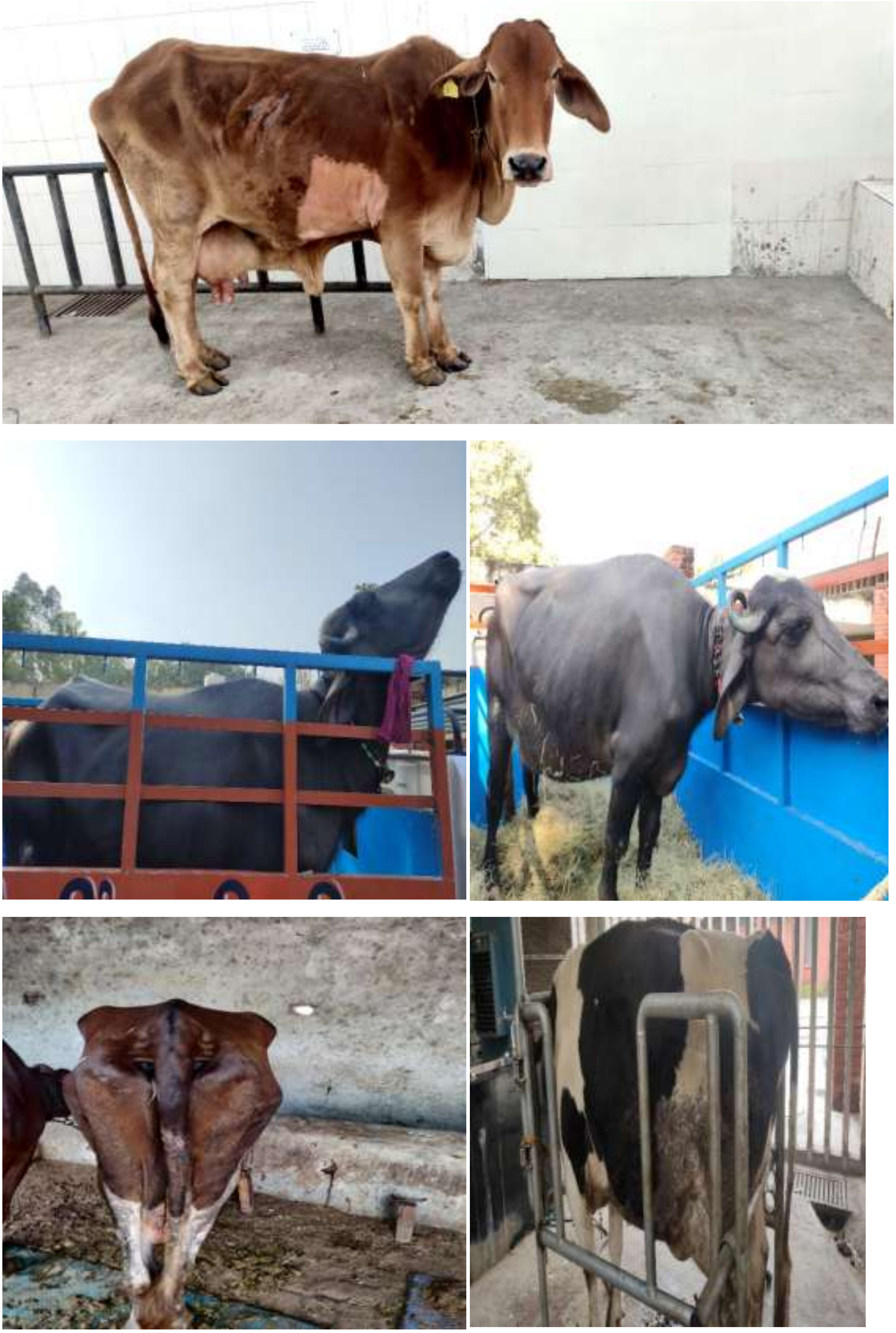


Fig. 5: Clincal representation of animals diagnosed with ketosis

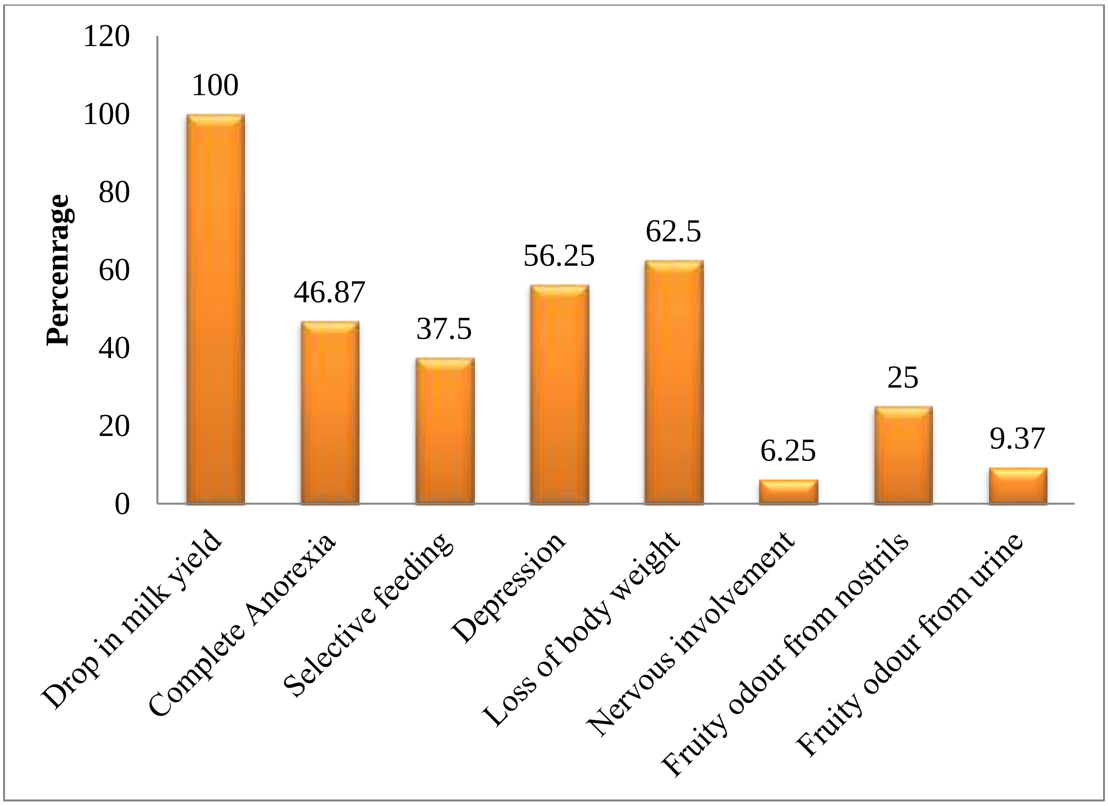


Fig. 4: Various clinical signs of disease in dairy animals

Other research workers have also recorded similar findings. Mir and Malik (2003) found that clinical signs like drop in milk yield, wasting and selective feeding were reported in 50% of animals and it is of diagnostic importance. Bali et al. (2016) reported the clinical signs in 18 lactating buffaloes and found that most common clinical sign was decrease in milk yield (100%), followed by selective feeding (79.14%), wasting (49.91%), and least signs observed was dry faeces (12.90%) and nervous signs (6%). This might be related to a high rate of lactose drainage via milk to satisfy the demands of high lactation, which relies on endogenous nutritional reserves and thus reduces milk production.

4.1.3.2. Clinical/general parameters

4.1.3.2.1. Temperature, Respiration rate and Heart rate

The mean value of temperature in buffaloes and cattle was 100.55±0.39 and 101.14±0.43 ^{o}F, heart rate was 60.33±2.41 & 66.10±3.56 bpm, and respiration rate was 23.67±5.08 & 19.68±2.01 bpm respectively (Table 10 and Table 11).

Thus, there was no significant difference in the temperature and heart rate between diseased and non-diseased animals, similar to the studies reported by other workers. Venkateshwarlu et al. (1993) reported that there was no significant difference in temperature, heart rate and respiration rate in control and diseased animals. Dar et al. (2014) conducted study on lactating buffaloes and found no significant difference in temperature (101-103F^o) however, there was slightly elevated respiration rate (30-40/min) and heart rate (70-80/min).

Kumar et al. (2015) found non-significant change in the temperature, heart rate, rumen motility and pulse rate in ketotic animals. Nazeer et al. (2019) found no significant change in temperature (101-102F^o) and pulse rate (50-60/min) but slight decrease in respiration rate (18-19/min). The variation in respiration rate could be due to climate difference and different management practices in different area.

Table 10: Vital parameters recoded in diseased and healthy buffaloes

Vital	**Diseased**	**Control**
Temperature (^{o}F)	100.55±0.39^{NS}	100.76±.39
Heart rate (bpm)	60.33±2.41^{NS}	59.37±2.26
Respiration rate (bpm)	23.67±5.08*	20.17±2.13

*Significant level at p<0.05, **Significant level at p<0.01, NS: non-significant

Table 11: Vital parameters recoded in diseased and healthy cattle

Vitals	**Diseased**	**Control**
Temperature (^{o}F)	101.14±0.43^{NS}	101.70±0.24
Heart rate (bpm)	66.10±3.56^{NS}	64.50±4.19
Respiration rate (bpm)	19.68±2.01^{NS}	20.16±2.13

*Significant level at p<0.05, **Significant level at p<0.01, NS: non-significant

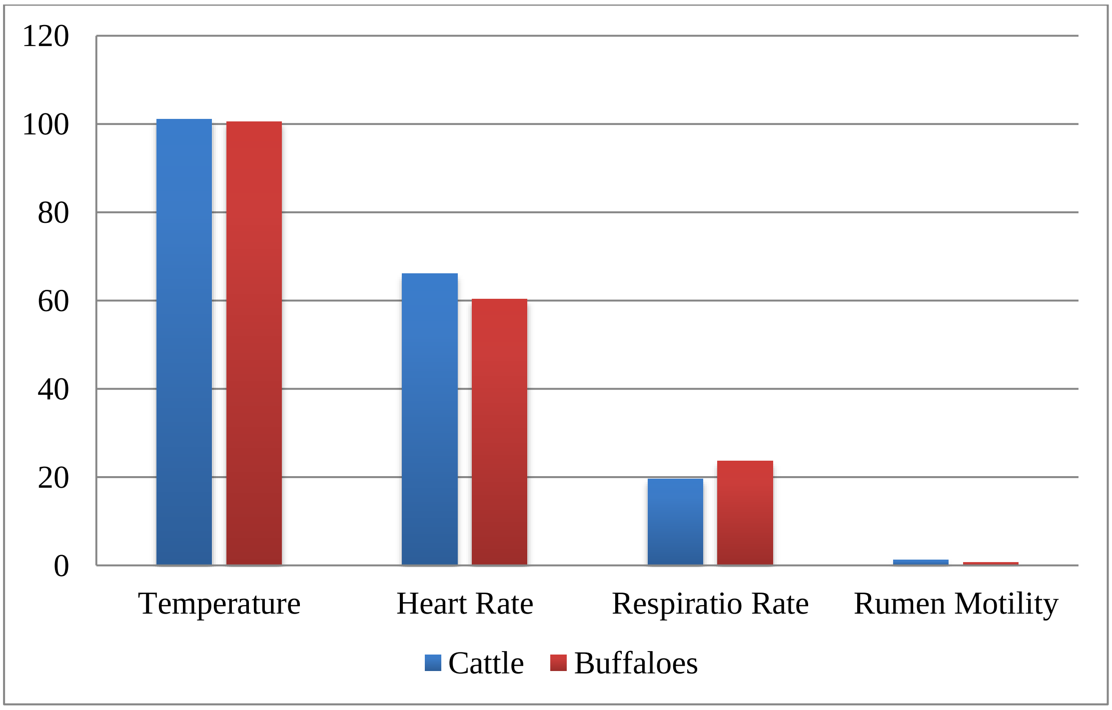


Fig. 6: Vitals in diseased cattle and buffaloes

4.1.3.2.2. Haematology

The haemoglobin was recorded in diseased animals (cattle and buffaloes) on the day of presentation and 5-7 days after treatment. The mean value of Hb in diseased cattle and healthy control was 9.03±0.41 and 9.08±0.29 g/dl respectively and in case of buffaloes the mean values of disease and control was 10.24±0.45 and 10.41±0.27 g/dl respectively (Table 12)

Table 12: Haemoglobin concentration analysis in ketotic animals and healthy

Parameter	Cattle		Buffaloes	
	Diseased	Control	Diseased	Control
Haemoglobin (g/dl)	9.03±0.41^{NS}	9.08±0.29	10.24±0.45^{NS}	10.41±0.27

*Significant level at p<0.05, **Significant level at p<0.01, NS: non-significant

The findings are in agreement with previous studies. Rajora and Pachauri (1994) concluded that there was no significant change in the haemoglobin concentration in pre and post-parturition cattle. Akhtar &Anjum (1997) conducted a study on 15 ketotic buffaloes and recorded the haematological parameters. They concluded that there was no significant change in the blood picture in diseased as compared to healthy buffaloes. Similar findings were recorded by Sahinduran et al.

(2010) and Elitok et al. (2010). A group of authors concluded that there was significant decrease in haemoglobin in diseased animals (Belic et al., 2010) that might be due to some other disease. The possible reason for no change in the Hb content in diseased animals is that ketosis is a metabolic disease, free from any kind of infection.

4.1.3.2.2.1 Total leukocyte count (TLC) and differential leukocyte count (DLC) estimation

The mean value of TLC in diseased cattle and healthy cattle was 8.63±0.52 and 8.59±0.46 × 10^3/µl respectively. In case of buffaloes the mean values of diseased and control was 8.75±0.20 and 8.95±0.52 × 10^3/µl respectively (Table 13) with no significant difference in the diseased group as compared to healthy control.

Table 13: Hematological indices in ketotic animals and healthy animals

Parameters	Cattle		Buffaloes	
	Diseased	Control	Diseased	Control
TLC (×10^3/µl)	8.63±0.52^{NS}	8.59±0.46	8.75±0.20^{NS}	8.95±0.52
Neutrophils (%)	42.20±3.07^{NS}	43.62±1.03	55.00±2.78^{NS}	53.54±2.04
Lymphocyte (%)	57.58±2.56^{NS}	58.12±1.08	44.12±1.89^{NS}	46.02±1.56

*Significant level at p<0.05, **Significant level at p<0.01, NS: non-significant

The results of present study are similar to previous findings. Akhtar & Anjum (1997) concluded that there was no significant change in the Total Leukocyte Count in diseased animals as compared to healthy animals. Sahinduran et al. (2010) reported significant decrease in total leukocyte count (TLC) in ketotic animals as compared to healthy ones.

The mean percentage of neutrophils in diseased and healthy cattle was 42.20±3.07 and 43.62±1.03 percent and lymphocyte were 57.58±2.56 and 58.12±1.08 respectively. In case of buffaloes, the mean percentage of neutrophils in diseased and healthy was 55.00±2.78 and 53.54±2.04 and lymphocyte was 44.12±1.89 and 46.02±1.56 respectively (Table 13).

The findings of present study are in agreement with previous studies in which there was no significant difference in the DLC count in diseased animals as compared to healthy animals (Elitok et al., (2010). No previous published report have indicated any correlation of DLC with ketosis in cattle and buffaloes. One study concluded that there was relative neutrophilia in diseased buffaloes and was significantly higher than normal animals (Akhtar &Anjum, 1997). Another study conducted by Seker (1989) reported the lymphocytosis in twenty seven percent of ketotic cattle. The results signify those chances of infection in the ketosis was not there and animals were free from infection.

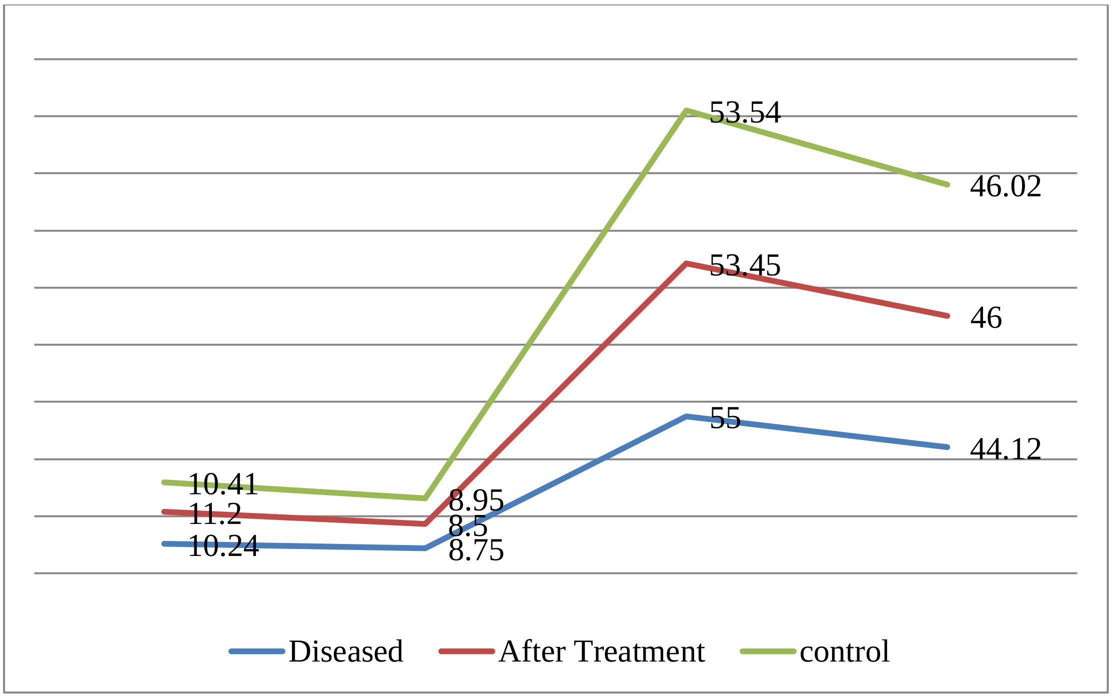


Fig. 7: Haematological parameter in diseased animals

4.1.3.2.3. Blood glucose

In the present study, a majority of cases were of type II ketosis in which the blood glucose was high.

In case of buffaloes, the blood glucose was slightly higher than that of cattle. The mean value of blood glucose in diseased buffaloes and diseased cattle was 163.33±13.60 and 128.6±17.7 mg/dl respectively (Table 14 and 15).

The findings are similar to previous studies reported by other authors (Holtieus & Holteius 1996; Oetzel 2004) who reported a high blood glucose in the diseased animals in type II ketosis. The high blood glucose was due to overfeeding in the dry period and also seen in the obese animals (Oetzel, 2007). The high level of blood glucose in diseased animals may be due to inadequate gluconeogenesis and ketogenesis due to inadequate mobilization of adipose tissue/fat in hepatic injuries.

Table 14: Mean value of blood glucose and potassium in ketotic buffaloes

Parameter	Diseased	Control
Blood glucose (mg/dl)	163.33±13.60**	86.5±7.81
Blood potassium (meq/L)	3.15±0.41**	4.22±0.13

*Significant level at p<0.05, **Significant level at p<0.01

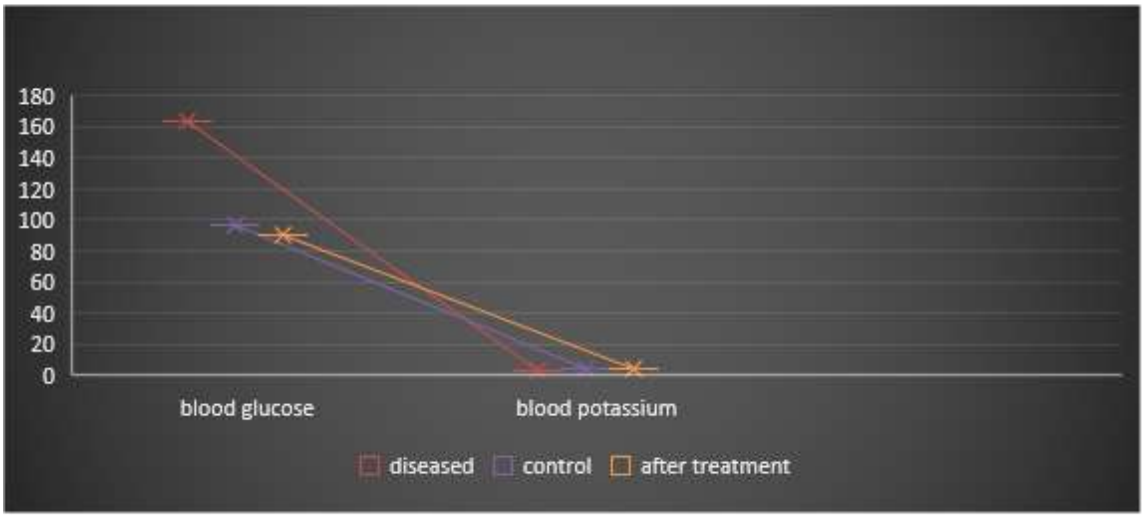


Fig. 8: Concentration of glucose and Potassium in diseased buffaloes

4.1.3.2.4. Blood potassium

The mean value of potassium was significantly decreased in diseased animals as compared to healthy animals (fig 6 and fig 7). In case of buffaloes, the mean value of blood potassium of diseased animals and control animals was 3.15±0.41 and 4.22±0.13 mEq/L respectively (Table 14). However, in case of cattle, the mean value of potassium in diseased animals and control was 3.01±0.19 and 4.16±0.11 mEq/L respectively (Table 15). There was more decline in the concentration of potassium in the buffaloes than cattle. However, no previous work has demonstrated the exact correlation between the hypokalaemia and ketosis.

Table 15: Mean value of blood glucose and potassium in diseased cattle

Parameter	Diseased	Control
Blood glucose(mg/dl)	128.6±17.7**	63.13±2.71
Blood potassium(mEq/L)	3.01±0.19**	4.16±0.11

*Significant level at p<0.05, **Significant level at p<0.01

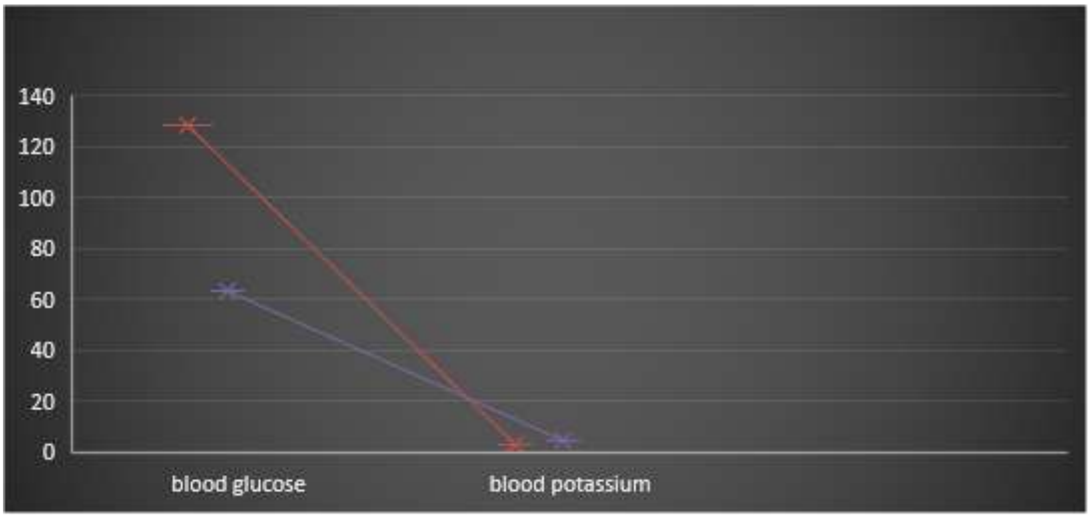


Fig. 9: Concentration of Glucose and Potassium in diseased cattle

Observed hypokalaemia in ketotic animals in this study could be due to greater mobilisation of adipose tissue or fat due to hepatic injuries (Divers & Peek, 2008) and increased cation transfer into milk (Jacob et al., 2011). In most of the dairy animals some degree of hypokalaemia occurs because they experience a decrease in feed intake during stage of early lactation and produce potassium-rich milk. In case of ketosis, the resulting decreased feed intake is rather more severe.

4.1.3.2.5. Estimation of blood b-hydroxy butyric acid (BHBA)

The mean value of blood BHBA in diseased animals was significantly higher than the control animals (fig 10).

The mean value of BHBA in diseased buffaloes and cattle was 1.77±0.09 and 1.60±0.15 mmol/L respectively and in case of healthy group the mean value of BHBA in buffaloes and cattle was 0.31±0.04 and 0.34±0.04 mmol/L respectively (Table 16).

Table 16: Mean value of blood BHBA in diseased buffaloes and cattle before treatment

Parameter	Cattle		Buffalo	
	Diseased	Control	Diseased	Control
Blood BHBA (mmol/L)	1.60±0.15**	0.34±0.04	1.77±0.09**	0.31±0.04

*Significant level at $p<0.05$, **Significant level at $p<0.01$

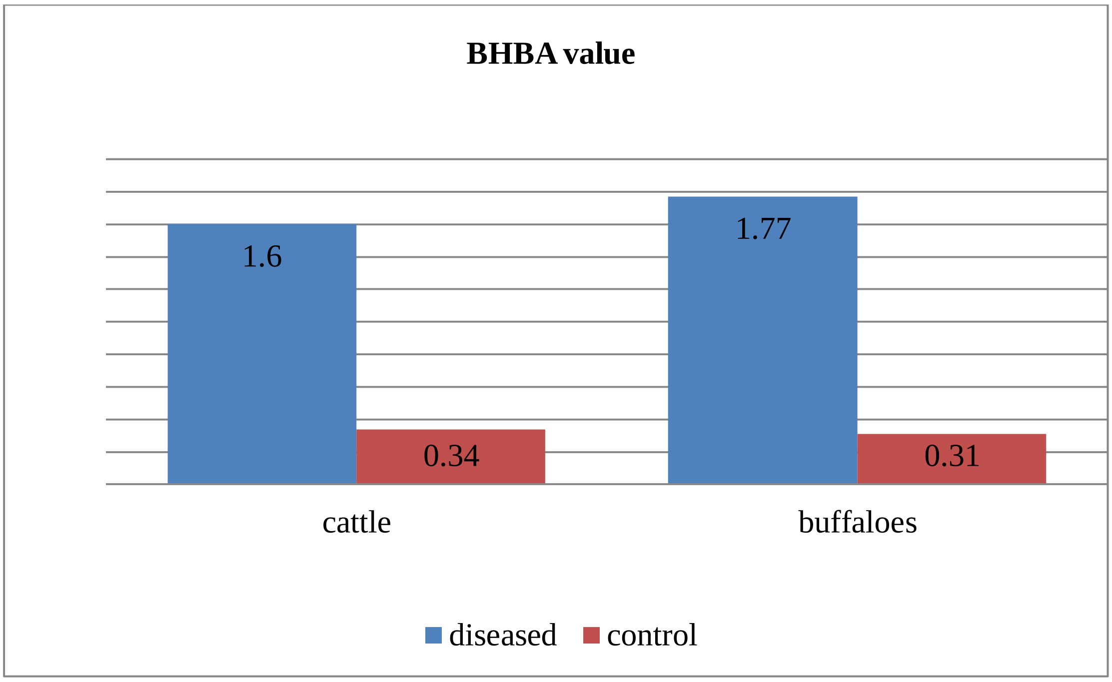


Fig. 10: Mean value of blood BHBA in diseased cattle and buffaloes

The findings of present study were similar to previous studies (Singh & Kasaralikar, 1988; Oetzel, 2007). Some authors classified animal affected with ketosis based on BHBA level of more than 1.2 mmol/L (Oetzel, 2004; McArt et al., 2012b). Oetzel (2007) used the cut value of 14.8 mg/dl for the estimation of blood BHBA concentration.

One of the most significant metabolic products of fatty acid metabolism is ketone bodies. One study concluded that value of BHBA in blood is eight time higher than the milk concentration (Elizabeth, 2006). When ketone bodies are found in excess of peripheral tissue's ability to utilise them, these accumulate in the blood circulation, causing ketone bodies to appear in the blood, milk and urine resulting in ketosis.

4.1.3.2.6 Urine specific gravity

The urine specific gravity was statistically analysed. The mean value of specific gravity in diseased buffaloes and healthy buffaloes was 1.015±0.01 and 1.020±0.02 respectively (Table 17). The mean value of specific gravity in diseased cattle and control was 1.018±0.02 and 1.021±0.01 respectively. However, the specific gravity was higher in diseased cattle than buffaloes.

Sharma et al. (2013) recorded specific gravity in ketotic cattle and the mean value was 1.015±0.0104.

Table 17: Mean value of urine specific gravity in diseased buffaloes and cattle

Group	**Cattle**	**Buffaloes**
Diseased	1.018±0.02^{NS}	1.015±0.01^{NS}
Control	1.021±0.01	1.020±0.02

*Significant level at p<0.05, **Significant level at p<0.01

4.1.3.2.6 Urine ketone bodies

The data presented in Table 18 show the value of urine ketone bodies (acetoacetate and acetone). The value was measured as one positive, two positive, three positive and so on.

Out of 32 positive cases, eight animals were in group of three positive, seven animals were in group of one and four positive each. The least number of animals were in group zero (negative for urine ketone bodies in dipstick test).

In case of buffaloes and cattle, maximum number of animals were in group of three positive and the least number of animals were in group of zero positive (Table 19 and Table 20).

Table 18: Concentration of urine ketone in diseased animals via dipstick test

Urine ketone bodies	**Animals positive**	**Percentage (%)**
0	2	6.25
+	7	21.8
++	4	12.5
+++	8	25
++++	7	21.8
+++++	4	12.5

Table 19: Concentration of urine Ketones in diseased buffaloes

Urine ketone bodies	Positive Animals	Percentage (%)
0	1	5
+	3	15
++	4	20
+++	6	30
++++	4	20
+++++	2	10

The findings of present study were similar to previous studies. Carrier et al. (2004) reported that dipstick test provides an acceptable result for the detection of ketosis in individual cow. Sharma et al. (2013) used dipstick test for the diagnosis of ketosis in animals. Based on ketone concentration in strip, the values were converted into mg/dl like negative (<5mg/dl), trace, mild, moderate, large and largest was 5, 15, 40, 80 and 160 mg/dl respectively.

Table 20: Concentration of urine Ketones in diseased cattle

Urine ketone bodies	Animals positive	Percentage
0	1	8.33
+	4	33.3
++	0	0
+++	2	16.6
++++	3	25
+++++	2	16.6

4.1.3.2.7 Urine Rothera's test

Out of 32 positive ketotic animals, the Rothera's test was positive in all the diseased animals. Among healthy controls, all animals were negative for Rothera's test in urine.

The findings were similar to previous reports (Venkateshwarlu et al. 1993; Oetzel 2004). When there was excretion of ketones in urine there was possibility of positive results. The primary goal of estimating ketones in diseased animals was to

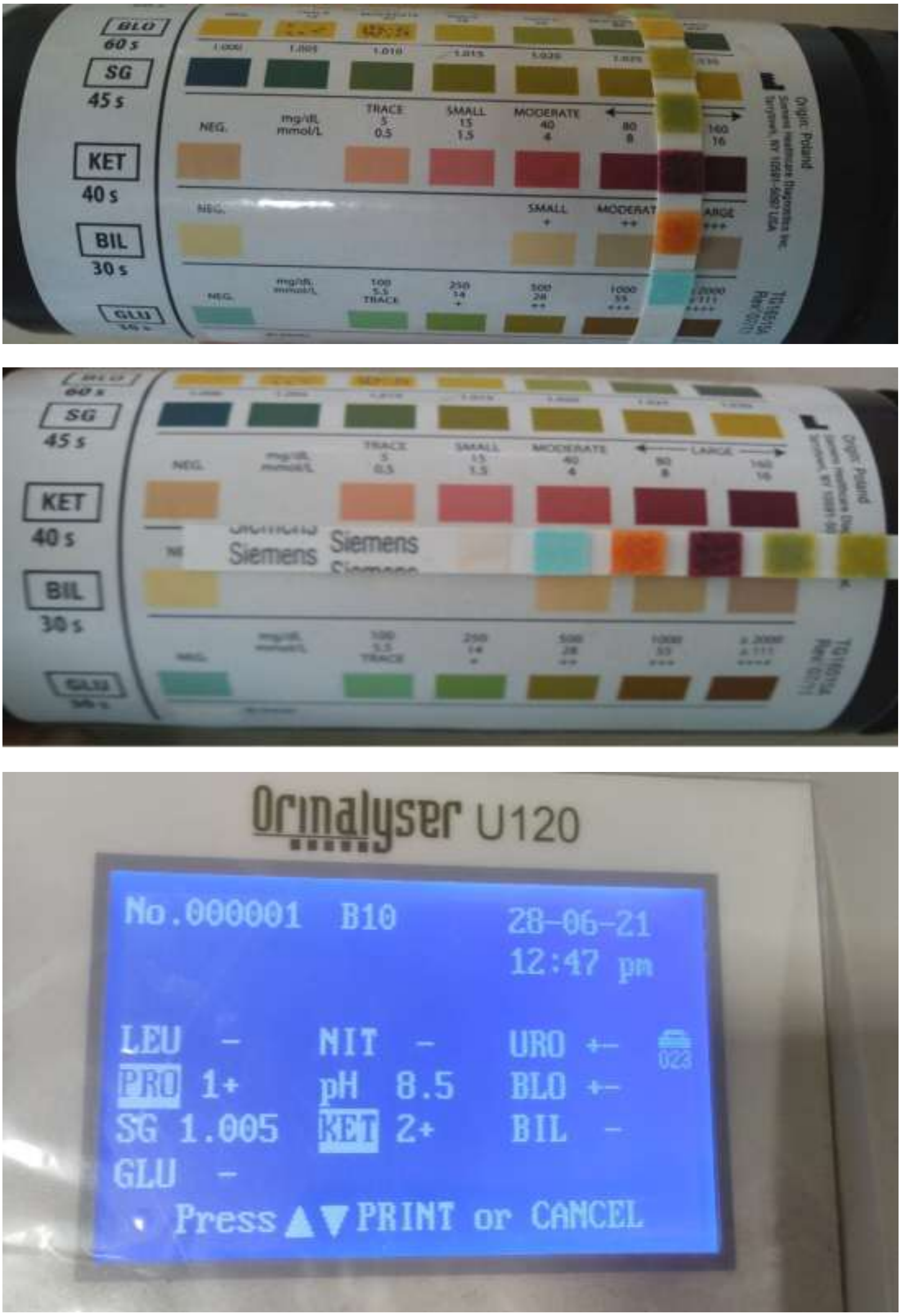
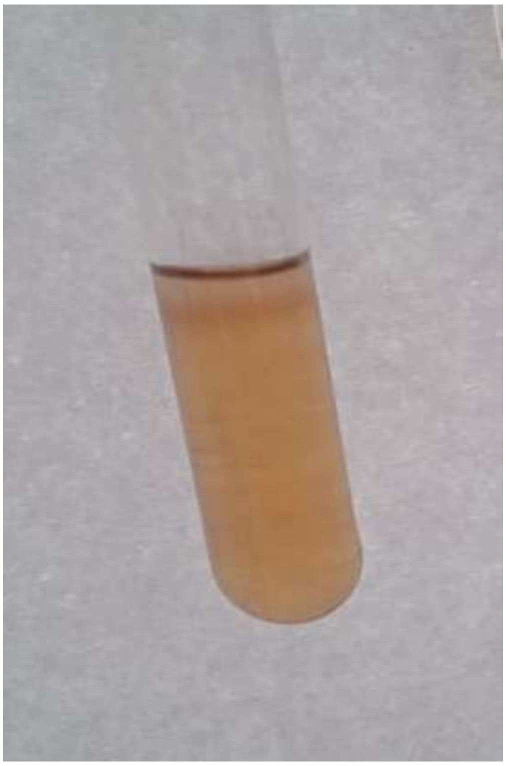
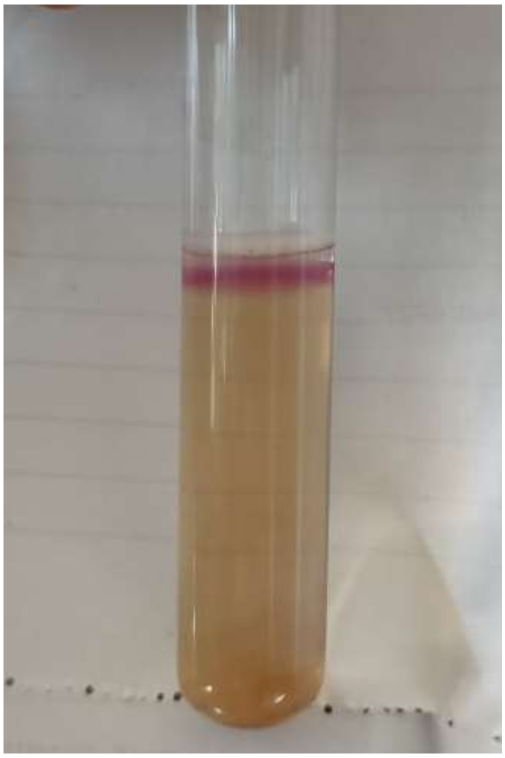


Fig. 11: ketone bodies estimation using colour chart and automatic analyzer



(a) **(b)**



(c) **(d)**

Fig. 12: Urine Rothera's test images (a) negative, (b) mild positive, (c) moderate positive and (d) severe positive

rule out how pathogenic ketosis was and how ketones affected the normal body's physiological processes.

4.1.3.2.8 Milk BHBA estimation

In present study, there was significant increase in the milk BHBA concentration in all the diseased animals.

The mean value of milk BHBA in diseased buffaloes and control animals was 512.50±79.56 and 68.75±21.22 µmol/L respectively. The mean value in diseased cattle and control was 405.0±68.08 and 45.50±11.78 µmol/L respectively. The mean value of milk BHBA in buffaloes was slightly higher than that of cattle (Fig. 9).

Similar findings were reported in previous studies. Carrier et al. (2004) and Oetzel (2007) reported that milk BHBA estimation was a useful diagnostic method for detection of ketosis when used at a cut point >100µmol/L, having higher specificity and sensitivity. The variation in the sensitivity and specificity in milk BHBA estimation was due to different cut values used by authors (Madreseh-Ghahfarokhi et al., 2018; LeBlanc, 2010). Geishauser et al. (2000) conducted a study on the sensitivity and specificity of milk BHBA using three different cut point value and reported higher sensitivity (80 percent) and specificity (76 percent) of milk BHBA when used at cut point value of either 100 or 200µmol/L.

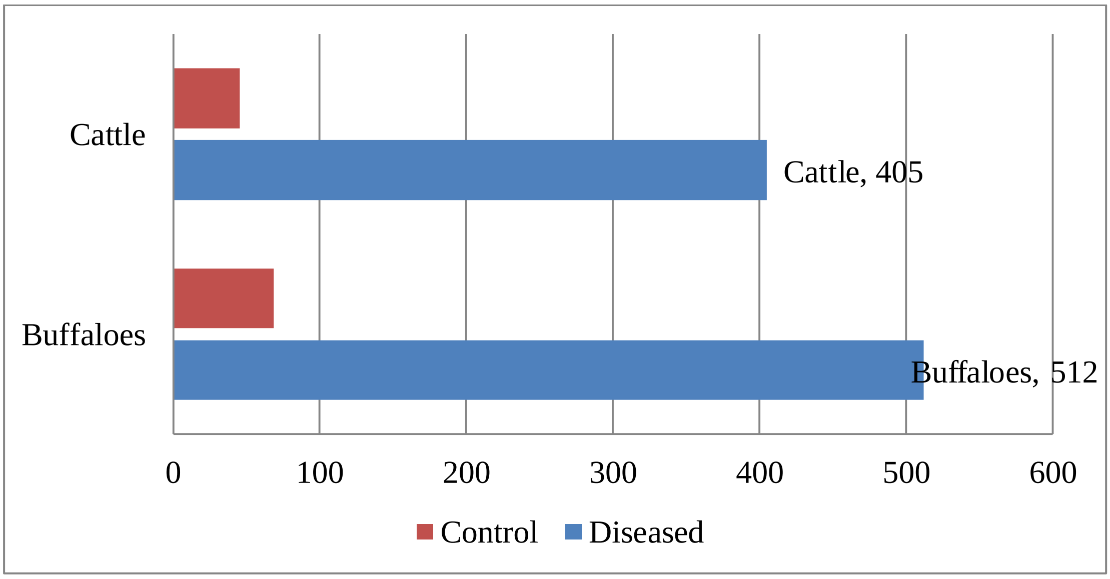


Fig. 13: Milk BHBA in ketotic cattle and buffaloes

4.1.3.2.8 Milk Rothera's test

Out of 32 positive cases of ketosis, only 10 percent of animals were positive for Rothera's test in milk while the rest 90 percent of animals were negative for the test on the day of presentation. In healthy controls, all the animals were negative for Rothera's test in milk. The Rothera's test in milk was of poor use in the diagnosis of ketosis in the present study.

Similar findings were reported by other authors (Carrier et al.2004; Oetzel 2004) who reported poor sensitivity and specificity of Rothera's test in milk as compared to that of blood.

4.1.3.2.9 Ultrasonography of liver

In the present study, the ultrasonography of liver was done to evaluate the status of liver which revealed that out of 32 cases, fatty liver was diagnosed in seven positive animals whereas in four animal, slight congestion was present along with the enlargement of caudal vena cava. Rest of animals showed normal liver morphology.

4.2 Therapeutic management

Thirty-two positive animals (20 buffaloes and 12 cattle) were taken for therapeutic management, out of which 20 animals responded to treatment and seven animals collapsed during the appropriate treatment protocol of three to five days.

In case of 20 positive buffaloes, 12 responded to treatment and three collapsed before the completion of appropriate treatment protocol (of five days). No follow up could be recorded in three animals due to owner incommunicado. Two animals remained irresponsive and were culled subsequently.

In case of 12 positive cattle, eight cattle responded to treatment and four animals collapsed before completion of appropriate treatment protocol. Overall recovery rate was 62.5 percent. In case of buffaloes the recovery rate was 60 percent, while in cattle the recovery rate was 66.67 percent.

Clinical improvement in the condition of treated animals was in agreement with previous studies (Talsma 1951; Blackburn et al. 1959), in one of which good response was recorded to potassium chloride in nine animals diagnosed with ketosis.

(a) (b) (c) (d)

Fig. 14: Milk BHBA test images (a) negative, (b) mild positive, (c) moderate positive and (d) severe positive

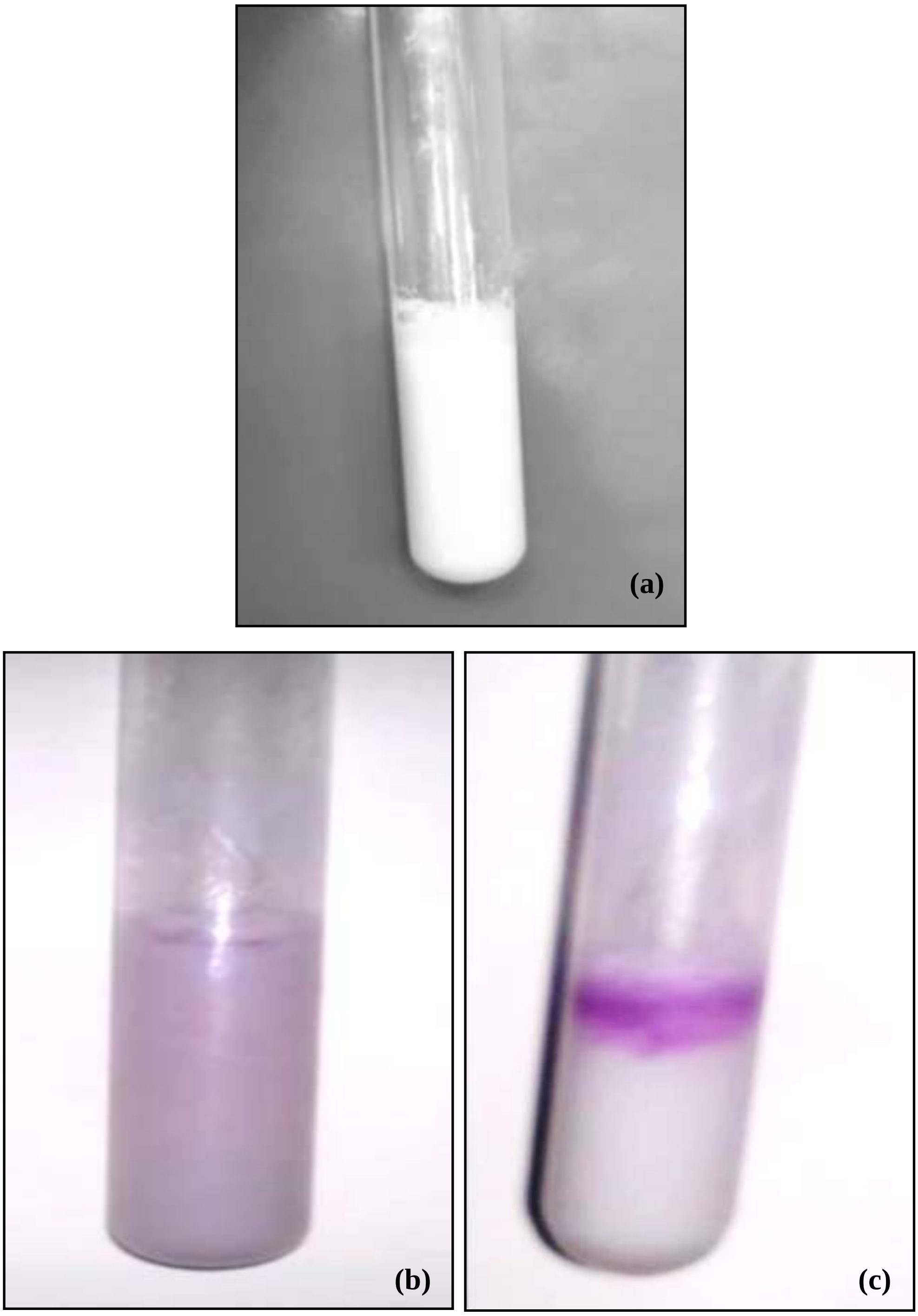


Fig 15: Milk Rothera's test (a) negative, (b) Mild Positive and (c) moderate Positive

Fig. 16: Ultrasonogram image of Enlarge Caudal Vena Cava of liver

Fig. 17: Ultrasonogram image showing congestion in liver

Fig. 18: Ultrasonogram image showing mild fatty deposition in liver

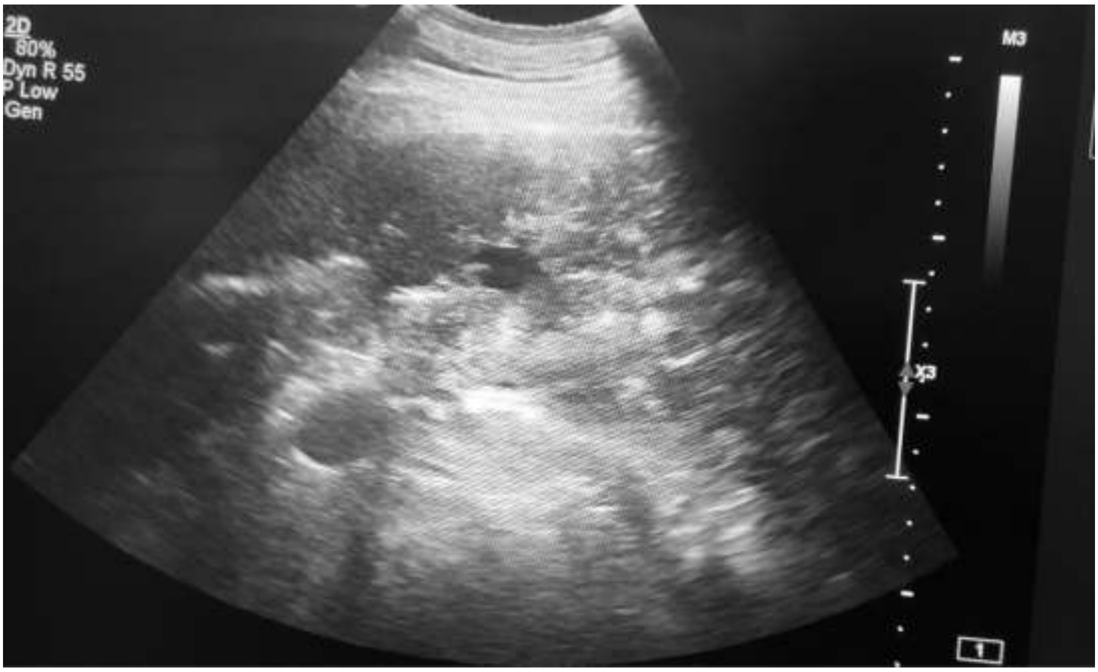


Fig. 19: Ultrasonogram image showing severe fatty deposition in liver

Potassium supplementation has been observed to raise the milk production of animals and to maintain the blood glucose concentration.

Good response to treatment was also observed in ketotic buffaloes supplemented with niacin. Foster (1988) reported the good effect of niacin feeding at daily dose rate of 12gm per animal in ketosis and resultant increase in the milk production as well as decrease in the concentration of blood BHBA and NEFA. Our findings disagree with some authors (Drackley et al., 2001) who observed increase in blood glucose concentration after giving niacin (6-12 gm per day) in ketotic animals as niacin promote gluconeogenesis. One similar study reported increase in milk yield of animals after giving niacin supplement (Fronk & Schultz, 1979).

4.2.1 Effect of treatment on milk yield

In the present study, there was significant increase in the milk yield of treated animals. The average increase in the milk of animals after treatment was recorded but it was less than actual milk yield before disease (Table 21 and Fig. 11).

The mean value of milk yield of animal before and after treatment was 5.25±2.47 and 8.83±2.39 litre per day respectively. The mean value of milk yield per day of animals before disease was 12.67±2.88 litre per day. Clinically there was 43.65 percent increase in milk yield of animals after treatment as compared to milk yield of animals during disease.

A similar study was performed by Teli and Ali (2007) who recorded the increase in milk yield of animals after treatment to 25.30 percent. Zialitis et al. (2007) reported a study on animals treated with supplementation of niacin and recorded the increase in milk yield of animals after treatment.

Table 21: Effect of treatment on the milk production in diseased animals

Parameter	Before disease	After disease	After treatment
Milk yield (per litre)	12.67±2.88**	5.25±2.47	8.83±2.39

*Significant level at p<0.05, **Significant level at p<0.01

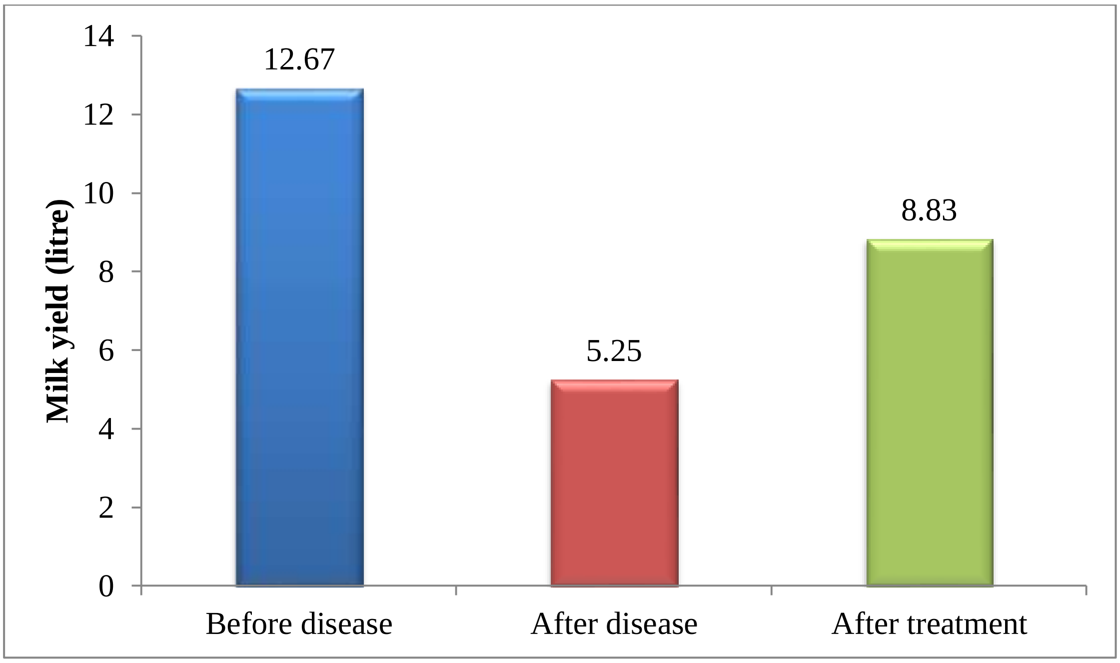


Fig. 20: Milk yield of animals before and after disease and after treatment

The reason behind decrease in production in diseased animal is attributed to lower efficacy of animal to provide lactogenic precursors to the mammary glands than the capacity of mammary glands.

4.2.2 Efficacy of treatment

The mean value of haematological and biochemical parameters after treatment in cattle and buffaloes is presented in Table 22 and 23, respectively.

Table 22: Mean value of haematological and biochemical parameter after treatment in buffaloes

Parameters	Diseased (N=20)	After treatment (N=14)	Control (N=10)
Haemoglobin	10.24±0.45*	11.29±0.59	10.41±0.27
Blood glucose	163.33±13.60**	89.91±3.55	86.5±7.81
Blood BHBA	1.77±0.09**	0.52±0.06	0.31±0.04
Blood potassium	3.15±0.15**	4.35±0.15	4.22±0.13
Milk BHBA	512.50±79.56**	39.58±12.86	68.75±21.22

*Significant level at $p<0.05$, **Significant level at $p<0.01$

Table 23: Mean value of haematological and biochemical parameter after treatment in cattle

Parameters	Diseased (N=12)	After treatment (N=8)	Control (N=10)
Haemoglobin	9.03±0.41	8.99±0.40	9.08±0.29
Blood glucose	128.6±17.7**	81.55±4.13	63.13±2.71
Blood BHBA	1.60±0.15**	0.49±0.09	0.34±0.04
Blood potassium	3.01±0.19**	4.38±0.18	4.16±0.11
Milk BHBA	405.0±68.08**	25.0±13.18	45.50±11.78

*Significant level at $p<0.05$, **Significant level at $p<0.01$

Among haematological parameters, there was significantly rise in the Hb value in treated animals as compared to diseased ones on the day of presentation in buffaloes. In case of cattle, no increase in the Hb values after treatment was observed.

Blood BHBA, blood glucose and milk BHBA value in both cattle and buffaloes was decreased significantly ($p<0.05$) post-treatment and were within normal physiological range.

Blood potassium level in both cattle and buffaloes increased significantly ($p<0.01$) post-treatment and was within physiological range.

CHAPTER V

SUMMARY AND CONCLUSIONS

Ketosis is an energy disorder characterised by elevated ketones in blood (ketonemia), milk (ketogalactia) and urine (ketonuria). The disease can cause a huge economic loss to the farmers. The disease mainly presents in peak lactation and when the demand for production is high. The disease is characterised as depressed milk yield, suboptimal feed intake, loss of body condition, depression, nervous involvement and fruity odour from breath as well as in urine. The early diagnosis of disease is important in dairy animals for prompt treatment and prevention of disease because of its higher economic impacts and thus prevention of other losses.

The present study was conducted to investigate the diagnosis of different forms of ketosis and its therapeutic management in both cattle and buffaloes. For an epidemiological aspect, 198 animals were screened, suspected for ketosis and presented at large animal hospital GADVASU, Ludhiana. The information of animals like age, sex, lactation, parity, detailed history, feeding behaviour, parturition status, loss of milk yield was observed. The physical examination was carried out followed by collection of milk, blood and urine samples. For therapeutic management of clinical cases, animals were differentiated into various types and proper treatment protocol was given for 5 days. Thirty-two clinical cases of type II ketosis were given Potassium Chloride @16gm/100kg bwt/day od and Niacin @ 12gm/day/animal od. Ten healthy cattle and buffaloes were taken as healthy control. Blood, milk and urine sample collection was done and various diagnostic tests were conducted on day 0 and post-treatment. The milk yield of ketotic animals was also recorded and compared with the milk yield of animals before disease. Based on the findings of different diagnostic tests, different treatments protocols were allotted to different forms of ketosis.

The overall occurrence of clinical ketosis in both cattle and buffaloes was observed as 18.18 percent (36/198), out of which four cases were of type I ketosis and 32 cases were of type II ketosis. The highest occurrence of clinical ketosis was in buffaloes of 2nd to 3rd parity and in cattle of 1st to 2nd parity. The highest cases of type II ketosis occurred in 0-14 days after parturition in both cattle and buffaloes. From the view point of clinical symptoms, the decrease in milk yield (100%) was reported in all the ketotic animals and the least signs observed was nervous signs (6.25%) in diseased animals. The drop in milk yield was severe (>60%) in 47 percent of diseased animals, moderate (30-60%) in 31 percent animals and mild (<30%) in 22 percent animals.

No significant difference was observed in temperature, respiration and heart rate of diseased animals as compared to healthy control in both cattle and buffaloes. However, hypomotile rumen was observed in diseased cattle as compared to healthy controls. Non-significant change in the haemoglobin concentration, total leukocyte count and differential leukocyte count was reported in diseased animals (both cattle and buffaloes) as compared to healthy ones. The blood glucose and potassium concentration showed significantly ($p<0.01$) change in diseased animals. The glucose concentration was significantly high in diseased animals as compared to healthy animals ($p<0.01$) and was within physiological range after five days of treatment. Also, the potassium concentration was significantly low in diseased animals and was in physiological range after treatment.

The blood as well as milk BHBA level was significantly high in ketotic animals as compared to healthy controls which returned within physiological range after five days of treatment. However, urine ketones were high in diseased animals than healthy ones. Rothera's test in urine was positive in all the examined animals but in milk only ten percent animals were found positive. The ultrasonography of liver showed mild to moderate changes in the diseased animals. Out of thirty-two positive animals, in four animal hepatic congestion was found and in ten animals mild to moderate fatty deposition was recorded. Rest of the diseased animals showed normal liver morphology.

Better response to treatment was recoded in animals and efficacy was recorded on the basis of diminished clinical signs, performance of animals, absence of ketones in urine as well as milk and by employing diagnostic tests. Twelve out of 20 buffaloes responded to treatment and eight out of 12 cattle responded to treatment after three to five days. The following conclusions may be drawn from the current research on bovine ketosis:

Conclusions:

- There is higher/greater prevalence of type II ketosis than type I ketosis in dairy cattle and buffaloes in Punjab.
- Type II ketosis occurred in animals after 0-14 days after parturition with maximum occurrence of disease in 2nd to 4rd parity.
- Hyperglycemia and low potassium level in type II ketosis was a consistent diagnostic finding.
- Milk and blood BHBA estimation was good indicator for diagnosis of type I and type II ketosis in cattle and buffaloes.
- Based on animal performance including rise in milk yield and absence of ketones in blood, urine and milk type II ketosis was amenable to treatment with potassium chloride and niacin for 3-5 days with 62.50 percent of animals showing complete recovery.

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