BIOCHEMICAL EVALUATION OF BUFFALO'S MILK ENZYMATIC ACTIVITY IN NORMAL AND MASTITIC ANIMALS

THESIS

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C O N T E N T S

I N T R O D U C T I O N

In the developing countries, shortage of available animal proteins with continuous increase in the annual population; is a highly complicated problem.

Trials are directed today for solving the important problems especially those concerned with the animal protein deficiency. Milk is considered one of the main foods containing sufficient amount of animal protein required for body building especially childrens.

Mastitis stands out as the most wide spread and destructive disease to milk production as well as mastitic milk itself is unfit for human consumption.

The serious effect exerted by mastitis is mostly due to its subclinical form during which the causative microorganisms act as invisible potential source for supreading infection in the herd.

Several methods for diagnosing mastitis have been reported. Bacteriological examination is expensive and time consuming, hence the need for simple, sensitive and reliable method sufficient to be applied on a large scale for herd testing is required.

This study was carried on, aming scientifically the following points:

1 . Biochemical Evaluation of buffalo's milk enzymatic activity in normal and mastitic animals compared with that of cow's milk.

2 . The incidence percentages of micro-organisms causing subclinical and clinical mastitis in both animals.

3 . The correlation between the different pathogenic isolates and the biochemical composition of mastitic milk in cases of subclinical and clinical mastitis.

4 . Use of some sensitive enzymes for a quick laboratory diagnosis especially subclinical form.

5 . The application of some modifications in the techniques used for assay of enzymes to obtain reliable rapid determinations.

Inflammation of the udder is more commonly known as mastitis, the name derived from the Greek Word "mastos", meaning mammary gland, than it is by the corresponding Latin term mammitis, which is derived from mamma, the Latin name for the same gland (Smith, 1974).

Parker (1976) added that this general term covering most disease conditions of the mammary gland which can affect the female of all species of mammals.

The effect of mastitis on lactating animals can provide the veterinarian with remarkable and valuable informations in relation to health and disease.

The review of literature on the effect of mastitis on the lactating animals may be classified as follows:

I - General effect of mastitis.

II- Effect of mastitis on the biochemical composition of milk.

I - General Effect Of Mastitis

Jordan (1930) reported that mastitic milk is unfit for human consumption because of its content of Staphylococcal enterotoxin which causes dizziness, loss of appetite, weakness, abdominal pain, headache, nausea, vomiting and diarrhea.

Shaw et al, (1935) stated that one of the obvious manifestations of mastitis is a decrease in the total amount of milk secreted.

Plastridge (1958) recoreded that mastitis reduces the milk yield and shortens the productive life of affected cows. Moreover, Moursy et al, (1972) reported that mastitis is the most wide spread and destructive disease to milk production.

Cockrill (1974) mentioned that mastitis is one of the scourges of milk production.

Beuche et al. (1975) stated that of all diseases of the udder causing losses in milk output the greatest harm is caused by subclinical and chronic mastitis. They also listed the different methods of determining reduction of output and concluded that the best one is that of comparing

output between healthy and infected quarters of the same udder.

Lampert (1975) stated that mastitis has important significance in the economy of milk production.

II- Effect Of Mastitis On Biochemical Composition Of Milk

A. Milk Enzymes

During the last 40 years a number of enzymes have been identified as being regularly present in fresh cow's milk.

All the enzymes of cow's milk so far investigated are also present in buffalo's milk. (Cockrill, 1974).

A clean milk produced under sanitary conditions from healthy animals contains a number of enzymes which are considered true constituents of milk (Rifaat et al. 1969).

The enzymes in raw milk must be considered in handling of product as they may influence undesirable changes in flavor (Lampert,1975).

(1) Effect of Mastitis on Glutamic oxalacetic and Glutamic pyruvic transaminases (GOT and GPT):

GOT and GPT are widely distributed in animal and plant tissues as well as in bacteria (Abderhalden,1961).

Cantarow (1960) mentioned that these enzymes had a wide tissue distribution. He stated that transaminases catalyze the transfer of an amino group (Glutamic) to a keto acid (oxalacetic or pyruvic). Pyridoxal phosphate being the co-enzyme involved in transamination processes.

Bogin and Ziv (1973) stated that milk GOT increases during the course of acute mastitis in Israeli Friesian dairy cows. They also recoreded that serum was not the sole source for this enzyme in mastitis secretions and the enzyme must have been liberated from cells composing the udder parenchyma, or from disintegrating leukocytes or both of them or from other sources.

Bogin et al. (1976) determined the GOT and GPT activity in slices of normal cow's udder as well as, udders with acute mastitis and udders characterized by extensive fibrosis and they recorded that the mean GPT activity in acute mastitis was 50% lower than in the normal udder while udder tissues characterized by extensive fibrosis contained significantly higher concentration of this enzyme , on the other hand GOT levels in the three types of udder tissue examined were nearly the same.

Frahm et al. (1977) determined the enzymatic activities of GOT and LDH in the plasma and homogenates of liver,kidney, heart, spleen, skeletal muscle of 7 young spotted bulls and they found that both enzymes seem to have no organ specificity. However, the literature seems to be defieient in data concerning the glutamic pyruvic transaminase (GPT) activity in normal and mastitic milk.

(2) Effect of Mastitis on Alkaline Phosphatase:

Phosphatases are enzymes that catalyze the hydrolysis of phosphate esters. There are numbers of kinds of phosphatases differing in their specificity towards the substrate that they will attack as well as in optimum pH and temperature requirements.

Alkaline phosphatase is one of the major enzymes which have been identified as being regularly present in milk, (Cockrill, 1974).

Dempsey et al. (1947) reported that in the nonpregnant animal the alkaline phosphatase reaction is most marked in the myoepithelial cells and nearly absent from the alveolar cells of the mammary gland but as pregnancy progresses the alveolar cells become rich in phosphatase activity.

Kalsall et al. (1959) recorded that alkaline phosphatase activity seems to be closely related to the DNA content of the gland and they believed that alkaline phosphatase is found whenever nucleic acid occurs.

Morton (1954) showed that the milk alkaline phosphatase occurs in small particles called microsomes and these particles are adsorbed on the surface of the fat globules.

Haab et al. (1956) reported that milk alkaline phosphatase varied among individual milkings.

Safwat et al. (1956) found that the alkaline phosphatase of buffalo's milk was much less than that of cow, while Rifaat et al. (1969) found that buffalo's milk has higher alkaline phosphatase (mean 10475 ug phenol/ml) in comparison to cow's milk (mean 302.3 ug phenol/ml).

Habb (1958) found a wide range variation in the content of alkaline phosphatase in cow's milk (from 238 ug phenol/ml up to 8778 ug phenol/ml milk).

Oser (1976) mentioned that cow's milk collected in mid lactation contains a high alkaline phosphatase content(80-120 King Armstrong Units/100 ml) as compared with human milk (0.7-16.2 King Armstrong Units/100 ml).

Arima (1962) observed that alkaline phosphatase in subclinical mastitic cow's milk is much greater than that for normal milk.

Abramyan et al. (1968) determined the alkaline phosphatase activity in milk from normal cows as well as from cows with experimental subclinical mastitis and they found that the alkaline phosphatase content of milk has increased by 60%, 24-48 hours after experimental infection of cows.

Taylor and Kitchen (1970) reported a negative correlation between alkaline phosphatase activity and cell count in samples of herd milk.

Bogin and Ziv (1973) found that when the Friesian dairy cows were infused with Escherichia coli endotoxin, the mean alkaline phosphatase level peaked at 50 hours and remained above the preinfection value for 300 hours.

Fuquay et al. (1975) reported that in cases of severe experimental coliform mastitis in cows, the level of plasma alkaline phosphatase increased and returned to the preinfection value by the 3rd week.

Bogin et al. (1976) determined the levels of alkaline and acid phosphatases in slices of normal cow's udders as well as udders with acute mastitis and udders characterized by extensive fibrosis and they found that the concentration of alkaline and acid phosphatases were somewhat higher in both the mastitic and fibrotic udders, they also added that the differences, however, were not significant.

Bozhkova and Tsvetkov (1976) stated that subclinical mastitis in cows resulted in an increase in the activity of alkaline phosphatase enzyme in milk.

Anderson (1977) recorded that the specific activity of all enzymes in milk including alkaline phosphatase was elevated as the cell count increased.

(3) Effect of Mastitis on Acid Phosphatase:

The acid phosphatase enzyme hydrolyzes phosphate esters just as does alkaline phosphatase, but its distinguishing feature is indicated by its name. The enzyme has its maximal activity in more acid solution; namely at about pH 5(Zittle, 1964).

Mullen (1950) studied the activity of acid phosphatase in cow's milk and observed that 80-90% of the acid phosphatase of whole milk remained after heating at pasteurization conditions (145 F "62.5°C" for 30 minutes. He also added

that, milk acid phosphatase enzyme does not associate itself with the fat globules but it is rather found in skim milk.

Andrews and Allchanidis (1973) stated that milk contains a single acid phosphatase isoenzyme with an isoelectric point close to pH 7.9 and a molecular weight of 42.000 ± 2000. They also found that the enzyme optimum pH was 4.9.

Bertran (1952) reported an average activity of 4.00 Bodansky units equal to 556.4 ug phenol/ml milk while Heyndrickx and Peeters (1958) reported an average of 370 ug phenol/ml milk.

Rifaat et al. (1969) stated that acid phosphatase activity in buffalo's milk ranged from 17.0 to 208 ug phenol/ml milk with an average of 57.5 ug phenol/ml milk, while in cow's milk it ranged from 25 to 606 ug/ml with an average of 175.8 ug phenol/ml milk.

Abramyan and Buniatyan (1968) determined, the acid phosphatase activity in milk of cows with experimental subclinical mastitis and normal cows. They found that the activity amounted to 0.80 ± 0.39 and 1.8 ± 0.46 Bodansky units respectively. They concluded that the acid phosphatase activity in mastitic milk decreases slightly than in normal

milk.

Andrews (1976) found that the acid phosphatase activity of milk samples from apparently healthy animals amounted to 3.5-11.5 U/liter. He also added that after 36 hours of experimental intramammary infection of Streptococcus agalactiae the acid phosphatase activity increased by a factor of 16.5, whereas, after 24 hours intramammary infusion of Escherichia coli endotoxin, the activity increased by a factor of 3.2.

Kitchen (1976) found that in bovine milk there exists a little correlation between the acid phosphatase activity and electronic cell count.

Bogin et al. (1976) determined the acid phosphatase activity in slices removed from normal cow's udder as well as from udders with acute mastitis and udder characterized by extensive fibrosis and they found that the acid phosphatase activity increased slightly in udders with acute mastitis and extensive fibrosis.

Mullen (1950) stated that acid phosphatase activity in milk from quarters infected with Streptococcus agalactiae was elevated.

(4) Effect of mastitis on 5'-Nucleotidase (5'-ribonucleotide phosphohydrolase):

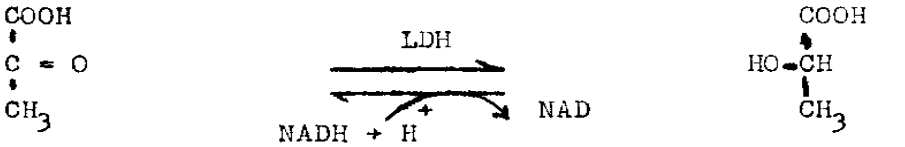
This enzyme is a phosphatase which acts only on nucleoside -5'-phosphatase, releasing inorganic phosphate. The substrate usually used in the assay of this enzyme is adenosine-5'-phosphate.

Schlotke (1976) stated that 5'-nucleotidase enzyme is present in the myoepithelial cells surrounding the alveolar tissues of the mammary gland.

Oser (1979) reported that 5'-nucleotidase enzyme was found in animal tissue and snake venom, and acte on nucleoside-5'-phosphate. However, data concerning 5'-nucleotidase enzyme in normal and mastitic milk are not available in the literature.

(5) Effect of Mastitis on Lactic dehydrogenase (LDH):

Oser (1976) stated that lactic dehydrogenase enzyme catalyzes the reduction of pyruvic acid into lactic acid in the presence of $NADH_2$ as a coenzyme as follows:



Pyruvic acid Lactic acid

Carlson et al. (1969) determined the distribution of lactic dehydrogenase in the mammary gland and milk of rat and they found that LDH activity is included in the fat globules of milk.

Karlsson et al. (1968) evaluated the level of lactic dehydrogenase (LDH) in rat milk at day 0 up to the day 22 of lactation and they found that the lactic dehydrogenase activity increases during the lactation period.

Frahm et al. (1977) stated that lactic dehydrogenase enzyme is one of the enzymes which have no organ specificity in the bovine.

Bogin et al. (1977) recorded that the orgin of elevated lactic dehydrogenase activity in mastitic milk is the leukocytes and the parenchyma cells of the udder.

Ziv et al. (1976) found that when the udders of cows were artificially infused with Escherichia coli endotoxin, the level of lactic dehydrogenase enzyme in mastitic milk increases 48 hours after infustion, whereas the level of the same enzyme in milk collected from uninfused quarters remained essentially unchanged .

Bogin et al. (1976) determined the lactic dehydrogenase activity in slices removed from normal cow's udder as well as from udders with acute mastitis and udders characterized by extensive fibrosis and they recorded that the lactic dehydrogenase increases slightly in mastitic udders.

Bogin and Ziv (1973) found that when the Friesian cows were infused with Escherichia coli endotoxin, the activity of lactic dehydrogenase in milk increased and amounted to 300 mu/ml at 0 hour and was highly elevated after 9 hours infusion (5525 mu/ml).

(6) Effect of Mastitis on Lipase:

Lipases are enzymes that hydrolyze fats. They are present in many organs, micrcorganisms and plant seeds. Under certain conditions, the lipases present in milk will liberate fatty acids from milk fat. (Summer and Nyrback, 1951).

El-Hagarawy and Sirry (1967) reported that the lipase was found to be higher in fresh buffalo's milk than in cow's milk, as its resulted in more liberation of free fatty acids.

Rifaat et al. (1969) found that the lipase activity of buffalo milk ranged from a minimum of 3.5 ml to a maximum of 68.5 ml (0.01 N NaOH) per 100 ml milk with an average of

16.5 ml, while in cow's milk it ranged from 3.0 ml to 33.5 ml (0.01 N NaOH) per 100 ml milk with an average of 14.8 ml per 100 ml milk.

Downey and Andrews (1969) found that there are several lipases in cow's milk.

Reichter and Randolph (1971) stated that the bovine milk lipase hydrolyses the triglycerides simple short chain fatty acids faster than those with long chain fatty acids and shows little specificity for natural oil emulsion.

Mendelson et al. (1977) reported that lipoprotein lipase is involved in the uptake of chylomicron triglycerides by lactating mammary gland. They also added that lipoprotein lipase activity is increased markedly in mammary gland and decreased in adipose tissue during lactation in the rat.

Wang and Randolph (1978) stated that temperature activation did not affect lipase activity of whole milk but altered the distribution of lipase in skim milk and cream fractions. They also added that, cooling-milk to 4°C caused migration of lipase activity to the cream fraction.

Heyndrick and Peeters (1958) reported an average lipase activity of 5.75 ml (0.01 N NaOH) per 100 ml milk.

Pavel (1960) found that the lipase activity for cow's milk ranged from 38.7-50 ml (0.01 N NaOH) per 100 ml milk.

Guthrie and Herrington (1960) stated that the lipase activity of mastitic milk is higher than that of normal milk.

(7) Effect of Mastitis on Amylase:

Amylases are enzymes which hydrolyse the α-1-4-D-glucosidic linkage in starch and glycogen. Two principal kinds of amylases called α-and B-are recognized. α-Amylase attack prefrentially central α-1,4-D glucosidic linkages in the starch molecule. α-Amylases are found in plants, microorganisms and in the pancreas, blood, urine and saliva of animals (Jenness and Patton, 1969).

Heyndrickx and Peeters (1958) determined the mean value of amylase enzyme content in cow's milk and found that the activity was 180 mg starch/100 ml milk.

Rifaat et al. (1969) found that the amylase activity in buffalo's milk ranged from 21 to 125 unit/100 ml milk with an average of 64.9 units, while in cow's milk it ranged from 47.5 to 66.5 unit/100 ml milk, with a mean value of 57.7 units.

Chrzaszcz and Goralowna (1925) stated that the amylase activity was elevated in mastitic milk as well as clostrum when compared with normal milk, and the enzyme was shown to concentrate in the cream. They also added that leukocytes in the milk had no influence on the activity of the enzyme. Guy and Genness (1958) reported that α-amylase content of milk varies from cow to cow and is high in milk from mastitic udders.

B. Effect Of Mastitis On Ascorbic Acid

Barakat and Abdel-Wahab (1961) reported the average content of ascrobic acid in human, cow's, goat's and buffalo's milk as 20.2, 7.6, 7.6 and 29.6 mg per litre milk respectively.

Oser (1979) stated that ascorbic acid content of cow's and goat's milk was 1 mg/100 gm whole milk. He mentioned also that this level was decreased by storage of milk several days.

Similarly, Jennes and Patton (1969) studied the content of ascorbic acid in cow's milk and they stated that fresh cow's milk contains an average of 20 mg/litre, while pasteurized milk contains 5 mg/liter whole milk.

Nani and Defranceshi (1957) reported that milk samples obtained during the clinical phase of Streptococcus agalactiae mastitis contained increased levels of ascorbic acid.

Kisza et al. (1966) reported that in cases of acute mastitis ascorbic acid content was decreased by 26% in summer and 45% in winter, while in chronic mastitis, the decrease reached about 20% in summer and 25% in winter.

C. Effect Of Mastitis On pH Value

Definition of pH:

pH is strictly defined as the negative logarithm of the hydrogen ion concentration (Plummer, 1978).

Zeich (1937) stated that milk from cows infected with Strept. mastitis has a pH value of about 6.8 and in chronic cases the pH may drop down to about 5.2.

Brayan (1939) investigated milk samples of 189 mastitic cases and found that from those, 112 samples gave negative bromothymol blue test and he concluded that, this test is not reliable.

Chu (1949) stated that the bromothymol blue test was of little value in detecting mastitis, the accuracy of the test being only 43.6%.

Wahby and Nasr (1957) determined the pH of 400 individual milk samples by means of Beckman's potentiometer and found that out of 100 mastitic milk samples detected, only 63 showed pH values above those recorded for normal samples, while the remaining 37 samples had pH values within the range of normal milk.

Kelly (1967) stated that the pH of milk of mastitic glands was found towards alkaline side.

Jenness and Patton (1969) reported that the pH of normal fresh cow's milk ordinarily falls between 6.5 and 6.7 and that values higher than 6.7 usually denote mastitic conditions in the udder, while those below 6.5 indicate the presence of colostrum of bacterial deterioration.

Renner (1975) and Beuche (1977) stated that the pH of mastitic milk was found towards the alkaline side.

Joshi et al. (1976) studied the pH values of milk samples, and reported that the pH of milk samples from healthy udders was found to lie between 6.1 and 6.7, whereas, in asymptomatic mastitic glands it ranges between 6.7 and 7.9.

Schalm et al. (1971) attributed the raished pH of mastitic milk to the increased permeability of the gland to blood components which permits movement of bicarbonate ions into the milk. They also added that, this process is not a passive dilution but is a selective transudation.

Incidence and causative agents of mastitis

Mastitis is a general name covering most diseases conditions of the mammary gland.

The inflammation arises as a response to infection or injury, but even the existing cause is an injury, an infection will usually follow. Therefore, it is difficult to be controlled since a maltitude of factors are directly or indirectly involved in its aetiology.

About 80% of mastitic cases are caused by the growth of micro-organisms in the udder, and the remainder by traumatic lesions.

Invasion of the udder by micro-organisms takes place in most cases by way of the test canal.

In septicaemic diseases some organisms are transferred to the mammary gland by way of the blood stream.

Slantez et al. (1940) recorded that Strept. agalactiae, Strept. uberis, Strept. dysagalactiae and Strept. faecalis were isolated from 84, 12, 2 and 1% respectively of mastitic cows. However, Ferguson (1944) found that 40.8% of cases were due to Strept. agalactiae and 25.4% to Staph. aureus. Moreover, streptococci were found by Narayanan et al. (1953) to be

responsible for about 30% of cases in cows and 80% in buffaloes. They added that the incidence percentage of mastitis in cows and buffaloes were found to be 45.5% and 30.8% respectively.

Nasr (1956) found that Strept. agalactiae was the prevalent organism in cases of subclinical mastitis .

Wahby and Nasr (1957) reported that Strept. agalactiae (75.0%), Strept. dysagalactiae (3.4%), Strept. uberis (7.96%), Strept. pyogenes (3.41%), Staph,aureus (4.54%), Corynebacterium pyogenes (3.41%) and Klebsiella pneumoniae (2.27%) were isolated from milk samples collected from mastitic cows.

Loftsgard et al. (1960) reported that mycological examinations were performed on 1460 milk samples of which 480 were from quarters showing symptoms of mastitis. Yeasts were demonstrated in 7 samples which were identified as Sacchromyces maxianus, Sacchromyces fragils, Candida krusei, Candida parapsilosis var intermedia and Trichosporon sp., while Candida tropicalis was isolated from cows with acute mastitis.

Arnaoout (1961) surveyed 3156 milk samples collected from 217 animals at Bahtim village. He found that the incidence of mastitis reached 22.5% and a variety of micro-organisms were isolated including strept. (63.80%), Staph. (0.61%), mixed infection with both Strept. and Staph. (18.40%), diplococci (9.81%) and Corynebacteria (7.36%) .

Heimonas (1961), in Northern Greece, examined 467 milk samples collected from mastitic cows. A wide variety of organisms were found to be incriminated including Staph . aureus (35%), Ps. pyocyanae (22%), Corynebacterium pyogenes (3.6%), Tubercle bacilli (2.3%), Alcaligenes faecalis (1.9%), Aspergillus fumigatus (0.2%) and Clostridium welchii (1.4%). On the other hand, mixed infections were recorded in 25% of cases.

The results obtained by Forst (1962) in a survey included 44 herds revealed that 29.7% of cows were infected with Strept. agalactiae while as much as 46.8% with Staph. aureus.

Latent bacterial flora was demonstrated and identified in 37.2% of cows by Muck (1963). He stated that out of 77 cases of acute mastitis, 20 cases were found to be due to coliforms, 4 cases due to Strept. agalactiae, 6 cases due

to Strept. uberis, 13 cases due to Micrococci, 9 cases due to other Streptococci and 10 cases due to Staphylococci.

In Egypt, the predominant organisms isolated from mastitic buffaloes and cows were found to be Staph. aureus and Strept. agalactiae (El-Guindy et al. 1964).

Khalil et al. (1968) tested bacteriologically 336 milk samples from apparently normal buffaloes. Different species of micro-organisms from 50 samples (14.9%) were isolated and Strept. uberis. They suggested that apparently normal infected quarters could be considered as the most serious from of mastitis.

Escherichia coli was reported by Rao et al. (1969) as the chief aetiological agent responsible for cases of mastitis in 85 Indian buffaloes and constituted an incidence percentage of 17.4, followed by Aerobacter aerogenes, Staph. pyogenes, beta-haemolytic streptococci, Pseudomonas aerogenosa, Alcaligenes faecalis and para-colon organisms.

Zakaria (1969) examined 510 individual milk samples (252 buffaloes and 258 cows) for detection of mastitis and found that the incidence of different causative organisms in subclinical mastitic cases include Strept. agalactiae (95.80%), Staph. aureus (2.10%), E. coli (1.05%) while mixed

infection of E. coli and Strept. agalactiae represented itself in only 1.05%.

Histological studies conducted by Mandal and Iyer(1970) on 325 quarters taken from slaughtered lactating buffaloes (Bos bubalis) revealed that 121 had histological lesions associated with mastitis caused by a variety of organisms . Morphological identification of tissue sections revealed streptococci in 28 cases, staphylococci in 12, Corynebacteria in 30 and fusiformis in only one case.

Khalil et al. (1972 II) reported that pathogenic agents isolated from quarter milk samples which were responsible for different forms of mastitis include Streptococci (64.40%), Coagulase positive staphylococci (20.6%), Corynebacterium pyogenes (12.3%) and E. coli (2.74%).

El-Nagar (1973) reported that Strept. agalactiae(23.25%), Staph. aureus (23.83%), E. coli (11.63%), Staph. epidermidis (14.53%), C. pyogenes (15.70%), Strept. dysagalactiae(6.40%) Strept. pyogenes (2.32%) and Yeast-like organisms (2.33%) were isolated from milk samples collected from mastitic bovine udder.

Kral et al. (1973) investigated 352351 milk samples and found that 33.8% was diagnosed as mastitic. On the other hand,

bacteriological investigations carried out on 627770 milk samples revealed that 15.9% of cases were due to bacterial agents, Strept. uberis and other streptococci were isolated from 2.8%, Strept. agalactiae from 9.0%, Staph. aureus from 3.3%, E. coli from 0.6% and Corynebacterium pyogenes from 0.21% of the samples.

Bacteriological studies performed by Chander et al. (1975) on 108 quarters showing manifestation of subclinical mastitis revealed the isolation of Staphylococci (46.2%), Streptococci (32.4%), Escherichia coli (7.4%),Corynebacteria (0.93%), pseudomonas species (2.8%) and unidentified organisms (10.2%).

Havelka (1975), over a 3-years period, examined bacteriologically 178853 milk samples collected from 72454 cows. Strept.agalactiae was isolated from 1.44%, while Staph. aureus from 4.19%, E.coli from 0.18%, Klebsiella species from 0.23% and Corynebacterium pyogenes from 0.20%.

Farid et al. (1975) found that out of 172 buffalo's milk samples collected from clinically normal udders, 33 (19.18%) were found to be bacteriologically mastitic; 11 (6.39%) due to Strapt. agalactiae, 2 (1.16%) due to Staph. aureus, 3 cases (1.75%) due to Staph. albus, one case (0.58%) due to

C. pyogenes, 2 cases (1.16%) due to unclassified Corynebacteria, 3 cases (1.75%) due to Coliformis, 5 cases (2.9%) due to anthracoids, one case (0.58%) due to Klebsiella species and 5 cases (2.90%) due to Yeast.

Jaffery et al. (1975) found that of 740 milk samples collected from 194 mastitic buffaloes in Lahore, 44% yielded Staphylococcus aureus, 42% Streptococci (30% Strept. agalactiae, 8.7% Strept. dysagalactiae, 3.2% Strept. uberis), 12.4% Coliforms (7.6% Aerobacter aerogenes, 4.3% E. coli and 0.5% E. intermedium), 5.1% Corynebacterium pyogenes, 3.2% paseudomonas aerogenosa, and 0.4% Yeast-cells resembling candida tropicalis.

Sinoussi et al. (1975) tabulated the percentage of different micro-organisms responsible for subclinical mastitis in buffaloes in upper Egypt. Haem. Staph. was isolated at a higher incidence (56.66%) than Strept. agalactiae (6.66%), dysagalactiae (6.66%) or other Streptococci (13.33%).

Aspergillus fumigatus pathogenic for laboratory animals was isolated from milk samples of 15 apparently healthy cows by Fenizia et al. (1976). They concluded that this species of pathogenic fungi could act as a primary invades in the pathogenesis of bovine mastitis. The milk produced was found

to contain relatively lower leucocytic count when compared with that produced under mixed infections with either Staphylococci or Streptococci.

Valenti (1976) reported that 18 strains of Staphylococci and 27 of Streptococci were detected in qurater samples of milk from mastitic cows, while mixed infection with both species was demonstrated in 12% of cases.

Misra (1976) concluded that, clinical cases of mastitis are mainly due to Staphylococcal infection, whereas subclinical and chronic mastitis were due to Streptococci.

Robinson et al. (1977) isolated haemolytic Escherichia coli from cases of gangrenous mastitis.

Weber et al. (1977) reported that 55 milk samples from heifers suffering from the so-called "summer mastitis" were examined under aerobic and anaerobic conditions. Corynebacterium poygenes together with the anaerobic Micrococus indolicus were isolated from 42 case, while Corynebacterium pyogenes was isolated from 2 samples, α-haemolytic Streptococci was isolated from 8 samples. M. sacchrolyticus and Staph. asacchrolyticus were isolated from one sample.

A total of 202 milk samples were collected from 140 lactating buffaloes (Bos bubalis) and 62 local breed cows at Edfina Veterinary Clinic and the Clinic of the Faculty of Veterinary Medicine, Alexandria University. Animals used in the sampling were tested to be free from external and internal parasites. Examined clinically to be sure in a healthy state. Their ages ranged between 4-10 years.

All the collected milk samples were subjected to both biochemical and bacteriological examinations.

Sampling

Collection of milk samples investigated was carried out early in the morning as recommended by Schalm et al. (1971).

The udder, teats and the milkers hands were perfectly washed with soap and water and dried with a clean towel just before sampling. The teat orifice was then cleaned with 70% ethyl alcohol before collection of samples. clean sterile screw-capped Mc Cartney bottles of 30 ml capacity were used for collecting milk samples.

All bottles were labelled to indicate the date, animal species, examined quarter as well as clinical findings.

Collected samples were transferred to the laboratory in an ice-box, without delay. Each sample was divided aseptically into 2 portions, one of which was incubated at 37°C for 18 hours for bacteriological work and the other portion was used for biochemical examination.

I. Biochemical Examination

Normal and mastitic milk samples were subjected to determination of:

1 . Glutamic oxalacetic transaminase (GOT)
2 . Glutamic pyruvic transaminase (GPT)
3 . Alkaline phosphatase (ALP)
4 . Acid phosphatase (ACP)
5 . 5'-Nucleotidase (5'-ND)
6 . Lactic dehydrogenase (LDH)
7 . Amylase
8 . Lipase
9 . Ascorbic acid
10. pH value

Dialysis

Dialysis is used to separate large molecules apart from small molecules and depends on the fact that semipermeable membrane will allow small molecules to pass throughout but

prevents the passage of large molecules.

The principles of dialysis as cited by Plummer (1978) was used in the present work as a guide. Cellophane membrane was the material most commonly used for dialysis. A suitable size and length of the membrane was selected and soaked in distilled water. The membrane was boiled for 30 minutes in alkaline EDTA (Na_2CO_3, 10 g/liter: EDTA, 1 mmol/liter) to avoid loss of activity of the molecules dialyzed. After boiling, the membrane was washed with distilled water.

Dialysis was best carried out with freshly prepared membrane since, once it is wet, it becomes very susceptible to be attacked by micro-organisms. If it has to be stored, the membrane is best kept by adding a trace of Benzoic acid to the solution.

The permeability limit of cellophane membrane depends on the size and any pretreatment, but as a rough guide, the membrane is said to be permeable to compounds whose molecular weights are below 30.000.

Moreover, the exact time required for dialysis differ according to the type of enzyme analyzed.

Dialysis was carried out on milk samples in order to obtain clear dialysates for the determination of:

(1) Glutamic oxalacetic transaminase (GOT)

(2) Glutamic pyruvic transaminase (GPT)

(3) Lactic dehydrogenase (LDH)

1. Determination of Glutamic oxalacetic transaminase

The determination of GOT in milk was carried according to Reitman and Frankel (1957) with a slight modification.

The oxalacetates formed in the reaction between GOT and its substrate decarboxylates spontaneously to pyruvate which is again reacts with 2:4-dinitrophenyl hydrazine(DNPH) to give a brown coloured hydrozone which is measured in the colorimeter at 510 nm.

R e a g e n t s

(1) Phosphate buffer (pH 7.4), prepared by dissolving 11.3 g of dry anhydrous disodium hydrogen phosphate and 2.7 g of dry anhydrous potassium dihydrogn phosphate in one liter of distilled water. The solution was checked up by a pH meter and stored in a refrigerator at 4°C.

(2) GOT substrate: (200 mM DL-aspartic acid, 2 mM α-keto-glutarate). The solution was prepared by dissolving 13.3 g of DL-aspartic acid in the minimum amount of N-sodium hydroxide (90 ml), then 0.146 g of α-keto-glutaric acid were added and dissolved by adding a little more sodium hydroxide solution. The pH was adjusted to 7.4 and the solution was made up to 500 ml with phosphate buffer solution. The substrate solution divided into 5-10 ml portions and stored frozen at -15°C.

(3) Stock pyruvate standard, (20 mM) 220 mg of sodium pyruvate were dissolved in 100 ml of phosphate buffer, this solution was stored at -15°C in ml aliquots.

(4) Working pyruvate standard: (4 mM) one part of the stock standard solution was mixed with 4 parts of phosphate buffer solution and stored at -15°C.
This was prepared fresh every week.

(5) 2:4-dinitrophenyl hydrazine (1 mM): 19.8 mg of dinitrophenyl hydrazine were dissolved in 10 ml of conc. hydrochloric acid and made up to 100 ml with distilled water and kept in a brown bottle at room temperature.

(6) 0.4 N-sodium hydroxide: 16 g of analar sodium hydroxide were dissolved in one liter distilled water.

P r o c e d u r e

(a) Test: 2.5 ml of substrate were warmed in a water-bath at 37°C for 3 minutes, 0.5 ml of milk was added and mixed gently. The mixture was incubated for exactly 60 minutes after which the tubes were removed from the bath and 2.5 ml of 2:4 dinitrophenyl hydrazine solution were immediately added and mixed well.

(b) Control: 2.5 ml of substrate were mixed with 2.5 ml of DNPH solution, 0.5 ml of milk was added.

(c) Standard: 0.5 ml of working pyruvate was mixed with 2 ml of substrate, 0.5 ml of water and 2.5 ml of DNPH solution.

(d) Blank: 2.5 ml of substrate were mixed with 0.5 ml of water and 2.5 ml of DNPH in a test tube.

The DNPH was allowed to react in all tubes for 20 minutes at room temperature, then 15 ml of 0.4 N NaOH were added and mixed well. The contents of all tubes were transferred into a cellophane bag (10 cm length) and placed in a beaker 50 ml capacity containing 5 ml 0.4 N NaOH. After 2 hours the colour was read at 510 nm.

C a l c u l a t i o n

The pyruvate formed by the milk is responsible for the difference between test and control (T-C) the pyruvate in 0.5 ml of the working standard (0.20 mole) produces the difference between standard and blank (S-B) so the pyruvate formed in 60 minutes by 0.5 ml of milk is

$$\frac{T - C}{S - B} \times 0.20 \text{ mole.}$$

Thus the pyruvate formed per minute/liter of milk is

$$\frac{T - C}{S - B} \times 0.20 \times \frac{1}{60} \times \frac{100}{0.5} = \frac{T - C}{S - B} \times 67$$

The calculated pyruvate is converted into I.U/liter by reference to a special table (Page 40).

2 . Determination of Glutamic pyruvic transaminase

The pyruvate produced by transamination by GPT reacts with 2:4-Dinitrophenylhydrazine (DPNH) to give a brown coloured hydrozone. Which is measured in the colorimeter at 510 nm.

R e a g e n t s

1 . Phosphate buffer (pH 7.4)

2 . GPT substrate: (200 mM-alanine, 2 mM ∝-ketoglutarate) 9.0 g of alanine were dissolved in 90 ml water with the addition of about 2.5 ml N-sodium hydroxide to adjust pH to 7.4.

To this solution 0.146 g of ∝-ketoglutaric acid were added and dissolved and the pH was again adjusted to 7.4 by addition of a little more sodium hydroxide solution. The volume was made up to 500 ml with phosphate buffer solution.

The substrate solution was divided into 5-10 ml portions and stored frozen at -15°C.

3 . Stock pyruvate standard (20 mM).

4 . Working pyruvate standard(4 mM).

5 . 2:4-dinitrophenyl hydrazine(1 mM).

6 . 0.4 N-sodium hydroxide.

P r o c e d u r e

The same procedure for determination of GOT was followed using GPT substrate instead of GOT substrate and reducing the incubation time to 30 minutes.

C a l c u l a t i o n

The pyruvate formed in 30 minutes by 0.5 ml milk is

$$\frac{T - C}{S - B} \times 0.20 \text{ mole.}$$

Thus the pyruvate formed per minute per liter of milk is

$$\frac{T - C}{S - B} \times 0.20 \times \frac{1}{30} \times \frac{100}{0.5} = \frac{T - C}{S - B} \times 133$$

The calculated pyruvate is converted into international units/litre by reference to the table (Page 40).

Table showing the correlation between the calculated pyruvate and the transaminases activity (I.U/liter).

Calculated pyruvate	GOT I.U/liter	GPT I.U/liter	Calculated pyruvate	GOT I.U/liter	GPT I.U/liter
2	2	1	52	55	22
4	3	2	54	60	23
6	5	2	56	-	24
8	6	3	58	-	25
10	7	4	60	-	26
12	9	4	62	-	27
14	11	5	64	-	29
16	13	6	66	-	30
18	15	7	68	-	31
20	17	7	70	-	33
22	19	8	72	-	34
23	20	8	74	-	35
24	21	9	76	-	36
26	23	9	78	-	37
28	25	10	80	-	38
30	27	11	82	-	39
32	29	12	84	-	40
34	31	13	86	-	42
36	33	14	88	-	44
38	35	15	90	-	46
40	37	16	92	-	48
42	39	17	94	-	50
44	41	18	96	-	52
46	44	19	98	-	54
48	47	20	100	-	56
50	51	21	102	-	60

3. Determination of Alkaline Phosphatase

The alkaline phosphatae activity was determined according to Folin and Ciocalteu method cited in Wotton (1974) .

The estimation of phosphatase depends upon measuring the amount of hydrolysis which takes place when the enzyme is allowed to act on a suitable substrate such as phenyl phosphate under standard conditions. The phenol released by enzymic hydrolysis can be determined by using Folin and Ciocalteu's reagent which precipitates milk proteins and produces a blue colour with phenol in presence of sodium carbonate.

R e a g e n t s

1 . Buffer. M/10 sodium carbonate-bicarbonate (6 Na_2Co_3: 4 $Na\ NCo_3$)

6.36 g anhydrous sodium carbonate and 3.36 g sodium bicarbonate were dissolved in distilled water and completed up to 1 liter.

2 . Substrate. M/100 disodium phenyl phosphate.

2.18 g were dissolved in 1 liter of water. The solution should be brought quickly to the boil to destroy any organism, cooled immediately and preserved with a little chloroform (4 ml per liter).

3 . Folin and Ciocalteu phenol reagent.

This is made by dissolving 100 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), and 25 g of sodium molybdate ($Na_2MO_4 \cdot 2H_2O$) in 700 ml of water, contained in a 1500 ml flask, 50 ml of phosphoric acid (sp. gr. 1.75) and 100 ml of concentrated hydrochloric acid were added. The flask is then connected to a reflux condenser by means of a glass ground joint and the mixture boiled for 10 hours; then 150 g of lithium sulphate, 50 ml of water and a few drops of bromine were added. The boiling was continued without a condenser for 15 minutes. The golden-yellow solution was allowed to cool, diluted to 1 liter, and preserved in a dark bottle. The reagent (1 volume) was diluted with water (2 volumes) for use.

4 . Sodium carbonate 15 per cent (w/v).

15 g of anhydrous sodium carbonate were dissolved in distilled water and the volume made up to 100 ml.

5 . Stock standard phenol (1 mg per ml).

1 g pure crystalline phenol was dissolved in, and made to 1 liter with 0.1 N HCl.

6 . Standard-phenol and Reagent (0.01 mg phenol per ml).

5 ml of the stock standard phenol (1 mg per ml) were accurately transferred into a 500 ml volumetric flask, 100 ml of dilute (1 in 3) Folin-Ciocalteu reagent were added and the

volume was completed up to the mark with distilled water. This solution keeps at least for six months, if preserved in the ice-chest.

Procedure

Test: In a conical centrifuge tube were placed 2 ml of buffer and 2 ml of substrate. The tube is allowed to remain in a water-bath at 37°C for 3 minutes. Without removal of the tube from the bath, 0.2 ml of diluted milk (1:10 times with distilled water) is added and mixed. The stoppered tube was allowed to remain in the bath for 15 minutes. At the end of this time 1.8 ml of dilute Folin-Ciocalteu phenol reagent were added and the mixture was centrifuged or filtered.

Control: In another tube 2 ml of buffer, 2 ml of substrate, and 1.8 ml of dilute Folin-Ciocalteu reagent were placed followed by 0.2 ml of milk and the whole mixture was centrifuged or filtered.

4 ml of filtrate from the test and control solutions were pipetted into test tubes. To each tube 2 ml of 15 per cent sodium carbonate were added and the tubes replaced in the water-bath for 10 minutes to bring up the colour.

Standard: The solutions were compared in the colorimeter with a standard made at the same time by taking 4 ml of

standard phenol solution and reagent, and 2 ml of 15 per cent sodium carbonate. Absorbance was read at 700 mu or red light filter "608".

Blank: 3.2 ml of water and 0.8 ml of dilute Folin-Ciocalteu were treated with 2 ml of sodium carbonate, at the same time as the standard.

Calculation

$$\text{Alkaline phosphatase activity} = \frac{\text{Reading of (test-control)}}{\text{Reading of(standard-blank)}} \text{ X30X10}$$

The King-Armstrong Unit of phosphatase is defined as the amount of enzyme which will set free 1 mg of phenol in the given time under the conditions of the test; and hence "units" per 100 ml = mg of phenol set free from the phenyl phosphate under the standard conditions.

5. Determination of 5'-Nucleotidase

5'-Nucleotidase enzyme was determined following the colorimetric method cited in Wotton (1974)* with slight modifications.

This enzyme is a phosphatase acts only on a nucleoside -5'-phosphate releasing inorganic phosphate. The substrate usually used in assays of the enzyme is adenosine-5'-phosphate. The optimal activity of milk 5'-nucleotidase enzyme is at pH 7.8. At this pH 5'-nucleotides are also hydrolysed by a non-specific alkaline phosphatase and since these enzymes almost always occur together, methods for the estimation of 5'-Nucleotidase activity must incorporate means of correction for this non specific hydrolysis. 5'-nucleotidase differs from alkaline phosphatase in being inhibited by nickel ions and this property is made of in the method described here to distinguish the two enzymes.

R e a g e n t s

1 . Veronal buffer (pH 7.5). 8.25 g of sodium diethylbarbiturate were dissolved in 140 ml of 0.2 N-HCl and the volume made up to 1 liter with distilled water.

2 . Adenosine-5'-phosphate (10 mM). 347 mg of Adenosine-5'-phosphate were dissolved in 18 ml of 0.1 N-sodium hydroxide and the volume made up to 100 ml with distilled water.

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* Campbell method.

3 . Manganese sulphate solution contains 320 mg of manganese sulphate per 100 ml distilled water.

4 . Nickel chloride solution contains 2.4 g of nickel chloride per 100 ml distilled water.

5 . Trichloroacetic acid solution contains 100 gm of trichloroacetic acid per one liter distilled water.

6 . Stock phosphate standard (100 mg P per 100 ml). 2.19 g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 500 ml distilled water, few drops of chloroform were added and the solution kept at 4°C.

7 . Working phosphate standard (1 mg P per 100 ml) contains 1 ml of stock standard per 100 ml of 5 per cent trichloroacetic acid. This is prepared fresh every few weeks.

8 . Acetate buffer pH 4.0. 2.5 g of copper sulphate ($Cu\ SO_4 \cdot 5\ H_2O$) and 46 g of sodium acetate ($CH_3 . CooNa . 3H_2O$) were dissolved in 1 liter of 2N-acetic acid. The pH was adjusted to 4.0.

9 . Rhodol solution. 2 g of Rhodol (paramethyl amino phenol sulphate) were dissolved in 80 ml of water, then 10 g of hydrated sodium sulphite ($Na_2SO_3 . 7H_2O$) was added and the volume made up to 100 ml and then filtered and stored in a dark bottle at 4°C.

10. Ammonium molybdate solution contains 5 g per 100 ml distilled water.

Procedure

Test. 1.5 ml of buffer was mixed with 0.1 ml of manganese sulphate.

Control. 1.3 ml of buffer was mixed with 0.1 ml of manganese sulphate and 0.2 ml of nickel chloride.

0.2 ml of milk was added to each tube, then placed in a water bath at 37°± 0.2°C for 3 minutes after which 0.2 ml of adenosine-5'-phosphate was introduced and the tubes left for exactly 30 minutes. The reaction was then stopped by the addition of 2 ml of 10 per cent trichloroacetic acid.The tubes were removed from the bath and shaked well.

The tube contents were either centrifuged or filtered through whatman No. 1 filter papers.

0.2 ml of the clear filtrate was pipettted into a test tube and 1.8 ml of 10 per cent trichloroacetic acid was added and mixed well.

Standard. 1 ml of working phosphate standard plus 1 ml of 10 per cent trichloroacetic acid.

Blank. 1 ml of water plus 1 ml of 10 per cent trichloroacetic acid.

To all tubes 3 ml of acetate buffer were added,followed by

0.5 ml of 5 per cent ammonium molybdate and 0.5 ml of Rhodol solution and mixed well.

After at least 5 minutes the blue colour obtained was compared at 880 nm or using Ilford 608 red filter.

C a l c u l a t i o n

The standard tube contains 10 ug of phosphate (as P), thus the phosphate produced in the enzymic reaction by 0.1 ml of milk is:

$$\frac{T - C}{S - B} \times 10 \text{ ug} = \frac{T - C}{S - B} \times 10 \times \frac{1}{31} \text{ U mole}$$

Therefore, 1 liter of milk in one minute produces

$$\frac{T - C}{S - B} \times \frac{10}{31} \times \frac{1000}{0.1} \times \frac{1}{30} = \frac{T - C}{S - B} \times 108 \text{ U mole}$$

$$\text{Milk 5'-Nucleotidase (I.U/liter)} = \frac{T - C}{S - B} \times 108 \times 10 \text{ (dilution)}.$$

6. Determination of Lactic Dehydrogenase

Lactic dehydrogenase (LDH) has been measured colormetrically using the method of Wroblewski (1959) with some modifications.

It depends on the reduction of pyruvate by incubation with the enzyme solution in the presence of the reduced Co-enzyme nicotinamide adenine dinucleotide ($NADH_2$). The reaction is stopped by adding dinitrophenylhydrazine solution which reacts with remaining pyruvate forming a hydrazone. The amount of untreated pyruvate is found by measuring the brown colour produced when the hydrazone is made alkaline.

R e a g e n t s

1 . Phosphate buffer pH 7.4 0.1 M.

This buffer was prepared by dissolving 13.97 g of anhydrous dipotassium hydrogen phosphate and 2.69 g of anhydrous potassium dihydrogen phosphate in 1 liter distilled water.

2 . Standard sodium pyruvate

21 mg sodium pyruvate were dissolved in 5 ml phosphate buffer (pH 7.4). The solution was stored in the refrigerator and if any signs of contamination appeared, the solution was discarded.

3 . Dinitrophenylhydrazine reagent

40 mg. 2,4-dinitrophenylhydrazine were dissolved in 8.5 ml concentrated HCl and diluted to 100 ml with distilled water.

4 . Substrate solution

4.9 ml phosphate buffer (pH 7.4) and 0.1 ml of standard sodium pyruvate were mixed.

5 . Reduced nicotinamide-adenine dinucleotide ($NADH_2$)

4 mg $NADH_2$ were dissolved in 0.5 ml phosphate buffer (pH 7.4). This solution was prepared fresh before use.

P r o c e d u r e

The technique was carried out by pipetting 2 ml substrate solution into a series of test tubes, then 0.2 ml of the milk samples under analysis was added and the mixture placed in a water-bath at 25°C. After few minutes the reaction was started by adding 0.2 ml of $NADH_2$ solution to every tube.

Incubation for exactly 15 minutes at 25°C was made, then the tubes were removed from the bath and immediately 2 ml of 2,4-dinitrophenylhydrazine solution was added and mixed.

A control tube was prepared by adding 2 ml substrate solution to 0.2 ml phosphate buffer and 2 ml of 2,4-dinitrophenylhydrazine.

A Blank tube was also prepared by mixing 2.2 ml of phosphate buffer with 2 ml 2,4-dinitrophenylhydrazine.

All tubes were allowed to stand for 20 minutes at room temperature. At the end of the incubation period, the contents

of each tube was placed in a cellophane bag (10 cm length) to which is added 15 ml of 0.4 N NaOH. The cellophane bags were tightly closed and placed in a 50 cc capacity beaker containing 5 ml of 0.4 N NaOH, and left for 2 hours.

After 2 hours the coloured solutions were measured spectrophotometrically at 510 nm.

C a l c u l a t i o n

The LDH activity of milk expressed in U mole pyruvate/ min/liter was calculated from the equation:

$$\frac{C - T}{C - B} \times 0.76 \times \frac{1}{15} \times \frac{1000}{0.1} = \text{U mole pyruvate/min/liter.}$$

W h e r e :

C = optical density of the control

T = optical density of the tested sample

0.76 = U mole pyruvate in the control tube

15 = time of incubation (enzymatic reaction)

$\frac{1000}{0.1}$ = For calculation the activity per liter.

7. Determination of Lipase

Milk lipase was determined following the rapid and sensitive method preivously cited by Parry et al.(1966) with some modifications.

In a lipase assay, the free fatty acids released cause a slight depression in pH which is corrected by addition of standard alkali. The amount of NaOH added with respect to time is recorded simultaneously, thereby giving a continuous record of lipase activity.

Apparatus: A pH meter "Beckman", microburet, magnetic stirrer and water bath adjusted at 37°C were used in this experiment for allowing sample agitation as well as control of temperature and pH.

Preparation of substrate and method of Assay

The substrate for the lipase assay consisted of an emulsion prepared by dispersing 10% (v/v) butter oil in a 10% aqueous solution of gum arabic. The mixture was warmed to 50°C and passed five times through a hand-homogenizer.

The assay mixture consisted of 5 ml of butter oil emulsion, 0.2 ml of 2.85 M NaCL and 0.5 ml of raw milk. Prior to mixing of the assay mixture, the pH of the emulsion and milk was adjusted to 8.8. Throughout this work the pH of assay

was maintained at 8.8 for raw milk through the microburet delivery of a 0.025 N sodium hydroxide solution. A constant temperature of 37 ± 0.1°C was maintained throughout the experiment.

The assay was completed within 5 to 7 minutes.

A control (boiled milk) was used and its reading was subtracted from the sample reading.

Calculation

One lipase unit is defined as the number of microequivalents of alkali (0.025 N NaOH) required per minute to titrate the free fatty acids released and to maintain a given pH at 37°C.

$$\frac{\text{MIS of 0.025 N NaOH used in sample} - \text{MIS of 0.025 N NaOH used in control}}{t} = \text{Lipase Unit/1 ml milk}$$

where "t" = time of titration in minutes (5-7 minutes).

To convert the lipase unit into 0.01 N NaOH/100 ml milk allow the following equation.

$$\frac{\text{Lipase Unit/ml}}{2.5} \times 100 = \text{MIS of 0.01 N NaOH/100 ml milk}$$

8. Determination of Amylase

The amylase activity of milk was determined according to the visual method cited by Wotton (1974)*

Amylase can be assayed by the loss of the blue colour formed due to presence of starch and iodine as the starch is degraded.

Reagents

(1) Substrate: prepared by dissolving 13.3 g of dry anhydrous Na_2HPO_4 and 4.3 g of benzoic acid in 250 ml of water and the solution was boiled. A mixture of 0.375 g of soluble starch in 5 to 10 ml of cold water was added with rinsing, to the boiling mixture. Boiling was further continued for one more minute, then the mixture was cooled to room temperature, diluted to 500 ml and kept at 4°C for no longer than one month.

(2) Iodine stock solution: obtained by dissolving 1 g of iodine in 20 ml of 10% KI and the volume made up to 300 ml with distilled water.

(3) Working solution (approx. 0.005N): obtained by diluting the stock solution 5 folds with distilled water.

Procedure

Samples were removed at time intervals from a starch-

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* Somogyi method.

milk reaction mixture and the colour formed between each sample and iodine solution in the depressions of a glazed tile was noted.

One drop of iodine solution was placed in each depression of the tile (iodine volatilizes readily so there should be no delay between dispensing the iodine and making the estimation). Milk is placed in a water-bath at 37°C.

2 ml of substrate were pipetted into a test tube and was maintained at 37°C for 5 minutes after which one drop "control" was removed to the first depression in the tile. 0.5 ml of milk was added immediately to the substrate at 37°C and mixed. Using a stop-watch and at time intervals listed below, one drop of the mixture was placed onto one drop of the iodine in successive depression and the colour noted immediately

Time for decolouration Minutes	Seconds	Amylase activity (Somogyi units/ 100 ml)
-	27	4000
-	36	3000
-	54	2000
1	48	1000
2	16	800
3	0	600
4	30	400
9	0	200
18	0	100

C a l c u l a t i o n

The somogyi unit is approximately equal to the amount of Amylase destroying 15 mg of starch (in the 2 ml of substrate) in 8 minutes.

Milk Amylase $\frac{8}{t} \times \frac{100}{0.5} = \frac{1600}{t}$ (Somogyi units/100 ml)

(where t = time in minutes for decoloration)

9. Determination of Ascorbic Acid

The determination of ascorbic acid in milk was carried out according to the N-Bromosuccinimide method reported by Barakat et al. (1961).

Reagents

(1) Metaphosphoric acid prepared by dissolving 15 g of Metaphosphoric acid in 450 ml of distilled water. Glacial acetic acid was added up to the mark in a 500 ml standard flask and the solution mixed well.

(2) N-Bromosuccinimide (NBS) solution. prepared by dissolving 200 mg of NBS in hot distilled water and the volume made up with distilled water to the mark in a 1000 ml standard flask. So the concentration of NBS solution used in the estimation will be 0.2 mg/ml.

(3) Aqueous potassium iodide. contains 4 gm pure potassium iodide per 100 ml distilled water.

(4) Starch solution prepared by dissolving 1 gm of soluble starch in hot distilled water and then the volume was completed to 100 ml.

Procedure

1. A known volume of the milk sample is well mixed with an equal volume of metaphosphoric acid, allowed to stand for 10 minutes and then filtered.

2 . Into a 50 ml capacity conical Erylenmeyer flask, 5 ml of the clear filtrate were introduced by means of a pipette, then 5 ml of 4% aqueous potassium iodide and 1 ml of starch solution were added and mixed.

3 . The mixture was titrated with 0.2% aqueous N-Bromosuccimide solution using a 1 ml micro-pipette graduated in hundreds of a milliliter. The NBS solution was added drop by drop with continuous shaking until a violet colour (end point) just appeared.

Simultaneously a blank experiment was performed without using the milk sample. The blank reading was subtracted from the titration.

C a l c u l a t i o n

mg Ascrobic acid/liter milk =

$$\text{mls NBS} \times 0.2 \times \frac{176}{178} \times \frac{2}{5} \times 1000$$

10. Determination of pH Value

The pH value of normal and mastitic milk samples was determined electrometrically within one hour after collection of samples (Bogin and Ziv 1973) by using "Beckman's pH" meter and buffer solution (pH 7.0).

II. Bacteriological Examination

Each milk sample was incubated for 18 hours at 37°C then centrifuged for 20 minutes at 3000 r.p.m.

(a) Microscopical examination. The morphological characters and staining reaction of the bacterial content of milk samples were studied. Films were prepared by spreading a loopful from each milk sediment onto a clean slide; which was air dried, fixed over the flame and stained with Loeffler's methylene blue.

(b) Cultural examination:

Loopfuls from both the sediment and cream layer, obtained from each sample were streaked onto 5% defibrined sheep's blood agar, Edward's agar, Mac Conkey's agar and nutrient agar plates as recommended by Solyts (1963) and Cruickshank et al.(1975). Inoculated media as well as uninoculated control plates were incubated aerobically at 37°C for 48 hours.

Moreover, four plates of Sabouraud's glucose agar were also used for each milk sample of which two plates containing chloramphenicol (0.05 gm/liter) while glycerol (6 gm/liter) was added to the other plates. One plate from each group was incubated at 37°C while the others at 25°C for a period up to 6 day.

Different developing colonies were picked up carefully and subcultured on separate plates and finally on agar slopes as pure cultures for further identification.

Identification of isolates

Identification of the isolated micro-organisms was performed according to Solyts (1963), Merchant and Packer(1969), Breed, et al. (1974) and Buchanon and Gibbons (1974) and based on:

1 . Cultural characters: By observing the type of growth on different solid media used.

2 . Morphological features: The staining reaction of each isolate to Gram's stain was studied microscopically.

The isolates were placed into one of the following groups:

(A) Gram-positive cocci:

Isolates of this group were first identified according to their cell arrangement as referred by Merchant and Packer (1969).

(1) Staphylococci:

Members of this genus were further identified according:

(a) Catalase production test, the presence of catalase enzyme in staphylococci and micrococci which helps in the

liberation of oxygen from hydrogen peroxide was used to differentiate them from streptococci and pneumococci (Finegold et al., 1978).

(b) Benzidine test: (Finegold et al. 1978).

(c) Mannitol fermentation: The ability of Staph. aureus to ferment this carbohydrate was used to differentiate it from Staph. epidermidis (Cruickshank et al. 1975).

(d) Coagulase test: The production of coagulase enzyme, which coagulates blood plasma is characteristic of Staph. aureus. The tube test was used to differentiate it from the other members of this genus.

(2) S t r e p t o c o c c i

They were identified according to Merchant and Packer (1969) by using the following tests:

(a) Blood haemolysis, in 5% defibrinated sheep's blood agar plates.

(b) Hydrolysis of sodium hippurate.

(c) Gelatin liquefaction.

(d) Sugar fermentation tests, including sucrose, glucose, lactose, mannitol, salicin, sorbitol and raffinose.

(e) Serological tests: B-haemolytic streptococci were serologically identified according to El-Kholy et al. (1974).

(B) Gram-positive rods:

They were identified according to Merchant and Packer (1969).

The following tests were performed:

a - Motility test.

b - Gelatin liquefaction test.

c - Sugar fermentation tests, including glucose, sucrose, maltose, salicin, lactose and galactose.

d - Urea hydrolysis test.

(C) Gram-negative rods:

Members of this group were identified according to the scheme proposed by Kovac (1956) and Edwards and Ewing(1972) which was based on:

1 . Triple sugar iron agar slopes:

The variation in pH values of the slant and butt, as well as the production of H_2S gas were used to differentiate between the various groups of enteric bacteria.

2 . Motility test.

3 . Sugar fermentation test:

The ability of each isolate to utilize glucose, sucrose, dulcitol, galactose, arabinose, mannose and salicin was studied.

4 . Indole production.

5 . Citrate utilization test:

Simmon's citrate agar slopes were inoculated. The production of a prussian blue colour indicated that the organism could utilize citrate as a sole source of carbon.

6 . Methyl red test.

7 . Voges-proskauer test.

8 . Gelatin liquefaction test.

9 . Pigment production in nutrient agar slopes.

(D) Yeast and Moulds:

Sabouraud agar plates were used for the primary isolation of fungi from milk samples examined. On the other hand, subcultures were made from growing colonies on czapek's agar media as adviced by Tom and Raper (1945) and Gilman (1975). Identification of isolated fungi was performed primarily by their gross and microscopic characteristics (Moss & Mc-Quown, 1969).

The gross characteristics were focussed on the following criteria:

1 - The rapidity of growth.

2 - Colony morphology.

3 - Texture of growth.

4 - Myc lium production.

5 - Pigmentation.

However, the microscopical examination was used to study the size, shape, septation and morphology of the specialized structures bearing the spores.

Moreover, sugar fermentation tests including glucose, galactose, sucrose, maltose and lactose incubated at 37°C for a period up to 6 days were also performed.

Statistical Analysis of the Results

The data obtained from the normal and mastitic milk samples were subjected to the following statistical analysis:

I . Preliminary statistical analysis showing:-

1. Sample Maximum.
2. Sample Minimum.
3. Sample Mean.
4. Sample standard error of the mean.

II. Detailed statistical analysis:

"t" test

*The standard error of the mean were calculated according to the following formula:

$$\text{Standard error of mean} = \sqrt{\frac{\frac{\sum X^2}{n} - \bar{X}^2}{n-1}}$$

where:

$\sum$ = sum

X = individual observations

$\bar{X}$ = mean of observations

n = number of observations

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* Snedicor (1956).

For comparison between the data obtained from normal and mastitic milk samples as well as between the data obtained from mastitic milk samples as affected by different causative micro-organisms and the normal milk samples, "t"test, was applied using the following formulae:

(1) For equal numbers:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{S.E_1^2 + S.E_2^2}}$$

and degree of freedom = $(n_1 + n_2 - 2)$

The significance of differences among the means were evaluated as being:

X significant at 0.05 level of probability.

XX significant at 0.01 level of probability.

(2) For unequal numbers:

$$t = \frac{(\bar{X}_1 - \bar{X}_2) - (U_1 - U_2)}{S_{\bar{X}_1 - \bar{X}_2}}$$

where:

$\bar{X}_1$ = mean of 1st observations.

$\bar{X}_2$ = mean of 2nd observations.

$(U_1 - U_2)$ = Zero

$S_{\bar{X}_1 - \bar{X}_2}$ = variance.

N.B.: The significant results were given in figures.

TABLE I

Levels of some Enzymes, Ascorbic acid and PH value of B. faloe's milk obtained from apparently normal animals

GOT, GPT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5'-Nucleotidase (5'-ND), Lactic dehydrogenase (LDH) Lipase, Amylase, Ascorbic Acid and PH value.

Case No	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5'-ND I.U/L.	LDH UM/L.	Lipase Unit/I00ML	Amylase Somo.U/I00ML	Ascorbic Acid mg/L.	PH value
1	2.00	0.00	4I.66	I.60	239.I8	I37.70	5I.50	66.00	I9.42	6.00
2	2.00	0.00	40.66	2.05	I58.90	I20.90	5I.20	60.66	20.42	6.30
3	9.00	0.00	I5.00	0.25	278.54	I26.60	40.90	40.00	23.60	6.20
4	6.00	0.00	39.66	2.82	239.27	79.40	40.95	80.00	25.60	6.50
5	6.00	0.00	I4I.33	0.90	I78.55	II3.20	4I.30	64.66	32.32	6.00
6	4.00	0.00	I22.42	2.43	II7.80	75.62	49.70	46.00	33.I0	6.20
7	4.00	0.00	33.93	I.60	I39.I8	I46.40	42.68	63.00	35.20	6.20
8	3.00	0.00	I48.00	0.70	I58.90	II3.00	48.60	70.00	24.80	6.50
9	5.00	0.00	II2.00	I.II	278.54	III.54	50.96	66.00	35.60	6.50
I0	5.00	0.00	95.33	4.30	I98.I8	79.40	46.40	40.00	32.22	6.70
II	3.00	0.00	25.00	I.II	I78.54	7I.I8	44.30	45.00	40.6I	6.60
I2	7.00	0.00	34.00	2.00	I78.54	II3.20	40.46	62.00	20.20	6.80
I3	4.00	0.00	42.73	I.II	I58.I8	79.40	49.48	86.00	I9.90	6.50
I4	5.00	0.00	5I.52	I.58	I58.90	80.20	42.65	40.00	20.00	6.40
I5	7.00	0.00	I7.50	0.25	I58.90	I06.70	4I.70	45.00	2I.2I	6.60
I6	6.00	0.00	25.00	0.26	278.54	I30.20	43.8I	60.00	22.42	6.70
I7	5.00	0.00	I42.30	2.36	I78.55	I46.75	40.26	73.00	27.60	5.80
I8	6.00	0.00	90.00	3.94	239.27	I20.I0	48.84	90.00	22.40	6.I0
I9	5.50	0.00	I5.00	4.2I	I58.90	I00.65	40.86	86.00	2I.60	6.30
20	4.00	0.00	98.25	2.05	I78.00	I20.65	44.85	74.00	3I.46	6.40

TABLE I (Continued)

GOT,GPT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5'-Nucleotidase (5'-ND), Lactic dehydrogenase (LDH)

Lipase, Amylase, Ascorbic Acid and PH value.

Case No	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5'-ND I.U/L.	LDH U.I/L.	Lipase Unit/I00ml	Amylase Somo.U/I00ml	Ascorbic Acid mg/L.	PH valu
2I	6.00	0.00	II4.90	4.60	I00.50	I50.60	50.86	62.00	20.I2	6.60
22	5.50	0.00	27.50	2.I0	I00.24	I20.82	4I.84	60.66	36.40	6.70
23	6.00	0.00	32.50	2.36	I90.65	II7.36	46.65	66.33	22.46	6.30
24	3.00	0.00	95.00	2.05	I98.I8	I08.26	42.40	62.00	3I.I2	6.50
25	3.00	0.00	37.50	I.II	I90.L0	I26.76	50.36	44.00	2I.06	6.I0
26	6.00	0.00	52.50	4.30	I39.27	I09.I2	4I.04	36.50	35.08	6.20
27	9.00	0.00	67.50	I.00	278.55	II2.65	43.86	32.50	20.08	6.60
28	3.00	0.00	I09.50	2.36	I90.I0	II6.75	40.98	64.54	40.26	6.50
29	I.00	0.00	44.25	2.82	I78.55	I20.66	5I.30	78.42	39.I2	6.70
30	4.00	0.00	45. 75	2.60	I06.24	I36.50	46.46	90.50	32.08	6.60
3I	6.50	0.00	48. 75	0.25	I90.00	I20.66	47.76	65.66	2I.00	6.70
32	3.00	0.00	I0I.25	0.26	I98.00	I06.76	4I.83	62.I6	33.3I	6.80
33	5.50	0.00	I9.50	I.60	239.27	89.60	39.90	82.00	3I.46	6.I0
34	2.00	0.00	46.50	2.43	239.I8	96.76	4I.63	46.00	23.37	6.40
35	4.00	0.00	44.25	6.45	190.I0	80.20	43.60	52.00	40.62	6.60
36	9.00	0.00	87.75	3.94	I98.I8	I26.26	40.64	65.00	43.06	6.50
37	6.00	0.00	82.50	2.36	I00.50	I40.80	42.54	63.00	23.20	6.40
38	3.00	0.00	80.62	2.I0	298.I8	I50.62	50.34	60.00	40.II	6.50
39	6.50	0.00	I02.30	2.00	I78.54	I40.45	5I.46	68.50	20.I9	6.40
40	5.00	0.00	68.50	3.94	II0.05	I08.64	46.46	64.50	I8.20	6.60

TABLE I (Ccontinued)

GOT,GPT,Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5'-Nucleotidase (5'-ND),Lactic dehydrogenase (LDH) Lipase, Amylase, Ascorbic Acid and PH value.

ase No	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5'-ND I.U/L.	LDH UM/L.	Lipase Unit/I00ml	Amylase Spmo.U/I00ml	Ascorbic Acid mg/L.	PH value
4I	4.00	0.00	96.80	2.I0	I79.I6	I43.20	42.64	56.50	I8.60	6.60
42	4.00	0.00	80.32	2.42	I39.27	I70.60	46.36	64.50	20.42	6.80
43	6.50	0.00	90.00	2.36	I78.80	I06.70	40.43	66.00	I9.60	6.50
44	3.00	0.00	98.25	2.05	II0.80	II6.72	52.26	70.00	25.42	6.40
45	5.50	0.00	76.25	0.94	II7.80	I28.2I	50.47	72.00	30.64	6.60
46	I.00	0.00	95.00	2.86	278.54	I36.46	43.49	66.50	33.22	6.20
47	2.00	0.00	40.66	2.82	I78.55	I53.60	42.50	58.50	40.00	6.30
48	6.00	0.00	95.52	4.36	I07.52	I64.68	45.64	69.00	39.42	6.20
49	6.00	0.00	84.75	3.00	I80.52	I70.60	44.86	70.00	20.6I	6.40
50	9.00	0.00	I00.00	2.I0	I78.54	I36.40	4I.64	66.00	22.40	6.30
5I	9.00	0.00	88.20	2.82	I98.I8	I35.40	42.68	64.00	20.82	6.20
52	2.00	0.00	I05.00	2.43	I58.90	I42.46	38.60	69.50	I8.92	6.I0
53	6.00	0.00	90.00	2.I0	I78.54	I34.45	5I.76	70.00	25.60	6.00
54	6.00	0.00	25.00	2.36	I58.90	I26.43	50.30	58.00	40.2I	6.70
55	I.00	0.00	95.00	2.20	II0.80	I09.20	40.89	66.25	36.32	6.60
56	2.00	0.00	I02.00	2.I6	II7.80	II6.64	52.40	64.66	33.22	6.40
57	I.00	0.00	I00.50	2.05	278.55	I26.45	42.0I	60.00	3I.6I	6.30
58	2.00	0.00	94.60	2.82	278.54	I36.48	44.40	56.50	30.40	6.20
59	3.00	0.00	79.50	2.36	I39.I8	I48.53	43.30	58.60	28.80	6.80
60	6.00	0.00	86.50	2.I0	II0.80	I52.60	5I.26	54.00	27.22	6.70

TABLE I (Continued)

GOT,GPT,Alkaline Phosphatase (ALP), Acid Phosphatase(ACP), 5'-Nucleotidase (5'-ND),Lactic dehydrogenase (LDH)

Lipase, Amylase, Ascorbic Acid and PH value.

Case No	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5'-ND I.U/L.	LDH UM/L.	Lipase Unit/I00ml	Amylase Somo.U/I00ml	Ascorbic Acid mg/L.	PH valu
6I	9.00	0.00	86.75	2.36	II7.80	88.65	5I.64	63.66	26.70	6.60
62	9.00	0.00	98.50	2.I0	278.55	I08.67	50.55	67.66	3I.62	6.Io
63	6.00	0.00	45.50	2.05	I80.52	I60.68	42.70	58.00	30.70	6.20
64	3.00	0.00	I02.50	3.94	I78.55	I40.80	43.32	59.56	36.I2	6.40
65	9.00	0.00	I00.00	2.20	239.I8	I20.43	42.40	72.00	39.30	6.00
66	2.00	0.00	I06.00	2.80	I70.60	II8.65	39.06	69.00	33.64	6.60
67	2.00	0.00	I05.50	2.I0	I82.46	I20.I0	44.70	68.56	3I.20	6.60
68	5.50	0.00	98.50	2.05	I39.I8	I40.30	46.84	67.50	28.I2	6.20
69	6.00	0.00	86.75	2.82	I80.52	I30.46	48.67	60.00	39.I6	6.40
70	4.00	0.00	42.73	I.60	I78.50	I20.I0	5I.56	64.66	38.I2	6.50
7I	9.00	0.00	98.25	4.60	I90.I0	II6.I7	53.32	62.66	36.I8	6.30
72	5.50	0.00	40.66	2.82	I98.I8	I20.60	42.LO	72.66	40.62	6.60
73	6.00	0.00	88.20	2.43	I00.50	I80.60	5I.86	70.00	22.40	6.50
74	7.00	0.00	I05.00	3.94	I90.65	I40.32	46.62	62.00	29.65	6.80
75	2.00	0.00	76. 25	2.36	I58.90	I64.65	44.46	67.66	26.8I	6.40
76	6.00	0.00	90.00	3.94	I98.I0	I70.32	5I.65	59.00	20.20	6.I0
77	5.50	0.00	96.80	2.05	II7.80	I86.40	43.84	60.00	2I.62	6.20
78	4.00	0.00	90.00	2.I0	I58.90	I76.20	4I.36	67.66	20.60	6.30
79	4.00	0.00	4I.66	4.36	I39.I8	I62.40	45.34	78.80	I9.62	6.50
80	3.00	0.00	86.75	0.94	I58.90	I40.50	46.46	66.00	20.28	6.40
8I	5.50	0.00	I00.00	4.20	I82.50	I62.65	40.86	67.66	2I.62	6.60
82	6.00	0.00	88.75	I.II	I78.55	I42.64	5I.96	65.66	20.86	6.70

Levels of some Enzymes, Ascorbic acid and PH value of Cow's milk obtained from apparently normal animals

GOT, GPT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5-Nucleotidase (5-ND), Lactic dehydrogenase (LDH) Amylase, Lipase, Ascorbic Acid and PH value.

Case NO	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5-ND I.U/L.	LDH UI/L.	Lipase Unit/I00ml	Amylase Somo.U/I00 ml	Ascorbic Acid mg/L.	PH value
I	5.50	0.00	I00.00	2.43	I82.50	80.65	4I.06	62.50	7.I0	6.40
2	2.00	0.00	83.75	0.90	I78.56	I20.04	40.89	58.00	7.60	6.50
3	4.00	0.00	I48.00	I.60	I70.60	I60.82	40.78	54.00	6.82	6.30
4	3.00	0.00	II2.00	2.80	I80.52	I70.76	3I.30	59.00	7.40	6.40
5	3.50	0.00	90.00	2.05	I58.90	I80.82	4I.60	60.66	7.80	6.50
6	3.00	0.00	96.80	2.36	I98.I0	I90.60	40.40	52.33	7.40	6.60
7	5.00	0.00	I42.50	3.94	I00.50	I00.80	42.32	50.66	7.90	6.50
8	3.00	0.00	I02.00	I.I0	I58.90	II0.60	38.60	48.00	9.00	6.70
9	2.50	0.00	I27.50	I.58	I39.I8	II7.63	36.09	56.00	6.90	6.40
I0	4.00	0.00	I00.00	2.I0	I70.60	I20.65	40.96	63.00	6.80	6.30
II	3.00	0.00	90.00	2.30	I78.50	I70.40	40.87	58.00	7.45	6.50
I2	4.50	0.00	95.00	2.60	I80.50	I60.65	37.3I	55.66	7.I0	6.50
I3	3.00	0.00	87.75	2.I0	II7.80	I54.60	38.I6	53.66	7.40	6.40
I4	5.50	0.00	I0I.25	2.43	I39.I8	I52.76	4I.20	52.00	7.32	6.60
I5	I.00	0.00	I08.00	2.82	I58.90	I40.65	40.03	50.66	7.00	6.70
I6	3.00	0.00	I09.50	2.80	I80.52	89.60	39.96	58.66	7.02	6.60
I7	I.00	0.00	I4I.33	2.I0	I79.20	86.46	4I.94	59.33	7.20	6.30
I8	6.00	0.00	95.33	2.82	I58.90	90.65	40.I3	62.46	7.62	6.50
I9	5.50	0.00	I52.50	2.20	II0.80	I64.60	4I.I0	56.66	7.00	6.40
20	5.00	0.00	I38.75	I.60	I80.52	I54.32	44.00	53.00	6.82	6.60

TABLE 3

Levels of some Enzymes, Ascorbic acid and PH value of Buffalee's milk obtained from subclinically mastitic cases

GOT, GPT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5'-Nucleotidase (5'-ND), Lactic dehydrogenase (LDH) Amylase, Ascorbic Acid end PH value.

Case NO	Causative Organisms	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5'-ND I.U/L.	LDH Um/L.	Lipase Unit/I00ml	Amylase Somo.U/I00ml	Ascorbic Acid mg/L.	PH va
I	Str.agalactiae	80.00	0.00	363.00	4.00	I9.50	546.I6	64.60	I45.40	3I.60	7.
2	" "	74.00	0.00	I87.00	2.05	0.00	654.00	55.20	II0.30	39.I0	7.
3	" " "	65.00	0.00	2I0.00	7.84	39.00	680.40	64.32	II5.I6	39.25	7.
4	" " "	I00.00	0.00	390.50	2.36	20.00	540.20	62.86	I02.I3	32.I0	7.
5	" " "	95.00	0.00	I48.00	6.20	0.00	620.00	54.30	I34.80	30.32	7.
6	" " "	80.00	0.00	208.00	2.05	0.00	760.60	63.I6	I36.00	40.36	7.
7	Staph.aureus	60.00	0.00	242.00	2.45	59.00	496.00	66.I0	78.80	29.60	7.
8	" " "	29.00	0.00	I98.50	2.36	78.50	5I0.60	62.I0	I60.30	30.35	7.
9	" " "	36.50	0.00	I62.50	I.36	58.90	520.I0	43.40	I33.40	36.40	7.
I0	" " "	60.00	0.00	I62.00	4.00	49.60	460.I0	55.40	I60.30	36.I4	7.
II	" " "	24.00	0.00	274.00	3.25	56.40	486.30	56.30	I26.I0	40.80	7.
I2	Str. + Staph.	65.50	0.00	260.50	4.30	46.50	520.40	64.36	I2I.20	25.30	7.
I3	" " "	60.00	0.00	205.00	4.2I	55.I0	530.I0	63.86	I40.60	40.30	7.
I4	" " "	95.00	0.00	3I8.70	I.58	40.65	5I6.30	53.43	98.20	44.80	7.
I5	" " "	74.50	0.00	260.00	4.30	36.40	540.62	53.2I	I34.80	26.35	7.
I6	" " "	70.00	0.00	I90.50	I.60	44.60	5I0.00	62.I6	I20.00	20.I0	7.
I7	Ps.aeruginosa	65.00	0.00	2I0.00	2.82	96.20	346.70	44.I5	I46.75	36.23	7.
I8	E. coli	4.50	0.00	I26.00	0.70	II0.64	2I6.00	43.I6	I40.60	30.62	7.

TABLE 4

Levels of some Enzymes, Ascorbic acid PH value of Cow's milk obtained from subclinically mastitis cases.

GOT, GPT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5-Nucleotidase (5-ND), Lactic dehydrogenase (LDH) Lipase, Amylase, Ascorbic Acid and PH value.

Case NO	Causative Organisms	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5-ND I.U/L.	LDH Um/L.	Lipase Unit/I00ml	Amylase Somo.U/I00ml	Ascorbic Acid mg/L.	PH value
I	Str.agalactiae	65.00	0.00	375.00	2.05	0.00	530.I0	53.40	I42.30	6.62	7.I0
2	" " "	60.50	0.00	272.50	5.00	20.50	525.65	53.20	I32.00	662I	6.70
3	" " "	60.00	0.00	3I8.70	0.90	I9.60	505.00	54.86	I33.33	5:I2	7.70
4	" " "	35.00	0.00	396.50	I.60	0. 00	520.25	53.I2	I64.I0	6.I0	7.60
5	" " "	38.50	0.00	402.00	2.80	26.I0	546.I0	62.I7	I60.I7	7.25	7.90
6	" " "	52.00	0.00	229.50	9.20	49.50	496.25	53.I6	I36.I0	5.3I	7.30
7	" " "	50.00	0.00	278.50	6.00	0.00	504.30	54.25	I26.I8	6.6I	7.60
8	" " "	64.50	0.00	372.00	1.58	0.00	560.I0	54.32	II6.32	7.06	7.00
9	Staph.aureus	I3.00	0.00	I0I.50	4.2I	60.00	476.50	55.I0	I43.2I	7.60	6.80
I0	" "	25.00	0.00	I88.50	2.2I	59.80	502.75	53.64	I06.66	8.60	7.30
II	" "	25.00	0.00	I98.25	2.30	48.30	496.35	55.I0	I46.75	6.74	7.20
I2	" "	40.50	0.00	220.50	2.00	56.I0	462.I5	44.32	I62.66	6.I8	7.00
I3	Str. + Staph.	27.00	0.00	205.50	2.I0	20.I0	496.36	54.48	I60.I0	8.I6	6.60
I4	" "	60.00	0.00	202.00	6.00	46.40	5I0.30	52.60	I26.I8	7.I6	7.50
I5	" "	46.50	0.00	204.50	4.00	56.40	482.30	53.I8	II8.62	7.25	7.60
I6	" "	40.00	0.00	227.00	2.20	76.I0	525.00	52.I9	90.I0	6.I0	7.7C
I7	" "	46.50	0.00	242.00	I.60	80.I2	498.I0	53.22	78.I2	6.I6	7.40
I8	E. Coli	50.00	0.00	I88.50	0.70	62.I0	436.I0	55.I0	I34.30	6.I2	7.20
I9	C. pyogenes	25.00	0.00	362.00	2.05	II7.00	482.28	43.87	98.I6	6.28	7.I0
20	Rh.nigricans	45.00	0.00	I50.00	2.45	80.50	420.25	44.60	I20.00	8.I6	7.30

TABLE 5

Levels of some Enzymes, Ascorbic acid and PH value of Buffaloe's milk obtained from clinically mastitic cases

GOT, GPT, Alkaline Phosphatase (ALP),Acid Phosphatase (ACP), 5-Nucleotidase (5-ND),Lactic dehydrogenase (LDH) Lipase, Amylase,Ascorbic Acid and PH value.

Case NO	Causative organisms	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5-ND I.U/L.	LDH Um/L.	Lipase Unit/I00ml	Amylase Somo.U/I00ml	Ascobic Acid mg/L.	PH val
I	Str.agalactiae	74.00	7.50	302.50	2.I0	40.25	795.I5	63.65	I85.40	54.40	7.50
2	" " "	65.00	0.00	380.00	0.65	I6.75	646.I0	64.60	I72.30	60.I0	7.60
3	" " "	85.00	5.50	260.50	9.20	0.00	700.20	66.82	I86.00	50.3I	7.00
4	" " "	80.00	I.50	209.00	I0.05	20.00	750.I0	64.60	I64.50	54.20	7.40
5	" " "	I00.00	2.00	362.00	0.50	40.75	682.75	73.96	I70.35	50.22	7.40
6	" " "	**65.00**	4.25	390.00	2.05	25.30	786.00	64.96	I64.30	60.20	7.50
7	" " "	72.00	0.00	20I.00	I.60	I9.50	800.60	53.86	I68.40	60.80	7.40
8	" " "	60.00	4.00	420.00	I.75	20.72	845.00	64.88	I26.50	56.40	7.70
9	" " "	54.00	I.50	382.25	0.85	30.40	765.72	56.I0	I74.65	54.I0	7.60
I0	Staph.aureus	60.00	0.00	I88.25	2.40	60.50	606.50	73.I0	I49.30	65.I0	7.70
II	" "	52.00	0.00	I90.50	0.30	20.I0	652.I0	73.60	I50.2I	73.20	7.80
I2	" "	35.00	0.00	270.00	4.40	0.00	586.25	64.48	I56.40	77.I8	7.40
I3	" "	44.00	4.50	208.00	0.60	I3.60	594.I0	64.52	I66.32	60.I0	7.40
I4	" "	30.00	2.00	I98.25	2.05	25.30	602.20	56.I0	I50.58	72.I6	7.40
I5	" "	35.00	0.00	240.00	4.60	40.I0	582.I0	65.20	I54.60	57.I0	7.50
I6	" "	35.00	0.00	272.00	4.I0	0.00	508.25	64.2I	I40.28	62.40	7.60
I7	C. pyogenes	40.00	0.00	I90.00	0.75	30.I0	505.25	53.I0	I46.50	36.2I	7.60
I8	" " "	45.00	0.00	I60.25	0.86	70.60	496.I0	72.I5	I52.46	40.60	7.7
I9	" " "	60.00	I.25	200.00	2.30	0.00	505.25	64.I0	I56.48	28.I4	7.60
20	" " "	35.00	I.50	I95.50	3.60	20.00	482.I0	63.22	I58.49	26.I6	7.40

TABLE 5 (Continued)

GOT, GPT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5'-Nucleotidase (5'-ND), Lactic dehydrogenase (LDH) Lipase, Amylase, Ascorbic Acid and PH value.

ase 0	Causative organisms	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5'-ND I.U/L.	LDH Um/L.	Lipase Unit/I00ml	Amylase Somo.U/I00ml	Ascorbic Acid mg/L.	PH value
21	G+ pyogenes	40.00	2.00	I82.25	4.I0	I9.25	608.20	63.46	I46.30	36.I0	7.30
22	" "	50.00	0.00	I90.50	0.65	0.00	546.20	62.50	I52.46	40.20	7.40
23	" "	38.00	0.00	206.00	0.70	20.20	540.00	63.65	I64.00	28.I3	7.80
24	" "	42.00	4.25	I72.00	4.20	58.25	440.00	54.82	I68.65	26.I0	7.70
25	E. coli	34.00	0.00	I50.00	2.05	20.50	482.50	74.60	I48.50	I7.48	7.60
26	" "	26.50	0.00	228.00	I.75	58.20	40I.20	63.22	I52.I6	27.28	7.50
27	" "	38.00	0.00	2I0.00	0.25	30.I0	482.00	72.90	I60.25	28.I0	7.40
28	Anthracoids	I3.50	0.00	I05.20	I.72	28.25	306.00	62.60	I58.50	23.60	7.40
29	Str. dysaga -	60.00	0.00	242.00	2.I0	20.25	505.25	63.2I	I60.80	58.I6	7.30
30	lactiae	50.00	4.25	228.00	3.20	40.I0	5I0.I0	62.70	I50.I2	49.I0	7.20
31	Str. bovis.	62.50	0.00	205.00	2.I5	20.50	542.25	63.42	I54.30	30.50	7.80
32	" "	54.00	0.00	2I0.00	4.20	60.30	560.00	53.I2	I56.00	38.I0	7.60
33	Ps. aeruginosa	4.00	0.00	96.50	I.20	30.I2	6I0.I0	63.I4	I49.60	22.I6	7.40
34	E. Coli +Str.	80.50	4.00	372.00	3.40	25.I0	402.00	73.I6	I44.00	50.I0	7.70
35	agalactiae	72.25	2.25	2I6.00	4.50	35.25	752.00	64.20	I64.35	54.20	7.40
36	Str. +Staph.	70.50	2.25	302.00	4.I0	50.I0	740.75	54.I0	I68.50	60.I0	7.40
37	" "	64.00	0.00	320.00	6.05	20.25	80I.20	64.60	I60.40	55.60	7.50
38	" "	58.50	I.50	280.00	3.08	36.I0	600.75	63.82	I56.30	49.I7	7.60
39	Staph.+E.coli	65.50	0.00	206.00	2.06	60.I0	665.00	73.20	I60.40	33.I8	7.70
40	" "	42.25	0.00	I86.00	I.50	25.20	482.75	63.62	I48.25	43.I0	7.50

Levels of some Enzymes, Ascorbic acid and PH value of Cow's milk obtained from clinically mastitic cases

GOT, GPT, Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), 5-Nucleotidase (5-ND), Lactic dehydrogenase (LDH) Lipase, Amylase, Ascorbic Acid and PH value.

se	Causative organisms	GOT I.U/L.	GPT I.U/L.	ALP K.A.U/I00ml	ACP K.A.U/I00ml	5-ND I.U/L.	LDH Um/L.	Lipase Unit/I00ml	Amylase Somo.U/I00ml	Ascorbic Acid mg/L.	PH value
I	Str. agalactiae	60.00	5.50	372.00	0.85	2I.50	630.20	65.48	I52.50	2I.50	7.I0
2	" " "	55.00	0.00	260.00	2.I0	0.00	625.65	65.I5	I49.60	I6.42	7.20
3	" " "	72.00	0.00	3I0.00	6.05	26.25	620.00	64.96	I80.20	30.I0	7.60
4	" " "	67.00	4.25	395.50	8.50	40.27	740.00	54.25	I72.00	I8.22	7.80
5	" " "	78.00	I.50	280.25	I.85	48.20	8I0.00	55.I2	I40.75	I9.38	7.60
6	" " "	85.00	2.25	206.50	2.95	20.00	586.70	45.I6	I54.00	I6.25	7.50
7	" " "	60.00	I.50	286.00	3.45	26.50	652.50	66.20	I40.00	I0.64	7.40
8	" " "	72.00	0.00	306.05	2.05	0.00	620.00	66.25	I50.70	20.I8	7.20
9	" " "	60.00	0.00	3I6.00	2.I0	I9.00	6I2.00	65.06	I46.40	23.I7	7.30
0	Staph aureus	45.00	0.00	I60.75	2.I0	65.00	605.00	64.32	I45.00	I7.40	7.50
I	" "	35.00	0.00	208.25	0.95	40.75	620.75	74.65	I42.30	I2.08	7.40
2	" "	42.00	2.50	I86.00	3.I0	50.42	5I2.00	64.82	I48.30	I8.78	7.40
3	" "	30.00	0.00	I73.50	2.00	30.80	508.00	53.46	I50.60	I7.52	7.30
4	C. pyogenes	27.00	0.00	I56.75	2.05	24.50	482.00	53.28	I40.30	7.40	7.40
5	" "	29.00	I.50	I68.20	0.96	30.40	496.I0	63.68	I38.25	7.90	7.50
6	" "	22.00	4:00	203.40	3.I0	58.I0	500.75	63.04	I4I.30	8.I0	7.60
7	" "	38.00	0.00	202.00	4.20	60.45	508.20	64.05	I46.I0	I0.50	7.70
8	" "	40.00	0.00	I98.00	2.00	20.I0	5I2.00	53.24	I32.28	8.65	7.40
9	Asp.Fumigutus.	22.00	0.00	260.40	2.35	23.I0	486.75	52.60	I40.25	7.24	7.40
0	Str. +Staph.	64.00	2.25	308.75	6.50	50.40	8I0.75	64.88	I62.70	I8.20	7.60
I	E.Coli.	23.50	0.00	280.70	2.05	I0.75	483.60	63.20	I49.I0	8.I0	7.40
2	E.Coli.	22.00	0.00	260.25	0.96	20.50	546.00	52.08	I52.30	7.2I	7.50

TABLE 7

Mean values of some Enzymes, Ascorbic acid and pH of apparently normal Buffaloe's milk.

Item	maximum	minimum	mean	S.E. ±
GOT	9.00	1.00	4.80	0.29
GPT	0.00	0.00	0.00	0.00
ALP[1]	148.00	15.00	76.09	2.80
ACP[2]	6.45	0.25	2.37	0.13
5'-ND	298.18	100.50	179.41	5.55
LDH	186.40	71.18	127.09	3.39
Lipase	53.32	38.60	45.22	0.46
Amylase	90.50	32.50	63.65	1.23
Ascorbic acid	43.06	18.20	28.30	0.82
pH Value	6.80	5.80	6.40	0.03

S.E. ± :- Standard error

1 . Alkaline phosphatase

2 . Acid phosphatase

TABLE 8

Mean values of some Enzymes, Ascorbic acid and pH of apparently normal cow's milk.

Item	maximum	minimum	mean	S.E ±
GOT	5.50	1.00	3.65	0.33
GPT	0.00	0.00	0.00	0.00
ALP[1]	152.50	88.75	111.35	4.89
ACP[2]	3.94	0.90	2.23	0.15
5'-ND	198.10	100.50	161.16	5.96
LDH	190.60	80.65	135.93	7.75
Lipase	44.00	31.30	39.94	0.60
Amylase	63.00	48.00	56.21	0.97
Ascorbic acid	9.00	6.80	7.33	0.11
pH value	6.70	6.30	6.49	0.02

S.E ± : Standard error.

1 . Alkaline phosphatase.

2 . Acid phosphatase.

TABLE 9

Test of significance of difference between mean values of some enzymes, Ascorbic acid and pH of apparantly normal milk of Buffaloes and cows.

Iteam	Mean Values	
	Buffaloe's milk	Cow's milk
GOT	4.80*	3.65
GPT	0.00	0.00
ALP[1]	76.09	111.35**
ACP[2]	2.37	2.23°
5'-ND	179.41	161.16°
LDH	127.09	135.93°
Lipase	45.22**	39.94
Amylase	63.65**	56.21
Ascorbic acid	28.30**	7.33
pH value	6.40	6.49°

° Non Significant

* Significant at $P < 0.05$

** Significant at $P < 0.01$

1 Alkaline Phosphatase

2 Acid Phosphatase

TABLE 10

Mean values of some Enzymes, Ascorbic acid and pH of Buffaloe's milk obtained from subclinically mastitic cases

Iteam	Maximum	Minimum	Mean	S.E ±
GOT	100.00	24.00	63.33	6.00
GPT	0.00	0.00	0.00	0.00
ALP[1]	390.50	126.00	228.68	16.98
ACP[2]	7.84	0.70	3.19	0.42
5'-ND	110.64	0.00	44.83	7.35
LDH	760.60	216.00	525.29	28.04
Lipase	66.10	43.16	57.34	1.81
Amylase	160.30	78.80	128.00	5.14
Ascorbic acid	44.80	20.10	33.87	1.53
pH value	7.80	7.00	7.40	0.06

S.E. ± : Standard error.

1 Alkaline Phosphatase.

2 Acid Phosphatase.

TABLE 11

Mean values of some Enzymes, Ascorbic acid and pH of Buffaloe's milk obtained from clinically mastitic cases

Iteam	Maximum	Minimum	Mean	S.E. ±
GOT	100.00	4.00	52.30	8.37
GPT	7.50	0.00	1.40	0.31
ALD[1]	420.00	105.20	238.18	12.25
ACP[2]	10.05	0.25	2.64	0.35
5'-ND	70.60	0.00	28.80	2.88
LDH	845.00	306.00	596.73	20.33
Lipase	74.60	53.10	64.18	1.86
Amylase	186.00	126.50	157.93	1.85
Ascorbic acid	77.18	17.78	46.78	2.10
pH value	7.80	7.00	7.51	0.03

S.E ±: Standard error.

1 Alkaline Phosphatase.

2 Acid Phosphatase.

TABLE 12

Test of significance showing the effect of mastitis on levels of some enzymes, Ascorbic acid and pH value of Buffaloe's milk.

Iteam	Mean Values					
	Normal	Subclinical	Normal	Clinical	Subclinical	Clinical
GOT	4.80	63.33**	4.80	52.30**	63.33	52.30°
GPT	0.00	0.00°	0.00	1.40**	0.00	1.40**
ALD[1]	76.09	228.68**	76.09	238.10**	228.68	238.10°
ACP[2]	2.37	3.19*	2.37	2.64°	3.19	2.64°
5'-ND	179.41**	44.83	179.41**	28.80	44.83	28.80°
LDH	127.09	525.29**	127.09	596.73**	525.29	596.73*
Lipase	45.22	57.34**	45.22	64.18**	57.34	64.18**
Amylase	63.65	128.00**	63.65	157.93**	128.00	157.93**
Ascorbic acid	28.30	33.87**	28.30	46.88**	33.87	46.88**
pH value	6.40	7.40**	6.40	7.51*	7.40	7.51°

° Non significant

* Significant at P < 0.05

** Significant at P < 0.01

1 Alkaline Phosphatase.

2 Acid Phosphatase.

TABLE 13

Mean values of some enzymes, Ascorbic acid and pH of cow's milk obtained from subclinically mastitic cases.

Iteam	Maximum	Minimum	Mean	S.E. ±
GOT	65.00	13.00	43.45	3.35
GPT	0.00	0.00	0.00	0.00
ALP[1]	402	101.50	256.75	19.42
ACP[2]	9.20	0.70	3.05	0.47
5'-ND	117.00	0.00	43.93	7.25
LDH	560.10	420.25	498.81	7.53
Lipase	62.17	43.87	52.79	0.94
Amylase	164.10	78.12	129.77	5.38
Ascorbic acid	8.60	5.12	6.74	0.21
pH value	7.90	6.60	7.28	0.08

S.E. ±: Standard error

1 Alkaline Phosphatase.

2 Acid Phosphatase.

TABLE 14

Mean values of some enzymes, Ascorbic acid and pH of cow's milk obtained from clinically mastitic cases.

Iteam	Maximum	Minimum	Mean	S.E. ±
GOT	85.00	22.00	47.66	4.33
GPT	5.50	0.00	1.15	0.36
ALP[1]	395.50	156.75	249.97	14.75
ACP[2]	8.50	0.85	2.83	0.41
5'-ND	65.00	0.00	31.23	3.91
LDH	810.00	482.00	589.50	21.20
Lipase	74.65	52.08	60.68	1.49
Amylase	180.20	132.28	148.86	2.36
Ascorbic acid	30.10	7.21	14.77	1.35
pH value	7.80	7.10	7.46	0.04

S.E. ±: Standard error

1 Alkaline Phosphatase.

2 Acid Phosphatase.

TABLE 15

Test of significance showing the effect of mastitis on some enzymes, Ascorbic acid and pH value of cow's milk.

Iteam	Mean Values					
	Normal	Subclinical	Normal	Clinical	Subclinical	Clinical
GOT	3.65	43.45**	3.65	47.66**	43.45	47.66°
GPT	0.00	0.00°	0.00	1.15**	0.00	1.15**
ALP[1]	111.35	256.75**	111.35	249.97**	256.75	249.97*
ACP[2]	2.23	3.05*	2.23	2.83°	3.05	2.83*
5'-ND	161.16**	43.93	161.16**	31.23	43.93	31.23°
LDH	135.93	498.81**	135.93	589.50**	498.81	589.50**
Lipase	39.94	52.79**	39.94	60.68**	52.79	60.68**
Amylase	56.21	129.77**	56.21	148.86**	129.77	148.86**
Ascorbic acid	7.33	6.74*	7.33	14.77**	6.74	14.77**
pH value	6.49	7.28**	6.49	7.46**	7.28	7.46*

° Non Significant

* Significant at P < 0.05

** Significant at P < 0.01

TABLE 16

Variations in the level of some **Enzymes, Ascorbic acid and PH** value of Buffaloe's milk **in cases of Subclinical mastitis** due to different causative organisms.

Causative organisms.		GOT	GPT	ALP	ACP	5-ND	LDH	Lipase	Amylase	Ascorbic acid	PH value.
Str. agalactiae	Max.	100.00	0.00	390.50	7.84	39.00	760.60	64.60	145.40	40.36	7.80
	Min.	65.00	0.00	148.00	2.05	0.00	540.20	54.30	102.13	30.32	7.00
	Mean	82.33	0.00	251.08	4.09	13.08	633.56	60.74	123.97	35.46	7.53
	S.E±	5.33	0.00	40.92	0.99	6.52	34.30	1.92	6.99	1.86	0.12
Staph. aureus .	Max.	60.00	0.00	274.00	4.00	78.50	520.10	66.10	160.30	40.80	7.70
	Min.	24.00	0.00	162.00	1.36	49.60	460.10	43.40	78.80	29.60	7.00
	Mean	42.30	0.00	207.80	2.68	60.48	494.62	56.66	131.82	34.66	7.26
	S.E.±	7.59	0.00	22.12	0.45	4.82	10.41	3.85	14.95	2.09	0.13
Str. agalactiae+Staph. aureus	Max.	95.00	0.00	318.70	4.30	55.10	540.62	64.36	140.60	44.80	7.60
	Min.	60.00	0.00	190.00	1.58	36.40	510.60	53.21	98.20	20.10	7.20
	Mean	73.00	0.00	246.94	3.20	44.65	523.60	59.40	122.96	31.37	7.34
	SE. ±	6.00	0.00	22.86	0.67	3.14	5.31	2.51	7.34	4.74	0.06

S.E ± :Standard error

TABLE 17

Test of significance showing the effect of the causative organisms in cases of subclinical mastitis on level of some enzymes, Ascorbic acid and PH value of Buffaloe's milk

Item	MEAN VALUES											
	Normal	Str. agalactiae	Normal	Staph. aureus	Normal	Mixed Str.+ Staph.	Str. agalactiae	Staph. aureus	Str. agalactiae	Mixed Str.+ Staph.	Staph. aureus	Mixed Str. + staph.
GOT	4.80	82.33 **	4.80	42.30 **	4.80	73.00 **	82.33 **	42.30	82.33	73.00 °	42.30	73.00 **
GPT	0.00	0.00	0.00	0.00	0.00	0.00 **	0.00	0.00	0.00	0.00	0.00	0.00
ALP	76.09	251.08 **	76.09	207.80 **	76.09	246.94 **	251.08	207.80 °	251.08	24 .94 °	207.80	249.94 °
ACP	2.37	4.09 **	2.37	2.68 °	2.37	3.20 °	4.09	2.68 **	4.09	3.20 °	2.68 *	3.20 °
5 - ND	179.41 **	13.08	179.41 **	60.48	179.41 **	44.65	13.08	60 .48 **	13.08	44.65 **	60.48	44 .65 *
LDH	127.09	633.56 **	127.09	494.62 **	127.09	523.60 **	633.56	494.62 **	633.56	523.60 *	494.62	523.60 °
Lipase	45.22	60.74 **	45.22	56.66 **	45.22	59.40 **	60.74	56.66 °	60.74	59.40 °	56.66	59.40 °
Amylase	63.65	123.97 **	63.65	131.82 **	63.65	122.96 **	123.97	131.82 °	123.97	122.96 °	131.82	122.96 °
Ascorbic acid	28.30	35.46 *	28.30	34.66 °	28.30	31.37 °	35.46	34.66 °	35.46	31.37 °	34.66	31.37 °
PH value	6.40	7.53 **	6.40	7.26 **	6.40	7.34 **	7.53	7.26 °	7.53	7.34 °	7.26	7.34 °

° Non Significant
* Significant at P < 0.05
** Significant at P < 0.01

Table 18

Variations in the level of some enzymes Ascorbic acid and PH value of Buffaloe's milk in cases of clinical mastitisdue to diff rent causative organisms.

Causative organisms.		GOT	GPT	ALP	ACT	5-ND	LDH	Lipase	Amylase	Ascorbic acid	PH value
Str. agalactiae	Max.	100.00	7.50	420.00	10.05	40.75	845.00	73.96	186.00	60.80	7.70
	Min.	54.00	0.00	201.00	0.50	0.00	646.10	53.86	126.50	50.22	7.00
	Mean	72.78	2.81	323.03	3.19	23.74	752.40	63.71	168.04	55.64	7.46
	S.E ±	4.69	1.18	27.48	1.23	4.19	21.38	1.95	5.83	1.35	0.06
Staph. aureus.	Max.	60.00	4.50	272.00	4.60	40.10	652.10	73.60	166.32	77.18	7.80
	Min.	30.00	0.00	188.25	0.30	0.00	508.25	56.10	140.28	57.10	7.40
	Mean.	41.75	0.93	223.86	2.64	22.80	590.21	65.88	152.51	66.75	7.54
	S.E ±	4.13	0.93	13.80	0.67	8.25	16.22	2.26	3.11	2.84	0.07
C.pyogenes	Max.	60.00	4.25	206.00	4.20	58.25	608.20	72.15	168.65	40.20	7.80
	Min.	35,00	0.00	160.25	0.65	0.00	440.00	53.10	146.30	26.10	7.30
	Mean	43.75	1.13	187.06	2.15	27.30	515.26	62.13	155.67	32.71	7.56
	S.E±	2.82	0.80	5.34	0.48	8.96	17.70	2.09	2.80	2.20	0.06

S.E.: Standard error

TABLE 19

Test of significance showing the effect of the causative organisms in cases of clinical mastitis on level of some enzymes, Ascorbic acid and PH value of Buffalee's milk.

Item	MEAN VALUES											
	Normal	Str. agalactiae	Normal	Staph aureus	Normal	C. Pyogenes	Str. agalactiae	Staph. aureus	Str. agalactiae	C. Pyogenes	Staph . aureus	C. Pyogenes
GOT	4.80	72.78 **	4.80	41.75 **	4.80	43.75 **	72.78 **	41.75	72.78 **	43.75	41.75	43.75 °
GPT	0.00	2.81 **	0.00	0.93 **	0.00	1.13 **	2.81	0.93 °	2.81	1.13 °	0.93	1.13 °
ALP	76.09	323.03 **	76.09	223.8 **	76.09	187.06 **	323.03 *	223.86	323.03 **	187.06	223.86 *	187.06
ACP	2.37	3.19 °	2.37	2.64 °	2.37	2.15 °	3.19	2.64 °	3.19	2.15 °	2.64	2.15 °
5 - ND	179.41 **	23.74	179.41 **	22.80	179.41 **	27.30	23.74	22.80 °	23.74	27.30 °	22.80	27.30 °
LDH	127.09	752.40 **	127.09	590.21 **	127.09	515.26 **	752.40 **	590.21	752.40 **	515.26	590.21 **	515.26
Lipase	45.22	63.71 **	45.22	65.88 **	45.22	62.13 **	63.71	65.88 °	63.71	62.13	65.88	62.13 °
Amylase	63.65	168.04 **	63.65	152.51 **	63.65	155.67 **	168.04 *	152.51	168.04	155.67 °	152.51	155.67 °
Ascorbic acid	28.30	55.64 **	28.30	66.75 **	28.30	32.71 °	55.64	66.75 **	55.64 **	32.71	66.75 **	32.71
PH value	6.40	7.46 **	6.40	7.54 **	6.40	7.56 **	7.46	7.54 °	7.46	7.56 °	7.54	7.56 °

° Non Significant
* Significant at P <0.05
** Significant at P <0.01

TABLE 20

Variations in the level of some enzymes, Ascorbic acid and PH value of cow's milk in cases of subclinical mastitisdue to different causative arganisms.

Causative organisms.		GOT	GPT	ALP	ACP	5-ND	LDH	Lipase	Amylase	Ascorbic acid	pH value
Str. agalactiae.	Max.	65.00	0.00	402.00	9.20	49.00	560.10	62.17	164.10	7.25	7.90
	Min.	35.00	0.00	229.50	0.90	0.00	496.25	53.12	116.32	5.12	6.70
	Mean	53.19	0.00	330.59	3.64	14.46	523.47	54.81	138.81	6.29	7.36
	S.E.±	4.07	0.00	22.98	1.01	6.36	7.75	1.08	5.76	0.27	0.14
Staph. aureus.	Max.	40.50	0.00	220.50	4.21	60.00	502.75	55.10	162.66	8.60	7.30
	Min.	13.00	0.00	101.50	2.00	48.30	462.15	44.32	106.66	6.18	6.80
	Mean	25.88	0.00	177.19	2.68	56.05	484.44	52.04	139.82	7.28	7.08
	S.E.±	5.64	0.00	26.10	0.51	2.73	9.30	2.60	11.83	0.53	0.10
Str. agalactiae + staph. aureus.	Max.	60.00	0.00	242.00	6.00	80.12	525.00	54.48	160.10	8.16	7.70
	Min.	27.00	0.00	202.00	1.60	20.10	482.30	52.19	78.12	6.10	6.60
	Mean	44.00	0.00	216.20	3.18	55.82	502.41	53.13	114.62	6.97	7.36
	S.E. ±	5.35	0.00	7.86	0.70	10.88	7.18	0.39	14.41	0.38	0.20

S.E. ± : Standard error

TABLE 21

Test of significance showing the effect of the causative organisms in cases of subclinical mastitis on level of some enzymes, Ascorbic acid and PH value of cow's milk.

Item	MEAN VALUES											
	Normal	Str. agalactiae	Normal	Staph. aureus.	Normal	Mixed Str.+staph.	Str. agalactiae	Staph. aureus.	Str. agalactiae	Mixed Str.+staph.	Staph. aureus	Mixed Str. +staph.
GOT	3.65	53.19 **	3.65	25.88 **	3.65	44.00 **	53.19 **	25.88	53.19	44.00 °	25.88	44.00 *
GPT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ALP	111.35	330.59 **	111.35	177.19 **	111.35	216.20 **	330.59 **	177.19	330.59 **	216.20	177.19	216.20 °
ACP	2.23	3.64 *	2.23	2.68 °	2.23	3.18 °	3.64	2.68 °	3.64	3.18 °	2.68	3.18 °
5 - ND	161.16 **	14.46	161.16 **	56.05	161.16 **	55.82	14.46	56.05 **	14.46	55.82 **	56.05	55.82 °
LDH	135.93	523.47 **	135.93	484.44 **	135.93	502.41 **	523.47 **	484.44	523.47	502.41 °	484.44	502.41 °
Lipase	39.94	54.81 **	39. 94	52.04 **	39.94	53.13 **	54.81	52.04 °	54.81	53.13 °	52.04	53.13 °
Amylase	56.21	138.81 **	56.21	139.82 **	56.21	114.62 **	138.81	139.82 °	138.81	114.62 °	139.82	114.62 °
Ascorbic acid	7.33 **	6.29	7.33	7.28 °	7.33	6.97 °	6.29	7.28	6.29	6.97 °	7.28	6.97 °
PH value	6.49	7.36 **	6.49	7.08 **	6.49	7.36 **	7.36	7.08	7.36	7.36 °	7.08	7.36 °

° Non Significant
* Significant at $P < 0.05$
** Significant at $P < 0.01$

TABLE 22

Variations in the level of some enzymes, Ascorbic acid and PH value of cow's milk in Cases of Clinical mastitis due to different causative organisms.

Causative organisms .		GOT	GPT	ALP	ACP	5′-ND	LDH	Lipase	Amylase	Ascorbic acid	PH value
Str. agalactiae	Max.	85.00	5.50	395.50	8.50	48.20	810.00	66.25	180.20	30.10	7.80
	Min.	55.00	0.00	206.50	0.85	0.00	586.70	45.16	140.00	10.64	7.10
	Mean	67.67	1.67	303.59	3.32	22.41	655.23	60.84	154.02	19.54	7.41
	S.E.±	3.29	0.68	18.82	0.81	5.32	24.06	2.21	4.52	1.80	0.09
Staph. aureus	Max.	45.00	2.50	208.25	3.10	65.00	620.75	74.65	150.60	18.78	7.50
	Min;	30.00	0.00	173.50	0.95	30.80	508.00	53.46	142.30	12.08	7.30
	Mean	38.00	0.63	182.13	2.04	46.72	561.44	64.31	146.55	16.45	7.40
	S.E.±	3.39	0.62	10.12	0.44	7.29	29.88	4.33	1.82	1.49	0.04
C. pyogenes.	Max.	40.00	4.00	203.40	4.20	58.10	512.00	64.05	146.10	10.50	7.70
	Min.	22.00	0.00	168.20	0.96	20.10	482.00	53.24	132.28	7.40	7.40
	Mean	31.20	1.21	185.67	2.46	38.71	499.81	59.46	139.65	8.51	7.52
	S.E.±	3.40	1.10	9.68	0.55	8.56	5.25	2.54	2.25	0.54	0.06

S.E. ± : Standard error

TABLE 23

Test of significance showing the effect of the causative organism in cases of clinical mastitis on level of some enzymes, Ascorbic acid and PH value of cow's milk.

Item	MEAN VALUES											
	Normal	Str. agalactiae	Normal	Staph. aureus	Normal	C. Pyogenes	Str. agala-ctiae	Staph aureus	Str. agalactiae	C. Pyogenes	Staph. aureus	pyo..
GOT	3.65	67.67 XX	3.65	38.00 XX	3.65	31.20 XX	67.67	38.00	67.67	31.20	38.00	31.
GPT	0.00	1.67 X	0.00	0.63 X	0.00	1.21 X	1.67	0.63 °	1.67	1.21 °	0.63	1.
ALP	111.35	303 .59 XX	111.35	102.13 XX	111.35	105.67 XX	303.59 XX	102.13	303.59 XX	105.67	102.13	105.
ACP	2.23	3.32 °	2.23	2.04 °	2.23	2.46 °	3.32	2.04 °	3.32	2.46 °	2.04	2.
5 - ND	161.16 XX	22.41	161.16 XX	46.72 X	161.16 XX	38.71	22.41	46.72 X	22.41	38.71 °	46.72	38.
LDH	135.93	655.23 XX	135.93	561.44 XX	135.93	495.81 XX	655.23 X	561.44	655.23 XX	495.81	561.44 X	495.
Li ase	39.94	60.84 XX	39.94	54.31 XX	39.94	59.46 XX	60.84	54.31 °	60.84	59.46 °	54.31	59.
Amylase	56.21	154.02 XX	56.21	146.55 XX	56.21	139.65 XX	154.02	146.55 °	154.02 X	139.65	146.55 X	139.
Ascorbic Acid	7.33	19.54 XX	7.33	16.45 XX	7.33	8.51 °	19.54	16.45 °	19.54 XX	8.51	16.45 XX	8.
PH Value	6.49	7.41 XX	6.49	7.40 XX	6.49	7.52 XX	7.41	7.40 °	7.41	7.52 °	7.40	7.

° Non significant
X Significant P < 0.05
XX Significant P < 0.01

TABLE 24

Incidence of infected cases in milk samples collected from apparently normal udders of buffaloes and cows

Animal species	Number of samples examined	Number of samples positive bacteriologically	%
Buffaloes	100	18	18
Cows	40	20	50
Total	140	38	68

TABLE 25

Frequency distribution of isolates from cases of sub-clinical mastitis

Micro-organisms isolated	Buffaloes		Cows	
	No. of cases	%	No. of cases	%
Str. agalactiae	6	33.34	8	40.00
Staph. aureus	5	27.78	4	20.00
Str. agalactiae + Staph. aureus	5	27.78	5	25.00
Pseud. aeruginosa	1	5.55	-	-
E. coli	1	5.55	1	5.00
C. pyogenes	-	-	1	5.00
Rhizopius nigricans	-	-	1	5.00
Total	18	100.00	20	100.00

TABLE 26

Percentage distribution of isolates from clinically mastitic animals.

Micro-organisms isolated	Buffaloes		Cows	
	No. of cases	%	No. of cases	%
Str. agalactiae	9	22.50	9	40.90
Staph. aureus	7	17.50	4	18.18
C. pyogenes	8	20.00	5	22.73
E. coli	3	7.50	2	9.09
Str. dysagalactiae	2	5.00	-	-
Str. bovis	2	5.00	-	-
Anthracoids	1	2.50	-	-
Pseud. aeruginosa	1	2.50	-	-
Str. agalactiae + E.coli	2	5.00	-	-
Str. agalactiae + Staph aureus.	3	7.50	1	4.55
Staph. aureus + E.coli	2	5.00	-	-
Asp. fumigatus	-	-	1	4.55
Total	40	100.00	22	100.00

Fig. (1)

GOT activity of Buffaloe's and Cow's milk

in Subclinical and Clinical mastitis

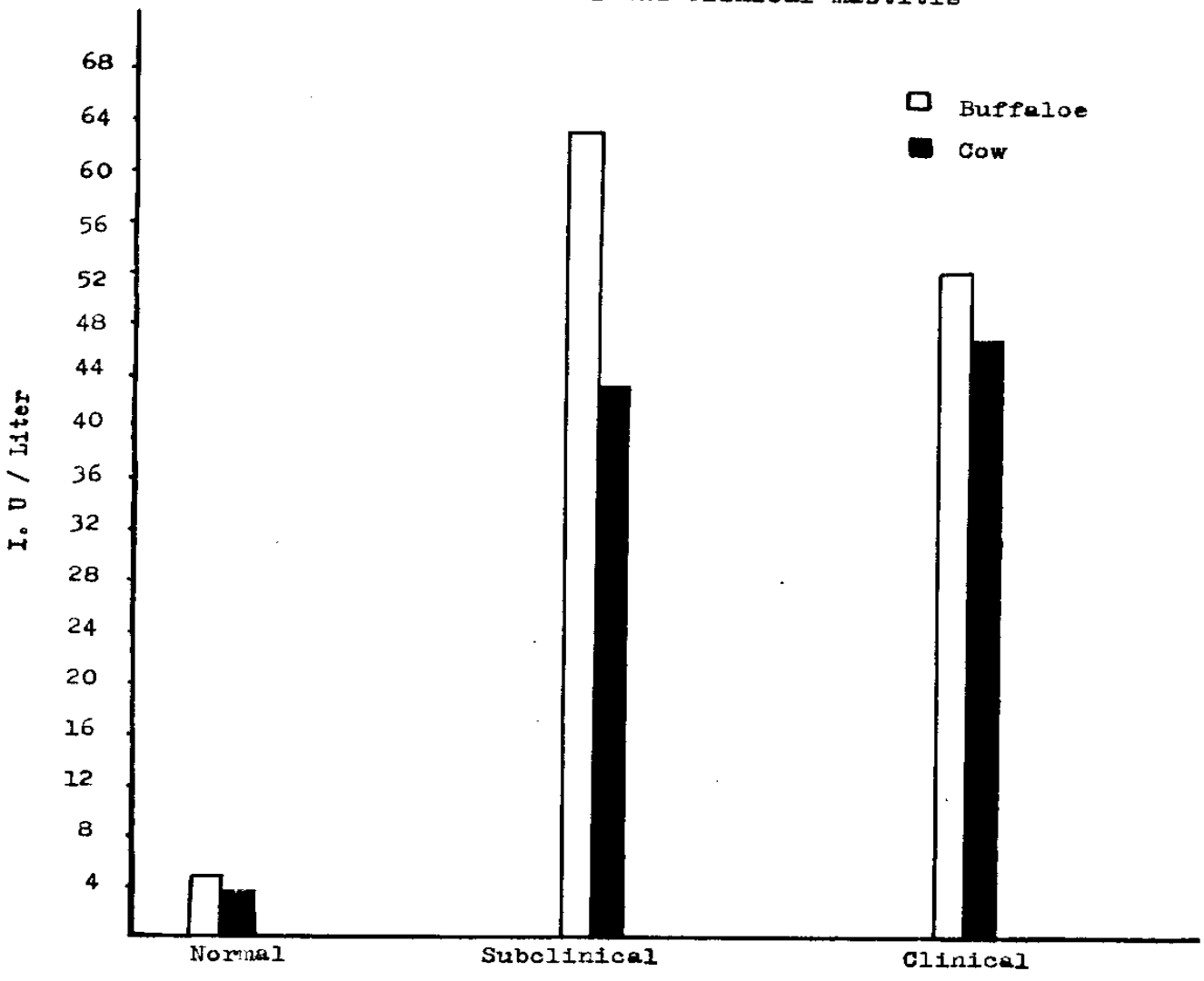


Fig. (2)
activity of Buffaloe's and Cow's milk
in Subclinical and Clinical mastitis

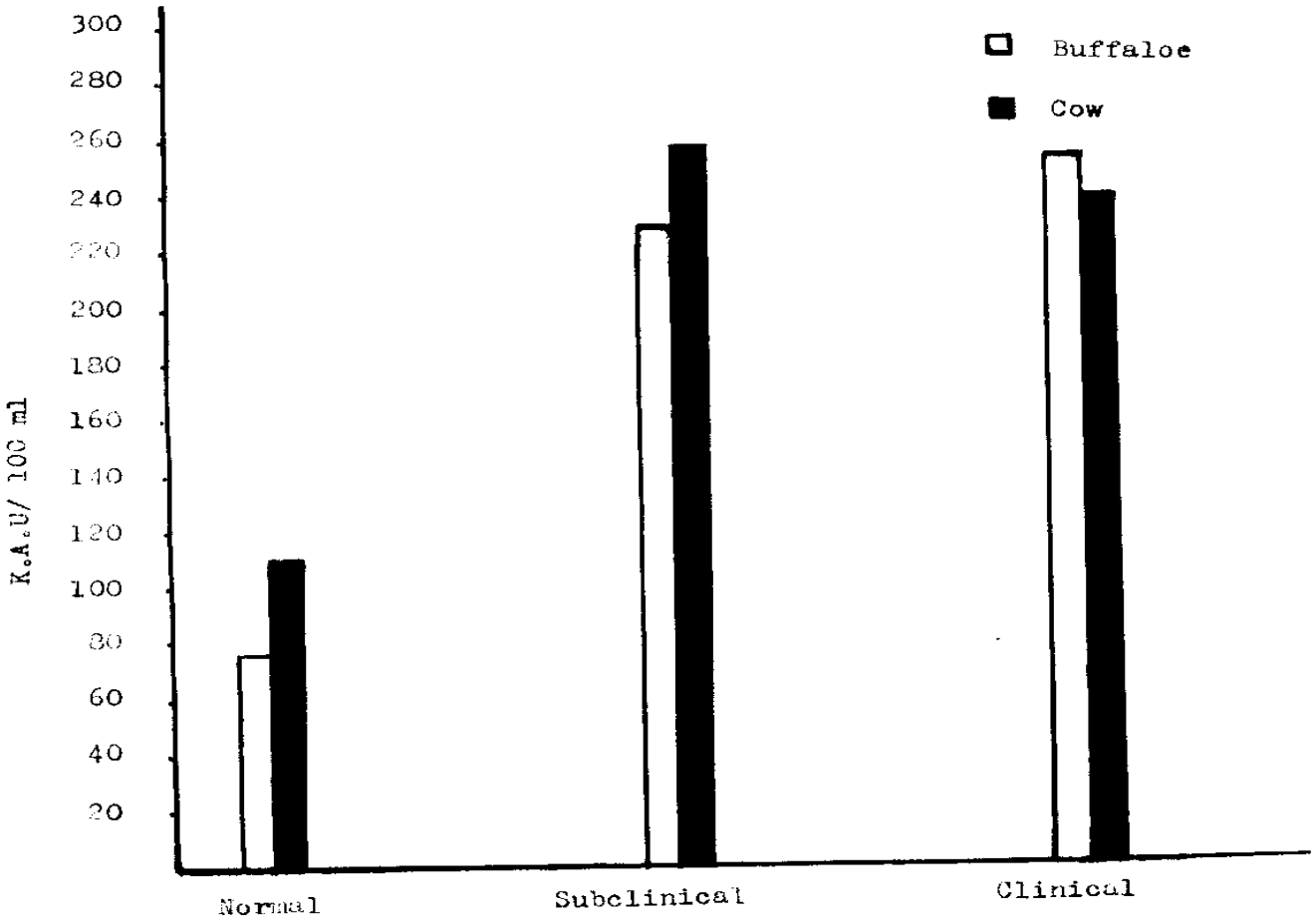


Fig. (3)
activity of Buffaloe's and Cow's milk
in Subclinical and Clinical mastitis

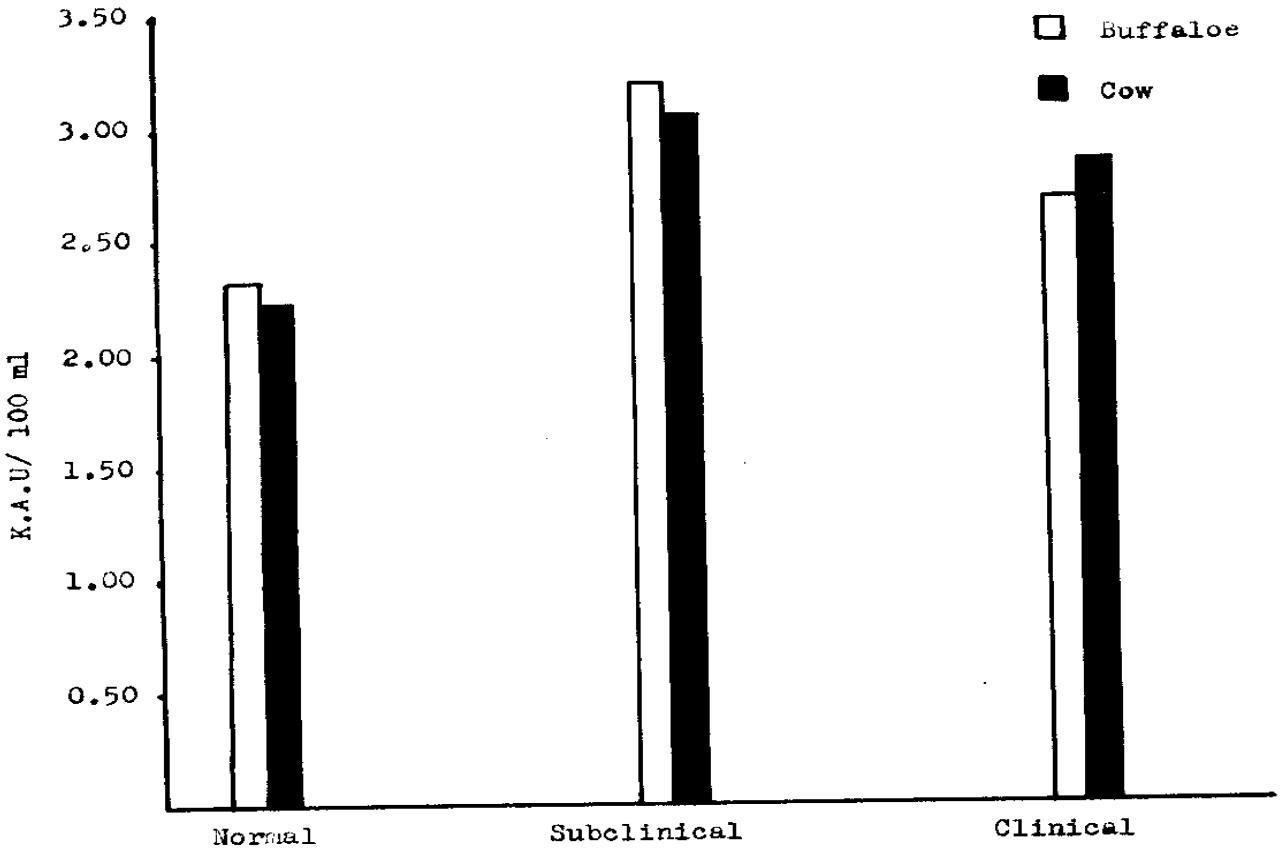


Fig.(4)

5- ND activity of Buffaloe's and Cow's milk in Subclinical and Clinical mastitis

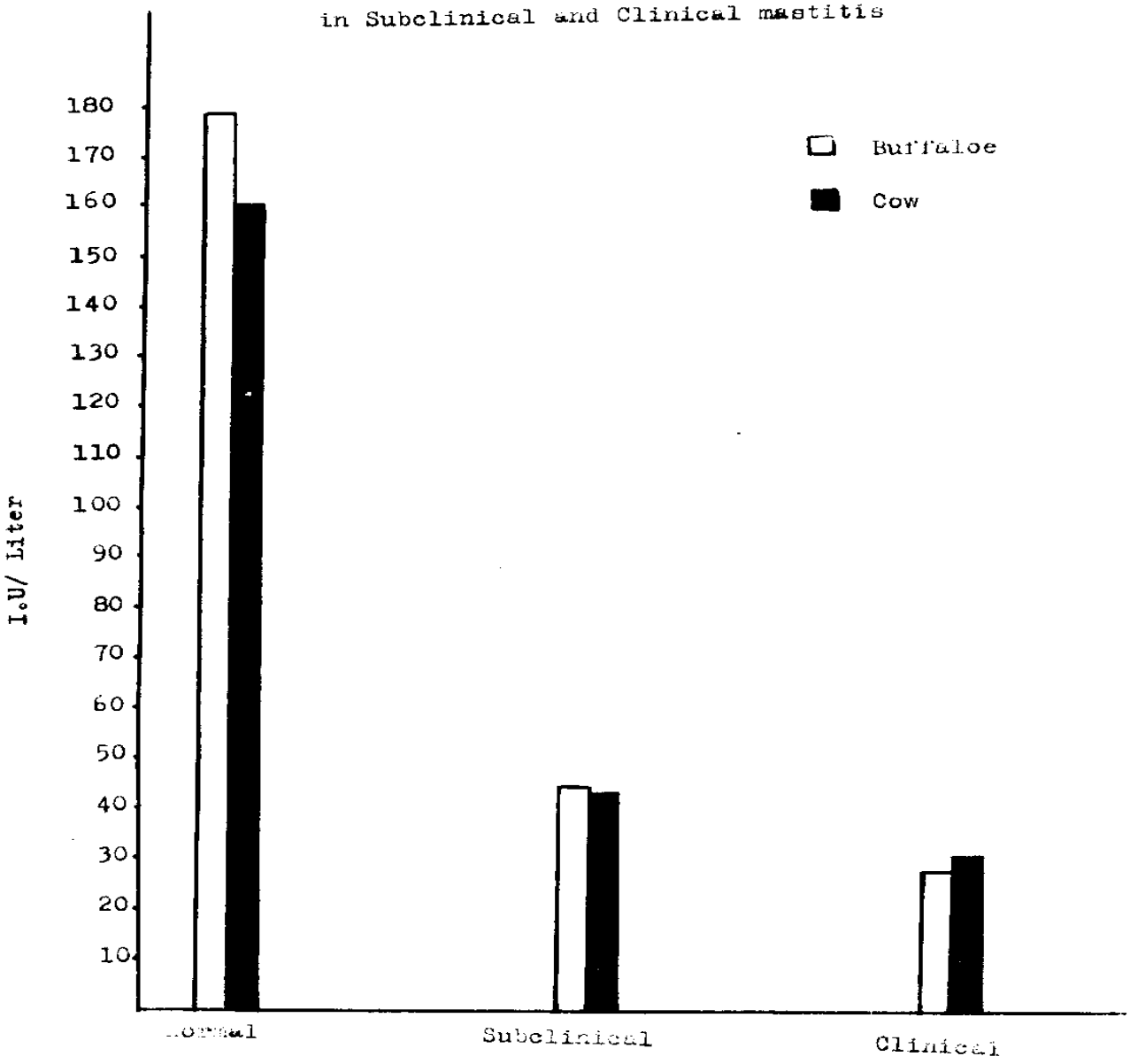


Fig. (5)

...l activity of Buffaloe's and Cow's milk
in Subclinical and Clinical mastitis

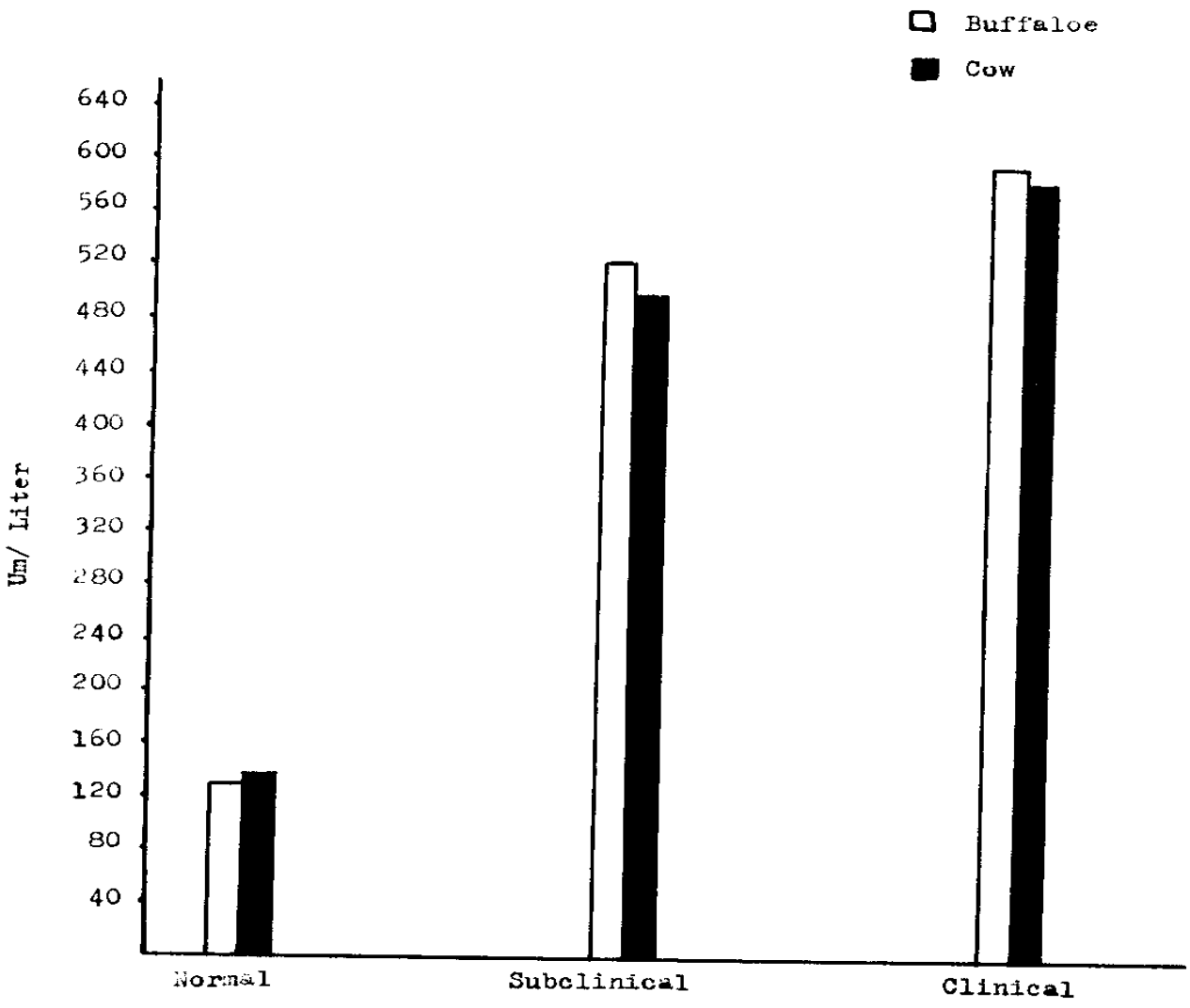


Fig. (6)

Lipase activity of Buffaloe's and Cow's milk

in Subclinical and Clinical mastitis

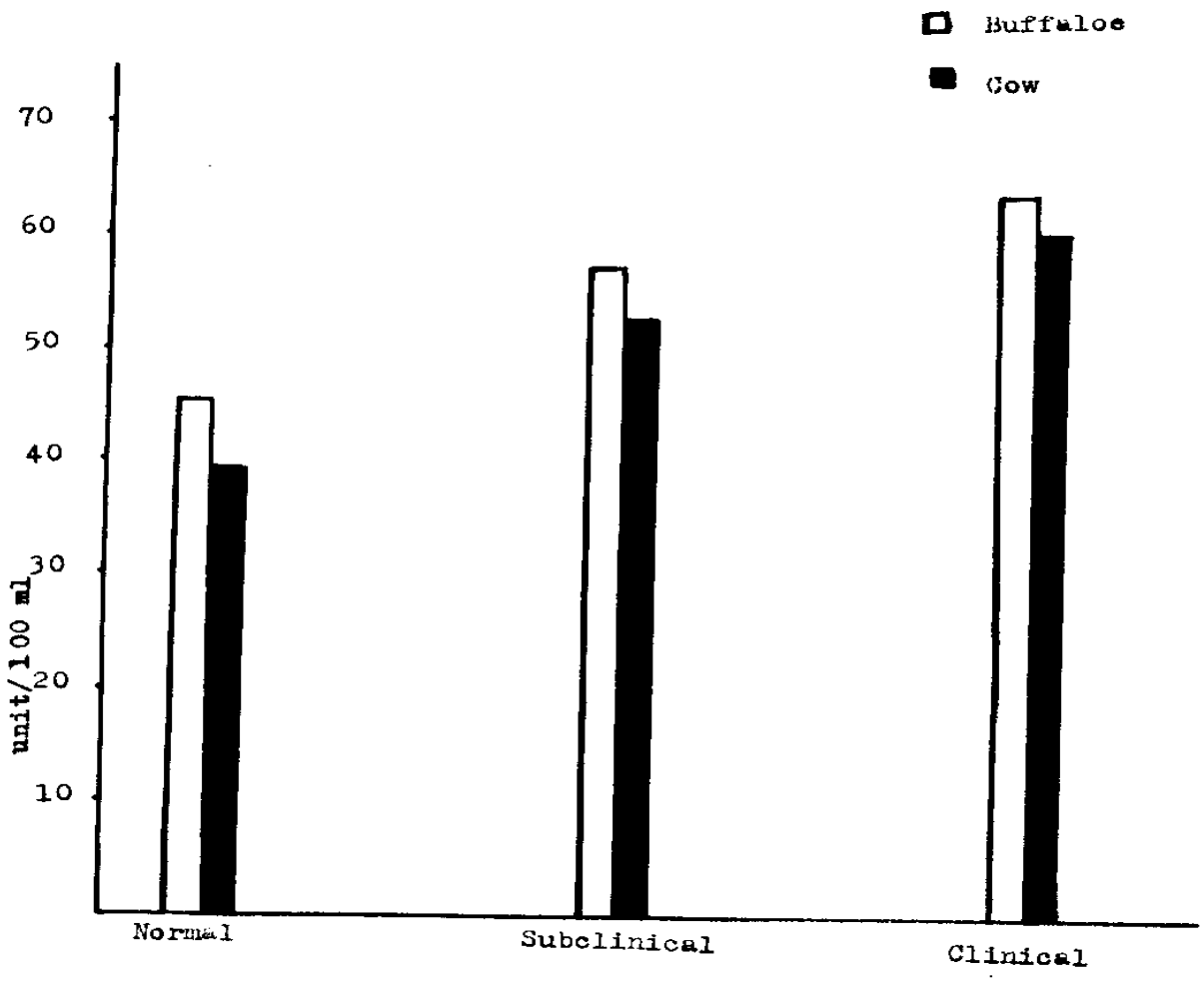


Fig.(7)

Amylase activity of Buffaloe's and Cow's milk in Subclinical and Clinical mastitis

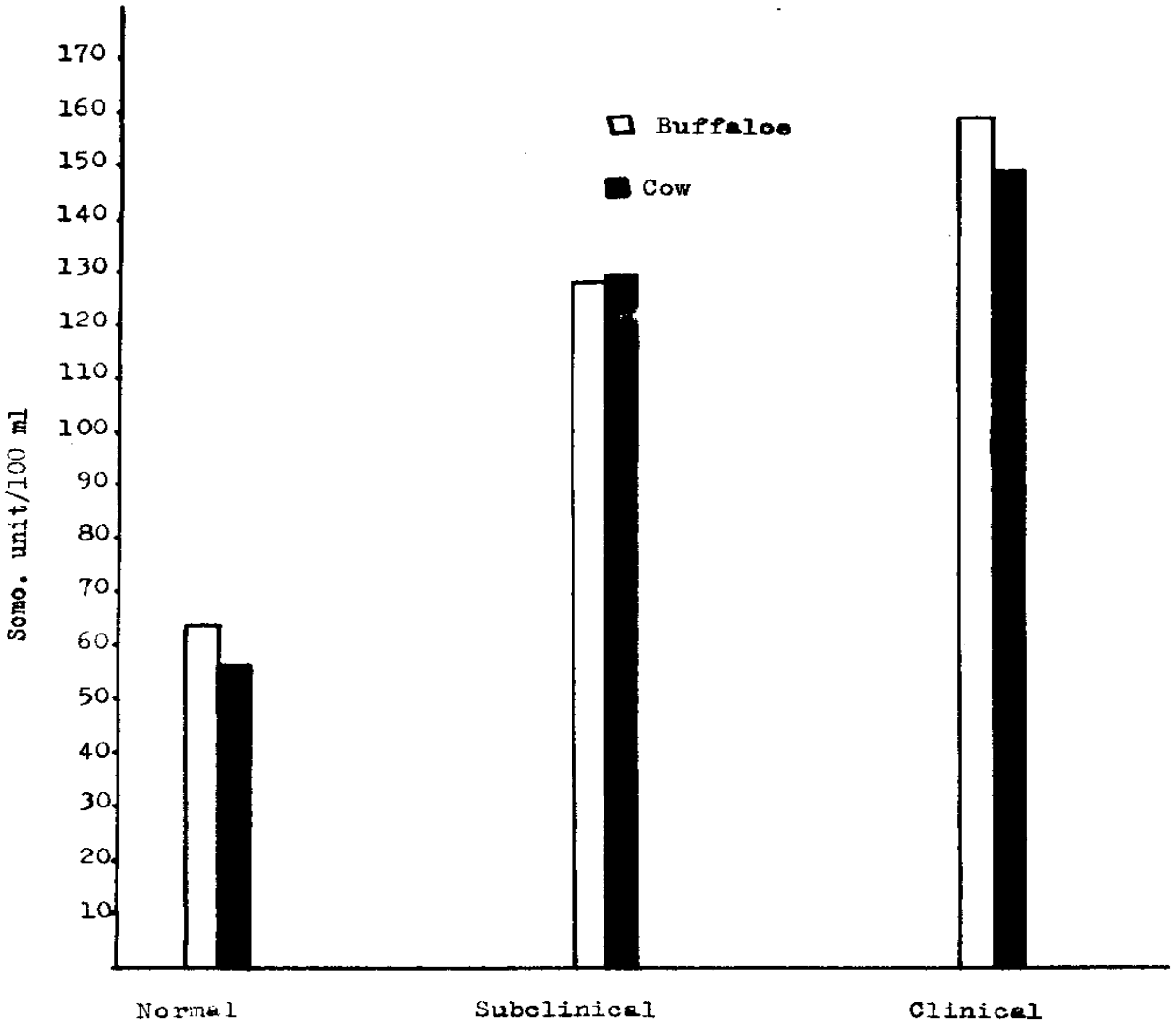


Fig.(8)

Ascorbic acid content of Buffaloe's and Cow's milk in Subclinical and Clinical mastitis

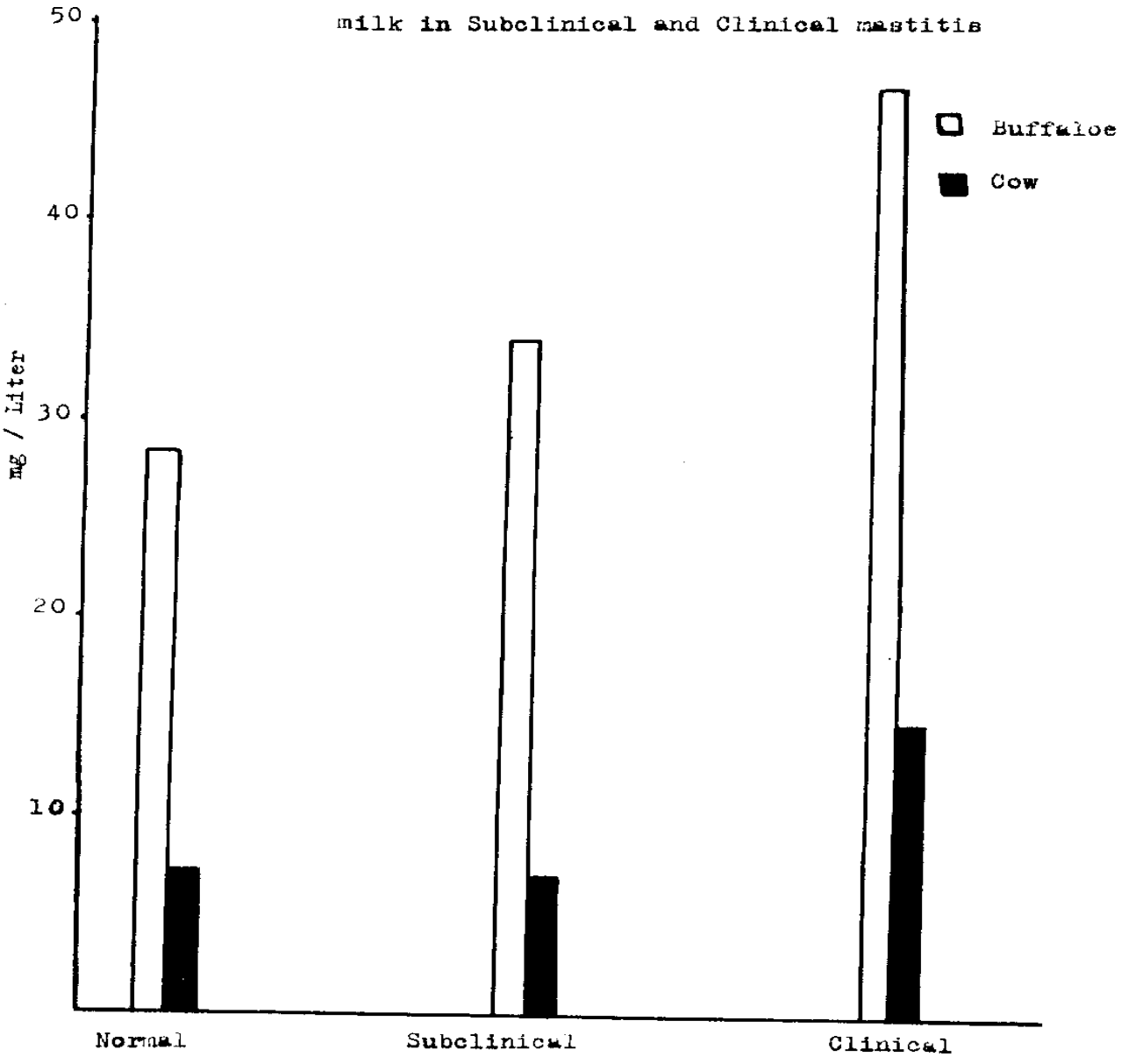
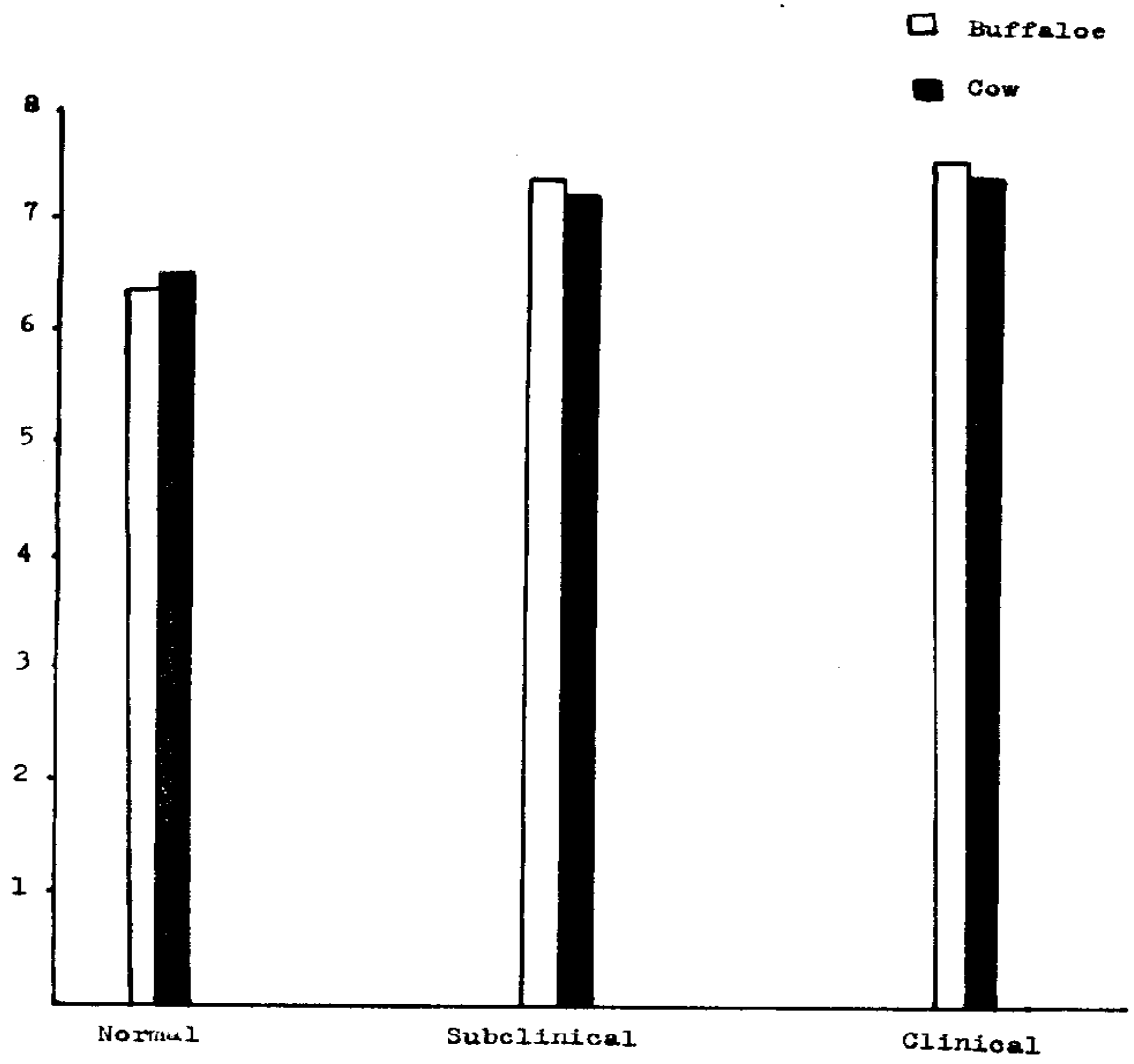


Fig. (9)

PH value of Buffaloe's and Cow's milk

in Subclinical and Clinical mastitis



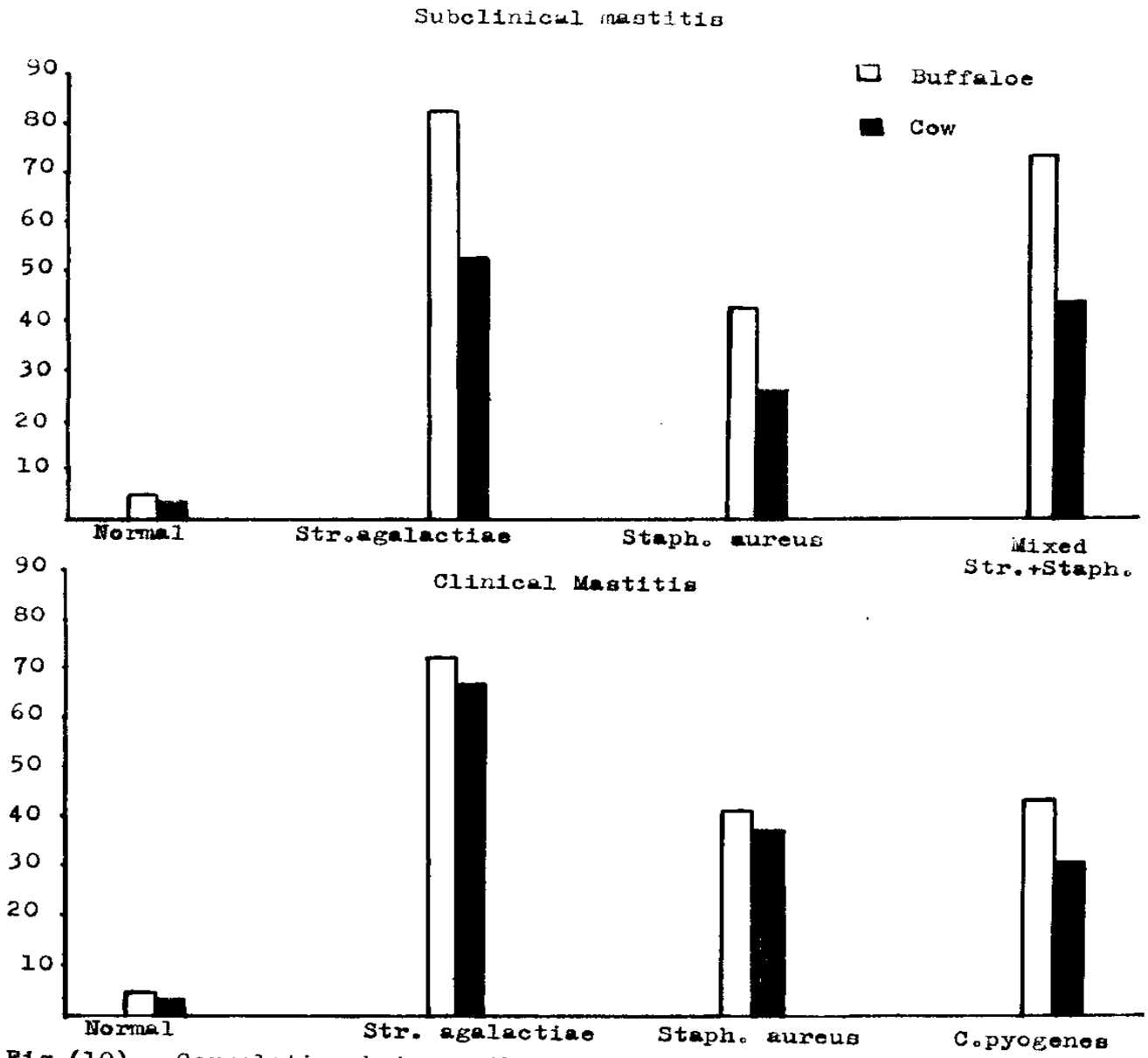
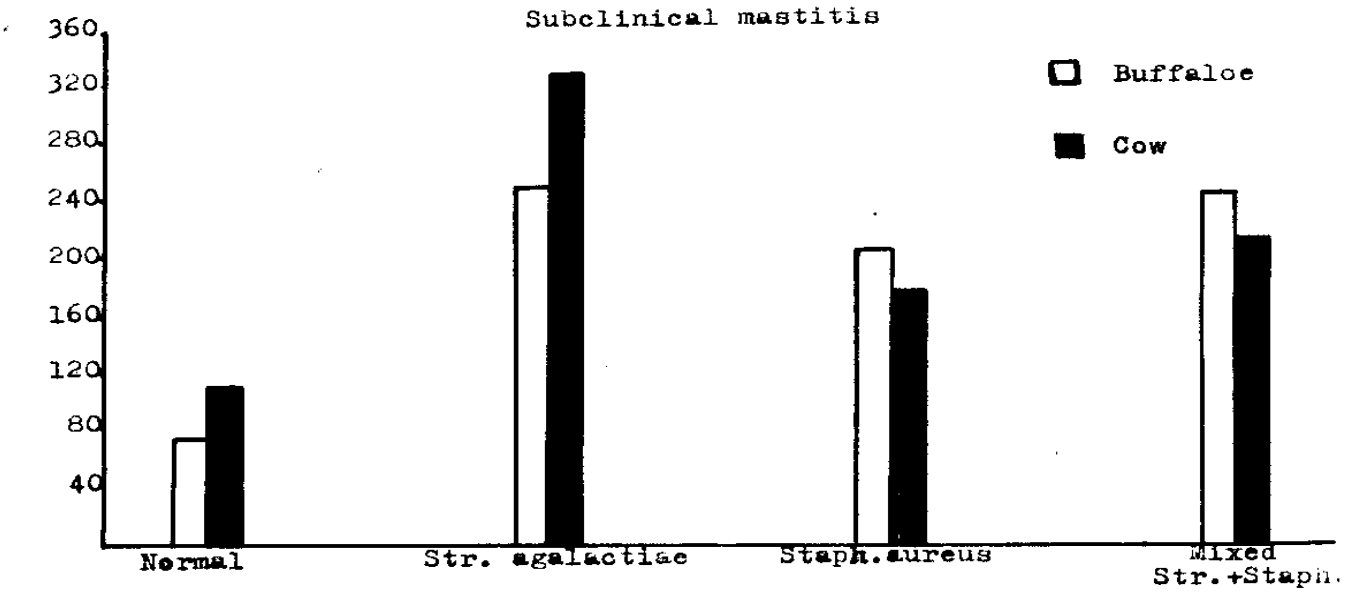


Fig.(10) Correlation between the different isolated organisms and GOT activity of buffaloe's and Cow's milk in Subclinical and Clinical mastitis.



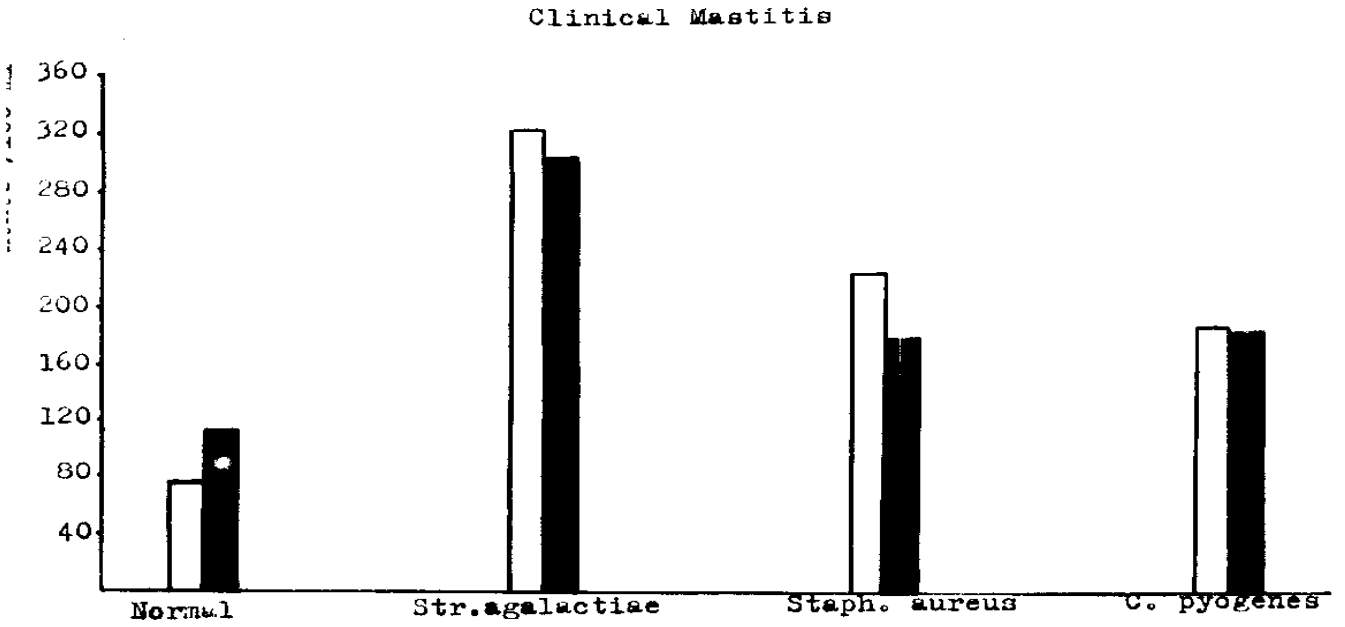
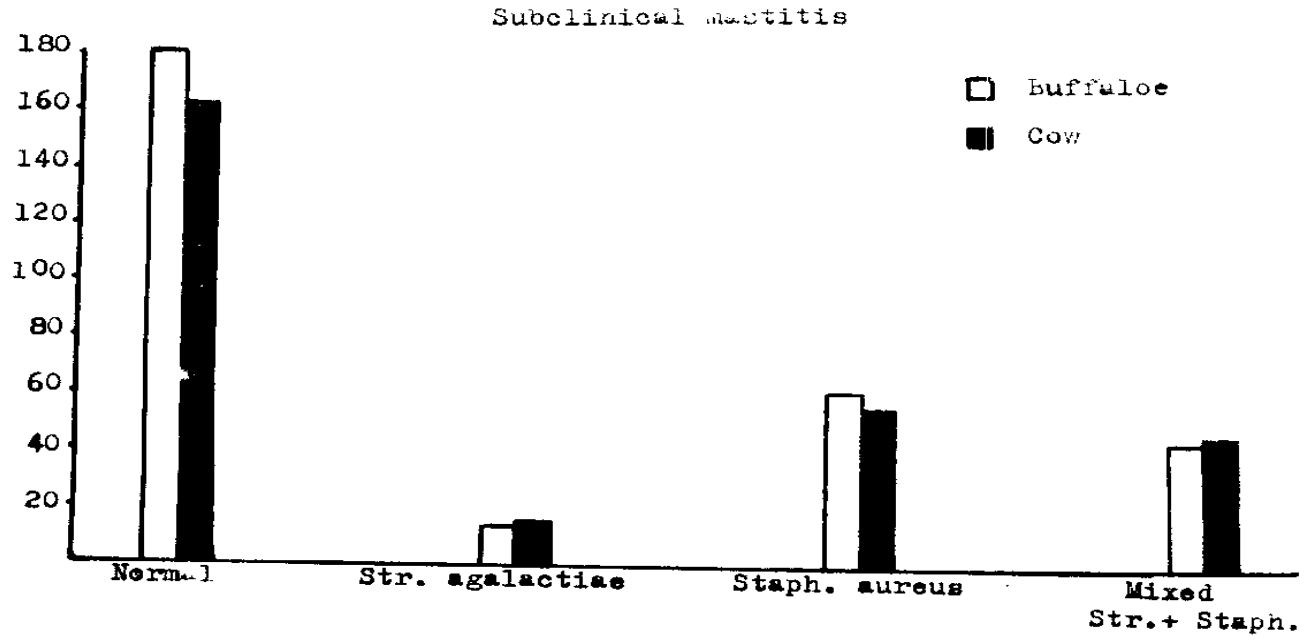


Fig. (11) Correlation between the different isolated organisms and ALP activity of buffaloe's and Cow's milk in Subclinical and Clinical mastitis.



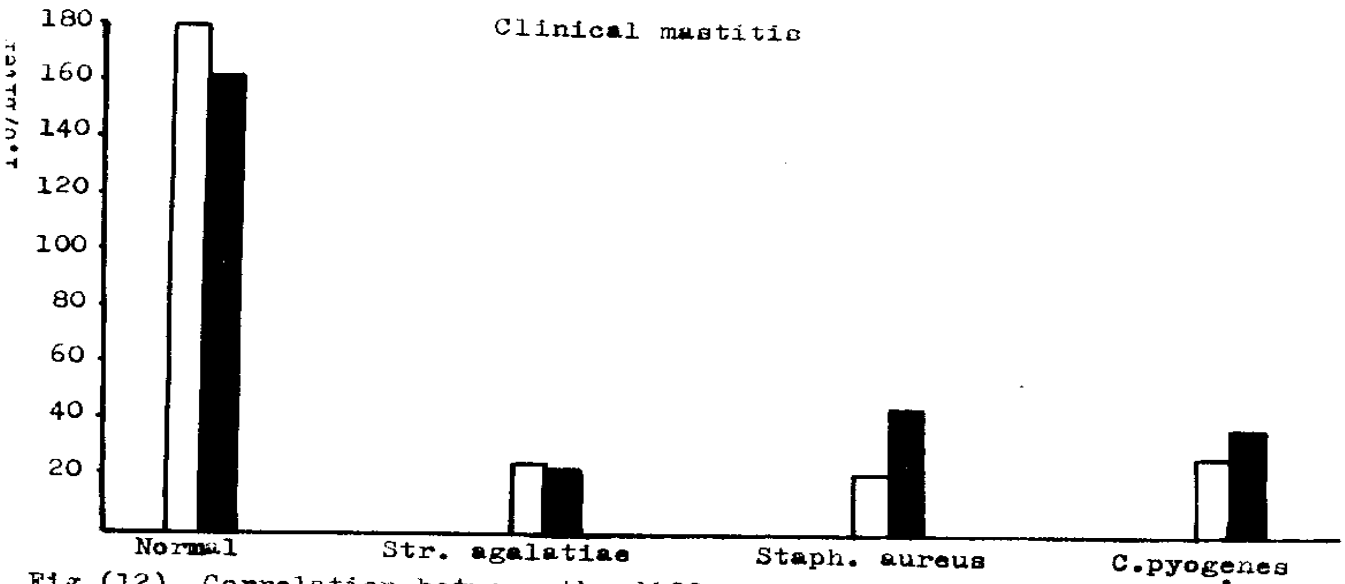


Fig.(12) Correlation between the different isolated organisms and $\frac{\beta}{5}$-ND activity of buffaloe's and Cow's milk in Subclinical and Clinical mastitis.

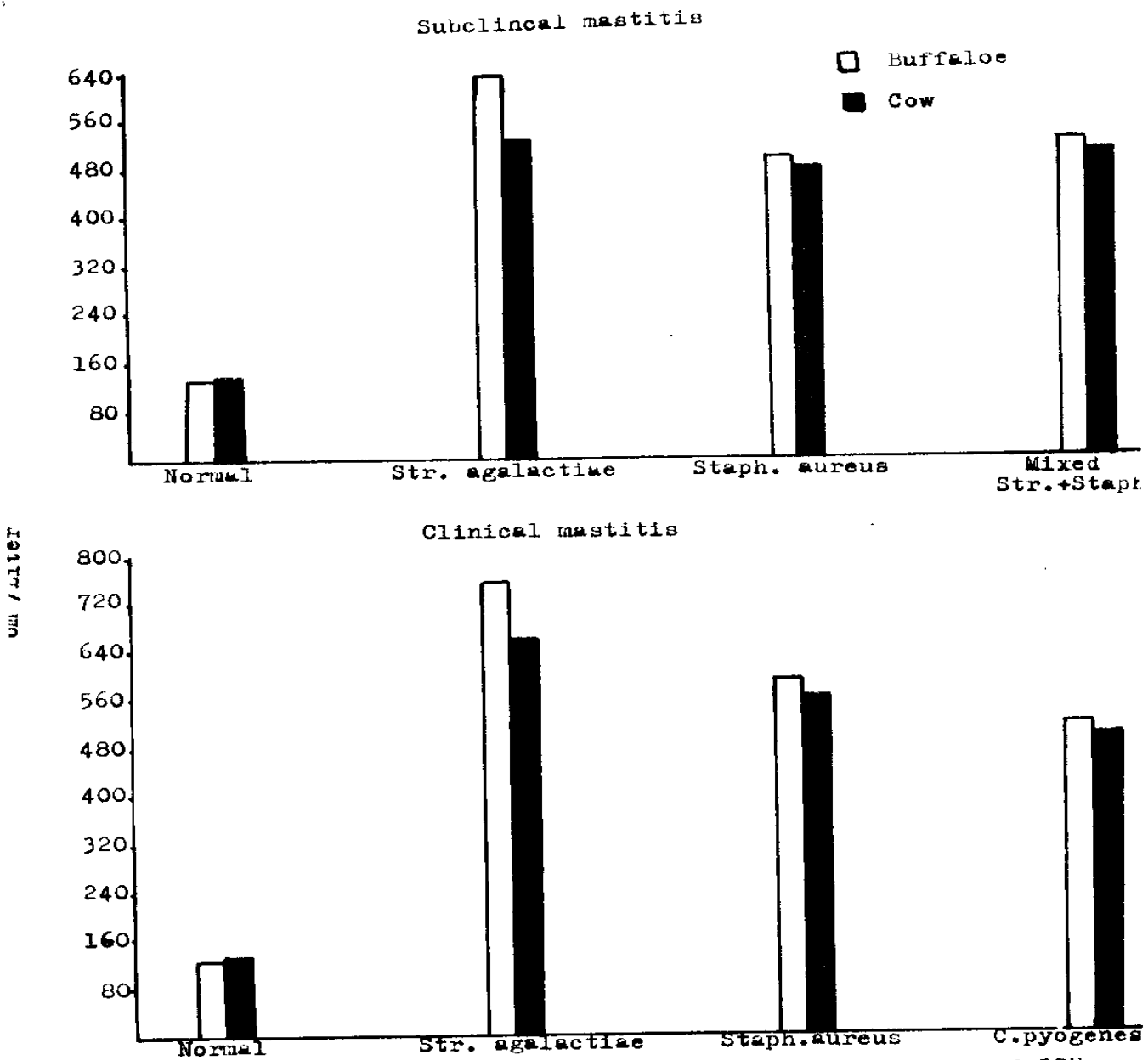


Fig.(13) Correlation between the different isolated organisms and LDH activity of buffaloe's and Cow's milk in Subclinical and Clinical mastitis.

Fig. (14)

Correlation between the different isolated organisms and Amylase activity of buffaloe's and Cow's milk in clinical mastitis

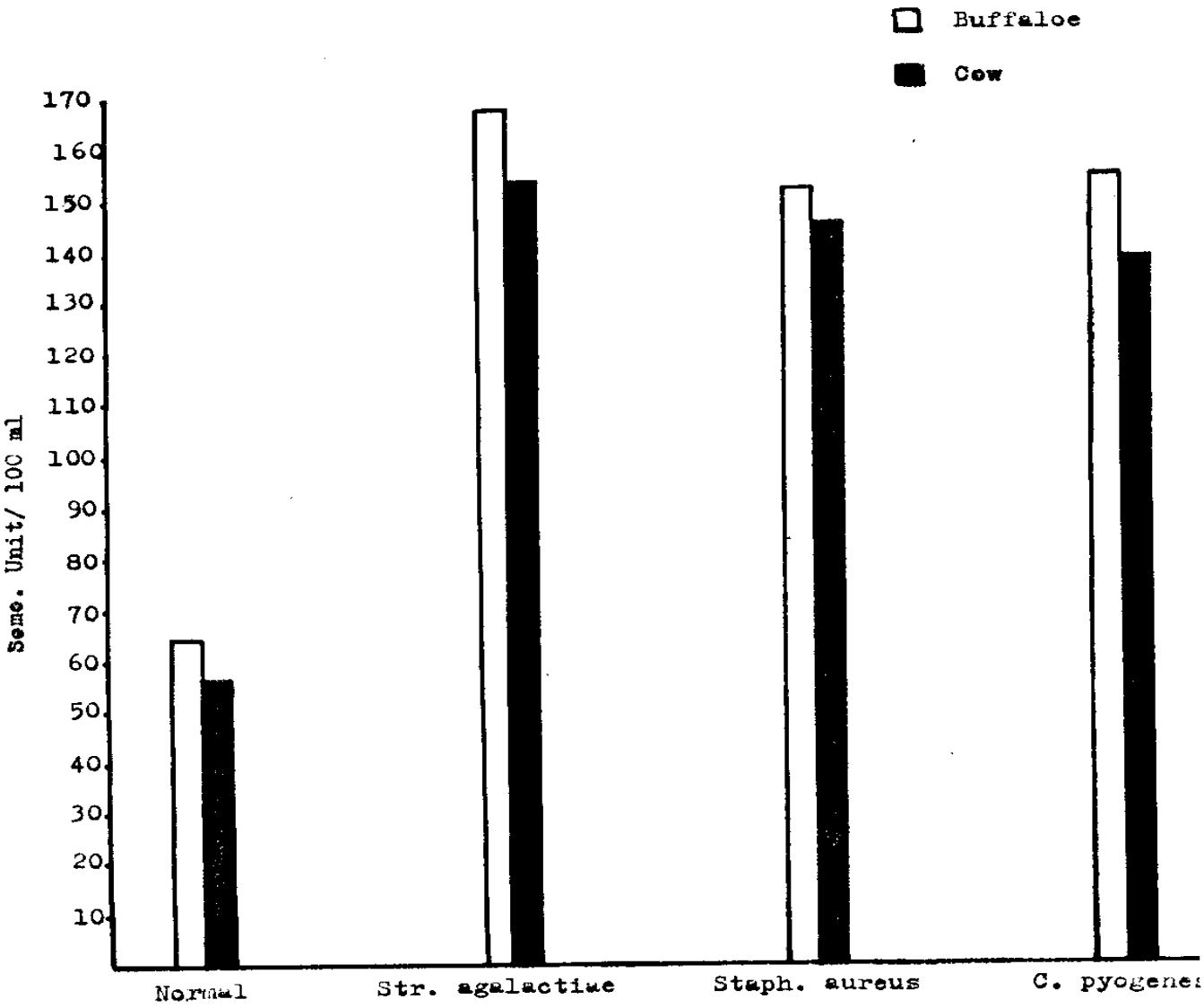
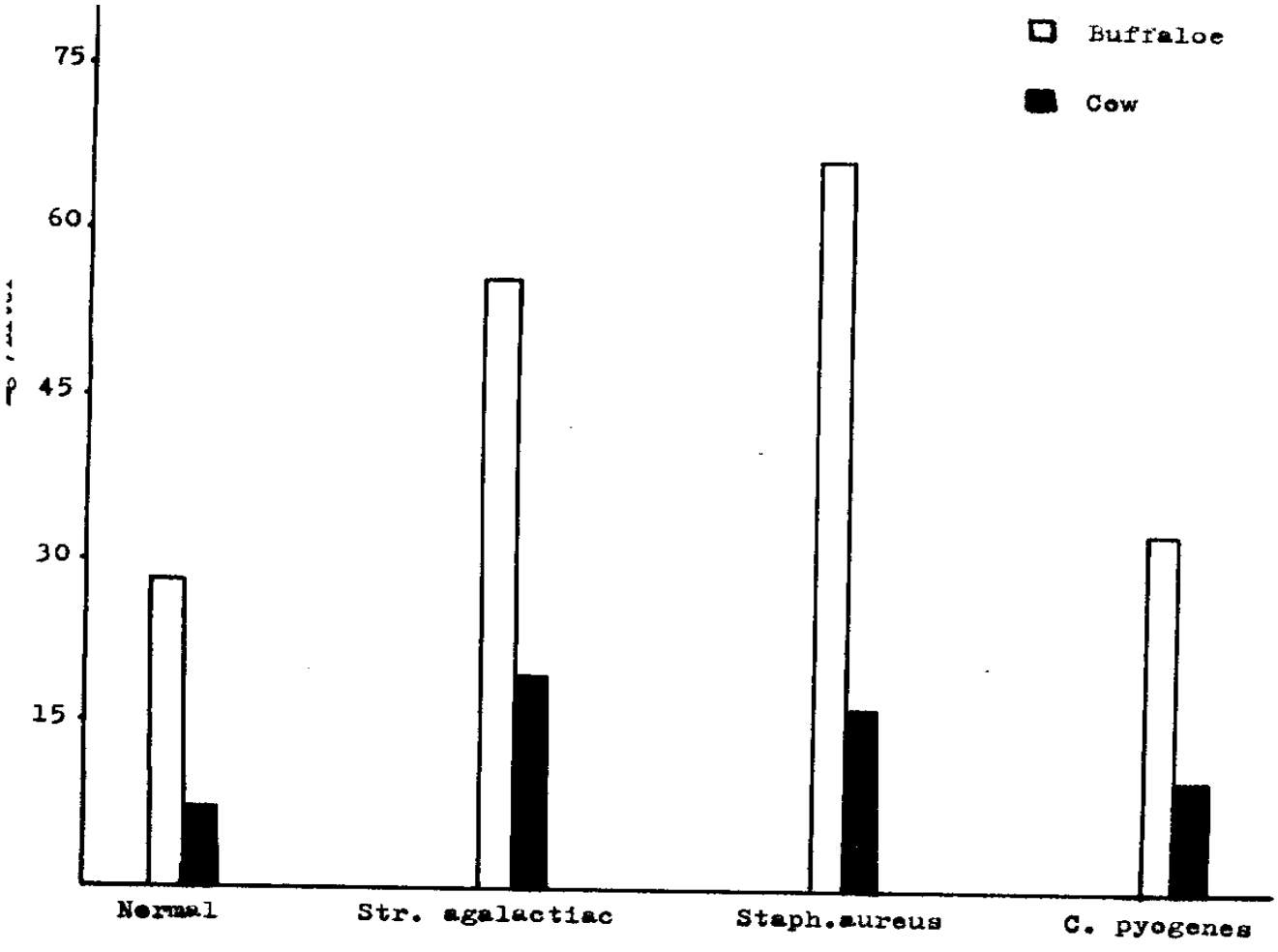


Fig. (15)

Correlation between the different isolated organisms and Ascorbic acid content of buffaloe's and Cow's milk in clinical mastitis



1. Incidence of causative micro-organisms

Mastitis is still one of the most serious diseases of dairy buffaloes and cattle as it reduces the milk yield and shortens the productive period of affected animals.

In this investigation 202 milk samples were collected from apparently normal and clinically mastitic buffaloes and cows (Tables 24-26).

As illustrated in Table (24), it can be noticed that 18 and 50% of milk samples collected from apparently normal udders of buffaloes and cows respectively were bacteriologically positive.

Milk-borne outbreaks have been recorded from time to time, which are usually of an explosive and wide spread nature. Milk may be the vehicle of a wide range of pathogenic bacteria, especially when coming from such unrecognizable apparently normal udders affected with subclinical mastitis.

The bacteriological examination of 100 and 40 buffalo's and cow's milk samples respectively revealed the isolation of the types shown in Table 25.

Str. agalactiae was isolated from 14 samples (73.34%), of which 6 strains were isolated from buffalo's and 8 strains from cow's milk.

on the other hand, Staphylococci were found to be still the organisms most commonly involved in cases of mastitis. Staph. aureus could be isolated in pure culture from 9 samples (47.78%), while mixed with Str.agalacti__ from 10 samples (52.78%).

In addition, sporadic isolates of C.pyogenes, Pseud. aeruginosa, E. coli and Rhizopius nigricans were recovered (Table 25).

From the data recorded in Table 25, it can be easily observed that Str. agalactiae and Staph. aureus were the predominant isolates from subclinically mastitic buffaloes and cows. These results confirm those previously reported by Nasr (1956), Arnaoout (1961) and Zakaria (1969). Moreover, nearly similar findings were reported by Kral et al. (1973) however, these results are considered higher than those found by Chander et al. (1975).

Such variation in the incidence percentage of pathogenic organisms isolated from subclinically mastitic milk may be attributed to the surrounding environmental conditions or hygienic measures adopted in animal habitations.

Table 26 revealed that 40 and 22 mastitic buffalo's and cow's milk samples respectively were investigated bacteriologically. It appears that Str. agalactiae, Staph. aureus and C.pyogenes constituted the predominant causative agent incriminated in clinical mastitis in buffaloes and cattle.

On the other hand, an evidence that E.coli has been implicated as aetiological agent of mastitis was provided in the present investigation by its isolation at an incidence of 7.5 and 9% from buffalo's and cow's milk samples respectively.

Moreover, mixed infections in which Str. agalactiae together with either Staph. aureous or E. coli as well as Staph. aureus with E. coli were also recorded (Table 26).

In addition, other infective agents were also recovered from cases of clinical mastitis inclduing Str.dysagalactiae, Str. bovis, Pseud. aeruginosa, anthracoids and Asp.fumigatus as illustrated in Table 26.

The incidence percentage of isolated micro-organisms from clinically mastitic buffalo's and cow's milk agree with the previous results reported by Khalil et al.(1972 II)El-Nagar, (1973), Jaffery et al. (1975) and Valenti (1976), however, lower incidence percentages were reported by El-Guindy et al. (1964) which might be due to varying environmental and

hygienic conditions.

Heimonas (1961) and Fenizia et al. (1976) identified Asp. fumigatus as a causative agent in mastitis, moreover, Farid, et al. (1975) isolated 5 strains of anthracoids as causative organisms in mastitic milk samples which confirm the results obtained in the present work.

II. Effect of mastitis on some enzymes, Ascorbic acid and pH value of buffalo's and cow's milk:

(1) Effect of mastitis on Glutamic oxalacetic transaminase (GOT)

Cantarow (1962) stated that liver and heart muscle are particularly rich in GOT activity which enter the blood as a result of tissue destruction, whereas Frahm et al.(1977) cited that GOT have no organ specificity.

In the present work, concerning the mean values of GOT activity in normal buffalo's milk as well as cow's milk are listed in Tables 7 and 8, which shows it to be 4.80 and 3.65 IU/liter of milk respectively.

Statistical analysis of results (Table 9) shows that GOT activity of buffalo's milk is significantly higher ($P < 0.05$) than that of cow's milk. Tables 10 & 11 show the mean GOT activity of milk obtained from mastitic buffaloes(subclinical

and clinical cases) as 66.33 and 52.30 IU/liter of milk respectively. In cow's milk the mean GOT activity in both mastitic cases amounted to 43.45 and 47.66 IU/liter of milk (Tables 13 and 14). Statistical analysis of results (Tables 12 & 15) show that GOT activity increases significantly ($P < 0.01$) in milk obtained from subclinical and clinical mastitic animals as compared with that of normal animals. Whreas no significant difference was noticed between values obtained from subclinical and clinical mastitic cases in both animals. In both cases of subclinical and clinical mastitic animals (Buffaloes and cows) a large variety of micro-organisms were isolated and cases were classified into 3 main categories according to the isolated micro-organism. Bacteriological examination of subclinical mastitic cases showed that the infection was either by Str.agalactiae alone or Staph. aureus alone or by both micro-organisms while in clinical mastitic cases, the infection was either by Str. agalactiae or by Staph. aureus or by C.pyogenes.

The levels of GOT activity was found to range widely according to the causative organism.

GOT levels of milk obtained from subclinical mastitic buffaloes (Table 16) ranged between 82.33 and 42.30 IU/liter milk. The highest value was shown in cases associated with Str.agalactiae, while the least value was shown in cases associated

with Staph. aureus only. Infection with both organisms showed an intermediate value 73.00 IU/liter.

It is quite clear from Table 17 that there is a significant difference between values obtained in cases of Str. agalactiae infection and Staph.aureus infection and also between Staph.aureus infected cases and mixed infection of Str. agalactiae + Staph.aureus ($P < 0.01$). On the other hand, GOT levels of milk obtained from clinical mastitic buffaloes (Table 18) ranged between 72.78 & 41.75 IU/liter. The highest value was obtained in cases associated with Str. agalactiae while the least values was shown in cases associated with Staph. aureus. Infection with C.pyogenes showed a mean value of 43.75 IU/liter. Statistical analysis of results (Table 19) reveals a significant difference ($P < 0.01$) between cases infected with Str. agalactiae and cases infected with either Staph. aureus or C.pyogenes.

In cows the GOT activity of milk obtained from subclinical mastitic animals (Table 20), ranged between 25.88-53.19 IU/liter. The highest value was shown in cases associated with Str. agalactiae, while the least value was shown in cases associated with mixed infection by Str. agalactiae and Staph. aureus. Infection with Staph. aureus alone showed an intermediate value of 44.00, such a result differs from that obtained in Buffaloes.

Statistical analysis shown in Table 21 reveals a significant difference between values obtained in cases of Str.agalactiae infection and Staph.aureus infection ($P < 0.01$) and also between Staph.aureus infected cases and mixed infected cases ($P < 0.05$).

On the other hand GOT levels of milk obtained from clinical mastitic cows (Table 22) ranged between 31.20-67.67. The highest value was shown in cases associated with Str.agalactiae while the least value was shown in cases associated with C. pyogenes.

Infection with Staph.aureus showed an intermediate value of 38 IU/liter. Statistical analysis (Table 23) reveale a significant difference between values obtained in cases infected with Str.agalactiae and cases infected with Staph. aureus ($P < 0.01$) and between cases infected with Str. agalactiae and C.pyogenes ($P < 0.01$).

The results of this study have shown that GOT activity of normal buffalo's milk was slightly higher than normal cow's milk and that both values were increased signficantly following subclinical and clinical mastitis whereas no significant change was observed between subclinical and clinical forms.It is also evident from this study that the rise effect on GOT

activity in both buffalo's and cow's milk obtained from subclinical cases of mastitis can be arranged according to the causative organisms in the following order Str.agalactiae > mixed infection of Str. agalactiae + Staph. aureus > Staph. aureus, whereas in clinical cases of mastitis in both animals, Str. agalactiae > C·pyogenes > Staph.aureus.

Kaker et al. (1966) reported that serum transaminases activity (GOT and GPT) were higher in buffaloes than cattle.

Bogin et al. (1976) found that GOT activity were nearly the same in tissue slices taken from apparently normal udder, udder tissue with acute mastitis and udder tissue characterized by extensive fibrosis. Our present data are in close agreement with those studied by Bogin&Ziv (1973), who stated that the mean GOT activity of normal Friesian cow's milk amounted to 4 ± 3 IU/liter of milk and increased after intramammary infusion with Escherichia coli endotoxin to reach a maximum level after 8 hours. This previous finding in combination with our present results can be considered as a diagnostic tool for early recognition of subclinical mastitis in buffaloes and cows. Bogin et al. (1973) attributed the increase in GOT activity of cow's milk obtained from mastitic animals to the disintegrating leukocytes or to the cells composing the udder parenchyma or from both.

Whereas Schalm et al. (1967) and Carroll et al. (1964) cited that intramammary infusion with bacterial endotoxin have a profound effect on vascular permeability and this altered permeability leads to an intense local edema characterized by maximum swelling of the gland and by transudation of serum components into milk.

(2) Effect of Mastitis on Glutamic pyruvic transaminase(GPT)

The results obtained from this study shows clearly that the milk from normal buffaloes or cows as well as milk obtained from subclinically mastitic animals is deficient in GPT, whereas milk obtained from clinically mastitic animals contained GPT activity. It is evident from table 11 & 14 that the GPT activity in mastitic buffalo's milk (1.40 IU/liter of milk) is higher than that of mastitic cow's milk (1.15 IU/liter of milk). GPT levels of milk obtained from clinical mastitic buffaloes (Table 18) ranged between 0.93 and 2.81 IU/liter of milk. The highest value was shown in cases associated with Str. agalactiae while the least values was shown in cases associated with Staph. aureus, infection with C. pyogenes showed a mean value of 1.13 IU/liter milk. Statistical analysis of results (Table 19) reveals a non significant difference between cases infected with Str. agalactiae, Staph. aureus or C. pyogenes.

In cows the GPT activity of milk obtained from clinical mastitic cases (Table 22) ranged between 1.67 and 0.63 IU/ liter milk. The highest value was shown in cases associated with Str. agalactiae while, the least values was shown in cases associated with Staph.aureus. Infection with C.pyogenes showed an intermediate value of 1.21 IU/liter milk. Statistical analysis of results (Table 23) revealed a non significant difference between values obtained in cases infected with Str. agalactiae, Staph. aureus and C. pyogenes. The available literature seems to be deficient in data concerning Glutamic pyruvic transaminase (GPT) activity in normal and mastitic milk. However, Bogin et al. (1976) recorded that the mean GPT activity in udder tissue characterized by acute mastitis was 50% lower than in the normal udder tissue. From the clinical picture and biochemical analysis done in this present thesis, it is probable that, the blood serum and parenchyma cells of the udder are the main sources of GPT in milk obtained from clinically mastitic buffaloes and cows.

The last fact can be used as a proper tool for diagnosis of clinical cases of mastitis in both animals.

(3) Effect of Mastitis on Alkaline Phosphatase

The data presented in Tables 7 and 8 show the mean values of alkaline phosphatase activity in normal buffalo's as well

as cow's milk which appears to be 76.09 and 111.35 K.A.U/100 ml milk respectively. Statistical analysis (Table 9) shows clearly that the alkaline phosphatase activity of cow's milk is significantly higher than that of Buffalo's milk. This result is in a good agreement with the previous work cited by Safwat et al. (1956) but contradictory to the work of Rifaat et al. (1969) who found that buffalo's milk has higher alkaline phosphatase activity than cow's milk. However, Haab (1958) and Rifaat et al. (1969) stated that the levels of alkaline phosphahtase activity in normal cow's and Buffalo's milk exhibited a wide range. The first authour cited a range in between 23.8-877.8 for normal cow's milk and the latter authour stated a range of 14-42 K.A.U./100 ml for normal cow's milk and a range of 24-3900 K.A.U/100 ml milk for normal buffalo's milk.

In this present work, Tables 10 & 11 show the mean values of alkaline phosphatase activity of milk obtained from mastitic buffaloes (subclinical and clinical cases) as 228.68 and 238.18 K.A.U/100 ml respectively. In mastitic cow's milk the mean alkaline phosphatase activity amounted to 256.75 and 249.97 K.A.U/100 ml of milk. (Table 13 and 14).

Statistical analysis, (Tables 12 & 15) show that alkaline phosphatase activity increases significantly ($P < 0.01$) in milk obtained from subclinical and clinical mastitic animals

(buffaloes and cows) as compared with that of normal animals, whereas no significant difference was noticed between values obtained from subclinical and clinical mastitic cases in both animals. In buffaloes with subclinical mastitis, the effect of the causative micro-organisms on alkaline phosphatase is not clear. The mean alkaline phosphatase activity differs only in a narrow range (207.8-251.08) as shown in Table 16.

Statistical analysis of the results obtained (Table 17) showed no significant difference between the values obtained in each of the 3 micro-organisms infection cases.

However, the effect of the causative micro-organisms manifested itself quite clear in cases of clinical mastitic buffaloes. The alkaline phosphatase activity of milk ranged between 187.06-323.03 as shown in Table 18, the highest value was shown in cases associated with Str. agalactiae infection,while, the least value was shown in cases associated with Staph. aureus infection.

Infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value (223.86).

Statistical analysis of the results obtained (Table 19) showed that there is a significant difference between values obtained in cases of Str. agalactiae infection and Staph.

aureus infection ($P < 0.05$) and also between Staph. aureus and C. pyogenes. In the same time there is a significant difference between values obtained from Str. agalactiae and C. pyogenes infected cases ($P < 0.01$).

In cows the alkaline phosphatase activity of milk obtained from subclinical mastitic animals (Table 20), ranged between 177.19 and 330.59 K.A.U/100 ml milk. The highest value was shown in cases associated with Str. agalactiae, while the least value was shown in cases associated with Staph. aureus. Infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value of 216.20 K.A.U/100 ml milk. Statistical analysis of the results obtained (Table 21) showed that there is a significant difference between values obtained in cases of Str. agalactiae infection and Staph.aureus infected cases ($P < 0.01$), and also between Str. agalactiae infected cases and mixed Str. agalactiae + Staph. aureus infected cases ($P < 0.01$). On the other hand, the ALP activity of milk obtained from clinically mastitic cows ranged between 182.13-303.59 K.A.U/100 ml milk as shown in Table 22. The highest value was shown in cases associated with Str. agalactiae, while the least value was shown in cases associated with Staph.aureus,whereas infection with C. pyogenes showed an intermediate value of 185-67 K.A.U/100 ml milk.

Statistical analysis of the results obtained (Table 23) showed that there is a significant difference between values obtained in cases of Str. agalactiae infection and Staph. aureus infected cases ($P < 0.01$), and also between Str. agalactiae infected cases and C. pyogenes infected cases ($P < 0.01$).

The present results agree well with that of Arima (1962), Bozhkova and Tsvekov (1976) and Anderson (1977). Kalsall et al. (1959) who offered a good explanation for the increased alkaline phosphatase activity in mastitic milk. They stated that alkaline phosphatase activity seemes to be closely related to the DNA content of the gland and it is believed that the enzyme is found whenever nucleic acid occurs. Also, Bogin and Ziv(1973) added that, leukocytosis and tissue regeneration processes are rich sources of additional DNA in the udder during mastitis which can perhaps explain the sustained high levels of alkaline phosphatase activity. However, the present results shows that alkaline phosphatase activity of some normal milk samples exceeds that obtained from subclinically or clinically mastitic animals. Therefore, the accurate diagnosis of mastitis should not depend completely on measurements of alkaline phosphatase activity, but generally it can be considered as an additive means for quick laboratory diagnosis. It is evident from this

st udy that the rise effect on alkaline phosphatase in both buffalo's and cow's milk obtained from subclinical cases of mastitis can be arranged according to the causative organisms in the following order Str. agalactiae > mixed infection by Str. agalactiae + Staph. aureus > Staph. aureus, whereas in clinical cases of mastitis in both animals, Str. agalactiae > C. pyogenes > Staph. aureus.

(4) Effect of Mastitis on Acid Phosphatase

The data presented in Tables 7 and 8 show the mean values of acid phosphatase activity in normal buffalo's and cow's milk as 2.37 and 2.23 K.A.U/100 ml milk respectively.

Statistical analysis (Table 9) show that milk from normal buffaloes and cows have approximately similar activity of acid phosphatase.

The present results are contradictory to the results obtained by Rifaat et al. (1969) as they found that the acid phosphatase activity of cow's milk was slightly higher than that of buffaloes.

Tables 10,11,13 and 14 show the mean acid phosphatase activity of milk obtained from mastitic buffaloes (subclinical and clinical cases) as 3.19 and 2.64 K.A.U/100 ml of milk for buffaloes and 3.05 and 2.83 for cows. Statistical analysis of

results of buffalo's milk (Table 12 and 15) show that acid phosphatase activity of milk obtained from subclinical mastitic buffaloes increases significantly ($P < 0.05$) as compared with normal milk. However, in both animals no significant difference was noticed between values obtained from subclinical and clinical cases of mastitis.

These results coinicde with the work of Andrews (1976) who reported a rapid and temporary increase of the enzyme in mastitic milk. Acid phosphatase levels of milk obtained from subclinically mastitic buffaloes (Table 16) ranged between 2.68 and 4.09 K.A.U/100 ml. The highest value was shown in cases associated with Str. agalactiae as compared with normal value, while the least value was shown in cases associated with Staph. aureus. However, infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value (3.20 K.A.U/100 ml).

In cases with clinical mastitis, the mean values of acid phosphatase activity in buffalo's milk ranged between 2.15-3.19 (Table 18).

The highest value was shown in cases associated with Str. agalactiae while the least value was shown in cases associated with C. pyogenes, while infection with Staph. aureus showed an intermediate value of 2.64 K.A.U/100 ml). The difference

between the 3 mentioned levels is non-significant (Table 19).

In cows associated with subclinical mastitis, acid phosphatase of milk ranged between 2.68 and 3.64 K.A.U/100 ml,the highest value was shown in cases associated with Str. agalactiae infection as compared with normal values and the least values was shown in cases associated with Staph. aureus.infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value 3.18 (Table 20). On the other hand, acid phosphatase activity of milk obtained from clinical mastitic cows ranged between 2.04-3.32 K.A.U/100 ml).

The highest value was shown in cases associated with Str. agalactiae while the least value was shown in cases associated with Staph. aureus, whereas, infection with C. pyogenes showed an intermediate range (2.46 K.A.U/100 ml), Table 22.

Concerning the effect of the causative organism, it is clear from the results obtained that Str. agalactiae was the sole organism responsible for the elevation of enzyme activity in subclinical mastitic cases.

This fact coincide with that reported by Mullen (1950)who stated that Str. agalactiae was capable of hydrolyzing phenyl phosphate at pH 4.1. Consequently, determination of acid phosphatase activity of milk can serve as an accurate reproducible

method for rapid and sensitive identification of Str. agalactiae as a causative organism in subclinical mastitis.

(5) Effect of mastitis on 5'-Nucleotidase (5'-ND)

The data presented in Tables 7 & 8 show the mean values of 5'-ND activity in normal buffalo's and cow's milk which appears to be 179.41 and 161.16 IU/liter of milk respectively. Statistical analysis (Table 9) shows clearly that the 5'-ND. Activity of normal buffalo's milk was approximately similar to those reported for cow's milk. No data in the literature had been met with concerning 5'-ND activity in normal as well as mastitic buffalo's and cow's milk.

Tables 10 & 11 show the mean values of 5'-ND activity of milk obtained from mastitic buffaloes (subclinical and clinical cases) as 44.83 and 28.80 IU/liter of milk respectively.

In cow's milk the mean 5'-ND activity amounted to 43.93 and 31.23 IU/liter of milk (Table 13 and 14).

Statistical analysis (Tables 12 and 15) show that 5'-ND activity decreases significantly ($P < 0.01$) in milk obtained from subclinical and clinical mastitic animals (buffaloes and cows) as compared with that of normal animals, whereas no significant difference was noticed between values obtained from

subclinical and clinical mastitic cases in both animals.

Schlotk● (1976) working on myoepithelial tissue surrunding the alveoli, and Baumer and Kennan (1975) stated that 5'-ND enzyme is mostly synthesized in the plasma membranes of mammary tissue.

The decrease in 5'-ND activity in matitic milk samples may be attributed to the destructive effect of causative organisms on the alveolar cells and surrounding myoepithelial cells especially the plasma membranes. Consequently their functions was disturbed. Therefore, determination of 5'-ND activity of milk can be used as an additive method in the diagnosis of mastitis.

5'-ND levels of milk obtained from subclinically mastitic buffaloes (Table 16) ranged between 13.08 and 60.48 IU/liter, the highest value was shown in cases associated with Staph. aureus, while the least value was shown in cases with Str. agalactiae, while infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value of 44.65. It is quite clear from Table 17 that there is a significant difference ($P < 0.01$) between values obtained in cases of Staph. aureus and Str. agalactiae, mixed Str. agalactiae + Staph. aureus and Str. agalactiae and also between Staph. aureus and mixed Str. agalactiae + Staph. aureus ($P < 0.05$).

On the other hand, 5'-ND levels of milk obtained from clinical mastitic buffaloes (Table 18) ranged between 22.80 and 27.30 IU/liter, the highest value was shown in cases associated with C. pyogenes, while the least value was shown in cases associated with Staph. aureus, whereas infection with Str. agalactiae showed an intermediate value (23.74). The difference between the 3 mentioned levels is non-significant (Table 19).

In cows the 5'-ND activity of milk obtained from subclinical mastitic animals (Table 20), ranged between 14.46 and 56.05 IU/liter, the highest value was shown in cases associated with Staph. aureus, while the least value was shown in Str. agalactiae infected cases, whereas infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value (55.82). Table 21 show that there is a significant difference between values obtained in cases of Str. agalactiae and each of Staph. aureus and the mixed Str. agalactiae + Staph.aureus infection ($P < 0.01$).

In cases with clinical mastitis, the mean values of 5'-ND activity in cow's milk ranged between (22.41 and 46.72 IU/ liter, (Table 22).

The highest value was shown in cases associated with Staph. aureus, while the least value was shown in cases associated with Str. agalactiae, whereas infection with C.poygenes showed an intermediate value of 38.71. Statistical analysis (Table 23) show that there is a significant difference ($P < 0.05$) between values obtained in cases of Str. agalactiae and cases of Staph. aureus infection.

It is evident from this study that the drop effect on 5-ND activity in both buffalo's and cow's milk obtained from subclinical cases of mastitis can be arranged according to the causative organisms in the following order Str. agalactiae > mixed infection by Str. agalactiae + Staph. aureus > Staph. aureus.

(6) Effect of Mastitis on Lactic dehydrogenase (LDH)

Frahm et al. (1977) stated that, lactic dehydrogenase have no organ specificity.

The data presented in Tables 7 and 8 show the mean value of LDH activity in normal buffalo's and cow's milk which appears to be 127.09 and 135.93 Um/liter of milk respectively. Consequently, the differences are not significant, (Table 9).

Tables 10 & 11 show the mean values of LDH activity of mastitic buffalo's milk (subclinical and clinical cases)as 525.29 and 596.73 Um/liter milk respectively. While in mastitic cow's milk the mean LDH activity amounted to 498.81 and 589.50 Um/liter of milk respectively (Tables 13 and 14).

Statistical analysis of results (Tables 12 & 15) show that LDH activity increases significantly ($P < 0.01$) in milk obtained from subclinical and clinical mastitic animals as compared with that of normal animals. Also in both animals, the LDH activity of milk obtained from clinical mastitic animals showed a significant increase when compared with that of milk obtained from subclinical mastitic animals.

In buffaloes, LDH activity of milk obtained from subclinical mastitic animals (Table 16), ranged between 494.62 and 633.56 Um/liter of milk. The highest value was shown in cases associated with Str. agalactiae, while the least value was shown in cases associated with Staph.aureus. Infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value of 523.60 Um/liter of milk. Statistical analysis of results obtained (Table 17) showed that there is a significant difference ($P < 0.01$) between values obtained in cases of Str. agalactiae and Staph. aureus infected cases, and also

between Str. agalactiae infected cases and mixed infection by Str. agalactiae + Staph. aureus ($P < 0.05$), as well as between values obtained in cases of mixed infection by Str. agalactiae + Staph. aureus and Staph. aureus infected cases.

In cow's milk collected from subclinical cases of mastitis, LDH activity ranged between 484.44 and 523.47 Um/liter of milk. The highest value was shown in cases associated with Str. agalactiae, whereas, the least value was shown in cases associated with Staph. aureus. But infection with mixed Str. agalactiae + Staph. aureus showed an intermediate value of 502.41 Um/liter (Table 20). Statistical analysis of results obtained (Table 21) showed that there is a significant difference ($P < 0.01$) between values obtained in cases of Str. agalactiae and Staph. aureus infected cases.

LDH activity of milk obtained from clinical cases of mastitis (Tables 18 and 22) ranged between 515.26 and 752.40 Um/liter for buffaloes milk and 499.81 to 655.23 Um/liter for cows milk. In both animals the highest value was shown in cases associated with Str. agalactiae, while the least value was shown in cases associated with C. pyogenes. Infection with Staph. aureus showed an intermediate value of 590.21 Um/liter for buffaloes and 561.44 for cows.

Statistical analysis of resutls (Tables 19 and 23) showed that in milk of both animals there is a significant difference between values obtained from Str. agalactiae infected cases and those obtained from (Staph.aureus + C.pyogenes)infected cases also between values of Staph. aureus and C. pyogenes infected cases.

Our results were in close agreement with those studied by Kova and Beseda (1975) and Ziv et al. (1976).

Bogin (1973) and Bogin et al. (1977) offered a good explanation for this consistent observation in mastitic milk. They stated that LDH activity was quickly elevated following infection and this explains that the blood serum was not the sole source for the enzyme in mastitic secretions, but it seems that the origin of the elevated LDH activity in mastitic milk is the leukocytes and parenchyma cells of the udder.

It is suggested that determination of LDH activity in milk can serve as a more accurate and objective method for evaluating the extent of udder damage and also the abrupt change in milk enzyme pattern after the inflammation especially at the time when the secretion was still unaltered(subclinical mastitis) and can possibly serve as a sensitive and quick tool for laboratory diagnosis as well as in the prognosis of acute mastitis.

(7) Effect of Mastitis on Lipase

In this present work, the mean values of lipase activity in normal buffalo's and cow's milk are shown in Tables 7 and 8 as 45.22 and 39.94 Unit/100 ml of milk respectively.

Statistical analysis of results (Tables 9) shows that lipase activity of buffaloes milk is significantly higher ($P < 0.01$) than that of cow's milk. Similar findings have been reported by El-Hagarawy and Sirry (1967) who stated that lipase activity was found to be higher in fresh buffalo's milk than in cow's milk as it resulted in more liberation of free fatty acids.

Tables 10 & 11 show the mean lipase activity of milk obtained from mastitic buffaloes (subclinical and clinical cases) as 57.34 and 64.18 Unit/100 ml of milk respectively.

In cows milk the mean lipase activity in both mastitic cases amounted to 52.79 and 60.68 Unit/100 ml of milk respectively (Tables 13 and 14).

Statistical analysis of results (Tables 12 & 15) show that lipase activity increases significantly ($P < 0.01$) in milk obtained from subclinical and clinical mastitic animals as compared with that of normal animals. Also a significant difference was noticed between values obtained from subclinical and

clinical mastitic cases in both animals ($P < 0.01$) .

In buffaloes, the lipase activity of milk obtained from subclinical mastitic animals (Table 16) ranged between 56.66 and 60.74 Unit/100 ml milk. The highest value was shown in cases associated with Str. agalactiae, while the least value was shown in cases associated with Staph. aureus, cases infected with both organisms showed an intermediate value of 59.40. On the other hand, lipase activity of milk obtained from clinical mastitic buffaloes (Tables 18) ranged between 62.13 and 65.88 Unit/100 ml of milk. The highest value was shown in cases associated with Staph. aureus, while the least value was shown in cases associated with C. pyogenes.

Infection with Str. agalactiae showed an intermediate value of 63.71 Unit/100 ml of milk.

In cow's the lipase activity of milk obtained from subclinical mastitic animals ranged between 52.04 and 54.81 Unit/100 ml milk. The highest value was shown in cases associated with Str. agalactiae, whereas the least value was shown in cases associated with Staph. aureus. Mixed infection of Str. agalactiae + Staph. aureus showed an intermediate value of 53.13 (Table 20). On the other hand, lipase activity of milk

obtained from clinical mastitic cows ranged between 59.46 and 64.31 Unit/100 ml milk. The highest and least value were shown in cases associated with Staph. aureus and C. pyogenes infected cases respectively, whereas infection with Str. agalactiae showed an intermediate value of 60.84 (Table 22).

Statistical analysis of results (Tables 17,19,21 & 23) indicate that there is no significant difference among the values obtained due to the effect of the different isolated organisms in both mastitic cases (subclinical and clinical mastitis) in buffaloes as well as in cows.

It is clear from this work that lipase activity of buffalo's and cow's mastitic milk obtained from subclinical and clinical cases was elevated generally in all infected animals, and this in close agreement with the results reported by Guthrie and Herrington (1960). The increase of lipase activity in mastitic milk may be attributed to the enormous amounts of causative organisms in the mastitic udder tissues.

(8) Effect of Mastitis on Amylase

In this present study the data presented in Tables 7 & 8 show the mean value of Amylase activity in normal buffalo's and cow's milk which amounted to 63.65 and 56.21 Somo. Unit/ 100 ml of milk respectively.

Statistical analysis of results (Table 9) show that normal buffalo's milk exhibit significantly increased ($P < 0.01$) amylase activity than cow's milk, in contradistinction to Rifaat et al. (1969) who reported that normal buffalo's and cow's milk exhibit approximately the same Amylase activity.

Tables 10,11,13 and 14 show the mean Amylase activity of milk obtained from mastitic buffaloes (subclinical and clinical cases) as 128 and 157.93 Somo. Unit/100 ml of milk respectively, and 129.77 and 148.86 for cows. Tables 12 and 15 show that there is a significant difference ($P < 0.01$) between the mean values of Amylase activity of normal milk and those of mastitic milk (obtained from subclinical and clinical cases) in both animals. Such results are in close agreement with those studied by Chrzaszcz and Goralowna (1925), and Guy & Genness (1958). The increase of Amylase activity in mastitic milk may be attributed to the presence of enormous amount of causative organisms in the mastitic udder tissues. In case of

buffaloes, the amylase activity of milk obtained from sub-clinically mastitic animals (Table 16) ranged between 122.96 and 131.82 Somo.Unit/100 ml milk, it appears therefore, that the type of infected organism had no significant effect on the amylase activity. On the other hand milk obtained from cases with clinical mastitis showed mean values of Amylase activity ranging between 152.51-168.04 Somo.Unit/100 ml (Table 18). The highest value was shown in cases associated with Str. agalactiae, while the least value was shown in cases associated with Staph.aureus,whereas C.pyogenes infected cases showed an intermediate value of 155.67. Statistically (Table 19), there is a significant difference ($P < 0.05$) between the values obtained from Str. agalactiae and Staph. aureus infected cases.

In cows, the amylase activity of milk obtained from sub-clinically mastitic animals ranged between 114.62-139.82 Unit/100 ml (Table 20). It appears, therefore, that the type of infected organism exhibited no significant effect on Amylase activity.

On the other hand, the Amylase activity of milk obtained from clinically mastitic cows (Table 22), ranged between 139.65-154.02 Somo.Unit/100 ml. The highest value was shown in

cases associated with Str. agalactiae, whereas, the least value was shown in cases infected with C.pyogenes. In Staph. aureus infected cases an intermediate value of 146.55 was noticed. Statistical analysis of results (Table 23) show that there is a significant difference ($P < 0.05$) between values obtained in cases of Str. agalactiae & C.pyogenes as well as between the latter and cases infected with Staph. aureus.

(9) Effect of Mastitis on Ascorbic Acid

The mean values of Ascorbic acid content in normal buffalo's and cow's milk are listed in Tables 7 and 8 which shows it to be 28.30 and 7.33 mg/liter milk respectively.

Statistical analysis of results (Table 9) shows that Ascorbic acid content of buffalo's milk is significantly higher ($P < 0.01$) than that of cow's milk. The present data coincide with those given by Barakat and Abdel Wahab (1961) as 19.5-39.5 mg/liter for buffalo's milk and 7.1-7.8 for cow's milk.

This increase in ascorbic acid content of normal buffalo's milk than normal cow's milk may be attributed to the high lactose content of normal buffaloes milk.

Tables 10 & 11 show the mean Ascorbic acid content of milk obtained from mastitic buffaloes (subclinical and clinical cases) as 33.87 and 46.88 mg/liter of milk respectively. With respect to cow's milk (Tables 13 & 14), the mean Ascorbic acid content in both mastitic cases amounted to 6.74 and 14.77. Statistical analysis of results, Table 12 show that Ascorbic acid content of mastitic milk (subclinical and clinical cases) increases significantly ($P < 0.01$) as compared with values of normal milk in both animals.

This finding coincide with those previously reported by Nani and Defranceshi (1957), but disagree with that cited by Kisza et al. (1966). This disagreement may be attributed to breed variation.

In buffaloes, the Ascorbic acid content of milk obtained from subclinical mastitic animals (Table 16) ranged between 31.37 and 35.46 mg/liter milk. Statistically no significant difference was found due to the type of infected organism. On the other hand, milk obtained from clinical mastitic animals, showed mean values of Ascorbic acid content ranging between 32.71 and 66.75 mg/liter milk, the highest value was shown in cases associated with Staph. aureus, while the least value was observed in cases infected with C.pyogenes.

Str. agalactiae infected cases showed an intermediate value (55.64 mg/liter, Table 18). It is quite clear from Table 19 that there is a significant difference ($P < 0.01$) between values obtained in cases of Staph. aureus infection and Str. agalactiae infection, and also between C. pyogenes infected cases and each of Str. agalactiae infected cases and Staph. aureus infection.

In cows the Ascorbic acid content of milk obtained from subclinical mastitic cases, ranged between 6.29 and 7.28 mg/liter of milk (Table 20). Statistically no significant difference was found due to the type of infected organism.

On the other hand, Ascorbic acid content of milk obtained from clinical mastitic cases ranged between 8.51 and 19.54 mg/liter milk. The highest value was shown in cases associated with Str. agalactiae infection, whereas, the least value was observed in cases associated with C.pyogenes infection. Infection with Staph.aureus showed an intermediate value (16.45 mg/liter), Table 22. Statistical analysis of the results (Table 23) show that there is a significant difference ($P < 0.01$) between values obtained from Str. agalactiae and C.pyogenes infected cases, as well as between values obtained from Staph. aureus and C.pyogenes infection.

The mechanism of this increasing of ascorbic acid content in mastitic milk is obscure and possibly complicated, the most probably mechanism seems to be the presence of enormous amount of causative organisms in the mastitic udder.

(10) Effect of Mastitis on pH value

In this present work, the mean pH value of milk of normal buffalo's and cow's milk are shown in Tables 7 and 8 as 6.40 and 6.49 respectively. Statistical analysis of the results obtained revealed no significant difference between the pH values of normal buffalo's and cow's milk.

Tables 10 & 11 show the mean pH values of milk obtained from mastitic buffaloes (subclinical and clinical cases) as 7.40 and 7.51 respectively. Statistical analysis of the results (Table 12) indicate that there is a significant difference ($P < 0.01$) between the mean pH values of normal milk and that of subclinically mastitic buffalo's milk and also between pH values of normal and clinically mastitic milk.

The pH value of milk obtained from subclinical mastitic buffaloes (Table 16) ranged between 7.26 and 7.53. The differences are found statistically to be non-significant.

In case of buffaloes, the pH value of milk obtained from mastitic cases ranged between 7.46 and 7.56 (Table 18).

In case of cows, the pH value of milk obtained from mastitic cases (subclinical and clinical cases) was found to be 7.28 and 7.46 respectively (Table 13 & 14). Satistical analysis of results (Table 15) indicate that there is a significant difference ($P < 0.01$) between pH value of normal cow's milk and that of mastitic milk, as well as between pH values of subclinically mastitic milk and clinically mastitic milk.

In cow's milk obtained from subclinical mastitic cases, the pH value ranged between 7.08 and 7.36 (Table 20). On the other hand, the pH value of cow's milk obtained from clinical mastitic cases, ranged between 7.40 and 7.52 (Table 22).

It is noticed from the results obtained that the pH value of mastitic buffalo's and cow's milk (subclinical and clinical cases) was found to shift towards the alkaline side more than normal milk. This result coincide with that reported by Kelly (1967), Renner (1975) and Beuche (1977) who stated that the pH value of mastitic milk is shifted towards the alkaline side. Schalm et al. (1971) attributed such a shift of the pH of mastitic milk to the increased permeability of the gland to blood components which permits movement of bicarbonate ions into the milk by selective transudation.

S U M M A R Y

Mastitis has important significance in the economy of milk production, it is the most serious disease problem confronting the dairy farmer. Several methods for diagnosing mastitis have been reported. Bacteriological methods is expensive and time consuming, hence the need for simple, sensitive and reliable method sufficient to be applied on a large scale for herd testing is required.

Quarter milk samples were collected from 140 lactating buffaloes (Bos bubalis) and 62 local breed cows at Edfina veterinary clinic and the clinic of the Faculty of Edfina vet. Med. Alex. University. Bacteriological examination of the milk samples collected from apparently normal animals revealed that 18% of buffaloes and 50% of cows were infected with specific microorganisms of mastitis. The most predominant organisms isolated from buffalo's and cow's milk in cases of mastitis (subclinical and clinical cases) were Str. agalactiae and Staph. aureus.

Milk samples collected from buffaloes and cows were analysed for determination of:

(1) Glutamic oxalacetic transaminase (GOT)
(2) Glutamic pyruvic transaminase (GPT)
(3) Alkaline phosphatase (ALP)

(4) Acid phosphatase (ACP)

(5) 5'-Nucleotidase (5' - ND)

(6) Lactic dehydrogenase (LDH)

(7) Lipase.

(8) Amylase.

(9) Ascorbic acid.

(10) pH value.

The results obtained showed:-

1 - The activities of Glutamic oxalacetic transaminase, lipase and Amylase as well as Ascorbic acid content of buffalo's milk were significantly higher than that of normal cow's milk. On the contrary, Alkaline phosphatase activity of normal cow's milk was significantly higher than that of buffalo's milk.

2 - The activities of Glutamic oxalacetic transaminase, Alkaline phsophatase, Lactic dehydrogenase, Lipase and Amylase as well as the pH value of milk obtained from mastitic buffaloes and cows (subclinical and clinical cases) showed a significant increase than that of normal animals milk.

3 - Milk obtained from normal buffaloes and cows as well as milk obtained from mastitic animals (subclinical cases) is absolutely deficient in Glutamic pyruvic transaminase

activity. While milk obtained from mastitic animals (clinical cases) showed a remarkable activity; a phenomen which can be used as a proper tool for diagnosis of clinical cases of mastitis in both animals.

4 - The acid phosphatase activity increased significantly only in buffalo's milk obtained from subclinical mastitic cases.

5 - In milk obtained from mastitic animals (subclinical & clinical cases) the activity of 5'-nucleotidase decreased significantly as compared with that of normal milk.

6 - Milk obtained from mastitic buffaloes (subclinical and clinical cases) as well as milk obtained from mastitic cows (clinical cases) showed a significant increase in Ascorbic acid content as compared with normal milk.

7 - The Glutamic pyruvic transaminase, lactic dehydrogenase, lipase, Amylase activity as well as, Ascorbic acid content of milk obtained from mastitic buffaloes and cows (clinical cases) increased significantly as compared with that obtained from subclinical cases of mastitis.

8 - pH value of milk obtained from mastitic cows(clinical cases) increased significantly as compared with that obtained from subclinical cases of mastitis.

9 - Milk obtained from mastitic buffaloes (subclinical cases) where Str. agalactiae was isolated showed an increased activity of both Glutamic oxalacetic transaminase and Lactic dehydrogenase enzymes.

10- Milk obtained from mastitic cows (subclinical cases) where Str. agalactiae was isolated showed an increased activity of Glutamic oxalacetic transaminase, Alkaline phosphatase and Lactic dehydrogenase.

11- Milk obtained from mastitic buffaloes and cows(subclinical cases) where Str. agalactiae was isolated showed an increased activity of acid phosphatase.

12- Milk obtained from mastitic buffaloes and cows (subclinical cases) where Staph. aureus was isolated showed a significant inhibition in the activity of 5'-nucleotidase.

13- Milk obtained from mastitic buffaloes (clinical cases) where Str. agalactiae was isolated showed a significant increase in Glutamic oxalacetic transaminase and Amylase activity.

14- Milk obtained from mastitic cows (clinical cases) where Str. agalactiae was isolated showed a significant increase in Glutamic oxalacetic transaminase and Alkaline phosphatase activity.

15- Milk obtained from mastitic cows (clinical cases) where Staph. aureus was isolated showed a significant decrease in 5-nucleotidase activity.

It can be concluded from the results of this present work that:-

(1) Accurate diagnosis of mastitis in both buffaloes and cows milk should not depend completely on Alkaline phosphatase activity due to the fluctuation of the activity of this enzyme but generally to some extent it can considered as an additive means for quick laboratory diagnosis.

(2) Determination of Acid phosphatase activity can serve as an accurate reproducible method for rapid and sensitive identification of Str. agalactiae as a causative organism in subclinical mastitis in both buffaloes and cows.

(3) Determination of Glutamic oxalacetic transaminase, 5-nucleotidase and Lactic dehydrogenase activity and to some extent Ascorbic acid content can considered as a diagnostic tool for early recognition of subclinical mastitis in both animals.

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موجز الرسالـــــة

التهاب الضرع مرض يصيب حيوانات المزرعة المدرة للالبان ويؤثر تأثيرا خطيرا على كمية اللبن المنتج ، وفى بعض الأحيان يمتد تأثيره الى توقف ادرار اللبن كلية .

والطرق المستخدمة لتشخيص هذا المرض عديدة أهمها الفحص البكتريولوجى الـــذى يعتمد على عزل وتصنيف الميكروبات المسببة للمرض . ولكن وجد أن الاعتماد على هـــذه الطريقة يحتاج الى وقت وجهد هذا بالاضافة الى أنه مكلف ماديا . لذا كان الاتجاه الـى استحداث طرق سريعة ودقيقة ورخيصة وفى نفس الوقت تصلح للفحص الدورى لمجموعة كبـيرة من الحيوانات فى آن واحد .

يتناول هذا البحث دراسة نشاط بعض الانزيمات ومحتوى حمض الاسكوربيك وأيضا تقدير الامين الايد روجينى فى :

١ ــ اللبن الجاموسى الطبيعى .
٢ ــ اللبن البقرى الطبيعـــى .
٣ ــ اللبن الجاموسى والبقرى فى حالة التهاب الضرع الخفى (تحت الاكلينيكى) .
٤ ــ اللبن الجاموسى والبقرى فى حالة التهاب الضرع الاكلينيكى .

كما يتضمن عزل وتصنيف الميكروبات المسببة لمرض التهاب الضرع الخفى والاكلينيكى فـــى كلا الحيوانين . لاجراء هذا البحث تم جمع عينات اللبن الجاموسى (١٤٠ عينة) والبقـرى (٦٢ عينة) للمقارنة ، من الوحدة البيطرية بقرية ادفينا ، ومن مستشفى كلية الطب البيطـرى بادفينا ــ جامعة الاسكندرية .

وبالفحص البكتريولوجى والاكلينيكى لجميع عينات اللبن أتضح أن هناك ١٠٢ عينة سليمـة وخالية من المرض (٨٢ جاموس ، ٢٠ أبقار) و ١٨ حالة التهاب ضرع خفى فى الجامـــوس

(١٨٪) و ٢٠ حالة التهاب ضرع خفي في الابقار (٥٠٪) أيضا أتضح وجود ٤٠ حالة التهاب ضرع اكلينيكي في الجاموس و ٢٢ في الابقار ولقد تم عزل ميكروبي الاستريتوكوكس في كلا الحيوانين . هذا بالاضافة الى عزل حالات فردية تتسبب عن ميكروبات أخرى .

تم تقدير النشاط الأنزيمي للخمائر التالية :

(١) انزيم الجلوتاميك اوكسالواستيك ترانس امينيز .
(٢) انزيم الجلوتاميك بيروفيك ترانس امينيز .
(٣) انزيم الفوسفاتيز القلوي .
(٤) انزيم الفوسفاتيز الحمضي .
(٥) انزيم ٥ نيكلوتيديز .
(٦) انزيم اللاكتيك ديهيدروجينيز .
(٧) انزيم الليبيز .
(٨) انزيم الاميليز .

كما تم تقدير محتوى حمض الاسكوربيك . وقياس الاس الايدروجيني .

١ — <u>خميرة الجلوتاميك اوكسالواستيك ترانس امينيز والجلوتاميك بيروفيك ترانس امينيز :</u>

تم قياس نشاط انزيم الجلوتاميك اوكسالواستيك ترانس امينيز والجلوتاميك بيروفيك ترانس امينيز بطريقة رايتمان وفرنكل (١٩٥٧) مع اجراء تعديل يتلخص في فصل المادة الملونة (المراد قياس تركيزها على جهاز الاسبكتروفوتومتر) من اللبن باستخدام طريقة الفصل الغشائي بواسطة ورق السلوفان مباشرة قبل القياس .

٢ — <u>خميرة الفوسفاتيز القلوي والحمضي :</u>

استخدمت طريقة فولن وسيوكلتيوفينول المستعملة لقياس نشاط انزيم الفوسفاتيز القلوي والحمضي في السيرم مع تعديل طفيف يتلخص في تخفيف اللبن عند قياس نشاط انزيم الفوسفاتيز القلوي بنسبة ١ : ١٠ بالماء المقطر وذلك لاحتواء اللبن الطبيعي على نسبة

عالية من هذا الانزيم .

٣ — خميرة ٥ — نيكلوتيديز :

تم قياس نشاط انزيم ٥ — نيكلوتيديز باستخدام طريقة كامبل .
مع اجراء التخفيف المناسب قبل قياس المادة الملونة .

٤ — خميرة الالكتك ديهيد روجينز :

تم تقدير نشاط انزيم الالكتك ديهيد روجينز بطريقة وريلسكى (١٩٥٩) مع استخدام طريقة الفصل الغشائى بورق السلوفان فى فصل المادة الملونة المراد قياس تركيزها على جهاز الاسبكتروفوتوميتر .

٥ — خميرة الليبيز :

استخدمت طريقة بيرى (١٩٦٦) لقياس نشاط خميرة الليبيز فى اللبن .

٦ — خميرة الاميليز :

تم قياس نشاط انزيم الاميليز فى اللبن بطريقة سوموجى بدون اجراء اى تعديل .

٧ — محتوى حمض الاسكوربيك فى اللبن :

تم تقدير محتوى حمض الاسكوربيك فى اللبن بمعايرته بواسطة N — بروموسكسيناميد .

٨ — الامس الايد روجينى :

تم قياس الامس الايد روجينى باستخدام جهاز قياس الامس الايد روجينى (بكمان) .

أسفرت نتائج التحليل البيوكيميائى على ما يلى :

(١) أتضح من الدراسة أن نشاط خميرة الجلوتاميك اوكسالواستيك ترانس امينار والليبيز والاميليز ومحتوى حمض الاسكوربيك فى اللبن الجاموسى الطبيعى اعلى من مثيلاتها فى اللبن البقرى . على العكس من ذلك وجد ان نشاط خميرة الفوسفاتيز القلوى اعلى فى

اللبن البقرى الطبيعى عن اللبن الجاموسى الطبيعى •

(٢) تبين أيضا من الدراسة ان نشاط خميرة الجلوتاميك اوكسالواستيك ترانس امينـــاز والفوسفاتيز القلوى واللاكتك ديهيد روجينز واللييز والاميليز وأيضا الاسيد روجينى قد ازدادت جميعها زيادة ملحوظة فى اللبن الجاموسى والبقرى فى حـــالات التهاب الضرع الخفى والاكلينيكى اذا قورنت بالمعدل الطبيعى فى كلا الحيوانين•

(٣) تبين من الدراسة ان نشاط خميرة الجلوتاميك بيروفيك ترانس امينار ينعدم تماما فى اللبن الطبيعى وأيضا فى حالات التهاب الضرع الخفى فى كلا الحيوانين • بينمـــا فى حالات التهاب الضرع الاكلينيكى لوحظ نشاط الانزيم مما يجعلنا ننصـــح باستخدام هذا الانزيم فى تشخيص الحالات الاكلينيكية لالتهاب الضرع فى كـــلا الحيوانين •

(٤) اتضح من النتائج أن نشاط خميرة الفوسفاتيز الحمضى ازدادت زيادة ملحوظة فى لبن الجاموس فى حالات التهاب الضرع الخفى فقط •

(٥) تبين من الدراسة ان نشاط خميرة ٥ ـ نيكلوتيديز فى اللبن نقصت نقصا ملحوظا فـــى حالات التهاب الضرع الخفى والاكلينيكى فى كلا الحيوانين اذا ما قورنت باللبـــن الطبيعى •

(٦) محتوى اللبن من حمض الاسكوربيك فى حالات التهاب الضرع الخفى والاكلينيكـــى (الجاموس) والاكلينيكى (الأبقار) قد ازدادت زيادة ملحوظة اذا قورنت باللبـــن الطبيعى فى كلا الحيوانين •

(٧) تبين أيضا ان نشاط خميرة الجلوتاميك بيروفيك ترانس امينار واللاكتك ديهيد روجينيـــز واللييز والاميليز وايضا محتوى حمض الاسكوربيك فى اللبن فى حالات التهاب الضـــرع الاكلينيكى فى كلا الحيوانين قد ازدادت زيادة ملحوظة اذا قورنت بمثيلاتها فى حالات التهاب الضرع الخفى •

(٨) أتضح أيضا من النتائج ان الاس الايدروجينى فى حالات التهاب الضرع الاكلينيكى فى لبن الابقار قد ازدادت زيادة ملحوظة عنه فى حالات التهاب الضرع الخفى فى نفس الحيوان .

(٩) تبين من الدراسة أن حالات التهاب الضرع الخفى التى تتسبب عن ميكروب الاستربتوكوكس اجلاكتيا تكون مصحوبة بزيادة ملحوظة فى نشاط خميرة الجلوتاميك اوكسالاسيتيك ترانس امينار والفوسفاتيز القلوى والاكتك ديهيدروجينيز فى اللبن الجاموسى .

(١٠) تبين ايضا ان حالات التهاب الضرع الخفى التى تتسبب عن ميكروب الاستربتوكوكس اجلاكتيا تكون مصحوبة بزيادة ملحوظة فى نشاط خميرة الجلوتاميك اوكسالواستيك ترانس امينار والفوسفاتيز القلوى والاكتك ديهيدروجينيز فى اللبن البقرى .

(١١) تبين من الدراسة ان حالات التهاب الضرع الخفى التى تتسبب عن ميكروب الاستربتوكوكس اجلاكتيا تكون مصحوبة بزيادة ملحوظة فى نشاط خميرة الفوسفاتيز الحمضى فى لبن الجاموس والابقار .

(١٢) لوحظ ايضا ان حالات التهاب الضرع الخفى التى تتسبب عن ميكروب الاستافيلوكوكس اوريس تكون مصحوبة بنقص ملحوظ فى نشاط خميرة هـ — نيكلوتيديز فى لبن الجاموس والابقار .

(١٣) تبين من الدراسة أيضا ان حالات التهاب الضرع الاكلينيكية التى تتسبب عن ميكروب الاستربتوكوكس اجلاكتيا تكون مصحوبة بزيادة ملحوظة فى نشاط خميرة الجلوتاميك اوكسالو آستيك والاميليز فى لبن الجاموس .

(١٤) اتضح أيضا من الدراسة ان حالات التهاب الضرع الاكلينيكية التى تتسبب عن ميكروب الاستربتوكوكس اجلاكتيا تكون مصحوبة بزيادة ملحوظة فى نشاط خميرة الجلوتاميك اوكسالو آستيك ترانس امينار والفوسفاتيز القلوى فى لبن الابقار .

(١٥) تبين أيضا من الدراسة ان حالات التهاب الضرع الاكلينيكية التى تتسبب عن ميكروب الاستافيلوكوكس اوريس تكون مصحوبة بنقصا ملحوظا فى نشاط خميرة هـ — نيكلوتيديز فى لبن الابقار .

نتيجة لعدم استقرار نشاط خميرة الفوسفاتيز القلوى فى لبن الايقار والجاموس فى حالات التهاب الضرع الخفى والاكلينيكى يتضح انه لا يمكن الاعتماد كلية فى التشخيص المؤسسى للمرض على قياس هذا الانزيم ولكن عموما يمكن ان يؤخذ به كطريقة اضافية للوصول الى التشخيص النهائى للمرض • ولكن بتقدير نشاط خميرة الفوسفاتيز الحمضى نستطيع ان نصل الى طريقة سريعة وحساسة للتعرف المباشر على ميكروب الاستربتوكوكس اجالاكتيا لمرض التهاب الضرع الخفى •

وبصورة موجزة ننصح باستخدام قياس نشاط خميرة الجلوتاميك اوكسالواستيك ترانس امينازو هـ — نيكلوتيد يز واللاكتك ديهيد روجينيز وايضا الى حد ما محتوى حمض الاسكوربيك فى التشخيص المبكر لمرض التهاب الضرع الخفى فى كلا الحيوانين •

قـــــرار لجنة الحكم والمناقشـــة

قـــــررت لجنـــة الحكم والمناقشـــة ترشـــيح السيد / طـ.ب ابراهيم فتوح حســـن للحصول علـــى درجـــة دكتوراه الفلسفـــه فى العلوم الطبيه البيطريـــه مادة :
(الكيمياء الحيويه وكيمياء التغذيه الحيوانيـــه)

الأســـتاذ الدكتور / عبد المقصود على عبد اللـــه
أستاذ الكيمياء الحيويه وكيمياء التغذيه الحيوانيـــه
كلية الطب البيطرى ـ جامعة القاهـــــــره

الأستاذ الدكتور / محمد رجاء رجب حســـنين
أستاذ الكيمياء الحيويه وكيمياء التغذيه الحيوانيـــه
كلية الطب البيطرى ـ جامعة الزقازيـــــــق

الأستاذ الدكتور / منير شاكر فلتاوؤس
أستاذ الكيمياء الحيويـــــــــه
كلية الطب البيطرى ـ جامعة الناهـــره

الأســـتاذ الدكتور / سمير عبد المجيد غنام
رئيس قسم الفسيولوجيا والكيمياء الحيويـــة
كلية الطب البيطرى ـ جامعة الأسكندريـة

تقييم بيوكيميائى للنشاط الانزيمى فى لبن الجاموس الطبيعى والتهاب الضرع

رسالة مقدمة من

ط. ب. ابراهيم فتوح حسن

للحصول على

درجة دكتوراه الفلسفة

فى العلوم الطبية البيطرية (كيمياء حيوية)

تحت اشراف :

الأستاذ الدكتور منير شاكر فلتاؤس

أستاذ الكيمياء الحيوية بكلية الطب البيطرى - جامعة القاهرة

الأستاذ الدكتور سمير عبد المجيد غنام
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الأستاذ الدكتور طه حسن مصطفى
رئيس مجلس قسم الميكروبيولوجيا وصحة الحيوان ووكيل الكلية

كلية الطب البيطرى - جامعة الاسكندرية

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