CLINICO-BIOCHEMICAL STUDIES ON KETOSIS IN CATTLE

गौ-वंश के कीटोसिस रोग में शयनिक एवं जैवरसायनिक अध्ययन

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INTRODUCTION

Ketosis is a multifactorial disorder of energy metabolism and represents incomplete combustion of fatty acids during periods of increased hepatic utilization of fatty acids. Biochemically it is characterized by ketonaemia, ketonuria, hypoglycaemia and low levels of hepatic glycogen (Radostits *et al., 1994).*

Bovine ketosis has been recognized since the nineteenth century and has been researched since the start of the twentieth century (Shaw, *1956;* Pehrson, *1966).* The condition is recognised as one of substantial economic significance (Littledike *et al., 1981)* and has been identified in both primitive and sophisticated dairy management systems.

High incidence of clinical and sub-clinical ketosis in dairy cattle has been reported from India and abroad (Bhuin and Chakrabarti, 1993; Duffield *et al., 1997;* Geishauser *et al., 1998).* The frequency of clinical cases has increased sharply in the recent past because of steep increase in milk production of individual cows.

Physiologically ketosis appears to be imbalance between input and output of energy due to dietary abnormalities especially when animals are lactating. Heavy milk production imposes a severe metabolic drain and creates negative energy and protein balance (Pehrson, *1966).* Half of the high producing dairy herd goes through borderline ketosis during early lactation (Emery *et al., 1964)* and it takes a small additional nutritional or metabolic insult for them to develop clinical ketosis.

Immediate peripartum period is at a greater risk for ketosis and most cases occur within approximately 60 days post calving (Grohn *et al.*, 1984, 1986; Dohoo and Martin, 1984). The incidence increases with age and that the peak incidence may be in lactation 3-6 (Lindstrom *et al.*, 1984; Bendixen *et al.*, 1987).

The pathogenesis of the condition has been a subject of controversy (Baird, 1982) and has not been fully elucidated. Ketosis was described as being a disorder of carbohydrate metabolism (Krebs, 1966) and this view was maintained until (Baird, 1982) suggested that ketosis may be better understood and managed in terms of the control of energy homeostasis in the bovine. Ketone bodies are important in metabolism (Robinson and Willanison, 1980) and the impact of bovine ketosis, related changes in lipid, carbohydrate, protein and steroid metabolism on the health and production of the periparturient cow may be substantial.

The treatment of ketosis is not only difficult but poses a challenge to veterinarians. Several lines of treatment for clinical ketosis like glucose injection, glucose plus insulin, corticosteroids, oral administration of sodium propionate and nicotinic acid have been reported.

Many authors agree that the first metabolic alteration to appear in primary ketosis is hypoglycaemia (Kronfeld, 1971; Bergman, 1973; Stamatovic *et al.*, 1983; Samanc and Damjanovic, 1987). This leads to a series of metabolic changes in the organism which are directed towards the securing of new sources of glucose and energy. Increased gluconeogenesis can supply more glucose but Krebs (1966)

showed that large quantities of ketone bodies are also formed at the same time. However, the causes of the original hypoglycaemia have not been clearly defined, although temporary hyperinsulinaemia has been suggested as one of the primary etiological factors potentiating hypoglycaemia (Kronfeld, 1971). Mitin and Kraljevic (1977) found higher concentrations of insulin in blood serum of ketotic cows than in healthy cows. In contrast to this, other authors (Hove, 1978; Hove and Halse, 1978) observed low insulin levels in ketotic cows. Moreover, there was a combined state of hypoglycaemia and hypoinsulinaemia in almost all cows exhibiting ketosis at the beginning of lactation (Schwalm and Schultz, 1976).

According to 1997 census, the state of Rajasthan has 12.15 million cattle out of which 0.21 million are crossbred. Bikaner has 0.55 million cattle out of which 5.63 thousand are crossbred. The total milk production for the state of Rajasthan during the year 1996-97 was estimated at 5873 million liters, which is increasing every year because of rising crossbred cattle and selection of high yielding indigenous cattle breeds. These cows require an intensive management and often develop certain metabolic disorders like ketosis resulting in heavy production losses.

Keeping in view the importance of high producing cattle, their feeding practices and management pattern adopted by farmers, present investigation on clinico-biochemical studies on ketosis in cattle has been undertaken with following objectives

1. To determine the prevalence of ketosis in cows in and around Bikaner in order to know its epidemiological significance.

2. To note clinical manifestations and biochemical changes in serum of ketotic cows and correlate them with the degree of ketonaemia.

3. To study glucose utilization in ketotic cows after intravenous administration of 50% dextrose to know the status of utilisation rate in individual case and decide the choice of treatment.

4. To evaluate the efficacy of treatment with 50% dextrose and triamcinolone acetonide.

5. To detect the insulin-dependent cases on the basis of decreased rate of glucose utilization in ketotic cows and determine whether the dose of zinc insulin can be decided on the rate of glucose utilization.

6. To conduct the glucose tolerance test in apparently healthy cattle to derive probably glucose utilization curve.

7. To determine the renal threshold of blood glucose in apparently healthy cattle.

REVIEW OF LITERATURE

Occurrence

Sjollema and Van Der Zande (1923) reported that ketosis occurs frequently during seventh to tenth day after parturition in cows.

Hutyra and Marek (1926) reported that ketosis in cows occurred most frequently after the birth of third to sixth calf.

Sjollema (1932) and Boddie (1935) reported that clinical ketosis usually occurs about a week or ten days after parturition and occasionally some what later.

Fincher (1936) reported that a large number of ketotic cases may occur at the first calving.

Duncan *et al.* (1939) observed that ketosis mostly occurred from 2^{nd} to 6^{th} week following parturition.

Knodt *et al.* (1942b) reported higher incidence of ketosis in the late winter and early spring because of the availability of grass silage treated with molasses during this period.

Allcroft (1947) observed that ketosis usually occurs from a few days to few weeks post-partum and highest incidence occurs at 3 weeks post-partum.

Shaw *et al.* (1952) reported that ketosis usually occurs from a few days to few weeks post-partum. The highest incidence appears to occur at about 3 weeks after parturition and a limited

number of cases suffer during the pre-partum and two or more months post-partum.

Blackburn *et al.* (1958) reported that incidence of ketosis was high in last 6 weeks of pregnancy when they are fed concentrate mixture, grass silage and dried grass.

Ford and Boyd (1960) reported that majority of cases of bovine acetonaemia occur in the second and third weeks of lactation and limited cases occur within few days post-partum.

Pehrson (1966) reported high incidence of ketosis in herds which were poorely fed and had a high lactation yield.

Rijpkema (1968) reported that ketosis occurs between first and sixth week post-partum.

Schultz (1968) observed that primary ketosis always occurred during the first three days to eight weeks after calving. The most critical period being three weeks after parturition with maximum frequency in older age. He further reported that secondary ketosis constitute one third of total ketotic cases in cows.

Fox (1971) reported highest incidence of ketosis during 3-6 years of age of animal (peak production years) with maximum frequency during two to three weeks to four months after parturition.

Halse and Mogstad (1975) observed that overfeeding before calving, underfeeding after calving, feeding of fermented silage and genetic factors were, possible causes of ketosis,

Henricon *et al.* (1977) reported that there is gradual fall in blood glucose level from first to fourth lactation. This indicated that cows in fourth lactation were much prone to ketosis.

Kronfeld (1980) reported that 10 per cent of cases appear within a week of calving, more than 70 per cent within a month and nearly all within 6 weeks.

Littledike *et al.* (1981) reported that majority of cases of primary ketosis occurs at six or eight weeks after parturition. Maximum incidence of disease was reported when the cows approach peak lactation. The average incidence has been about 4 per cent in United States and 2 per cent in United Kingdom. They further reported that breed had no influence on incidence but more cases were seen in winter.

Baird (1982) reported that clinical ketosis occurred spontaneously in susceptible high yielding dairy cows between 2 to 4 weeks of lactation. The condition was not common in cows lactating for the first time.

Lebeda *et al.* (1982) analysed the presence of ketone bodies on urine samples and reported higher rate of ketonuria in winter than summer.

Kauppinen (1983) studied the prevalence of bovine ketosis in relation to number and stage of lactation. He reported 3, 7, 20, 22 and 13 per cent incidence at first, second, third, fourth, fifth or sixth and later calvings, respectively.

Dohoo and Martin (1984) found that approximately 90 per cent of clinical or subclinical ketosis cases occurred within approximately 60 days following parturition.

Grohn *et al.* (1984) reported high percentage of ketotic cases during September to May than during June to August. They recorded 89 per cent of cases within 8 weeks with highest occurrence in 3 to 5 weeks after parturition.

Andresson and Emanuelson (1985) measured milk acetone level during first three months post-partum in 3078 dairy cows. The prevalence of hyperketonuria was 8-9 per cent in first month, 4-7 per cent in second month and 1-2 per cent in third month.

Grohn *et al.* (1986) observed that 90 per cent of ketosis cases were within 8 weeks postpartum with highest occurrence in 3-5 weeks.

Bendixen *et al.* (1987) reported 4.41 per cent and 1.87 per cent clinical ketosis in Swedish Red and Swedish Holstein cattle, respectively.

Kalita *et al.* (1987) observed clinical ketosis in high yielding Jersey crossbred cows between second and fifth lactation.

Simensen *et al.* (1987) reported high risk of ketosis in winter which increased with number of lactations.

Deluyker (1989) reported 21.5 per cent incidence of clinical ketosis in a herd of high producing Holstein cows.

Syed Ziauddin *et al.* (1992) recorded the maximum incidence of ketonuria in cows having age between 5 to 7 years. He further reported that Holstein Friesian breed of cow was more prone to ketonuria as compared to Red Dane and Jersey.

Bhuin and Chakrabarti (1993) reported an incidence of 4.54 per cent and 4.23 per cent in crossbred Holstein and other breeds of cows respectively. They further reported that the incidence of ketosis was higher in 8-9 years and lower in 4-5 years old cows. The greater frequency of ketosis was recorded during third lactation and less in first lactation. They reported a significant reduction (15.2 to 41.1 per cent) in milk yield in ketotic cows.

Radostits *et al.* (1994) reported that bovine ketosis occurs most commonly during the first month of lactation, less commonly in second month and only occasionally in late pregnancy.

Singh (1994) reported an overall incidence of 11.26 per cent in crossbred cows. He observed 12.50, 8.33, 33.33, 25.00 and 20.83 per cent incidence in months of September, October, November, December and January,respectively.

Duffield *et al.* (1997) observed that the prevalence of ketosis for cows in early lactation, mid-lactation, late lactation and dry cow was 14.1, 5.3, 3.2 and 1.6 per cent ~respectively. There was a trend of increasing prevalence with increasing parity across all stages of lactation.

Geishauser *et al.* (1998) reported that the prevalence of subclinical ketosis in cows was highest during the first 6 weeks of lactation, and a peak was detected during the second week of lactation.

Clinical Manifestations

Sjollema (1932) reported loss of appetite, pica, dry faeces, deep breathing with sweetish smell, irragular rumination and decreased milk production as clinical signs of ketotic cows.

Sampson (1952) recorded anorexia, decrease in milk yield, rapid loss of body weight, elimination of dry mucus-coated faeces and unsteady gait in bovine ketosis.

Kronfeld *et al.* (1966) classified various types of ketosis as (i) subclinical (ii) mild (iii) severe or acute and (iv) chronic. The clinical type was classified as (i) nervous (ii) digestive and (iii) wasting.

Belschner (1967) observed the clinical findings of bovine ketosis which included loss of appetite, decreased milk production, rapid loss of condition, firm dung covered with mucus, listlessness, unusual walking movements, grinding of teeth, constant licking and acetone-like odour in the breath and sometimes in milk and urine.

Rijpkema (1968) reported pica, reduced appetite especially for concentrate, reduced milk yield and loss of body weight in bovine ketosis.

Schultz (1968) reported loss of appetite, refusal of concentrate, depression, constipation and urine showing characteristic colour responses to RRthera's test.

Cote *et al.* (1969) reported hypophagia in 85 per cent, hypolactia in 87 per cent, salivation in 63 per cent, turgid uterus in 63 per cent, vulvar discharge in 50 per cent and poor condition in 40 per cent cases of bovine ketosis. Firm mucus coated faeces were found in 16 per cent cases of bovine ketosis.

Kronfeld and Emery (1970) noticed some behavioural changes such as lack of alertness, blank expression, lack of responsiveness to stimuli along with usual signs of anorexia, hypolactia, ketonuria, abnormal chewing movements, excessive salivation, exaggerated licking and depression in milk production.

Rajan and Ganapathy (1973) found rectal temperature within normal range, reduced milk yield, complete anorexia, suspended rumination and mucus-coated faeces in cows suffering from ketosis.

Baird *et al.* (1974) observed anorexia, reduced milk yield, ketolactia, ketonuria, refusal of concentrate and loss of weight in ketotic cows.

Baird (1982) reported that bovine ketosis appeared suddenly and signs included loss of appetite (particularly for concentrate), decreased milk production and rapid loss of body condition. Some cows became excitable although the majority were apathetic. Body temperature was normal and milk gave a positive reaction to Rothera's test.

Grohn *et al.* (1983) recorded clinical signs of spontaneous ketosis as loss of appetite, decreased milk production and rapid deterioration of body condition.

Lean *et al.* (1991) reported weight loss, decreased dry matter intake and selective intake of forage, particularly hay, in preference to concentrates in ketotic cows. He also reported a normal temperature, respiration and heart rate but depressed ruminal contraction. Dry scanty faeces, decreased milk production and acetone like odour in breath of cows were detected.

Radostitis *et al.* (1994) described `wasting' and `nervous' forms of bovine ketosis. The wasting type was characterised by moderate decrease in appetite and milk yield over four days with loss of weight and firm and dry faeces. The nervous form of ketosis was suggestive of delerium and characteristic signs included walking in circle, staggering, head pushing, apparent blindness, vigorous licking of skin and inanimate objects, depraved appetite and chewing movements with salivation.

Strip test

Nash *et al.* (1954) reported that ketostix and acetest are superior than the original Rothera's nitroprusside test.

Chertack and Sherrick (1958) reported that ketostix and acetest can be used with serum or plasma.

Free *et al.* (1958) studied the clinical samples and observed that the tablet and strip test were essentially equivalent.

Popof (1979) assessed pH, ketone, glucose, protein, bile pigment and blood in urine in ketotic sheep using the reactive strip and stated that it is a rapid albeit rough diagnostic technique and can be used complementary to more precise but more time consuming urine test.

Singari *et al.* (1988) evaluated the comperative efficacy of strip test with Rothera's test and found positive correlation between strip and Rothera's test to the extent of 88 per cent while 10 per cent of samples gave false positive and 2 per cent gave false negative reaction to ketone bodies.

Veenhuizen *et al.* (1991) determined the onset of clinical ketosis by keto-diastix (Ames Division, Miles Laboratories Inc., Elkhart-in) in presence of high concentration of the ketone bodies in the urine.

Singh (1994) reported that in ketotic cows keto-diastix as well as Rothera's test produced similar results.

Gupta (1999) studied the comparative efficacy of keto-diastix reagent strip and modified Rothera's test on 120 crossbred and 80 Rathi cows and concluded that there was no discrepancy between the two tests.

Rothera's test

Le Nobel (1884) observed the formation of grey colour on reaction of nitroprusside with acetoacetic acid resulting in alkalinization. Acidification with acetic acid turns the colour to magnetic.

Rothera's (1908) reported that when acetone and acetoacetic acid react with sodium nitropruside they produce violet colour.

Duncan and Huffman (1940) reported that in Rothera's test, intensity of permanganate colour depends upon the amount of ketone bodies present in urine or milk. They classified intensity of colour into faint (+), more pronounced (++, +++) and intense colour ( ) and stated that this test did not detect 13 - hydroxybutyric acid in urine and milk.

Knodt *et al.* (1942) reported that milk ketone levels were half of the body levels whereas the urine levels exceeded the blood levels by about four times. The average composition of urinary ketone bodies in ketotic cows was 61.72 per cent 13-hydroxybutyric acid and 38.28 per cent acetone and acetoacetic acid. They also reported that Rothera's test did not measure 13-hydroxybutyric acid in urine.

Emery *et al.* (1964) reported that Rothera's test can detect ketone up to 2 mg per cent in milk wheres Kronfeld *et al.* (1966) showed its presence up to 10 mg per cent.

Kronfeld (1972) reported that the nitroprusside test gave a purple colour with acetoacetic acid and acetone but not with 13-hydroxybutyric acid. He further added that test was sensitive to acetoacetate (1 mg per cent), acetone (2 mg per cent) and pyruvate (20 mg per cent).

Henry (1975) stated that sensitivity of Rothera's test was about 1-5 mg/dl acetoacetate or 10-25 mg/dl acetone. He further

reported that after 10-15 minutes the reaction should be read as trace purple : 5 mg/dl acetoacetic acid or 20 mg/dl acetone; moderate purple 50 mg/dl acetoacetic acid or 250 mg/dl acetone and strong purple : 50 mg/dl acetoacetic acid or 800 mg/dl acetone. He further added that sodium nitroprusside did not react with B-hydroxybutyric acid. Acetone produced mild acidosis, whereas acetoacetic acid and B-hydroxybutyric acid produced more severe acidosis.

Radostits *et al.* (1994) described that Rothera's test measured only the acetoacetic acid content of the urine and not the B-hydroxybutyric acid but a very little acetone.

Biochemical Parameters

Sjollema and Van Der Zande (1923) reported abnormally high levels of acetone bodies in blood and urine for the first time in cows exhibiting symptoms of ketosis.

Hupka (1928) was first to demonstrate the hypoglycaemia associated with ketosis in cows and found that sugar administration was beneficial.

Sjollema (1932) reported that in typical nervous form of ketosis there may be some disturbance of mineral metabolism together with ketonaemia and levels of acetone in blood and urine were lower in animals with the nervous form of acetonaemia than in other forms of ketosis.

Boddie (1935) observed high level of acetone bodies in blood and urine of cows exhibiting symptoms of ketosis. The total

blood ketone concentration was almost invariably more than 20 mg/dl and sometimes exceeding to 100 mg/dl.

Sampson and Hayden (1935) recorded that blood calcium was reduced in cows during acetonaemia.

Duncan *et al.* (1939) reported mean blood ketone of ketotic cows as 23.9 mg/dl (ranging from 1.55 to 79.05 mg/dl). The values obtained for plasma calcium, magnesium and chloride did not indicate a disturbance in the metabolism of these constituents in ketosis.

Eden and Green (1940) classified ketosis as mild when the blood ketones were 5-15 ml/dl and pronounced when the level was above 15 mg/dl.

Sampson and Boley (1940) measured calcium in the serum of 8 cows during the course and after recovery from ketosis. The difference between the average values was only 0.46 mg/dl.

Saarinen and Shaw (1950) observed hypophosphataemia in bovine ketosis whereas plasma sodium and potassium were normal in most cases of ketosis.

Shaw (1956) recorded low concentration of blood glucose in bovine ketosis. He further added that level of blood ketone bodies of cows with primary ketosis was usually higher than fasted cows at a similar levels of blood glucose.

Halse and Velle (1958) reported hypocalcaemia in ketosis. The lowering of blood calcium was apparently secondary and due to reduced feed intake.

Bach and Hibbitt (1959) recorded high concentration of serum ketone bodies and very low concentration of blood glucose in bovine ketosis.

Pehrson (1966) recorded lower concentration of blood sugar and serum protein, whereas serum ketone bodies, transaminase and bilirubin were elevated.

Schultz (1968) recorded hypoglycaemia and ketonuria in bovine ketosis. The hypoglycaemia was presumably due to large amount of glucose removed by mammary gland to make lactose, whereas plasma-free fatty acid and blood ketone were elevated. The ratio of 13-hydroxybutyric acid to acetoacetate plus acetone in blood ranged from 4:1 at lower levels, to 2:1 at the higher level in bovine ketosis.

Cote *et al.* (1969) analysed blood samples of ketotic cows and found that plasma acetone plus acetoacetate and plasma free fatty acids were elevated. No significant changes were found in serum magnesium and phosphorus content.

Schultz (1971) recorded high concentration of ketone bodies in blood, urine and milk but decreased levels of blood glucose ~---end serum calcium.

Rajan and Ganapathy (1973) recorded blood ketone values in ketotic cows from 7.211-26:041 mgldl with a mean of

12.46±0.28 mg/dl. He also observed blood ketones in 6 healthy dairy cows aged 2 to 6 years, from 3 days to 6 months after parturition : The mean blood ketones were 1.5 mg/dl (1.3 to 1.9 mg/dl).

Payne *et al.* (1974) observed a high concentration of urea during summer due to high intake of protein from pasture.

Hewett *et al.* (1975) observed that the urea - N values were directly affected by protein level. They concluded on the basis of their observation that serum urea-N was a fairly exact reflection of protein intake when energy and roughage remained constant.

Panduranga *et al. (1975)* reported mean blood glucose levels of ketotic, anorexic and normal cows as 40.54±2.8, 54.34±3.8 and 60.54±3.62 mg/dl respectively. Ketone bodies were detected in milk, urine or both in anorexic cows when blood glucose level was 40.50 mg/dl.

Rowlands *et al.* (1975) observed that urea concentration decreased from 16.5 mg N/ 100 ml before calving to 14.7 mg N/ 100 ml after calving. The fall was most pronounced in summer month when normal urea-N concentration was higher due to grazing summer pasture. In winter, the fall during calving was not significant.

Kitchenham and Rowlands (1976) observed a downward trend of urea-N with increasing age. They also found a breed difference in urea-N concentration, being higher in Friesian Ayrshire cows than either of both breeds.

Schwalm and Schultz (1976) studied relation of insulin concentration of blood metabolites in dairy cows. They reported a

depressed milk yield and serum insulin and elevated levels of free fatty acids and cholesterol.

Zhabolenko (1976) reported low concentration of blood sugar, albumin and high concentration of total lipids, ketone bodies and total protein in clinical cases of bovine ketosis.

Hove (1978) observed hypocalcaemia associated with hypoglycaemia and ketonuria in bovine ketosis.

Lee *et al.* (1978) reported lower concentration of serum magnesium, whereas plasma free fatty acids and blood ketones were elevated.

Sarode *et al.* (1981) recorded hypoglycaemia, ketonaemia and ketonuria in clinical cases of Sahiwal cows.

Schierka and Filar (1981) reported decreased levels of calcium, potassium and sodium in induced ketosis with administration of butyric acid.

Hamada *et al.* (1982) observed hypoglycaemia and hypomagnecaemia associated with increased concentration of blood acetoacetate and free fatty acids in clinical cases of ketosis in cows.

Ghergariu *et al.* (1986) found an increase in urea-N in summer (26.1 mg/ 100 ml) than winter (16.9 mg/ 100 ml).

Fatur and Jazbec (1990) recorded chemical changes with hyperphosphataemia in 25 per cent and decreased blood volumes of standard bicarbonate in 76 per cent, sodium in 49 per cent and glucose in 88 per cent of the ketotic animals.

Smith (1990) described that in clinical ketosis blood glucose concentration were 20 to 40 mg/dl, total blood ketones were greater than 30 mg/dl and total urine ketones were greater than 80 mg/dl.

Radostits *et al.* (1994) described hypoglycaemia, ketonuria and increased levels of plasma fatty acids in bovine ketosis. Blood glucose levels were reduced from the normal levels of 50 mg/dl to 20-40 mg/dl and blood ketone levels were elevated to 10-100 mg/dl.

Rao and Suryanarayana (1996) reported that mean±S.E. of blood glucose levels in cows were 36.25±2.1; 39.4±1.2 and 45.5±2.1 mg per cent during first, second and third month and beyond post-partum, respectively.

Gupta (1999) reported that there was a highly significant decrease in calcium and glucose concentration in both crossbred and Rathi cows suffering from ketosis. Highly significant increase was observed in alanine amino-transferase, aspartate amino-transferase and urea-nitrogen values in both crossbred and Rathi cows. Highly significant increase was also observed in magnesium, alkaline phosphatase, total protein and globulin values in ketotic crossbred cows. Significant increase in albumin values in crossbred and total protein in Rathi cows were recorded. Non-significant difference was found in inorganic phosphorus and A:G ratio in both crossbred and Rathi cows. Magnesium, alkaline phosphatase, albumin and globulin values in ketotic Rathi cows did not reveal any significant changes.

Treatment

Hupka (1928) was the first to use glucose for the treatment of ketosis in cows and it proved beneficial.

Sjollema (1932) first treated a case of bovine ketosis with glucose plus insulin and found that glucose utilization increased with insulin treatment.

Duncan *et al.* (1939) reported decrease in blood and urinary acetone after intravenous infusion of glucose.

Gingras (1947) reported that 40 per cent of ketotic cases did not respond to one injection of 500 ml of 50 per cent glucose solution.

Hatziolos and Shaw (1950) injected cortisone acetate (900 mg im) in ketotic cows. The cows regained their appetite in two hours, all signs of paresis disappeared and blood glucose became normal in 48 hours.

Shaw *et al.* (1952) reported that hydrocortisone acetate (300-600 mg) was an effective treatment for ketosis. The appetite improved and blood glucose and acetone bodies returned to normal levels.

Goetsch *et al.* (1956) reported that 50 per cent glucose is successful treatment in bovine ketosis. They reported that 10 per cent of injected glucose gets eleminated through urine.

Bouckaert (1958) treated cases of bovine ketosis glucose plus insulin. The combined treatment was very effective removing ketone bodies from blood.

Bergman and Roberts (1967) studied urinary excretion d blood concentration in cattle after treatment with 200 g glucose Nee iv. They reported that about 10 per cent of glucose was excreted in urine.

Schultz (1968) reported that 500 ml of 50 per cent glucose solution is a successful treatment in bovine ketosis. Hs further reported that blood glucose fell below normal levels within two hours.

(1969) reported that intravenous administration pf 50 per cent dextrose in a doss of 250 g provided a short-term relief of hypoglycaemia in ketosis and glucose utilization increased with insulin treatment.

Ksdsnburg and Mulling (1971) treated cases of starvation I induced ketosis with glucose plus insulin. The combined treatment decreased blood acetoacetate and D (-) 3- hydroxybutyrats.

Whitskar (1981) reported that single dose treatment with glucose (50%) plus insulin was very effective in treating clinical cases of bovine ketosis.

Rings (1985) reported loss of glucose in urine after infusion of concentrated glucose solution. Hs suggested that moderate irate of infusion with insulin provides rapid utilization of glucose with speedy recovery.

Blood and Radostits (1989) reported that repeated reatments with 500 ml of 50 per cent solution of glucose given intravenously showed marked improvement in, most ketotic cows.

Sakai *et al.* (1993) evaluated therapeutic efficacy of glucose and glucose plus insulin and reported that combined therapy was very effective in treating clinical ketosis.

Singh (1994) studied the comperative efficacy of 25% Dextrose, Prednisolone and 25% Dextrose plus insulin and concluded that glucose plus insulin was most effective in ketotic cows.

Shpigel *et al.* (1996) reported that treatment of ketotic cows with dexamethasone (40 mg im) and 50 per cent glucose solution (500 ml iv) was more efficacious than treatment with dexamethasone alone.

Glucose Tolerance test

Sakai *et al.* (1996) conducted glucose and xylitol tolerance tests for ketotic and healthy dairy cows. They reported an increase of glucose concentration by 6.4 and 8.1 fold in healthy and ketotic cows immediately following administration of 500 ml of 50% glucose from base line value. There was a gradual decline in blood glucose from 0 to 15, 30, 45, 60 and 120 minutes in both normal and ketotic cows.

Samanc *et al.* (1996) administered 500 ml of 50% glucose solution in 10 healthy and 10 ketotic cows and reported an increase in glucose concentration of both healthy and ketotic cows immediately following administration. The rate of decline was similar in both

groups of cows indicating that the net rate of glucose utilization did not differ between them. The mean values of glucose at the end of experiment at 240 minutes were higher than the initial values in both groups.

Anderson *et al.* (2000) conducted intravenous glucose tolerance testing of healthy bulls. They administered 500 ml of 50% glucose solution to bulls weighing between 911.5 and 1035.5 kg body weight (258 mg/kg bat .). The base line serum glucose concentration was 69±6. At 30, 60, 120 and 240 minutes it was 183±29, 138±35, 103±20 and 71±16 respectively.

MATERIAL AND METHODS

Animals :

Urine simples of 505 post-parturient cows belonging to the college diary firm, outdoor patients brought for treatment it medicine clinic of College of Veterinary and Animal Science, Baking and individual animals shown by owners it their holdings an and around Bakiner were examined for prevalence of ketosis during November 19 X,gto October 1999.

Fifty cows showing clinical signs of ketosis and the urine of which was positive for Modified Rothgi's test/urane multidiagnostic strap were included for biochemical studies (Before and after treatment).

For comparison of biochemical status of clinically positive ketotic cows, i total of ten apparently healthy lactating cows were included an this study. Sax healthy heifers for studying glucose tolerance and sax for determining renal threshold were also included an the study.

Prevalence

Prevalence of the diseases was determined across

(a) Different months of year.

(ii) Different stages of lactation.

(iii) Different age groups.

(iv) Different parities.

History and Clinical Examination

(i) History of parturition, feeding, stage of lactation and milk-yield was taken from owner.

(ii) Observation was made regarding general symptoms and clinical observations including rectal temperature, pulse, respiration rate and ruminal movements.

Clinical Diagnosis

Clinical diagnosis was carried out through history of the case, symptoms, biochemical estimations and laboratory tests for ketone bodies in urine.

The various techniques applied in relation to sampling procedure and laboratory investigations for confirmation of diagnosis have been described as under

Sampling Procedure

After clinical examination of the animals, the blood samples were collected from jugular vein from 50 ketotic, 10 healthy cows and 12 apparently healthy heifers with all aseptic precautions in sterilized test tubes. The blood slants were made and incubated for 1 hour at 30°C. Blood clots were broken and tubes were centrifuged at 2300 r.p.m. for 30 minutes. The serum was pipetted out in small pyrex tubes and was kept immediately in the deep freeze at -20°C till analysis.

Fresh urine samples were collected directly in sterile vials after massaging the perineal region for the presence of ketone bodies.

Testing for ketone bodies and other urine multidiagnostic strip parameters

Urine samples of all the 505 cows were subjected to urine multidiagnostic strip (Ames) and Modified Rothera's test.

(a) Urine multiple diagnostic strip test

Multiple reagent strips for urinalysis are firm plastic strips to which are affixed several separate reagent areas. Depending on the products being used, the multiple reagent strip provide tests for glucose, bilirubin, ketone (Acetoacetic acid), specific gravity, blood, pH, protein and urobilinogen in urine.

Procedure

The reagent areas of strip were immersed completely in freshly collected urine and the strip was removed immediately. The strip was held in horizontal position to prevent possible mixing of chemicals from adjacent reagent areas. After dipping, the reagent areas on the strip were compared with the corresponding colour chart on the bottle label at the time specified.

Glucose and Bilirubin	-	at 30 second after dipping
Ketone	-	at 40 second after dipping
Specific gravity	-	at 45 seconds after dipping
pH and Protein	-	at 60 seconds.

Principles of the procedure

Glucose

The test is based on a double sequential enzyme reaction. One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose. A second enzyme peroxidase, catalyzes the reaction of hydrogen peroxide with a potassium iodide chromogen to oxidize the chromogen to colours ranging from green to brown.

Bilirubin

This test is based on the coupling of bilirubin with diazolized dichloroaniline in a strongly acid medium. The colour ranges through various shades of tan.

Ketone

This test is based on the development of colours ranging from buff-pink, for a negative reading, to purple when acetoacetic acid reacts with nitroprusside.

Specific gravity

This test is based on the apparent Pka change of certain pretreated polyelectrolytes in relation to ionic concentration. In the presence of an indicator, colours range from deep blue-green in urine of low ionic concentration through green and yellow-green in urine of increasing ionic concentration.

Blood

This test is based on the peroxidase like activity of haemoglobin, which catalyzes the reaction of disopropylbenzene dehydroperoxide and 3, 3, 5, 5 - tetramethyl benzidine. The resulting colour ranges from orange through green.

pH

This test is based on a double indicator principle that gives a broad range of colours covering the entire urinary pH range. Colours range from orange through yellow and green to blue.

Protein

This test is based on the protein error of indicators principle. At a constant pH the development of any green colour is due to the presence of protein. Colours range from yellow for "Negative" through yellow - green and green to green - blue of "Positive" reactions.

Urobilinogen

This test is based on modified Ehrlich reaction, in which P-diethylaminobenzaidehyde in conjunction with a colour enhancer reacts with urobilinogen in a strongly acid medium to produce a pink-red colour.

Modified Rothera's Test

Principle

Nitroprusside in alkaline solution react with acetoacetic acid and to a lesser extent acetone to form a purple colour. The test is insensitive to 13-hydroxybutyrate (Bruss, 1989).

Procedure

Approximately 2 g of Modified Rothera's reagent was taken in a dry clean test tube and urine was run down the inside of the test tube so that it forms a layer on the top of the reagent. Without mixing the contents, the tube was set aside for one minute. The presence of acetoacetic acid and acetone was indicated by the development of a violet colour.

The Modified Rothera's test was read on the basis of development of colour intensity as per Henry (1975) in milligrams of acetoacetic acid per decilitre of urine as follows

Grade	Colour intensity	Acetoacetic Acid (mg/dl)
	Buffy pink	Nil
Trace	Very light purple colour	5
+	Light purple colour	15
++	Purple colour	40
+++	Deep purple colour	80
++++	Very deep purple colour	80

Composition of Modified Rothera's reagent

Ammonium sulphate	100 g
Sodium carbonate anhydrous	50 g
Sodium nitroprusside	3 g.

Urine multidiagnostic strip and Modified Rothera's test on urine was carried out every 24 hourly to record the response of treatment.

Biochemical estimations

F.

Biochemical estimations of blood (serum) samples were done before and after treatment in each ketotic cow. Biochemical analyses was also done in 10 apparently healthy cows. Serum glucose analysis was carried out after every 24 hours to record the response of treatment in ketotic cows.

The blood/serum of apparently healthy, ketotic and recovered ketotic cows was analysed for

(a) Glucose by GOD/POD method by Tietz (1976).

(b) Serum alanine aminotransferase (ALT) by 2,4 - DNPH method of Reitman and Frankel (1956).

(c) Serum aspartate aminotransferase (AST) by 2,4 - DNPH method of Reitman and frankel (1956).

(d) Serum and urine ketone by method of Henry (1969).

(e) Calcium by Trinder's method (1960).

(l) Inorganic phosphorus by method of Gomorri (1942).

(g) Magnesium by titan yellow method of Niel and Neely (1956).

(h) Total protein by modified Biuret method of Doumas *et al.* **(1981).**

(i) Albumin by modified Biuret method of Doumas *et al.* (1971).

(j) Globulin was estimated in g/100 ml as difference between total protein and albumin.

(k) Urea nitrogen by DAM method of Coulambe and Favrean (1965).

(l) Serum sodium by modified method of Trinder (1951).

(m) Serum potassium by turbidometric method of Oscer (1979).

Treatment and Glucose utilization test in ketotic cows

Treatment consisting of 500 ml of 50% dextrose i.v. was given to each ketotic cow. Glucose utilization in blood/serum was conducted at 0, 2 and 4 hours interval after administration of 50% dextrose. Injection Triamcinolone Acetonide (Vetalog, Sarabhai Chemicals) 2 ml intramuscularly was given to each ketotic cow after glucose utilization test. Thereafter 50% dextrose (500 ml) was given after every 24 hours till the urine became negative to Modified Rothera's test and urine multidiagnostic strip (ketone).

Non-responding ketotic cows showing hyperglycaemia were treated with injection zinc insulin.

Glucose Tolerance Test

Glucose tolerance test was studied in six apparently healthy heifers weighing between 300 to 350 kg bat. These animals were fasted for 24 hours and were given intravenous injection of 50% dextrose (Sakai *et al.*, 1996) at the rate of 1. g/kg bat. Blood samples were collected at the beginning of the experiment (pre-treatment), immediately after administration of 50% dextrose and then every hour until 6 hours. Samples were placed on ice until clotted and then centrifuged. Serum was decanted into small pyrex tubes and stored at

-20°C and analysed for serum glucose immediately after the experiment.

Renal Threshold Test

Renal threshold test was done to determine renal threshold' of cattle. Six apparently healthy heifers were given 50% dextrose solution intravenously at the dose rate of 1 g/kg b.wt. The voided urine samples were collected in sterilized test tubes and urine glucose was monitored by urine multidiagnostic strip. Blood samples were collected simultaneously from jugular vein with every voided urine sample. The blood samples were placed on ice until clotted and then centrifused. Serum was decanted in small pyrex tubes and stored at -20°C and analysed for serum glucose. The experiment ended when urine multidiagnostic strip was negative for urine glucose.

Statistical Methods

The data obtained in the research work undertaken were statistically analysed and compared using standard formula given for mean, standard error, t-test and analysis of variance as per the procedures explained by Snedecor and Cochran (1968). Multiple comparisons of the means were done by using Duncan's new multiple range test as described by Steel and Torrie (1980).

RESULTS

Prevalence of bovine ketosis

In the present investigation entitled "clinico-biochemical studies on ketosis in cattle" 505 cows were screened for ketosis using Modified Rothera's test in and around Bikaner in the year 1998-99 (November - October). Fifty cows out of the total of 505 were diagnosed as ketotic. The overall prevalence of ketosis was 9.90 per cent. The disease mainly emerged in cows after they partur,cted. About one-fifth animals suffered from ketosis from the age of first parturition to the age of about 7 years. Thereafter the prevalence among such cows (aged between 8 and 9 years) increased and ketosis was found to affect around one-third of the studied population and abruptly dropped to less than 10 per cent above the age of 9 years. Thus the prevalence increased with the history of increasing milk yield. Similarly more than the double number of cows developed ketosis in their second to fifth parity as compared to the first and sixth when the lactation yield was higher. The maximum number of cases occurred during first and second month after parturition. The prevalence exhibited increased trend in colder months (winter) particularly following parturition in September-October. Results are presented in Table - 1.

Clinical manifestations in ketotic cows

All the 50 ketotic cows were reported to be sick from last 3 to 6 days with additional history of cessation of feeding on concentrate

TABLE 1 : PREVALENCE OF KETOSIS IN COWS

S.No.	Occurrence of Ketosis		Total Number of affected cows	Per cent Prevalence
1.	**Age of cows in**	< 6	10	20
	year	6-7	9	18
		7-8	11	22
		8-9	16	32
		>9	4	8
2.	Parity of cows	First	4	8
		Second	10	20
		Third	12	24
		Fourth	12	24
		Fifth	10	20
		Sixth	2	4
3.	Stage of Lactation	First	18	36
	in months	Second	22	44
		Third	5	10
		Fourth	3	6
		Fifth	2	4
4.	Month of the year	November	7	14
		December	5	10
		January	6	12
		February	4	8
		March	6	12
		April	4	8
		May	3	6
		June	3	6
		July	4	8
		August	3	6
		September	2	4
		October	3	6

(cottonseed cake and ground pulse mix), decreased consumption of fodder, marked decrease in milk yield, change in taste of milk and loss of body condition. The clinical examination of these animals revealed loss of elasticity of skin, grinding of teeth, absence of rumination, mucus-coated faeces. Acetone-like odour in urine was detected in 60 per cent advanced cases (30 out of 50). The mean values for rectal temperature, pulse rate, respiratory rate and ruminal movements for diseased and healthy cows and milk-yield before the onset of disease and during disease are given in Table-2. These were the cases of wasting form of ketosis. No case of nervous form was encountered during the period of study.

Biochemical parameters in healthy and ketotic cows

The biochemical parameters in 50 ketotic and 10 healthy cows were estimated during disease and after recovery, i.e. pre and post-treatment are given in Table - 3.

The mean values of serum ketone (Acetone + Acetoacetic acid) in healthy cows was 1.21±0.10 mg/dl whereas its mean values in ketotic cows during disease and after recovery were 10.46±0.32 mg/dl and 1.36±0.29 mg/dl, respectively. Such values for urine ketones were 1.66±0.12, 26.67±1.13 and 1.92±0.04 mg/dl, respectively.

The mean value of serum glucose in mg/dl in healthy cows was 55.47±1.15 whereas its mean values in ketotic cows during disease and after recovery were 33.18±1.09 and 51.36±0.32, respectively.

The mean values of total serum protein in healthy cows and in ketotic cows during disease and after recovery were 7.64±0.11

TABLE 2 : MEAN±SE OF CLINICAL PARAMETERS OF HEALTHY AND KETOTIC COWS.

S.No.	Type of cows	Body temperature in °F	Pulse/min.	Respiration/ min.	Ruminal movements/2 min.	Reported milk yield in litres/day	
						Before illness	During illness
1.	Healthy (n=10)	101.4±0.23	51.5±1.57	18.6±0.58	3.0±0.21	-	-
2.	Ketotic (n=50)	100.78±0.33	59.96±0.24	20.42±0.32	1.64±0.09	15.06±0.30	8.22±0.14

Table 3 : Mean ± SE of biochemical parameters in healthy and ketotic cows,

S.No.	Parameters	Healthy Cows (n=10)	Ketotic Cows (n=50)	
			Before treatment	After treatment
1.	Serum Ketones (mg/dl)	1.21±0.10	10.46±0.32**	1.36±0.29
2.	Urine Ketones (mg/dl)	1.66±0.12	26.67±1.13 * *	1.92±0.04
3.	Blood glucose (mg/dl)	55.47±1.15	33.18±1.09**	51.36±0.32*
4.	Total Serum protein (g/dl)	7.64±0.11	10.68±0.20**	8.14±0.09
5.	Serum albumin (g/dl)	3.71±0.14	4.68±0.15**	3.67±0.12
6.	Serum globulin (g/dl)	3.92±0.10	5.99±0.13**	4.46±0.11
7.	A/G Ratio	0.95±0.05	0.79±0.02*	0.88±0.05
8.	Blood urea nitrogen (mg/dl)	16.79±1.59	29.86±1.31**	20.13±0.71
9.	Serum calcium (mg/dl)	10.10±0.25	8.24±0.15**	9.98±0.11
10.	Serum inorganic phosphorus (mg/dl)	5.11±0.19	4.79±0.13	5.45±0.10
11.	Serum magnesium (mg/dl)	2.77±0.25	3.83±0.07**	2.76±0.12
12.	Serum sodium (mEq/1)	139.42±1.69	95.47±2.28**	128.57±2.05*
13.	Serum potassium (mEq/1)	4.68±0.14	2.59±0.06**	3.87±0.09**
14.	Serum ALT (U/ml)	26.20±2.02	56.86±2.75**	36.43±1.88*
15.	Serum AST (U/ml)	47.20±3.42	95.22±1.56**	71.95±0.90**

* Variations in mean values were significant ($p<0.05$) when compared with the mean values of healthy cows.

** The variations in mean values were highly significant ($p<0.01$) when compared with the mean values of healthy cows.

g/dl, 10.68±0.20 g/dl and 8.14±0.09 g/dl, respectively. The mean values for serum albumin and serum globulin in healthy cows were 3.71±0.14 and 3.92±0.10 g/dl, respectively. The mean values of these in ketotic cows during disease were 4.68±0.15 and 5.99±0.13 g/dl, respectively. Such values in ketotic cows after recovery were 3.67±0.12 and 4.46±0.11 g/dl, respectively. The mean serum albumin globulin ratio in healthy and ketotic cows during disease and after recovery was 0.95±0.05 and 0.79±0.02 and 0.88±0.05, respectively.

The mean values for blood-urea -nitrogen in healthy cows was 16.79±1.59 mg/dl, whereas its mean values in ketotic cows during disease and after recovery were 29.86±1.31 mg/dl and 20.13±0.71 mg/dl, respectively.

The mean values for serum calcium, serum inorganic phosphorus and serum magnesium in healthy cows were 10.10±0.25 mg/dl, 5.11±0.19 mg/dl and 2.77±0.25 mg/dl, respectively. Such values in cows affected with ketosis were 8.24±0.15, 4.79±0.13 and 3.83±0.07 mg/dl, respectively. The means for such values after recovery in the affected cows were 9.98±0.11, 5.45±0.10 and 2.76±0.12 mg/dl, respectively.

The mean values for serum sodium and serum potassium in healthy cows were 139.42±1.69 mEq/1 and 4.68±0.14 mEq/l, respectively. Such values for these parameters during ' disease were 95.47±2.28 and 2.59±0.06 mEq/1, respectively, and after recovery were 128.57±2.05 and 3.87±0.09 mEq/1, respectively.

The mean values of serum alanine aminotransferase and serum aspartate aminotransferase in healthy cows were 26.20±2.02 and 47.20±3.42 U/ml, respectively. The mean values of these parameters during disease were 56.86±2.75 and 95.22±1.56 U/ml, respectively. Such values after recovery in affected cows were 36.43±1.88 and 71.95±0.90 U/ml, respectively.

Biochemical status of ketotic cows as per Rothera's classification

The cases in which urine gave Rothera's reaction for trace were not incorporated in the study. The results on biochemical status of the fifty studied ketotic cows before and after treatment and ten healthy animals are presented in Table - 4.

Biochemical changes in ketotic cows as per degree of ketonaemia

All the fifty ketotic cows subjected to present investigation were grouped according to Rothera's reaction of urine as +, ++, +++ and ++++. The biochemical changes in these groups, were determined in relation to degree of ketonaemia in them. The degree of ketonaemia was positively correlated with ketonuria and intensity of Rothera's reaction of urine. When mean value of ketonaemia was 7.06±0.17, 9.35±0.25, 11.53±0.11 and 12.84±0.12 mg/dl, the mean value of ketonuria was 13.44±1.06, 26.98±1.05, 29.15±0.60 and 34.01±0.53 mg/dl and the Rothera's reaction +, ++, +++ and ++++, respectively in the four groups of ketotic cows. The results are presented in Table-4 and Figure 1 to 14.

TABLE 4 : BIOCHEMICAL STATUS OF HEALTHY AND KETOTIC COWS AS PER MODIFIED **ROTHERA'S TEST** CLASSIFICATION (BEFORE TREATMENT)

S.No.	Parameters	Healthy Cows (n=10)	Ketotic Cows (n=50)			
		Rothera's -	Rothera's + (n=11)	Rothera's ++ (n=11)	Rothera's +++ (n=13)	Rothera's ++++ (n=15)
1.	Serum Ketones (mg/dl)	1.21±0.10[8]	7.06±0.17[b]	9.35±0.25`	11.53±0.11[d]	12.84±0.12`
2.	Urine Ketones (mg/dl)	1.66±0.12[a]	13.44±1.06[b]	26.98±1.05[c]	29.15±0.60[4]	34.01±0.53[e]
3.	Blood glucose (mg/dl)	55.47±1.15[°]	37.97±1.59[b]	37.45±1.12[b]	31.61±1.28[8]	27.88±2.51a
4.	Total Serum protein (g/dl)	7.64±0.1 I[a]	9.28±0.30[b]	10.74±0.38[c]	10.80±0.37[°]	11.56±0.35[°]
5.	Serum albumin (g/dl)	3.71±0.14[8]	4.29±0.13[8bc]	4.16±0.30[8b]	4.81±0,28[bcd]	5.25±0.34[4]
6.	Serum globulin (g/dl)	3.92±0.10[8]	4.99±0.22[b]	**6.58±0.28[4]**	5,98±0.16[c]	6.30±0.51[cd]
7.	A/G Ratio	0.95±0.05	0.86±0.03'[bc]	0.65±0.06[a]	0.80±0.04'	0.84±0.06'
8.	Blood urea nitrogen (mg/dl)	16.79±1.59[8]	28.72±1.67[bc]	32.27±2.96[bc]	25.07±2.42[b]	33.06±2.65[c]
9.	Serum calcium (mg/dl)	10.10±0.25[b]	8.68±0.29[b]	8.66±0.36[b]	8.38±0.25[b]	7.47±0.21[8]
10.	Serum inorganic phosphorus (mg/dl)	5.11±0.19[b]	4.84±0.32[b]	4.65±0.32[8]	4.79±0.19[b]	4.85±0.2[Ib]
11.	Serum magnesium (mg/dl)	2.77±0.25[a]	3.62±0.19[b]	4.09±0.15[b]	3.84±0.17[b]	3.80±0.10[b]
12.	Serum sodium (mEq/l)	139.42±1.69[c]	110.79±2.30[b]	88.31±7.12[8]	97.56±2.14[a]	87.68±2.94[8]
13.	Serum potassium (mEq/1)	4.68±0.14[c]	2.55±0.26[ab]	2.40±0.07[8]	2.49±0.07[ab]	2.85±0.06[b]
14.	Serum ALT (U/ml)	26.20±2.02[8]	36.09±4.48[8]	53.45±5.62[b]	65.84±4.00[c]	66.80±3.81[c]
15.	Serum AST (U/ml)	47.20±3.42[8]	89.81±1.71[b]	98.00±3.80[b]	99.38±3.68[b]	93.53±2.48[b]

TABLE 5 : BIOCHEMICAL STATUS OF HEALTHY AND KETOTIC COWS AS **PER MODIFIED ROTHERA'S TEST CLASSIFICATION (AFTER TREATMENT)**

S.No.	Parameters	Healthy Cows (n=10)	Ketotic Cows (n=50)			
		Rothera's -	Rothera's + (n=11)	Rothera's ++ (n=11)	Rothera's +++ (n=13)	Rothera's ++++ (n=15)
1.	Serum Ketones (mg/dl)	1.21±0.10$	1.39±0.06 abcd	1.25±0.06ab	1.32±0.04 abc	1.46±0.04cd
2.	Urine Ketones (mg/dl)	1.66±0.12 ab	1.90±0.08 abc	1.66±0. 1Oa	1.87±0.06 abc	2.17±0.07d
3.	Blood glucose (mg/dl)	55.47±1.15c	50.77±0.66ab	52.50±0.52a	50.73±0.33ab	51.49±0.83ab
4.	Total Serum protein (g/dl)	7.64±0.11ab	7.62±0.28a	8.07±0.19abc	8.28±0.13c	8.46±0.11c bcd
5.	Serum albumin (g/dl)	3.71±0.14 abcd	3.34±0.14a	3.64±0.24abc	3.52±0.28ab	4.09±0.24
6.	Serum globulin (g/dl)	3.93±0.108	4.27±0.16ab	4.43±0.22ab	4.76±0.23b	4.37±0.26ab
7.	A/G Ratio	0.95±0.05a	0.78±0.02a	0.86±0.09a	**0.80±0.1 ı**a	1.04-0.13a
8.	Blood urea nitrogen (mg/dl)	16.79±1.59$	19.54±1.39ab	20.54±1.59ab	17.69±1.05a	22.40±1.39b
9.	Serum calcium (mg/dl)	10.10±0.25b	10.35±0.13b	**10.30±0.2 ı**b	10.07±0.23b	9.39±0.21a
10.	Serum inorganic phosphorus (mg/dl)	5.11±0.19a	5.96±0.11b	5.27±0.28a	5.30±0.18a	5.35±0.17a
11.	Serum magnesium (mg/dl)	2.77±0.25a	3.49±0.46b	2.52±0.15a	2.32±0.13a	2.57±0.09a
12.	Serum sodium (mEq/1)	139.42±1.69b	130.68±2.80ab	126.63±6.46a	129.46±1.24ab	127.67±4.64ab
13.	Serum potassium (mEq/1)	4.68±0.14b	3.86±0.24a	3.69±0.05a	3.95±0.20a	3.94±0.22a
14.	Serum ALT (U/ml)	26.20±2.02a	23.09±2.29a	31.54±2.99a	43.38±3.12b	43.79±3.08b bc
15.	Serum AST (U/ml)	47.20±3.42a	67.72±1.52b	75.36±2.15c	73.53±1.96bc	71.19±1.17

The mean serum glucose levels exhibited a declining trend with the increase in degree of ketonaemia in the four groups of ketotic cows.

The total serum protein rose with the rise in ketonaemia in all the four group of ketotic cows. Serum albumin and globulin levels after fluctuating on either side finally rose substantially in the last group that had highest degree of ketonaemia and had Rothera's reaction ++++. There were similar changes in serum albumin and globulin ratio without any substantial change in last group of the ketotic cows.

Blood-urea-nitrogen concentration after showing a mixed trend attained peak in the last group of ketotic cows that had developed highest degree of ketonaemia.

The serum calcium levels had a declining trend with the increase in degree of ketonaemia whereas serum phosphorus and magnesium levels followed mixed trend in these four groups of ketotic cows. Such mixed trends were also observed for serum sodium and potassium.

The serum alanine aminotransferase and aspartate aminotransferase activities exhibited increasing and mixed trend respectively, in the four groups of ketotic cows.

Biochemical changes **in ketotic cows as per degree of ketonuria**

Biochemical changes in ketotic cows as per degree of ketonuria were identical to those already mentioned according to

Fig. 1 : Relation Between Serum Ketones and Urine Ketones

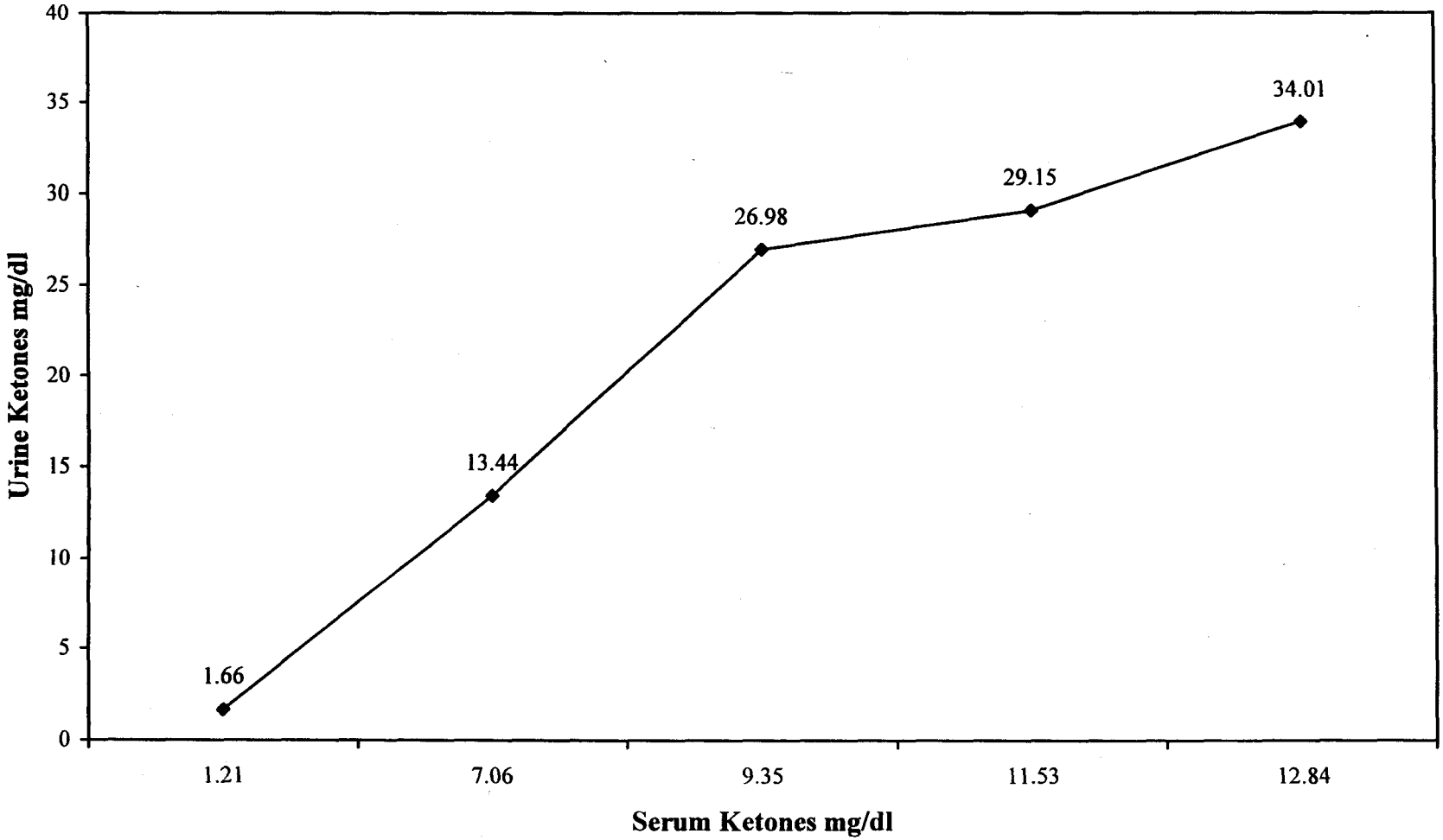


Fig. 2 : Relation Between Serum Ketones and Serum Glucose

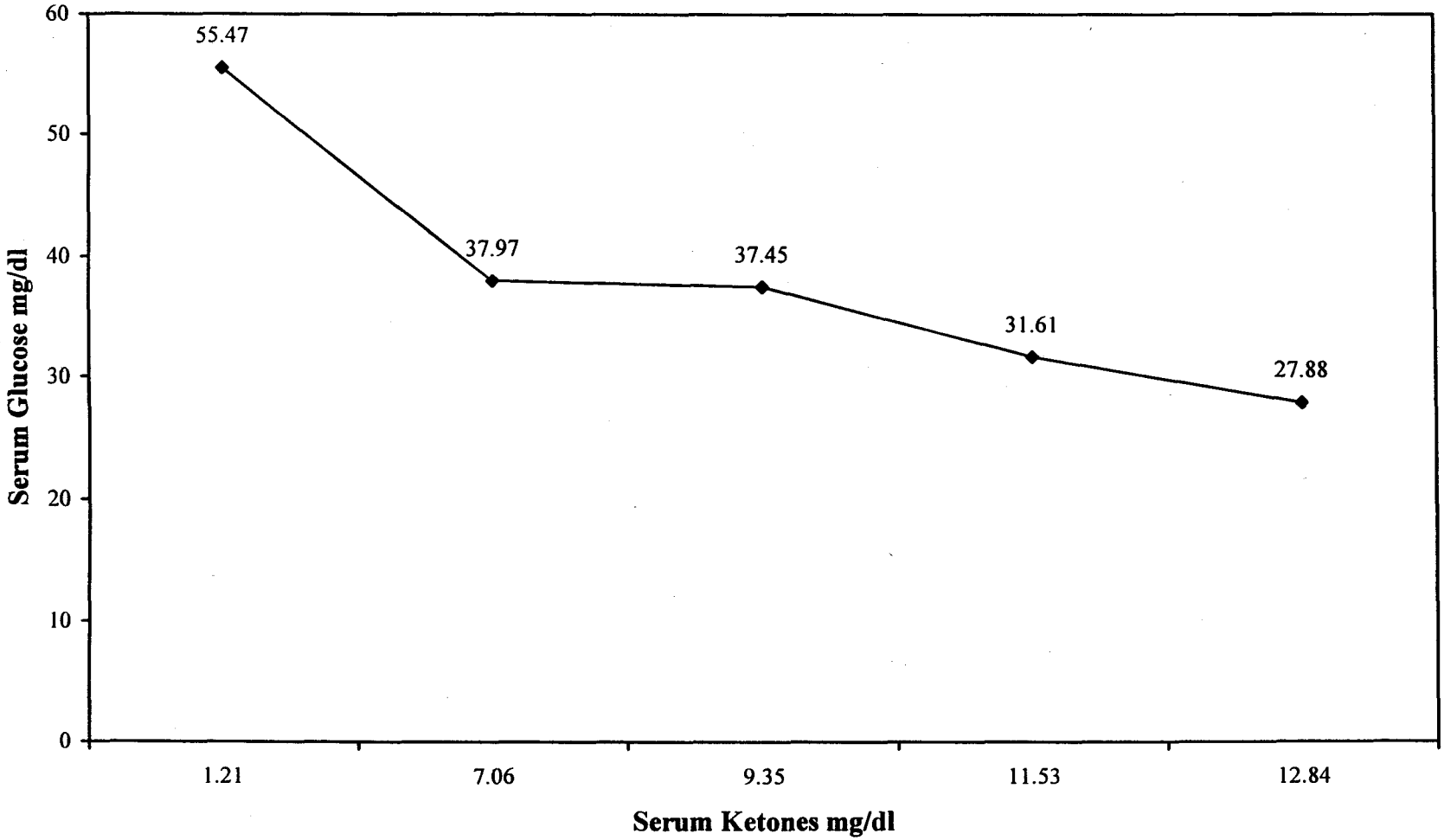


Fig. 3 : Relation Between Serum Ketones and Total Protein

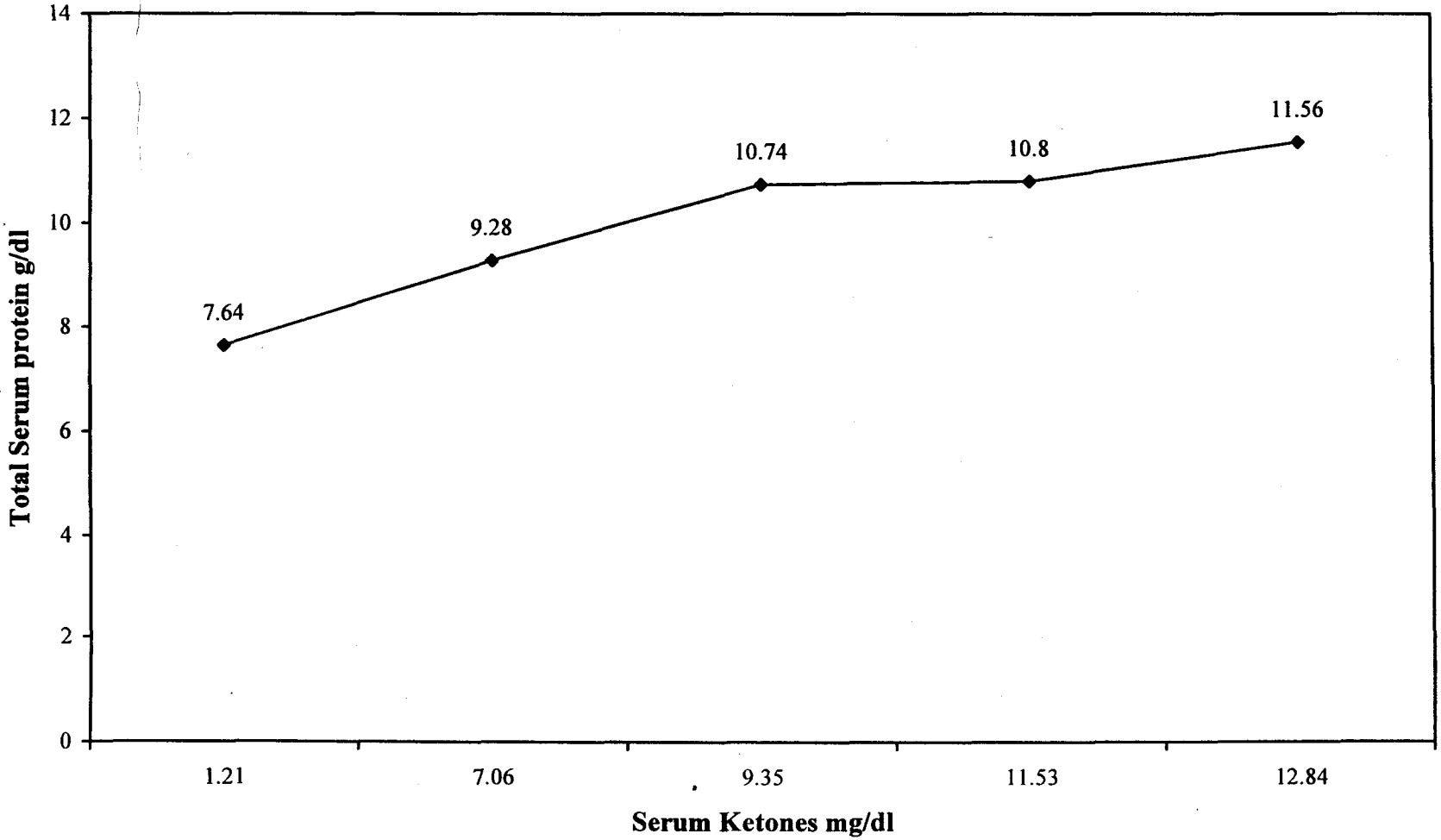


Fig. 4 : Relation Between Serum Ketones and Serum Albumin

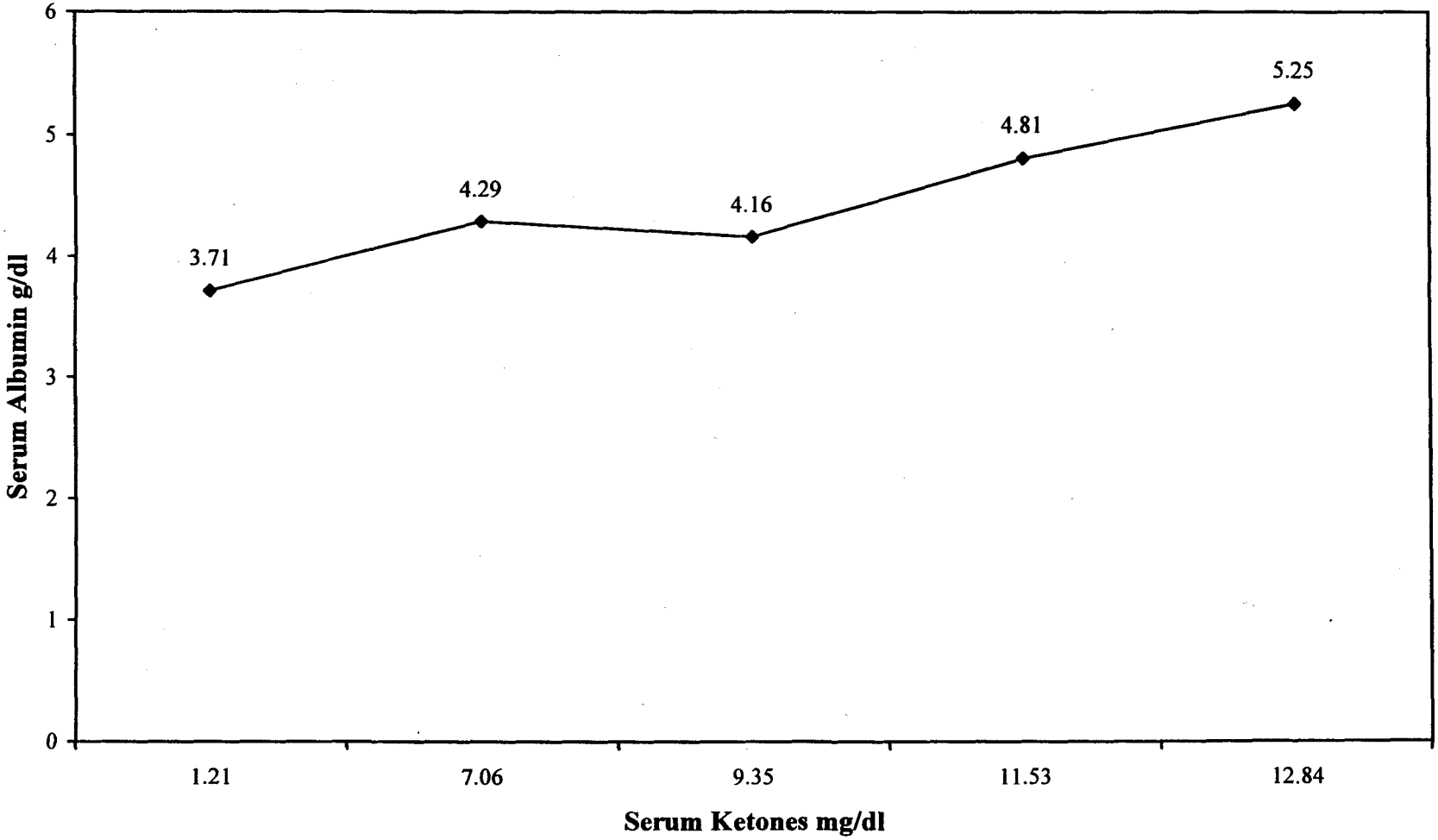


Fig. 5 : Relation Between Serum Ketones and Serum Globulin

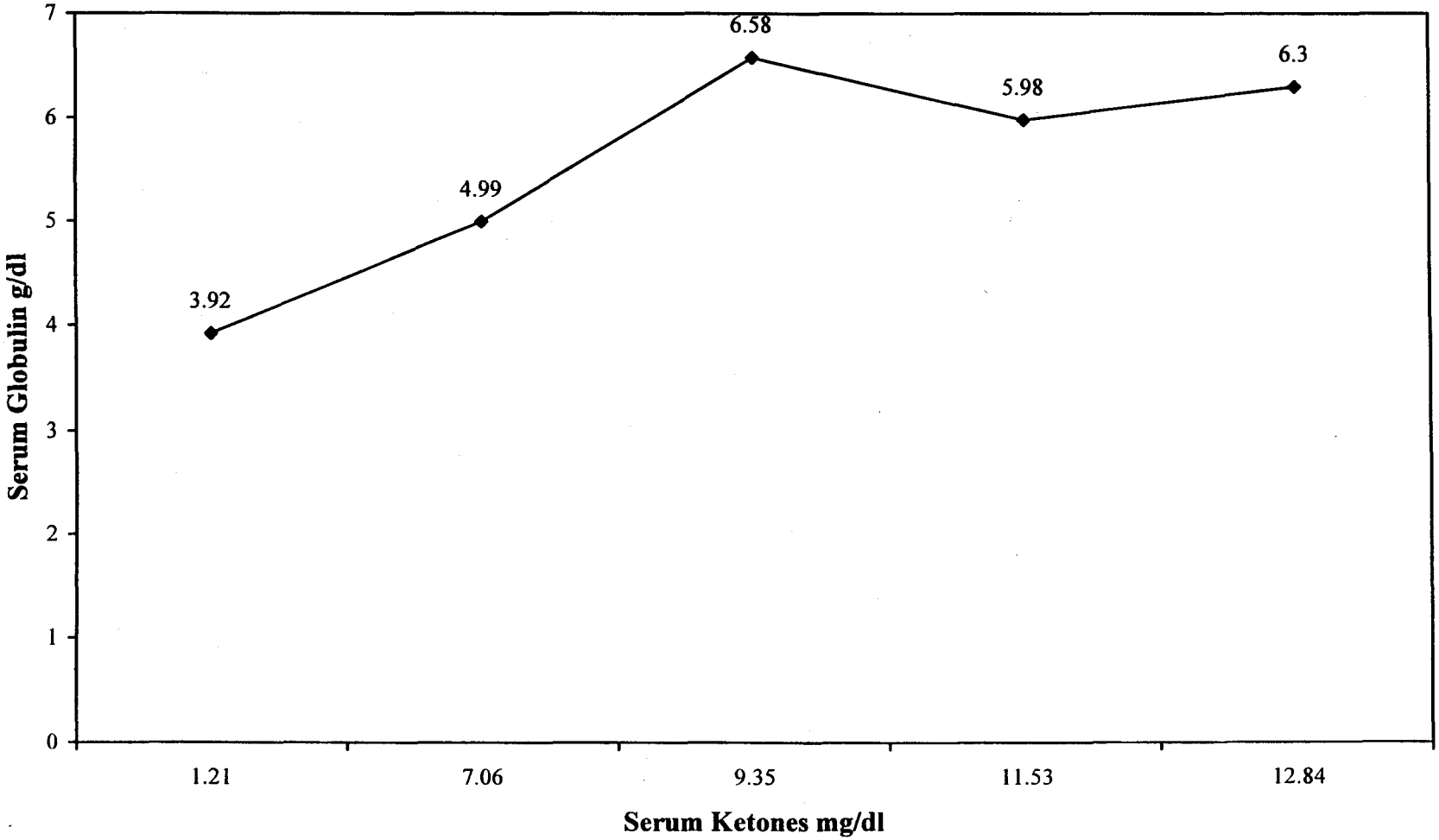


Fig. 6 : Relation Between Serum Ketones and A : G Ratio

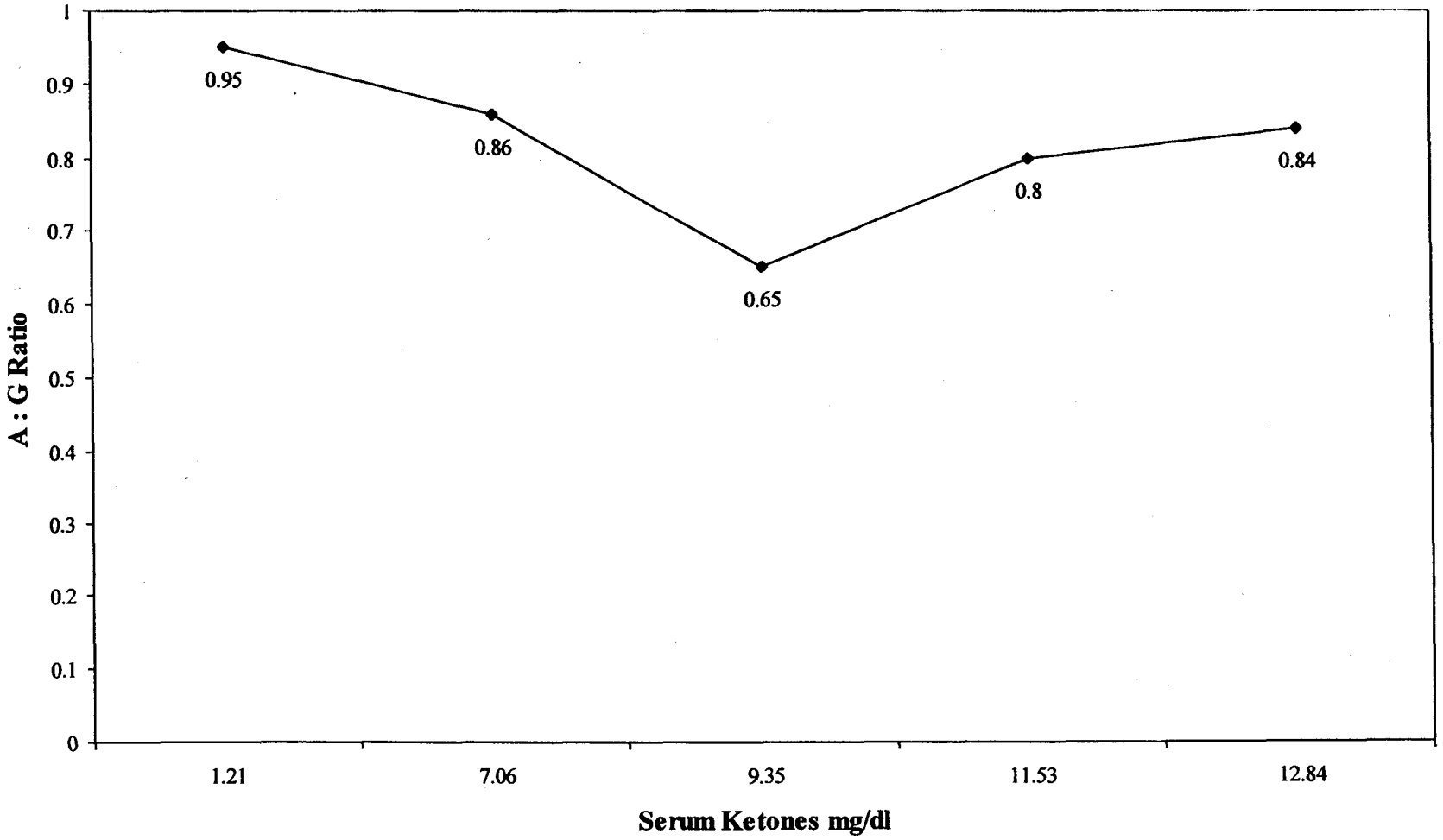


Fig. 7 : Relation Between Serum Ketones and BUN

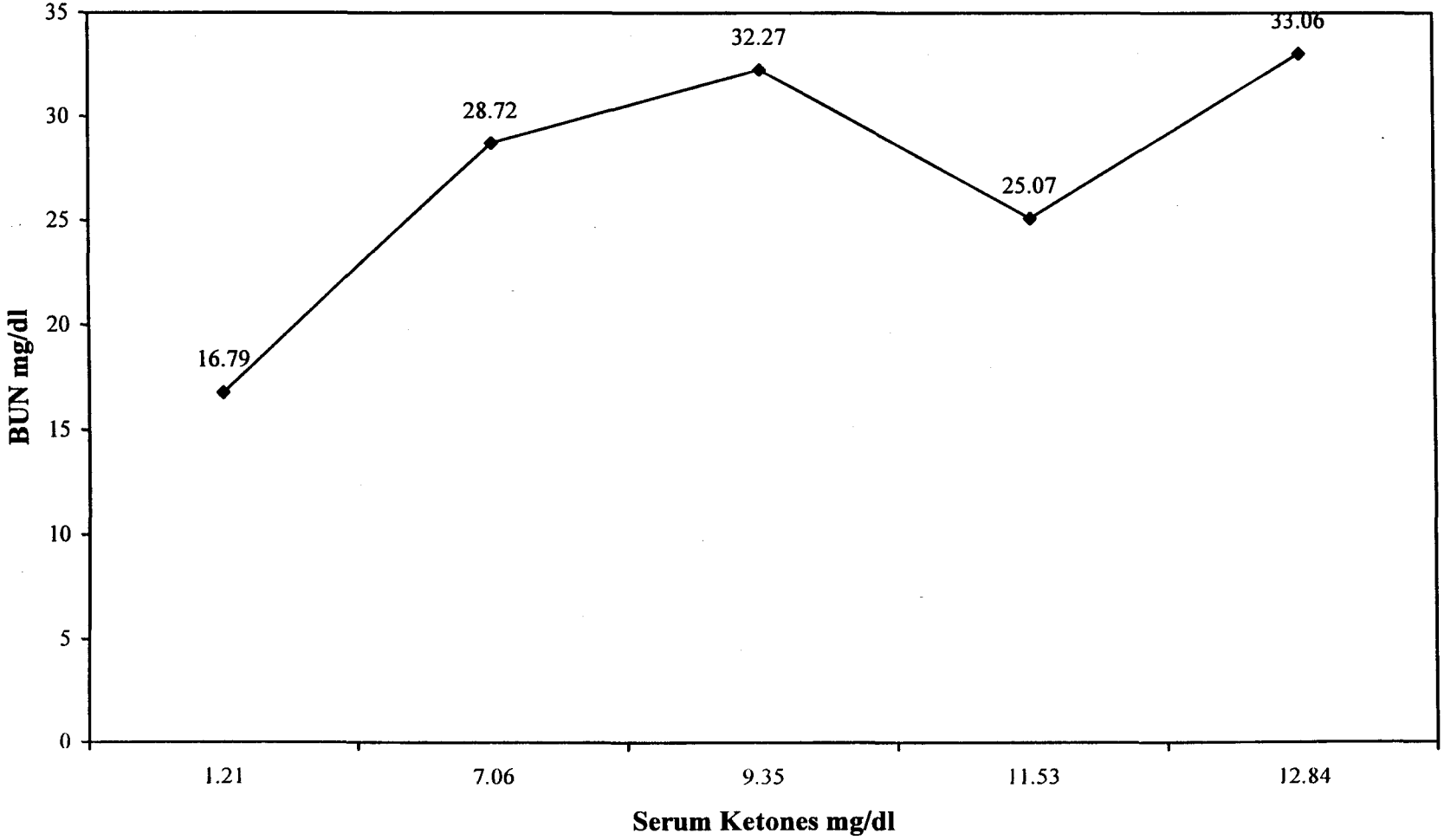


Fig. 8 : Relation Between Serum Ketones and Serum Calcium

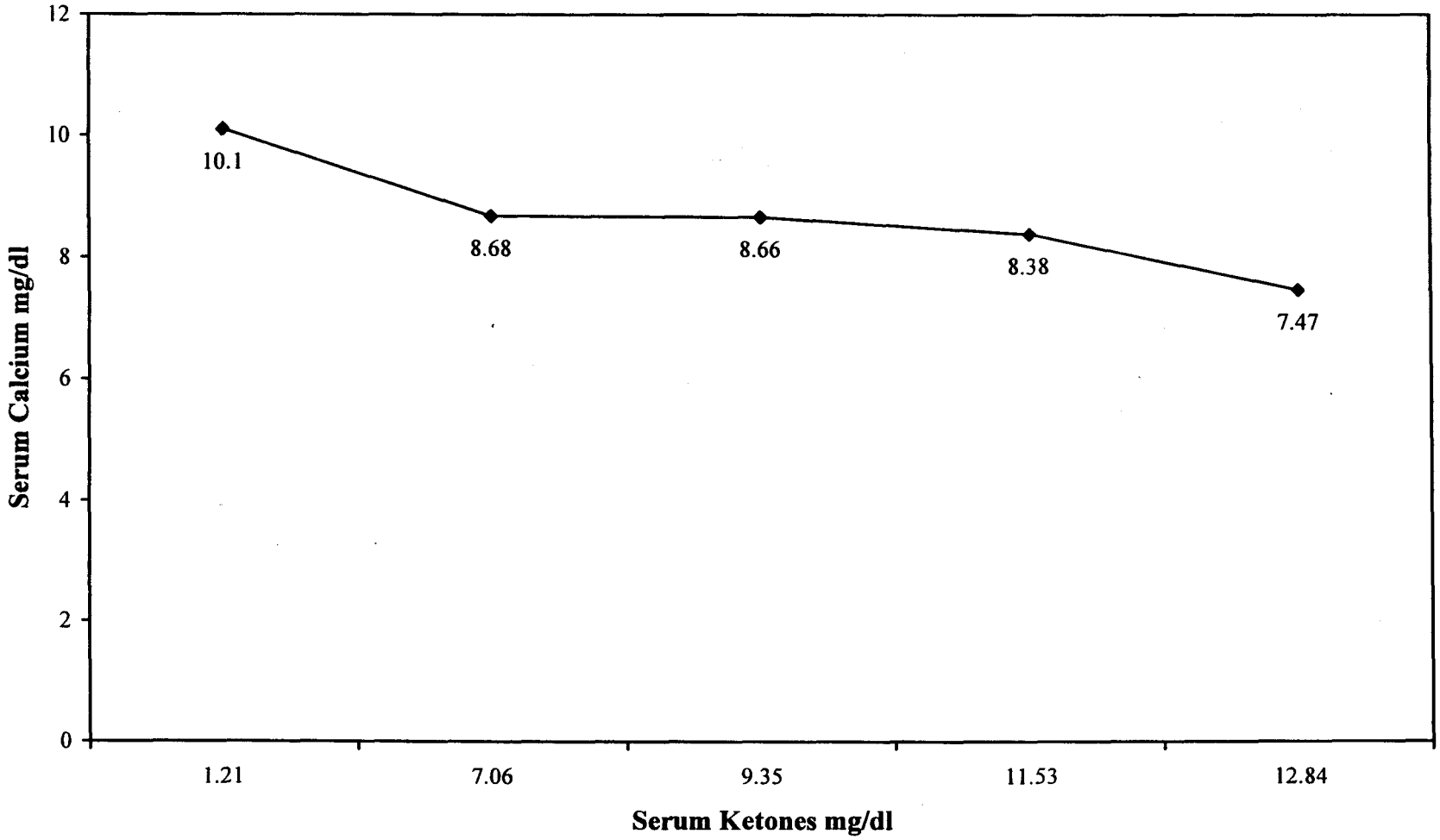


Fig. 9 : Relation Between Serum Ketones and Serum Phosphorus

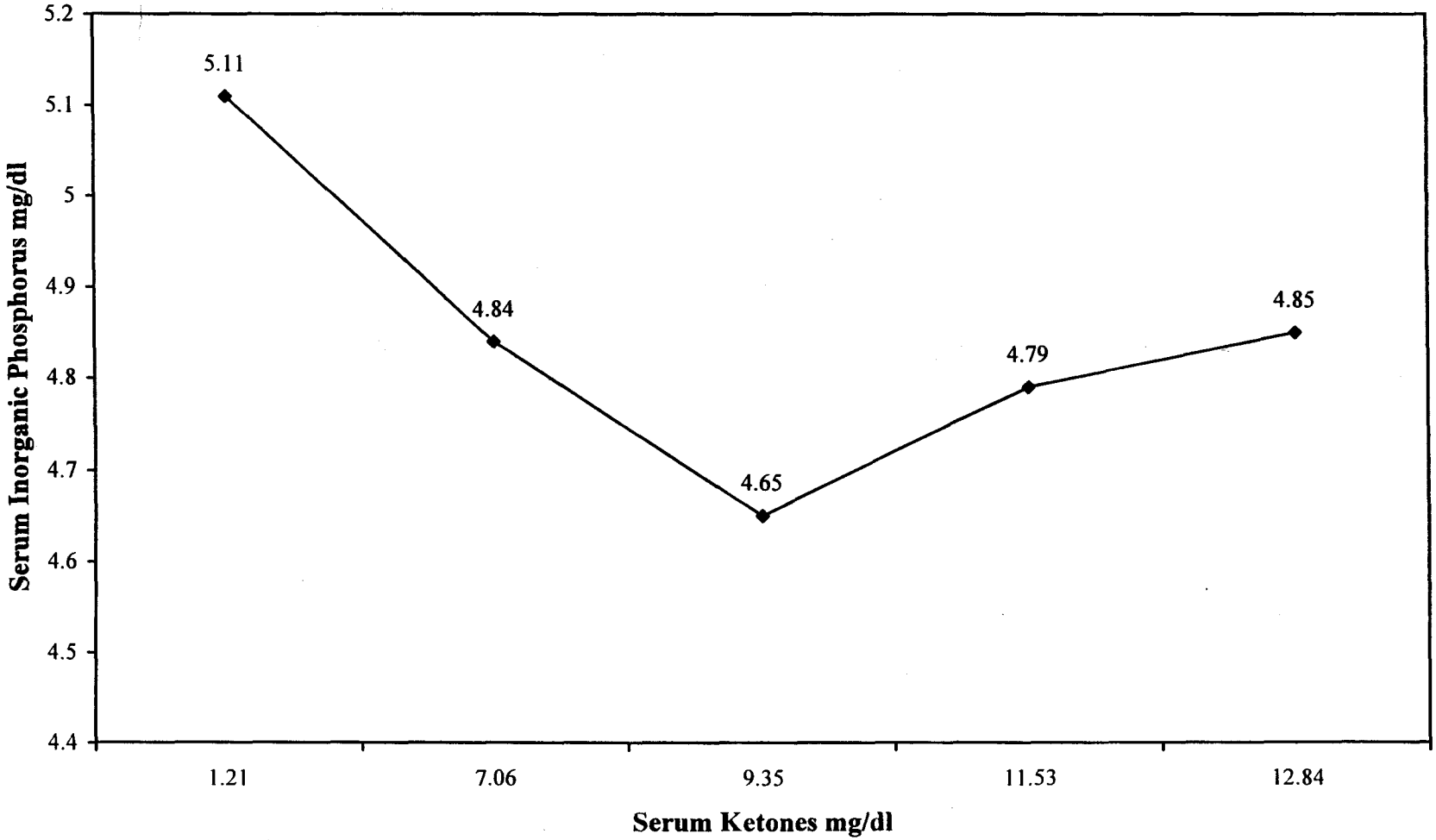


Fig. 10 : Relation Between Serum Ketones and Serum Magnesium

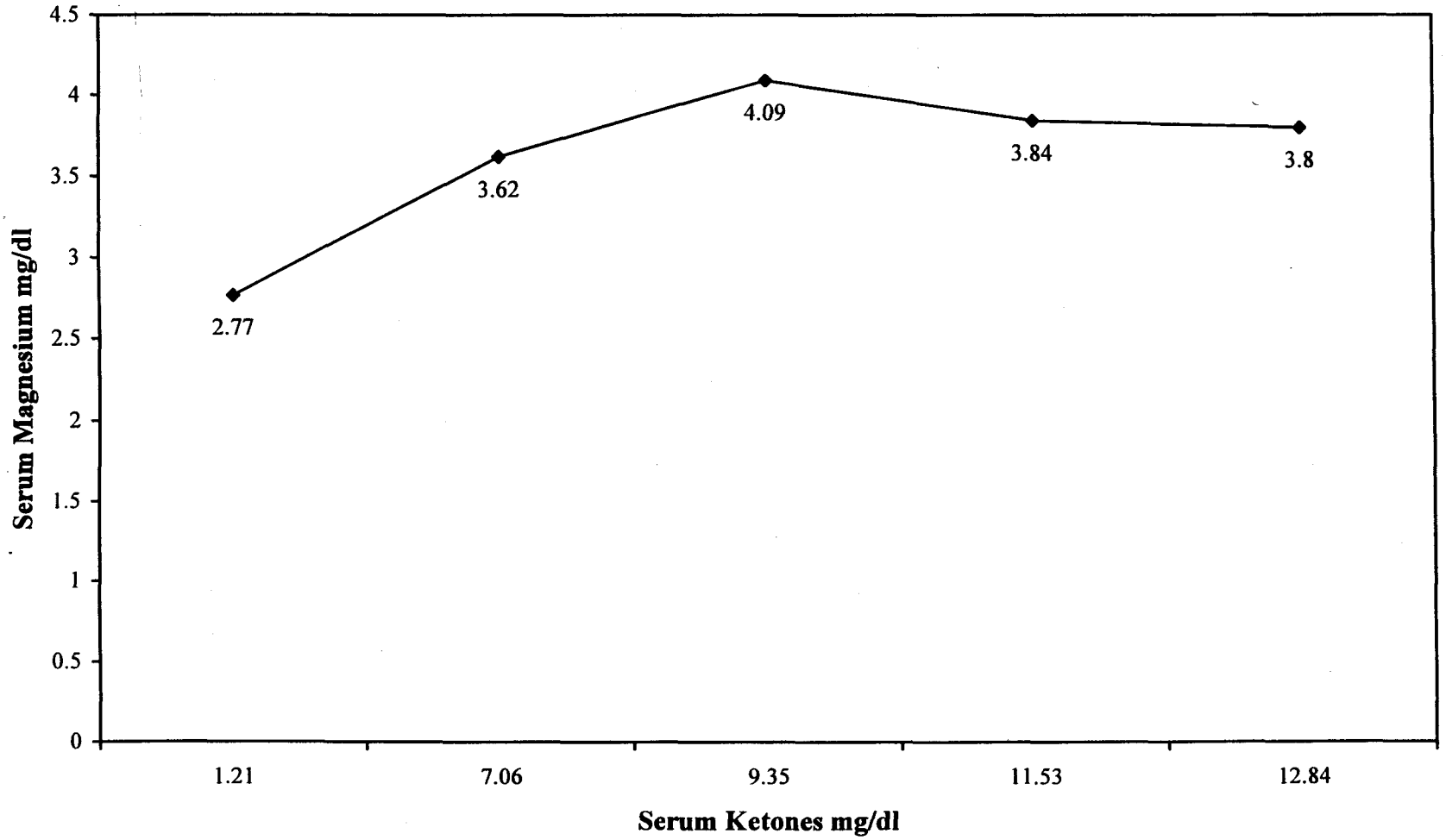


Fig. 11 : Relation Between Serum Ketones and Serum Sodium

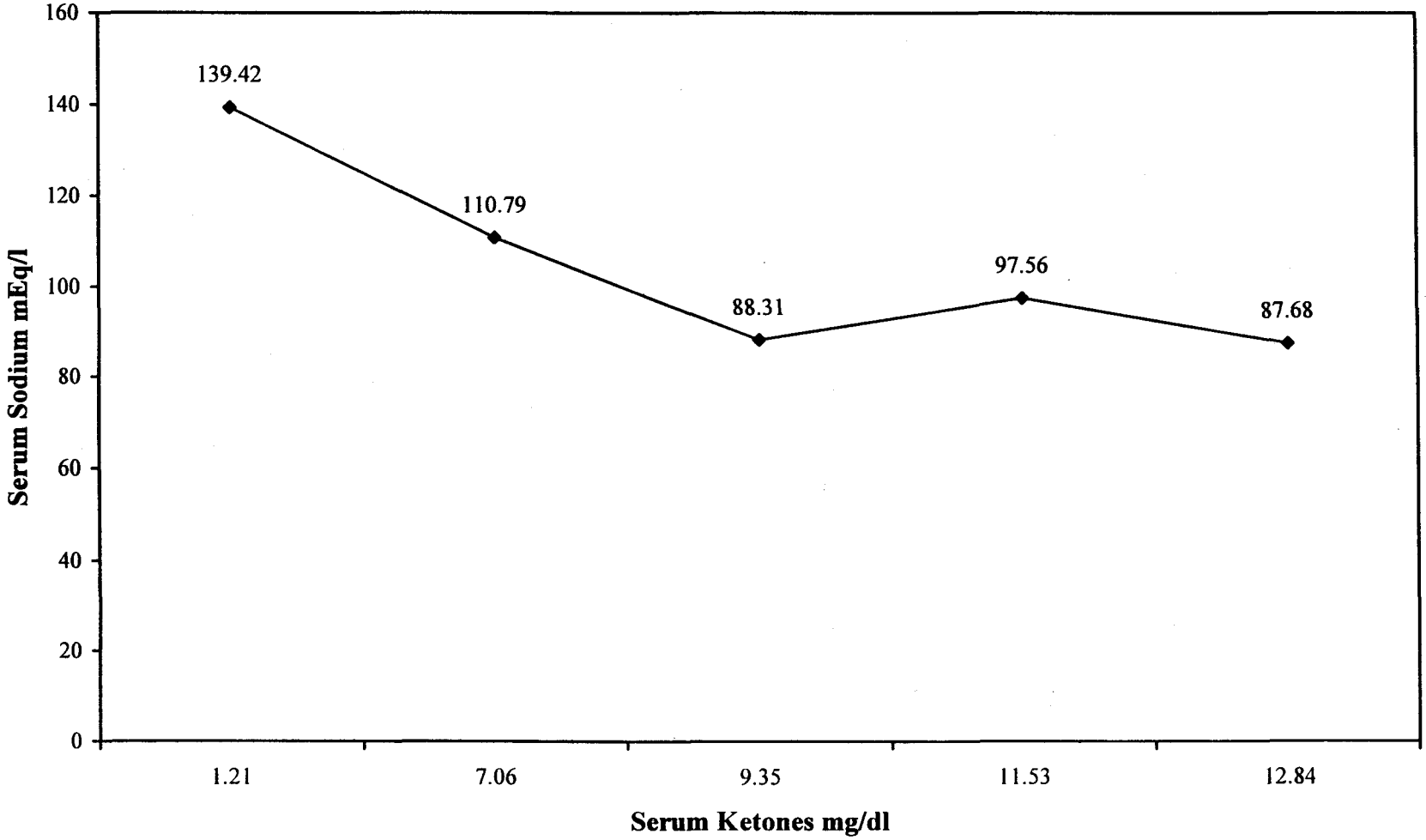


Fig. 12 : Relation Between Serum Ketones and Serum Potassium

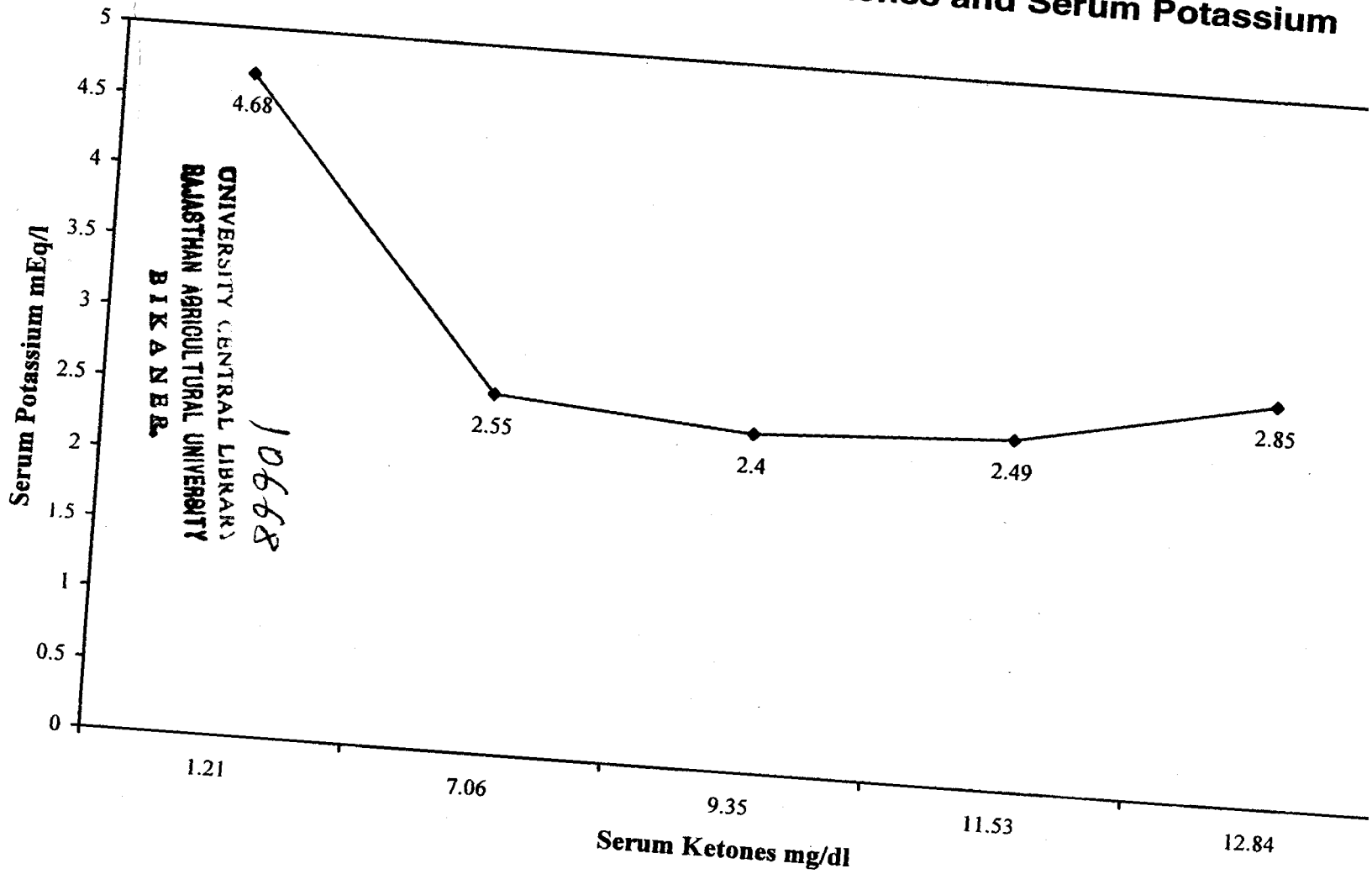


Fig. 13 : Relation Between Serum Ketones and Serum ALT

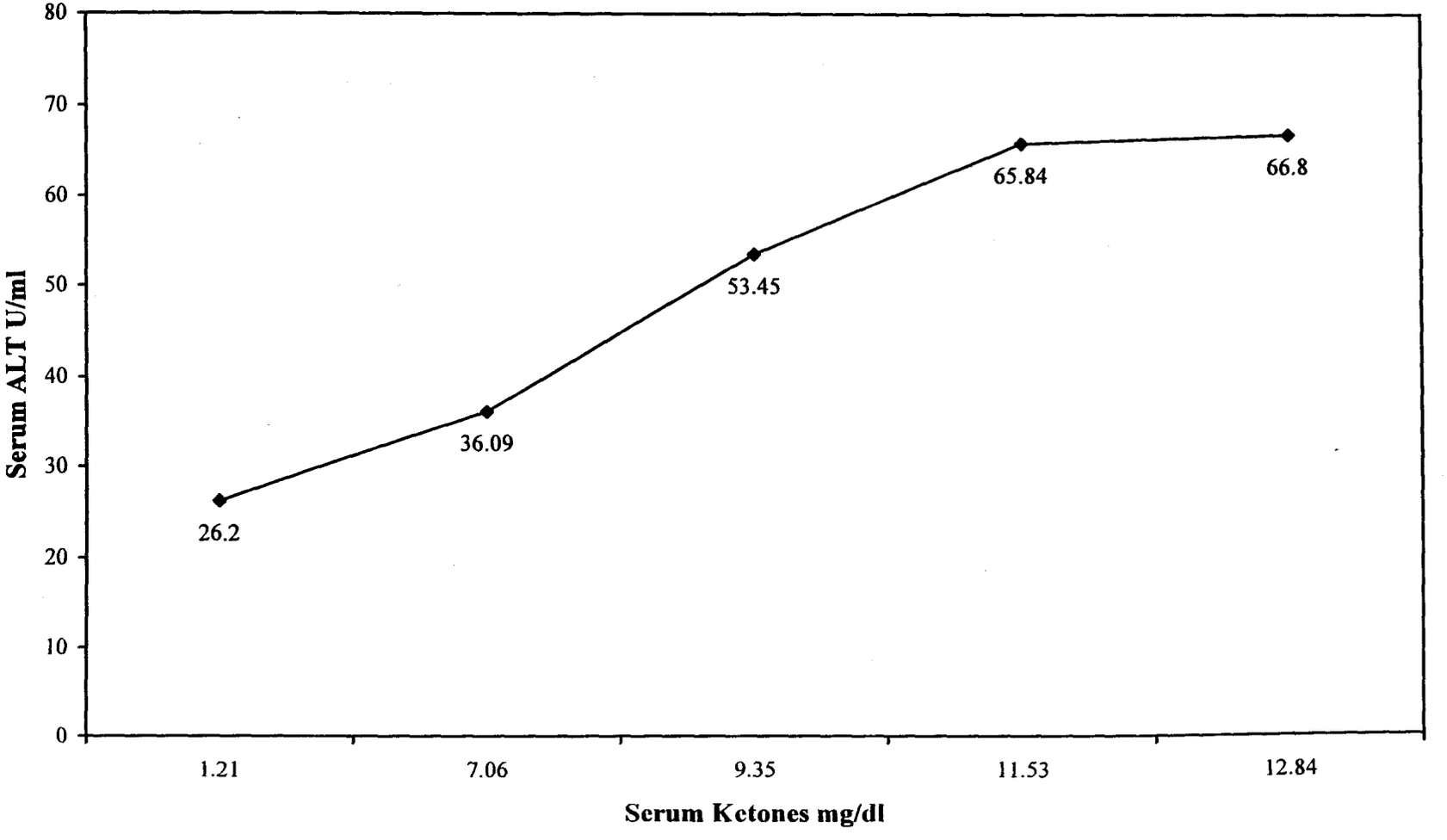


Fig. 14 : Relation Between Serum Ketones and Serum AST

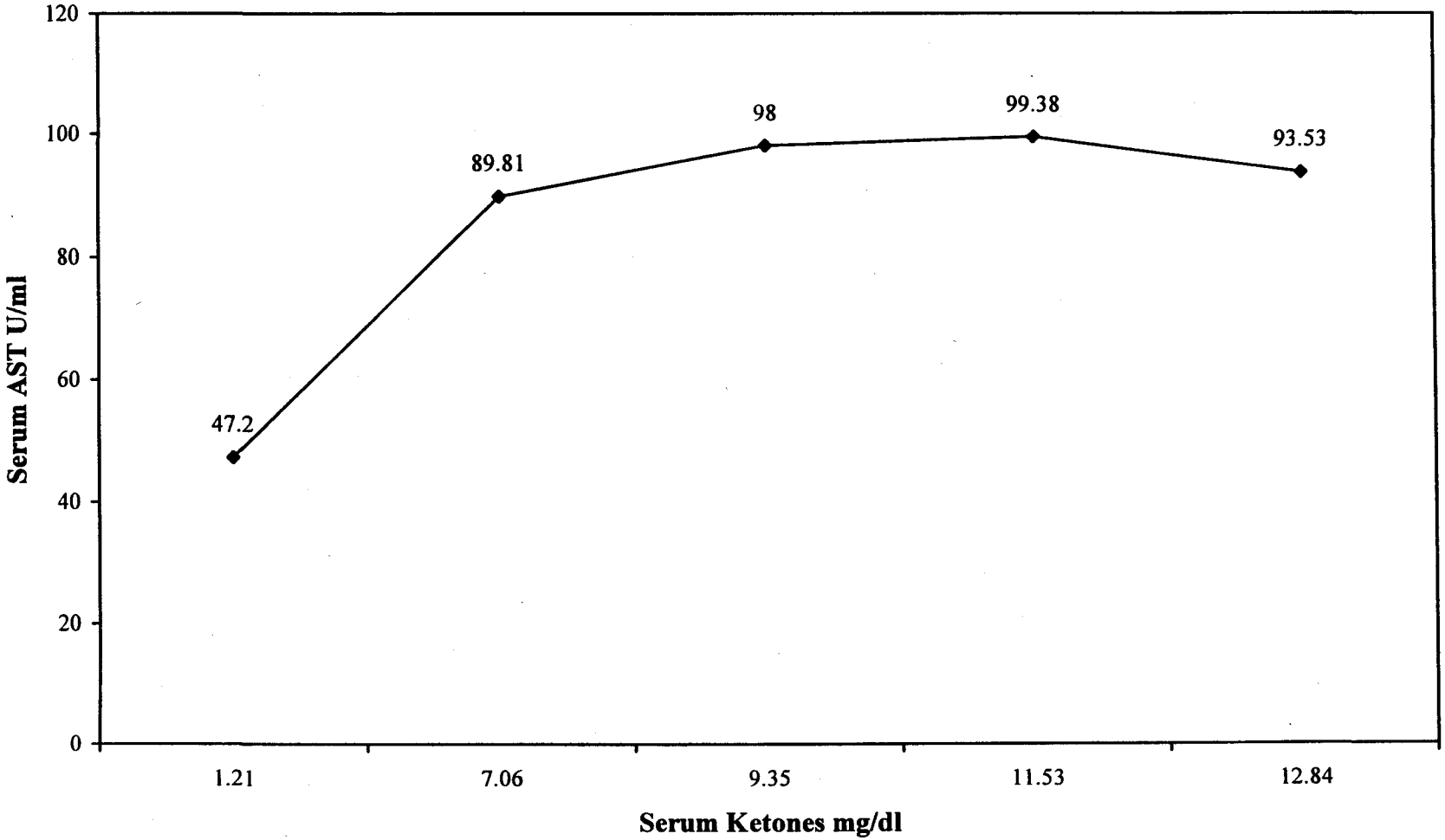


Fig. 15 : Relation Between Urine Ketones and Serum Ketones

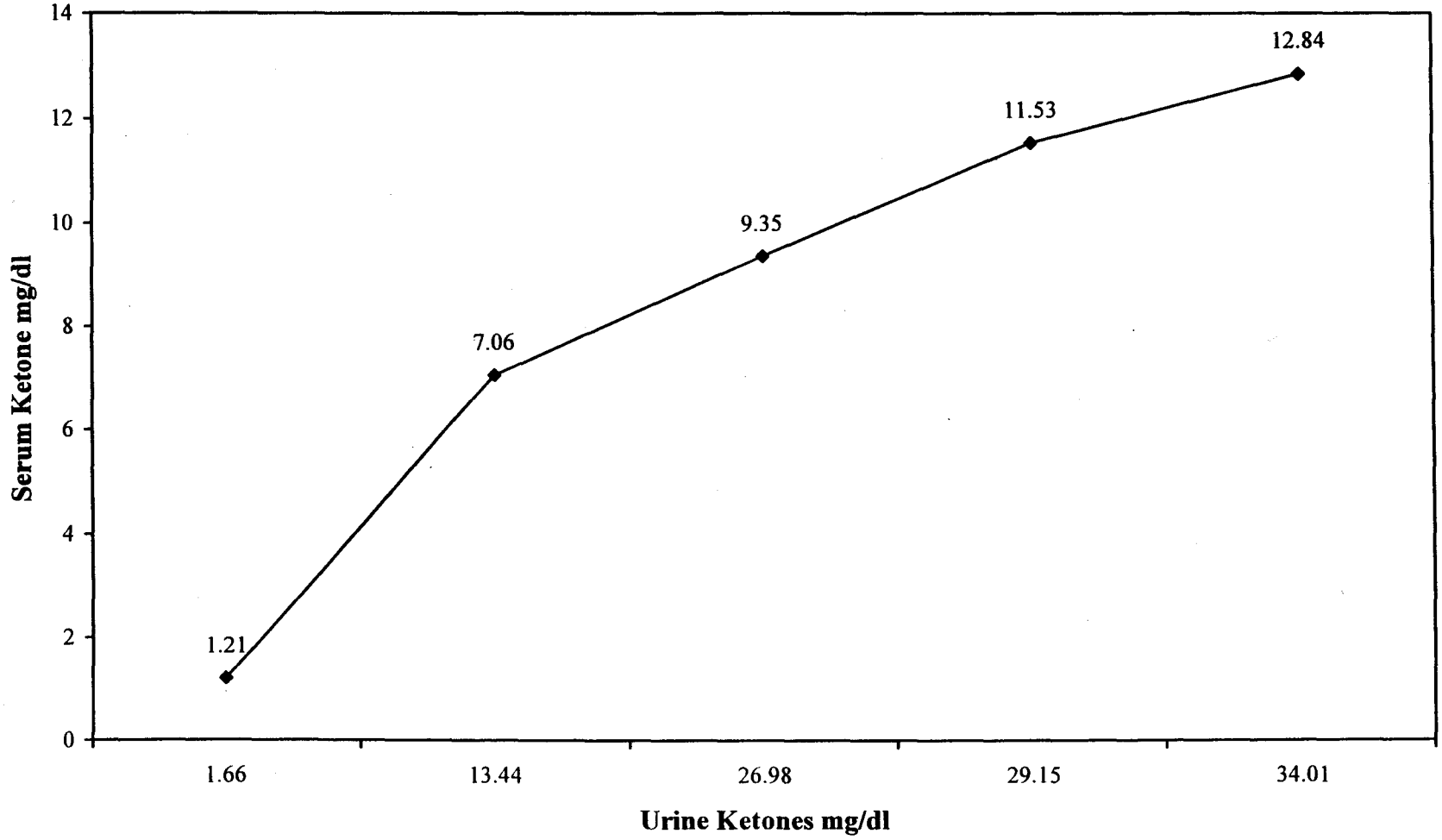


Fig. 16 : Relation Between Urine Ketones and Serum Glucose

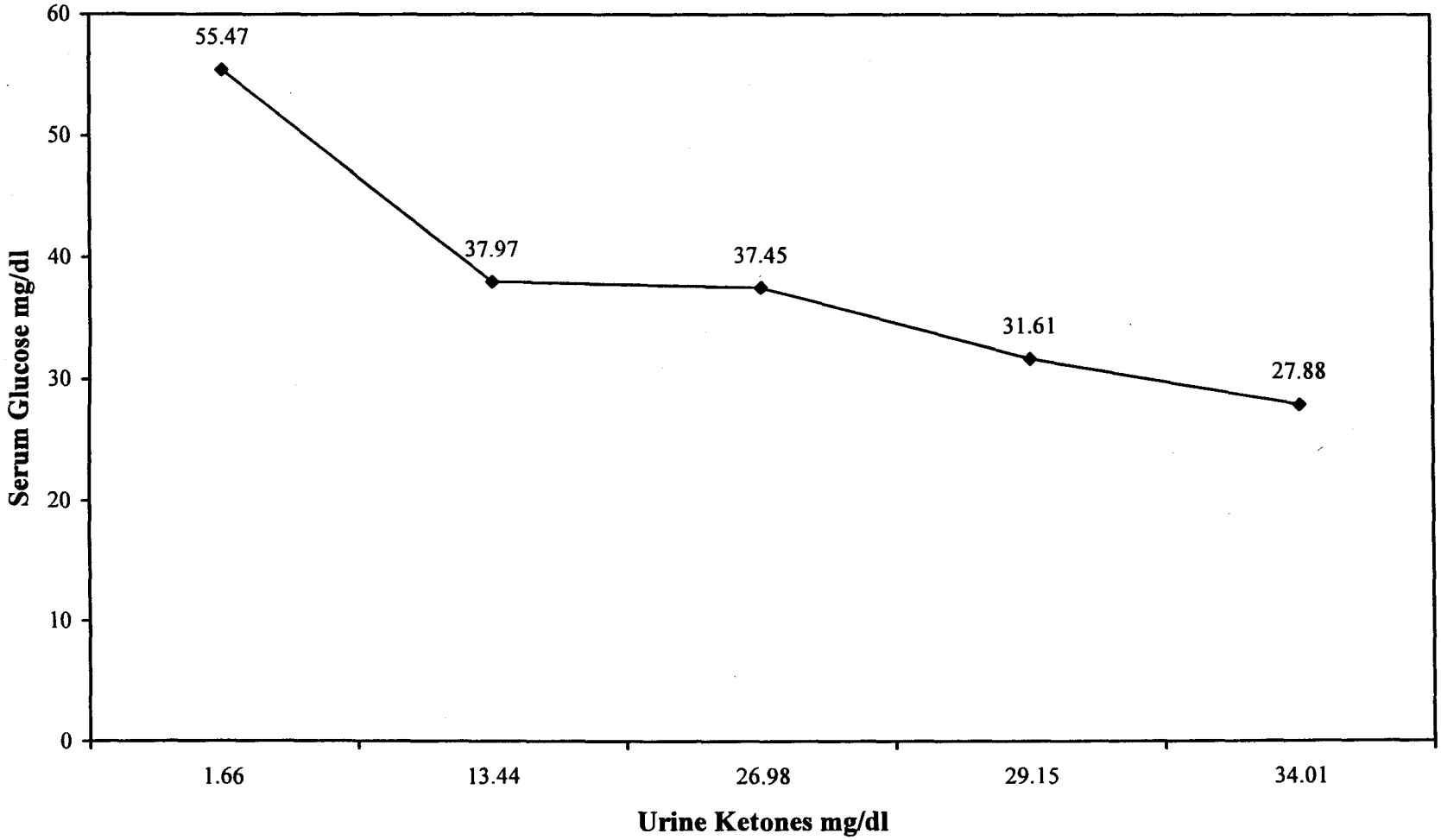


Fig. 17 : Relation Between Urine Ketones and Total Protein

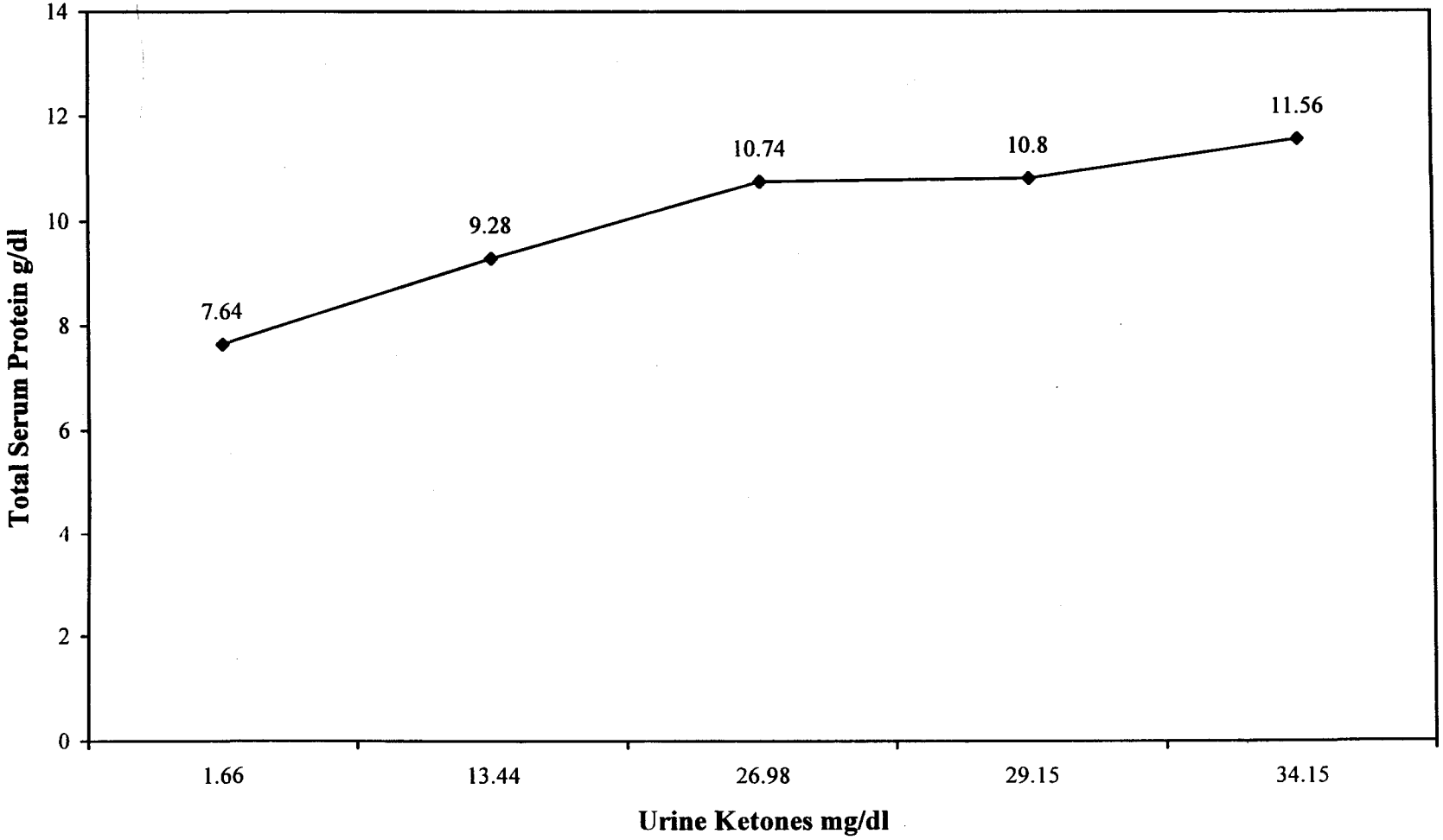


Fig. 17 : Relation Between Urine Ketones and Total Protein

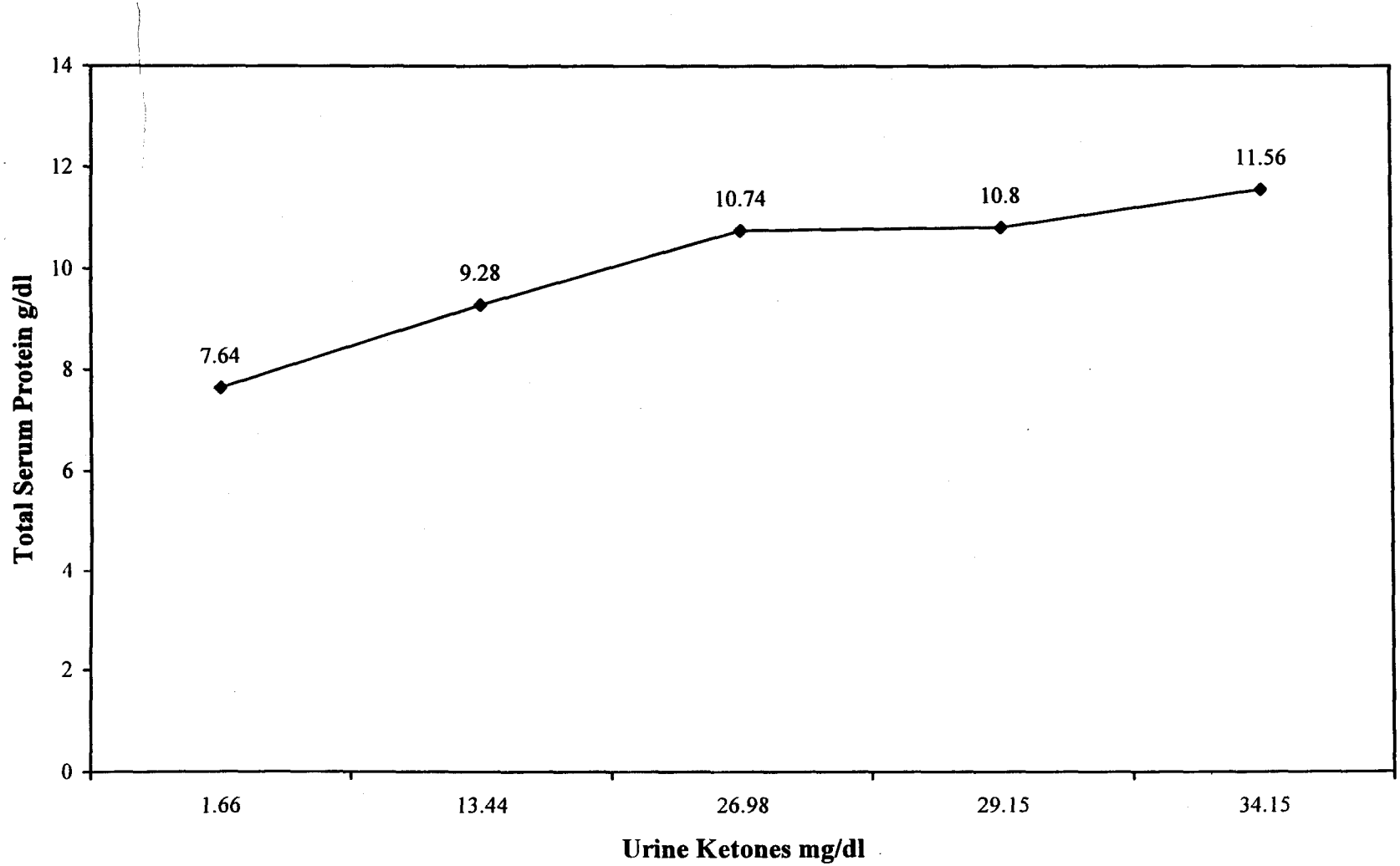


Fig. 19 : Relation Between Urine Ketones and Serum Globulin

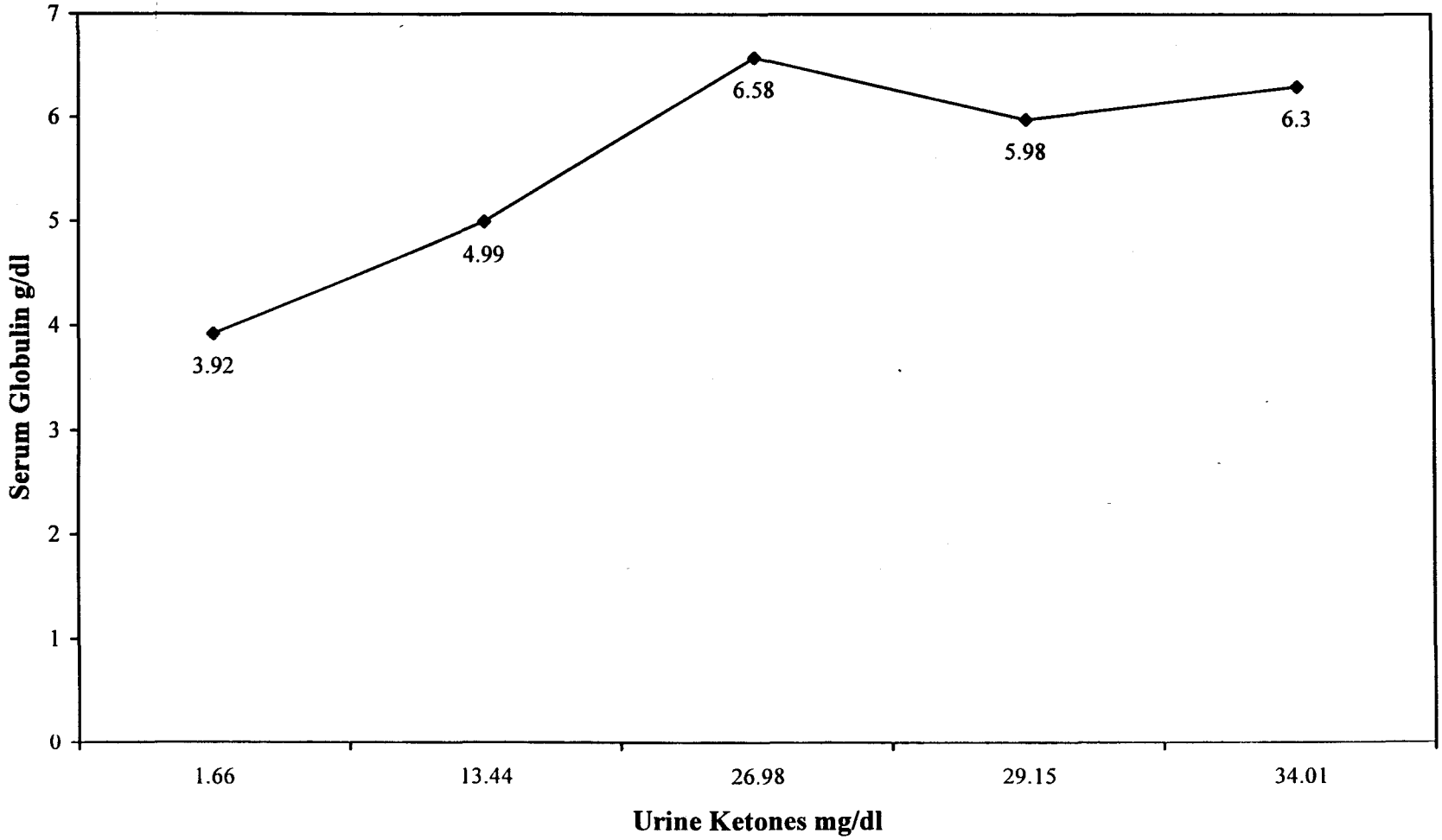


Fig. 20 : Relation Between Urine Ketones and A : G Ratio

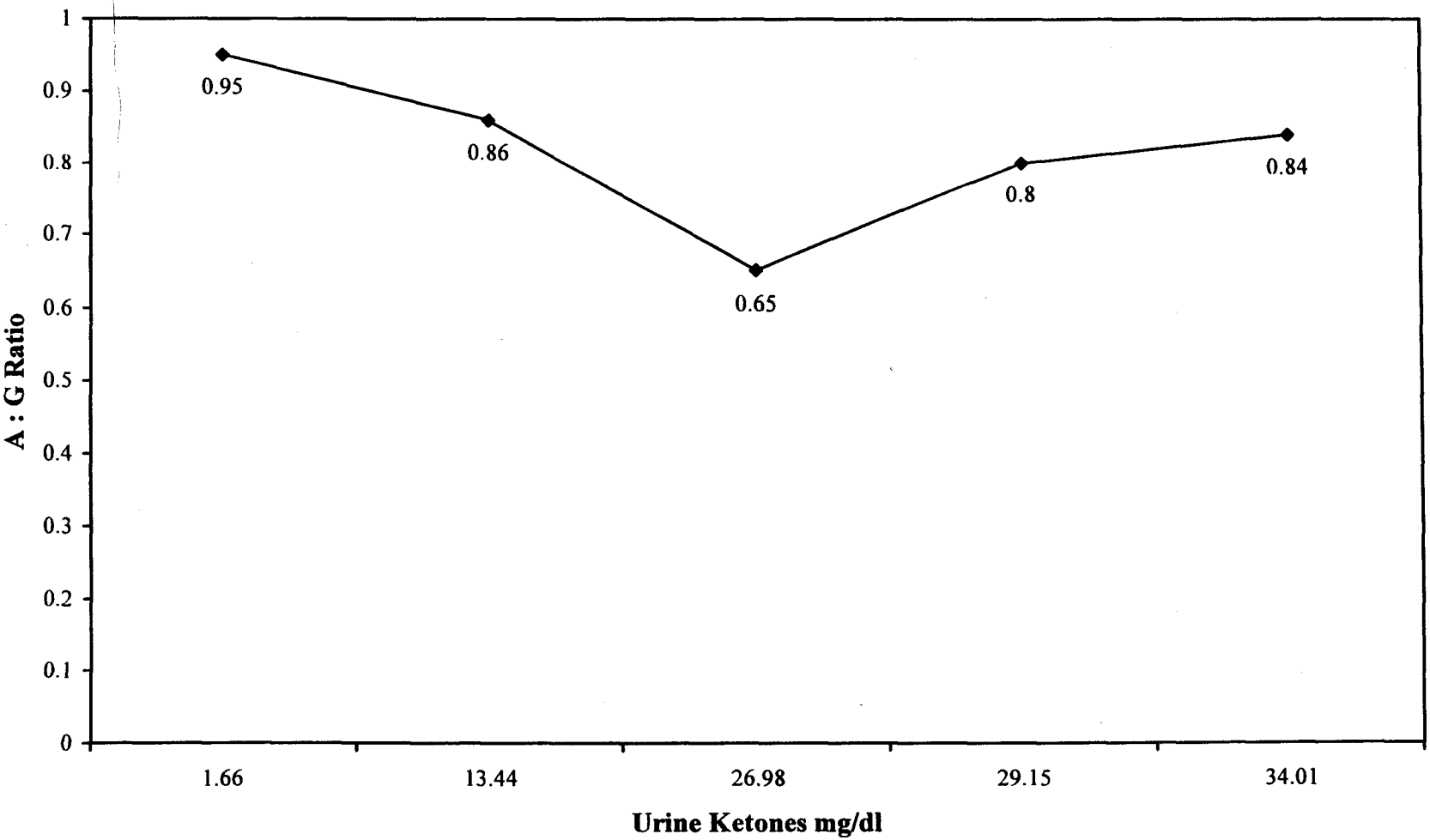


Fig. 21 : Relation Between Urine Ketones and BUN

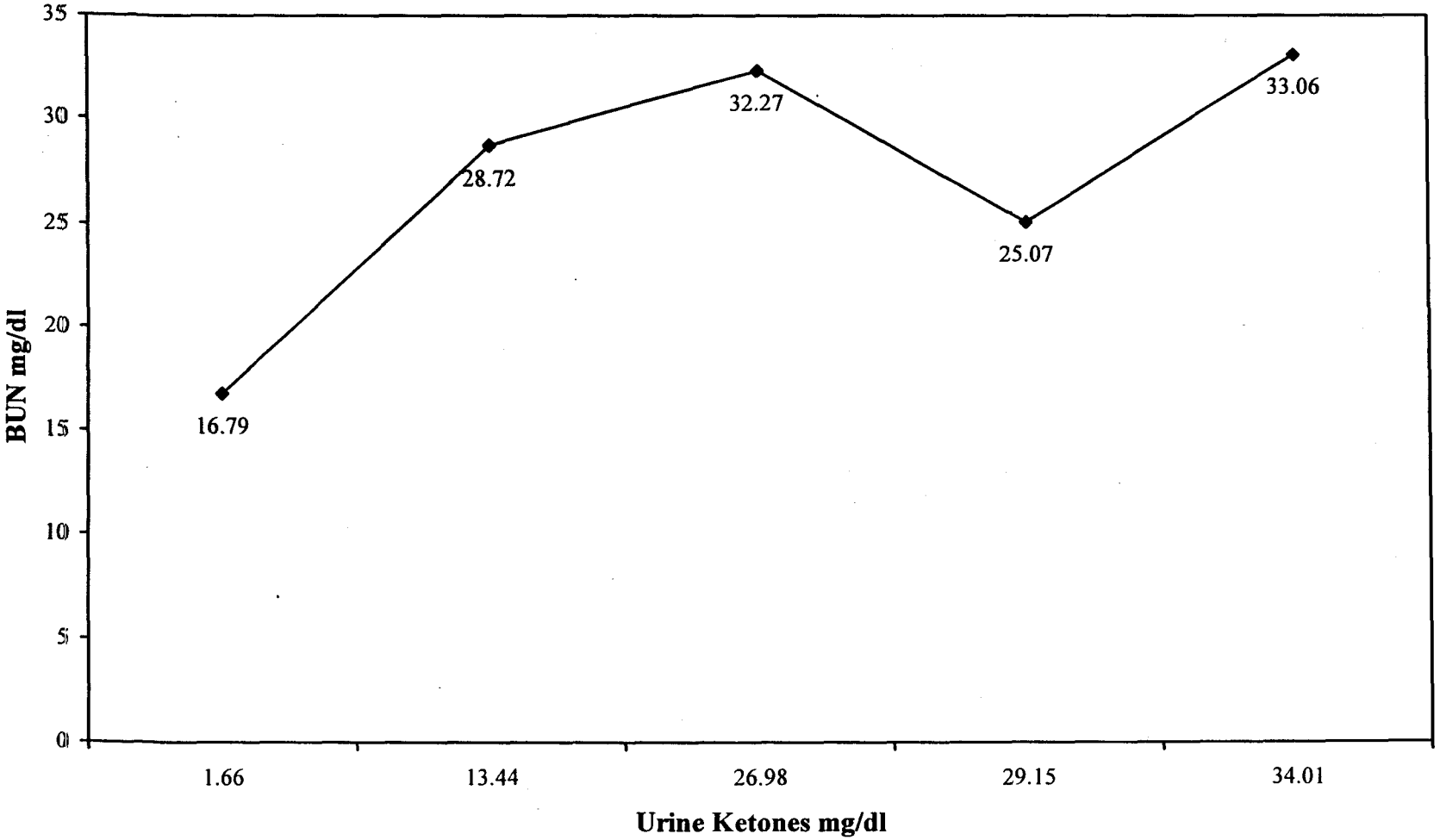


Fig. 22 : Relation Between Urine Ketones and Serum Calcium

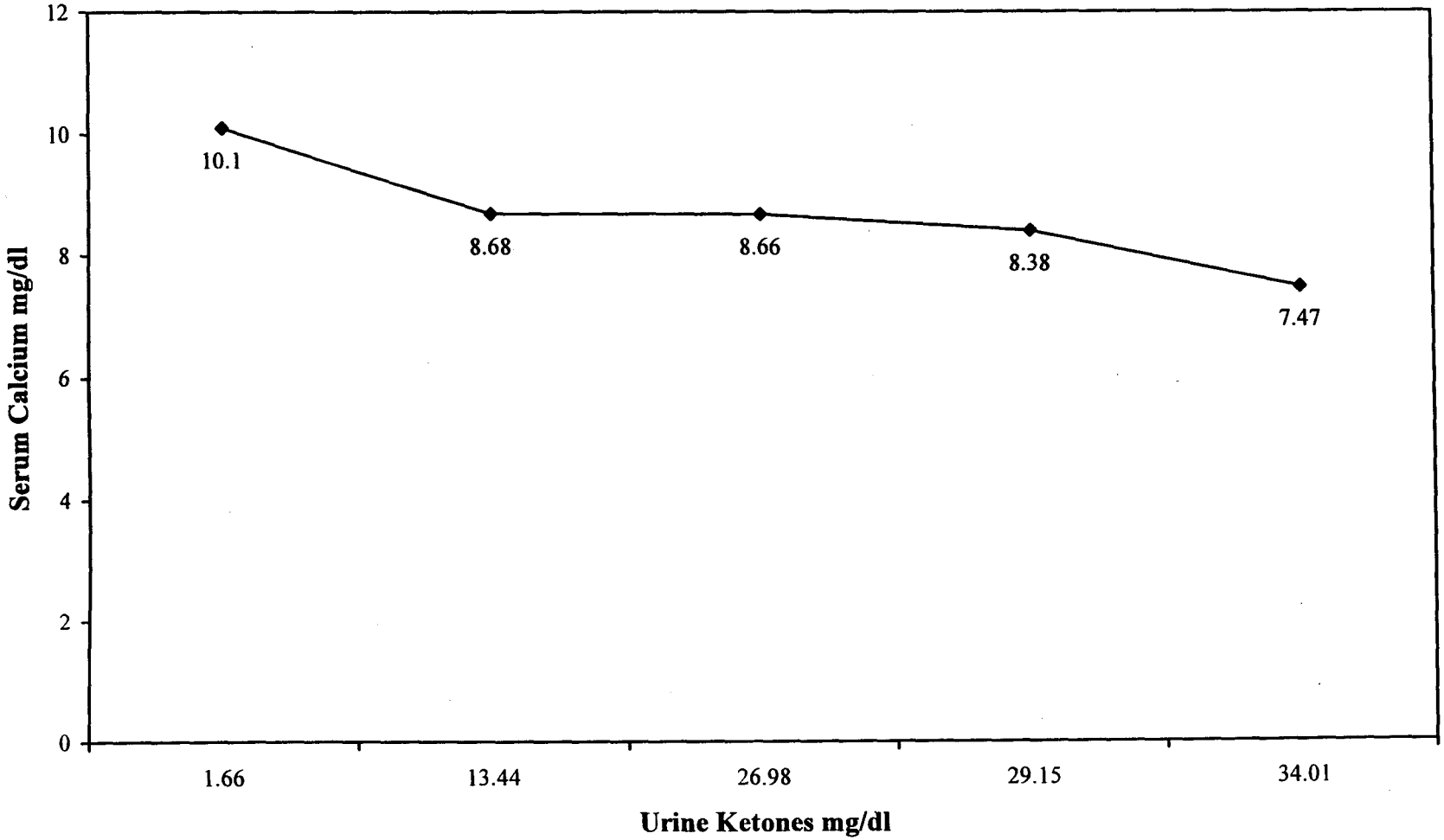


Fig. 23 : Relation Between Urine Ketones and Serum Phosphorus

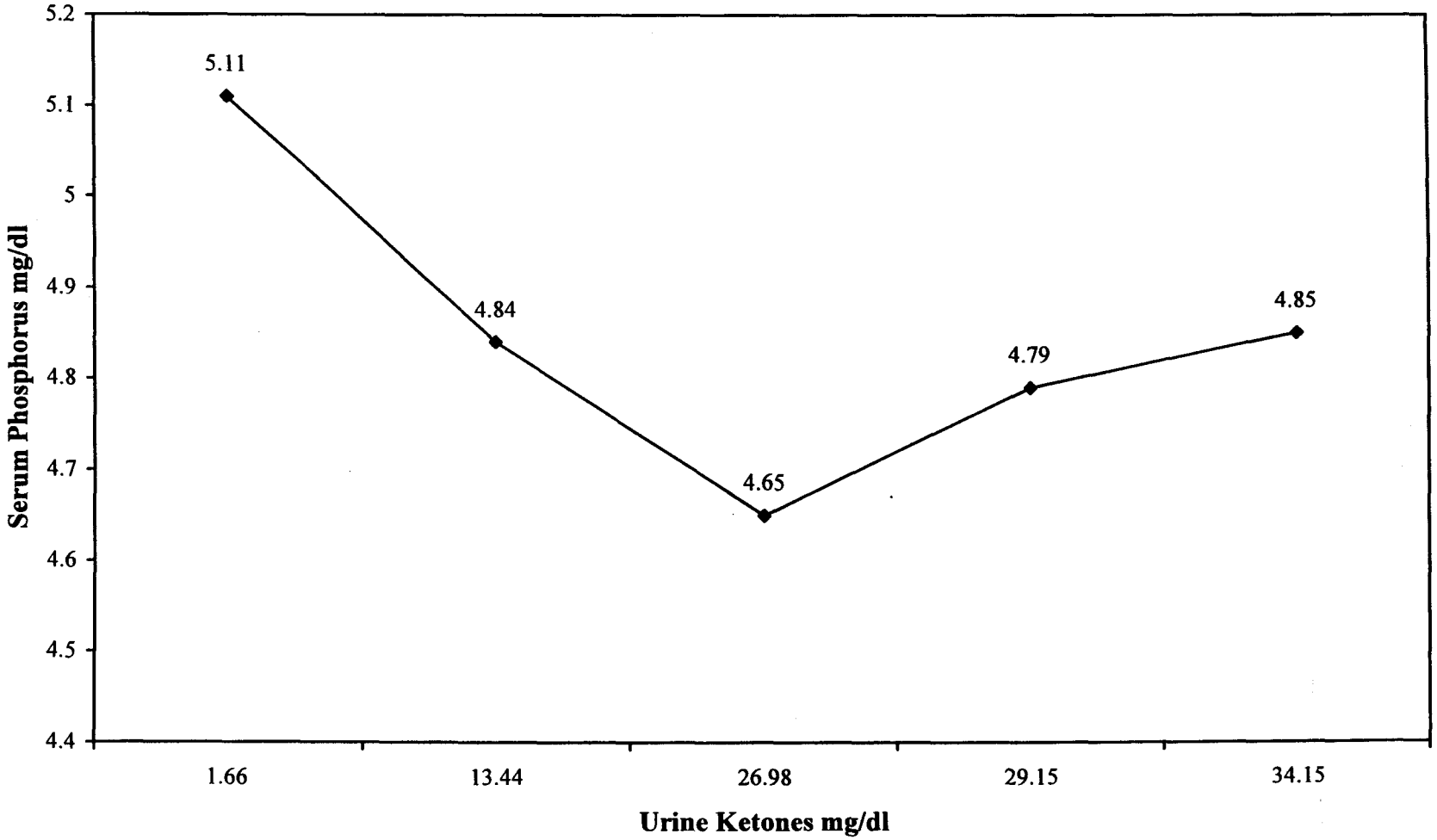


Fig. 24 : Relation Between Urine Ketones and Serum Magnesium

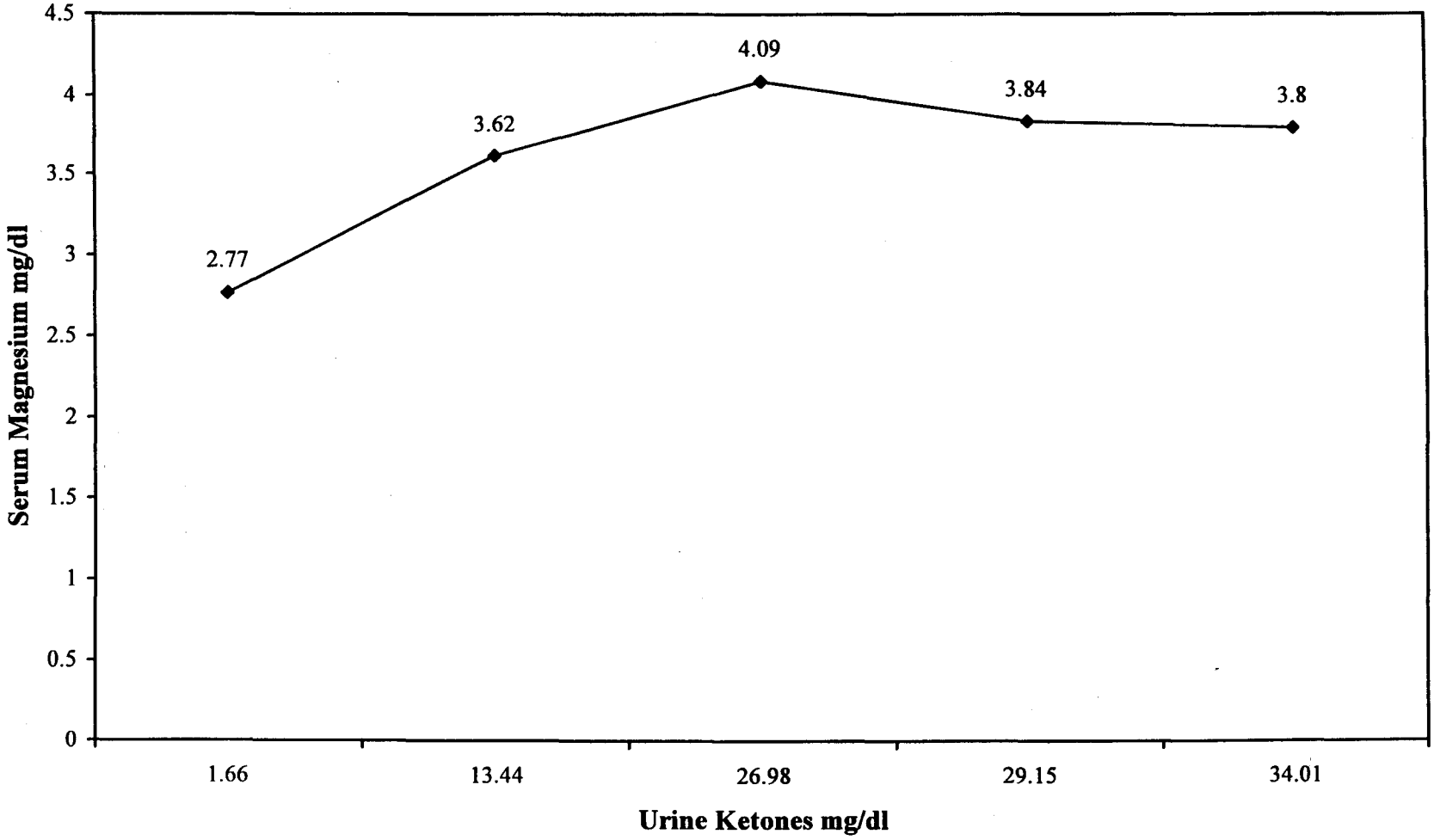


Fig. 25 : Relation Between Urine Ketones and Serum Sodium

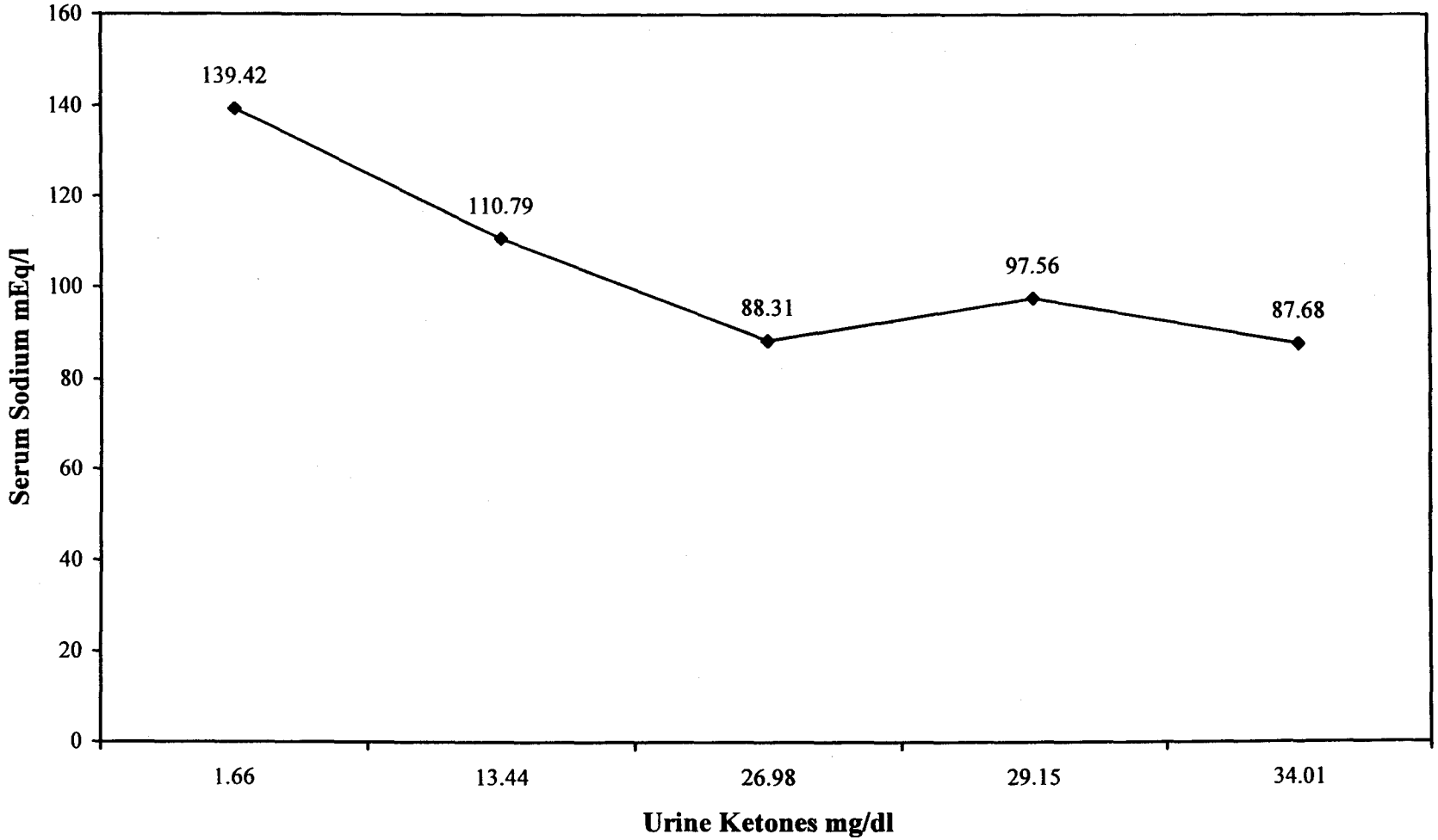


Fig. 26 : Relation Between Urine Ketones and Serum Potassium

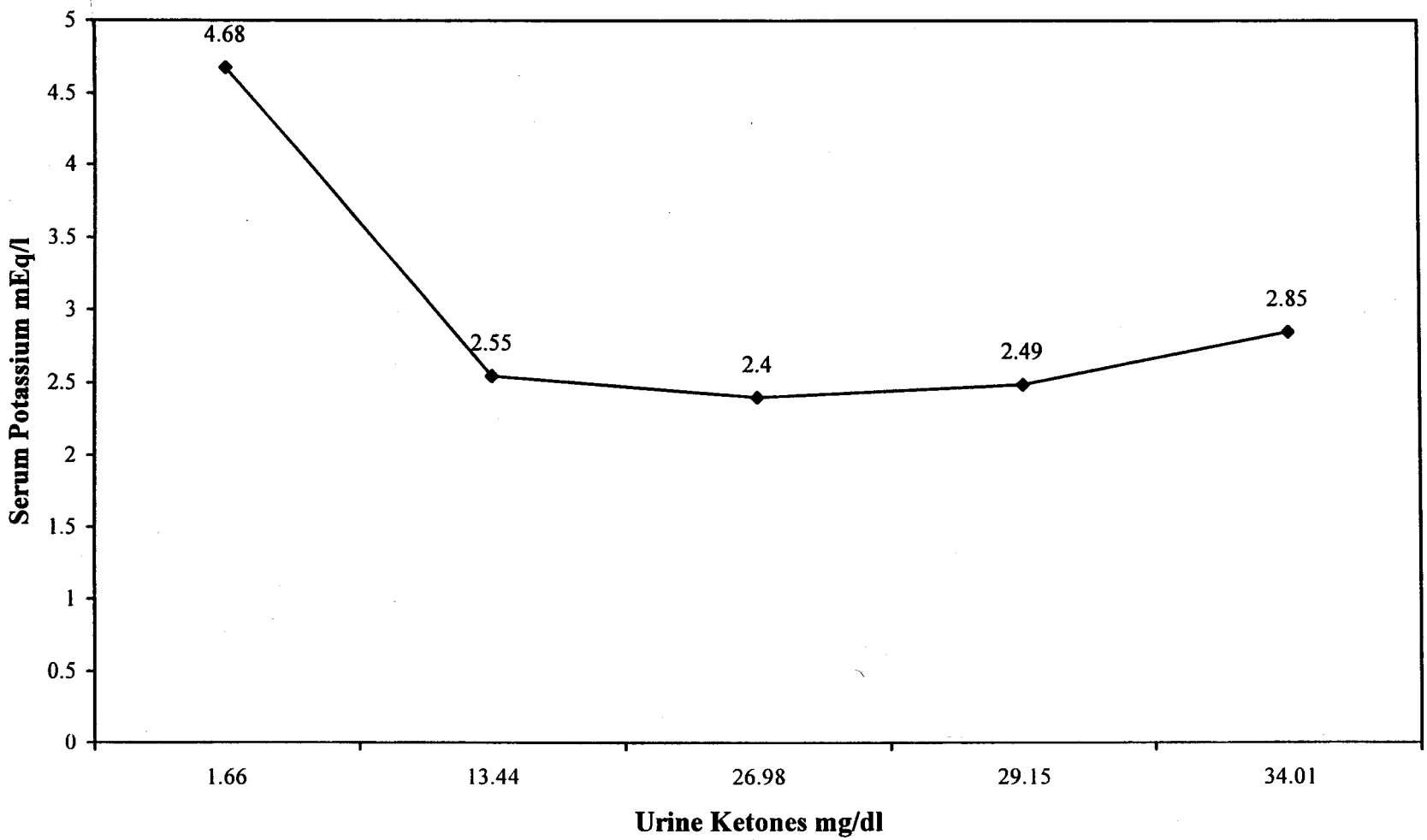


Fig. 27 : Relation Between Urine Ketones and Serum ALT

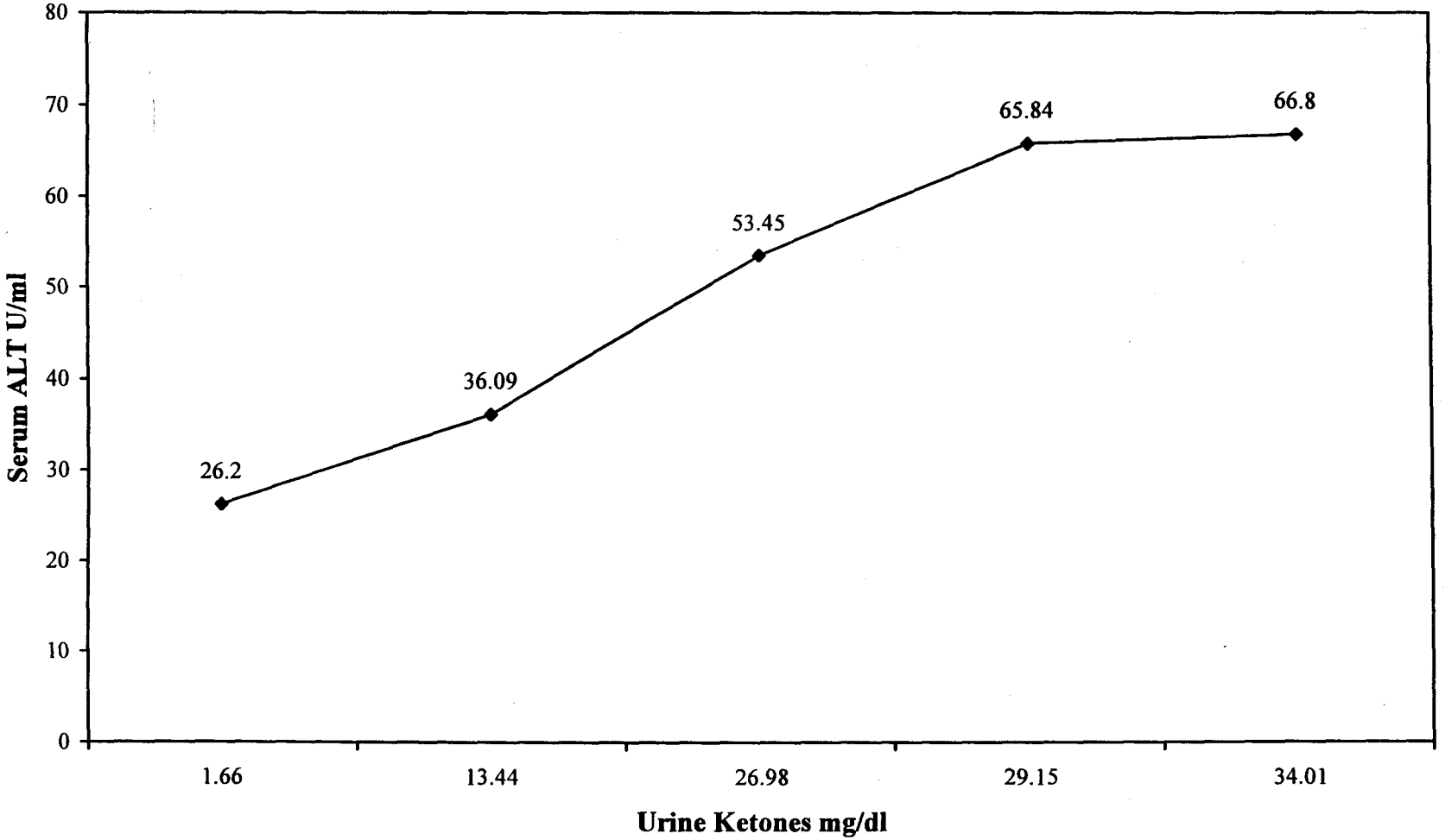
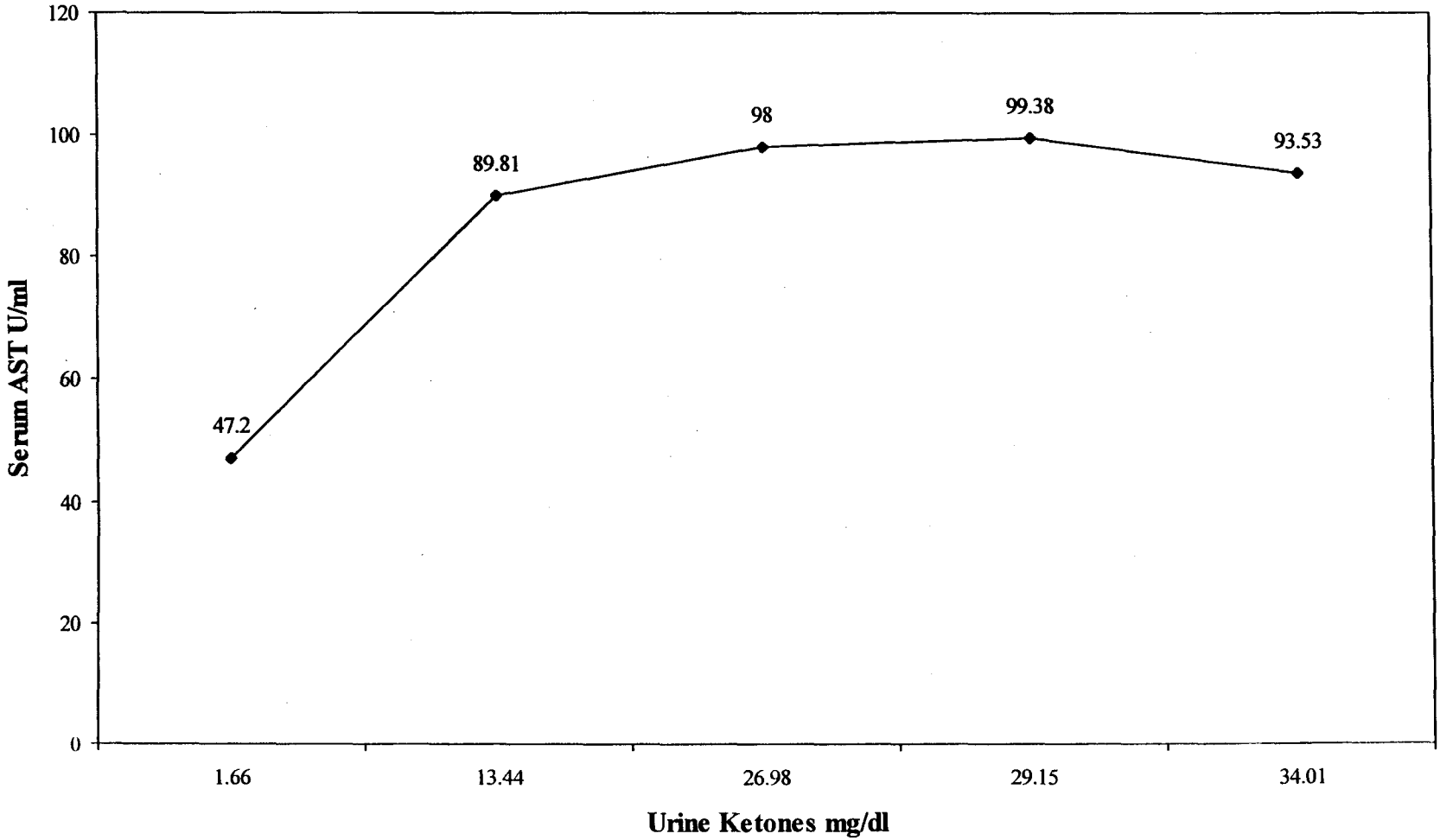


Fig. 28 : Relation Between Urine Ketones and Serum AST



degree of ketonaemia. Results are presented in Table - 4 and Figure 15 to 28.

Glucose utilization (tolerance) in healthy and ketotic cattle

Prior to planning the treatment for ketotic cows, glucose tolerance test was conducted in healthy cattle so as to study glucose utilization after intravenous administration of 50 per cent dextrose at the rate of 1 g/kg bat. First of all base value of blood glucose was determined before administration of 50 per cent dextrose. Thereafter the blood glucose levels were continuously monitored at 0 hour (immediately after administration), 1, 2, 3, 4, 5 and 6 hours. The results are presented in Table - 6.

On the basis of glucose tolerance test conducted on healthy animals, glucose utilization was studied in ketotic cows during the course of treatment. Blood samples of these animals for glucose determination at different intervals were drawn before administration of 50 per cent dextrose (base value), immediately after administration (at 0 hour), and at 2 and 4 hour. Results are presented in table number 6. The glucose utilization curves for both healthy and ketotic cattle are illustrated in Figure - 29.

Renal threshold of glucose in healthy cattle

Renal threshold of glucose was determined in six healthy cattle after administration of 50 per cent dextrose at the rate of 1 gm/kg b.wt. in each animal. Blood glucose levels were determined and the presence of glucose in urine by multistix was monitored. Both the levels of blood glucose and reaction of multistix of glucose in urine

TABLE 6 : GLUCOSE UTILIZATION (TOLERANCE) IN HEALTHY AND KETOTIC CATTLE ON INTRAVENOUS ADMINISTRATION OF 50 PER CENT DEXTROSE.

Types of animals and % fall of blood glucose	Mean ± SE of glucose mg per dl at planned intervals.							
	Base value (Before administration)	Value at 0 hr. (immediately after administration)	Value at 1 hour	Value at 2 hour	Value at 3 hour	Value at 4 hour	Value at 5 hour	Value at 6 hour
1. Healthy (n=6)	55.16±2.11	391.83±2.59	267.16±2.29	206.00±7.16	165.16±2.77	122.5±6.11	74.66±2.34	57.16±2.04
fall from 0 hr. value	-	-	36.90%	55.00%	67.00%	80.05%	94.34%	99.40%
2. Ketotic (n=50)	33.18±1.09**	370.43±0.51	-	161.67±5.55*	-	106.80±4.93	-	-
fall from 0 hr. value	-	-	-	62.00%	-	78.33%	-	-

* Variation in mean value was significant ($p<0.05$) when compared with the mean value of healthy animals.

** Variation in mean value was highly significant ($p<0.01$) when compared with the mean value of healthy animals.

Fig. 29 : Glucose Utilization Curve

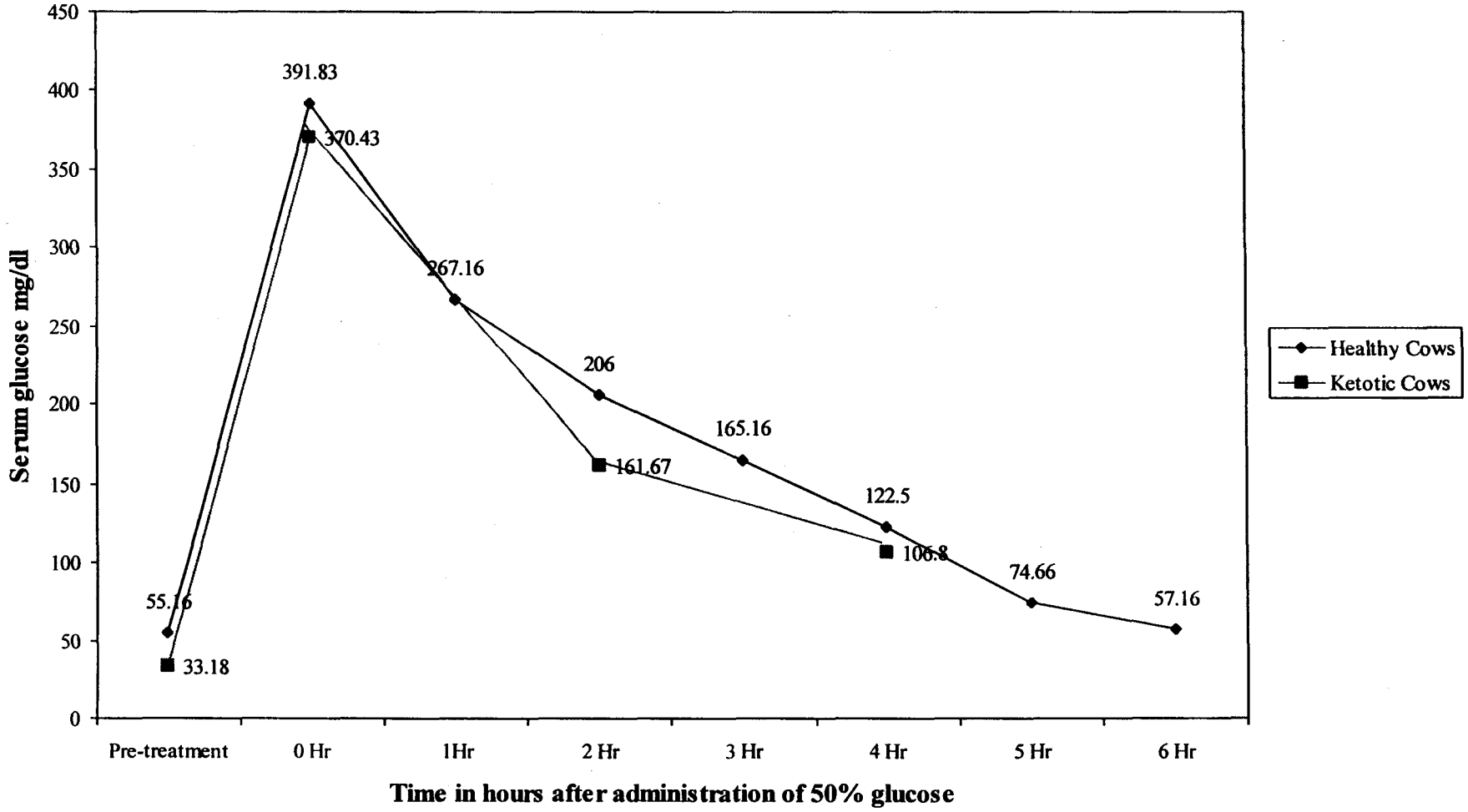


TABLE 7 : RENAL THRESHOLD OF GLUCOSE IN HEALTHY CATTLE

Animal No.	Parameters	Sample number (2-10 Sample value between 0 to 5 hours depending upon urination)									
		Base Value	Value. at 0 hour								
	1	1	2	3	4	5	6	7	8	9	10
I.	Blood Glucose (mg/dl) Glucose in urine (By multistix)	48 -	391 ++++	285 ++++	281 ++++	266 ++++	207 ++++	168 +++	110 ++	74 f	69 -
2.	Blood Glucose (mg/dl) Glucose in urine (By multistix)	58 -	394 ++++	231 ++++	140 +++	112 ++	99 +	75 +	70 -		
3.	Blood Glucose (mg/dl) Glucose in urine (By nultistix)	55	392 ++++	203 ++++	137 +++	118 ++	80 +	73 -			
4.	Blood Glucose (mg/dl) Glucose in urine By multistix)	50 -	380 ++++	190 ++++	112 ++	84 +	74 f	72 -			
5.	Blood Glucose (mg/dl) Glucose in urine (By multistix)	60 -	396 ++++	185 ++++	106 ++	80 +	70 -				
6.	Blood Glucose (mg/dl) Glucose in urine (By multistix)	60 -	395 ++++	290	271 ++++	232 ++++	170 ++++	132 +++	80 +	71 -	

declined continuously to the point when glucose ceased to appear in urine. The levels of blood glucose were determined just before the disappreance of glucose from urine. This point represented the renal threshold of glucose. The results are presented in Table - 7.

Efficacy **of treatment**

All the 50 affected cows treated for ketosis were divided into four groups according to results of Rothera's test conducted on their urine as (1) +, (2) ++, (3) +++ and (4) . These ketotic cows on first day were treated with 500 ml of 50 per cent dextrose and Triamcinolone acetonide 12 mg/animal. The cases out of these that developed glycosuria (by multidiagnostic strip test) on administration of this treatment were considered to be insulin dependent, constituted the fifth group irrespective of intensity of Rothera's reaction. The line of treatment adopted and the efficacy of treatment for each of these five groups of ketotic cows are presented in separate Table from 9 to 13.

A profile of serum glucose and urine analysis was determined for 10 healthy cows in the same way as it was estimated for ketotic cows and is presented in Table - 8.

TABLE $_4$: PROFILE OF SERUM GLUCOSE, MODIFIED ROTHERA'S AND MULTIDIAGNOSTIC STRIP REACTION IN HEALTHY COWS

	No. of Animals	Biochemical estimation	URINALYSIS								
		Serum glucose	Rothera's reaction	Multidiagnostic Strip Reaction							
				Urobilinogen	Protein	pH	Blood	Sp.:gravity	Ketone	Bilirubin	Glucose
	10.	55.47±1.15 (50.00-60.12)	-	0.2±0.00 (0.2-0.2)	-(2/10) - Trace (7/10) +(1/10)	8.20±0.13 (7.5-8.5)	-	1.024±0.01 (1.015±1.030)	-	-	-

- Ranges for parameters have been given in parenthesis below the readings.
- Responses in number of animals have been given in parenthesis against the reading for the parameter.

TABLE - 9 : TREATMENT OF KETOTIC COWS, URINE **OF WHICH** WAS "+" **FOR MODIFIED ROTHE"'S TEST CLINICALLY MONITORED DAILY WITH MULTIDIAGNOSTIC STRIP REACTION AND BLOOD GLUCOSE DETERMINATION.**

No. of Cows	Estimation & Treatment	Day of Treatment			Remarks
		1	2		
10.	(A) S6rum : Glucose	37.31±1.61 (28.14-42.75)	44.72±1.18 (35.12-47.80)	50.52±0.62 (46.91-52.17)	Levels attained normalcy after two treatments, i.e. on 3rd day.
	(B_1) Urine : Rothera's reaction	+(10/10)	+(3/10) Trace (6/10) -(1/10)	-(9/9)	One animal became negative to Rothera's test on 2nd day and the rest nine on 3rd day.
	(B2) Urine Multidiagnostic strip reaction.				
	1. Urobilinogen	0.2(10/10)	0.2(10/10) one case recovered	0.2(9/9)	Levels remained unaltered throughout the duration of treatment like in healthy cows.
	2. Protein	-(4/10) Trace (6/10)	-(8/10) Trace (2/10) one case recovered	-(9/9)	The reaction was similar to healthy cows throughout the period of treatment.
	3. pH	8.10±0.06 (8.0-8.5)	8.15±0.10 (7.5-8.5)	8.22±0.09 (8.0-8.5)	PH remained in normal range throughout the period of treatment

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	4. Blood	-(10/10)	-(10/10) one case recovered	-(9/9)	As also in healthy cattle, there was no presence of blood in urine of ketotic cows.
	5. Specific gravity	1.013±0.01 (1.005-1.020)	1:018±0.01 (1.010-1.030)	1.022±10.11 (1.020-1.030)	Attaind normalcy on 3rd day, normal range being 1.015 to 1.030.
	6. Ketone	+(10/10)	-(3/10) Trace(6/10) -(1/10)	-(9/9)	Reaction of urine for ketone by multidiagnostic strip exactly matched the Rothera's test as shown above in B 1.
	7. Bilirubin	-(10/10)	-(10/10) one case recovered	-(9/9)	Absent in urine, as also noticed in healthy cattle.
	8. Glucose	-(10/10)	-(10/10) one case recovered	-(9/9)	Absent in urine of all these "+" ketotic cows.
	(C) Treatment	T and D (10/10)	D (9/10) Recovered (1/10)	Nil Recovered (9/9)	One out of 10 ketotic cows recovered after one treatment, i.e. on 2nd day and the rest on 3rd day.

- Ranges for parameters have been given in parenthesis below the readings.
- Responses in number of animals out of treated population have been given in parenthesis against the reading for the parameters.
- Reading/values presented in the table were determined before administration of treatment on every concerned day.

T = Triamcinolone Acetonide 12 mg/animal I/M.

D = 50 per cent dextrose 500 ml I/V per animal.

rASLE – to _ TREA i MENT OF KETOTIC COWS, URINE **OF WHICH WAS ++ FOR MODIFIED ROTHEI3A'S TEST** CLINICALLY **MONITORED DAILY WITH MULTIDIAGNOSTIC STRIP REACTION AND BLOOD GLUCOSE DETERMINATION.**

No. of Cows	Estimation & Treatment	Day of Treatment				Remarks
		1	2	3		
7.	(A) Serum : Glucose	38.60±1.29 (34.53-43.17)	43.04±1.71 (38.24-51.11)	49.01±1.26 (46.36-56.27)	51.88±0.40 (50.64-53.41)	Levels attained normalcy after three treatments, i.e. on 4rd day.
	(B1) Urine : Rothera's reaction	++(7/7)	+(7/7)	Trace (6/7) -(1/7)	-(6/6)	One animal became negative on 3rd day and the rest 6 on 4th day.
	(B2) Urine Multidiagnostic strip reaction.					
	1. Urobilinogen	0.2(7/7)	0.2(7/7)	0.2(7/7) One case recovered	0.2(6/6)	Levels remained unaltered throughout the duration of treatment like in healthy cows.
	2. Protein	-(1/7) Trace (5/7) +'(1/7)	-(3/7) Trace (4/7)	-(5/7) Trace (2/7) One case recovered	-(5/6) Trace (1/6)	The reaction was similar to healthy cattle throughout the period of treatment.
	3. pH	8.50±0.00 (8.5-8.5)	8.21±0.10 (8.0-8.5)	8.00±0.00 (8.0-8.0)	8.00±0.00 (8.0-8.0)	PH remained in normal range throughout the period of treatment

Contd

	4_ Blood	- (7/7)	- (7/7)	-(7/7) One case recovered	**-(6/6)**	**As also in healthy cattle, there was no** presence of blood in urine of ketotic cows.
	5. Specific gravity	1.010±0.01 (1.005-1.015	1.018±0.01 (1.010-1.025)	1.022±0.01 (1.020-1.025)	1.025±0.01 (1.020-1.030)	Attaind normalcy from 3rd day onwards.
	6. Ketone	++(7/7)	+(7/7)	Trace (6/7) -(1/7)	-(6/6)	Reaction of urine multidiagnostic strip exactly matched the Rothera's test as shown above in B_1.
	7. Bilirubin	-(7/7)	-(7/7)	-(7/7) One case recovered	-(6/6)	Absent in urne, as also noticed in healthy cattle.
	8. Glucose	-(7/7)	-(7/7)	-(7/7) One case recovered	-(6/6)	Glucose absent in urine of all these "++" ketotic cows.
	(C) Treatment	T and D (7/7)	D (7/7)	D (6/7) Recovered (1/7)	Nil Recovered (6/6)	One out of 7 ketotic cows recovered after 2 treatment, i.e. on 3rd day and the rest on 4th day.

- Ranges for parameters have been given in parenthesis below the readings.
- Responses in number of animals out of treated population have been given in parenthesis against the reading for the parameters.
- Reading/values presented in the table were determined before administration of treatment on every concerned day.

T = Triamcinolone Acetonide 12 mg/animal I/M.

D = 50 per cent dextrose 500 ml I/V per animal.

TABLE -11 : TREATMENT OF KETOTIC COWS, URINE OF WHICH WAS "+++" FOR MODIFIED ROTHERA'S TEST CLINICALLY MONITORED DAILY WITH MULTIDIAGNOSTIC STRIP REACTION AND BLOOD GLUCOSE DETERMINATION

No. of Cows	Estimation & Treatment	Day of Treatment				Remarks
		1	2	3	4	
10.	(A) Serum : Glucose	30.66±1.52 (24.34-38.26)	38.72±0.70 (36.12-42.26)	43.92±0.48 (42.26-46.35)	50.88± 0.33 (50.00-52.25)	Levels attained normalcy after 3 treatments, i.e. on 4rd day.
	(B_1) Urine : Rothera's reaction	+++ (10/10)	++(8/10) +(2/10)	+(8/10) Trace (2/10)	-(10/10)	All animals became negative on 4th day.
	(B_2) Urine Multidiagnostic strip reaction.					
	1. Urobilinogen	0.2(10/10)	0.2(10/10)	0.2(10/10)	0.2(10/10)	Levels remained unaltered throughout the duration of treatment like in healthy cows.
	2. Protein	+(8/10) Trace (2/10)	Trace (10/10)	Trace (6/10) -(4/10)	"Trace (7/10) -(7/10)	As in healthy cattle, reaction ranged between - ve and + but the cases of "+" were more.
	3. pH	8.40±0.06 (8.0-8.5)	8.25±0.08 (8.0-8.5)	8.15±0.07 (8.0-8.5)	8.15±0.07 (8.0-8.5)	pH remained in normal range throughout the period of treatment.

Contd

	4. Blood	-(10/10)	-(10/10)	-(10/10)	-(10/10)	As also in healthy cattle, there was no presence of blood in urine of ketotic cows.
	5. Specific gravity	1.005±0.01 (1.000-1.010)	1.013±0.01 (1.005-1.020)	1.020±0.01 (1.015-1.025)	1.024±0.01 (1.020-1.030)	Attained normalcy on 3rd day, normal range being 1.015 to 1.030.
	6. Ketone	+++ (10/10)	++(8/10) +(2/10)	+(8/10) Trace (2/10)	-(10/10)	Reaction of urine multidiagnostic strip exactly matched the Rothera's test as shown above in B_1.
	7. Bilirubin	-(10/10)	-(10/10)	-(10/10)	-(10/10)	Absent in urine, as also noticed in healthy cattle.
	8. Glucose	-(10/10)	-(10/10)	-(10/10)	-(10/10)	Absent in urine of all these "+++" ketotic cows.
	(C) Treatment	T and D (10/10)	D (10/10)	D (10/10)	Nil Recovered 10/10	All the 10 ketotic cows recovered after three treatments i.e. on 4th day.

- Ranges for parameters have been given in parenthesis below the readings.
- Responses in number of animals out of treated population have been given in parenthesis against the reading for the parameters.
- Reading/values presented in the table were determined before administration of treatment on every concerned day.

T = Triamcinolone Acetonide 12 mg/animal IIM.

D = 50 per cent dextrose 500 ml IN per animal.

TABLE - 12 : TREATMENT OF KETOTIC COWS, URINE OF WHICH WAS "++++" FOR MODIFIED **ROTHERA'S TEST CLINICALLY MONITORED DAILY WITH MULTIDIAGNOSTIC STRIP REACTION AND BLOOD GLUCOSE DETERMINATION.**

No. of Cows	Estimation & Treatment	Day of Treatment				Remarks
		1	2	3	4	
14.	(A)~ Serum : Glucose	25.65±1.24 (20.14-34.31)	34.51±1.40 (26.22-41.36)	44.97±1.13 (40.91-56.09)	51.77±0.61 (49.15-58.32)	Levels attained normalcy after 3 treatments, i.e. on 4rd day.
	(B,) Urine : Rothera's reaction	++++ (14/14)	+++ (11/14) ++(1/14) +(2/14)	+(11/14) Trace (2/14) -(1/14)	-(13/13)	One animal became negative on 3rd day and the rest 13 on 4th day.
	(B2) Urine Multidiagnostic strip reaction.					
	1. Urobilinogen	0.2(14/14)	0.2(14/14)	0.2(14/14) one case recovered	0.2(13/13)	Levels remained unaltered throughout the duration of treatment like in healthy cows.
	2. Protein	++(6/14) +(3/14) Trace (4/14) -(1/14)	++(2/14) +(4/14) Trace (7/14) -(1/14)	Trace (13/14) -(1/14) one case recovered	-(13/13)	Protienuria became prominent affecting about 43 per cent cases and started disappearing after 2 days of treatment i.e. from 3rd day onwards.
	3. pH	8.42±0.05 (8.0-8.5)	8.25±0.07 (8.0-8.5)	8.10±0.06 (8.0-8.5)	8.15±0.07 (8.0-8.5)	pH remained in normal range throughout the period of treatment.
	4. Blood	-(14/14)	-(14/14)	-(14/14) one case recovered	-(13/14)	As also in healthy cattle, there was no presence of blood in urine of ketotic cows.

Contd

		5. Specific gravity	1.003±0.01 (1.000-1.010)	1.010±0.01 (1.005-1.025)	1.020±0.01 (1.010-1.030)	1.026±0.01 (1.020-1.030)	Ketotic cows had low specific gravity which attained normalcy on 3rd day after treatment, normal range being 1.015 to 1.030.
		6. Ketone	++++ (14/14)	+++ (11/14) ++(1/14) +(2/14)	+(11/14) Trace (2/14) -(1/4) one case recovered	-(13/13)	Reaction of urine multidiagnostic strip exactly matched the Rothera's test as shown above in B1.
		7. Bilirubin	-(14/14)	-(14/14)	-(14/14) one case recovered	-(13/13)	Absent in urine, as also noticed in healthy cattle.
		8. Glucose	-(14/14)	-(14/14)	-(14/14) one case recovered	-(13/13)	Absent in urine of all these "++++" ketotic cows.
		(C) Treatment	T and D (14/14)	D (14/14)	D (13/14) One case recovered	Nil Recovered 13/13	One out of 14 ketotic cows recovered after 2 treatments i.e. on 3rd day and the rest on 4th day.

- Ranges for parameters have been given in parenthesis below the readings.
- Responses in number of animals out of treated population have been given in parenthesis against the reading for the parameters.
- Reading/values presented in the table were determined before administration of treatment on every concerned day.

T = Triamcinolone Acetonide 12 mg/animal I/M.

D = 50 per cent dextrose 500 ml I/V per animal.

TABLE - 13: TREATMENT OF INSULIN DEPENDENT KETOTIC COWS, URINE OF WHICH GAVE MODIFIED ROTHERA'S REACTIN FROM "+" TO "++++" CLINICALLY MONITORED DAILY WITH MU TIDIAGNOSTIC STRIP REACTION AND BLOOD GLUCOSE DETERMINATION.

No. of Cows	Estimation & Treatment	Day of Treatment						Remarks
		1	2	3	4	5	6	
9.	(A) Serum : Glucose	38.87±2.88 (31.73-59.23)	54.77±5.57 (40.00-82.52)	78.97±4.18 (63.33-101.90)	62.60±12.00 (43.35-157.32)	66.11±17.01 (46.51-100.00)	53.00±2.00 (51.00-55.00)	Initial hypoglycaemia change to hyperglycaemia with the administration of glucose for the treatment.
	(B1) Urine : Rothera's reaction	++++ (1/9) +++ (3/9) ++(4/9) +(1/9)	+++ (2/9) ++(1/9) +(5/9) Trace (1/9)	++(6/9) +(3/9)	+++ (1/9) ++ (1/9) +(1/9) Trace (1/9) -(6/9)	+(2/3) -(1/3)	-(2/2)	Sole administration of glucose remained ineffective in treating ketosis i.e. ketonuria.
	(B2) Urine Multidiagnostic strip reaction.							
	1. Urobilinogen	0.2(9/9)	0.2(9/9)	0.2(9/9)	0.2(9/9) 6 case recovered	0.2(3/3) one case recovered	0.2(2/2)	The levels remained unaltered in these insulin dependent cases like healthy cows.
	2. Protein	++(1/9) +(3/9) Trace (5/9)	+(2/9) Trace (7/9)	Trace (3/9) -(6/9)	Trace (2/9) -(7/9) 6 cases recovered	-(3/3) one case recovered	-(2/2)	Protienuria exhibited an uncertain trend during development of disease in such type of cases.
	3. pH	7.44±0.17 (7.0-8.5)	7.33±0.17 (6.5-8.0)	7.05±0.27 (6.0-8.5)	7.61±0.25 (6.0-8.5)	7.66±0.60 (6.5-8.5)	8.50±0.00 (8.5-8.5)	Lowered pH with hyperglycaemia accompanied with ketonuria revealed existance of Diabetic ketoacidosis (DKA).
	4. Blood	-(9/9)	-(9/9)	-(9/9)	-(9/9) 6 cases recovered	-(3/3) one case recovered	-(2/2)	As also in healthy cattle, there was no presence of blood in urine.

Contd

	5.' ,Specific gravity	1.025±0.00 (1.025-1.025)	1.026±0.01 (1.025-1.030)	1.028±0.01 (1.025-1.030)	1.025±0.01 (1.020-1.030)	1.025±0.00 (1.025-1.025)	1.027±0.01 (1.025-1.030)	There was no alteration in specific gravity of urine.
	6. ketone \	++++ (1/9) +++ (3/9) ++(4/9) +(1/9)	+++ (2/9) ++(1/9) +(5/9) Trace 1/9)	++(6/9) +(3/9)	+++ (1/9) +(1/9) Trace (1/9) -(6/9)	+(2/3) -(1/3)	-(2/2)	Reaction of urine multidiagn-ostic strip exactly matched the Rothera's test as shown above in B_1.
	7. Belirubin	-(9/9)	-(9/9)	-(9/9)	-(9/9) 6 cases recovered	-(3/3) one case recovered	-(2/2)	Absent in urine, as also noticed in healthy cattle.
	8. Glucose	-(9/9)	+(2/9) -(7/9)	++(1/9) +(5/9) - (3/9)	+++ (1/9) -(8/9) 6 cases recovered	++(1/3) -(2/3) one case recovered	-(2/2)	Administration of glucose for treatment resulted in glycos-uria confirming to diabetic ketoacidosis.
	(C) Treatment	T and D (9/9)	D (7/9) D and I (2/9)	D (1/9) D and 1(8/9)	D (1/3) D and I (1/3) 1(1/3) 6 cases recovered	D (1/2) 1(1/2) One case recovered	Nil Recovered (2/2)	Insulin treatment was incorporated because of resultant glycosuria under the condition (presence) of hyperglycaemia with the objective to help the body in overcoming the pancreatic insufficiency.

- Ranges for parameters have been given in parenthesis below the readings.
- Responses in number of animals out of treated population have been given in parenthesis against the reading for the parameters.
- Reading/values presented in the table were determined before administration of treatment on every concerned day.

T = Triamcinolone Acetonide 12 mg/animal I/M.

D = 50 per cent dextrose 500 ml IN per animal.

I = Zinc insulin 40 units/ml S/C at dose rates of 2, 3, 4 and 5 ml for blood glucose levels 60-80, 80-100, 100-120 and above 120 mg/dl, respectively in the concerned animal.

DISCUSSION

Prevalence of bovine ketosis

A total of 505 cows that were included in this clinico-biochemical studies on ketosis had a history of anorexia and drop in milk-yield following parturition. All these animals were suspected for ketosis and were subjected to field diagnostic tests for confirming ketosis. Only 50 animals out of these were detected as ketotic. Thus, the overall prevalence of ketosis was 9.90 per cent in these animals. The disease emerged after parturition and the prevalence was higher mainly in (a) colder months, (b) during first and second month after parturition (c) in second to fifth parity, and (d) in cows aged up to 9 years.

The prevalence of ketosis in indigenous cows, crossbred cows and pure exotic cows has been reported to range between 11.26 and 13.00 per cent (Kauppinin, 1983; Singh, 1994; Gupta, 1999). This indicated that the prevalence of ketosis in and around Bikaner was almost the same as detected by these workers in this region and elsewhere. However, Bhuin and Chakrabarti (1993) and Deluyker (1989) reported much lower and much higher prevalence of clinical ketosis in crossbred and exotic cows, respectively as compared to the findings of the present investigation. Further, the findings of Bhuin and Chakrabarti (1993) are in agreement with the observations of the present study that the prevalence of ketosis was higher in 8-9 years and lower in 4-5 year old cows. Similarly Singh (1994) and Gupta (1999) reported that the prevalence was highest in cows aged between 8-9 years.

The prevalence of ketosis was higher from second to fifth parity. Kalita *et al.* (1987) also observed clinical ketosis in high yielding jersey crossbred cows between second and fifth lactation. Bhuin and Chakrabarti (1993) reported a greater frequency of ketosis during third lactation and less in first lactation. Their findings are also similar to those recorded in the present study. So was true for the observations made by Henricon *et al.* (1977) who observed that the cows were more prone to ketosis in their fourth lactation because of gradual fall of blood glucose level from first to fourth lactation.

The incidence of ketosis was higher in first and second months of lactation. The other workers have also reported that large number of ketotic cases occurred during this period. Sjollema and Van Der Zande (1923) and Hutyra and Marek (1926) reported that ketosis occurred frequently during 7th to 10th day after parturition in cows. Schultz (1968) observed that primary ketosis occurred from third day to eight week after calving. Ketosis has been reported to affect lactating cows between first and sixth week (Pehrson, 1966), between second and third week (Ford and Boyd, 1960), between second and sixth week (Duncan *et al.*, 1939) and mostly during third week post-partum (Alicroft, 1947; Shaw *et al.*, 1952).

The prevalence of ketosis in cows was higher in colder months, highest being in November. This finding is in agreement with Schultz (1968) and Lebeda *et al.* (1982) who noticed that ketosis mostly occurred in cows during winter. However, Grohn *et al.* (1984) reported that the disease could affect the cows between the months of September and May. During present investigation it was observed that

the disease could affect four to 'eight per cent high yielding cows between April to October.

Clinical Manifestations

The symptoms noticed in clinical cases of ketosis in cows were loss of appetite and approximately 45 per cent fall in milk-yield (as reported by owner). Most of the ketotic cows first refused to eat concentrate and then showed reduced appetite for green and dry fodder. There was loss of body condition, dry firm mucus-coated faeces, loss of elasticity of skin, dullness, grinding of teeth, absence of rumination and acetone-like odour in urine of severe cases. Most of the ketotic cows had a normal temperature, marginal increase in pulse and respiration rate with reduced ruminal movements. All the diseased cows exhibited the common symptom of constipation due to ruminal stasis. Mills *et al.* (1986) postulated that accumulation of ketone bodies in blood and liver might decrease intracellular pH, thus could result in metabolic acidosis and in turn increased pulse and respiration. Their clinical observation is similar to that recorded in present study. The other observations noticed in the studied cows are also in agreement with those reported by Sjollema (1932), Schultz (1968), Fox (1971), Baird (1982), Bhuin and Chakrabarti (1993), Radostits *et al.* (1994), Singh (1994), Choudhuri (1994, 1996) and Gupta (1999).

Approximately 45 per cent reported decrease in milk yield in the studied ketotic cows is similar to that reported by Sampson (1952). He recorded half the normal amount of milk reduction in ketotic cows after illness. Bhuin and Chakrabarti (1993) also reported a significant reduction (15.2 to 41.2 per cent) in milk yield in ketotic

cows. According to Schwalm and Schultz (1976) and Baird (1982) the drop in milk yield in clinical ketosis was due to decreased feed intake and insufficient glucose to meet out demand of high yielders.

Biochemical study on healthy and ketotic cows

One of the major findings of the biochemical study in 50 ketotic cows was a highly significant increase ($p<0.01$) in ketone (Acetone plus Acetoacetic Acid) levels in both serum and urine of the affected cows when compared with 10 healthy animals. The concentration of ketone bodies both in serum and urine of ketotic cows fell down to attain normalcy on recovery following treatment.

Sjollema and Van Der Zande (1923) and Boddie (1935) reported abnormally high levels of acetone bodies in blood and urine of ketotic cows, as recorded in the present study. Cote *et al.* (1969) also reported elevated plasma acetone plus acetoacetate in ketotic cows.

The mean values of serum ketones (10.46±0.32 mg/dl) in ketotic and healthy cows (1.21±0.10 mg/dl) detected in this study are in agreement with Rajan and Ganapathy (1973) who reported such mean values as 12.40±0.28 mg/dl and 1.50 mg/dl (1.3 to 1.9 mg/dl), respectively. Ketonuria recorded in the present study is in agreement with Hove (1978) who also detected ketonuria in bovine ketosis.

The development of ketonaemia could be understood by knowing the sequence of following biochemical changes affecting metabolism in ketotic cows. Fat mobilization increases in immediate periparturient period (Metz and Vanden Bergh, 1977) and is associated with elevated levels of free fatty acids (Radloff *et al.*, 1966; Erfle *et al.*,

1974; Roberts *et al.*, 1981). Fatty acids produced from the mobilization of fat are transported to the liver and oxidised to produce acetyl - COA and NADH. Acetyl - COA is either oxidised in TCA cycle or is metabolised to acetoacetyl - COA and subsequently to acetoacetate and b - hydroxybutyrate in relative deficiency of oxalo-acetate (Brockman, 1978; Baird, 1982; Rings, 1985). There is also endogenous production of acetate from acetyl - COA by the liver (Reynolds *et al.*, 1989). Insulin levels influence ketone metabolism in number of ways and reduced insulin has been reported both in animals developing ketosis (Schwalm and Schultz, 1974) and animals with ketosis (Colhoun *et al.*, 1962; Hove, 1974; Hove, 1978). Low insulin levels promote a high rate of lipolysis in tissues which increase supply of free fatty acids to liver resulting in hyperketonaemia (Baird, 1982) and also decreased utilization of ketone bodies by peripheral tissues (Jarrett *et al.*, 1974; Robinson and Williamson, 1980). The net effect of low insulin is to increase ketone levels through increased synthesis and decreased utilization. Glucagon plays a gluconeogenic role in bovines (Brockman, 1984; De Boer *et al.*, 1986) but may not stimulate lipolysis to the same extent as in non - ruminants (Etherton *et al.*, 1977). However, if low ratio of insulin to glucagon were present lipolysis will be stimulated thus providing ketogenic precursor to the liver (Lean *et al.*, 1992).

Development of hypoglycaemia was another major finding recorded in the studied ketotic cows. The mean values of blood glucose reported by Radostits *et al.* (1994) were 20-40 mg/dl and 50 mg/dl in ketotic and healthy cows were in approximation with such mean values recorded in the present study, i.e. 33.18±1.09 mg/dl and

55.47±1.15 mg/dl, respectively. Decreased blood glucose levels ($p<0.01$) in ketotic cows almost attained normalcy after treatment. Hypoglycaemia in ketotic cows has also been noticed by several workers like Pehrson (1966), Schultz (1968), Hove (1978), Horber *et al.* (1980), Kronfeld (1980), Sarode *et al.* (1981) and Radostits *et al.* (1994).

Hypoglycaemia could develop in ketotic cows because of disorder of carbohydrate metabolism in the following way. Hypoglycaemia in clinical cases of ketosis is due to large amount of glucose being removed by the mammary glands to make lactose coupled with insufficient food intake to replenish the glucose supply (Schullz, 1968; Baird, 1982; Radostits *et al.*, 1994). The demand of glucose by mammary glands in high yielders during their first few weeks of lactation is greater than the available glucose. This causes negative carbohydrate balance leading to hypoglycaemia (Grohn *et al.*, 1983) The depletion of liver glycogen in ketotic cow can also lead to hypoglycaemia (Kronfeld *et al.*, 1960). Kronfeld (1971), Schwalm and Schultz (1976) and Hove (1978) studied the relationship of insulin concentration of blood to occurrence of ketosis in dairy cows. They reported that insulin concentration of blood first rises in response to hyperketonaemia and then falls once more as hypoglycaemia becomes established. Baird *et al.* (1968) reported a reduction in hepatic oxaloacetate levels in ketotic cows. Oxaloacetate is preferentially utilized for gluconeogenesis. When demand of glucose is high, the liver either makes maximum amount of glucose which in turn interferes with normal function of TCA cycle as a result of which ketone bodies appear as a byproduct of liver respiration or some ox

aloacetate is used for cell respiration and this results in lower rate of gluconeogenesis (Kreb, 1966). Gluconeogenesis is decreased significantly during ketosis (Mills *et al.*, 1986) and this is because of lack of gluco-corticoid hormone from adrenal insufficiency (Shaw, 1956) or decreased levels of propionate or amino acids which are precursors of oxaloacetate.

There was a highly significant ($p<0.01$) increase in total protein, serum albumin and serum globulin concentration in ketotic cows during disease with a significant ($p<0.05$) decrease in albumin globulin ratio. The means of these parameters reverted to the normal levels after treatment.

Hyperproteinemia along with increased albumin and globulin accompanied by hypoglycaemia might be due to energy deficient and protein-rich ration given to the high yielders though such a ration could provide enough energy and protein requirements to the low yielding cows. Similar findings have been reported by Singh (1994) and Gupta (1999). On the contrary Pehrson (1966) observed slightly lower concentration of total serum protein. This could have been due to high dietary proteins provided at the farmer's holdings. According to Hibbit (1979) high protein intake exacerbate an energy deficit because of energy losses resulting from its metabolism and excretion. This energy deficit was responsible for development of ketosis. In the light of available literature and the present study post parturient ketosis be taken up as a managemental disease for which the management of the farm or the animal owner should introduce a practice of increasing energy in ration by addition of more cereals in the diet of recently parturited cows.

The decrease in albumin globulin ratio as recorded in the prevent study might have been due to less increase in serum albumin as compared to globulin in ketotic cows. Larson and Hays (1958) and Rowlands *et al.* (1975) reported a rapid rive in globulin level after parturition.

There was a highly significant increase ($p<0.01$) in the mean blood urea nitrogen levels in cows affected with ketosis. This level substantially decreased after treatment but remained higher ($p<0.05$) when compared with the mean level found in healthy cows. The increase in BUN levels might be because of high protein intake as has also been discussed by Hewett *et al.* (1975). They observed that the blood urea nitrogen values were directly affected. by protein level. They concluded that blood urea nitrogen was a fairly exact reflection of protein intake when energy and roughage remained constant. Significantly elevated values of BUN were also detected by Brattier *et al.* (1972), Shaffer *et al.* (1981), Harada (1986), Swain *et al.* (1986) and Subs Raja (1989) as was observed in the prevent study.

Highly significant decrease ($p<0.01$) was recorded in mean serum calcium level in ketotic cows whereas the difference between the mean values of serum inorganic phosphorus was non-significant. The mean values of these parameters reached to normal levels after treatment. Serum magnesium although increased highly significantly ($p<0.01$) during disease but attained normalcy after treatment. Sampson and Hayden (1935) recorded similar decrease in serum calcium level of cows during acetonaemia. Halve and Belle (1958) also reported hypocalcaemia in ketosis and suspected that the

lowering of blood calcium was due to reduced feed intake. Schultz (1971) recorded hypoglycaemia accompanied with hypocalcaemia similar to that observed in the present investigation. As per Radostits *et al.* (1994) the cause of decreased serum calcium level was due to increased loss of base in the urine to compensate for the acidosis in cattle. The changes in serum calcium and magnesium in the studied cows might have been the effect of above dietary and urinary factors. Gupta (1999) also noticed increase in serum magnesium levels in crossbred ketotic cows and not in Rata cows. However, significantly decreased serum magnesium levels during ketosis have been reported by Lee *et al.* (1978) and Armada *et al.* (1982). The serum inorganic phosphorus levels although did not exhibit significant changes in ketotic cows in this study as well as the study conducted by Gupta (1999) but were observed to increase in a study reported by Fate and Jazbec (1990) and to decrease in another study by Saarinin and Shaw (1950).

A highly significant decrease ($p<0.01$) in mean serum sodium and potassium concentration occurred during disease in ketotic cows. The mean levels of both of these parameters shots and remained little lower than normal after treatment. The results similar to these clinical cases were obtained in experimental study conducted by Stiller and Filar (1981) when they produced ketosis in cows by administration of butyric acid. The observed decrease in both serum sodium and potassium might have been caused by such factors that the ketotic animals were off feed, that there was increased loss of bases in urine, that the renal tubular function was stressed and that there

existed the effect of corticosteroids of stress during disease as per the above description.

Enzymatic clinics pathological study revealed that ketotic cows developed hepatic damage which was evident through highly significant increase ($p<0.01$) in serum alanine aminotransferase (ALT) and serum aspirate aminotransferase (AST) activity. Although observed indirectly, the liver damage still existed even after treatment as was indicated by the levels of these enzymes following treatment. These observations recorded in the present study are in agreement with those reported by Persons (1966) and Benkateshwarulu *et al.* (1994). Severity of liver damage was found associated with the levels of serum asparate aminotransferase by Ford and Boyd (1960), Forenbacher and Srebocan (1963) and chronicled (1980).

Biochemical status of ketotic cows as per Modified Rotherham's classification

In Modified Rotherham's test the acetone and acetoacetic acid in urine react with sodium nitroprusside of Rotherham's reagent to produce violet colour. The intensity of colour depends upon the amount of ketone bodies present in urine. The intensity of colour thus produced is classified as trace, +, ++, +++ and The major alterations in biochemical status of the studied 50 ketotic cows as per Modified Rotherham's classification as follows.

Table - 14 : Major alterations in biochemical status *(approximate)* and manifestations of ketotic cows as per Modified' Rotherham's classification-.

S. No.	Parameters	Classification as per Modified Rotherham's test			
		+	++	+++	++++
1.	Serum Ketones	T 700%	T 900%	T 1100%	T 1300%
2.	Urine Ketones	T 800%	T 1500%	T 1750%	T 2000%
3.	Serum Glucose	30%	4 35%	40%	L 50%
4.	Alanine aminotransferase	T 150%	T 200%	T 250%	T 250%
5.	Acetone like odour in urine	Undetectable	Undetectable	Detectable	Pronounced

The mean values of all the studied parameters in ketotic cows after treatment reverted to normal ranges except alanine aminotransferase in the case of +++ and | , and aspartate | inotransferase throughout from + to ||||. The mean values of ALT and AST though started decreasing during treatment but remained much higher than the mean values of healthy cows. This revealed that hepatic damage existed till the time of termination of the study, i.e. when the animals were considered apparently recovered. These observations pointed out that the vitamin - B complex with liver extract should be included in the recipe for treatment of ketosis in cows in future. The resultant changes in recovery of biochemical status after treatment in ketotic cows have already been discussed in the earlier section entitled "Biochemical study on healthy and ketotic cows".

Biochemical changes in ketotic cows as per degree of ketonaemia

Both ketonuria and alanine aminotransferase activity were positively correlated with degree of ketonaemia whereas blood glucose and ketonaemia had a negative correlation between them. With a unit rise in ketonaemia, ketonuria increased by 2.48 units. When ketonaemia rose by one unit, alanine aminotransferase increased by 5.43 units. Per unit elevation of ketonaemia resulted in 3.57 units decrease of serum glucose. Per unit rise in ketonaemia resulted in 0.86 unit fall in calcium levels as well as 1.07 units rise in serum protein concentration. The other studied parameters (Serum albumin, globulin, albumin globulin ratio, blood urea nitrogen, serum inorganic phosphorus, serum magnesium, serum sodium, serum potassium and serum asparatate aminotransferase activity) were found to have no correlation with degree of ketonaemia.

Observations similar to those recorded in the present study have also been reported by other workers. Shaw (1956) recorded that when blood ketone bodies increased during ketosis in cows, the blood glucose dropped to low concentration. Such observations were also recorded by Hamada *et al.* (1982) in clinical cases of ketosis in cows. Gupta (1999) found a highly significant increase in the activities of alanine aminotransferase in serum of cows suffering from ketosis.

Biochemical changes in ketotic cows as per degree of ketonuria

Both ketonaemia and alanine aminotransferase activity were positively correlated with degree of ketonuria, whereas blood glucose and ketonuria were negatively correlated with a unit rise in ketonuria, ketonaemia increased by 0.40 units. When ketonuria rose

by one unit, alanine aminotransferase activity increased by 2.21 units. Per unit elevation of ketonuria caused blood glucose to decrease by 1.52 units. With per unit rise in ketonuria, there occurred 0.36 unit fall in serum calcium levels along with 1.78 unit rise in total serum protein. Ketonuria had no correlation with the other studied parameters viz. serum albumin, serum globulin, albumin globulin ratio, blood urea nitrogen, serum inorganic phosphorus, serum magnesium, serum sodium, serum potassium and serum aspartate aminotransferase activity.

Boddie (1935) observed high level of acetone bodies in both blood and urine of cows exhibiting symptoms of ketosis. Schultz (1968) recorded hypoglycaemia and ketonuria in bovine ketosis. Gupta (1999) observed increasing activity of alanine aminotransferase in the serum of ketotic cows with increasing ketonuria.

Glucose utilization (tolerance) in healthy and ketotic cattle

Glucose utilization in both healthy and ketotic cattle was studied after intravenous administration of 50% dextrose. The peak levels were achieved immediately after glucose administration in both healthy and ketotic cattle. The glucose utilization in healthy cattle was such that its 36.90 per cent amount was utilized by the body at one hour, up to 55.00 per cent at 2 hours, up to 67 per cent at 3 hours, up to 80.05 per cent at 4 hours, up to 94.34 per cent at 5 hours and 99.40 per cent at 6 hours. The study of glucose utilization in ketotic cattle revealed that up to 62 per cent amount was utilized at 2 hours after administration of 50 per cent dextrose and up to 78.33 per cent amount got utilized at 4 hours after administration, the moment when the

experiment was terminated. The trend of utilization of glucose was similar in both types of animals and the mean level of glucose in ketotic cows remained lower throughout the period of test as compared to healthy cattle. There was highly significant ($p<0.01$) difference between mean base values of blood glucose (before administration of dextrose) of healthy and ketotic cattle. The peak (at 0 hour) value had no significant difference between these groups. However, a significant ($p<0.05$) difference at 2 hours of glucose utilization was seen between healthy and ketotic cattle. The values of glucose utilization at 4 hours had no significant difference and the percent utilization was almost similar in healthy and ketotic cattle.

Sakai *et al.* (1996) administered 500 ml of 50 per cent glucose in healthy and ketotic cows. He reported an increase of glucose concentration by 6.4 and 8.1 fold in healthy and ketotic cows respectively immediately following administration. He reported a gradual decline in blood glucose from 0 to 15, 30, 45, 60 and 120 minutes in both normal and ketotic cows.

Samanc *et al.* (1996) reported that net rate of glucose utilization did not differ between healthy and ketotic cattle, as has also been seen in this study. They observed that the mean values at the end of experiment were higher than the initial values in both groups.

Anderson *et al.* (2000) administered 500 ml of 50% glucose solution to bulls weighing between 911.5 and 1035.5 kg body weight (258 mg/kg bat.). The glucose utilization took 240 minutes. In the present experiment the glucose utilization took longer duration because of less body weight of healthy and ketotic cows.

Renal threshold of glucose in healthy cattle

Along with determination of glucose utilization (tolerance) in cattle prior to finalising the line of treatment, it was also decided to judge renal threshold of glucose in six healthy cattle after administering 50% dextrose at a rate of 1 g/kg b.wt. It was considered essential to know about the amount of glucose an animal could retain in blood circulation without passing glucose in urine, i.e. to observe renal threshold of glucose for each of the aforesaid six animals by examining presence of glucose in urine by multistix and simultaneously determining the blood glucose levels continuously for about 5 hours following the intravenous administration of glucose.

The base values for blood glucose in these animals ranged between 48-60 mg/dl and the peak values after administration of glucose ranged between 380 and 396 mg/dl. The urine multidiagnostic strip at these two moments of study exhibited negative results for former and ++++ results for latter, respectively. Thereafter the blood glucose levels started declining continuously upto the observation period of 5 hours and reaction of multistix for the presence of glucose in urine also came down to show negativity. The renal threshold was 74.00 mg/dl after which urine became negative for glucose.

Kaneko (1963) considered urinary loss of glucose as an additional factor in maintenance of the blood glucose concentration. Bell and Jones (1945) reported the range of renal threshold of glucose in cattle higher than the values determined in the present study. They reported such values to fall between 98 and 102 mg/dl.

During the present study the renal threshold was 74.00 mg/dl which is close to the upper limit of normal blood glucose level. The method used in the present study for the determination of renal threshold of glucose correlated with urine glucose just before its disappearance from urine while the blood glucose was towards higher limit of normal levels of blood glucose. Whereas, the physiological studies of determining renal threshold levels in human being as well as in other species of animals (Kataria, 2000 in camels) were ssed on the appearance of glucose in the urine samples of catheterized individuals while the blood glucose levels were rising. As such, the blood glucose levels went on decreasing because the urine was stored in the urinary bladder till the natural micturition reflex initiated urination and sample collection. Furthermore, catheterisation was purposefully avoided in order to alleviate any effect of stress on blood glucose levels. Nevertheless, the renal threshold of cattle can be considered some where between 74.00 to 102.00 mg/dl, considering the lower and higher limits of the ranges arrived at during the present study and reported by Bell and Jones (1945) respectively.

Efficacy of treatment

The study conducted on glucose tolerance in healthy animals and that on glucose utilization in ketotic cows revealed that total amount of glucose present in a bottle of 500 ml of 50% dextrose administered intravenously was utilized within 6 hours in both types of these animals. Further, an investigation conducted on renal threshold in healthy animals by using same amount of glucose as mentioned above and also administered intravenously showed that the cattle could retain about 74.00 mg glucose/dl in their circulation whereas extra

amount got excreted in urine within 5 hours after administration. These recorded findings made the basis for designing the line of treatment for treating the ketotic cows.

All the ketotic cows were treated by administering Triamcinolone acetonide 12 mg intramuscularly and 500 ml of 50% dextrose intravenously on the first day. This helped in raising blood glucose levels and a drop in ketonuria. As per the need the ketotic cows were given one or two more treatments on second and third day which constituted only IN dextrose administration. Parameters of urine (Multidiagnostic strip reaction, Modified Rothera's test) and serum (glucose) were monitored daily so as to judge the efficacy of treatment. Estimation of these parameters were also made in ten healthy cattle for comparison of their status with that of ketotic cows. During the course of treatment it was found that nine animals out of 50 ketotic cows developed glycosuria during the course of treatment and had to be treated with zinc insulin in addition to the regular treatment as mentioned above.

For judging the efficacy of treatment all the 50 affected cows that were treated for ketosis were divided, into four groups, according to the results of Modified Rothera's test conducted on their urine as (1) +, (2) ++, (3) +++, (4) ++++. Nine cases out of these fifty cows developed glycosuria on administration of 50 per cent dextrose were considered to be insulin-dependent and constituted the fifth group. The line of treatment adopted and the efficacy of treatment for each of these five groups has been discussed as follows.

Before discussing the efficacy of treatment for these five categories of ketotic cattle and comparing these with the control group, it is worth while to mention that (1) Blood and bilirubin were absent in the urine of all such animals (healthy and diseased), (2) the amount of urobilinogen excreted in urine was same in all such animals, and (3) the intensity of Modified Rothera's reaction exactly matched the reaction given by multidiagnostic strip for ketone in ketotic cows read as +, ++, +++ ++++ (4) glucose in urine was absent in all the forty-one non-insulin dependent ketotic cows and (5) the nine insulin-dependent cases developed glycosuria during the course of treatment.

(1) Treatment of ketotic cows, urine of which was "+" for Modified Rothera's test

Ten ketotic cows of this category ("+") were treated with Triamcinolone acetonide 12 mg/animal intramuscularly and 500 ml of 50 per cent dextrose intravenously on the first day. One animal recovered on second day and the rest nine were administered only dextrose on second day that resulted in recovery of all these animals. Modified Rothera's reaction on urine of these animals exhibited that one animal became negative after one treatment, i.e. on second day and the rest nine animals on third day. The presence of protein in urine of affected cows ranged from negative to trace as was also seen in healthy cattle. The pH of urine of all these animals remained in normal range throughout the period of treatment. The specific gravity of urine before treatment was lower than normal and attained normalcy on third day. The treatment administered elevated blood serum glucose levels to a limit lower than the limit of renal threshold resulting in total glucose utilization in the body after each such treatment.

(2) Treatment of ketotic cows, urine of which was "++" for Modified Rothera's test

Seven ketotic cows of this category ("++") were treated with Triamcinolone acetonide 12 mg/animal intramuscularly and 500 ml of 50 per cent dextrose intravenously on first day. This was followed by 500 ml of 50 per cent dextrose administration on second day. One animal recovered on third day and the rest six animals were administered 50 per cent dextrose again which resulted in recovery of all these animals on fourth day. Blood glucose levels attained normalcy mostly after three treatments. Modified Rothera's reaction of urine of these animals exhibited that one animal became negative after two treatments i.e. on third day and the rest six animals on fourth day. The presence of protein in urine of affected cows ranged from -ve to +ve as was also seen in healthy cattle. The pH of urine of all these animals remained in normal range throughout the period of treatment. The specific gravity of urine before treatment was lower than normal and attained normalcy on third day. The treatment administered elevated the blood serum glucose levels to a limit lower than the limit of renal threshold resulting in total glucose utilization in the body after each such treatment.

(3) Treatment of ketotic cows, urine of which was "+++" for Modified Rothera's test

Ten ketotic cows of this category were treated with Triamcinolone acetonide 12 mg/animal intramuscularly and 500 ml of 50 per cent dextrose intravenously on the first day. Blood glucose levels attained normalcy after three treatments. All the animals

recovered on fourth day. Modified Rothera's reaction on urine became negative for all these animals on fourth day. The presence of protein in affected cows ranged from -ve to +ve as was also seen in healthy cattle but the +ve cases were more (8/10). The pH of urine of all these animals remained in normal range throughout the period of treatment. The specific gravity of urine before treatment was lower than control group and attained normalcy on third day. The treatment administered elevated the blood serum glucose levels to a limit lower than the limit of renal threshold resulting in total glucose utilization in the body after each treatment.

(4) Treatment of ketotic cows, urine of which was for Modified Rothera's test

Fourteen ketotic cows of this category were treated with Trimcinolone acetonide 12 mg/animal intramuscularly and 500 ml of 50 per cent dextrose intravenously on the first day. One animal recovered on third day and the rest thirteen animals recovered on fourth day. Blood glucose levels attained normalcy after three treatments. Modified Rothera's reaction of urine of these animals exhibited that one animal became negative on third day and rest thirteen became negative on fourth day. The presence of protein in affected cows ranged from - to ++. Proteinuria became prominent affecting about 43 per cent cases before treatment and started disappearing after two days of treatment. The pH of urine of all these animals remained in normal range throughout the period of treatment. The specific gravity of urine before treatment was lower than control group which attained normalcy on third day. The treatment administered elevated the blood serum glucose levels to a limit lower

than the limit of renal threshold resulting in total glucose utilization in the body after each such treatment.

(5) Treatment of insulin dependent ketotic cows, urine of which gave Modified Rothera's reaction from "+" to "++++"

Nine ketotic cows of this category were treated with Triamcinolone acetonide at the dose of 12 mg/animal intramuscularly and 500 ml of 50 per cent dextrose intravenously on the first day. Initial hypogylcaemia changed to hyperglycaemia accompanied with glycosuria in two of the nine cases with the administration of glucose for the treatment of ketosis on first day of treatment. Therefore these two cases were treated with 500 ml of 50 per cent dextrose I/V and zinc insulin subcutaneously on second day and the rest seven cases were treated only with 50 per cent dextrose (500 ml) intravenously. Thus administered second dose of dextrose resulted in glycosuria in a total of six cases out of nine on third day. Single case that did not develop glycosuria was treated only with dextrose, whereas the rest eight cases were treated with zinc insulin in addition to dextrose because it was necessary to treat ketosis which was still present (two cases which were treated with zinc insulin earlier did not have glycosuria on third day but showed hyperglycaemia with ketosis). The treatment given on third day could cure six cases out of nine and of the remaining three cases, one required only treatment with glucose, required treatment only with zinc insulin (because of hyperglycaemia), and the last required treatment with both dextrose and insulin. This treatment given on fourth day resulted in recovery of seventh case. Out of the remaining two cases on fifth day, one was treated only with dextrose and the other only with zinc insulin. These

two cases recovered on sixth day i.e. after five days treatment. Zinc insulin for the treatment of this category was used at a dose rate of 80 U (2 ml), 120 U (3 ml), 160 U (4 ml) and 200 U (5 ml) for blood glucose levels 60-80, 80-100, 100-120 and above 120 mg/dl, respectively in the concerned animal.

It was observed that administration of glucose and Triamcinolone acetonide remained ineffective in treating ketonuria in these insulin -dependent ketotic cases. Proteinuria exhibited an uncertain trend in these animals. Lowered pH of urine with hyperglycaemia accompanied with ketonuria revealed existence of diabetic ketoacidosis. The specific gravity of urine remained almost unaffected. Administration of glucose during treatment resulted in glycosuria confirming to diabetic ketoacidosis. Insulin was incorporated in the line of treatment because of resultant glycosuria under the condition of hyperglycaemia with the objective to help the animals in overcoming the pancreatic insufficiency. Glucose utilization was poor and glucose administration resulted in hyperglycaemia crossing the limit of renal threshold in these animals during the course of treatment. The study on each animal was terminated as it cured (negative for Modified Rothera's test/multidiagnostic strip-ketone).

The findings recorded in the present study on treatment given to above mentioned five groups of ketotic and diabetic ketoacidosis have been compared with the research work conducted by other scientists. One of the important fording that intensity of Modified Rothera's reaction exactly matched the reaction given by multidiagnostic strip for ketone in ketotic cases read as +, ++, +++ and was in agreement with that reported by Singh (1994) who stated

that keto-diastix as well as Modified Rothera's test produced similar results in ketotic cows. Gupta (1999) concluded that there was no discrepancy between Modified Rothera's test and keto-diastix in bovine ketosis. A positive correlation was also found between strip and Rothera's test by Singari *et al.* (1988). Absence of bilirubin in urine, negativity of urine for haematuria and haemoglobinuria, and unaltered amount of urobilinogen excreted in urine of ketotic cows were the observations similar to those recorded in healthy controls.

The treatment in all the 50 ketotic cows was started with the administration of Triamcinolone acetonide 12 mg/animal intramuscularly and 500 ml of 50 per cent dextrose/animal intravenously. The administration of 50 per cent dextrose was continued till recovery. Meanwhile it was observed that such treatment could achieve cure in 41 (82 per cent) cases out of 50. The rest nine (18 per cent) cases developed glycosuria during the course of treatment of ketosis without relieving ketonuria. In these nine cases it was essential to administer glucose to treat ketosis and zinc insulin to treat hyperglycaemia.

Out of the total of 50 ketotic cows, the disease was mild in ten (20 per cent) cases urine of which was + for Modified Rothera's test, moderate in seven (14 per cent) cases urine of which was ++, and severe in 24 (48 per cent), the urine of which was +++ and ++++ for Modified Rothera's test. The urine of nine (18 per cent) insulin dependent cases was independent of intensity of Modified Rothera's reaction, that varied between + and . The treatment advocated in mild, moderate and - severe cases of ketosis remained same but recovery period varied in number of days. The animals were declared

cured on achieving negativity of Modified Rothera's and multidiagnostic strip, reactions for ketone in urine accompanied with progressing levels of serum glucose near normalcy. All the 41 non-insulin dependent cases got cured after treatment of one to three days. Two treatments were enough to cure mild ketosis, the moderate cases of ketosis required mostly three days treatment. Severe cases of ketosis got cured after three treatments. Appetite started improving after first treatment and animals started consuming complete ration by the time of complete clinical recovery.

From the past several decades ketosis was considered as a disease of immense economic importance and much efforts were made to find out its proper treatment. Hupka (1928) was the first to use glucose for the treatment of ketosis in cows and it proved beneficial. Duncan *et al.* (1939) reported decrease in blood and urinary acetone after intravenous infusion of glucose, a finding similar to that recorded in present investigation. Hatziolos and Shaw (1950) injected cortisone acetate (900 mg im) in ketotic cows. The cows regained their appetite in two hours, all signs of paresis disappeared and blood glucose became normal in 48 hours. In the present study the non-insulin dependent ketotic cows achieved recovery between 24 and 72 hours.

Protein was either absent or present in urine of mild cases of ketosis. The position of proteinuria in moderate cases was also same except that one case out of seven ketotic cows developed proteinuria amounting to "+". The number of cases and intensity of proteinuria increased in severe cases of bovine ketosis. Mismanagement of post-parturient nutrition of cows resulting in decreased availability of carbohydrate and thus over intake of protein

in ration could trigger the pathogenesis of bovine ketosis with proteinuria clinically exhibited with the advancement in severity of disease. Benjamin (1978) and Anon (1999) reported that proteinuria occurred due to ingestion of excessive amount of protein. This was also true in healthy and diseased cattle studied under this investigation.

There was no significant alterations in pH of urine of mild, moderate and severe cases of disease when compared among these and with the readings recorded for healthy control. The specific gravity was observed to have negative relation with the severity of the disease. The specific gravity fell with the advancement of disease. Comparatively it was more in milder cases than severe cases, moderate cases occupying middle position, but it was all the times lower in diseased animal in comparison with healthy animals. Decreased renal function might have been a factor causing this phenomenon as has also been reported by Benjamin (1978).

The urine of insulin-dependent ketotic cows also gave identical reaction when subjected to Modified Rothera's test and multidiagnostic strip test as was also seen while conducting urine analysis of other categories of ketotic cows. Similarly observations made for the presence of urobilinogen and absence of blood and bilirubin in urine of these animals were similar to those of the other categories of ketotic and healthy cows. Proteinuria exhibited an uncertain trend in such type of cases. It was also observed that appearance_ of glucose in urine resulted in increase in specific gravity of urine in these animals when compared with the other groups of ketotic cows. Lowered pH with hyperglycaemia accompanied with

ketonuria revealed existence of diabetic ketoacidosis in these insulin-dependent cases.

Melendez (1997) found that urinalysis reagent strip were reliable for diagnosis of diabetic ketoacidosis by identifying glycosuria and ketonuria. The cases in the present study were also clinically monitored by such a method. Increased specific gravity in urine was seen in diabetes mellitus and primary renal glycosuria by Benjamin (1978) and Anon (1999). Their findings were in agreement with those recorded in this investigation. Further Kaneko (1963) postulated that urinary pH was of little value in detecting acidosis in diabetes mellitus, for only in extreme cases the pH varied beyond normal limits. In the present study also the urinary pH though decreased during diabetic ketoacidosis but did not exhibit prominent fall.

Bouckaert *et al.* (1958) treated cases of bovine ketosis with glucose and insulin. This combined treatment was very effective in removing ketone bodies from blood, a finding which was similar to that recorded in present investigation. Whitekar (1981) also reported that single dose treatment with 50 per cent glucose and insulin was very effective in treating clinical cases of bovine ketosis. As glucose utilization was enhanced by administering zinc insulin in the cases of diabetic ketoacidosis in this study. Rings (1985) also observed rapid utilization of glucose with speedy recovery following administration of glucose and insulin during bovine ketosis. Sakai *et al.* (1993) also recommended such a combined therapy for effective treatment of bovine ketosis.

SUMMARY

Bovine ketosis was clinically studied in 50 ketotic cows in and around Bikaner town located in northwestern semiarid part of Rajasthan in the year 1998-99. The disease affected cows mostly after parturition. The disease emerged in cows aged up to 9 years mainly during first and second month after parturition, in second to fifth parity and mostly in colder months. The major clinical manifestation were abrupt decrease in milk yield, cessation of feeding on concentrate, decreased fodder intake and acetone like odour in urine of severe cases and constipation. One of the major findings of the biochemical study was that there was a highly significant increase in ketone (acetone and acetoacetic acid) levels in both serum and urine of affected cows when compared with ten healthy cows. Hypoglycaemia was another major finding recorded in studied ketotic cows. There was a highly significant increase in total serum protein, serum albumin and serum globulin concentration during disease with a significant decrease in albumin globulin ratio. There was highly significant increase in the blood-urea-nitrogen levels in cows affected with ketosis. Highly significant decrease was recorded in mean serum calcium level in ketotic cows whereas the difference between the mean values of serum inorganic phosphorus was non-significant. There was a highly significant increase in serum magnesium levels. A highly significant decrease in the mean sodium and potassium concentration occurred during disease in ketotic cows. There was a highly significant increase in serum alanine aminotransferase and serum aspartate aminotransferase activity. As a general finding it was recorded that all

the biochemical parameters attained normalcy after treatment in each of the 50 studied ketotic cows except ALT, AST and BUN.

Major alteration in biochemical status and manifestations of ketotic cows grouped as per Modified Rothera's classification as + (mild), ++ (moderate), +++ and ++++ (severe) cases were that the serum and urine ketones as well as alanine aminotransferase increased whereas blood glucose decreased in the animals of all these groups with the increase in intensity of Modified Rothera's reaction. Acetone-like odour was prominent in severe cases of ketosis.

Biochemical changes in ketotic cows as per degree of ketonaemia were that ketonuria, alanine aminotransferase and total serum protein were positively correlated with ketonaemia. Such a relation of these parameters was also observed to exist between these and degree of ketonuria. The degrees of ketonuria were dependent on degree of ketonaemia. Blood glucose and serum calcium levels decreased with the increase in ketonaemia and ketonuria.

Glucose utilization in diseased cows and glucose tolerance in healthy animals remained identical in terms of initial rise in blood glucose levels and total period by which the administered glucose was eliminated from blood circulation (6 hours). Further the renal threshold of glucose in healthy animals was 74.00 mg/dl on administration of 50 per cent dextrose in blood serum.

All the ketotic cows were treated by administrating triamcinolone acetonide 12 mg/animal intramuscularly and 500 ml of 50 per cent dextrose per animal intravenously on the first day of

treatment. As per the need the ketotic cows were given one or two more treatment on second and third day which constituted only intravenous dextrose administration. This resulted in recovery of 41 cases out of 50 which belonged to all the studied four groups i.e. from "+" to "++++". The rest nine animals started developing glycosuria without disappearance of ketonuria and were classified under the fifth group of insulin dependent ketotic cases (Diabetic ketoacidosis). All these nine animals were successfully treated by supplementing the above line of treatment with zinc insulin during the course of treatment.

CONCLUSION

The prevalence of bovine ketosis was 9.90 per cent among the suspected clinical cases. Based on anamnesis the milk yield abruptly decreased up to about 40 per cent in untreated animals. A study on grouping of cases according to the intensity of Modified Rothera's reaction of their urine as +, ++, +++ and ++++ revealed that the veterinary physician could have an idea about approximate levels of some important related biochemical parameters in the cases of concerned group for ketonaemia, ketonuria, serum glucose, serum protein, ALT and AST. Reaction of multidiagnostic strip under urine examination exactly matched Modified Rothera's reaction in terms of intensity expressed as +, ++, +++ and ++++. Further, the use of multidiagnostic strip proved advantageous over Modified Rothera's test because the strips could exhibit changes in additional parameters like urine pH, amount of glucose present in urine, specific gravity along with ketonuria whereas Modified Rothera's test could only detect ketonuria (acetate + acetoacetic acid only). Thus it became very easy to detect insulin-dependent cases of ketosis i.e. diabetic ketoacidosis among the patients undergoing treatment for ketosis because the resultant glycosuria following glucose administration indicated the existence of hyperglycaemia. Therefore, the daily monitoring of blood glucose and examination of urine by multidiagnostic strip helped in assessing the efficacy of treatment under hospital condition.

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CLINICO - BIOCHEMICAL STUDIES ON KETOSIS IN CATTLE

Ph.D. THESIS

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ABSTRACT

This study was conducted on bovine ketosis in and around Bikaner in the year 1998-99. The prevalence was higher between second and fifth lactation particularly in colder months in cows aged between 6 and 9 years, the span of life when milk-yield was at peak. The major clinical manifestations were abrupt decrease in milk-yield, cessation of feeding on concentrate, decreased fodder intake, constipation and acetone-like odour in urine of severe cases. The disease was mainly characterized by ketonaemia, hypoglycaemia, proteinemia and increased enzymatic activity of ALT and AST in blood serum and ketonuria with changes in specific gravity and pH of urine. The clinico-biochemical aspects of disease were recorded according to degree of (1) ketonaemia and (2) ketonuria expressed as +, ++, +++ and ++++ by Modified Rothera's test and urine multidiagnostic strip. Treatment with 50 per cent dextrose intravenously and Triamcinolone acetonide intramuscularly could successfully cure 82 per cent affected cows. The rest 18 per cent animals were detected to be insulin dependent cases of diabetic ketoacidosis and required administration of zinc insulin subcutaneously during the course of treatment for recovery.

गौ-वंश के कीटोसिस रोग में शयनिक एवं जैवरसायनिक अध्ययन

पी.एच.डी.-शोध ग्रन्थ

पशु औषध विभाग

पशु चिकित्सा एंव पशु विज्ञान महाविघालय

राजस्थान कृषि विश्वविघालय, बीकानेर-३३४००१

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अनुक्षेपण

गौ-वंश के कीटोसिस रोग पर यह शोध कार्य बीकानेर शहर एवं इसके आस-पास के क्षेत्र में वर्ष १९९८-९९ में किया गया। इस रोग ने गायों को उनके जीवन काल के सर्वोच्च दुग्ध उत्पादन के समय, दूसरे से पाँचवें ब्यांत के मध्य और विशेष रूप से वर्ष के ठण्डे महीनों में उनकी आयु के ६ से ९ वर्षो के बीच अधिक प्रभावित किया। अधिक प्रभावित पशुओं के लक्षणों में प्रमुख थे - अचानक दुग्ध उत्पादन घट जाना, बाँटा न खाना, चारा कम खाना, कब्ज होना और मुत्र में असीटोन जैसी गन्ध आना। इस रोग के मुख्य नैदानिक गुण थे - कीटोनरक्ता, अवग्लूकोनरक्ता, उच्चप्रोटीनरक्ता एवं ए.एल.टी. और ए.एस.टी. की सीरम में बढ़ी हुयी इन्जाईमी क्रिया तथा मुत्र में किटोनमेह के साथ आपेक्षिक घनत्व व पी. एच. परिवर्तन। इस रोग के शयनिक एवं जैवरसायनिक पहलुओं को (१) कीटोनरक्ता (२) कीटोनमेह के श्रेणी के अनुसार +, ++, +++, ++++ की प्रकट सीमाओ में रोथरा परिक्षण तथा मुत्र बहुनिदान सूचक स्ट्रीप द्वारा अंकित किया गया। ५० प्रतिशत डेक्स्ट्रोस घोल अंतः शिरा में और ट्राइमसीनोलोन एसीटोनाईड अंतः माँस पेशी में दे कर की गई चिकित्सा द्वारा ८१ प्रतिशत रोगी गाये सफलता पूर्वक उपचारित की जा सकी। शेष १८ प्रतिशत रोगी पशु इन्सूलीन निर्भरता के कारण मधुमेह-कीटोअम्लता से प्रभावित पाये गये और उनकी चिकितसा के समय उन्हें जिंक इन्सूलीन (सबक्यूटेनियसली) देने पर ही उनका उपचार संभव हुआ।