سم الله الرحمن الرحيم

Effects of Fattening on some Biochemical Parameters in Sheep Infected With Morel's Disease

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Abstract

This study was conducted in the department of Biochemistry Faculty of Veterinary Medicine University of Khartoum. The aim of the study is to investigate the effects of fattening, on Sheep infected with Morel's disease. The work targetd changes in parameters of lipids and protein metabolism that influenced by infection. Ten Hammary sheep of (8-10) month age, were selected for this study. They were divided into two groups, one group was subjected to fattening program and the other one fed roughages for maintenance. Then both groups were infected with *Saph aureus sub sp anaerobeus*, the causative agent of Morel's disease. Blood samples collected twice before infection and after the ripening of the abscesses and also adipose tissue biopsy was taken once from around the ripen abscess. The study showed clear effects of acute infection on most of the parameters measured and fattening significantly influenced these effects.

Blood lipids values were significantly affected:

1. Total lipids, total cholesterol and free fatt acids levels, were greatly decreased after infection and the difference was significant ($P<0.05$) in the fattened group.
2. Triacylglycerols levels, in contrast to other lipids, increased significantly ($P<0.05$) in both groups after infection.
3. Phospholipids were the only lipid parameter that remained constant in both groups after infection, it was not affected by fattening or infection.

Blood proteins values were also significantly affected:

1. Total proteins level decreased significantly ($P<0.05$)in both groups after infection.
2. Albumin values were found to be very low after infection, this effect was significant ($P<0.05$) in the fattened group.
3. In contrast to albumin, total globulins values were increased after infection and the difference was significant ($P<0.05$) in the fattened group.

The PH, of the extract of the adipose tissue biopsy, in both groups, was acidic as 5.96 and 6.29 in the fattened and unfattened groups respectively, but the difference was not significant.

The abscesses size increased following the grade of the body weight in both groups but the largest ones were found in the fattened group.

The body temperature followed the same manner during abscess formation in both groups.

ملخص الاطروحة

في هذه الدراسة والتي إجريت بقسم الكيمياء الحيوية – كلية الطب البيطري – جامعة الخرطوم لمعرفة أثر التسمين في الضأن المصاب بمرض الدمامل علي بعض القياسات الكيموحيوية وقد كانت النتائج كالآتي:

1- انخفاض تركيز كل من الأحماض الدهنية الحرة، البروتين الكلي الزلال، الدهن الكلي والكولسترول في الدم المصاب بجرثومة الاستافلوكوكس الذهبية تحت السلاله اللاهوائية سالبة إنزيم الكتاليز انخفاضها معنوياً بل واكثر انخفاضاً في حالة التسمين مقارنة مع الغير مسمنه من نفس الضأن.

2- انخفاض تركيز البروتين الكلي نتيجة الاصابة كان نتيجة انخفاض تركيز الزلال حيث أن تركيز القلوبيولين الكلي لم ينخفض في المجموعة الغير مسمنه بل زاد معنوياً في المجموعة المسمنة.

3- ارتفاع تركيز الحامض الدهني ثلاثي الجلسرين في الدم المصاب عموماً بل أكثر ارتفاعاً في حالة التسمين مقارنة بتركيزه قبل الاصابة.

4- لم يتأثر تركيز الدهون الفسفورية في الدم المصاب عموماً بل انخفاضها في السائل المستخاص من الانسجة الدهنية للحيوانات المصابة واكثر انخفاضاً في المصابة غير مسمنة.

5- الازدياد النسبي في حجم الدمامل للضأن المصاب كان مصاحباً للزيادة في وزن الجسم الحي للحيوان.

6- الاس الهيدروجيني للسائل المستخاص من الانسجة الدهنية للحيوانات المصابة كان في المسمنة المصابة أكثر حمضية من غير المسمنة وفي كليهما حمضياً.

Introduction

Morel's disease is commonly known as abscess disease, this is because abscess was the only signs of the disease. It is first discovered by, Morel, (1911) but later it was studied by many authors and reported as abscess disease, encountered in young sheep at ages between 8-10 months old, (Morel, 1911, Funete and Suarez, 1985). The disease is also known to be endemic in nature with high morbidity and frequent relapses but with no mortality (Bajmocy *et al*., 1984). This has an economical importance because it endangers exportation of sheep. It was observed that the disease mainly affects animals of good health, which were kept for fattening (Aynaud, 1928 and Hassan , 1996). The causative agent of the disease is thought to be *Staphylococcus aureus sub sp. Anaerobius*, it is a catalase and oxidase-ve, gram +ve bacteria (Schliefer, 1985 and Sneath, *et al*., 1986). This organism is known to be affected by addition of saturated and unsaturated fatty acids as this depress the growth and entero-toxin –B formation also its membrane mutant can be dependent. (Robert, 1977; Jay-Naidoo, 1980 and Noble, 1990). Studies on the effect of the addition of cholesterol to the bacteria showed that cholesterol alone has no effect, but its addition to media containing linoleic acid reduced the inhibitory effect of the fatty acid at pH 7, but at pH 5.5 cholesterol showed no effect. However, Hassan , (1996) reported higher serum cholesterol levels in fattened sheep compared to unfattened ones. These findings together with the higher rate of infection in fattened sheep imply that there is some

relationship between the infection by the disease and lipids metabolism especially fat deposition and mobilization, and levels of the free fatty acids in the blood and adipose tissue. The present study is designed to clearify this relationship and to determine the changes in blood lipids accompany the infection in fattened sheep compared to infected poorly fed animals. The study will be performed by infecting two groups of sheep with the bacteria, one will be subjected to fattening program and the other to maintenance ration, blood samples will be collected from all animals for the estimation of total lipids, free fatty acids, triglycerides, total cholesterol, phosphlipids, total protein, total globulins, albumin. Adipose tissue biopsy will be taken from the two groups by the end of the experimental period and will be subjected to the same chemical analysis, plus the pH of adipose tissue of both fattened and unfattened animals to discren a relationship between the etiology and the incidence of the disease in the altered conditions by the fattening process.

Chapter 1

Literature Review

1-1 Sheep abscess disease (Morel's disease)

This disease was firstly recognized in the world in France by Morel, (1911) and latter reported by several other French scientists (Aynaud, 1922, 1927) (Carre, 1923a, 1923b, 1927) and Benito and Borrel, 1957). In 1983 the disease was diagnosed in Hungary in a flock of sheep imported from France (Bajmocy *et al*., 1984). The first incidence of the disease was reported in Spain by Blanco Loizelier, (1958). Fuente and Suarez, (1985), reported new outbreak of the disease in Spain.

In 1987 the disease appeared in Iran (Afnan and Hedjazi, 1978) In Aflock of native breed (Shirlaw and Ashford, 1962). The disease was diagnosed in goats in the Sudan in 1987 by El-Sanousi *et al.,* (1989). Also the disease was reported in Valley of the Cunea area (Valenti and Bieler, 1984). The disease was reported in Saudi Arabia by El-Sanousi, *et al.,* (1988) in Najdi sheep, other workers reported the same disease under different names mainly Pseudo-tuberculosis.

The disease commonly affects young sheep and their symptoms are similar to those of caseous lymphadenitis (Morel, 1911; Joubert, 1958; Shirlaw and Ashfored. 1962, Bajomcy *et al*; 1984; Fuente and Suarez, 1985. The main pathological character of the disease is the formation of abscesses close to or within the superficial lymphnodes

and not around them. Hamad, (1989) found that abscesses can appear at inoculation and adjacent to lymph nodes in those animals which were inoculated subctaneolsly (S/C), while it appeared only at site of inoculation, in animals inoculated intramuscularly (I/M). In other instances abscesses were observed in the subcutaneous tissue (Shirlaw and Ashfored 1962 and Bajomcy *et al*; 1984) and rarely in lungs (Aynaud, 1922, Bajmocy *et al*., 1984 and Hamad, 1989). Santa Rosa, *et al*., (1989) found one case of coagulase positive *Staph*yocous from 17 livers abscesses examined in goats at postmortem.

Lymph nodes most commonly affected are the prescapula, popliteal, inguninal and parotid lymph nodes (Morel, 1911). Aynaud, (1927) and Joubert, (1958) considered the angle of the Jaw, the Shoulder Joint and the scrotum to be the predilection sites for abscesses. Shirlaw and Ashfond, (1962) claimed that abscesses occurred in order of frequency close to the prescapular., popliteal, parotid and anterior cervical lymph nodes Bajomcy *et al*. (1984) noticed suppurtion occured most frequently in the mandibular perscapular and subiliac lymphnodes.

Fuente and Suarez, (1985) mentioned that abscesses more frequently located in the lymph nodes of the mandibular region (Mandibular, Parotid and Iateral retropharyngeal) followed by the superficial cervical, subiliac popliteal, suprammmary and scrotal lymph nodes respectively.

Hamad, (1989) mentioned that the frequency of lymph nodes were as follow: (Parotid. Mandibular, prescupular, and popliteal lymph nodes respectively).

The sizes of abscesses are variable, it could be as small as a pigeon's egg or as large as an orange (Morel, 1911, Shirlaw and Ashford, 1962). Aynaud mentioned that the size of an abscess was as a big as two-hand's fist. However, Bajmocy *et al*., (1984) found the size to be as a hen's egg or as a man's fist. The size of an abscess may reach the size of a football (Al.hendi, *et al*., 1993).

The lesions usually appear small and then gradually increase in size. When mature, they rupture and expel a thin greenish yellow pus and healing takes place after along time (Morell, 1911; Aynaud, 1922) Bajmocy *et al*., 1983) Fuaute and Suarez, 1985; Hamad, 1989). However, Aynaud, (1922) mentioned that ruptured abscesses might proliferate in other point adjacent to the first one. Shirlaw and Ashford, (1962) reported the occurrence of more than two abscess simultaneously on the same animals.

Bajmocy *et al*., (1984) and Hamad, (1989) Noticed that the adjacent lymph nodes were usually attacked few weeks after the old onc's and that affected ruptured and healed.

Shirlaw and Ashford, (1962) reported that histopathological sections prepared from lymphnodes close to abscesses showed slight hyperaemia of lymphoid tissue, oedema of lymph channels and hyperplasia of lymphoid cords. Hamad, (1989) described the

hyperplasia of the lymphoid tissue and at the centre of each lesion there was aliquified as homogenous material in which polymorphnuclear leucocytes were seen at different stages of destruction with debris of necrotic tissue.

An abscess disease has usually been encountered in lambs at ages between eight to ten months old was described by Morel, (1911). Bajmocy *et al*., (1984) reported the disease in almost all lambs that had been in an affected flock. Fuent and Suarez, (1985) considered it as a disease of young sheep up to four months old and only rarelely affect adult ones.

The disease is endemic in nature with high morbidity and frequency relapses, but no mortality is directly attributed to it (Bajmocy *et al*., 1984) Aynaud, (1928) found that the disease was mainly seen in animals of very good health which were kept for fattening. Bajmocy *et al*., (1983) reported the spread among ewe hoggs 4-5 month old, and in 10 one-year old rams of the lacaune breed (French in origin), the disease affected 2-3 animals initially, spreading to 150 animals in 2 months and 391 sheep in 4 months.

Animals recovered without impairment of growth but relapses occurred after 6-10 weeks lambs borne contracted the disease within few weeks.

1-2 Causal Agent of Morel's Disease

The causal agent was described by Morel, (1911), as being micrococci, arranged in pairs, tetrads and in clusters. Aynaud, (1922-

1923) observed gram-positive cocci in films of pus from affected sheep.

Joubert, (1958) described the organism as Gram positive cocci, 0.6 to 0.8 mm in diameter, arranged singly or in groups, non-motile, uncapsulated and non-sporing- Shirlaw and Ashford, (1962) found the coccus to be 1.0 to 1.5 mm in diameter Bajmocy *et al*., (1984) showed that cells of the organism were arranged more frequently in tetrads and occasionally in conglomerates.

Fuente, (1985) described the organism of abscess disease as a gram positive coccus, 0.8 to 1.0 mm in irregular clusters which occurred singly, in pairs, tetrads and predominantly as irregular clusters and non-motile. The organism was found by El-Sanousi, (1989) to be partially acid-fast. Morel, (1911) Aynaud, (1922) and Carre, (1923a,b) mentioned that the organism did not grow on simple media or when incubated aerobically. Carre, (1927) seperated this coccus from *Staph*ylococci on the basis of its cultural characteristics. But Aynaud, (1928) found many biochemical characters in common with *Staphylococci* and considered the organism to be *Staphyloccocus* adapted to sheep. Bentio and Borrel, (1957) and Joubert, (1958) considered the causative agent of abscesses disease to be an autonomous species that had not yet be described, for which they proposed the name *Micro coccus pyogoues ovis* and *Micrococcus* abscedens ovis respectively. On the basis of anaerobic growth of this organism, the eight edition of Bergey's manual determinative

bacteriology, list "Micrococcus abscedens Ovis" to the genus peptoccus Blanco Loizelier, (1958) and Fuente and Suarez, (1985) demonstrated that the aetiological agent of abscess disease was acatalse-negative and benzidine negative *Staph*ylococcus, they considered it to be a respiratory deficient *Staph*ylococcus aureus.

However, more recently Fuente *et al*., (1985) reported that respiratory deficient *Staph aureus* exhibited a cell wall composition typical of *Staph aureus* ATCC 12600^{T} in addition, DNA_DNA hybirdzation indicated that the organism was very closely related to *Staph aureus* at the species level. However, because of biochemical distinctiveness (Catalase and Benzidine negative, no or only weak growth under aerobic conditions) and the aetiological importance of this organism, they classified it as *Staph*ylococcus aureus *sub sp. anaerobius* this name was adopted in the ninth edition of Bergey's manual of determinative bacteriology (Schliefer, 1985 and Sneath *et al*., 1986).

1-2-1 Growth and Characteristics

Generally authers agreed that good growth occurs when cultures of the oganism are incubated anaerobically or under Co_2 tension, but not aerobically (Shirlaw and Ashford, 1962 and Bajmocy *et al*; 1984 and Fuente and Suarez, 1985). Co_2 is usually generated by the candle Jar system. Shirlaw and Ashtord, (1962) Bajmocy *et al*; (1984) used 10% Co_2 where as Fuente and Suarez, (1989) used 2.5% Co_2 Aynaud, (1928) reported that the organism could grow aerobically

when cultivated in eggyolk agar. However, Shirlaw and Ashrord, (1962) showed that the orgaism did not grow aerobically even after incubation for five days. Bajmocy *et al* ,. (1984) noticed that the organism was micro-aerophilic and underaerobic condition pin-point colonies appeared on the fifth day of incubation or latter. Hamad, (1989), showed that after serial of aerobically subcultures (6^{th} and 7^{th}). Most of the tested isolates gave growth after 48 hours of incubation, comparable to that obtained under increase Co_2 tension or under anaerobic conditions and appeared golden in colour which become more evident. All isolates colonies become golden in colour at the tenth subculture.

Fuente and Suarez, (1985) noticed that after a few subcultures on sheep blood, Streaks of confluent growth in those areas that were heavily inoculated.

Fuente *et al* ,. (1985) found that on primary isolation growth occurred when pus samples were directly inoculated into nutrient broth. However, slow growth appeared after 48-72 hours, as white granules deposits without turbidity of the medium, when nutrient broth and barin-heart infusion were inoculated from plate cultures. Shirlaw and Ashford, (1962) Bajmocy *et al* ,. (1984) Fuente and Suarez, (1985) and Fuente *et al* ,. (1985) reported that the growth of the organism was slow and appeared after incubation for 48-72 hours at 37℃.

They described the colonies on sheep blood ager as small, white, Circular, smooth, glistening, opaque, low convex and entire. The diameter of colonies on was described by Carre, (1923 a,b) and Aynaud, (1928) to be 1mm, whereas shirlaw and Ashtord, (1962)

described it to be only 0.5mm. However, Fuente and Suarez, (1985) reported that colonies of the organism ranged between 1 to 3mm in diameter.

Growth of the organism in serum broth occurred within 48-72 hours with formation of deposition without turbidity of the medium (Aynaud, 1928). According to Fuente *et al.,* (1985) a granular deposit in broth culture was formed after three days of incubation at 37°c. They added that when broth media were supplemented with 5% horse serum, the typical deposits appeared after 24 hours of incubation.

Shirlaw and Ashford, (1962) described the growth on Dorset Egg Medium to be in the form of a thin filim with no digestion of the medium, moreover, the organism remained viable on this medium even after 14 months of storage at room temperature. Fuente *et al.,* (1985), reported luxuriant growth on Dorset Egg Medium, with a colony diameter of 4 to 6mm on Baird-Parker medium the organism produced very small black colonies after 3days of incubation at 37°c - it also grow in the specific medium of Schlieter and Kramer, (1980) for *Staph*ylococci (Fuente and Suarez, 1985) but not on Maconkey Agar (Shirlaw and Ashford, 1962).

Most auther agreed that colonies were white in colours and the organism did not produce pigment (Carre, 1923b and Jourbert, 1958 and Shirlaw and Ashford, 1962 and Bajmocy *et al* ,. 1984 and Fuente and Suarez, 1985 and Fuente *et al* ,. 1985). However Aynaud, (1923) reported that serial subculturing of the organism resulted in production

of agolden pigment. There is a general agreement between most authers that *Staph. aureus sub sp. anaerobius* produces B- haemolysis on sheep blood ager (Shirlaw and Ashtord, 1962 and Bajmocy *et al.*, 1984 Fuente and Suarez, 1985). In addition this organism lyses bovine, rabbit and human erythrocytes Fuente *et al* ,. (1985).

However, Cruickshank *et al* ,. (1975) mentioned that B- haemolysis cuased partial haemolysis of rabbit and human erythrocytes erythrocytes this haemolysis has been called ("hot – cold') lysin to emphasize its enhanced haemolytic activity when incubation at 37°c is followed by replacement at 4°c ambient temperature (Elek and levy, 1950). Hamad, (1989) mentioned that the organism produced awide zone of partial haemolysis around colonies on 5% sheep Blood Ager. This zone become clear and complete when the culture was further placed at 4°c for 24 hours, while on 5% human blood ager, the organism produced narrow zones of complete haemolysis with sharply demarcated boarder the organism did not produced haemolysis on horse blood ager.

1.2.2 Biochemical properties

Generally *Staph. aureus sub sp. anaerobius* is Catalase and oxidase negative organism (Fuenete and Suarez, 1985) and Fuenete *et al* ,. (1985) reported that this organism produced coagulase, heat labile nucleases, phosphatase, gelatinase and eggyolk factors. However,

Shirlaw and Ashford, (1962) mentioned that the organism did not produced coagulase or gelatinase enzymes.

Bajmocy *et al*; (1984) and Fuente and Suarez (1985) reported that the organism was negative for methylred and Voges – Proskaeur tests and not utilize citrate, produced urease or reduce nitrates to nitrites. However Aynand, (1928) showed that the organism reduced nitrates to nitrites.

The organism fermented glucose, sucrose and fructose without gas production but not fermented mannitol and lactose (Joubert, 1985). Moreover, Shirlaw and Ashford (1962) reported that this organism fermented mannose but not maltose, but Bajmocy *et al*; (1984) demonstrated that this organism fermented maltose, but not mannose.

Shirlaw and Ashford, (1962) observed that the organism fermented dulcitol, inulin, leavulose, raffinose, glycerol, inositol arabinose, dextrin, rhamnese, xylose and trehalose. Fuente and Suarez, (1985) and Fuente *et al*., (1985) reported that the organism did not ferment dulcitol, inulin, laevulose, raffinose, glycerol, inositol, arabinose, dextrin, rhamnose xylose or trehalose.

Hamad, (1989) mentioned that the organism is catalase, oxidase and clumpingfactor negative, coagulase positive, ferment glucose and sucrose. Most of the isolates were positive for DNase, phosphatase, gelatinase, fructose, maltose and mannose. All isolate gave negative results for methyl red, urease and citrate tests and failed to ferment

mannitol, Lactose, galactose, rhamnose, xylose, arabinose, trehalose glycerol, inositol, sorbitol, dulcitol or inulin.

1-3 Effect of Lipids and pH on *Staph aureus* growth

Noble, (1992) showed attempts that have been made to discren a relationship between skin flora and free fatty acids in man (The dog skin lipids eg. About 60% of them is cholestrol and its derivatives) plus other esterol (Nicolaides *et al*; 1968 and Nikhari, 1974).

Although *S. aureus* is rarely found on normal human skin, it is often present on diseased skin even on area regarded as clinically normal (Noble, 1981). Fatty acids have often been included amongest the factors thought likely to suppress organisms on skin (Jay – Naidoo., 1980).

Heczho and Kasprowiez, (1976) have shown that *Staphylococci* have higher minimum inhibitory concentration values for various fatty acids including linoleic acid than have propionibacteria, some other mechanism presumably governs the varity of *Staph. aureus* on normal skin. Robert, (1977), mentioned that the growth and enterotoxin –B–formation by *Staph aureus* and its membrance mutant can be depressed or stimulated by addtion of grade amount of saturated or unsaturated fatty acid mixtures. Under some conditions depression of *Staph enterotoxin-B* formation is separate from growth inhibition.

Small amount of unsaturated fatty acids mixtures not only antagonize some effect of growth and *Staph enterotoxin–B* formation, but also appear to potentiate inhibition of growth and *Staph enterotoxin–B* formation by the unsaturated fatty acid mixture. (Robert, (1977).

The inhibition of *Staph aureus* by fatty acids was pH– dependent (Jay – Naidoo-, 1980). Lacey and Lord, (1981) found that linoleic acid was much more inhibitory to *Staph aureus* than coagulase negative *Staphylococci* from human skin and that *Staph aureus* strains of animals origin sensitive were more than those of human origin. Oleate is more inhibitory to *Staph aureus* than to all cagulase-negtive species (Ushaijiwa *et al*., 1984).

Also Noble, (1990) mentioned that *Staph epidermids Stahp hominis* tolerate higher levels of propionate than *Staph aureus* or other ceagulase-negative species. Jay – Naidoo, (1980) mentioned that cholesterol alone had no significant effect on the growth of *Staph aureus* at either 5.5 or 7 pH. However when cholesterol (10mg/1m) was added to medium containing linoliec acid (4mg/m1) the inhibitory effect of the fatty acid was reduced. But at pH 5.5 cholesterol play no advantageous effect against inhibitory action of linoleic acid. Cholesterol was also able to reduce the inhibitory effect of myristic acid. The various cholesterol esters had no effect on growth of sensitive and resistant variants at ether pH 5.5 or 7 values. The *Staph aureus* TCA cycle is depressed upon depletion of rapidly catabolizable carbon sources, this coincides with the transition to producing only formulated d-toxin and results in an increased inflammatory response. The pro-inflammatory response should increase host cell damage and this results their establish that, there is an important linkage between bacterial metabolism and pathogenesis (Dtto and Musser 2003).

1-4 Pathogenicity tests of Morel's disease

1-4-1 Laboratory animals

Various workers have confirmed resistance of laboratory animals to experimental infection.

Aynaud, (1928) injected 100 guinea pigs through the I/M and S/C routes, non of them developed signs or lesions of the disease. He also found mice to be resistance to experimental infection, Jubert, (1958) reported that mice and guinea pigs were refractory to experimental infections irrespective of the routes of administration or size of dose administrated, Shirlaw and Ashford, (1962) showed that guinea pigs were referction to intradermal (I/d), intravenous (I/V) and intraperitoneal (I/P) inoculation of 0.1 ml culture suspension of the organism which was equivalent in density to Brown tube No. 4

Fuente and Suarez, (1985) inoculated four groups of mice intra-peritoneal (I/P) or S/C with increasing dose of 0.25, 0.5, 0.75ml of a culture suspension containing 10^7 cells/mI. They also tried the I/d route with 0.05, 0.1 and 0.2mI of the culture suspension. They found all mice were resistant to the disease regardless of the inoculation route of dose administered. The same authors found guinea pigs resistant to experimental infection.

Aynaud, (1928) and Jouibert, (1958) found rabbits resistant to experimental infection through all routes of administration. However, Shirlaw and Ashford, (1962) inoculated four rabbits I/d with 0.2ml of culture suspension of the organism adjusted to Brown tube No. 4 two

rabbits developed a patch of cellulites at sites of inoculation within two days. At post mortem, lesions were found to extend to adepth of 0.5 mm beneath the skin and the organism was rcovered in pure culture from these lesions.

Fuente and Suarez (1985) injected the groups of rabbits I/m, S/C and I/V with a culture of the organism (10^7cell/mI). Only one rabbit, which was inculated S/C, developed a papule after 24 hours, Hamad, (1989) mentioned that mice and guinea-pigs injected via I/d route and rabbits which injected I/m and S/C routes, all were found to be refractory and the organism was not recovered from inoculation sites or form their lesions in livers, spleens or lungs.

1-4-2 Sheep

There is a general agreement between the investigators that *Staph aureus subsp, anaerobius* is pathogenic for sheep and goats. Aynaud, (1923, 1927, 1928) found that the organism was pathogenic for sheep and goats when the administration via the I/M, S/C, I/P and intra testicular routes. The lesions appeared in the form of abscesses at the sites of inoculation, I/P injection of the organism resulted in development of abscesses in the abdominal muscles. He noticed that the organism was not pathogenic when given via the oral or the intratracheal routes. Shirlaw and Ashford, (1962) reported that sheep were susceptible to infection when the organism was administrated via the I/P or the S/C routes. Inoculated animals developed abscesses at sites of inoculation after two days post infection, I/P and after 12 days on these infected S/C.

they observed Abscesses in adjacent regional lymph nodes at post-mortem six weeks following infection. The same authors noticed that scarification of the organism did not reproduce the disease in sheep.

However, Bajmocy *et al.,* (1984) were able to reproduce the disease by scarification as well as by the I/P and I/V routes. Fuente and Suarez, (1985) showed that sheep inoculated S/C or I/M with a culture suspeion of the organism developed abscesses both at the inoculation sites and adjacent lymphondes. Hamad, (1989) mentioned that in the animals infected with S/C routes the abscesses appeared at the inoculation sites and adjacent laymph nodes, in addetion some of the inoculated animals developed pulmonary abscesses.However, animals inoculated I/M developed abscesses only at the inoculation sites. He also mentioned the course of the experimental infection starting as swelling at inoculation site increase in size, ripened and ruptured expelling athin greenish yellow pus. After about seven day's the adjacent regional lymph nodes enlarged in animal that had been inoculated by the S/C route. He also mentioned that, histopathological section prepared from the infected lymph nodes showed hyper plasia of the lymphoid tissue and multiple abscesses in which debri of necrotic cells and polymorphonuclears leucocytes at different stages of destruction were seen. The majority of the infiltrating cells were polymorphonuclears cells and also varying number of mononuclear cells were seen. In case of pulmonary abscesses section, similarty in

microscopic appearance to that of the affected infiltrations were seen, and alveoli adjacent to the lesions were emphysematous.

1-5 The effect of infection on plasma protein

Plasma contains many proteins with a variety of function. Most are synthesized in the liver and are glycosylated. The levels of certain proteins in plasma increase during acute inflammatory states or secondary to certain types of tissue of tissue damage: These proteins are called "acute phase protein" (or reactants) and include C-reactive protein (CRP, so-named because it reacts with the C polysaccharide of pneumococci), α1-antitrypsin, haptoglobin, α1-acid glycoprotein, and fibrinogen. The elevations of the levels of these proteins vary from as little as 50% to as much as 1000-fold in the case of CRP. Their levels are belived to play a role in the body's response to inflammation. For example, C-reactive protein can stimulate the classical complement pathway, and α1-antitrypsin can neutralize certain proteases released during the acute inflammatory state. Interleukin 1 (IL-1), a polypeptide released from mononuclear phagocytic cells, is the principal-but not the sole-stimulator of the synthesis of the majority of acute phase rectants by hepatocytes. Additional molecules such as IL-6 are involved, and they as well as IL-1 appear to work at the level of gene transcription. (Murray *et al*., 2000).

Robert, (1999) reported that during the severe injury or infection an overall metabolic response occurs that results in aloss in lean body mass. However, each tissue has specific response that may be unique, and net protein synthesis may even be increased in some tissue, thus protein

synthesis is accelerated in the liver for the production of acute phase proteins, the immune system, and wound repair requires rapid protein synthesis.

The catabolic response largely occurs in the Skeletal muscle, over a short period of time, the muscle has one adequate reserve of protein maintain function despite accelerated catabolism, (Bams and Miranda, 1985). The net synthesis or catabolism of muscle protein depend on the balance between rate of protein synthesis and breakdown. The precursors for protein synthesis are derived from either protein breakdown or from transmembrane transport from the plasma. The amino acids given in nutrition can only be incorporated into protein after being transported into the muscle cells from the blood. Thus, the processes of protein synthesis, breakdown, and transmembrane amino acids transport are linked, and it is necessary to evaluate the respone to stress by quantifying these three related processes (Biolo, *et. al.* 1995).

The negative protein balance caused by severe injury results from a large increase in the rate of protein breakdown. Although synthesis is also increased, the increase is insufficient to offset the increased rate of breakdown. The increase in muscle protein breakdown is coupled with an increase in the outward transport of amino acid precursors for synthesis elsewhere in the body. The negative amino acid balance persist across the muscle even for a person in the fed state. Further more, increasing the amount of protein intake has no effect on the rate of muscle protein synthesis. (Patterson, *et. al.* 1997).

1-5-1 Albumin

Albumin, is the protein synthesized in the liver which is not glycosylated, is the major protein and is principal determinant of intravascular osmotic pressure; it also binds many ligands, such as drugs, bilirubin and free fatty acids. The liver produces about 12g of albumin per day, representing about 25% of total hepatic protein synthesis and half its secreted protein. Albumin is initially synthesized as a preproprotein. Its signal peptide us removed as it passes into the cisternae of the rough endoplasmic reticulum, and a hexapeptide at the resulting amino terminal is subsequently cleaved off farther along the secretory pathway. The synthesis of albumin is depressed in a variety of diseases, particularly those of the liver. The plasma of patients with liver disease often shows a decrease in the ratio of albumin to globulins (decreased albumin/globulin ratio). The synthesis of albumin decreases relatively early in conditions of protein malnutrition. (Murray *et al*., 2000).

1-5-2 Immunoglobulins

α_2- Macroglobulin is a major plasma protein that neutralizes many proteases and targestcertain cytokines to specific organs. Immunoglobulins play a key role in the defense mechanisms of the body, as do proteins of the complement system; some of the principal features of these proteins were described. (Murray *et al*., 2000).

The blood cells which are responsible for the production of the immune system in general are the lymphocytes.

There are two types of lymphocytes:

B-lymphocytes (B-cells).

These are mainly derived from bone marrow in higher animals, they were produce from the stem cells and will migrate with the lymphoid tissue and later will be colonized to mature B-lymphocytes.

T-lymphocytes (T-cells).

These are also produced in the bone marrow but migrate early in liver to the thymus and will be colonized there. All immunoglobulin molecules consist of two identical light chains and two identical heavy chains, held together as atetramer by disulfide bonds forming (Y) shape. Each chain is divided in specific regions or domains, this according to their structure and function. Immunoglobulin bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. Immunoglobulins also have effector functions that binding to various cell types, and activate the cells to perform some function. (Roitt, 1993).

1-6 The effect of infection on plasma lipids

Since lipids are insoluble in water, nonpolar lipids must be combined with amphipathic lipids and proteins to make water-miscible lipoprorteins for transport between the tissues in the aqueous blood plasma.Four major groups of lipoproteins are recognized:Chylomicrons transport lipids resulting from digestion and absorption. Very low density lipoproteins (VLDL) transport triacylglycerol from the liver. Low-density lipoproteins (LDL) are cholesterol-rich lipoproteins resulting from the metabolism of VLDL, and high-density lipoproteins (HDL) are alls cholestrol from the tissues and in the metabolism of other lipoproteins. Chylomicrons and VLDL are first metabolized by

hydrolysis with lipoprotein lipase in extrahepatic tissue. Most of the triacylglycerol is removed, and a lipoprotein remnant is left in the circulation. These remnants are taken up into the liver by receptor-mediated endocytosis, but some of the remnants (IDL) resulting from VLDL form LDL and are finally taken up by the liver and other tissues via the LDL receptor. Triacylglycerol is the main storage lipid in adipose tissue. It is released after hydrolysis by hormone-sensitive lipase to free fatty acids and glycerol. Free fatty acids are bound to serum albumin for transport to the tissues, where they are used as an important fuel source. Hormone-sensitive lipase is stimulated by epinephrine and norepinephrine and inhibited by insulin (Murray *et al*., 2000).

Transitory changes in the plasma levels of lipids, cholesterol and triglycerides have been observed since a long time by many authors, in the course of bacterial infections, with hypocholesterolemia, hypertriglyceridemia in the acute phase, increasing the third day of clinical evolution. Their decrease accompanies the return to normal. The authors propose the hypothesis of a correlation between plasma cholesterol levels and the acute phase response during sepsis, which could be induced by the mediators or effectors of inflammation. Bentz Magnette,(1998).

Allen, (1988) reported that in young broiler chicks inoculated with 2 x 10(6) sporulated oocysts of Eimeria acervulina per bird, total plasma lipids were significantly depressed compared with controls in

the first week after inoculation. The lowest level observed was at 5 days post-inoculation (d.p.i.), at which time the chick host is known to experience malabsorption in the chick host (Ruff and Wilkins, 1980). Analysis of plasma components of infected chicks at 4 and 7 d.p.i. showed that triglycerides, total cholesterol, free fatty acids, pigments and total protein were significantly decreased compared with controls. At 7 d.p.i., reduction of total cholesterol reflected mainly reduction in high density lipoprotein (HDL) cholesterol. However, the ratio of HDL cholesterol/total plasma cholesterol was not significantly different from the control ratio. Density gradient ultracentrifugation of chick plasma separated lipoproteins into three main fractions: portomicrons plus very low density lipoproteins (PM + VLDL), low density lipoproteins (LDL) and HDL. These fractions were analyzed for lipid content. Infection with E. acervulina caused (1) significant reduction in the triglyceride and cholesterol contents of the PM + VLDL fraction at 3 and 5 d.p.i., (2) significant reduction of LDL cholesterol at 9 d.p.i. and LDL phospholipid at 5-9 d.p.i., and (3) significant reduction of HDL cholesterol at 3-9 d.p.i. and HDL phospholipid at 5-9 d.p.i. Starvation of uninfected chicks for 48 h caused significant reduction in plasma triglycerides and phospholipids, but an increase in total cholesterol. Density gradient ultracentrifugation showed that the changes in these components reflected mainly reduction of the lipids in the PM + VLDL fraction. The LDL fractions, however, appeared more intense than those of the controls and contained more cholesterol and

phospholipids. These results suggest that changes at 3 and 5 d.p.i. in the plasma lipoprotein pattern of chicks infected with E. acervulina most closely resemble changes seen in chicks starved for 48 h as far as PM + VLDL fraction is concerned. However, changes seen from 7 to 9 d.p.i. involve the LDL and HDL fractions and may reflect alterations in lipid and/or lipoprotein synthesis in the liver and intestine.

(USA NCEP Recommendations) reported that acute bacterial and viral infection leads to temporarily altered cholesterol levels which return to the usual levels upon recovery.

Katunguka-Rwakishaya, (1997) mentioned that the pathophysiology of Trypanosoma congolense infection was studied in two breeds of sheep, the Scottish Blackface (SB) and Finn Dorset (FD), which were known from previous studies to differ significantly in their susceptibility to haemonchosis, in which anaemia is also the primary pathophysiological effect. It was found that infected SB and FD lambs developed similar intensities of parasitaemia. However, infected SB lambs developed a higher degree of anaemia, more severe thrombocytopaenia and hypoalbuminaemia than infected FD. Following infection, the concentrations of plasma cholesterol, serum phospholipids and total lipids decreased. The decline in these lipid components appeared to be greater in infected SB than in infected FD lambs.

Also Katunguka Rwakishaya, *et. al.*,(1997) reported that in Scottish Blackface sheep infected with Trypanosoma congolense, and given either a low protein (51.5 g digestible crude protein per day) or a high protein (116 g digestible crude protein per day) diet. Both low and high protein diets were isocaloric and animals were monitored for 10

weeks after infection. It was observed that infection was associated with marked reduction in the concentrations of plasma total lipids, cholesterol, phospholipids and non-esterified fatty acids in both dietary groups.Control animals on a high protein diet had higher concentrations of plasma total lipids and cholesterol than those on a low protein diet. Infection caused severe hypoalbuminaemia and reduction in total iron-binding capacity only in the low protein infected group compared to their controls while the changes in the high protein infected and control groups were similar. Control animals receiving a high protein diet had higher concentrations of plasma albumin and total iron-binding capacity than those receiving a low protein diet. These observations suggest that Trypanosoma congolense infection in sheep is associated with marked changes in blood biochemical parameters, some of which are influenced by dietary protein.

Meraihi, *et. al.*,(1990) noticed that the influence of a gram-positive sepsis on the metabolism of circulating lipids, fasted rats were injected with saline (control group) or with a suspension of heat-killed or live *Staph*ylococcus aureus. 18 h later, body temperature was increased, while albuminemia and ketonemia were decreased in the group injected with heat-killed bacteria, as opposed to the control group. Passing from these groups to the group injected with live bacteria, more differences appeared: increase of triglyceridemia and free cholesterolemia; decrease of esterified cholesterol levels and especially of the in vitro activity of diaphragm, heart and adipose tissue

lipoprotein lipase and of hepatic lipase. The decrease of lipolytic activities occurred whether they were measured on a fat emulsion containing long-chain or medium- and long-chain triglycerides. The fact that for the latter the activity was always higher than for the former suggests that the host infected with gram-positive bacteria would clear exogenous fat more easily in the case of medium-chain triglycerides.

Lanza-Jacoby *et. al.,* (1982) found that serum triglyceride levels were significantly elevated in the E coli-treated rats. Adipose tissue LPL and FAS activity was significantly decreased by 50% in E coli-treated rats compared with the control rats. These results suggest that the elevated serum triglyceride levels associated with sepsis maybe caused by a decreased rate of clearance of lipids from the blood and an increased rate of hepatic lipid synthesis.

Lamas *et. al.,* (2002), described the increasing worldwide prevalence of obesity is a major health problem since excessive body weight constitutes a risk factor in a number of chronic diseases. It has been reported that obese individuals are more susceptible to infection than lean subjects; however, the underlying factors are not fully understood.

Khovidhunkit *et. al.,* (2004) reported that infection and inflammation induce the acute-phase response (APR), leading to multiple alterations in lipid and lipoprotein metabolism. Plasma triglyceride levels increase from increased VLDL secretion as a result of adipose tissue lipolysis, increased de novo hepatic fatty acid

synthesis, and suppression of fatty acid oxidation. With more severe infection,VLDL clearance decreases secondary to decreased lipoprotein lipase and apolipoprotein E in VLDL. In rodents, hypercholesterolemia occurs attributable to increased hepatic cholesterol synthesis and decreased LDL clearance, conversion of cholesterol to bile acids, and secretion of cholesterol into the bile.

Hassan, (1996) mentioned that the cholestrol concentration in fattened sheep was significantly higher ($P<0.05$) than unfattened sheep and its founded to be 74.06±5.56mg/dl in fattened sheep and 43.00±4.66mg/dl in unfattened sheep.

Chapter 2

Materials and Methods

2-1 Animals

Ten, male Hamari sheep were used in this experiment all animals are about eight month old their weight was 21.5 kg. They brought from Abu zaid market. Grouped in two groups A and B, and kept in closed system-housing left about 3 weeks to adapte, and an anti coccidial drug (Amprolium) was given orally.

Also broad spectrum anti–parasitic drug (Ivermection 1%) dosed ½ cc s/c/head. by An–Ear– Tag Applicator for each animal of the group numerated ear tag were applied.

Group A was fattened on concentrate (Dura –grain " Feterita" 52%) cotton seed cake (csc) 47% and salts as 1%), while group B was left on roughages *Sorghum vulgene* for maintenance and each animal was followed carefully for any abnormality the body temperature was measured daily using Digetal thermometer.

2-1-1 Tagging using an Ear – Tag Applicator

First the area disinfected with sprit, shaved and then an enumerated ear – tag applicator was applied, for group A and group B.

2-1-2Weighing

The sac–bag balance was applied at the flank, hocked in enumerated balance and then read for each animal.

2-2 Sample Collection

2-2-1 Sera for the biochemical examination

It was taken twice, first as the initial sample after adaptation period, then they were subjected to different diets, group A continued with fattening ration and B supplied with raphages for maintenance. After seven weeks both groups were infected with *Staph aurous sup sp. Anaeroius*. Then one week later the second sample was collected from both groups.

Parameters measured were (total lipids, triglycerides, free fatty acids (FFA) cholesterol, phospho lipids , total Proteins, total globulins and Albumins).

Jagular vein region was shaved, disinfected and 5cc syring was used to take 5cc blood sample in a test tube placed in an angle position to ease separation of clot from sera which was kept in the refreigrator for use.

2-2-2 Extraction of Volume from adipose tissue biosy

From adipose tissue around the abscess about 1 gram was taken using the scisor and forceps and then kept in avial filled with normal saline in the refriegrator for the extraction of volume from adipose tissue which was done later as follow:

One gram of the sample plus 10mL chloroform methanol 2:1 was heamogenized , 10 mL of the mixture of the extraction solution was then taken, left to stand for 30 mintues , filtered in a measuring cylinder or graduated test tube , then 4 mL of 0.4 % Nacl was added

and left over night , the upper phase which contains water was removed and the volume of extracted solution was removed which contains the fats and then proceed latter as in serum .

2-3 Experimental procedure

Inoculation with *Stephylococcus aureus sub sp. anaerobius* which is highly haemolytic catalase negative, was done after seven weeks of fattenning when the weight of group A (fattened sheep) reached 23–25 kg. but that of group B unfattened was only 13–15 kg Bwt. Animals were Inoculated at the left site near the parotid lymph node I/d and slightly S/C the shaving of the inoculated areas was carred out two days before Inoculation to avoid infection through ulceration. Then the animals were closely observed for the appearance of abscesses for two weeks for taking the second sample and the biobsy.

Temperature was taken before Inoculation and daily Post–Infection for 12 dayes for all groups. Inoculation sites were examined daily for size by Vernir and for ripening by palpation. When ripened, adipose tissue biopsy was taken from each, then extraction of volumes was performed FFA, PH, phospholipids and cholesterol were measured in the samples.

2- 4 Biochemical measures

Tests were performed to the serum and for the adipose tissue sample, similar volume were taken from the extract.

2-4-1 Plasma Total Protein Determination

Plasma total proteins were determined by method of Henry, (1957).

Principle

Proteins together with a basic copper-sulphate solution containing tartrate (biuret reagent) form a violet blue colour complex).

Reagents

Reagent 1	Potassium – Sodium – Tartrate	15 mmol/L
Biuret	Sodium Iodide	100 mmoI/L
	Potassium Iodide	15 mmoI/L
	Copper-sulphate	5 mmoI/L
Standard	Concentration	7 g/dL

Prepation and Stability

The reagent is ready for use .

It's stable up to the date of expiration as specified .

Sample

Serum or plasma

Procedure

	Blank	Standard	Sample
Standard	-	25 µL	-
Sample	-	-	25 µL
R.1 Biuret	1.0mL	1.0 mL	1.0 mL

Mix and incubate 15 min at 30 – 37c

Wait 5 min . at room temperature

Measure the extintion at 540nm against Blank

The colour stable for 30 min .

Calculation

E. Sample

_____ x standard conc. (g/dL) = Total Protein (g/dL)

E. Standard

G/dl = 10g/L

2 -4- 2 Determination of Albumin BCG(Bromcresol Green Method

According to the method described by Doumas and Waston, (1971).

Principle

Colorimetric determination of serum albumin using bromcrosol green (BCD) att pH 4.2

Reagent concentration

Succinato buffor , pH 4.2	75mmol/L
Bromcrosol green	0.15 mmol/L
Brij 35	7 ml/l

Reagent R4 (Standard)

Sheep albumin	5 g / 100ml

Preparation and Stability

All reagents are rady to use.

The reagent is stable at room temperature up to the date of expiration spocifiod . Avoid direct sunlight .

Sample

Serum or plasma collected in heparin .

Procedure

Wavelength	620 nm, Hg 623 (620-640 nm)		
Temperature	+29 to +37C		
Cuvette	1cm light path		
Zero adjustment	Reagent blank		
	Blank	Standard	Sample
Sample	-	-	20µl
Standard / R4	-	20µl	-
Reagent / R1	4000 µl	4000 µl	4000 µl

Mix well and measure after 10 minutes Read result

Aganist reagent blank. The color is stable for 30 minutes.

Calculation

$$\frac{E\ sample}{E\ Standard} \times \text{Standard conc} = \text{Albumin in g/dl}$$

Standard conc 5 g/100 ml

2-4-3 Plasma total Globulins Determination

This was obtained by substraction of albumins from the total proteins concentration.

Total proteins – The albumins = The total globulins

2-4-4 Determination of total lipids

Total lipids concentration was determined according to

Frings , and Dunn , (1970)

Principle

Determination is based on the colour obtained by phosphovaniline after digestion in H_2So_4 .

Reagents

1- Conc. H2 So4

2- Vanilin 0.6% in water (w/v).

3- Phospho – vanilin reagent

200 ml 0.6% vanilin + 800 Conc.

Phophoric acid – storage in a brown bottle at room temp.

4- Stock standard: 1 gr. Olive / 100 ml absolute ethanol

5- Working std: 200, 400, 600, 800 mg %

And 1000 mg % for STD cure the dilution is made by absolute ethanol.

Procedure

- Serum 0 – 1 ml
- Conc . H_2 So_4 2 – 0 ml mix well .
- Place the tubes in a boiling water bath for 10 min . Cool for about 5 min.
- Transfere 1 ml of the mixture into a clean test tube .

- 5 ml Phospho – vanilin reagent is added , the tube are will mixed . and incubated in water bath at 37°c for 15 min .
- Cool for 5min. read absorbance at 540 nm

Calculation

$$\frac{\text{E. sample}}{\text{E. standard}} \times \text{conc standard} = \text{mg \%}$$

2-4-5 Determination of Cholesterol

According to the method described by Gordon, (1968).

Principle

Cholesterol and its esters are released from lipoproteins by detergents. cholesterol . esterase hydrolizes the esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol – oxidase according to the following equation :

Esters cholesterol $+ H_2O \xrightarrow{CHE}$ cholesterol + fatty acids

Cholesterol $+ O_2 \xrightarrow{CHOD}$ cholest – 4 – en – one $+ H_2O_2$

H_2O_2 + 4 – AP + phenol $\xrightarrow{POD}$ Quinonimine $+ H_2O$

The quantity of this red dye quinonimine formed is proportional to the cholesterol concentration .

Reagents

Reagent 1	Pipes pH 6.9	90mmol/L
	Phenol	26 mmol/L
Reagent 2	Peroxidase	1250 u/ L
Vial of enzymes	Cholesterol esterase	300 U/L
	Cholesterol oxidase	300 U/L
	4 – Aminophenazone	0.4mmol/L
Standard	Cholesterol sol .	200 mg /dL

Preparation and stability

Dissolve the contents of one bottle R.2 to the contents of one bottle Buffer Reagent R.1

This working reagents is stable 4 months at 2 – 8ºC or 40 days at room temperature when stored in dark bottle .

Sample

Serum or EDTA plasma .

Stable for up to 3 months at – 20ºC for up to 78 days at 2 – 8ºC.

Procedure

	Blank	Standard	Sample
Standard	-	10 µL	-
Sample	-	-	10µL
Working Reagent	1.0 ml	1.0 mL	1.0mL

Mix , incubate 5 min at 37c or 10 min at 15 – 25c

Measure the extintion (E) of standard and sample against

Blanck reagent at 505 nm (500 – 550) or Hg. 546 nm

The colour is stable for 60 min .

Calculation

$$\text{Cholesterol conc} = \frac{\text{E. sample}}{\text{E. standard}} \times \text{conc standard}$$

Mg/ dl x 0.0258 = mmol / L

Standard conc : 200 mg /dl

2 –4- 6 Quantitative Determination of Triglycerides

According to the method described by Buccolo, *et al* (1973).

Principle

Sample triglycerides incubated with lipoproteinlipase (LPL) liberate glycerol and free fatty acids . Glycerol is converted to glycerol – 3 – phosphate (G3P) and adenosine – 5 – diphosphate (ADP) is then converted by glycerol kinase and ATP. Glycerol – 3 – phosphate (G3P)

is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2) .

In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4–aminophenazone (4–AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye :

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty} + \text{acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + \text{O2} \xrightarrow{\text{GPO}} \text{DAP} + \text{H2O2}$$

$$\text{H2O2} + 4 - \text{AP} + \text{p} - \text{chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the triglycerides concentratration in the sample.

Reagents

R 1	GOOD pH 7.5	50 mmol/L
Buffer	P – chlorophenol	2mml/L
	Lipoprotein Lipase	150000 U/L
	Glycerolkinase (Gk)	500 U/L
R 2	Glycerol – 3 oxidase (GPO)	2500 U/L
Enzymes	Peroxidase (POD)	440 U/L
	4 – Aminophenazone (4 – AP)	0.1 mmol/L
	ATP	0.1 mmol/L
Triglycerides cal	Triglycerides aqueous primary standard	200 mg/dl

Preparation

Working reagent (WR): Dissolve (→) the contents of one vial R2 Enzymes into one bottle of R 1 Buffer .

Ref 1001310 Working reagent (WR) Dissolve (→) the contents of one vial R2 Enzymes in 10 ml of R 1 Buffer .

Storage And Stability

All the components of the kit are stable until the expiration date on the label when stored tightitly closed at 2 – 8ºC protected from light and contamination prevented during their use . Do not use reagents over the expiration date .

Once open is stable up to 1 month when stored tightly closed at 2 – 8ºCprotected from light and contamination prevented during their use .

- Spectrophotometer or colorimeter measunring at 505 nm (490-550) .
- General laboratory equipment .

Sample

Serum or plasma! Stability of the sample: 5 days at 2 – 8ºC

Procedure

1. Assay condition :

   Wavelength …….……505 nm (490 –550)

   Cuvette: ……..……1 cm light path

2. Adjust the instrument to zero with distilled water .
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard UL	-	-	-
Sample (uL)	-	-	10

4. Mix and incubate for 5 min . at 37ºC or min at room temperature .
5. Read the absorbance (A) for at least 30 minuts .

Calculation

(A) Sample / (A) Standard X 200 (standard conc) = mg/dl triglycerides in the sample

Conversion factor mg/dl x 0.0113 = mmol/L

2 –4- 7 Determination of phospholipids

According to the method described by Correvty , *et al.,* (1961).

Reagents

1- T.C.A 5% w/v in D-W-

2- Digestion Mixture :-

1- D . W 50 ml

2- Conc . H2 So4 25ml

3- hydrochloric acid 20% 25ml

mix 1,2 and 3

4- Sodium Acetate :-

50% of the trihydrate w/v in DW.

2.5 % w / v ammonium molybdate

5- metol : 1 gr / 100ml 3 %

soduim metabisulphite

6- Stock std:

0.439 gr anhydoraus $KH_2 PO_4$ in

100 md D .W containing 0.2 ml H_2SO_4

7- Working STD

Dilute Stock 1ml in 250 ml DW.

1- Procedure

2- in a test tube marked at 10 ml level :-

3- Serum 0.2 ml .

4- Add 5.0 ml of 5% T CA drop by drop with shaking.

5- Centrifuge to give a tightly Packed PPT .

6- Decart of supernatant .

7- Stand the tube inverted on a filter Paper .

8- Wipe round the inside of the tube .

9- Add 1mL Digestion mixture.

10- Heat gently utill the liquid becomes colourless .

11- Allow To cool .

12- Add carefully 1.0 DW. and boil for 15 sec . to convert phosphate into orthophophate .

13- Add after cooling 1.0 mL D.W 50% Sodiumactate

14- Make up to 10.0 mL mark with DW.

15- Add 1 mL ammonium molypdate mix .

16- Add 1 mL metol and mix

17- Stand 15 min .

18- Measure O.D. against blank at 700 nm or red filter .

The colour is stable for several hours.

Regent Blank

0.25 ml Conc . H_2SO_4 , 1.0 ml 50%

Na Acetate , 1.0 ml molypdate , 1ml

Metol and 8.75 ml DW.

Standard

5 ml working std .

0.25 ml H_2So_4

1.0 ml A date + 1 ml molybdate + 1.0 mL metol + 5. 75 D.W.

Calculations

$$\frac{T}{std} \times 10 \quad \text{mg \%}$$

2 – 4 -8 Free Fatty Acids Determination

Free fatty acids were determined in plasma according to the method described by Mikac-Devic *et al* , (1973).

Principle of the methds

The method is based on transfer of free fatty acids – coppersoaps from coppernitrate – triethanol – amine reagent to the chloroform phase, and the determination of copper with the 1,5 diphenyl carbohydrazide.

Reagents

1- copper – triethanolamine Solution

i. 6.45% W/V copper nitrate (6.45 g/100 m1)

distilled water .

ii. IM aqueous triethanolamine solution:

triethanolamine (13.2 ml) were dissolved in 100 ml DW .

mix equal volumes of (i) and (ii) daily before use .

2- 5% w/v sodium chloride solution.

3- 1.5 diphenyl carbohydrazide 1.5 w/v in acetone.

4- Chloroform.

5- Palmitic acid 51.2 mg/ 100 ml chloroform (2000 µ mole / L)

Procedure

To 1 ml sodium chloride solution 5% in a glass – stoppered test tube. 1 ml copper-triethanolamine solution was added. Plasma (0.1 ml) was added and the stoppered tubes were shaken for 3 min.

Chloroform (4ml) was added and the tubes were shaken for 5 min. to 2 ml of the chloroform phase was added 0.2 ml of 1.5 diphehyl-carbohydrazide reagent. After mixing and standing for 10 min, the violet colour was read at 550 nm in spectrophotometer {pye unicam sp6-200)

Calculations

$$\frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 51.2 = \text{mg /100 ml plasma}$$

2 – 5 Statistical analysis

Using T-test design (Nie, 1983) Statistical Analysis System (SAS) was performed, which analyzed the mean values of all parameters with each other and compared the significant difference between the groups and the controls.

Chapter 3

Results

3-1 Effects of fattening on blood biochemical measures in sheep infected with Morel's disease

The effect of fattening on free fatty acids proteins, total lipids, cholesterols, triglycerids, Albumins and phospholipids in sheep infected with Morels' disease, is presented in table (1).

3-1-1 Total proteins

The effect of fattening on total proteins in sheep infected with Morel's disease is presented is table (1) the mean values of total proteins were significantly ($P<0.05$) low after infection in both fattening and unfattening groups compared to values before infection.

3-1-2 Albumins

The effect of fattening on Albumin in sheep infected with Morel's disease is presented in table (1) the mean values of Albumin were significantly ($P<0.05$) low in the infected fattened sheep and showed lower value in the infected unfattening sheep compared to the start level.

3-1-3 Total Golbulins

The effect of fattening on the total globulins in sheep infected with Morel's disease is presented in table (1) the mean values of the total golbulins were significantly ($P<0.05$) high values in both groups compared to the start levels.

Table (1)

The effect of fattening on total protein, Albumins, total golbulins, total lipids, cholesterols, triglycerids, phospholipids and free fatty acids, in sheep infected with Morel's disease (N=5).

	T. Proteins Mg/100ml	Albumins Mg/100ml	T. Golbulins Mg/100ml	T. Lipid Mg/100ml	Cholesterol Mg/100ml	T.G Mg/100ml	Phospholipid Mg/100ml	F.F.A Mg/100ml
Day one Group A	6.3a ± 0.57	4.18a ± 0.89	2.23a ± 0.93	272.29a ± 55.28	62.19a ± 24.54	24.75a ± 10.89	4.9a ± 1.45	30.78a ± 14.92
Day one Group B	7.84a ± 1.5	4.5a ± 0.46	3.22a ± 1.61	250.99a ± 6.66	99.25a ± 38.91	33.04a ± 11.78	5.84a ± 0.54	34.75a ± 17.64
A After infection	6.29b ± 0.61	2.51b ± 0.55	3.66b ± 0.93	144.57b ± 19.98	57.92b ± 14.24	37.19b ± 22.	4.46a ± 1.31	19.96b ± 8.2
B After infection	5.39b ± 0.27	2.29b ± 0.28	3.05a ± 0.55	150.59b ± 11.08	81.65b ± 15.06	36.83b ± 18.78	4.5a ± 1.12	23.59b ± 11.08

Means within columns followed by different letters are significantly different at ($P<0.05$).

N = Number of replicates.

3-1-4 Total Lipids

The effect of fattening on total lipids in sheep infected with Morel's disease is presented in table (1) the mean values of total lipids decreased significantly ($P<0.05$) in the infected fattened group A, and only very low level in the infected unfattening sheep compared to values of time zero.

3-1-5 Cholesterol

The effect of fattening on cholesterol in sheep infected with Morel's disease is presented in table (1) the mean values of total cholesterol were lower in both groups after infection and the differnce was significant ($P<0.05$) in group A the fattened sheep.

3-1-6 Triglycerides

The effect of fattening on triglycerides in sheep infected with Morel's disease is presented in table (1) the mean values of triglycerides are increased significantly ($P<0.05$) in both the infected fattening and unfattening sheep compared to the levels before infection.

3-1-7 Phospholipids

The effect of fattening on phospholipids in sheep infected with Morel's disease is presented in table (1) the mean values of phospholipids showed similar levels in the two groups, after infection with slightly lower levels compared to day one.

3-1-8 Free Fatty Acids

The effect of fattening on free fatty acids in sheep infected with Morel's disease is presented in table (1) the mean values of FFAs after infection were significantly ($P<0.05$) lower in the fattened group and just low in the unfattening – sheep compared to the samples of day one for each group before infection.

3-2 The effects of fattening on adipose tissue composition of sheep infected with Morel's disease.

3-2-1 Free fatty acids concentration

The effect of fattening on free fatty acids concerntration is presented in Fig (1) the Concentration of FFA in adipose tissue biobsy from both fattened and unfattened infected groups were found to be simillar, only the unfattened groups showed slightly higher value compared to the fattened group.

3-2-2 Cholesterols

The effect of fattening on cholesterol concentration presented in Fig (2) the concentration of cholesterol in adipose tissue biobsy from both fattened and unfattened infected groups were found to be simillar, only the unfattened groups showed slightly higher value compared to the fattened group.

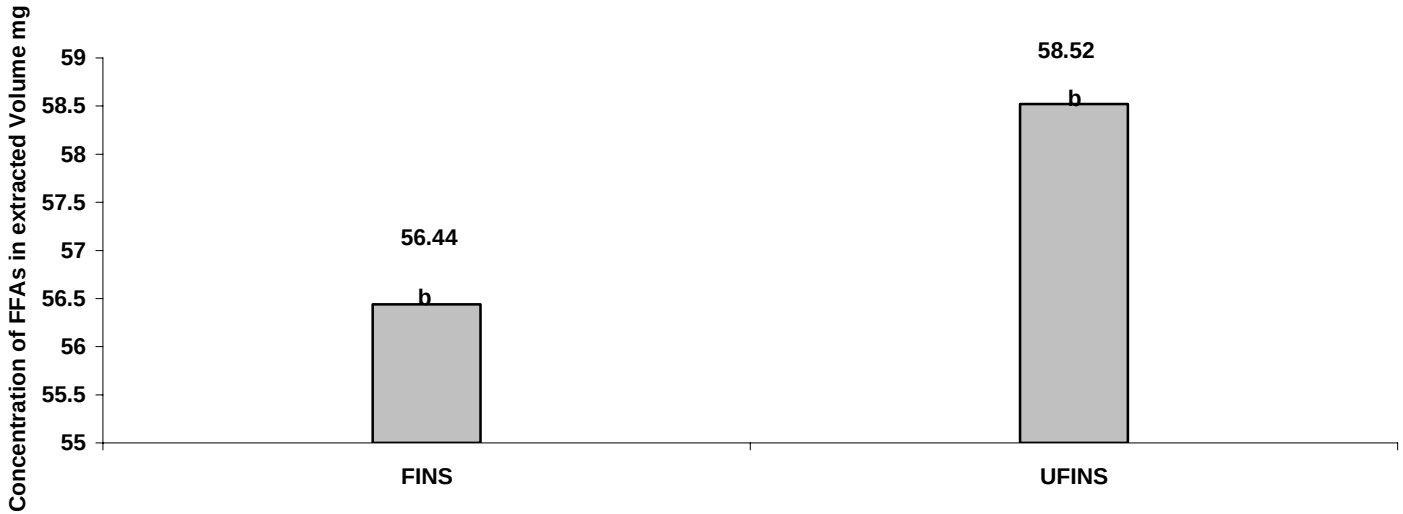
3-2-3 The PH

The PH of adipose tissue biobsy of fattened and unfattened is presented in Fig (3) they were found to be more acidic for the the fattened group compared to the unfattened infected one.

3-2-4 Phospholipids

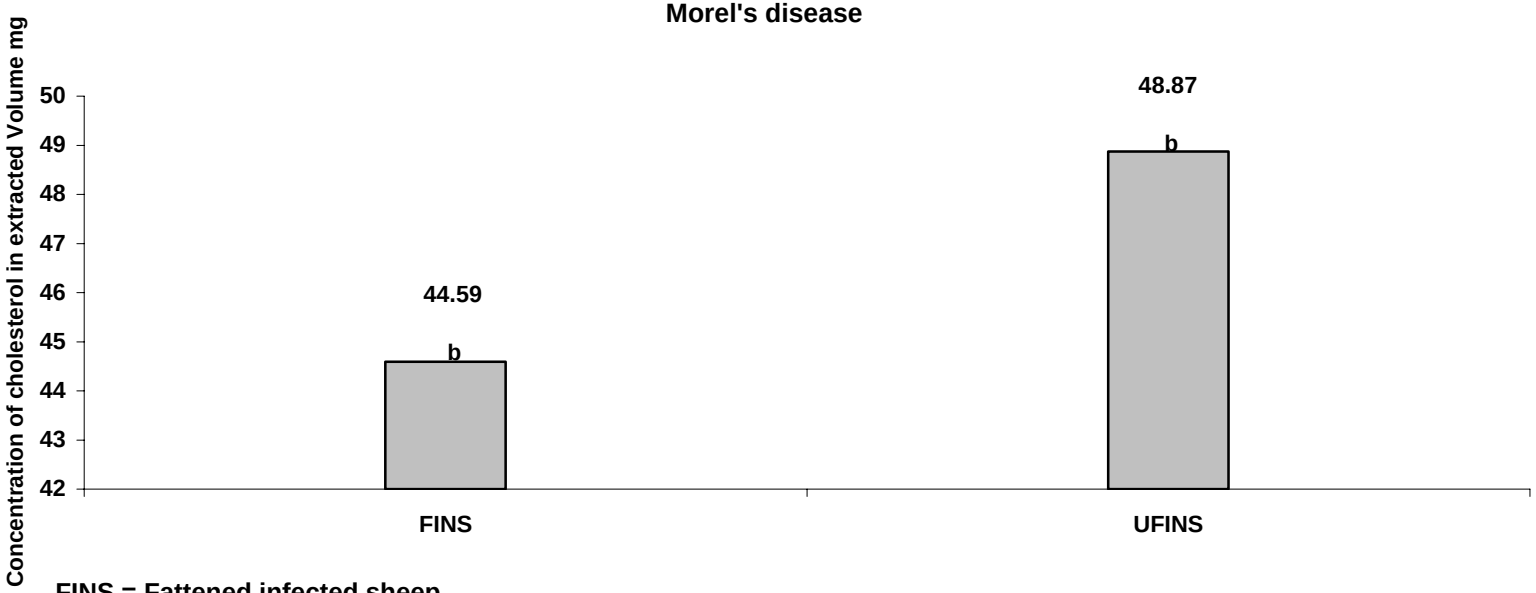
Phospholipids concentration is presented in Fig (4) the concentration of phospholipids in adipose tissue biobsy from fattened infected sheep was significantly ($P<0.05$) high than in the unfattened infected group.

Fig (1)The Effect of fattening on free fatty acids of adipose tissue biopsy in sheep infected with Morel's disease



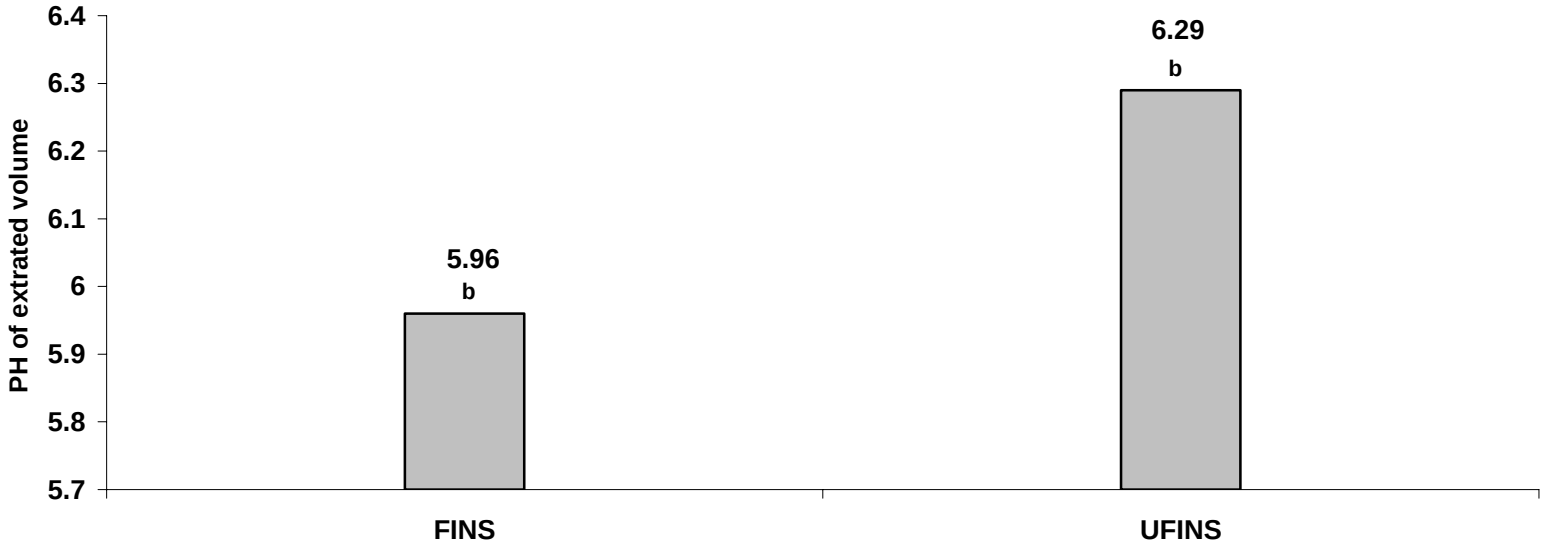
FINS = Fattened infected sheep.
UFINS = UnFattened infected sheep.
n = Number of replicates.
n = 5
Means above Columns followed by different letter, are significantly different.

Fig (2)The Effect of fattening on total cholesterol of adipose tissue biopsy in sheep infected with Morel's disease



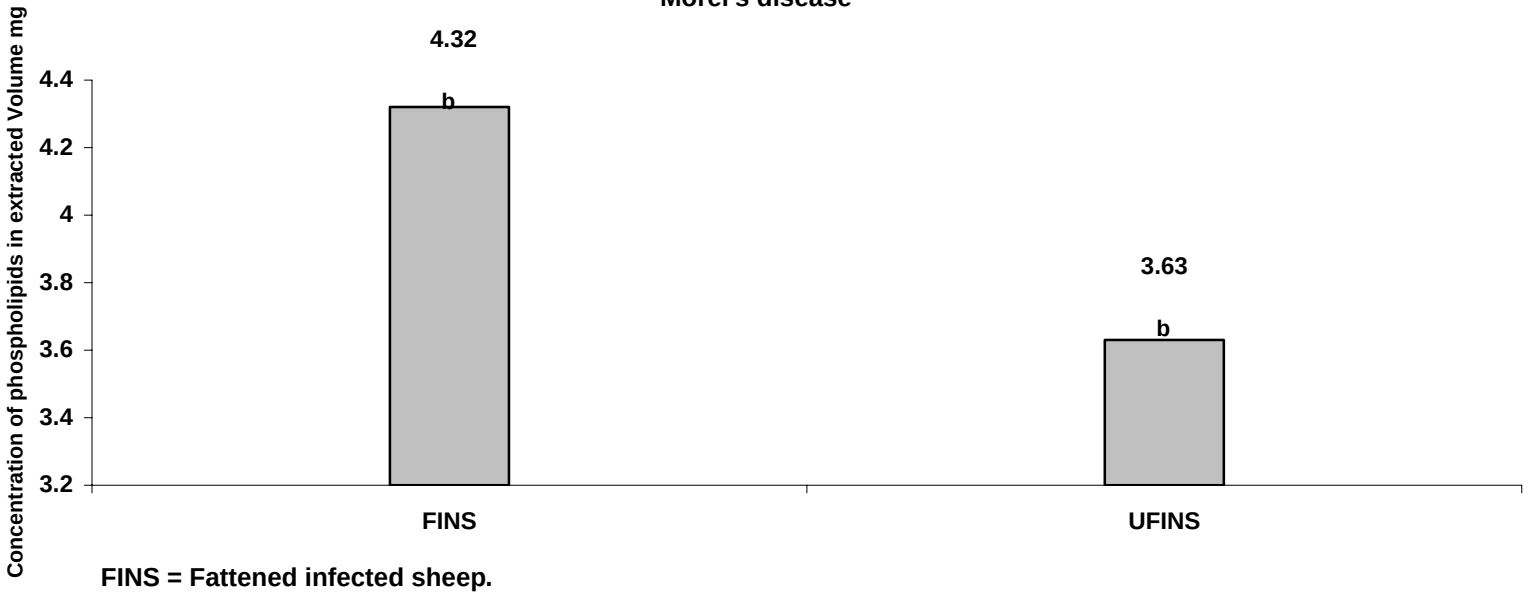
FINS = Fattened infected sheep.
UFINS = UnFattened infected sheep.
n = Number of replicates.
n = 5
Means above Columns followed by different letter, are significantly different.

Fig (3)The Effect of fattening on PH of adipose tissue biopsy in sheep infected with Morel's disease



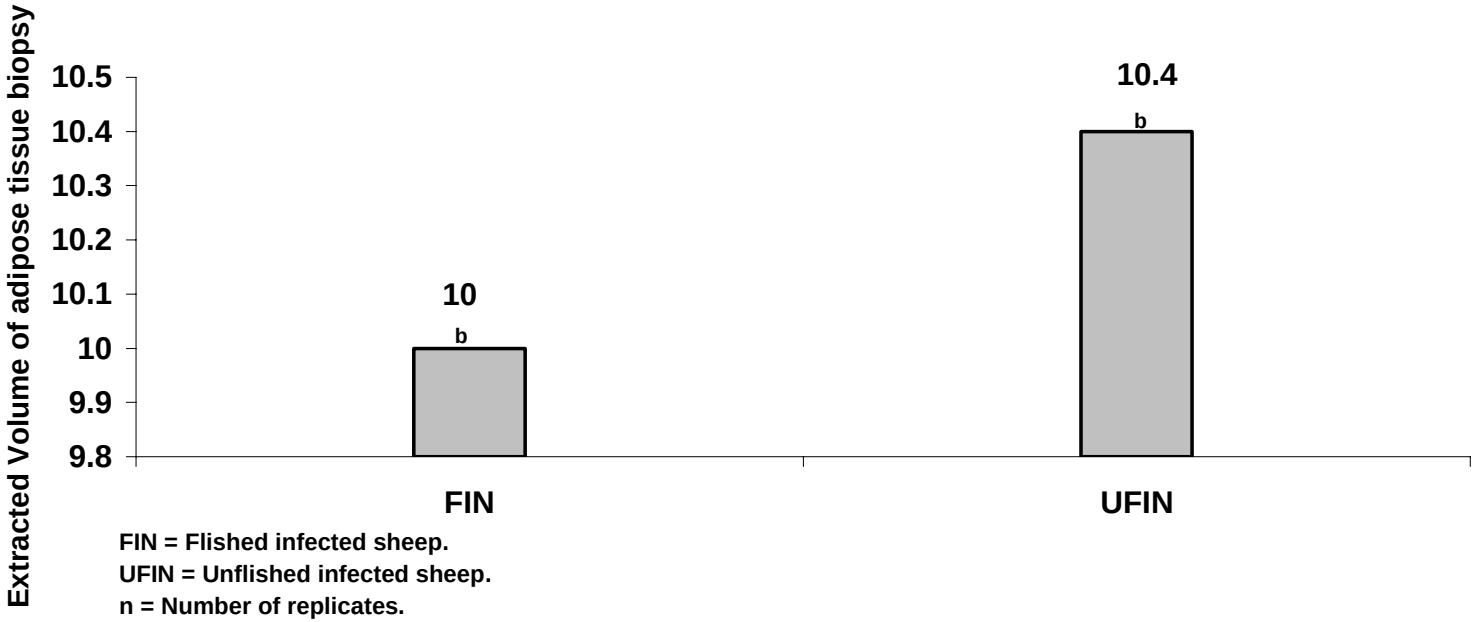
FINS = Fattened infected sheep.
UFINS = UnFattened infected sheep.
n = Number of replicates.
n = 5
Means above Columns followed by different letter, are significantly different.

Fig (4)The Effect of fattening on phospholipids of adipose tissue biopsy in sheep infected with Morel's disease



FINS = Fattened infected sheep.
UFINS = UnFattened infected sheep.
n = Number of replicates.
n = 5
Means above Columns followed by different letter, are significantly different.

Fig (5) Extracted volume of adipose tissue biopsy in sheep infected with Morel's disease



FIN = Flished infected sheep.
UFIN = Unflished infected sheep.
n = Number of replicates.
n = 5
Means above Columns volume of different extracted tissue biopsy.

3-3 The Body weight effect

3-3-1 The Body weight and abscess size

The correlation between the abscess size (cm^2) and the body weight (kg) of fattened and un fattened groups was shown in table (2) where the abscess size increased after infection proportionally to the body weight.

Table (2)

The correlation between abscess size (cm^2) and body weight (kg) of both groups (A and B).

	Days post infection	3	5	7
Group A	Size of Abscesses (cm^2)	23.2	23.6	24.9
	Body weight (kg)	22.4	22.8	23.3
Group B	Size of Abscesses (cm^2)	12.9	14.0	16.8
	Body weight (kg)	17.5	19.4	21.1

3-3-2 The food conversion Ratio (FCR)

The food conversion Ration (FCR) for group A while group B was fed roughages for maintanance

Average daily intake from concentrates	1.5 kg/day/head
Average daily gain in weight	71.4 gm/day
Average total gain weight (49 days)	3.5 kg
Initial average live weight	21.85
Fattening average live weight FCR	0.05

3-3-3 The correlation between the fattened and body weight (kg).

Table (3)

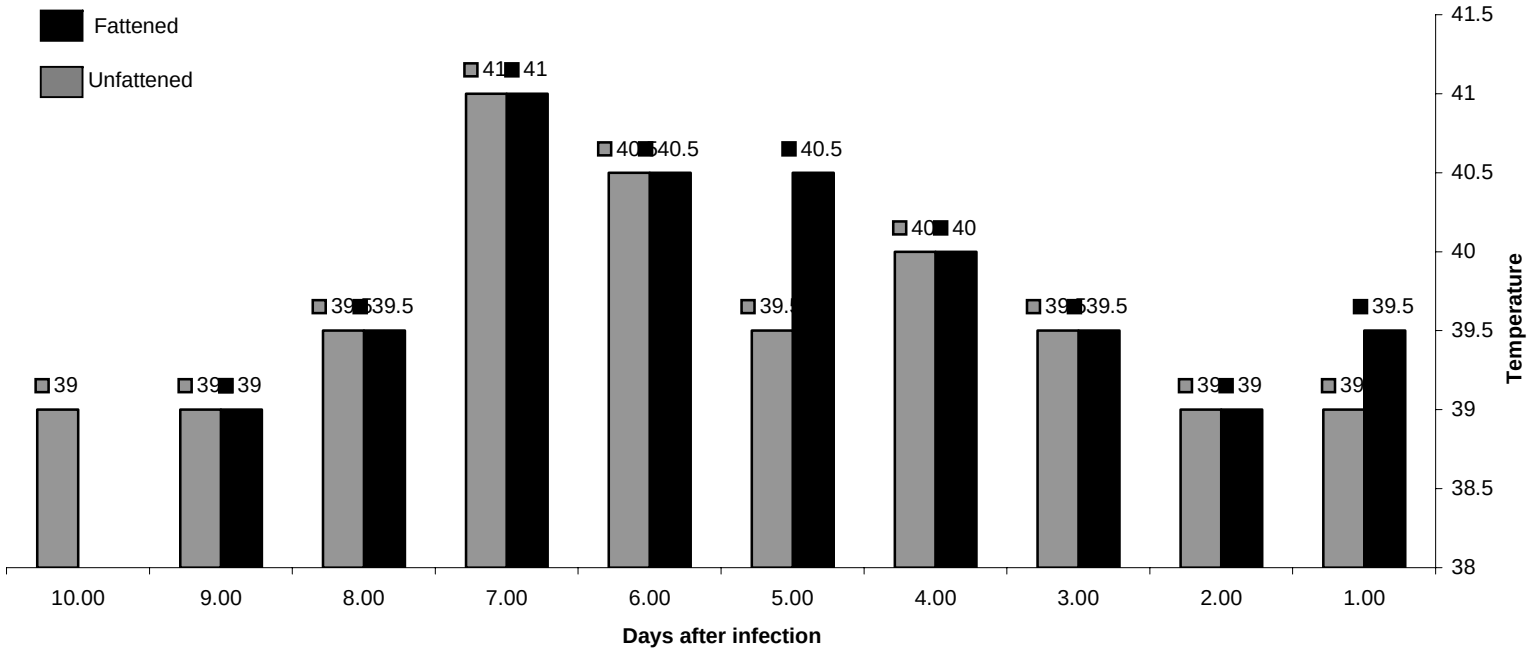
Number of animal	Fattened infected sheep Group A	unfattened infected sheep Group B
Intial BWT (kg)	21.7 ± 2.36	22.0 ± 2.32
Final BWT (kg)	25.2 ± 1.35	17.5 ± 3.52

The final and intial body weights for fattened and unfattened infected sheep, were presented in table (3) group A increased in weight by 16.1% and group B decresed in weight by 22.7% compred to their intaial weights.

3-4 Body Temperature

The effect of infection in fattened and unfattened body temperature was presented in Fig (6). It was raised in post infection and then came to the normal range by day 3 and then the raised again by day 7 when the abscesses were opened. This could be due to reabsorption of toxin from discharging abscesses causing a sort of toxaemia.

Fig (6)Body temperature of fattened and unfattened infected sheep (Cº)



Chapter 4

Discussion

This study was conducted to investigate the effects of pathogenicity of *(Staph aurous sup sp. Anaeroius)* on fattened sheep compared to poor fed group. The effect on some important biochemical parameters related to lipids metabolism in blood and adipose tissue were measured.

Hassan, (1996) reported that incidence of sheep Abscess disease was found to be about 5.8% in natural areas in Watish and Hamari breeds and reached 62.5% among feedlots areas in hamaric breed, morever the occurrence of the disease in natural grazing areas was low. He suggested that this could be due to the type and quality of feed in the pasture which is enough only for maintenance, and cases that showed the disease were mainly those been subjected to concentrated feed (dura) in the drought seasons, since the pasture was very poor. Moreover, the less contact between animals in open pasture minimizes the infection.

In contrast to that, the high percentages of the disease in feedlots areas that were confinded to an intensive system, the concentrated feed provided changes in the body conditions i.e. gaining weight, and the excretions from the skin of traces amount of lipids like cholesterol and fatty acids, that lead to concentration of certain organism and suppress others.

4.1 The effects of fattening on plasma biochemical values measured in sheep infected with Morels disease.

4-1-1 Plasma proteins

Plasma proteins were affected significantly ($P<0.05$) in the fattened group after infection this was manifested as clear reduction of the albumin fraction from 4.18 ± 0.89 to 2.51 ± 0.55 and significant increase of total globulins fraction from 2.23±0.93 to 3.66±0.93. this effect is well known of the liver as it shifts from albumin synthesis to the proteins of acute inflammatory response (Murry, *et al.,* 2000), (Mac Sween and Whaley, 2001). Similar effects were observed in the unfattened group except that the total globulins fraction was not increased after infection. Subfraction of the total globulins might clearify this result.

The level of albumin in both fattened infected and unfattened infected was low but it was lower in the fattened ones compared to the level before infection. This could be explain as that hepatic protein biosynthesis, shift during inflammation from albumin synthesis to the synthesis of proteins involved in the acute inflammatory response such as C-reactive protein, coagulation compounds, fibrinogen and complement components (Mac Sween and Whaley, 2001).

4-1-2 Plasma Lipids

All plasma lipids fractions studied showed siginificant changes in the present work except the phospholipids, lower values were observed in total lipids, free fatty acids and

cholesterol in both groups after infection, but the differences were significant ($P<0.05$) in the fattened group. In contrast triacyle glycerols showed higher levels after infection in both groups and the differences were significant ($P<0.05$). The effcts of infection on the different fractions of plasma lipids was previously studied by many authers,

These findings not agree with Hassan, (1996) who reported higher values of blood cholesterol after infection as 74.06±5.56 compared to 43.00±4.66 in unfattened sheep.

In table (1) the FFA showed very low levels in both infected unfattened and fattened groups compared to the levels before infection. It is well known that free fatty acids, either that arize in the plasma from lipolysis of triacylglycerol in adipose tissue or as a result of the action of lipoprotein lipase during uptake of plasma triacylglycrols into tissues, could be found in low levels in the fully fed condition compared to fully fasting state. This condition is in monogastric animals. The case in continuous feeders as ruminants, where there is uninterrupted influx of nutrient from the intestine, the free fatty acids remain at a very low level, (Murry, *et al.,* (2000).

In the present work, sheep which are ruminant animals showed very low levels of fatty acids after infection, and the difference was siginificant ($P<0.05$), this effect was explained previously by Robert, *et al.,* (1979) who reported that, during pneumococcal sepsis, there is reduced ketogenic capacity of the liver and a possibly decreased hepatic supply of fatty acids. The

reduccion of fatty acid supply to the liver appeared to be a secondary concequence of a severe reduction in circulating plasma albumin. In the present work plasma albumin reduced significantly ($P<0.05$) in both groups. Table (1) and this might explain the low level of free fatty acids after infection.

The effect of infection on the levels of plasma lipids was studied extensively by many authers. Allen, (1988) measured plasma lipids in broiler chicks inculated with Emeria acervulina and found that, total plasma lipids were significantly depressed. Analysis of plasma components of infected chicks, showed significant decrease of triglycerides, total cholesterol and the free fatty acids. Also Katunguka, (1997) measured the levels of blood lipids in Scottish Black face sheep infected with Trypanosoma congolense. He observed marked reduction in the concentrations of plasma total lipids, cholestrol, phospholipids and non-esterified fatty acids. Also Bentz and Magnette, (1998) reported that, transitory changes in plasma levels of lipids, cholesterol, and triglycrides have been obsereved previously, in the course of bacterial infections, with hypocholesterolemia, hypertriglyceridemia in the acute phase, increasing in the third day. Their decrease accampanies the return to normal. The (USA NCEP Recommendations) mentioned, the acute bacterial and viral infection, as leading factors to temporarily altered cholesterol levels which return to the usual levels upon recovery. Also Katunguka, *et al.* (1997) studied Trypanosoma congolense infection in two breeds of sheep, Scottish Black face

and Finn Dorset. Following infection, the concentration of plasma cholesterol, serum phospholipids and total lipids decreased. The decline was greater in the infected Scottish Black face than in Finn Dorset.

It is clear in the present work, that fattening influenced the changes of plasma lipids due to infection, as manifested by the significant reduction of plasma total lipids, total cholesterol in the fattened infected sheep. The decrease was not significant in the unfattened group for the same parameters. Bentz and Magnette, (1998) researched the correlation between the intensity of the acute phase response, represented by the C-reactive protein levels, and reduced cholesterol levels, or hypertriglyceridemia, or lymphocytopenia, they proposed a hypophthesis of correlation between plasma cholesterol levels and the acute phase response during sepsis, which could be induced by the mediators or effectors of inflammation.

One can suggest from the findings in the present study, that fattening increased the effect of infection with *Staph. aureus*, on the acute phase of the inflammatory reaction.

4-2 The effects of fattening on adipose tissue values measured of sheep infected with Morel's disease

4-2-1 Lipids

All lipid parameters studied in the adipose tissue biopsy, from both group showed no significant differences in the present work except phospholipids, lower values only, were observed in free fatty acids, and cholesterol in the fattened

group after infection. In contrast the difference was significantly ($P<0.05$) high for the phospholipids of the fattened group compared to unfattened.

However, since group B in the present work lost weight from 22.0±2.32 kg_{Bwt} to 17.5±3.52 kg_{Bwt} , then the adipose tissue could be affected. It is well known that triacylglycerols undergoes hydrolysis by hormone sensitive lipase to form free fatty acids and glycerols, and when the rate of fatty acids re-estrification is not sufficient to match the rate of lipolysis, free fatty acids accumulate and diffuse into the plasma to act as important source of fuel for many tissues (Murry, *et al.,* 2000).

The effect of the concentration and type of fatty acids, in vitro on the growth of the causative agent of Morel's disease the *Staph. aureus*, was studied extensively previously, Noble, (1992) showed attempts that has been made to discren a relationship between skin flora and free fatty acids in man. Jay-Nidoo, (1981) reported that fatty acid has often been inculated amongest the factors though likely to suppress organisms on skin. Heczho and Kasprowics, (1996) showed that *Staphlyococci,* have higher minimum inhibitory concentration values for various fatty acids including linoleic acid than have propiono bacteria.

Also Robert, (1977) mentioned that the growth and entrotoxcin B formation by *Staph. aureus* and its membrance mutants can be depressed or stimulated by addition of grade amounts of saturated or unsaturated fatty acid mixures, this not

only antagnize some effect of growth and *Staph.* enterotoxin-B formation, but also apear to potentiate inhibition of growth and *Staph*. enterotoxin-B formation by the unsaturated fatty acid mixture, these effects were under given pH. (Robert, 1977).

Also many studies were made to study the effect of different fatty acids on *Staph speicis*, they showed that the type of fatty acids affect the growth also the origin of the bactera either from human or animal can influence the result, (Lacey and Lord (1981); Ushaijna, *et al* (1984), Nobel, (1999) and Dutto and Musser, (2003). However, Jay-Naidoo, (1980) reported that, the inhibition of *Staph* by fatty acid is pH dependent. In the present work, lower total lipids, cholesterol, free fatty acids and pH, in the adipose tissue biopsy of the fattened group were seen compared to the unfattened one. Together with significantly ($P<0.05$) phopholipids increased levels in the biopsy from the fattened one. This would suggest clearly that, fattening affected lipids metabolism, at the area of the infection which lead to increase abscess size. This is most propably by increasing the amount and type of fatty acids, that promote the growth of the organism and the suitable pH for that growth. For the inoculation site, the left side was preferred by Hassan, (1996). He reported that the occurrence of the abscessation in the left side was more than in the right side, this may be due to the distribution of lymphatic vessels and trunks in the left side, where the lymph goes to the left tracheal trunk which may join the thoracic duct and terminate in the external

jugular or the common jugular vein. This distribution of the lymphatic vessels may occur to a lesser extent in the right side where the tracheal trunk may join the right lymphatic duct or the thoracic duct and then terminate in the external jugular or common jugular vein (Sisson and Grossman's, 1975). The size of abscess in fattened sheep reached 4.5×5.25 cm and that of ufattened reaches 4.05×3.2cm. The abscesses formed in fattened and unfattened sheep reached a size that is proportional to the animal body weight where it is 23.2cm^2 for 22.4 kg Bwt, 23.6 cm^2 for 22.8 kg Bwt and 24.9 cm^2 for 23.3 kg Bwt. For fattened. Where it was 12.99 cm^2 for 17.5 kg Bwt, 14.0 cm^2 for 19.4 kg Bwt and 16.8 cm^2 for 21.1 kg Bwt for the unfattened ones. The great correlation between the fattening and the onset of the disease was noticed earlier by Aynaud, (1928). The fattening process in the present works enhanced the occuerance of the abscess disease where the response in the fattened infected sheep was greater than the unfattened infected ones. Moreover the fattened infected groups showed a larger abscesses size than the unfattened infected ones. This was explained as to be due to the depletion of rapidly catabolizable carbon source in the area of infection, is more in the fattened infected than the unfattened infected ones, (Dtto and Musser 2003). The close contact between animals plus the habbit of scratching the head area by the hooves of the hind limbs, may create an entrance for the causative agent. The similarity of the disease to caseous lymphdenitis was noticed in the appearance of the abscesses

externally, and sometimes it is difficult to differentiate the two diseases by the clinical signs, but the rapid onset for Morel's disease and its relation to the fattening process is very clear which was reported as (62.5% in feedlot areas) Hassan, (1996) and for caseous lymphdenitis the incidence was found to be about 15.8%. Caseous lymphdenitis is also choronic in nature and usually discovered in the slaughter house.

4-2-2 pH.

The pH of adipose tissue was acidic for both groups, but more acidic for group A. Group B under going adipose tissue lipolysis due to the reduction of its weight, whereas group A was increasing in weight. This may create favarable media to the causative agent to reproduce formulated d-toxin which is very potent chemottracant agent for the neutrophill cells and results in an increase of an inflammatory response. This is in agree with (Dtto and Musser, 2003).

4-3 Body Temp.

The body temp. of both groups was raised as the result of infection but came to the normal level by day 3 and then raised again by day 7 when the abscesses were opened. This cauld be due to reabsorption of toxin from discharging abscesses resulting in toxaemia.

Conclusions

The present work is conducted to examine the effect of fattening on some biochemical parameters related to lipids metabolism in sheep infected with Morel's disease.

Ten Hamari sheep were infected with the *Staph aureus sub sp anaerobius,* five of them were subjected to fattening program and the other group poorly fed for maintenance.

The results obtained after infection were as follows: It showed significant decrease in total proteins as a result of the decrease of albumins concentration in both infected groups.

Also the two infected groups showed significant decrease in all lipids fractions except the triglycerites which showed high serum concentration specially in the fattened group. The adipose tissue composition of sheep infected with Morel's diseases showed similar level of free fatty acids and cholesterol concentration for both infected groups were found to be similar, only the unfattened ones showed slightly higher values compared to the fattened group. But phospholipids concenteration very high in the fattened group. The pH of adipose tissue of both groups was found to be acidic but more acidic for the fattened compared to the unfattened infected group. Whereas the abscesses size increased along infection days proportionally to the body weight. Also the body temperature raised post infect and came to the normal range by

day 3 and then raised again at day 7, when the abscesses were ruptured.

Clear differences were observed in all values measured in the present work. This was mainly due to fattening as it was the only difference between group A and group B.

The changes in plasma lipids profile together with plasma protein levels can be well studied in future work, to be moniter as early diagnostic values for the disease before the apearance of the abscess. So as to subject the herd for treatment to prevent abscess formation.

Future studies on the effects of fattening on some biochemical parameters (FFA, TGs, cholesterol, total lipid, phospholipid, total protein, Total globulin and albumin on sheep infected with Morel's disease should be carried with regarding to the causative dose of the *Staph. aureus sub sp. anaerobius* and the other strain of *Staph*. will result in more accurately diagnostic significances.

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