THE RELATIONSHIP BETWEEN BIOCHEMICAL CONSTITUENTS AND BACTERIAL COUNT IN MILK OF CATTLE

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List of Abbreviations

ADP = Adenosine Diphosphate
ANOVA= Analysis of variance
APHA = American Public Health Association
ATP = Adenosine Triphosphate
ATPase = Adenosine Triphosphate hydrolyzing enzyme
cfu = colony forming unit
CPC = Calcium phosphate citrate
DNPH = Dinitrophenyl Hydrazine
Eds = Editors
EDTA = Ethylene Diamine Tetraacetic Acetic Acid (sodium salt)
FFA = Free Fatty Acid(s)
fg = femtogram
fig = figure
FRG = Federal Republic of Germany
GAPD = Glyceraldhyde 3-phosphate dehydrogenase
GLDH = Glutamate dehydrogenase enzyme
HACCP = Hazards Analysis and Critical Control Points
IDF = International Dairy Federation
LDH = Lactic Dehydrogenase enzyme

NAD^+ = Nicotinamide Adenine dinucleotide (oxidized form)

NADH = Nicotinamide Adenine dinucleotide (reduced form)

$NADP^+$ = Nicotinamide Adenine dinucleotide phosphate(oxidized form)

NADPH = Nicotinamide Adenine dinucleotide phosphate(reduced form)

nm = nanometer

PCA = Perchloric acid

PGK = 3-phosphoglyceric phosphokinase enzyme

pH = [G,fr.potenz (power) + H (symbol for hydrogen)]

TCA = Trichloroacetic acid

TCC = Total colony count

V/V = volume in volume

W/V = weight in volume

Xg = Gravity force of centrifuge

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INTRODUCTION

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The importance of microbiology to dairy industry has been demonstrated by recent outbreaks of food borne illness associated with consumption of milk and dairy products that had been contaminated with pathogenic organisms or toxins. Undesirable microorganisms constitute the primary hazard to safety, quality, and wholesomeness of milk and dairy foods. Consequently, increased emphasis has been placed on the microbiological analysis of milk and dairy products designed to evaluate quality and to ensure safety and regulatory compliance. The focus of dairy microbiology, however, remains largely on conventional methods: plate counts, most probable numbers, and dye reduction tests. These methods are slow, tedious, intensive in their requirements for material and labor, and often not suitable for assessing the quality and shelf-life of perishable dairy foods. With the exception of Coliforms, Salmonella, and Staphylococcus aureus, isolation and characterization of various organisms occurring in milk and milk products are seldom a part of the routine microbiological analysis in the dairy industry. Recent emphasis on the programs based on HACCP (Hazards Analysis and Critical Control Points) for total quality management in the dairy industry and increased demand for surveillance of products, process, and environment have led to increased interest in rapid methods and automation in microbiology. Several methods for rapid isolation, enumeration, and characterization of microorganisms are being adapted by the dairy industry. During study of these methods, metabolic behavior of microorganisms through the biochemical composition of milk was studied

and certain biochemical constituents were found to be closel related to the bacterial number, activity and pathogencity. Among these constituents are Adenosine Triphosphate (ATP), Ammonia, Pyruvic Acid, and Lactate Dehydrogenase enzyme (LDH), so our study aimed to find a relationship between the level of these compounds and colony forming unit in milk by rapid, easy and cheap test.

REVIEW OF LITERATURE

A rapid test for monitoring the bacteriological quality of stored bulked raw milk would be useful in the control and management of supplies and in determining whether there might be a risk to product quality because high bacterial counts in the raw milk ***(Law et al,1976; Law et al.,1977).***

The rapid control of the hygienic and bacteriological quality of raw milk is essential both for the assurance of an efficient real time selection of milk for various technological treatments. Ex-farm refrigerated bulk tank milk arriving at the factory at 4-7°C may be processed within 24 hours or stored for three days or more before processing ***(Roberts, 1979)***.During storage psychrotrophic bacteria multiply and the mesophilic and psychrotrophic colony count may exceed 10^7/ ml of milk ***(Muir et al ,1978; Cousins et al ., 1977) .***

The introduction of refrigerated storage of milk has resulted in a change of the bacterial flora and tests previously used to determine bacterial quality are now too time consuming (as plate count) or fail to yield a result (as dye reduction method) ***(O'Toole,1983).*** This has led to an investigation of alternate methods to rapidly determine the bacterial quality of raw milk ***(Pettipher, 1981)***.

For about 15 years, metabolites of bacterial catabolism have been intensively and controversially discussed as parameters

of the saprophytic bacteriological quality of milk, especially under psychrotrophic conditions. The enzymatic breakdown of carbohydrates, fat and protein produces beside others, the metabolites lactate, pyruvates, free fatty acids (FFA) and ammonia. With low milk bacterial count, the variability of the physiological lactate, pyruvate and ammonia content of milk is of increasing importance. Accruing this base several biochemical indices were used to estimate bacterial quality of milk. ***(Suhren & Heeschen, 1991).*** Accordingly the following biochemical indices were used for assessment of bulk milk samples in relation to bacterial count,

1-Determination of ATP in milk

2-Determination of Pyruvic acid in milk

3-Determination of Ammonia in milk

4-Determination of LDH in milk

Adenosine 5" Triphosphate (ATP):

In order to maintain living processes, all organisms must obtain supplies of free energy from their environment. Autotrphic organisms couple their metabolism to some simple exergonic process in their surroundings; e.g., green plants utilize the energy of sunlight, and some autotrophic bacteria utilize the reaction $Fe^{2+} \longrightarrow Fe^{3+}$. On the other hand, Heterotrophic organisms obtain free energy by coupling their metabolism to the breakdown of complex organic molecules in their environment. In all of these processes, ATP plays a central role in the transference of free energy from exergonic to endorgonic processes.

The importance of phosphates in intermediary metabolism became evident with the discovery of the chemical details of glycolysis and the role of ATP, ADP and inorganic phosphate (Pi) in this process. ATP was considered to be a means of transferring phosphate radicals in the process of phosphorylation. The role of ATP in biochemical energetic was indicated in experiments demonstrating that ATP and creatine phosphate were broken down during muscular contraction and their resynthesis depends on supplying energy from oxidative processes in muscle. ***(Murray et al., 1996)***

ATP History & Chemistry

Lohmann, Fiske & Subbarow discovered ATP in 1929. ATP is a high energy source that is a product of oxidative phosphorylation and serves as the major intracellular transducer of free energy. Important also is the finding, as shown by many tests mainly carried out in the USA that the ATP concentration per cell is almost constant within the same group of organisms: thus an ATP determination also permits quantitative investigation of the biomass. ***(D'Eustachio & Johonson, 1968 ; Chappelle and Levin, 1968)***

Nomenclature of ATP:

ATP is specialized nucleotide containing ribose, adenine and three phosphate groups. The position of the phosphate in the nucleotide is indicated by a numeral, e.g., adenosine with phosphate attached to carbon (5) of the sugar ribose would be designated Adenosine 5'Triphosphate. The prime mark after the numeral is required to differentiate the numbered position on the sugar moiety from the numbered position on the purine base; the latter would not be followed by the prime mark. The abbreviation A used to designate the nucleoside that contains adenine. The 3 phosphates that are attached to the sugar moiety, are represented by abbreviation AT(Triphosphate) and the abbreviations are added to the abbreviation for the corresponding purine nucleotide. Finally adenosine triphosphate with 3 phosphate residues attached to the 5' carbon of adenosine would be abbreviated ATP ***(Murray et al., 1996)***

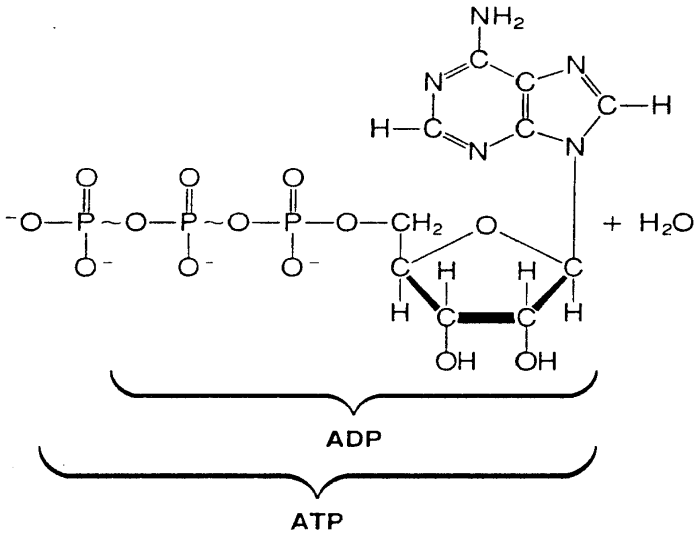


Figure (a): ATP structure

Bacterial ATP:

Electron transport systems are found in the cytoplasmic membrane of numerous of aerobic bacteria. Electron flow from reducing substrates to O_2 is coupled to transmembrane proton movements in these species, just as it is in the mitochondrial respiratory chain. Protons are pumped out of the bacterial cell, leaving the cytoplasm alkaline and with a net negative charge relative to the external solution. The cell membrane also contains an ATP synthase or ATPase similar to the mitochondrial ATP-synthase, and protons moving back into the cell through the ATPase drive the formation of ATP. A proton-translocating ATPase is also found in many species of anaerobic bacteria that do not have electron-transport systems. The role of the ATPase in these species is to pump protons out the cell at the expense of ATP made by fermentative pathways as glycolysis . The proton efflux creates an electrochemical potential gradient which the bacteria use to drive the uptake of sugars and other nutrients ***(Geoffrey, 1989).***

Bauchop and Elsden,(1960) established that the amount of cellular material synthesized during growth of microorganism is related to the amount of ATP produced by catabolism of the energy source; growth -yield coefficient (YATP) is 10 mg (dry weight) of cellular material per mol of ATP. This coefficient has usually been determined by growing organisms in batch culture with growth limited by energy source.

Kinetic experiments in batch culture also indicate that, the same proportionality between ATP and cellular material synthesized applies throughout the period of growth. The relationship of course,is based on calculation of the amount of ATP produced during catabolism of a substrate by a specific biochemical pathway ***(Forrest and Walker,1964)***

It would appear that, the measurements of ATP pool in an organism would exhibit regularities related to the rate of production of ATP. It would also be expected that, the pool level would be related to the energy requirements of the organism, so that comparisons of the ATP pool with the synthetic activity of an organism would give an indication of its energetic behavior***(Krebs,1962)***

From measurements of ATP pool in Escherchia coli it has been calculated that, the concentration of ATP is not affected by the growth rate of organisms***(Franzen and Binkley, 1961)*** whereas in Aerobacter aerogenes it has been shown that the main factor affecting the ATP pool in resting cells of this organism is the oxygen tension in the suspending media and that the pool level is not necessarily related to the energy requirements***(Strange et al.,1963)***

It seems that the behavior of the pool may be more complex than would be suggested by a simple steady-state balance between well-

regulated rates of production and utilization for synthetic reactions***(Krebs,1962)***

Refrigeration of milk containing E. aerogenes (a mesophil) exposes the organism to cold stress and inhibits growth which could explain the decline in ATP cell values ***(Cole et al., 1967 and Strange et al.,1963),*** but replacing the cells at optimal temperature allows microorganisms to regain the ATP levels during stress ***(Chapman and Atkinson, 1977)***

Bush et al.,(1975) proved that changes in metabolic rate will affect the level of ATP and consequently sub optimal and stress situations may change the ATP content. E. Aerogenes, in chilled milk should be activated at 30 °C for at least 30 min. Before enumeration by ATP measurement, so further is required to determine whether is necessary for other bacteria commonly found in chilled milk***(Theron et al., 1983)***

Karl (1980) proved that intracellular content of ATP must be constant in all the bacteria, the average bacterium contains around femtogram (1 fg or 10^{-15} g) of ATP. Figures for bacteria ranging from 0.1 to 5.5 fg have been found. There are a variety of reasons for the variation in ATP content per bacteria ***(Quesneau, 1983)***

Nordlund et al., (1980) said that it is known that the ATP concentration varies throughout the microbial growth cycle. It is lower in the stationary phase. *VanCrombrugge & Waes,(1991)* found that The variation also may originate due to the assay procedures, different ATP extraction procedures are used, certain extraction reagents can give stress situations and consequently low ATP values. They also added that the variation can also arise from the fact that different enzyme preparation and ATP standard solutions are used to determine and quantify the extracted ATP. for further explanation said that in relating the ATP to the colony forming units (cfu), when using the plate count method, one has to take into consideration the clumping of bacteria, ignoring this fact can give a high ATP value for a bacterial cell. Nevertheless, under controlled conditions, ATP concentration within the viable cell is reasonably constant.

Stollenwerk et al., (1998) reported that, cells of strains of the same species were shown to differ significantly in their basal ATP content. Since the ATP concentration of bacterial strains of different species varies and is also influenced by the growth conditions of bacteria (solid or liquid culture medium), a species standard curve has to be established for bacteria grown under the same culture conditions.

ATP in Milk:

Little is known about adenine nucleotides of bovine milk. In an early investigation, ***(Kay & Marshall,1928)*** reported the presence of up to 33 mg adenine nucleotides/ L. of goat's milk. However, these workers were not able to demonstrate adenine nucleotides in milk from cows or human.

However for reasons discussed subsequently, it is possible that small amounts of ATP could exist sequestered in the colloidal calcium phosphate citrate (CPC) complex that is intimately associated with the casein micelles in bovine milk ***(Mc Gann&Pyne, 1960). Brunner,(1974); Johnson, (1974)*** suggested that in view of the presence of phosphatases and ATPases in raw bovine milk, it is unlikely that ATP could exist free in milk serum.

Zulak et al.,(1976) studied the behavior of ATP in goat's milk and stated that milk left in the goat's udder overnight had less ATP than fresh secreted milk. In similar experiments the skim milk derived from whole milk accumulated in the udder overnight was lower in ATP than skim milk from freshly secreted milk. In comparison ATP also found that, the ATP concentration of all the human and bovine milk samples were less than of goat's milk even for milk accumulating in the goat's udder overnight. Reasons for these

variations are not known and will require a more extensive species-comparative study.

Richardson et al., (1980) studied the level and location of ATP in bovine milk. Approximately 0.2 µmol ATP/l milk serum were evident both in whole milks and the corresponding skim-milks. ATP was not detectable in skim-milk ultra filtrates. These findings indicated that ATP was present in a non-dialysable portion of skim milk. However, the ATP in the skim-milks decreased by less than 20% compared with the whole milks indicating that the calcium phosphate-citrate (CPC)--caseinate micelles were the source of the ATP. ATP was not detectable in colloidal phosphate-free milk, from which CPC had been removed, confirming that the ATP was sequestered in the constituent CPC. Likewise, the occurrence of significant amounts of Mg, another potent stabilizer of amorphous calcium phosphate (ACP) in other biological systems, was confirmed in the colloidal phosphate of milk.

Emanuelson et al.,(1988) studied effect of parity and stage of lactation on ATP and reported that, in relation to lactation stage , the concentrations were high shortly after parturition, fell rapidly during the first 30 days and then showed a slight increase towards the end of lactation. In relation to lactation number, it can be seen that, the concentrations during second lactation were invariably lower than during third and later lactation. This pattern is similar in both

apparently normal milk and surely healthy subset except that in the healthy subsets the initial drop in concentrations was slightly more pronounced and the levels in mid lactation were somewhat lower.

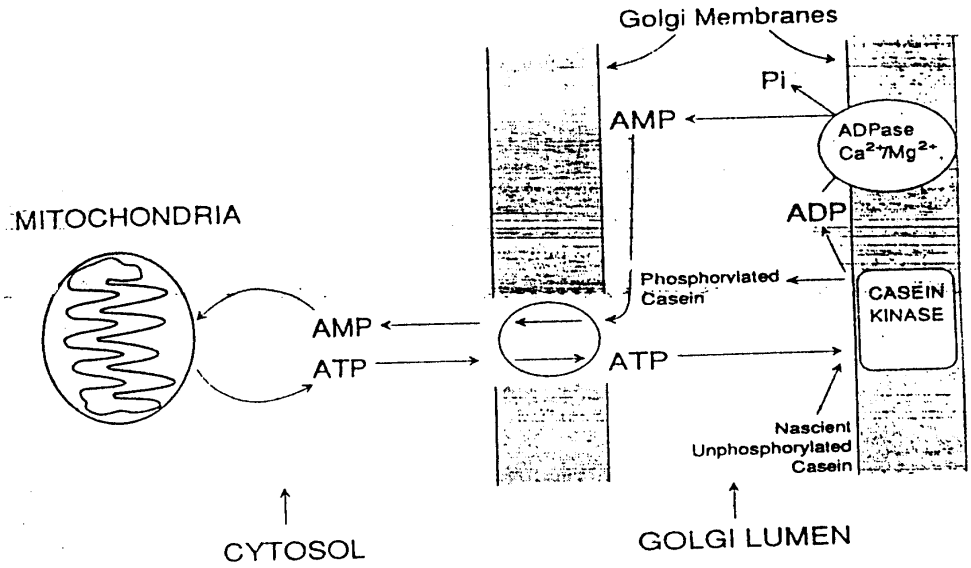
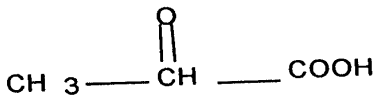


Figure (2): *The ATP cycle in lactating mammary gland* ***(in Basch et al.,1992).*** *The adenosine-5'-diphosphatase (ADPase) described in this manuscript completes the cycle necessary to transport the ATP required to phosphorylate casein in Golgi apparatus The ATP↔ AMP transport* ***(Capasso et al., 1982)*** *and casein kinase* ***(Bingham et al., 1972 ; Szymanski and Farrell, 1982)*** *have previously been described for Golgi Apparatus from lactating mammary glands.*

Pyruvic Acid:

Chemistry:

Pyruvic acid is also named (2-oxopropionic acid) and its formula is



so it is a typical ∝-keto acid.

Pyruvic acid may be prepared by hydrolysis of the corresponding nitrate or dihalosubstituted acid, or by oxidation of the corresponding hydroxy acid:

$CH_3\ Co\ CL \xrightarrow{Cu\ CN} CH_3\ Co\ CN \xrightarrow{H\ H_2O} CH_3\ Co\ Co_2\ H$

$CH_3\ C\ Br\ Co_2\ H \xrightarrow{H2O} CH_3\ Co\ Co_2\ H$

$CH_3\ CH\ (OH)\ Co_2\ H \xrightarrow{Cr\ O3} CH_3\ Co\ Co_2\ H$

Pyruvic acid is, however, most conveniently obtained by hydrolysis of tartaric acid, a preparation not available for other ∝-keto acids. Pyruvic acid is a viscous liquid, has boiling point of 165°C, miscible with water, it also has the normal properties of carboxylic acids, forming salts, esters, amides, etc., and also exhibits many normal properties forming an oxime and phenylhydrazone. Pyruvic acid reacts with halogens readily, substitution occurring via formation of (the enol tautomer) which also of biological importance ***(Taylor,1983)***

Pyruvic acid is a central intermediary metabolite of most bacteria in milk. It is produced in glycolysis through Embden-Meyerhof Parnas, the Dickens-Horecker and the Entner-Doudoroff pathways, the former being primary for homofermentative lactic acid and the latter being primary for many psychrotrophs. Pyruvate is also produced from deaminated amino acids and from free fatty acids*(Marshall& Harmon, 1978).*

Tolle et al., (1976) constructed a model of pyruvate formation, excretion and utilization as may occur within microbial cells (Fig. 3).

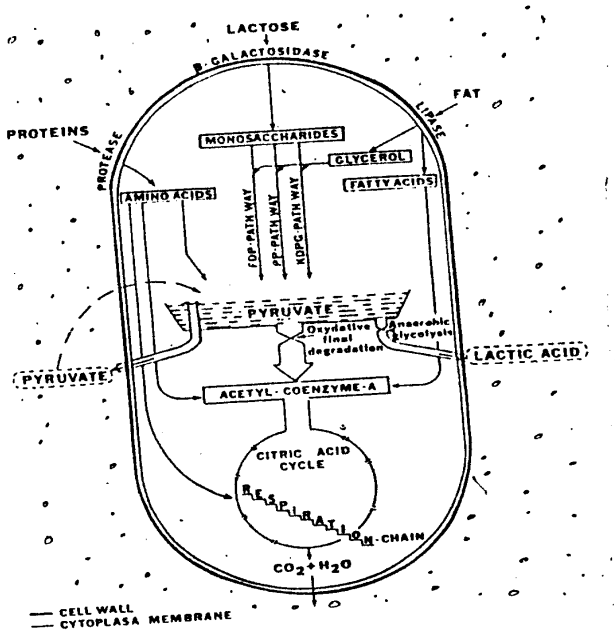


Figure (6): *Model of Pyruvate formation, utilization and excretion by the microbial cell in milk(After:* ***Tolle and Heeschen,*** *Commission of the European Communities, No., 21. September, 1976)*

Pyruvic acid and Bacterial count in milk:

Immediately after production milk contain 0.5 - 1.5 mg/ l ***(Tolle et al., 1972; Zandstra & de Vries, 1977; Marshall& Harmon, 1978).*** Also higher concentrations (5 mg/ l) were recorded ***(Marshall & Harmon, 1978).***

Tolle et al., (1972) have already studied that, to take the extreme cases, a high initial contamination without multiplication of organisms can not be measured as an increase in the basic value of the pyruvate content, whereas on the other hand relatively small numbers of bacteria which are metabolically active during along period lead to an obvious increase in the concentration of pyruvate.

The same author also published a report of extensive studies in which they examined numerous test of milk quality. These include pyruvate, dye reduction, alcohol coagulation and clot-on-boiling as well as total and selective bacterial plate counts. They concluded that the automated test for pyruvate gave promise of being highly useful in determining the quality of milk. They also found that pyruvate not destroyed by pasteurization. ***Heeschen et al., (1975)*** also studied thoroughly the determination of pyruvate in milk and proposed it for evaluation of the hygienic quality of milk.

For individual farm milk stored at refrigeration temperatures, there is no close relationship between pyruvate

values and viable bacterium counts determination at intervals during storage ***(Toll et al., 1976;Zandstra & de Vries, 1977).*** Pyruvate is related to bacterial activity and not to bacterial count and the initial viable bacterial count of individual farm milk ***(Zandstra & de Vries, 1977)***

Weisner, (1975);Zandstra &de Vries, (1977) found an important phenomenon that during first days of storage of milk at low temperatures the pyruvate content increase whereas the colony count remains at about the same level.

Slavchev, (1979) carried out a comparative study on the pyruvate and reductase tests applied in milk of varying hygiene properties. It was established that: just after milking the pyruvate and reductase tests have a low correlation with bacterial quality of milk; in milk cooled at 3 --5 °C for a longer period (72 hours) the pyruvate test has a good correlation with bacterial infestation of milk; following storage of milk at temperature above 10 °C the reductase test is better correlated with bacterial infestation of milk; the pyruvate test is appropriate for evaluation of bacterial activity in milk cooled at low temperature and transported every second day; the reductase test is appropriate evaluation of bacterial activity in not well cooled milk that is transported one day.

Cousins et al., (1981) said that during storage of milk at temperature permitting bacterial growth, pyruvate, an intermediary metabolite of most bacteria capable of multiplying in milk, is produced in excess of the metabolic requirement and accumulates in milk.

Slavchev, (1981) after testing of 302 samples, stated that the pyruvate test could not be used alone as a method in the evaluation of the clinical state of the udder, but could be referred to as an adjunct in the complex study of mammary gland.

Soderhjelm et al., (1982) carried out a comparative study between pyruvate test, ammonia test and bacterial count in milk and concluded that ammonia and pyruvate content vary within wide limits and that the correlation between these two chemical parameters and the total count of bacteria or the number of psychrotrophs is low. This fact is strengthened by the correlation between ammonia, pyruvate and bacterial counts in pure culture isolated from the raw milk.

Grappin & Dromard, (1982) found also low correlation between pyruvate and colony count in milk with low bacterial count but suggested that it can be used in developing countries whereas milk with high bacterial count.

Stahlhut & Rojahn, (1987) found that pyruvate level in milk affected by other physiological parameters rather than bacteria such as udder health, feeding and differences in the level of metabolic activity in the course of lactation, especially in high yielding cows, but not affected by seasonal variation ***(Sugeil et al., 1988).***

The pyruvate content was officially approved as a method for determination of the bacteriological quality of farm milk in the FRG, where the limit of first class milk decreased from 500 000 cfu/ ml (1980) to 300 000 cfu/ ml (1986) but it is expected to be no longer be applied in the milk quality payment scheme when the limit of first class milk decrease to 100 000 cfu/ ml ***(IDF, 1991).***

LDH Activity and Bacteria in Milk:

Lactic dehydrogenase (LDH) was firstly crystallized from rat muscle in 1943 ***(Kubowitz&Ott, 1943)***. LDH is a tetramer Mr 140 000. The enzyme is widely distributed in tissues and plays a key role in energy metabolism. LDH is a bisubstrate enzyme; the substrates consist of either lactate or pyruvate and a derivative of the hydrogen-carrying coenzyme, NAD. Most vertebrates possess at least two genes for LDH, which make similar but nonidentical polypeptides called M and H. In embryonic tissue, both genes are equally active, resulting in equimolar amounts of the two genes products and a stastistical array of tetramers

(M_4,M_3H_1, M_2H_2, H_3M_1 and H_4 in the ratios of 1:4:6:4:1). These forms are called isozymes, or isoenzymes. They can usually be detected by differing electrophoretic mobilities. As embryonic tissue multiplies and differentiates, the relative amounts of the M and H forms change. In pure heart tissue, which is considered aerobic, the H4 tetramer predominates. In skeletal muscle, which functions anaerobically, the M4 isozyme predominates***(Geoffrey, 1989).***

The finding of multiple molecular LDH forms in psychrotrophic bacteria is not surprising since the existence of subunit components of LDH in animal tissue has been demonstrated by several investigators ***(Chilson et al., 1965; Fritz , 1965; Hochachka, 1965 and Kaplan, 1963)*** . Also ***Yoshida, (1965)*** has reported that four LDH subunits exist in bacterium Bacillus subtilis.

LDH molecular behavior in animal tissues has been reported to be influenced by temperature, aeration, and the presence of citric acid intermediates. ***Fritz, (1965)*** working within rabbit muscle and the isoenzyme LDH 5, demonstrated that a change in the citric acid cycle occurred when citric acid intermediates accumulated. ***Hochachka,(1965)***using gold fish demonstrated that lower temperatures (i.e. 5°C vs. 20°C) appeared to increase the liver metabolic rates and specifically the activity of LDH.

Hohorst et al.,(1965) has reported LDH to be a key enzyme metabolic control of the oxidation-reduction potential of cells for glycolysis. These workers emphasized the necessity for control of reduction state of nicotinamide-adenine-dinucleotide (NAD)-dependent dehydrogenase systems which involve such key metabolites as pyruvate, oxaloacetate, and 1,3diphosphoglycerate.

The adaptive role of LDH in the bacterial cell has not been as well documented as it has been with animal cells. However, the work of ***Dennis & Kaplan,(1960); Ingraham & Stokes(1959)*** and ***Brown,(1957)*** did investigate the possible mechanisms involved in the adaptive response of specific microorganism. ***Dennis &Kaplan, (1960)*** investigating *Lactobacillus plantarium* reported the presence of a NAD independent LDH system as well as multiple substrate-LDH enzyme systems. ***Ingraham&Stokes(1959)***investigated a psychrophilic strain of *Pseudomonas* and mesophilic *E.coli* strain for possible enzyme systems involved with the adaptive mechanisms to low temperatures. He was unable to find significant enzyme activity difference from the effect of the growth temperature 10 °C for these organisms on the cell free dehydrogenase systems for malic acid, citric acid, and glucose-6-phosphate. ***Brown,(1957)*** observed a decrease in enzymatic activities of "psychrophiles" by lowering the temperature of growth which was less than the decrease observed for mesophils under similar conditions.

Vessel, (1968) has suggested that because the enzyme LDH is heterogeneous, it affords the organism metabolic advantages such as the existence of isozymes at different locations within the cell which may subserve various cellular metabolic requirements. Conceivably, by altering the environment, the cell has, in effect, altered the metabolic equilibrium within its interior, as an aid in the adaptation to the new environmental condition. One possible explanation for this adaptive response may be the availability of different balance of metabolites to the cell for forming its cellular substances and obtaining cellular energy.

While it is recognized that protein synthesis is under the control of the gene, it follows that the isozyme formation is also directly related to gene control. In this context, ***Markert, (1968)*** stated that gene controlling isozymees not only exhibit extreme specificity of cell function but the degree of their activity varies in a controlled fashion over a wide range. It was observed that the degree of environmental influence on the LDH enzyme behavior appeared to be species related with possible genus implication since both *Pseudomonas species* demonstrated similar type adjustments in their LDH pattern ***(Chambers&Harper,1975)***.

Bogin & Avidar, (1976) examined udder slices removed from cows with normal udders, udders with acute mastitis and udders

characterized by extensive fibrosis to determine the activity of lactate dehydrogenase (LDH).

They found that the activity of LDH in abnormal udders was slightly elevated, and was far higher than that of blood serum. This explains that the blood not the origin of the high levels of LDH in mastitic milk.

Bogin et al., (1977) presumed that LDH was released into milk by leukocytes, udder tissues and bacteria.

Hassan, (1980) subjected 202 milk samples collected from 140 lactating buffaloes and 62 cows to biochemical and bacteriological examinations. He found a significant increase in LDH content in milk obtained from subclinically mastitic animals especially when the isolated bacteria was Strept. agalctiae. .

Hambitzer&Sommer, (1987) developed a new method to determine the activity of LDH in milk. The determination of LDH was performed with milk serum after high-speed centrifugation of skimmed milk. The method yields a high recovery, accuracy and linearity over a wide range of LDH activity. They found a high and significant correlation between the LDH activity and cellular content in milk.

Kato et al., (1989) studied the patterns of LDH isoenzymes in mastitic milk and in granulocytes, monocytes, platelets and lymphocytes (T&B). They found that the patterns of lymphocytes and granulocytes were similar to those of mastitic milk. It is suggested that granulocytes and lymphocytes at least partly contribute to the origin of LDH isoenzymes in mastitic milk.

Nizamlioglu & Ergains, (1991) found a significant positive correlation between LDH activity and somatic cell count in ewe's milk. They found that the mean activity of LDH in normal milk was 57.08 ±16 U/ ml and 875±140.7 U/ ml in mastitic ewe's milk. Values higher than 85 U/ L in thawed and centrifuged quarter milk samples indicate pathological process ***(Anderrson, 1991).***

Lipperheide et al., (1995) reported that values of LDH higher than 85 U/L in bovine milk indicates a pathological process in the examined udders.

Ammonia and Bacterial Count in Milk:

Ammonia is an end product of protein catabolism. The NH_3 resulting from deamination of amino acids is converted to ammonia either directly or indirectly (e.g., by means of a transamination to yield a readily deaminated product such as glutamate). In microorganisms using a single amino acid as a nitrogen source, the ammonia so liberated is assimilated and used to form other nitrogen-containing cell components. When the amino acid is carbon source, much more ammonia is liberated than is needed for biosynthesis, and it is disposed of by excretion to the medium. This simple disposal mechanism is adequate for free-living microorganisms, since the ammonia is carried away in the surrounding medium or escapes into the atmosphere. In the other hand ammonia is the form in which nitrogen is incorporated into organic material, but it is less often available to plants or bacteria for biosynthesis than other forms of nitrogen. When present for any length of time in nature, ammonia will either be assimilated into organic materials or be oxidized by nitrifying bacteria (such as Nitrosmonas and Nitrobacter) to nitrite and nitrate. Reduction of nitrate by plants or by bacteria seldom yields ammonia in excess. Nitrogen fixation, by which nitrogen of the atmosphere is reduced to ammonia, occurs in a very limited number of microorganisms and plants. Thus most plants must assimilate ammonia at the low levels that are generated by their own nitrate and nitrite reductases or from the reduction of nitrogen by microorganisms ***(Geoffrey, 1989)***

Storgards & Lindqvist, (1962) demonstrated that the ammonia content of milk changed more rapidly during the storage of milk than did any of the numerous ninhydrin positive substances present. Due to the rather in convenient methods available at that time for ammonia determination no further work was done. The advent of the ammonia electrode made it possible to measure the ammonia content directly in milk in a few minutes. ***Helaine,(1979)*** reported its successful use in connection with the reception of milk to the dairy.

Lately for the determination of the bacteriological quality of raw milk among the numerous proteolytic metabolites' ammonia is discussed ***(Soderhjelm & Lindqvist,1980)***.Freshly drawn milk has an ammonia content among 3.0 and 6.0 mg/kg (0.20 mM: o.35 mM) ***(Heeschen, 1972; Soderhjelm & Lindqvist 1980).*** Ammonia value greater than 8.5 mg/kg (over o.5 mM) indicates inferior quality ***(Suhren et al., 1976).*** The studies on lactating black and white cows have shown that the ammonia in colostrum and milk of cows is higher in winter and spring ***(Sugeil et al., 1988)***

According to results from experiments with raw milk and with pure cultures, the bacterial count must be very high (10^7 - 10^8 cfu /ml) before ammonia content increases significantly ***(Soderhjelm et al.,1982).Zangerl e (1986)*** also reported that ammonia content was considered to be not useful indicator for the bacteriological quality of raw milk with less than 10^8 cfu/ml.So in those areas where higher

limits of grading are in use, the application of this practicable parameter might be good tool in quality Payment scheme.*(Suhren & Heeschen, 1991)*

After determination of ammonia by both specific electrode and segmented flow system in 20 large e bulk milks (10 000: 25 000 liters), ***Pinelli,(1991)*** concluded that ammonia content of large bulk milk is an index of its total hygienic quality (freshness), useful for real time milk selection and quality improvement both for raw and pasteurized milk also of unknown hygienic history of production, storage and transport. The ammonia is rapid, efficient, simple, cheap and easy to automate.

MATERIALS & METHODS

Plan of The work

Twenty farm bulk tank milk samples ***(Richardson, 1985)*** were obtained from Mehalet Mousa dairy farm (belonging to Animal production Research Institute). The samples under study were transferred directly to the laboratory in the Faculty of Vet. Med. at Kafr El-Sheikh city for analysis.

The analysis was performed at the following time intervals:

1- At the time of arrival .

2- After 24 hours at 8 °C .

3- After 48 hours at 8 °C .

4- After 72 hours at 8 °C .

The obtained farm bulk tank milk samples were tested for determination of :

a - Bacterial ATP in milk .

b - Ammonia in milk .

c - Pyruvic acid in milk .

d - Lactate dehydrogenase activity (LDH) in milk.

e - Total Colony Count (TCC).

Sampling Procedure .

A - Equipment for collecting samples :

1 - Thermometer : mercury -filled , dial type , graduation intervals not to exceed 1°C.

2 - Sample transfer instruments : stainless steel metal dipper with long handle.

3-Sample containers: 100 ml-bottles,with tightly closed and leak-proof, non toxic , non bacteriostatic, nonbactericidal, and sterilized by dry heat

4-Sample case: rigid plastic construction, (ice box) completely insulated, and tightly covered leaving a space for crushed ice to cool samples and to maintain samples at 0 °C to 4.4°C until reaching to the laboratory. .

B - Cleaning , Sterilizing , and Sanitizing equipments:

1-At first all equipments were washed by detergent (Potassium Hydroxide).

2-Equipments were rinsed by pure water to remove residues of the detergent

3- Glassware were sterilized by hot dry air in the hot air oven .

4- Other objectives were sterilized in the autoclave .

5- Equipments were removed immediately before sample collection

C - The procedure :

1-Before measuring and sampling milk, hands were washed and dried and kept clean during the sampling operation

2-In a farm holding cooling tank milk was agitated at least 5 min. immediately before sampling .

3-when using dipper, it was removed from the sanitizing solution, completely drained, and rinsed at least twice in milk before transferring the sample.

4-Sample container was handled aseptically, not drop, or lay down .

5-pre-cooling multi-use sterile glass containers in sampler case .

6-Sample container filled not more than 3/4 full to permit proper mixing of sample at the laboratory.

7- All tanks were sampled individually and labeled.

8-Samples were placed immediately in sample case , surrounded by crushed ice but not frozen and transferred to the laboratory.

9-Part of each sample was taken for chemical tests, the other part was taken for TCC.

METHODS

I - Determination of bacterial ATP .

(A) Hydrolysis of non bacterial ATP :

Principle:

Hydrolysis of ATP was done according to ***Theron e t al .,(1986)***

Triton x -100 makes somatic cell wall permeable which enables releasing of ATP but the microbial cells [yeast, molds, bacteria] are normally not affected by this reagent, then the released ATP is then hydrolyzed by ATP- ase [Apyrase] with other free ATP in milk.

Reagents:

(1) Triton x - 100: 0 . 2 % (v/v)

triton ..... o .2 ml (Sigma)

dist. water ..... 100 ml

(2) EDTA : 10 mM (w/v)

EDTA ..... 3.722 g [Titriplex III (Merck)]

triton 0 .2 % ..... 1000 ml

(3) Apyrase : 0.5 or 1 unit

Apyrase ..... 200 unit [Grade I, (Sigma)]

triton 0.2%..... 5 ml

Procedure :

Milk sample (0.1 ml) was mixed with 0.5 ml [Triton x-100 (0.2 %)- EDTA-Apyrase]mixture. This mixture was vigorously shaken for 15 min. at 24° C on vortex mixer to aid release and hydrolysis of non bacterial ATP.

(B) Extraction of Bacterial A.T.P :

The extraction procedure was done according to ***Bagnara& Finch,(1972)***

Reagents :

(1) perchloric Acid ($HCLO_4$) : (AR) 1.2 M (V/V)

$HCLO_4$..... 12.1 ml

dist. water ..... add to 1 Lit

(2) Potassium Hydroxide (KOH) : 0.72 M (W / V)

KOH ..... 40.4 g .

dist .water.....add to 1 lit .

* KOH was dissolved in water

(3) Potassium Bicarbonate ($KHCO_3$) : 0.16 M (W / V)

$K HCO_3$ ..... 16.2 g .

K OH 0.72 M ..... add.to 1 lit .

Procedure :

A 200µl of sample were pipetted directly into 100 µ l of 1.2 *M* $HCLO_4$ at 0 °C in a centrifuge tube. The tube was shaken on vortex mixer for 10 sec., left to stand in ice for a further 15 min., and then centrifuged in the cold for 10 min. at 2500g. A200µl from the supernatant was then taken and immediately neutralized with 100µl of 0.72 *M* K OH containing 0.16 *M* K HCO_3. This mixed solution allowed 90 % neutralization of the $HCLO_4$ by K OH so that the remaining H CLO_4 required approximately half of the K HCO_3 to complete its neutralization. This procedure gave effective precipitation of the perchlorate and left the residual solution buffered near pH 7. The K CLO_4 was precipitated by a further short centrifugation.

C - Assay of The Extracted ATP :

Principle

The ATP in milk was measured spectrophotometrically following the method of ***Bucher, (1947) & Adams, (1963).*** During assay of ATP the spectrophotometric technique was done which is highly sensitive , can detect as 10^{-8} moles ATP, other authors used bioluminescence but it is not widely available in our laboratories.

In the described procedure , the enzyme , phosphoglycerate phosphokinase (PGK) , is used to catalyze the following reaction :

$$\text{ATP} + \text{3-Phosphoglycerate} \xrightarrow{\text{PGK}} \text{ADP} + \text{1,3-Diphosphoglycerate}$$

The enzyme glyceraldhyde phosphate dehydrogenase (GAPD) is also present in the reaction mixture to catalyze the following :

1,3-Diphosphoglycerate + NADH $\xrightarrow{GAPD}$ Glyceraldehyde-3-P+ NAD + P

By determining the decrease in absorbency at 340 nm that results when NADH is oxidized to NAD , a measure of the amount of ATP originally present is obtained .

Reagents :

All reagents were obtained from Sigma Chemical co ., USA .

(1) NADH , Prweighed Vials , (0.3 mg / vial)

* Vials are stored at room temperature.

(2) PGA buffered solution .

buffered solution of 3- phosphoglyceric acid , 18 mmol / L , magnesium ions and EDTA. Chloroform added as preservative .

* solution is stored refrigerated (2-8 °C).

(3) GAPD / PGK enzyme mixture .

Suspension in ammonium sulfate of glyceraldhyde3-phosphate dehydrogenase (rabbit muscle) , 800 units / ml and 3-phosphoglyceric phosphokinase (yeast), 450 unit / ml when prepared .

* solution is stored refrigerated (2-8°C)

(4) ATP , disodium .

* stored below 0 °C

All reagents are supplied ready for use .

procedure :

(1) Into a 0.3 mg NADH vial , pipette in the order indicated :

1.0 ml PGA buffered solution .

1.5 ml dist.water .

0.5 ml sample .

The vial was capped and inverted several times to dissolve NADH .

(2) Entire contents were decanted into a cuvette . Read and recorded the INITIAL absorbency A Vs water as reference at 340 nm .

*The INITIAL should not be less than 0.6 . A Lower value , indicates NADH decomposition.

(3) Into the cuvette, 0.04 ml GAPD/PGK enzyme mixture was pipetted and mixed by inversion and cuvette was replaced in the instrument.

(4)The absorbency was read and recorded Vs water as reference at 340 nm. readings continued until the minimum absorbency reading was reached (usually requires less than 10 minutes) .This is record as FINAL reading.

Calculation :

$$ATP\ (\mu mol/ml) = \frac{\Delta A \times 3.04}{6.22 \times V}$$

Where ΔA = INITIAL - FINAL

3.04 = volume of liquid in the cuvette

6.22 = Millimolar absorptivity of NADH at 340 nm

V = volume of sample

ATP mg /ml = ATP µmol / ml x 0.611

(mg = 10^{-12} fg)

Based on molecular weight of ATP , disodium ,with 3 molecules of water and 99% purity .

II-Determination of Pyruvic Acid in Milk :

The Pyruvic Acid in milk was measured colorimetrically following the Dinitrophenylhydrazine (DNPH) method of ***Friedman & Haugen, (1943)***

Principle :

Deproteinated sample is treated with 2,4 DNPH forming 2,4dinitrophenyl-hydrazone which reacts with strong alkali to form a reddish compound that is estimated photometrically.

Other ketoacids react to form similar hydrazones but the pyruvic hydrazone can be preferentially extracted with sodium carbonate .

Reagents:

(1) Hydrochloric acid (H CL) (2 N) : (V/V) 100 ml

H CL..... 18 ml (Merck)

Dist Water .....add to 100 ml

(2) 2,4 DNPH solution : (W/V) 100 ml

DNPH ..... 100 mg (Aldrich)

H CL 2 N ..... add to 100 ml

DNPH was dissolved in volume of acid then add to 100 ml acid

(prepared freshly each week)

(3) Trichloroacetic Acid (TCA) 10 % : (W/V) 100 ml

TCA ..... 10 g (Merck)

dist water ..... add to 100 ml

Acid crystals were dissolved in volume of water then added to 100ml.

(4) Sodium Carbonate ($Na_2 CO_3$) 10 % : (W/V) 100 ml

$Na_2 CO_3$ ..... 10 g

dist water..... add to 100 ml.

$Na_2 CO_3$ was dissolved in volume of water then added to 100 ml

(5) Sodium Hydroxide (Na OH) 1.5 N: (W /V) 100ml

Na OH ..... 6 g

dist water ..... 100 ml

Na OH was dissolved in water volume then added to 100 ml

(6) Sodium Hydroxide 2.5 N : (W/V) 100 ml

Na OH ..... 10 g

dist water..... 100 ml

Na OH was dissolved in volume of water and added to 100 ml .

(7) Standard pyruvic acid solution 0.02 M : (W/V) 100 ml

Pyruvic acid ..... 10 mg

dist water ..... add to 1000 ml

Pyruvic Acid was dissolved in volume of water and added to 1000 ml

Procedure :

Two ml of the sample were poured to 10 ml of 10 % TCA in centrifuge tub, stoppered, mixed well and centrifuged at 3000 r.p.m/ min. Three ml of clear supernatant were pipetted into 18 x 150 mm test tube and the temperature was adjusted to 25°C. One ml of DNPH was added and allowed to react at room temperature for exactly 5 min. Three ml of xylene were added (or toluene or benzene) and a stream of air was passed through the the mixture for 2 min. After settling has occurred, the lower layer was removed and discarded by means of dropper with capillary tip. Six ml of 10 % sod . carb. was added and again mixed by bubbling air through the mixture for 2 min. After permitting the mixture to settle, 5 ml of the aqueous (lower) layer was mixed with 5 ml of 1.5 N Na OH soln. The obtained colour was measured colorimetrically after 5 min. at 520 nm.

Calculation:

$$\frac{\textit{The reading of sample}}{\textit{The reading of standard}} \times 1 = \text{mg Pyruvic acid/}$$

III - Determination of LDH Activity in Milk:

LDH in milk was measured enzymatically following the method of ***Wroblewski & La Due ,(1955)***

Principle:

The principle of the test is based on LDH mediated reaction

$$Pyruvate + NADH + H^{+} \xrightarrow{LDH} Lactate + NAD^{+}$$

Sample is mixed with sodium pyruvate in the presence of NADH ,the rate of oxidation of the NADH to NAD is measured by reduction of optical density of mixture at 340 nm .

Reagents:

1 - Buffer Solution : (w/v)

Anhydrous dipotassium phoshate (K_2HPO_4) ..... 13.97 gm.

Anhydrous monopotassium phosphate (K H2 PO4) ..... 2.69 gm.

Dist. Water ad to ..... 1000 ml.

2 - NADH Solution : (w/v)

NADH ..... 5 mg (Grade I : BDH)

Buffer solution ..... 2 ml.

*Prepared within one hour before use.

3- Sodium pyruvate Solution : (w/v)

Sodium pyruvate ..... 5 mg. (Merck)

Buffer solution .....2 ml.

Procedure :

*** Milk sample preparation :** ***(Hambitzer&Sommer 1987)***

To determine LDH activity in milk samples , skim milk was prepared by centrifuging (2000 xg, 15 min.), 500 µl of the skimmed milk were diluted with 500 µl redistilled water and centrifuged for 40 min. at 3000 xg to obtain a slightly opalescent milk serum in the supernatant*(2) .

Assay procedure:

A - Blank :

In the cuvette, the following were mixed

2.4 ml Buffer solution ,
0.1ml Sample ,
0.1 ml NADH solution.

Permitted to stand at room temperature for 20 minutes , then placed in the spectrophotometer and zero the instrument at 340 nm .

NOTES

*(1) The sedimentation of light scattering lipid-protein complexes is facilitated by the dilution of skim milk with redistilled water due to the reduced viscosity and specific density. Furthermore recommended dilution of skim milk samples , since very high LDH activities were often found in the non diluted skim milk , which exceed the measuring range . However in no case was the LDH activity below the measuring range ,even in the diluted milk samples , therefore , the sensitivity of the method is not reduced by this dilution step *(Sommer, 1987)*.

*(2) The extinction of the NADH-buffer -solution decreases immediately after the skim milk sample is added and remains constant after 10 minutes for at least the following 30 minutes . This decrease of the extinction results either from the NADH-buffer-solution and the sample being different in their optical density , from the dissociation of the protein-lid-complexes in milk, or from the consumption of NADH before the addition of the starting reagent . In the latter case, the reaction is started by trace amounts of pyruvate in the milk , particularly in bacterially contaminated samples . The small loss of NADH does not impair the following determination of the LDH activity , since the samples of NADH remains well above the concentration which is necessary for the LDH-maximum activity .

B - Test :

In the cuvette mix , 2.4 ml Buffer solution ,
0.1 ml Sample ,
0.1 ml NADH solution.

Allow to stand at room temperature for 10*(3) minutes, then add
0.1 ml Sodium pyruvate solution ,

Read the optical densityat 30 seconds intervalfor a total 10 readings.

Calculation :

LDH activity (U/L) of sample at 340 nm.

= Δ E/min. X 10 000 (acc.to *Worblewski & La Due, 1955)*[4]

= Δ E/min. X 4820 (acc.to *Racker et al., 1958)*[5]

Where Δ E = Decrease in the optical density

*(4) According to Wroblewski and La Due a unit is the amount of LDH which changes the optical density of NADH at 340 nm.by 0.001 in 1 minute at 27°C

*(5) According to Racker et al., a unit is the amount of enzyme which enverts 1μ mol of substrate/minute at 25°C

IV - Determination of Ammonia

The ammonia in milk was measured spectrophotometrically following the method of ***Zanger & Ginger, (1986)***

priniple:

Ammonia combines with *a*-ketoglutarate and NADPH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NADP+ . The corresponding decrease in absorbance at 340 nm is proportional to the ammonia concentration in the sample.

a-ketoglutrate + NH_3 + *NADPH* $\xrightarrow{\text{GLDH}}$ *glutamate* + *NADP*$^+$

Reagents :

Reagents are obtained from **Randox Lab Ltd. UK .**

Contents	Initial Concentration Solutions
1- Reagent	
NADPH	O.26 mmol / l
a-ketoglutarate	3.88 mmol / l
2- Buffer	
Triethanolamine	0.15 mol / l, pH 8.6
3- GLDH	≥ 1200 U / ml
4- Standard	294 µmol / l (5 mg / dl)

Preparation of solutions:

1- Reagent

The contents of one vial 1 were reconstituted with 5 ml buffer 2 . Stable for 5 days at +15 to +25 °C or 3 weeks at +2 to +8 °C , in the absence of bacterial contamination .

2- Buffer

Contents ready for use . Stable up to the expiry date specified when stored at +2 to +8 °C

3- GLDH

Contents ready for use . Stable up to the expiry date specified when stored at +2 to +8 °C

4- Standard

Contents ready for use . Stable up to the expiry date when stored at +2 °C to +8 °C .

Procedure :

Wavelength:	340 nm
Cuvette :	1 cm light path
Temperature :	37 °C
Measurement:	against air

Pipette into cuvette :

	Reagent Blank	Standard	Sample
Sample	---	---	0.1 ml
Distilled water	0.1 ml	---	---
Standard	---	0.1 ml	--
Reagent	1.0 ml	1.0 ml	1.0 ml

The contents in each tube were mixed and allowed to stand for 5 min.Read initial absorbance of sample and blank (A_1).

GLDH 3	0.01 ml	0.01 ml	0.01 ml

Mix, and incubate for 5 min. Read final absorbance of sample and blank
(A_2)

Calculation:

A_{blnk} = Blank A1 - Blank A2

$A_{standard}$ = Standard A1 - Standard A2

A_{sample} = Sample A1 - Sample A2

$$\frac{A_{sample} - A_{blank}}{A_{standard} - A_{blankV}} \times 294 = \mu mol / l$$

VI-Total Colony Count:(Richardson,1985)

Medium:

Plate count agar medium was used, it consists of:

*Bacto-yeast extract 2.5 gm
*Bacto-yeast tryptone 5.0gm
*Dextrose 1.0gm
*Agar 15gm
*Dist.Water up to 1000 ml.
pH 6.8-7.0

Procedure:

Serial milk dilutions (1/10, 1/100, 1/1000, ... etc.) were done . One ml from each dilution was mixed with melted plate count agar(45°C) in a sterile petri dish. Inoculated plates were left to solidify before being incubated 32°C for three days. Plates showing 25 to 250 colonies were chosen for counting.

Calculation:

Total colony count/ml milk sample =

Number of colonies X rate of dilution.

Statistical Analysis:

The data obtained in this study representing the different variables were statically analyzed according to the methods described by ***Robert and James (1960) and Snedecor and Cochran (1969)*** showing:-

1-Sample maximum (Max.)
2-Sample minimum (Min.)
3-Sample mean
4-Sample standard error (SE)

The mean value of X was obtained from the sum of individuals (ΣX) divided by the number of samples (n) as follows

$$\bar{X} = \frac{\sum X}{n}$$

The standard error was calculated according to the formula :

$$S.D. = \sqrt{\frac{\sum X^2}{n-1} - \frac{(\sum X)^2}{n(n-1)}}$$

where :-

S .D . = standard diviation .

x = individual values

n = number of samples

$$\text{Coefficient of variation (C . V .)} = \frac{S.D.}{X} \times 100$$

$$\text{standard error (S. E .)} = \frac{S.D.}{X}$$

RESULTS

Table(1): bacterial ATP content (fg/ml) in raw bulk milk in relation to bacterial count (cfu/ml)

	ATP			B. C.		
	M ± SE.	Min.	Max.	M ± SE.	Min.	Max.
0	A $35.35X10^9$ ±3.98	$12X10^9$	$67X10^9$	A $0.38X10^8$ ±0.06	$0.06X10^8$	$1.45X10^8$
24	B $49.15X10^9$ ± 4.62	$24X10^9$	$91X10^9$	B $0.70X10^8$ ±0.06	$0.23X10^8$	$1.48X10^8$
48	C $75.15X10^9$ ± 6.44	$36X10^9$	$128X10^9$	C $1.93X10^8$ ±0.44	$0.31X10^8$	$6.45X10^8$
72	D $111.9X10^9$ ±7.15	$54X10^9$	$146X10^9$	D $6.92X10^8$ ±0.11	$0.62X10^8$	$6.92X10^8$

*Means with the same letter are not significantly different.

Fig. (1): Effect of storage time on ATP as related to bacterial count.

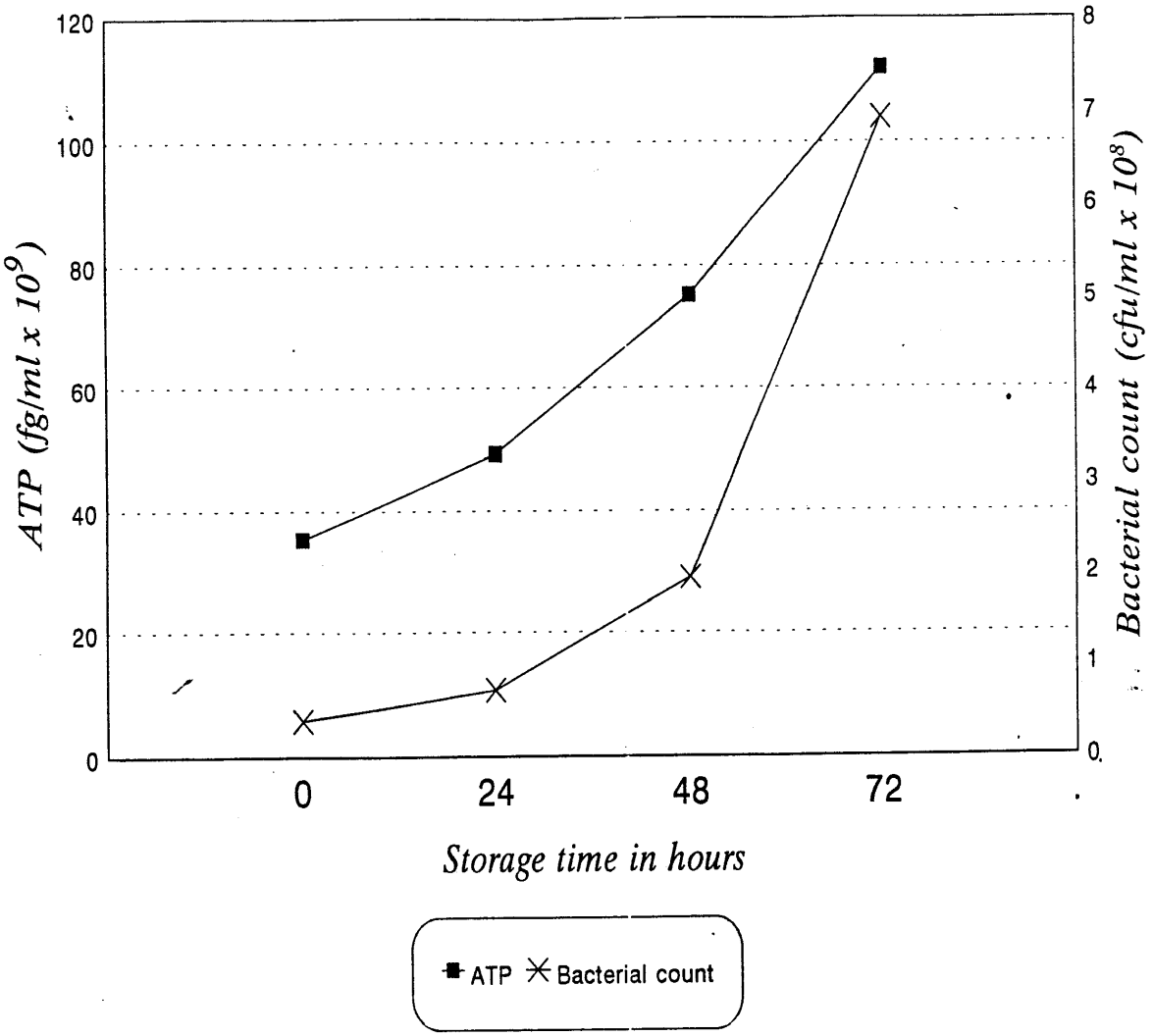


Fig. (2): Effect of storage time on ATP.....

Table (2): Pyruvic acid content (mg/l) in raw bulk milk in relation to bacterial count (cfu/ml)

	Pyr. A.			B. C.		
Time/Hours	**M ± SE.**	**Min.**	**Max.**	**M ± SE.**	**Min.**	**Max.**
0	A 7.34 ± 055	4.12	12	A $0.38X10^8$ ± 0.06	$0.06X10^8$	$1.45X10^8$
24	A 7.22 ± 0.53	4.32	12	B $0.70X10^8$ ± 0.06	$0.23X10^8$	$1.48X10^8$
48	B 8.75 ± 0.50	5.1	13.2	C $1.93X10^8$ ± 0.44	$0.31X10^8$	$6.45X10^8$
72	C 9.19± 0.53	4.96	13.2	D $6.92X10^8$ ± 0.11	$0.62X10^8$	$6.92X10^8$

*Means with the same letter are not significantly different.

Fig. (3): Effect of storage time on pyruvic acid as related to bacterial count.

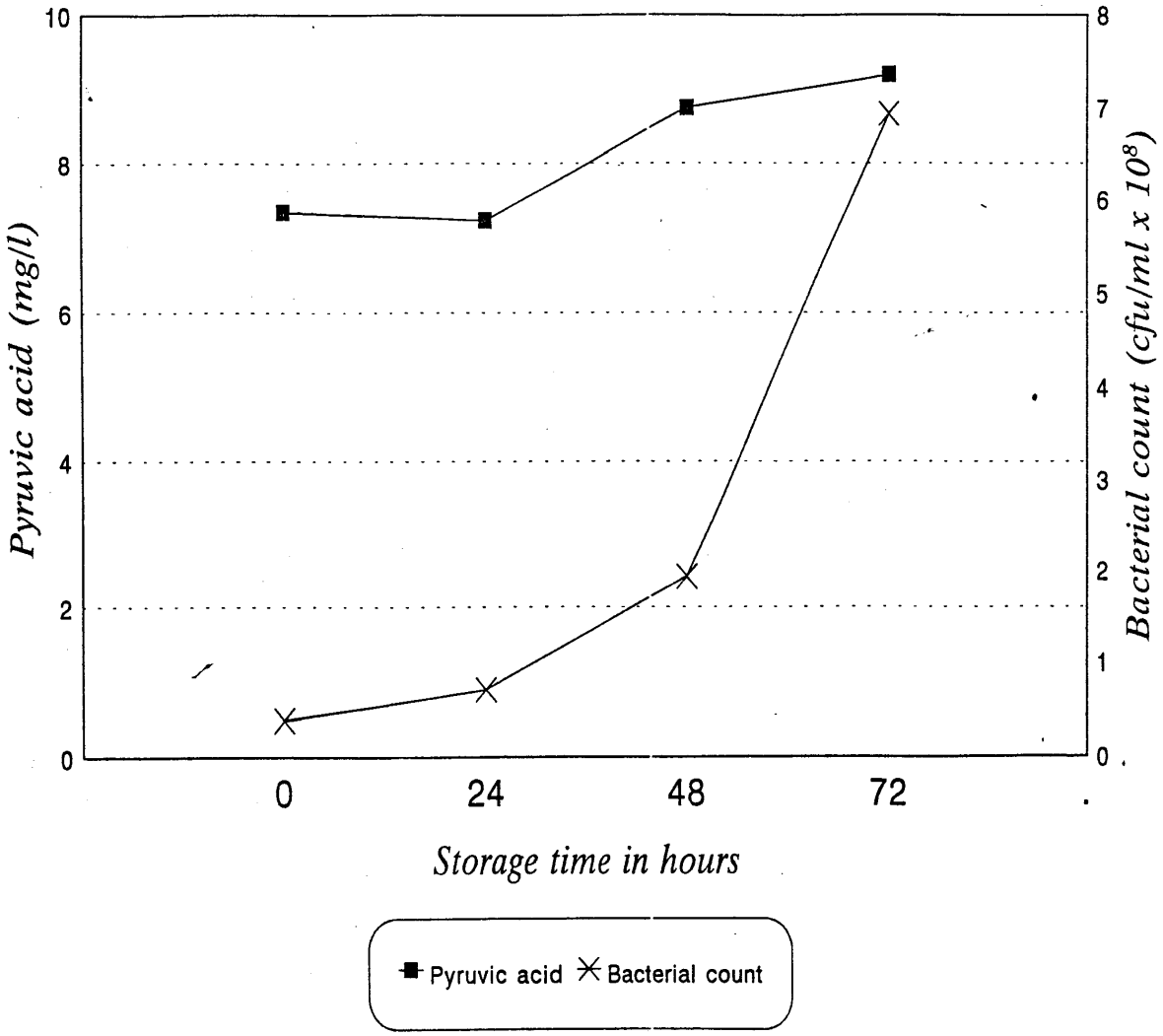


Fig. (4): Effect of storage time on pyruvic acid....

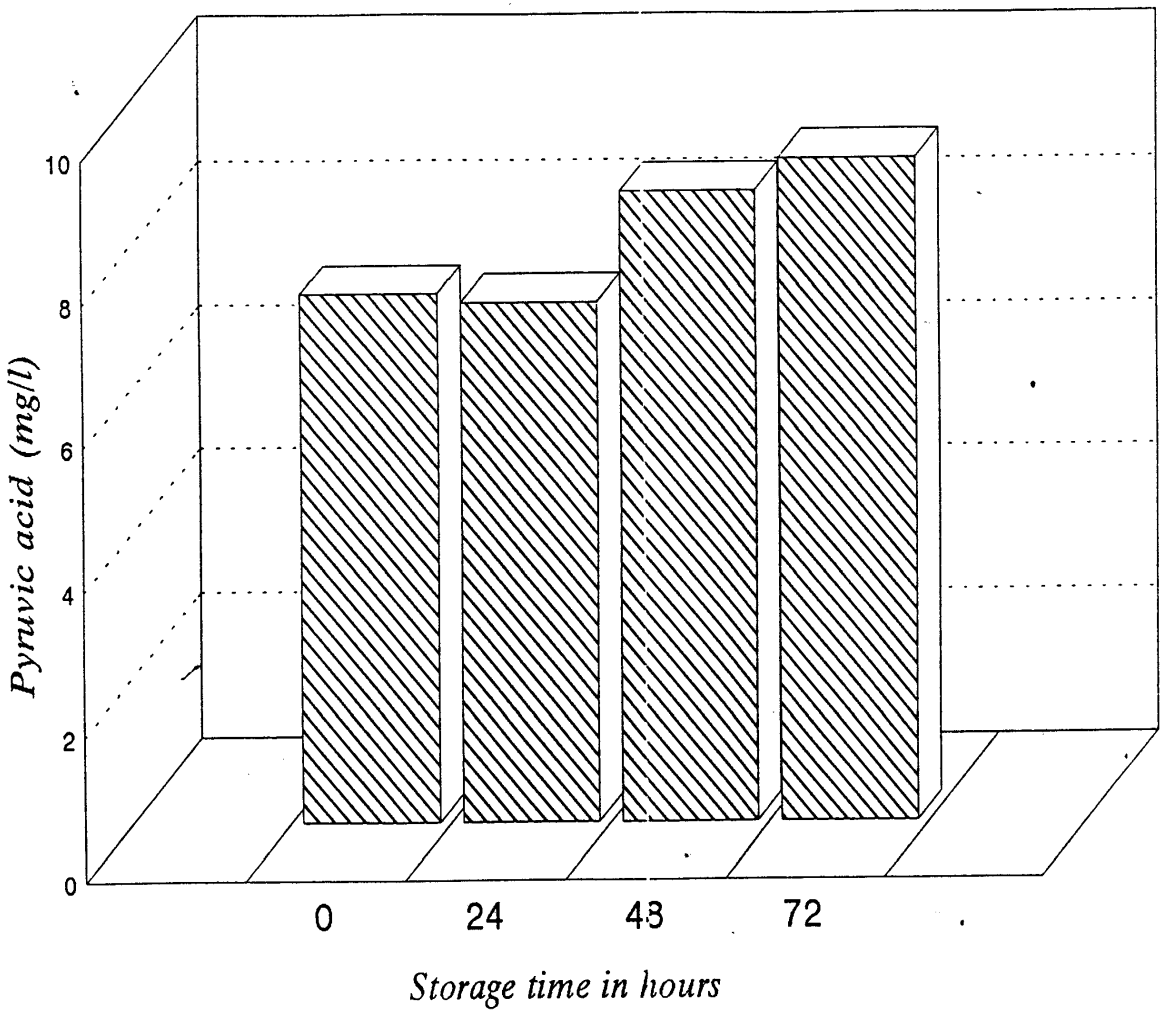


Table (3): LDH activity (U/l) in raw bulk milk in relation to bacterial count (cfu/ml)

	LDH			B. C.		
Time/Hours	M ± SE.	Min.	Max.	M ± SE.	Min.	Max.
0	A 80.46 ± 6.97	36.60	174.72	A $0.38X10^8$ ± 0.06	$0.06X10^8$	$1.45X10^8$
24	A 92.73 ± 8.90	33.60	177.79	B $0.70X10^8$ ± 0.06	$0.23X10^8$	$1.48X10^8$
48	A 72.81 ± 5.64	26.88	146.00	C $1.93X10^8$ ± 0.44	$0.31X10^8$	$6.45X10^8$
72	A 86.37 ± 6.79	33.60	151.50	D $6.92X10^8$ ± 0.11	$0.62X10^8$	$6.92X10^8$

*Means with the same letter are not significantly different.

Fig. (5): Effect of storage time on lactic dehydrogenase as related to bacterial count.

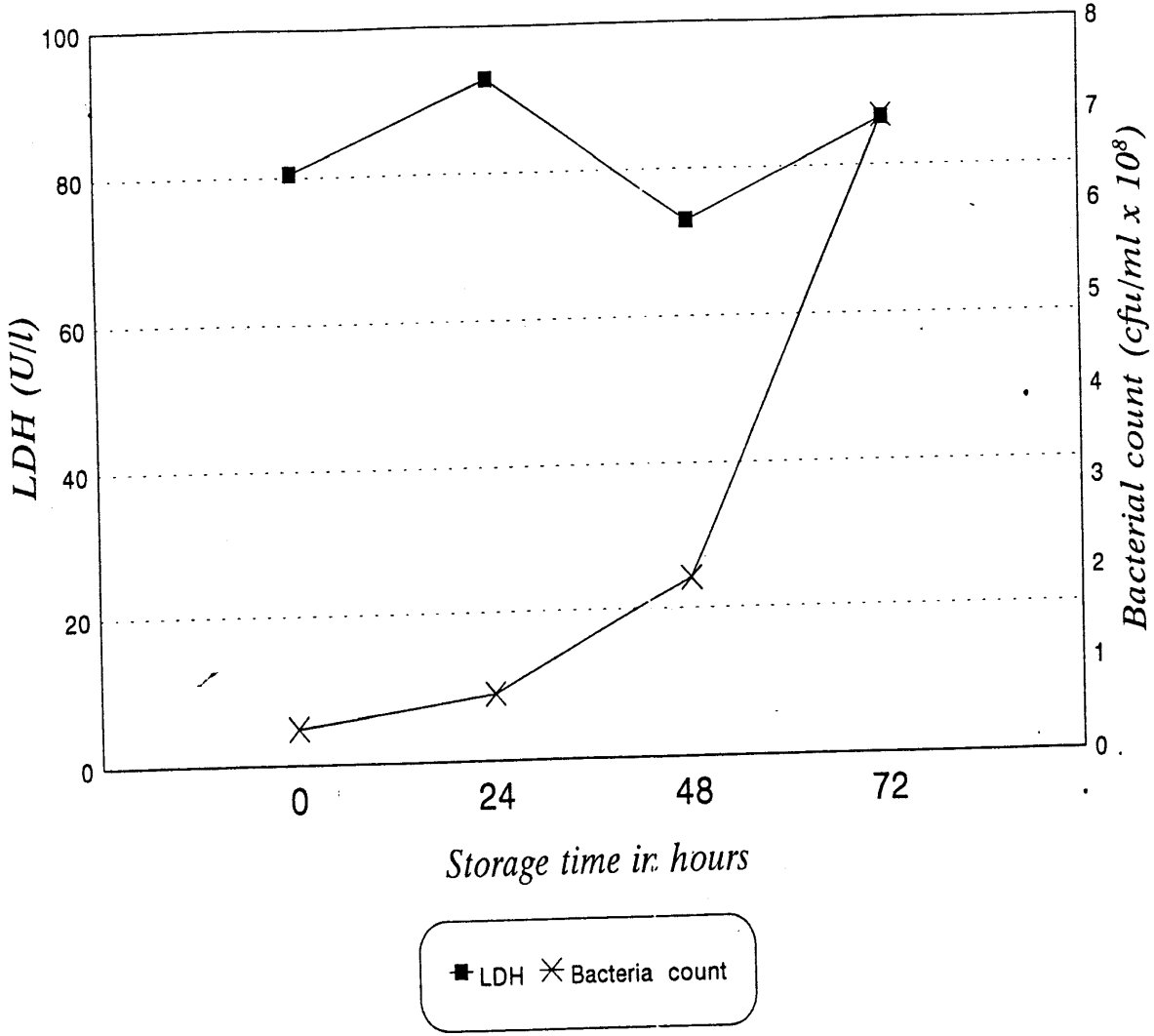


Fig. (6): Effect of storage time on lactic dehydrogenase...

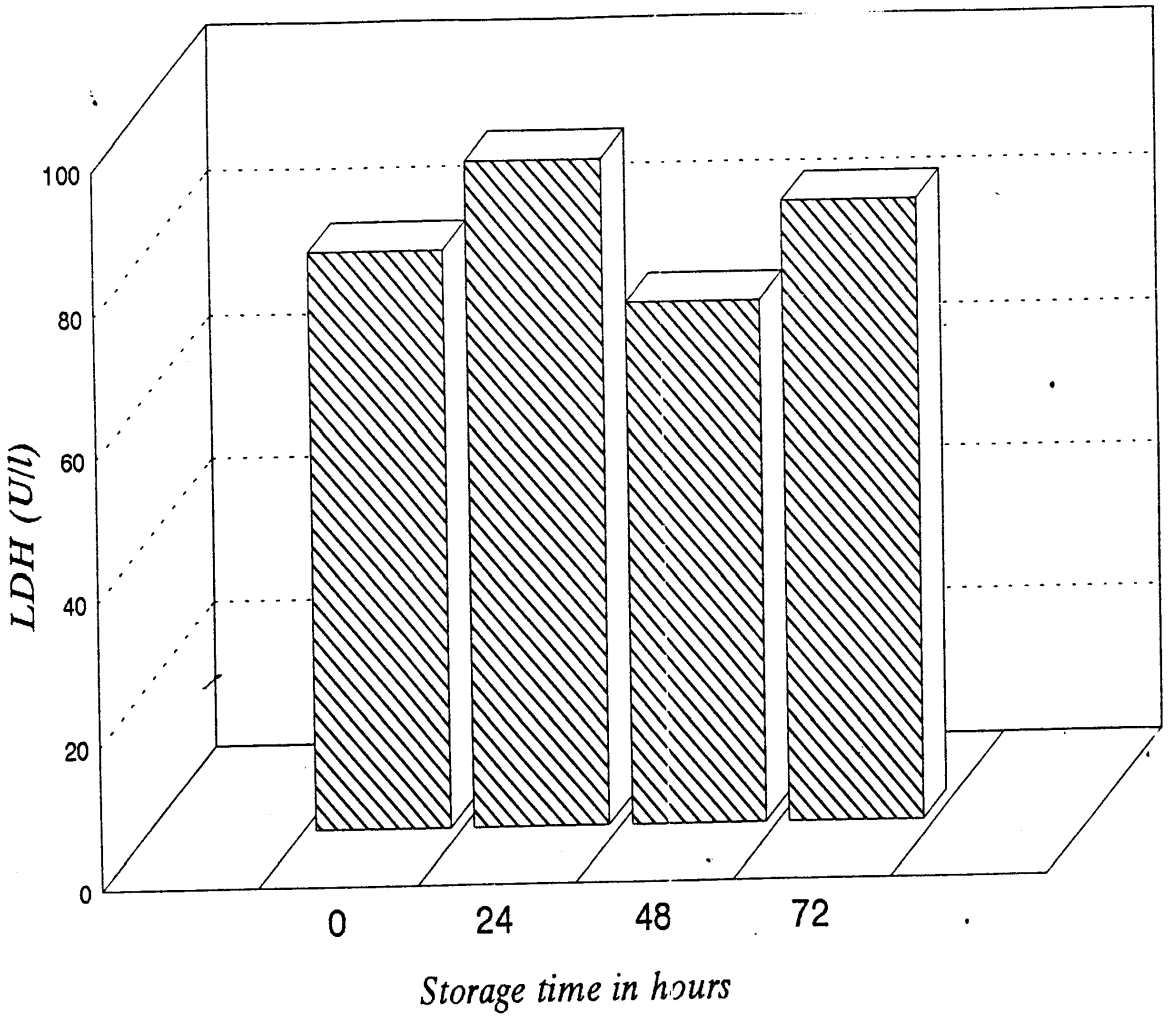


Table (4): Ammonia content (mM/l) in raw bulk milk in relation to bacterial count (cfu/ml)

	Amm.			B. C.		
Time/Hours	M ± SE.	Min.	Max.	M ± SE.	Min.	Max.
0	A 0.29 ± 0.08	0.02	1.65	A $0.38X10^8$ ± 0.06	$0.06X10^8$	$1.45X10^8$
24	A 0.38 ± 0.09	0.05	1.67	$0.70X10^8$ ± 0.06	$0.23X10^8$	$1.48X10^8$
48	B 0.77 ± 0.12	0.09	1.70	B $1.93X10^8$ ± 0.44	$0.31X10^8$	$6.45X10^8$
72	B 0.61 ± 0.10	0.13	1.65	C $6.92X10^8$ ± 0.11	$0.62X10^8$	$6.92X10^8$

*Means with the same letter are not significantly different.

Fig. (7): Effect of storage time on ammonia as related to bacterial count.

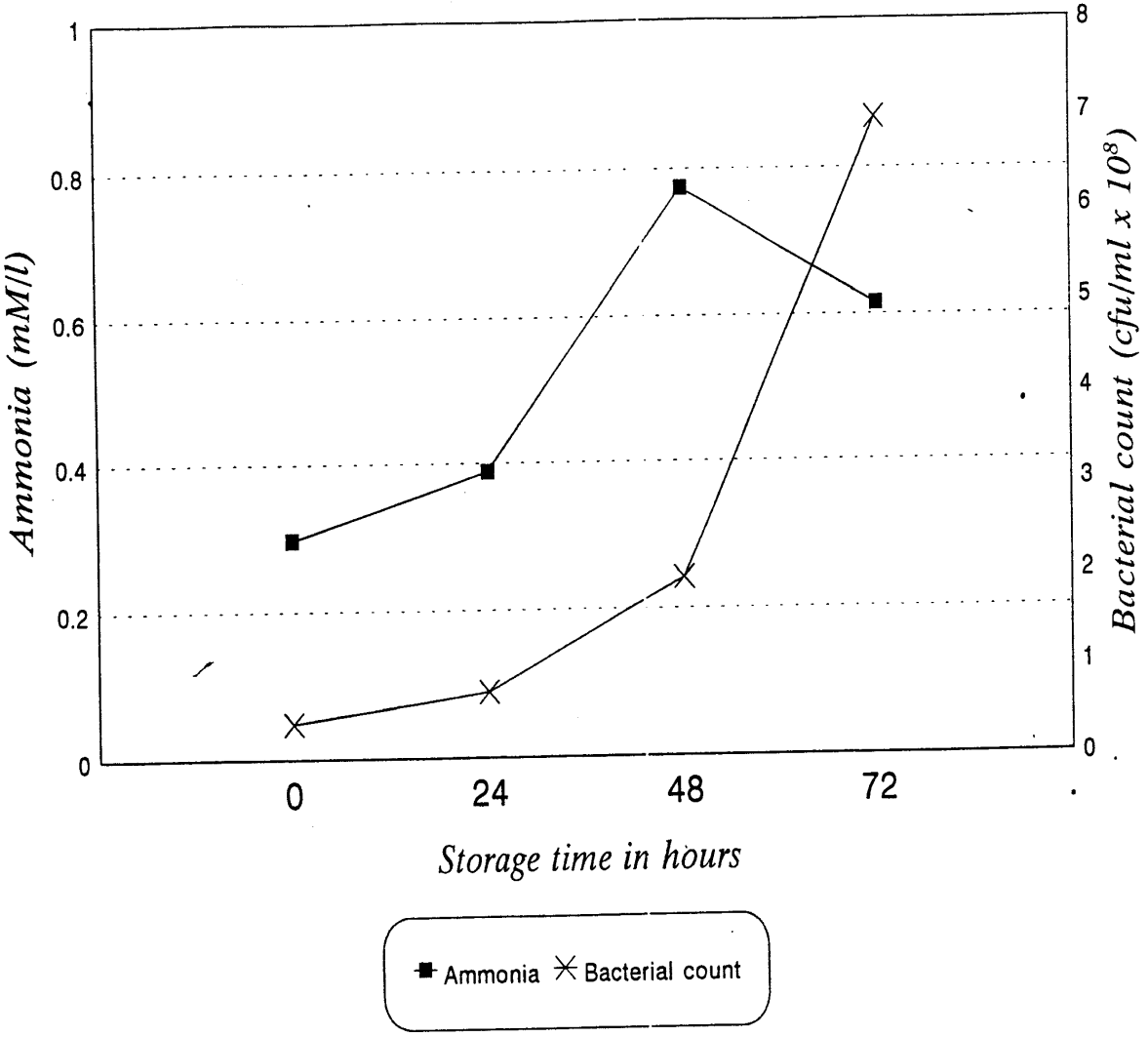


Fig. (8): Effect of storage time on ammonia...

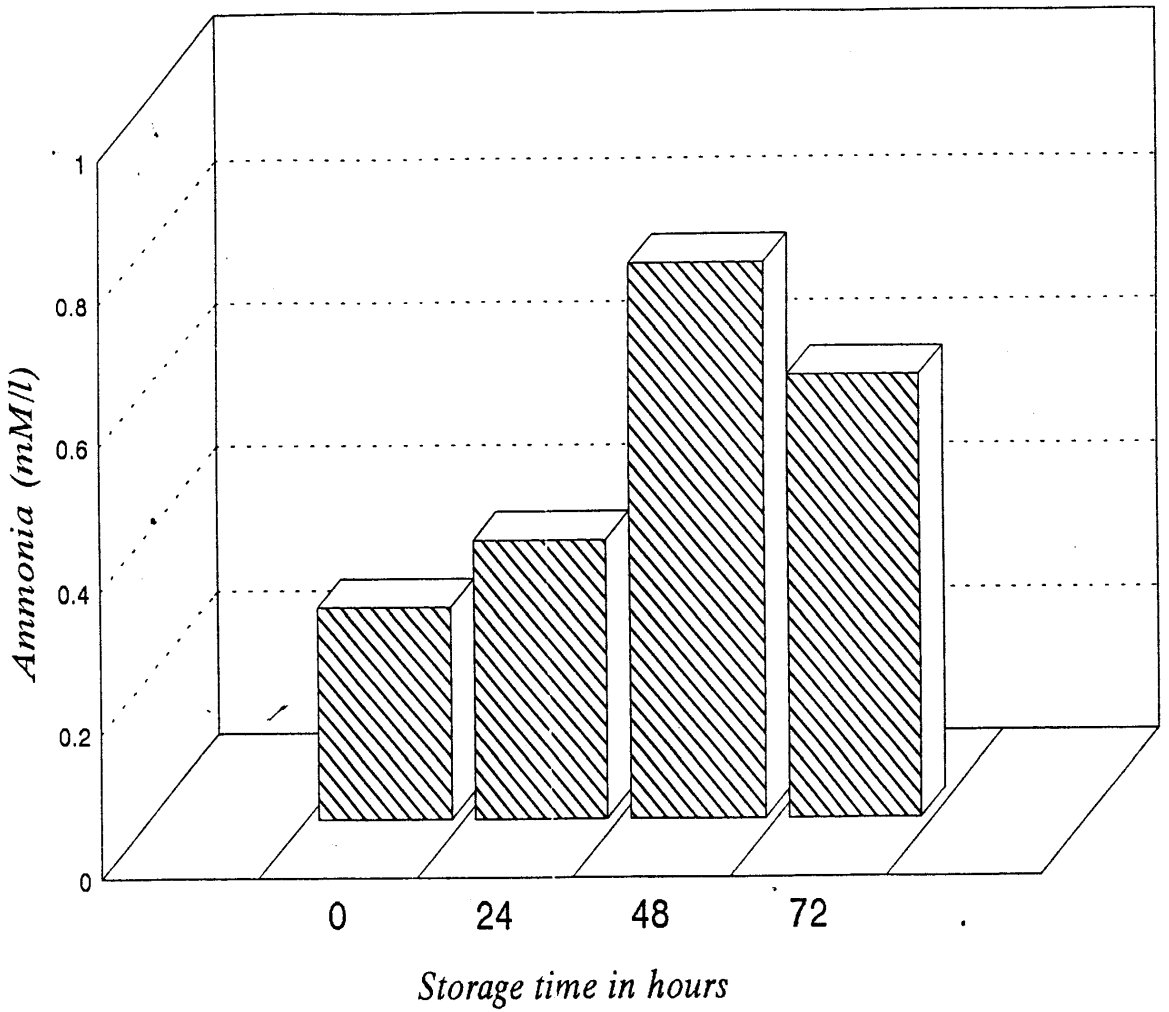


Fig. (9): Effect of storage time on bacterial count....

Table (5): Correlation coefficient between biochemical constituents and bacterial count at ***zero time***

	ATP	Pyr.	LDH	Amm.	BC
ATP		0.27	-0.007	-0.34	-0.26
Pyr.	0.27		-0.05	-0.08	0.13
LDH	-0.007	-0.05		-0.03	-0.14
Amm.	-0.34	-0.08	-0.03		0.03
BC	-0.26	0.13	-0.14	0.03	

*Significant at P<0.05
** Significant at P<0.01

Table (6): Correlation coefficient between biochemical constituents and bacterial count at ***24 hours storage***

	ATP	Pyr.	LDH	Amm.	BC
ATP		0.30	-0.06	-0.39	-0.18
Pyr.	0.30		-0.15	-0.44	0.03
LDH	-0.06	-0.15		0.22	0.22
Amm.	-0.39	-0.44	0.22		0.30
BC	-0.18	0.03	0.22	0.30	

*Significant at P<0.05
** Significant at P<0.01

Table (7): Correlation coefficient between biochemical constituents and bacterial count at ***48 hours storage***

	ATP	*Pyr.*	*LDH*	*Amm.*	*BC*
ATP		0.17	0.41	-0.27	0.53**
Pyr.	0.17		0.04	-0.27	0.26
LDH	0.41	0.04		0.14	0.38
Amm.	-0.27	-0.27	0.14		-0.88
BC	0.53**	0.26	0.38	-0.88	

*Significant at P<0.05
** Significant at P<0.01

Table (8): Correlation coefficient between biochemical constituents and bacterial count at ***72 hours storage***

	ATP	*Pyr.*	*LDH*	*Amm.*	*BC*
ATP		0.43	0.27	-0.17	0.76**
Pyr.	0.43		0.27	-0.50	0.15*
LDH	0.27	0.27		-0.16	0.38
Amm.	-0.17	-0.50	-0.16		-0.13
BC	0.76**	0.15*	0.38	-0.13	

*Significant at P<0.05
** Significant at P<0.01

Table (9): Overall Correlation coefficient between biochemical constituents and bacterial count

	ATP	Pyr.	LDH	Amm.	BC
ATP		0.43	0.09	0.05	0.75**
Pyr.	0.43**		-0.02	-0.16	0.40**
LDH	0.09	-0.02		0.002	0.18
Amm.	0.05	-0.16	0.002		0.08
BC	0.75**	0.40**	0.18	0.08	

*Significant at P<0.05
** Significant at P<0.01

DISCUSSION

The bacteriological quality of milk is an arbitrary concept.The level of hygiene varies widely from region to region. Often the requirements for bacteriological quality depend on the specific purpose for which the milk is required. The method chosen for judging milk should give the information which is relevant to the special case. Under different circumstances the use of different methods can be done.

Bacteria like any living organism react biochemically with the surrounding media consuming their needed compounds and releasing their metabolites to the media, and also conserve their specific constituents that cannot be released except by special extraction procedure e.g., ATP. The released constituents may be also present normally in milk but at certain level which changes in correlation to bacterial number and growth. ***IDF report (1991)*** summarized different methods evaluating bacterial count in raw milk, among these methods is the measurement of certain biochemical compounds which were suggested to be closely related to bacterial count in milk and consequently can be used to evaluate bacterial quality of raw milk as rapid, accurate and cheap methods instead of other traditional methods that are too time consuming (as plate count) or fail to give results (as dye reduction test).

In our work, milk samples that subjected to study were collected from dairy farm following milk hygiene system represent the most common system in milk collection and industry in Egypt, then these samples were subjected for determination of four biochemical constituents suggested by IDF to be mostly related to bacterial count. These constituents were ATP, Pyruvic acid, Ammonia and LDH activity. Finally the work aimed to relate these biochemical constituents and bacterial count in milk of cattle.

ATP :

The present study showed that, the mean values of bacterial ATP in raw bulk milk were $35.53X10^9 \pm 3.48$; $49.15X10^9 \pm 4.62$; $75.15X10^9 \pm 6.44$ and $111.9X10^9 \pm 7.15$ fg/ml at o, 24, 48 and 72 hours storage time respectively. Meanwhile the recorded mean values of bacterial count at the same storage time were $0.38X10^8 \pm 0.06$; $0.70X10^8 \pm 0.06$; $1.93X10^8 \pm 0.44$ and $6.92X10^8 \pm 0.11$ respectively (Table 1; Figure 1)

Statistically, our results demonstrated that, an increase in the storage time accompanied by similar increase in the mean values of ATP and bacterial count (Table 1). Concerning the correlation coefficient (ANOVA test) between mean values of ATP an bacterial count in relation to storage time revealed that there were no significant

correlation at zero time and after 24 hours of storage followed by highly significant increase ($P<0.01$) at 48 and 72 hours of storage (Tables 5-9).

These findings seem to coincide with the results obtained by ***Sharp et al., (1970)*** who said that for the result to be significant in terms of, say, bacterial contribution to total must be as great or greater than intrinsic ATP in the substrate material i.e. when bacterial ATP became greater than intrinsic ATP, and in overall correlation became highly significant.

Also in most of samples bacterial ATP were relatively high although great trials were done to exclude all non bacterial ATP by using (Triton-EDTA-Apyrase mixture) which also coincide with trials of ***Theron et al., (1986)*** who also suggested that not all somatic cell ATP not destroyed by the somatic cell treatment or there may even be other sources of ATP present in milk. Also this procedure is non specific and will yield values for many types of contaminant, e.g. pus , yeast fungal or dead bacterial cells. If important or required the method only yield values for specific type of contaminant preliminary separation of contaminant from substrate can be done e.g. filtration of bacteria by bacterial filter as done during ATP f test ***Van, et al ., (1986)*** .

Finally ATP can give, in principle a better estimate of bacterial contamination in milk than can the convential plate count .Provided that its concentration in living organisms is reasonably constant ***D'Eustachio & Johnson, (1968)*** the measurement of ATP, and therefore of biomass , may be a better indicator of biological activity than the number of colony forming units, particularly where clumped or filamentous. But also require a good separation from the substrate.

Pyruvic Acid:

Like ATP, Pyruvic acid is an important compound in bacterial life, Pyruvic acid produced as accentual product from metabolism of protein, carbohydrate and lipid so considered as an important indicator of bacterial activity, and can say that it may be related to bacterial count. Pyruvic acid also produced by body cells and excreted normally in milk at certain levels recorded previously at range between 1-5 mg /l ***(Marshall & Harmon, 1978)*** but represented by bacterial count of (500 000:110 000 cfu/ml).

The present study showed that the mean values of Pyruvic acid in raw milk were 7.34 ± 0.55; 7022 ± 0.53; 8.75 ± 0.50 and 9.19 ± 0.53 mg/ml at 0,24,48 and 72 hours storage time respectively. Meanwhile the recorded mean values of bacterial count at the same storage time were

$0.38X10^8$ ±0.06; $0.70X10^8$ ±0.06; $1.93X10^8$ ±0.44 and $6.92X10^8$ ±0.11 respectively (Table 3 and Figure 3).

Statistically our results demonstrated that, an increase in the storage time was accompanied by similar increase in the mean values of Pyruvic acid and bacterial count (Table 3). Concerning the correlation coefficient (ANOVA test) between mean values of Pyruvic acid and bacterial count in relation to storage time revealed that there were no significant correlation at zero time and after 24 hours of storage time followed by significant increase ($P<0.10$) at 48 and significant ($P<0.05$) after 72 hours of storage and in overall correlation showing high significant correlation ($P<0.01$) (Tables 5-9).

Our results seem to coincide with the results of ***(Tolle et al., 1976)*** who concluded that, the automated test for Pyruvate gave promise of being highly useful in determining the quality of raw milk. Supporting our findings, ***Marshall& Harmon, (1978)*** done a hard and extensive work on Pyruvate values and bacterial count and types in milk; finally concluded that, Gram +ve bacteria are the most contaminants of raw milk but storage help psychrotrophs to dominate, however Pyruvate seems to be closely related to the two categories. Also they added that samples need to be tested initially then incubated at temperature and

time that allow significant growth of undesirable bacteria before being tested again, and this is suitable for small volumes as well as large tanks of milk.

These findings seem to interfere with the results obtained by ***Soderjhelm et al., (1982)***who concluded that the pyruvate method fails to give any direct information about the quality of the milk because it is in fact, a test of bacteriological activity and one single measurement can not be used as a sorting out method at the dairies, also with ***Cousins et al., (1981)*** who reported that there is a poor relationship between Pyruvate content and bacterial count of 4 and 5 days old milks stored at 5°C and 7°C or when the count of samples exceeded $1x10^7$ cfu/ml was possibly due to catabolism of Pyruvate already produced. ***Arold,(1977)*** also found that ability to produce Pyruvate in milk differed widely among strains of psychrotrophs

Also the findings seem to be interfering with the results of ***Zandstra & de Vries, (1977)*** who said that for individual farm milks stored at refrigeration temperature, there is not close relationship between Pyruvate values and viable bacterial counts determined at intervals during storage. However is seemed possible that because of

large volumes involved (80 000: 120 000 Lit.), silo tanks, derived from 20 or more farm refrigerated tanks, might be of more uniform bacteriological quality than that from individual farm tanks and that the Pyruvate contents of the silo milk might be closely related to their bacterial count.

In my opinion, and because Pyruvic acid is closely related to metabolism and essential source of energy in the bacterial cell (Kreb's cycle), Pyruvate can be used as a good indicator for bacterial activity and count if daily tests of Pyruvate content could be fed into a computer which would collate the data and present it in tabular or graphic form. From these data, management could make decisions regarding time and temperature constrains on distribution and on the sanitary status of fillers and milk lines. Also Pyruvate can be measured by tests that can be automated permitting a great number of samples to be examined.

LDH Activity

The present study showed that, the mean values of LDH activity in raw milk were 80.46±6.97; 92.73±8.90; 72.81±5.64 and 86.37±6.79 U/L at 0, 24, 48, and 72 hours storage time respectively. Meanwhile the recorded mean values of bacterial count at the same storage time were $0.38X10^8$ ±0.06; $0.70X10^8$ ±0.06; $1.93X10^8$ ±0.44 and $6.92X10^8$ ±0.11 respectively (Table 4; Figure 4).

Statistically, our results demonstrated that an increase in the storage time was accompanied by similar increase in the mean values of bacterial count , but in a fluctuated manner regarding LDH activity compared to bacterial count at the same storage time.

Concerning the correlation coefficient (ANOVA test) between the mean values of LDH activity and bacterial count in relation to storage time revealed that there was nonsignificant correlation at 0, 24, 48 and 72 hours respectively with slight non overall correlation ($P<0.10$) (Tables 5-9).

These findings seem to coincide with results obtained by ***Kato et al., (1989)*** who found that LDH isozymes patterns in granulocytes and lymphocytes correspond to the LDH activity detected in mastitic milk

and concluded that LDH is mainly secreted by leukocytes. ***Hambitzer & Sommer, (1987)*** also reported that the storage affect the LDH activity which may decreased due to damage to enzyme protein conformation or increased due to disruption of intact plasma membrane of milk cells and the release of cellular LDH into the milk samples. Also ***Lipperheide et al., (1995)*** reported that the level of LDH activity increased up to 30% by addition of Triton X-100 to fresh milk and after incubation of 10 minutes at 25°C and could not be further increased by addition of the detergent after thawing, i.e. no cells to be destroyed and release additional LDH.

But unlike our findings, ***Bogin et al., (1977)*** presumed that LDH was released into milk by leukocytes, udder tissues and bacteria. Also ***Hassan, (1980)*** subjected 202 milk samples collected from 140 lactating buffaloes and 62 cows to biochemical and bacteriological examinations. He found a significant increase in LDH content in milk obtained from subclinically mastitic animals especially when the isolated bacteria was Strept. agalactiae.

Finally, in my opinion it can be said that when virulent bacteria are present, lead to irritation of udder tissues and then excretion of large numbers of leukocytes into the milk, by the time and storage, rapture and

elevate the level of LDH activity in milk but unsuitable preservation of enzyme protein lowering its activity values and so on. Consequently, the values of LDH activity can be used as an indicator of presence of pathogenic bacteria which propose to their danger but not as indicator to the bacterial number in milk.

Ammonia:

Ammonia is probably formed as a final product from proteolytic breakdown of proteins and simple nitrogen compounds. the present study showed that, the mean values of ammonia in raw bulk milk were 0.29±0.08; 0.38±0.09; 0.77±0.12 and 0.16±10 mM/l at 0,24,48 and 72 hours storage respectively. Meanwhile the recorded mean values of bacterial count at the same storage time were $0.38X10^8$ ±0.06; $0.70X10^8$ ±0.06; $1.93X10^8$ ±0.44 and $6.92X10^8$ ±0.11 respectively (Table2 and Figure2).

Statistically, our results demonstrated that an increase in the storage time was accompanied by similar increase in the mean values of bacterial count but ammonia mean values not did so (Table 3). Concerning the correlation coefficient (ANOVA test) between the mean values of ammonia and bacterial count in relation to the storage time revealed that although ammonia values were relatively normal at zero

time with slight correlation with the bacterial count , this correlation decreased after 48 and 72 hours respectively and in overall correlation there was a non significant correlation with the bacterial count in raw bulk milk (Tables 5-9).

These findings seem to coincide with results obtained by ***Soderjhelm et al., (1982)*** who found that with raw milk and with pure cultures the bacterial count must be very high, up to 10^7-10^8 cells/ml, before there are any significant increase in the ammonia contents, however this method is impractical and expensive since the milk very rarely is of such low quality. Also results seem to coincide with results of ***Storgards & Lindqvist, (1962)*** who demonstrated that the ammonia content of milk changed more rapidly during the storage of milk than did any of the numerous ninhydrinpositive substances present.

But the results seem to be disagree with the results of ***Pinelli,(1991)*** who concluded that ammonia content of large bulk milk is an index of its total hygienic quality (freshness), useful for real time milk selection and quality improvement both for raw and pasteurized milk also of unknown hygienic history of production, storage and transport. The ammonia is rapid, efficient, simple, cheap and easy to automate.

In my opinion the ammonia values that did not follow the same pattern in relation to the bacterial count during storage may be due to ammonia resulted from action of proteolytic strains ,these proteolytic strains may be present more or less than non-proteolytic types. Also ammonia is unstable compound may be already produced by action of endogenous milk proteineases but not supported by production of proteolytic strains. Finally, we found ammonia values up and down in relation to the bacterial count during storage.

ENGLISH SUMMARY

Milk is a rich source of essential nutrients for healthy and strong human body but also is a good media for growth of many health hazards microorganisms. These organisms proposing for many dangerous diseases or industrial faults of dairy products, so these organisms must be excluded or at least minimized in milk For this object, previously used methods were too time consuming or not accurate. Many trials have been done to study relationship between microbial count and certain biochemical constituents in milk to develop another rapid, accurate and rapid methods. In this work twenty bulk milk tanks samples were obtained from Mehalet Mousa dairy farms and analyzed for bacterial ATP, Pyruvic acid, ammonia, LDH activity in relation to bacterial count at time of collection and after storage at 8◦C for 24, 48 and 72 hours to permit bacterial growth. The obtained findings were summarized as below:

Bacterial ATP in milk:

It has been proved that ATP value is constant for each living cell. So ATP can be used to evaluate the biomass. In this work the free ATP in milk was destroyed using [Triton X-100 - EDTA- Apyrase]. Then bacterial ATP was extracted and measured selectively in relation to storage time and bacterial count. Statistically, the findings showed that an increase in the storage time accompanied by similar increase in mean values of bacterial ATP and bacterial count . Concerning correlation coefficient (ANOVA test) between mean values of ATP and bacterial count in relation to storage time revealed that, there were no

significant correlation at zero time and after 24 hours of storage time followed by highly significant increase at 48 and 72 hours of storage time and at overall correlation. Also the findings proved that bacterial ATP can be used as a good indicator for bacterial count in milk if bacterial ATP could be extracted and measured selectively by a specific method as bacterial filtration of sample.

Pyruvic acid in milk:

Like ATP, Pyruvic acid is an important compound in bacterial life. Pyruvic acid is produced as a central product during the metabolism of carbohydrate, protein and lipid. So Pyruvic acid may be related to bacterial number and activity. The samples were treated as done during analysis of bacterial ATP. The obtained results demonstrated that an increase in the storage time was concomitant to similar increase in mean values of Pyruvic acid and mean values of bacterial count. Concerning the correlation coefficient (ANOVA test) between mean values of Pyruvic acid and bacterial count in relation to storage time revealed that, there were no significant correlation at zero time and after 24 hours of storage followed by insignificant increase at 48 and significant after 72 hours and in overall correlation showing highly significant correlation. Pyruvic acid can be used as a good indicator for

bacterial count if daily tests of Pyruvic acid content could be fed into a computer which would collate the data and present it in tabular or

graphic form. From the previous data, management could make decisions regarding time and temperature constraints on distribution and on the sanitary status of fillers and milk lines.

LDH Activity in milk:

LDH is a key enzyme during metabolism of bacteria. LDH has been reported as a key enzyme metabolic control of the oxidation-reduction potential of cells for glycolysis. Our results demonstrated that irregular pattern of LDH activity in relation to bacterial count. LDH can not be used as an indicator for bacterial number but for bacterial pathogenisity and healthy state of udder.

Ammonia in milk:

Ammonia is a non protein nitrogenous compound produced after catabolism of protein. Proteolysis may be produced by indigenous milk proteinases or by proteolytic bacteria. Statistically our results demonstrated that the mean values of ammonia were not related to bacterial count, in this respect they did not follow the same pattern of ATP and Pyruvic acid when compared to bacterial count during storage time. The irregularity of ammonia mean values may be due to presence of non-proteolytic bacteria by large number than proteolytic types. Also ammonia is unstable compound, if not continuously produced, it is lost. So ammonia content considered as a weak indicator for bacterial count in milk.

CONCLUSION

For the present study, it could be concluded that,

- The levels of ATP and Pyruvic acid are milk intimately related bacterial count. Therefore, their determination is valuable for evaluation bacterial quality of raw milk.
- Since LDH activity is mainly somatic in origin released under effect of pathogenic bacteria, its determination can be used to indicate healthy state of udder tissues and also the presence of pathogenic bacteria in raw milk
- The level of Ammonia in milk is not closely related to bacterial count, hence can not be used for judgement of the bacterial quality of raw milk.

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ARABIC SUMMARY

[نبذة عن حياة الباحث]

ولد الباحث فى الخامس عشر من يوليو سنة ١٩٧٠ ببلقاس –محافظة الدقهلية

أكمل البـــاحث الدراسة الإبتدائية بمدرسة المـــغـــازى الإبتدائيـــة سنة ١٩٨٢

أكمل البـــاحث الدراسة الإعدادية بمدرسة المـــغـــازى الإعدادية سنة ١٩٨٥

أكمل البـــاحث الدراسة الثانوية بمدرسة بـــلقاس الثانويـــة بنين سنـــة ١٩٨٨

حصل البـــاحث على بكالوريوس العلوم الطبية البيطرية من جامعة الزقازيق

فى ديسمبر سنة ١٩٩٣ بتقدير عام جيد جدا

سجل البـــاحث للحصول على درجة المـــاجستير فى العلوم الطبيـــة البيطرية

(كيمياء حيوى) عام ١٩٩٥

يعتبر لبن الماشية السائل الثالث من حيث الأهمية فى حياة الإنسان بعد الماء و الـــدم حيـــث يشكل مصدرا هاما من مصادر العناصر الغذائية الهامة لنمو و صحة الجسم البشرى ولكن و على الرغم من هذه الأهمية أيضا يعتبر وسط جيد لنمو كثير من الميكروبات التى ربما تكون ضارة بصحة الإنسان كسبب لكثير من الأمراض الوبائية التى تنتشر بعد تناول اللبـــن أو أن هذه الميكروبات تؤدى إلى تغيرات غير مستساغة فى منتجات الألبان تجعلها غير صالحـــة للاستخدام عن طريق التأثير على خوصها التجارية و التخزينية بتقليل فترة صلاحيتها ممـــا دفع الإنسان إلى البحث عن وسيلة دقيقة و سريعة للكشف عن هذه الميكروبـــات ودراســـة سلوكها فى اللبن .

و لذلك استخدمت العديد من الطرق لهذا الغرض و لكنها إما أن تستغرق وقت طويـل للحصول على نتيجة (مثل الزرع على الأطباق) أو أنها لا تعطى نتائج دقيقة (مثـــل اختبـــار الاختزال للصبغات) ومن حيث انه لا يستلزم عزل بكتيريا بعينها إلا فى قليل من الأحيان (مثل الكلوستريديوم) فقد أدي ذاك إلى استحداث طرق اكثر سرعة و دقة مـــن الطـــرق السابقة .

بعد خمسة عشرة عاما من دراسة المخلفات الكيميائية الناتجة عن التمثيـــل الغذائـــي للبكتيريا أثناء تكاثرها فى اللبن أدى ذلك لاكتشاف وجود علاقة بين مســـتوى بعـــض هـــذه المركبات فى اللبن و عدد البكتيريا أيضا فى اللبن و من هذه المركبات الادينوسين ثلاثـــى الفوسفات , حمض البيروفيك , الامونيا , إنزيم اللاكتيك ديهيدروجيناز.

و امتدادا لذلك أجريت هذه الدراسة علي عدد عشرين عينة لبن تم الحصول عليها من التنكات الرئيسية لجمع اللبن من مزارع الجاموس الحلاب بمنطقة محلة موسى بمحافظة كفر الشيخ و التابعة للمركز القومى لبحوث إنتاج الحيوان. بعد ذلك تم نقل هذه العينات إلى معامل كلية الطب البيطري بمدينة كفر الشيخ و تحليلها لقياس

١- الادينوسين ثلاثي الفوسفات البكتيري في اللبن.

٢- حمض البيروفيك في اللبن.

٣- الامونيا في اللبن.

٤- إنزيم اللاكتيك ديهيدروجيناز في اللبن.

٥- العدد البكتيري في اللبن.

و ذلك بعد حفظها ثم تحليلها فى الأوقات آلاتية:

١- فور تجميع اللبن عند (٨° درجة مئوية).

٢-بعد ٢٤ ساعة من الحفظ عند (٨° درجة مئوية)

٣- بعد ٤٨ ساعة من الحفظ عند (٨° درجة مئوية)

٤- بعد ٧٢ ساعة من الحفظ عند (٨° درجة مئوية)

و قد تم استخلاص آلاتي

١-مركب الادينوسين ثلاثي الفوسفات:

هذا المركب اثبت العلماء أن له قيم ثابتة خاصة بكل خلية حية و كل نوع من الميكروبات ولذا يمكن استخدامه في عد البكتيريا بعد استخلاصه منها بطرق معينة.و لذلك تم التخلص من جميع المصادر الأخرى له فى اللبن ثم استخلاص الأدينوسين ثلاثى الفوسفات البكتيرى

و قياسه . وبالتحليل الإحصائي للادينوسين ثلاثي الفوسفات و العدد البكتيرى بالنسبة لمدة التخزين وجد أن الادينوسين ثلاثي الفوسفات البكتيري عند وقت التجميع وبعد ٢٤ ساعة من الحفظ عند درجة حرارة (٨°درجة مئوية) ليس له علاقة معنوية بعدد البكتيريا في اللبن . ولكن بعد٤٨ و ٧٢ ساعة من الحفظ عند درجة حرارة (٨°درجة مئوية) و بعد تزايد عدد البكتيريا بصورة ملحوظة وجد أن له علاقة معنوية بعدد البكتيريا في اللبن .و من هذا يمكن القول أن الادينوسين ثلاثي الفوسفات البكتيري يمكن استخدامه كمؤشر للعدد البكتيرى فى اللبن

٢ – حمض البيروفيك:

هذا الحمض يمثل مركب مركزي في عملية التمثيل الغذائي في البكتيريا ولذا يمكن استعماله كدليل على نشاط البكتيريا في اللبن.

و من الدراسة تبين أن مستوى هذا الحمض يزيد مع زيادة عدد البكتيريا في اللبن و لكن هذه الزيادة لا تكون معنوية آلا بعد ٧٢ ساعة من الحفظ عند درجة حرارة (٨°درجة مئوية) و لا يمكن أيضا ربط هذه الزيادة بعدد ثابت أو معين من البكتيريا.ولكن بتتبع نتائج هذه العلاقة ثم تغذيتها لأجهزة الكمبيوتر وعمل رسوم بيانية لها يوميا, يمكن بعد ذلك استخدامها أيضا كمؤشر لعدد البكتريا فى اللبن

٣-الامونيا:

الامونيا تعتبر مركب نهائي لعملية التمثيل الغذائي للبر وتين في البكتيريا و الخلية الجسمية على السواء . و تنتج الأمونيا بتأثير البكتريا المحللة للبروتين على اللبن . و لذلك وجد من الدراسة الإحصائية أن لها علاقة ليست معنوية بالعدد البكتيري في اللبن عند وقت التجميع وبعد ٢٤ ساعة من الحفظ عند درجة حرارة (٨°درجة مئوية) بل أن مستواها قد قل بعد ٤٨ و٧٢ ساعة من الحفظ رغم الزيادة الملحوظة في عدد البكتيريا في اللبن.أى أن الأمونيا لم تسلك مسلك حمض البيرو فيك والادينوسين ثلاثي الفوسفات البكتيري و لذلك لا يمكن الاعتماد على مستوى المونيا فى اللبن كمؤشر للعد البكتيرى فى اللبن.

٤ – إنزيم اللاكتيك ديهيدروجيناز في اللبن:

إنزيم اللاكتيك ديهيدروجيناز في اللبن يفرز من البكتيريا و خلايا الجسم أيضا و لكن بعض العلماء اثبتوا أن المصدر الرئيسي له في اللبن هو كرات الدم البيضاء و الخلايا الليمفاوية و تأكيدا لذلك فان مستوى هذا الإنزيم على عكس المركبات الثلاثة السابقة لم يبدو له أي علاقة بعدد البكتيريا في اللبن. لكن مستوى هذا الإنزيم يتعلق بوجود بكتيريا مرضية مسببة لالتهاب الضرع و يرتفع عند وجودها.

*و من خلال هذه النتائج تم التوصل إلى إمكانية استخدام قياس المركبات الكيميائية (حمض البيروفيك و الادينوسين ثلاثي الفوسفات البكتيري) لإعطاء فكرة سريعة و تقدير سطحي لعدد البكتيريا في اللبن للحكم على صلاحيته في مراكز التجميع ثم بعد ذلك يتم التأكد من هذه النتائج بطريقة الزرع في الأطباق.

*الأمونيا ليس لها علاقة بالعد البكتيرى ولا يمكن استعمالها لذلك.

* يمكن الاعتماد على إنزيم اللاكتيك ديهيدروجيناز في اللبن فى إعطاء فكرة عن الحلة الصحية للضرع و وجود بكتريا مرضية فيه

جامعة طنطا–فرع كفر الشيخ
كلية الطب البيطرى
قسم الفسيولوجيا و الكيمياء الحيوية

قرار لجنة الحكم و المناقشة

قررت لجنة الحكم و المناقشة بجلستها المنعقدة فى يوم السبت ١٩٩٩/١/١٦م (٢٨ رمضان١٤١٩ هـ) ترشيح السيد **ط.ب/ نصر السيد محمد نصر** للحصول على درجة الماجستير فى العلوم الطبية البيطرية (تخصص كيمياء حيوية)

اللجنة

اسم العضو	التوقيع
١– أ.د/ نبيل محمد طه	.....
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٢– د/ إبراهيم محمد أمان	.....
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د/ خالد عبد الحليم كحيلو	.....
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العلاقة بين المكونات الكيميائية الحيوية
و
العد البكتيرى فى لبن الماشية

رسالة مقدمة من

طب / نصر السيد محمد نصر
بكالوريوس العلوم الطبية البيطرية ١٩٩٣ (جامعة الزقازيق)
للحصول على درجة الماجستير فى العلوم الطبية البيطرية
(كيمياء حيوية)

تحت إشراف

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أستاذ و رئيس قسم الفسيولوجيا و الكيمياء الحيوية
وكيل الكلية للدراسات العليا و البحوث
كلية الطب البيطرى بكفر الشيخ – جامعة طنطا

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١٩٩٩