بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Graduate Studies

Clinical, Haematological and Biochemical Studies on Induced Frothy Bloat in Goats in South Darfur State, Sudan

الدراسات السريرية والمخطط الدموي والكيميائية الأحيائية للنفاخ الرغوي المسبب في الماعز في ولاية جنوب دارفور، السودان

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By

Entisar Mohamed Hussain Ibrahim

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Supervisor:

Dr. Siham Elias Suliman Mohammed

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

﴿ فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ إِلَيۡكَ وَحۡيُهُۥۖ وَقُل رَّبِّ زِدۡنِي عِلۡمٗا ﴾

صدق الله العظيم

(سورة طه الآية 114)

قال رسول الله صلى الله عليه وسلم:

(من سلك طريقا يبتغي فيه علما سهل الله له طريقا إلى الجنة وان الملائكة لتضع أجنحتها لطالب العلم رضى بما يصنع وان العالم ليستغفر له من في السماوات ومن في الأرض حتى الحيتان في الماء وفضل العالم على العابد كفضل القمر على سائر الكواكب وان العلماء ورثة الأنبياء وان الأنبياء لم يورثوا دينارا ولا درهما وإنما ورثوا العلم فمن أخذه أخذ بحظ وافر**) أخرجه مسلم.**

Dedication

This Thesis work is dedicated to my dear father Engineer Mohamed Hussain Ibrahim, my mother Teacher Laila Mahmoud Yacoub, My sister Dr. Sara Mohamed hussain, My husband Khaled Adam Hamed, My son Mohamed Khaled Adam.

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First and foremost, I thank Allah for letting me live to see this thesis through.

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Table of contents

List of tables

List of Figures

List of Abbreviations

EDTA	Ethylene diamine-tetra-acetic acid
No.	Number
PCV	Packed cell volume
SD	Standard deviation
RBCs	Red blood cells
WBCs	White blood cells
PH	Potential hydrogen

Abstract

The current study was conducted to examine the potential of fresh lush alfalfa to induce frothy bloat in goats and to evaluate some physical, hematological and biochemical parameters. The study was done from September 2017 to February 2018 in the Department of Clinical Studies, Faculty of Veterinary Science, Nyala University, South Darfur State. A total number of 25 clinically healthy male goats (6- 12 months age and 20-25 kg body weight) were utilized in the current investigation. 25 control blood samples and rumen fluid samples were taken from these animals before feeding alfalfa. The animals were feed the plant after fasting for 16 hours and the samples were taken every 2 hours for 24 hours (300 samples). Physical (pulse and respiratory rates, temperature, eye mucous membranes, percussion and auscultation), hematological (Packed cell volume, total red blood cell count, total white blood cell count and differential of white blood cell count) and biochemical (Serum total protein, albumin, urea, calcium, sodium, and potassium) parameters were measured using standard methods. The changes of rumen fluid (color, odor, consistency and pH) were also recorded using standard methods. The clinical signs of induced frothy bloat appeared after 6 hours in goats, there were tachycardia, tachypnea and congested mucous membranes. Percussion and auscultation revealed tympanic sound and decreased ruminal motility. Pulse rate 40-120/min, and respiratory rate 10-68/min were significantly increased from the normal rate in bloated goats. Rectal temperature 36.5-41.1C^{o} was significantly decreased from the normal rate. TRBCs 7-20×10^{6} were significantly increased. PCV and TWBCs were significantly decreased. Lymphocytes, eosinophils and monocytes were significantly decreased ($P \leq 0.05$). Neutrophils were significantly increased, but no effect on Basophils was observed in bloated goats. Rumen fluid showed green

colour, foul odor and frothy consistency with medium to large bubbles. Rumen PH was significantly decreased after 6 hours of induction, after 14 hours rumen pH was significantly increased. Serum total protein was significantly increased after 4 hours, after12 hours total protein was significantly decreased. Serum albumin was significantly decreased. Serum urea, Potassium, calcium and sodium were significantly increased. In conclusion fresh lush alfalfa has a high ability to induce a frothy bloat in goats (6-12 months age), because of rapid severe changes of physical, hematological and biochemical parameters and this leading to death of the animal.

المستخلص

أجريت هذه الدراسة لفحص إمكانية حدوث النفاخ الرغوي للماعز بواسطة البرسيم الغض وتقييم المعالم السريرية والدموية والكيميائية الحيوية، وذلك بقسم الدراسات السريرية كلية العلوم البيطرية، جامعة نيالا، ولاية جنوب دارفور. في الفترة من سبتمبر 2017 حتى فبراير 2018.

تم إجراء التجربة علي عدد 25 ذكر ماعز أصحاء سريريا في عمر 6 الى 12 شهر ووزن 20 الى 25 كيلو جرام. أخذت 25 عينة دم و سائل الكرش للتقييم والتحكم قبل حدوث النفاخ. تم إعطاء الحيوانات البرسيم الغض بعد فترة تجويع لمدة 16 ساعة ثم أخذت العينات كل 2 ساعة لمدة 24 ساعة (300 عينة).

القراءات للمعالم السريرية (معدل النبض، التنفس، درجة الحرارة، الأغشية المخاطية للعين، الطرق، التسمع) والدموية (حجم الخلايا المتكدسة، العدد الكلي لكريات الدم الحمراء والبيضاء والعدد التفريقي لكريات الدم البيضاء). والكيميائية الإحيائية (البروتين الكلي، الألبيومين، اليوريا، الصوديوم، الكالسيوم والبوتاسيوم في المصل)، والتغيرات في سائل الكرش (اللون، الرائحة، المحتوى، الأس الهيدروجيني) قد سجلت بإستعمال طرق قياسية.

الأعراض السريرية في الماعز قد ظهرت بعد 6 ساعات من حدوث النفاخ الرغوي وهي إرتفاع معنوي في معدل النبض والتنفس، إحتقان الأغشية المخاطية، الصوت الطبلي في عملية طرق الكرش مع إنخفاض في حركة الكرش.

معدل النبض 40-120 في الدقيقة والتنفس 10-68 في الدقيقة إرتفعا بصورة معنوية من المعدل الطبيعي في الحيوانات المصابة بالنفاخ الرغوي بينما قلت درجة الحرارة 36.5-41.1 درجة مئوية بصورة معنوية من المعدل الطبيعي ، العدد الكلي لخلايا الدم الحمراء 7- 20×10^6 ارتفع بصورة معنوية، حجم الخلايا المتكدسة والعدد الكلي لخلايا الدم البيضاء انخفضوا بصورة معنوية، الخلايا اللمفاوية والحمضية والوحيدة انخفضوا بصورة معنوية ($P \leq 0.05$)، الخلايا العدلة ارتفعت بصورة معنوية بينما لم يلاحظ اختلاف معنوي في الخلايا القاعدية.

تغير لون سائل الكرش الى الاخضر مع رائحة كريهة ومحتوى رغوي وفقاعات متوسطة الى كبيرة الحجم، الأس الهيدروجيني للكرش انخفض بصورة معنوية بعد 6 ساعات ثم ارتفع بصورة معنوية بعد 14 ساعة من احداث النفاخ.

بروتين المصل الكلي ارتفع بصورة معنوية بعد4 ساعات من احداث النفاخ ثم انخفض بصورة معنوية بعد 12 ساعة، ألبيومين المصل انخفض بصورة معنوية بعد احداث النفاخ الرغوي، مستوي الصوديوم والبوتاسيوم والكالسيوم واليوريا إرتفع بصورة معنوية.

وفي الختام ان البرسيم الغض له فعالية عالية لكي يحدث النفاخ الرغوي في الماعز عمر(6-12شهر) والذي يسبب الإرتفاعات السريعة للمعالم السريرية لهذا المرض و قد تؤدي للنفوق.

Introduction

Ruminant animals carry an active population of micro-organisms; bacteria, fungi, and protozoa in the forestomach of their digestive system. Without these organisms, the animal would be unable to digest fibrous feeds, such as grasses and legumes. In the process of digesting these materials, the microorganisms produce large quantities of gas that must be expelled (Majak *et al*., 2003). Bloat is an over distention of the rumenoreticulum with the gases of fermentation, either in the form of a persistent foam mixed with the ruminal contents, called primary or frothy bloat, or in the form of free gas separated from the ingesta, called secondary or free-gas bloat (Dana *et al*., 2005). Bloat is most common at the beginning of the rainy season when animals are exposed to fast growing lush pasture after being on a diet of dry feed. Salivary mucin is antifoaming, but saliva production is reduced with succulent forages. Bloat-producing pastures are more rapidly digested and may release a greater amount of small chloroplast particles that trap gas bubbles and prevent their coalescence. The immediate effect of feeding is probably to supply nutrients for a burst of microbial fermentation. However, the major factor that determines whether bloat will occur is the nature of the ruminal contents. Protein content and rates of digestion and ruminal passage reflect the forage's potential for causing bloat. Over a 24-hr period, the bloat-causing forage and unknown animal factors combine to maintain an increased concentration of small feed particles and enhance the susceptibility to bloat (Majak *et al*., 2003 and Dana *et al*., 2005). Alfalfa harvested at the vegetative stage caused the highest incidence of bloat (Thompson *et al*., 2000).

Objectives are:

1. To examine the potentiality of fresh lush alfalfa in induction of frothy bloat in goats.
2. To manage the problems associated with frothy bloat.
3. To study the clinical signs and changes in rumen fluid associated with frothy bloat.
4. To study haematological and biochemical changes of the diseases.

Chapter One

Literature review

1. Frothy bloat:

Ruminal tympany is abnormal distention of the rumen and reticulum caused by excessive retention of gases of fermentation, either on the form of a persistent foam mixed with the rumen contents or as free gas separated from the ingesta. Normally gas bubbles produced in the rumen coalesce, separate from the rumen contents to form pockets of free gas above the level of the contents and finally are eliminated by eructation (Radostits *et al*., 2007). According to the etiology can define different form of ruminal tympany: a primary ruminal tympany or a secondary ruminal tympany. Secondary ruminal tympany is characterized by physical obstruction to eructation like esophageal obstruction caused by a foreign body, by stenosis of the esophagus, by pressure from enlargement outside the esophagus, such as tubercuolous involvement of bronchial lymphnodes, or by obstruction of the cardia (Radostits *et al*., 2007). Primary ruminal tympany (also named frothy bloat) is caused by the production of stable foam that traps the normal gases of fermentation in the rumen. The essential feature is that coalescence of the small gas bubbles is inhibited and intraruminal pressure increases because eructation cannot occur (Radostits *et al*., 2007). Both types of bloat can occur simultaneously (Boda *et al*., 1956).

Frothy bloat is the over distension of the rumen caused by the accumulation of fermentation gases in a stable protein foam or froth (Tanner *et al*., 1995).Bloat is defined as a severe enlargement of the abdomen due to an over-accumulation of gasses trapped within the rumenoreticulum. This condition can affect both sheep and cattle (Dana *et al*., 2005).

1.1. Risk factor associated with frothy bloat:

The major factor that determines whether bloat will occur is the nature of the ruminal contents. Protein content and rates of digestion and ruminal passage reflect the forage's potential for causing bloat (Dana *et al*., 2005).

1.1.1. Animal species:

Based on grazing behavior, it would be expected that sheep might affected more severely than cattle because they selectively choose to eat leaves over stems and chew what they ingest more frequently than cattle. Furthermore, sheep appear to select legumes over grasses because the legumes can be eaten more rapidly (Ayre-Smith,1971; Clarke *et al*.,1974 and Colvin and Backus, 1988).

1.1.2. Age of the animal:

Young animals are considered more susceptible to acute and severe bloat than older animals (Mendel and Boda, 1961 and Howarth *et al*., 1991) suggested that with experience, individual animals can cope with bloat-provoking condition. Learning of grazing skills early in life from experienced mothers may have an impact on the off spring's subsequent bloat susceptibility (Ramos and Tennessen,1992).

1.1.3. Fasting:

Fasting has also been shown to predispose animals to pasture bloat (Mendel and Boda, 1961 and Howarth *et al*., 1991).

1.1.4. Animal susceptibility:

Animal susceptibility to bloat is related to the clearance of small feed particles from the rumen. Frequent bloaters have a slower clearance than non-bloaters, which has been demonstrated in both feedlots and pastures. Susceptibility may also be related to the mineral ion balance (the relative concentration of minerals) in the rumen. A predisposition to bloat appears to be associated with a low concentration of sodium together with a high concentration of potassium in the rumen fluid; however, this finding has little practical significance at present (Majak *et al.,* 2003).

1.1.5. Stage of plant growth:

Stage of plant growth should be monitored since, the likelihood of bloat decreases with advancing maturity. Plants in the pre-bud stage are the most

bloat-prone, so grazing should be kept to a minimum at this time point (Coulmen *et al*., 2000 and Majak *et al*., 2003). Bloat has historically been associated with lush growth of pasture forage. Reports of grasses that cause bloat are rare and are restricted to vegetative and lush pastures, where initial digestion rates are high. Alfalfa's potential for causing bloat is highest when moisture conditions are optimal for vegetative growth. Under these conditions, the stems become turgid and fleshy but not fibrous; the leaves are soft and easily crushed between the fingers (Majak *et al*., 2003).Frost and growth of alfalfa at low temperatures have been shown to increase bloat risk by increasing the leaf cell constituents (soluble protein and polysaccharides) implicated in pasture bloat (MacAdam and Whitesides, 1996).

1.1.6. Eructation:

Bloat is characterized by a ruminal dysfunction which results in the accumulation of excessive gas within rumen and is the most common and frequent digestive ailment affecting the ruminants. The slime extracellular bacterial mucopolysaccharides releases and starts accumulating inside the rumen because of rapid microbial growth rates and subsequent cell lysis and increase in quantity of fermented carbohydrates. As a result eructation mechanism is impaired or inhibited because of gas entrapped in high viscous rumen liquor and also forms stable foam that leads to bloat (Cheng *et al*., 1998).

1.1.7. Moisture:

The bloat potential of alfalfa is reduced when soil moisture is insufficient because of either drought or soil salinity. The bloat potential of alfalfa is not significantly affected by irrigation that maintains the soil moisture at 50 per cent of field capacity. At Kamloops, alfalfa is grown under irrigation, and the impact of seasonal rainfall patterns on the incidence of bloat cannot be assessed (Majak *et al*., 2003).

1.1.8. Soil type:

Pasture bloat is more frequent in the moist areas of the parkland regions, especially in Gray Wooded soil zones, becoming progressively less frequent in Dark Brown to Brown soil zones. Legume bloat has been reported in 40 per cent of the livestock farms in the Peace River region of northern Alberta. Many attempts have been made to relate bloat to the mineral nutrition of the plant, generally with conflicting and inconclusive results. The mineral status of the crop is not a reliable predictive factor in the occurrence of pasture bloat (Majak *et al*., 2003).

1.2. Causes of frothy bloat:

Bloat ensues as a chronic manifestation of disease, a dysfunction of the upper digestive tract, or from the consumption of a bloat-provoking feed (Cole *et al*., 1945; Johns, 1954; Cole and Boda, 1960; Howarth *et al*., 1978 and Garry, 1990), the cause is entrapment of the normal gases of fermentation in a stable foam. Coalescence of the small gas bubbles is inhibited, and intraruminal pressure increases because eructation cannot occur. Several factors, both animal and plant, influence the formation of a stable foam. Soluble leaf proteins, saponins, and hemicelluloses are believed to be the primary foaming agents and to form a monomolecular layer around gas rumen bubbles that has its greatest stability at about pH 6 (Dana *et al*., 2005). Bloat results from the accumulation of gas in the rumen. Normally, gas produced during fermentation of feed rises through the rumen contents and forms a gas pocket in the dorsal sac. During frothy bloat the gas trapped within the liquid and particulate contents of the rumen continues to accumulate. Continued accumulation of gas within the rumen increases the pressure within the rumen, eventually causing death by asphyxiation as the rumen exerts pressure on the diaphragm and lungs (Dougherty, 1956).

1.2.1. Legumes causing frothy bloat:

Consumption of legume forages in large quantities is one of the primary causes of frothy bloat; however, not all legumes cause frothy bloat. Legumes can be classified as either bloat-causing, or bloat-safe. Bloat-causing legumes include alfalfa, sweet clover, red clover, ladinoclover, white clover, and alsike clover (Majak *et al*., 2003 and Dana *et al*., 2005). Bloat-safe legumes include sainfoin, birdsfoot trefoil, cicer milk vetch, and lespedeza (Austin, 1981).

Corollaries to the foam hypothesis include theories that protein, saponins, lipids, cations, polysaccharide slimes and cellular fragments of alfalfa stabilize the gas bubbles that are generated during digestion (Johns, 1954; Mangan,1959; Pressey *et al*., 1963; McArthur *et al*., 1964; Miltmore *et al*., 1970; Clarke and Hungate,1971; Gutek *et al*., 1974; Cheng *et al*.,1976; Howarth *et al*., 1977; Majak *et al*., 1980; Majak and Hall, 1990; MacAdam *et al*., 1995 and Mathison *et al*., 1999).

Howarth (1975) suggested that the distribution of surface active substances like proteins and lipids in the rumen liquor may affect their ability to stabilize or destabilize the rumen froth. Subsequent investigations (Howarth *et al*., 1978) led to the suggestion that the lipid membranes of chloroplasts fragmented from mastication and bacterial maceration acted as nucleation sites for bubble formation.

1.2.1.1. Legumes cell walls:

There are many hypothesized reasons why some legumes cause bloat and others do not. It seems that a combination of factors, both plant, animal and microbial ultimately contribute to the condition (Austin, 1981). Bloat-safe legumes have thick cell walls which prevents mechanical disruption while animals are chewing. This means that when bloat-safe legumes enter the rumen it will take longer for rumen microorganisms to invade plant tissues, and consequently are they digested very slowly (Howarth *et al*., 1979). In contrast, bloat-causing legumes are more vulnerable to being broken down

during chewing because of weaker cell walls, allowing rumen microorganisms easier access to cellular constituents. One constituent released upon degradation of the mesophyll cell wall is chlorophyll. It is hypothesized that heightened chlorophyll concentrations within the rumen is the main cause of legume-associated bloat (Majak *et al*., 1986). Once chloroplasts are released into the rumen they are subject to digestion themselves. Digestion results in disruption of the lamellar membranes of chloroplasts releasing soluble proteins, namely fractions I and II, which are believed to be the major foaming agents in the rumen (Howarth, 1975).

1.2.1.2. Legumes soluble proteins:

Legume forages contain high concentrations of soluble proteins that are surface-active foaming agents. Initially, a protein known as Fraction I or 18s (ribulosebisphosphate carboxylase) was examined, but it was soon realized that Fraction II proteins also had foaming properties (Howarth *et al*., 1973). Soluble proteins alone do not account for the extreme viscosity of frothy rumen contents. Because soluble protein concentrations in rumen fluid do not correlate with the occurrence of bloat in cattle that are fed fresh alfalfa, they cannot be the culprits (Majak *et al*., 2003).

1.2.1.3. Saponins:

Saponins are other surface active foaming agents in alfalfa that were considered to be a potential cause of bloat (Lindahl *et al*., 1957). No significant differences in the occurrence of bloat and frothy rumen contents were found in animals fed high versus low saponin alfalfas (Majak *et al*., 1980). Saponins are glycosides; that is, they are composed of carbohydrate and noncarbohydrate, or aglycone, portions. The aglycones are often referred to as sapogenins. The sapogenin nucleus may be either of steroid or triterpenoid structure (Robinson, 1963 and Farnsworth, 1966): Saponins in common forage legumes are of the triterpenoid type (Lindahl *et al*., 1957). Mangan (1959) investigated the foaming properties of various legume

saponins, a "cytoplasmic protein" from red clover, and rumen liquor taken from bloated cattle. The cytoplasmic protein and the rumen liquor showed very similar in vitro relationships of foam strength to pH, leading (Magan, 1959) to conclude that the cytoplasmic protein fraction was the primary foaming agent in bloating forages.

A correlation between calcium content of alfalfa and bloating incidence has been observed (Miltimore *et al*., 1970) calcium is a constituent of saponin foam (Mangan, 1959). A correlation between alfalfa forage zinc and bloat incidence was also observed (Miltimore *et al*., 1970) it is of interest that a relationship between soil zinc and forage saponin levels has been suggested (Henrici,1952). Support for a possible saponin involvement in bloat comes from the fact that administration of legume saponins to ruminants does in fact cause bloating (Lindahl *et al*., 1957). However, they were unable to correlate a bloat severity index with the level of total saponins in ladino clover forage (Jackson *et al*., 1962).

1.2.1.3.1. Saponins inhibition of smooth muscle activity:

In an extensive series of studies that examined the effects of alfalfa saponins on the motiliy of smooth muscle (Lindahl *et al* ., 1957). Intraruminal administration of saponin to sheep resulted in a pronounced reduction in rumen motility. Intravenous administration of saponin also resulted in reduced rumen motility. In these experiments, the site of action was not identified; not all the inhibition could be explained by direct effects on the ruminal musculature. Alfalfa saponin was also found to inhibit eructation, with evidence that direct effects on the central nervous system were involved . The significance of these observations lies in their relationship to the bloat situation, besides a possible rule in stable foam formation, saponins may interfere with gas loss from the rumen by inhibiting eructation.

1.2.1.3.2. Saponins effects on nutrient absorption:

Some effects of ingested saponins on nutrient absorption have been suggested by Ewart (1931) and Sollman (1957) that saponins favor the absorption of sugars. Apparently, no definitive studies have been undertaken to verify this. Saponins reduce the absorption of cholesterol, as has been already discussed, by forming an insoluble addition product. It might be anticipated that saponin would combine with other sterols of similar structure such as those with vitamin D activity and interfere with their absorption. Ewart (1931) stated that saponins favor the absorption of salts of calcium and magnesium.

1.2.1.3.3. Erythrocyte hemolysis:

Saponins have pronounced hemolytic properties (Sollman, 1957). Reaction of the saponin with cholesterol in the erythrocyte wall, resulting in permeability changes, may be responsible for the hemolytic activity (Glanert *et al*., 1962), although a number of hemolytic saponins do not form cholesteroids (Jones and Elliott, 1969). For a number of species has classified susceptibility of erythrocytes to hemolysis as follows guinea pig, horse > dog, rat rabbit > man, pig > goat, sheep, cattle (Ewart, 1931).

The hemolytic effect of saponins is apparently not correlated with other properties; no relationship has been noticed between foam strength (Woodward and Alsberg,1916 and Lindahl *et al*., 1957), bloat producing ability (Lindahl *et al*., 1957), or acute toxicity in monogastrics and the hemolytic properties of saponins (Lindahl *et al*., 1957).

1.2.1.4. Tannins:

In addition to mechanical strength of cell walls, bloat-safe legumes have large amounts of condensed tannins which inhibit frothy bloat. Condensed tannins are plant poly phenols capable of binding soluble proteins responsible for foam production. Condensed tannins have also been shown to reduce digestive activity of rumen microorganisms, slowing clearance of feed

particles from the rumen. This can be beneficial since rapid passage of legume particles from the rumen has been linked with a higher incidence of bloat; however, tannins are only advantageous to a certain degree. It has been shown that if tannins reach concentrations in excess of 20 to 30 g/kg of DM intake, digestion can be negatively affected (Coulman *et al*., 2000).

1.2.2. Microbial factors:

There are also microbial factors which contribute to the stability of foam inside the rumen. In bloated animals it has been noted that rumen bacteria produce an overabundance of mucopolysaccharides that form into a slime in the rumen. This slime increases the viscosity of rumen contents, in addition to stabilizing the gaseous foam (Cheng *et al*., 1998 and Majak *et al*., 2003). According to Coulmen *et al*. (2000) it is the increase in viscosity which leads to formation of gas into the characteristic bubbles.

1.2.2.1. Gas production:

In healthy cattle approximately 30 to 50 liters of gas are produced every hour (Bowen, 1996). Gas is produced as a result of microbial fermentation of ingested feeds and it accumulates at the top of the rumen. Animals rid their bodies of this gas through eructation, or belching. Eructation is initiated when receptors in the dorsal sac of the rumen and those in the area surrounding the cardia, the junction between the rumen and the esophagus, are exposed to free gas. The cardia remains tightly closed if exposed to anything other than gas, preventing liquid and particulate matter from entering the lungs. A complex series of rumenoreticular muscle contractions then forces liquid material away from the cardia, creating an empty space for the dorsal sac to push gas forward (Findlay, 1998). Once surrounded by gas the cardia will relax, which triggers the animal to breathe deeply, drawing gas upwards into the esophagus and lungs, ultimately being expelled through the mouth. This sequence of events should occur approximately once every minute if normal fermentation processes are occurring inside of the rumen (Majak *et al*., 2003). In frothy

bloat the eructation mechanism fails to occur because gas becomes trapped in small bubbles. These bubbles form a frothy or foamy mass inside the rumen (Howarth, 1975).

1.2.3. Feedlot bloat:

Frothy bloat also is seen in feedlot cattle, and less commonly in dairy cattle, on high-grain diets. The cause of the foam in feedlot bloat is uncertain but is thought to be either the production of insoluble slime by certain species of rumen bacteria in cattle fed high-carbohydrate diets or the entrapment of the gases of fermentation by the fine particle size of ground feed. Fine particulate matter, such as in finely ground grain, can markedly affect foam stability, as can a low roughage intake. Feedlot bloat is most common in cattle that have been on a grain diet for 1–2 months. This timing may be due to the increase in the level of grain feeding or to the time it takes for the slime-producing rumen bacteria to proliferate to large enough numbers (Dana *et al*., 2005). Frothy feedlot bloat has been attributed to small feed particles in grain rations that cause slime to form in rumen contents. The slime is made up primarily of polysaccharides secreted by rumen bacteria and released from ruptured bacterial cells. The bacterial polysaccharides increase the viscosity of rumen fluid and subsequently trap small gas bubbles in the rumen fluid, leading to frothy rumen contents and bloat (Majak *et al*., 2003).

1.2.4. Ruminal atony:

Ruminal tympany also can be secondary to the acute onset of ruminal atony that occurs in anaphylaxis and in grain overload; this causes a decrease in rumen pH and possibly an esophagitis and rumenitis that can interfere with eructation. Ruminal tympany also develops with hypocalcemia. Chronic ruminal tympany is relatively frequent in calves up to 6 months old without apparent cause; this form usually resolves spontaneously (Dana *et al*., 2005).

1.3. Diagnosis of frothy bloat:

Diagnosis of rumen dysfunction diseases is difficult using only routine clinical methods of examination. So, in order to establish an objective diagnosis, can examine the rumen fluid adjacent to the blood analysis (Hofirek *et al*., 1989). Animals die if fails to diagnose the bloat and not treated (Radostitis *et al*., 2007).The clinical diagnosis of frothy bloat is obvious (Dana *et al*., 2005). Correct diagnosis of the causes of bloat are made with the expectation that a reliable course of action can be taken to eliminate it (Garry, 1990).

1.3.1 Case history

Normal response after a bloat incident is to examine the animals and the conditions they were in, including the plants they were eating, to see if something can be learned that will help predict and prevent another occurrence. If they can find a common factor responsible for bloat perhaps they can diagnose the problem early, treat it before there is a death, or at least make it occurrence more predictable (Berg, 2000).

1.3.2 Clinical signs and parameters

Bloat commonly begins within 1 hour after being turned onto a bloat producing pasture, the rumen becomes obviously distended suddenly and the left flank may be so distended that the contour of the para lumbar fossa protrudes above the vertebral column; the entire abdomen is enlarged. As the bloat progresses, the skin over the left flank becomes progressively more taut and, in severe cases, cannot be tented. Dyspnea and grunting are marked and are accompanied by mouth breathing, protrusion of the tongue, extension of the head, and frequent urination. Rumen motility does not decrease until bloat is severe. If the tympany continues to worsen, the animal will collapse and die. Death may occur within 1 hour after grazing began but is more common ~3–4 hour after onset of clinical signs. In a group of affected cattle, there are

usually several with clinical bloat and some with mild to moderate abdominal distention (Dana *et al.*, 2005).

Distention is the first clinical symptom used to detect bloat but it is generally insufficient to ascertain the severity of bloat or to verify the onset of acute bloat(Lindahl *et al*., 1957 and Garry, 1990).

The early symptoms include standing up and lying back down repetitively, kicking at the belly, frequent defecation and urination, grunting, and extension of the neck and head. As bloat severity worsens animals have difficulty breathing because of pressure that is exerted on the diaphragm by the gas filled rumen. The animal protrudes the tongue, salivates, and extends the head. Occasionally, projectile vomiting occurs, and the animal may expel soft feces in a stream. Ruminal movements are usually much increased in the early stages and may be almost continuous, but the sounds are less audible because of the frothy nature of the ingesta. Later, when the distension is extreme, the movements are decreased and may be completely absent. The tympanic note or drum sound produced by percussion (tapping on the distended rumen) is characteristic. Before severe bloat (known as clinical tympany) occurs, a temporary increase in eructation and rumination can be noted, but both disappear with severe bloat (Boda *et al*, 1956; Garry, 1990 and Majak *et al*., 2003).

Animals vary in their physical ability to adapt to the pressure and in their individual response to discomfort. A change in girth is not linear with respect to changing ruminal pressure (Waghorn, 1991). As the rumen expands, it fills the abdominal cavity, stretching the muscles and exerting pressure on the internal organs. Discomfort will be more severe in animals that have small body cavities, larger internal organs, or layers of non-elastic fat, connective tissue and muscle. Thus the only objective measure of severity is intraruminal pressure (Waghorn, 1991) and for intact animals the recommended procedure is palpation of the left flank (Lippke *et al*., 1972).

Death may occur quickly, but usually does not take place until 2 to 4 hours after the onset of bloat. When the bloat becomes severe enough, the animal collapses and dies quickly, almost without a struggle. Death is likely caused by suffocation, when the distended rumen pushes against the diaphragm and prevents inhalation (Majak *et al*., 2003).

Animal respiratory rate increases to up to 60 inhalation-exhalation cycles per minute (Majak *et al*., 2003). Frothy bloat increases respiratory rates and pulse rate (Baraka *et al.*, 2000; Chakrabarti, 2001; Mohamed and Selim, 2001; Radostits *et al*., 2007; Anderson and Rings, 2008; Smith, 2014 and Saber, 2016).

Respiratory rate and pulse rate increases could be explained on basis of progressive increment of heart rate as diaphragm and lungs were compressed due to severe distention interfering with venous return to the heart and lung ventilation leading to elevation of pulse and respiratory rates (Anderson and Rings, 2008).

Frothy bloat decreases rectal temperature and conjunctival mucous membrane became congested (Saber, 2016). In severe cases cyanosis of mucous membrane bulging of the eyes and death may occur due to respiratory failure (Mohamed and Selim, 2001)

Frothy bloat decreases Rumen motility (Baraka *et al*., 2000; Mohamed and Selim, 2001; Ismail *et al*., 2007; Radostits *et al*., 2007; Anderson and Rings, 2008 and Saber, 2016) and this could be explained on basis of activation of high threshold tension receptors in reticulo-rumen increasing the inhibitory inputs to gastric center (Leek, 1969 and Leek, 1983).

1.3.3 Rumen fluid examination

Examination of rumen fluid should establish the colour, odour, consistency, pH. The normal colour of rumen fluid is olive-green to greenish-brown with a sweet and fermentative smell (often described as aromatic and non-repellent). This partially resembles the composition of the diet. Cattle fed

on maize silage or straw have yellowish to brown coloured rumen fluid. Cattle on pasture have green coloured rumen fluid. Cattle fed on high concentrations often have olive-brown rumen fluid (Petrovski, 2017).

The normal consistency of rumen fluid is slightly viscous, samples of rumen fluid which contain medium to large bubbles that do not coalesce are highly indicative of frothy bloat. Sample of rumen fluid with pasty consistency that contain a large number of small bubbles are indicative of vagus indigestion (Petrovski, 2017).

The physiological pH of the rumen fluid and contents varies significantly depending on the type of diet, time between feeding and sampling, and the portion of the rumen where the sample has been obtained from. The normal pH of the rumen fluid collected by rumenocentesis in cattle on an exclusively pasture diet is 6.0 to 7.2. A rumen pH of 5.0 to 6.0 may be normal in cattle on a high, easy-digestible carbohydrate diet. A rumen pH of 6.0 to 6.8 is normal for cattle on a diet rich in crude fibre. Rumen pH may increase and become alkaline when microflora is inactivated. However, a high rumen pH (>8.0) may be detected in cattle where the rumen samples mixed with a high volume of saliva (Petrovski, 2017).

1.3.4 Palpation

Palpation of the left flank is an external diagnostic procedure to establish whether the rumen contents are abnormally uniform, due to the presence of foam (Garry, 1990).

1.3.5 Gastric intubation

Gastric intubation can be used to expel gas and some rumen contents to confirm the diagnosis (Berg, 2000). The existence of frothy and free-gas bloat can be determined by passing a stomach tube into the rumen. When the rumen contents are frothy, the tube fills with froth and the gas cannot be relieved (Majak *et al*., 2003).

1.4 Clinical pathology

1.4.1 Haematological analysis

Packed cell volume (PCV) is used for estimation of dehydration degree after excluding of anaemia and acute blood loss, as long as PCV increases because of either spleenic contraction or dehydration, the use of this variable alone as indicator of hydration status is unreliable, so PCV has to be measured with total protein concurrently, for accurate estimation of dehydration (Blood *et al.*, 1989).

Frothy bloat decreases packed cell volume (Mohamed, 1984; Baraka *et al*., 2000; Kamal, 2008 and Saber, 2016) and this could be referred to varied degrees of dehydration concurrently with the disease (Saber, 2016).

Decrease of total red blood cells (TRBCs) was noticed in frothy blood (Baraka *et al*., 2000 and Kamal, 2008). Total white blood cells (TWBCs) was increased in cases of frothy bloat (Baraka *et al*., 2000; Ismail *et al*., 2007 and Saber, 2016). Increase of Neutrophils, monocytes and decrease of lymphocytes and Eosinophils was noticed in frothy blood (Baraka *et al*., 2000; Ismail *et al*., 2007; Kamal, 2008 and Saber, 2016).

1.4.2 Biochemical analysis

Bloat indigestion can be attributed to a decrease in both intake and absorption of calcium due to anorexia or gastrointestinal atony usually associated with primary indigestion and other diseases (Parsad, 1977 and Blood *et al*., 1989).

Frothy bloat decreases serum calcium levels (Mohamed, 1984; Baraka *et al*., 2000 and Kamal, 2008) which could be explained on basis of anorexia accompanied with the disease and disturbance in absorption and utilization of calcium (Mohamed, 1984).

Frothy bloat increases serum total protein (Baraka *et al*., 2000; Ismail *et al*., 2007; Kamal, 2008 and Saber, 2016).

Saber (2016) noticed that frothy bloat decrease serum albumin and Kamal (2008) in camel recorded insignificant decrease.

Serum electrolyte concentrations are influenced by the amount of secreted saliva, the rate of sodium and potassium secretion and / or sequestration in the abomasum and the rate of renal excretion and absorption as affected by acid-base imbalance (Melvin, 1970 and Blood *et al*., 1989). This may explain the unusual behavior of the individual electrolyte concentrations. Kamal (2008) noticed that bloat decrease sodium and increase potassium levels.

1.5 Treatment of frothy bloat

The treatment of frothy bloat by emergency rumenotomy that using of trocarization. Antizymotic agents are also used (Polymerized methyl silicon, Poloxalene, Polyethylene, Sodium sulfosuncinate). Salivation and purgative drugs are also used. All these drugs and emulsion are administered to reduce the surface tension (Brander *et al*., 1991 and Radostits *et al*., 2007).

Chapter Two

Materials and Methods

2.1. Area of the study:

The study was conducted in south Darfur state, Nyala University, Faculty of Veterinary Science, Department of Clinical Studies.

2.2. Experimental animals:

A total of 25 male goats (6- 12 months age and 20- 25 kg body weight) were examined in the study. Before the start of the experiments the animals were subjected to clinical examination.

2.3. Monitoring tools:

Stethoscope, digital thermometer, stomach tube and stop watch.

2.4. Experiments:

The animals were subjected to the use of alfalfa after 16 hours of fasting to induce frothy bloat. Physiological parameters and samples were taken before induction and then every 2 hours following induction for 24 hours.

2.5. Clinical examination:

2.5.1. Clinical signs:

Clinical signs were documented for each individual animal according to Kelly (1984).

2.5.2. Physical parameters:

All animals were examined clinically for estimation of respiratory rate, pulse rates, rectal temperature, eye mucous membrane, percussion and auscultation according to Kelly (1984) and Radostits *et al*. (2000).

2.6. Blood samples collection:

Ten ml of whole blood were collected (25 samples before feeding alfalfa, 300 samples after feeding alfalfa) from Jugular vein by disposable syringe, after following of aseptic technique procedures, 5ml was mixed with Ethylene Di amine Tetra Acetic acid (EDTA) as an anticoagulant in plastic container

for hematological indices. The remaining part (5 ml) was used for separations which were needed for biochemical analysis.

2.7. Rumen fluid samples:

Ten ml of Rumen fluid samples were collected (25 samples before feeding alfalfa, 300 samples after feeding alfalfa) using a stomach tube from the caudo-ventral sac of the rumen. The first jets of sample were discarded to avoid the effect of saliva according to the method described by Kleen (2004).

2.8. Haematological indices:

The whole blood was tested for the determination of red blood cell count (RBCs$\times 10^6$ cell/ml), packed cell volume (PCV%), total white blood cells (WBCs$\times 10^3$ cell/ml), and differential count of the white blood cells according to Jain (1986) as follows:

2.8.1. Red blood cells (RBCs):

For counting the red blood cells (RBCs), the whole blood was diluted by Gower's solution (consisting of sodium sulfate 12.5 g, glacial acetic acid 33.3 ml and 200 ml distilled water) to 1:200. Haemocytometer was used for counting the red blood cells using lens objective 40. The counted number was multiplied by 10000 for obtaining numbers of red blood cells in micro litre of the blood sample.

2.8.2. Packed cell volume (PCV):

Three-fourth of plain capillary tube was filled by blood using capillary traction, the same end of capillary tube used for blood traction was sealed by sealing material, and then blood in capillary tube was separated by centrifugation at 1500rpm for five minutes. Packed cell volume was measured in millimeter, which usually expressed in percentage (%).

Centrifugation separated blood in the capillary tube into three parts:

-The clear upper part was blood plasma which exceeded 50% of the whole blood in the capillary tube.

-In the middle under plasma directly, white to gray coloured thin layer, called buffy coat consisting of white blood cells and thrombocyets.

-The last part under buffy coat was mass of red blood cells, called packed cell volume (PCV).

2.8.3. Total WBCs:

For counting of the white blood cells, the blood was diluted by Turkey solution to 1:20. Haemocytometer was used for counting the white blood cells using objective 10. The counted number in one was multiplied by 50 for obtaining numbers of white blood cells in micro litre of the blood.

2.8.4. Differential count of White blood cells:

One layer blood film was made on glass slide, after drying the film it was fixed by absolute methyl alcohol and stained by Giemsa stain, then 100 of white blood cells was counted using objective 100, percentage of each neutrophils, basophils, acidophils, monocytes, and lymphocytes were counted.

2.9. Blood biochemical parameters:

The following blood biochemical parameters: total protein, albumin, urea, sodium, potassium, calcium, were measured using spectrophotometer (*Biosystem* –BTS-302) in the Physiology Laboratory, Faculty of Veterinary Science University of Nyala.

2.9.1. Total protein:

Total proteins were measured according to the method describted by King and Wooton (1956), in alkaline media protein form intensive violate blue complex with copper salt. This intensity was directly related to the concentration of protein in the sample. Sample concentration of protein was calculated by the equation:

$$\text{Sample concentration of protein} = \frac{\text{sample}}{\text{standard}} \times 7(\text{standard concentration})$$

2.9.2. Albumin:

At pH 4.2 bromocresol green binds to the albumin. The intensity of the formed colour was proportional to the concentration of the albumin in the sample; the concentration of the albumin in the sample was calculated by the equation according to the method described by Bartholomew and Delany (1966) as follows:

$$\text{Albumin concentration in sample} = \frac{\text{sample}}{\text{standard}} \times 5(\text{standard concentration})$$

2.9.3. Urea:

Urea was converted to indophenol as following equation

$$\text{Urea} + H_2O \text{—urease} \rightarrow (NH^+_4)_2 + CO_2$$

$$NH^+_4 + \text{salicylate} + NaClO \text{— Nitroprusside} \rightarrow \text{indophenols}$$

The produced colour intensity was directly related to the concentration of urea in the sample , and it was calculated according to the method described by Fawcett and Scott (1960) by the equation:

$$\text{Urea concentration in sample(mg/dl)} = \frac{\text{sample}}{\text{Standard}} \times 50$$

2.9.4. Calcium:

Calcium was measured according to manufacturer (spinreact, Girona Spain) instructions. Colour produced following the reaction between calcium and o-Cresolphtalein in alkaline medium, was directly related to the concentration of calcium in the sample. The final result was calculated as following :

$$\text{Concentration of calcium in the sample (mg/dl)} = \frac{\text{Sample}}{\text{Standard}} \times 10(\text{standard con}).$$

2.9.5. Sodium:

Sodium was measured according to manufacturer (spinreact, Girona Spain) instructions, it reacts with chromogento produce chromophore which its colour absorption refers to the sodium concentration in the sample. The final result was calculated as following:

Concentration of sodium in the sample (mEq/L) = Sample × 5(standard conc).
Standard

2.9.6. Potassium:

Potassium was measured according to manufacturer (spinreact, Girona Spain) instruction, its concentration was measured by using tetraphenylboron which produce turbid colloid suspension. Resulted turbidity directly refers to the concentration of the potassium in the sample, this concentration was calculated by equation

Sample concentration of potassium (mEq/L)= Sample ×5(standard conc).
standard

2.10. Rumen fluid analysis:

The rumen fluid pH, color, odour and consistency were determined according to (Alonso, 1979; Dirksen and Smith, 1987 and Radostits *et al*., 2007).

2.10.1. Colour and odour:

The colour and odour of rumen fluid were assessed by visual inspection and smell. The colour was assessed in a glass tube of a smaller diameter (<1.5cm). The odour was assessed after closed the glass tube for five minutes and then opened the tube.

2.10.2. Consistency:

Rumen fluid consistency was assessed by slowly turning a glass tube half-filled with rumen fluid 45-60° left and right from an upright position.

2.10.3. Ruminal pH:

Ruminal pH was determined immediately after collection with a pH-meter AP-17 (Hungary).

2.11. Statistical analysis:

Complete randomized design was used for running the experiment. One-way analysis of variance was used to generate the analysis of variance

table (ANOVA). Moreover, descriptive statistics in tubular form was used for description different parameters obtained from goats.

Chapter Three

Results

3.1. Clinical signs:

Clinical signs of experimentally induced frothy bloat in goats began to appear after 6 hours of induction, these signs included tachypnea, tachycardia and increase in rumination and congested mucous membranes as showed in Figure (1). Examination of digestive tract revealed reduction in rumen motility and left-side abdominal distention as showed in Figure (2), The tympanic or drum sound on percussion, extension of the head and neck (Figure 3), frequent urination, grunting, diarrhea (Figure 4).

3.2. Changes in physical parameters in bloated goats:

In comparison with control group, respiratory rate was significantly increased after 6 to 8 hours and 22 to 24 hours (31 ± 9.05) of induction, pulse rate was significantly increased after 6 to 12 hours, 24 hours (63 ± 14.66) of induction, while rectal temperature was significantly decreased after 2 to 4 hours, 12 to 24 hours (38.95 ± 0.65) of induction (Table 1).

3.3. Haematological changes in bloated goats:

From table 2 PCV was significantly decreased after4 hours, 8 to 10 hours and 14 to 24 hours (25 ± 7.17) of induction. TWBCs was significantly decreased after 6 to 8 hours and 12 to 24 hours (11 ± 2035.99) after induction, while TRBCs was significantly increased after 2 to 8 hours, 12 to 16hours, 20 hours and 24 hours (12.6 ± 1977107.91) after induction, these parameters were compared with control group.

Lymphocytes were significantly decreased after 6 to 12 hours, 16 hours and 20 to 24 hours (55 ± 6.06) of induction, neutrophils were significantly increased after 6 to 12 hours, 16 to 24 hours (39 ± 4.83) of induction, eosinophils were significantly increased after 2 to 4 hours of induction, after 14, 18, 22 hours eosinophils were significantly decreased (3 ± 1.30), monocytes were significantly decreased after 2 to 24 hours (1 ± 1.23) of

induction, no differences in basophils were found between control group and bloated goats (Table 3).

3.4. Biochemical changes in bloated goats:

In table (4) total serum protein was significantly increased after 4 hours (9.38 ± 3.12) of induction, but at 12 to 22 hours (6.84 ± 1.09) it was significantly decreased. Serum albumin was significantly decreased after 6 to 22 hours (2.53 ± 0.51) of induction. Serum urea was significantly increased after 14 to 22 hours (50.56 ± 12.89) of induction. While serum calcium was significantly increased after 2 to 4 hours (9.35 ± 2.61) of induction. Also sodium was significantly increased after 8 hours (124.64 ± 6.10), 14 to 16 hours and potassium was significantly increased after 12 to 18 hours (6.79 ± 1.56).

3.5. Rumen fluid changes in bloated goats:

The physical changes of freshly rumen fluid that the colour changed from yellowish to green colour (Figure 5and 6), rumen fluid odor was changed from aromatic to foul odor. The consistency of rumen fluid was changed from slightly viscous to frothy with medium to large bubbles (Figure 5and 6).

In comparison with control group, rumen pH was significantly decreased after 6 to 10 hours of induction, after 14 to 24 hours (6.61 ± 0.24) rumen pH was significantly increased (Table 5).

Table 1: Physical parameters [Respiratory rate/min(means± SD),Pulse rate/min (means ± SD) , Temperature C^o (means ±SD)] of experimental goats (n= 25) under frothy bloat in South Darfur State.

Parameter	Respiration	Pulse	Temperature
Time	Means± SD	Means± SD	Means± SD
0	25± 7.01	54± 13.81	39.52± 0.33
2	23± 7.02	58± 15.20	37.81± 0.66*
4	27± 8.14	62± 13.74	38.76± 0.77*
6	32± 7.84*	65± 12.72*	39.49± 0.45
8	32± 10.88*	67± 18.09*	39.38± 0.67
10	29± 10.90	67± 16.36*	39.37± 0.51
12	24± 8.71	65± 17.43*	38.95± 0.70*
14	20± 5.28	58± 14.84	37.79± 0.64*
16	22± 5.29	57± 13.08	37.90± 0.70*
18	25± 7.29	57± 11.65	38.24± 0.76*
20	26± 6.33	61± 13.26	38.48± 0.63*
22	32± 8.67*	62± 13.84	38.89± 0.54*
24	31± 9.05*	63± 14.66*	38.95± 0.65*

SD = standard Deviation

Means within the same columns carrying stars are significant at ($P \leq 0.05$)

Table 2: Haematological indices [Packed cell volume: PCV % (means± SD), Total white blood cells (WBCs) 10^3 cell/µl(means± SD), and red blood cell count (RBCs) 10^6 cell/µl(means± SD)] of experimental goats (n= 25) under frothy bloat in South Darfur State.

Parameter	PCV	TWBCs	TRBCs
Time	Means± SD	Means± SD	Means± SD
0	30± 5.29	12.4± 1724.65	11± 1922678.95
2	28± 4.94	12.7± 1963.62	13.4± 1311256.01*
4	27± 4.96*	11.7± 2178.29	14.8± 1434192.22*
6	28± 4.73	10.1± 2945.04*	13.1± 1560922.48*
8	23± 5.29*	9.3± 2206.43*	14.3± 1006581.34*
10	24± 3.04*	11.8± 1229.66	10.7± 2542161.94
12	29± 4.76	9.9± 1328.38*	12.6± 2819052.62*
14	23± 3.45*	10.8± 1453.52*	12.6± 1235963.59*
16	22± 3.48*	10.2± 1041.30*	13.4± 1870178.51*
18	27± 3.60*	10.3± 1178.28*	10.3± 1294833.58
20	24± 1.88*	8.3± 1202.33*	13.1± 563741.37*
22	24± 1.73*	11.3± 1084.268*	11± 2115116.47
24	25± 7.17*	11± 2035.99*	12.6± 1977107.91*

SD = standard Deviation

Means within the same columns carrying stars are significant at ($P \leq 0.05$).

Table 3: Differential count of the White blood cells % (means± SD) of experimental goats (n=25) under frothy bloat in South Darfur State.

Parameter	Iymphocytes	Neutrophils	Eosinophils	Monocytes	Basophils
Time	Means± SD	Means± SD	Means± SD	Means± SD	Means± SD
0	62± 4.56	29± 5.53	4± 2.30	4± 2.89	0± 0.57
2	60± 3.29	33± 3.25	5± 1.43*	2± 1.41*	0± 0.40
4	60± 4.61	32± 4.48	5±1.73*	2± 1.06*	0± 0.63
6	57± 9.82*	36±8.07*	4±1.67	2± 1.60*	0± 0.69
8	57± 11.16*	36± 9.51*	5± 1.62	2± 1.41*	1± 1.11
10	57± 11.63*	37± 11.79*	4± 1.33	2± 1.41*	1± 1.00
12	56± 8.47*	38± 7.42*	4± 1.41	2± 1.28*	1± 0.98
14	63± 4.87	32± 4.34	2±1.70*	2± 1.05*	1± 0.95
16	57± 5.38*	36± 5.79*	4± 1.58	2± 1.29*	0± 0.75
18	59± 5.45	36± 4.41*	2± 1.70*	2± 1.47*	0± 0.75
20	55± 9.05*	39± 7.42*	4± 1.94	2± 1.56*	0± 0.82
22	54± 6.46*	41± 5.92*	3± 1.30*	2± 1.05*	1± 0.82
24	55± 6.06*	39± 4.83*	3± 1.25	1± 1.23*	0± 0.98

SD = standard Deviation

Means within the same columns carrying stars are significant at ($P \leq 0.05$).

Table 4: Blood biochemical parameters [Total protein mg/dl(means± SD),Albumin mg/dl(means± SD),Urea mg/dl(means± SD),Calcium mg/dl(means± SD),Sodium mg/dl(means± SD) and Potassium mg/dl(means± SD)]of experimental goats (n=25) under frothy bloat in South Darfur State.

Parameter	T.protein	Albumin	Urea	Calcium	Sodium	Potassium
Time	Means± SD	Means± SD	Means± SD	Means± SD	Means± SD	Means± SD
0	8.39± 2.31	3.04± 0.87	37.18± 15.72	8.02± 2.39	120.00± 7.36	6.20± 1.26
2	8.52± 2.07	3.09± 0.61	33.20± 11.90	9.26± 1.91*	121.48± 4.12	6.52± 1.01
4	9.38± 3.12*	2.87± 0.64	32.08± 9.11	9.35± 2.61*	121.44± 6.71	6.28± 0.83
6	8.37± 1.87	2.57± 0.42*	33.12± 4.89	8.20± 2.48	122.88± 7.75	6.68± 0.95
8	8.31± 1.88	2.46± 0.37*	36.73± 10.15	9.07± 2.24	124.64± 6.10*	6.28± 1.10
10	8.18± 1.65	2.63± 0.63*	34.34± 6.47	8.66± 1.76	123.76± 7.32	6.40± 1.04
12	6.45± 1.58*	2.64± 0.55*	39.79± 10.43	8.21± 2.60	120.76± 6.88	7.32± 1.18*
14	5.78± 0.93*	2.53± 0.53*	44.54± 8.81*	7.64± 1.79	125.21± 6.90*	7.48± 1.01*
16	6.49± 1.31*	2.60± 0.47*	49.96± 14.82*	7.66± 2.24	126.28± 10.89*	8.04± 0.84*
18	6.46± 1.03*	2.72± 0.44*	45.60± 8.87*	8.20± 2.41	120.04± 8.23	6.79± 1.56*
20	6.94± 2.25*	2.49± 0.56*	46.00± 11.58*	8.20± 1.44	119.96± 8.57	6.20± 1.00
22	6.84± 1.09*	2.53± 0.51*	50.56± 12.89*	8.49± 1.81	122.96± 6.85	6.04± 0.97
24	7.87± 1.14	2.84± 0.76	39.27± 12.27	7.93± 1.91	123.80± 9.83	6.52± 1.23

SD = standard Deviation

Means within the same columns carrying stars are significant at ($P \leq 0.05$).

Table 5: Rumen pH of experimental goats (n=25) under frothy bloat in South Darfur State.

Parameter	Rumen pH
Time	Means± SD
0	6.36± 0.15
2	6.32± 0.22
4	6.25± 0.22
6	6.12± 0.21*
8	6.14± 0.17*
10	6.13± 0.18*
12	6.38± 0.31
14	6.50± 0.18*
16	6.55± 0.16*
18	6.58± 0.21*
20	6.52± 0.29*
22	6.57± 0.22*
24	6.61± 0.24*

SD = standard Deviation

Means within the same columns carrying stars are significant at ($P \leq 0.05$).

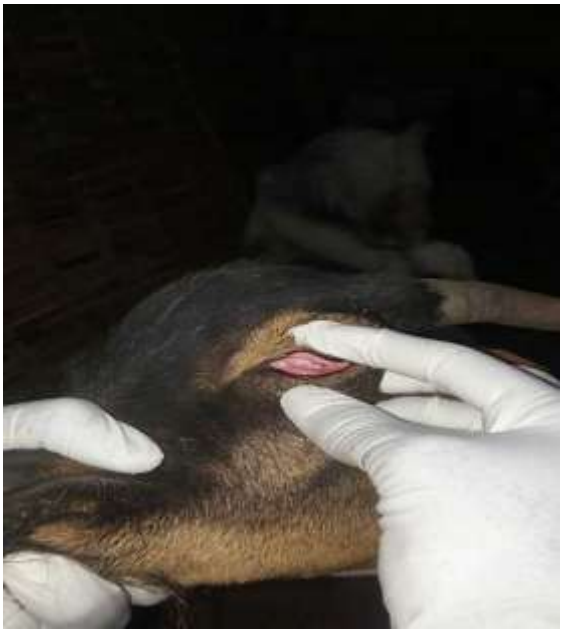

Figure1: Conjunctival congested M .membrane in goat

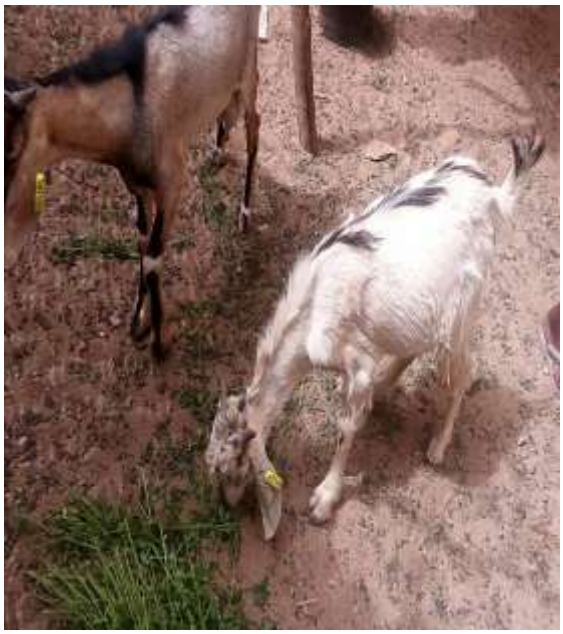

Figure 2: Left-side abdominal distention in goat

Figure3: Extension of the head and neck

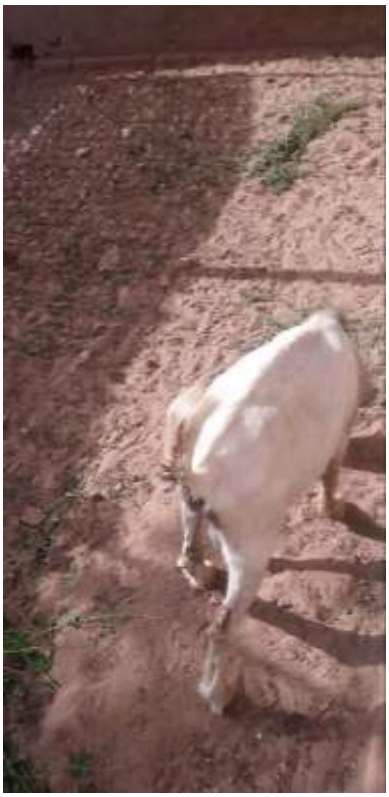

Figure 4: Diarrhea

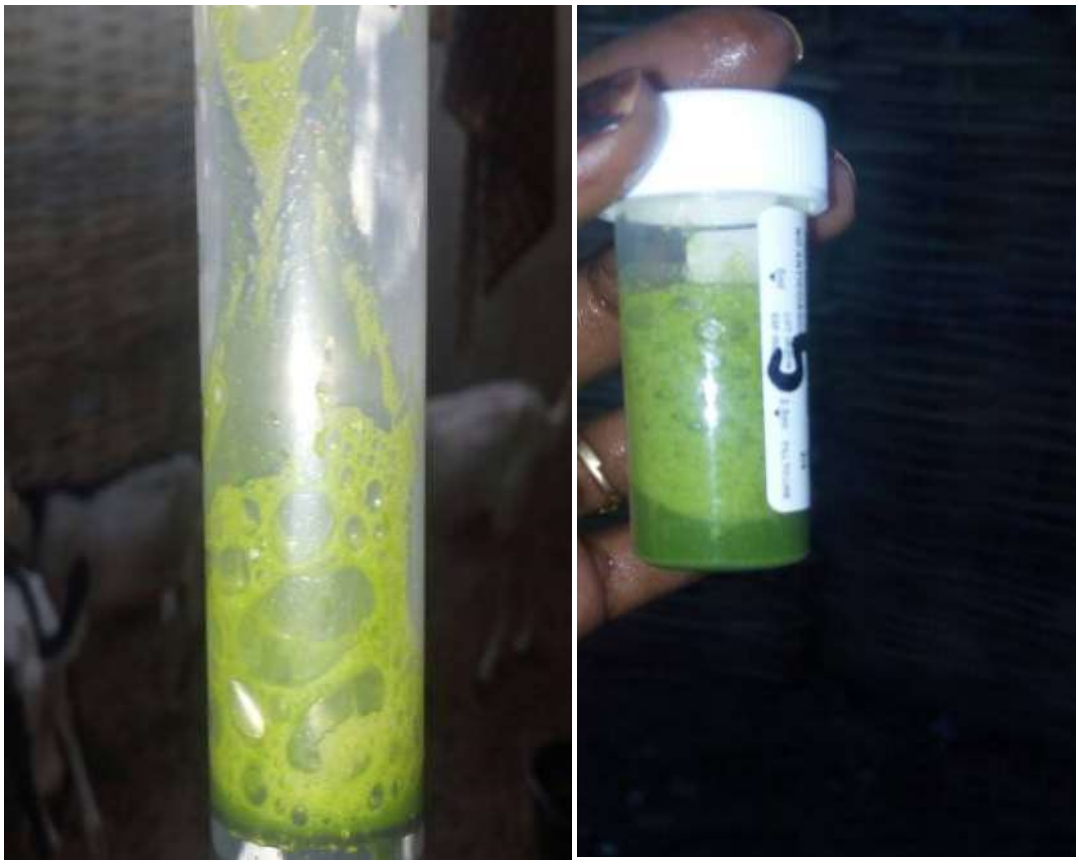

Figure 5,6: Frothy rumen fluid with medium to large bubbles collected from cases of bloat in goats

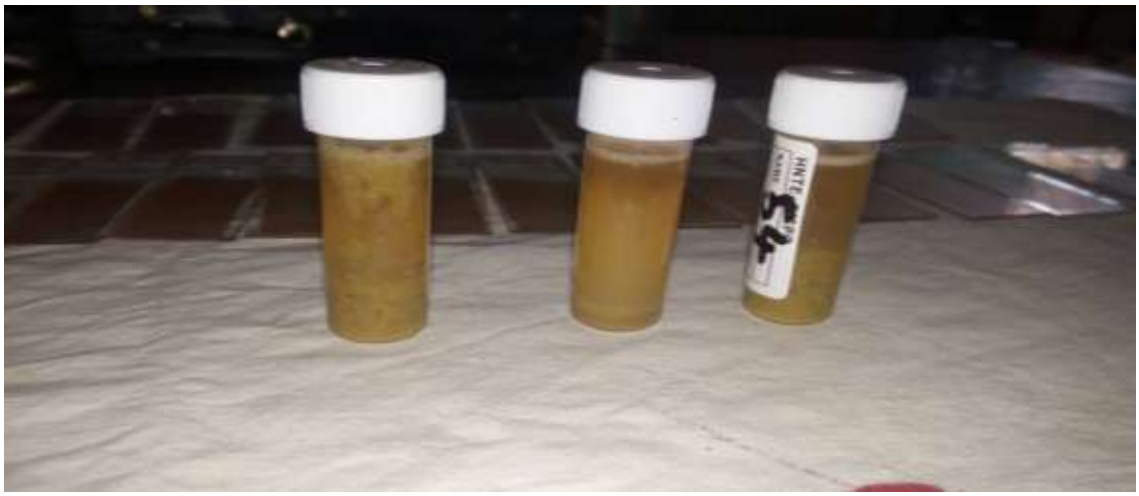

Figure 7: Normal control animals rumen fluid

Chapter Four

Discussion

The present study revealed that the fresh lush alfalfa is highly potential to induce frothy bloat in goats. This finding is in agreement with that reported by Majak *et al*. (2003) and Saber (2016).

Different clinical signs were observed in bloated goats included: tachypnea, tachycardia, increase in rumination, congested mucous membranes, reduction in rumen motility, left-sided abdominal distention, the tympanic or drum sound on percussion, extension of the head and neck, frequent urination, grunting, diarrhea .These signs were also reported by Majak *et al*. (2003) and Saber (2016). Reduction of rumen motility can be explained on basis of activation of high threshold tension receptors in reticulo-rumen increasing the inhibitory inputs to gastric center (Leek, 1969 and Leek, 1983).

Pulse and respiratory rates were significantly increased in bloated goats this finding was in agreement with Baraka *et al.* (2000) in camel, Radostits *et al*. (2007), Anderson and Rings (2008) and Smith (2014). Also these findings are found in cattle and sheep (Chakrabarti, 2001; Mohamed and Selim, 2001 and Saber, 2016). This could be explained on basis of progressive increment of heart rate as diaphragm and lungs were compressed due to severe distention interfering with venous return to the heart and lung ventilation leading to elevation of pulse and respiratory rates. Decrease in rectal temperature recorded in this study was similar to that recorded by Saber (2016).

Rumen fluid colour showed green colour this also was mentioned by Radostits *et al*. (2007) and Saber (2016). Foul odor and frothy consistency with medium to large bubbles of rumen fluid noticed in this study was also recorded by Kubesy (1983). Rumen pH was significantly decreased after 6 to 10 hours of induction this finding was also recorded by Kubesy (1983) and

Baraka *et al*. (2000) in camel. Also Kamal (2008) and Smith (2014) recorded the same results of pH . This can be explained as a result of ruminal atony affecting the rate of fermentation and/or hydrolysis and thereby the production of acid or alkaline intermediates (Bradford, 1990 and Baraka *et al*., 2000). Increasing of rumen pH in 14-24 hours is in accordance to the finding of Saber (2016). However, the high rumen pH (>8.0) detected is due to rumen fluid mixed with volume of saliva (Petrovski, 2017).

TRBCs was significantly increased, while Kamal (2008) in camel and Saber (2016) in sheep recorded the decrement. PCV was significantly decreased in cases of frothy bloat according to: Mohamed (1984), Baraka *et al*. (2000) and Saber (2016). While in dehydrated animals the packed cell volume, hemoglobin level and total erythrocyte count increase. The total serum protein concentration increases if there is water loss from extra cellular fluid according to Ismail *et al*. (2007) and Kamal (2008). TWBCs was significantly decreased in goats affected with frothy bloat, Baraka *et al*. (2000) and Saber (2016) noticed significantly increased. Lymphocytes were significantly decreased this finding was in agreement with Saber (2016) in sheep. Neutrophils were significantly increased this finding was in agreement with Ismail *et al*. (2007), Kamal (2008) and Saber (2016). Monocytes were significantly decreased, while Baraka *et al*. (2000), Kamal (2008) and Saber (2016) noticed them to be increased. Eosinophils were significantly increased after 2 to 4 hours and after 14, 18, 22 hours eosinophils were significantly decreased this finding was in agreement with Baraka *et al*. (2000) and Saber (2016) in sheep. In this study pathological leukocytosis is associated with increase of segmented neutrophils granulocytes due to stress reaction. Also alteration of the number of other cells resulted from the action of the disease as stress factor according to Coles (1986).

Serum albumin was significantly decreased this finding is in agreement with Saber (2016). Serum total protein was significantly increased after 4 hours of induction of frothy bloat this finding was in agreement with Ismail *et al*. (2007) in cattle and Saber (2016) in sheep. After 12 to 22 hours total protein was significantly decreased. According to Coles (1986) alteration in the total protein due to decrease in the quantity of albumin and this decreasing is often accompanied by hyperglobulinemia and this cannot usually sufficient to maintain the total protein concentration and hypoproteinemia results. Serum urea was significantly increased this finding was in agreement with Saber (2016) and this can be explained on basis of reduced renal perfusion and hence decreased renal glomerular filtration (Smith *et al*., 1992). Serum calcium was significantly increased, while Mohamed (1984), Baraka *et al*. (2000), Kamal (2008) and Saber (2016) reported significant decrease which could be explained on basis of anorexia accompanied with the disease and disturbance in absorption and utilization of calcium. Sodium was significantly increased, while Kamal (2008) in camel reported significant decrease. Potassium was significantly increased this finding was in agreement with Kamal (2008) in camel. Blood *et al*. (1989) and Melvin (1970) recorded that increasing of sodium and potassium as result of secreted saliva and / or sequestration in the abomasum and the rate of renal excretion and absorption is affected by acid–base imbalance.

Conclusion and Recommendations

Conclusion

Fresh lush alfalfa has a great ability to induced frothy bloat in goats in age 6 to 12 months. Respiratory rate, pulse rate, mucus membrane colour, rumen percussion, rumen motility are the most important clinical parameters to evaluate the bloated goats. Examination of the colour, odour and consistency of rumen fluid is important in diagnosis of frothy bloat. Rumen pH should be corrected during treatment of frothy bloat. Serum albumin levels is an important prognostic indicator, low serum albumin levels correlate with an increased risk of mortality rates. Calcium, sodium and potassium should not be given to bloated goats as they were found high in them.

Recommendations

1. Further investigations are highly recommended from aspects of rumen methane, rumen enzymes and rumen ciliates for therapeutic and productive purposes.
2. A legume content of 50% of the grazable yield is suggested as the maximum bloat-safe level. In this situation, the animals are able to graze or consume grass and alfalfa at the same time.
3. Frothy bloat develops rapidly so rapid treatment is required.
4. At the beginning of autumn the movement of animals should be controlled in order to avoid frothy bloating due to presence of fast growing legumes in pre flowering stage.

References

Alonso, A. (1979). Diagnostic Analysis of Rumen Fluid. The Veterinary clinics of North America. Large animal practice, **1**(2):363-376.

Anderson, D.E. and Rings, M. (2008). *Current Veterinary Therapy*: Food Animal Practice, Elsevier Health Sciences. 715 p.

Austin, A. R. (1981). Legume use in relation to animal health. Legumes in Grassland: Proceedings of the 5th Study Conference of the Scottish Agricultural Colleges, Peebles, Scotland.

Ayre-Smith, R.A.(1971). Pasture bloat in cattle. *Aust. Vet. J.* **47**:162-169.

Baraka, T; El-Sherif, M; Kubesy, A. and Illek, J. (2000). Clinical Studies of Selected Ruminal and Blood Constituents in Dromedary Camels Affected by Various Diseases. *ActaVeterinaria Brno*,**69**(1):61-68.

Bartholomew, R.J. and Delany, A.M. (1966). Blood albumin determination proceeding of Australian Association of Clinical Biochemists.1-214.

Berg, B.P. (2000). *An evaluation of initial rates of digestion in a strain of Alfalfa selected to prevent bloat in grazing ruminants.* PHD thesis, p:5-8.

Blood, D.C; Radostits, O.M; Arundel, J.H. and Gay, C.C. (1989). *Veterinary Medicine. A text Book of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses.* 7th Ed, W.B. Saunders. London.

Boda, J.M; Cupps, P.T; Colvin, H.J. and Cole, H.H. (1956). The sequence of events preceding death of a cow in acute experimental bloat on fresh alfalfa tops. *Journal of American Veterinary Medicine Assoc.* **128**:532-535.

Bowen, R. (1996). Rumen physiology and rumination. <http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion /herbivores /rumination.html>. Accessed 4/19/2007.

Bradford, P.S. (1990). Large Animal Internal Medicine. *C. V. Mosby Comp,* p:747-780.

Brander, G.C; Pugh, D.M; Bywatr, R. J. and Jenkins, W.L. (1991). *Veterinary Applied Pharmacology and Therapeutics,* fifth edition. Bailliere Tindal, London, Philadelphia, Toronto, Sedney, Tokyo, PP. 212-214.

Chakrabarti, A. (2001). *Text Book of Clinical Veterinary Medicine.* 2nd Ed., Taj Prss. India. p: 707.

Cheng, K.J; Hironaka, R; Jones, G.A; Nicas, T. and Costerton, J.W. (1976). Frothy feedlot bloat in cattle: production of extracellular polysaccharides and development of viscosity in cultures of streptococcus bovis. *Can. J. Microbiology*.**22**: 450-459.

Cheng, K.J; McAllister, T.A; Popp, J.D; Hristov, A.N; Mir, Z. and Shin, H.T. (1998). A review of bloat in feedlot cattle. *Journal of Animal Science*, **76**:299-308.

Clarke, R.T.J. and Hungate, R.E. (1971). Bloat in Cattle. XXXV. Microbial activities in the reticulo-rumens of cows differently susceptible to legume bloat. *N. Z. J. Agric. Research*,**14**: 108-121.

Clarke, R.T.J; Jones, W.T. and Reid, C.S.W (1974). Bloat in Cattle: XLI. Proteins of saliva as possible genetic markers in breeding programmes to reduce bloat susceptibility in cattle. *N. Z. J. Agric. Research* **17**: 411-415.

Colvin, H.W. and Backus, R.C. (1988). Bloat in sheep(Ovis aries). *Comparative Biochemistry and Physiology Part A*: Physiology, **91**(4):635-644.

Cole, H.H; Huffman, C.F; Kleiber, M; Olson, T.M. and Schalk, A.F. (1945). A review of bloat in ruminants. *Journal of Animal science*,**4**:183-236.

Cole, H.H. and Boda, J.M. (1960). Continued progress toward controlling bloat. A review. *Journal of Dairy science*,**43**:1585-1614.

Coles, H. E. T (1986). *Veterinary Clinical Pathology*. Fourth edition. W.B. Saunders Company, Philadelphia, London, Toronto, Montreal, Sudney, Tokyo. PP. 139-140.

Coulman, B. E; Gruber, M; McAllister, T.A; Majak, W. and Thompson, D. (2000). American Forage and Grassland Council. *Proceedings*, p. 351-358.

Dana, G.A; Dave, P.A;Leo, B.J; Katherine, E.Q; Otto, M. R; Philip, T.R. and Alice, M.W. (2005). *The Merck Veterinary Manual.* 9th ed. Merck& CO; INC. U.S.A. ISBN: 0-911910-50-6.

Dirksen, G. and Smith, M. (1987). Acquisition and Analysis of Bovine Rumen Fluid. *The Bovine practitioner,* **22**: 108-116.

Dougherty, R.W. (1956). Physiology of the rumen as related to bloat. In: A review of bloat in ruminants. *NAS-NRC Publ*. No. 388.

Ewart, A .J. (1931). The poisonous action of ingested saponins. *Commonw. Aust. counc. Sci. Ind. Res. Bull.* No.50.

Farnsworth, N.R. (1966). Biological and phytochemical screening of plants. *Journal of pharm. Sci.* **55**:225-276.

Fawcett, J.K. and Scott. J.E.(1960). A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*.**13**:156-159.

Findlay, A. L. R. (1998). Motility of the gastrointestinal tract of ruminants. <http://www.chu.cam.ac.uk/~ALRF/motility.htm>. Accessed 4/30/2007.

Garry, F. (1990). Managing bloat in cattle. *Vet. Med*. June, p 643-650.

Glanert, A. M; Dingle, J.T. and Lucy, J.A. (1962). Action of saponin on biological cell membrane. *Nature,***196**:953-955.

Gutek, L.H; Goplen, B.P; Howarth, R.E. and McArthur, J.M.(1974). Variations of soluble proteins in alfalfa, sainfoin, and birds foot trefoil. *Journal of Crop Science*. **14**:495-499.

Henrici, M. (1952). Comparative study of the contents of starch and sugars of Tribulusterrestris, lucerne, some Gramineae and Pentzia incana under different metereological and physiological conditions. *Onderstepoort Journal of Veterinary Researches.* **25**:45-92.

Hofirek, B; Jagas, P. and Dvorak, R. (1989). A Metabolic Profile Test of Rumen Fluid and Its Application in the Diagnosis of Rumen Dysfunction. *Cornell Veterinarian Journal*, **80**(4, 10):659-662.

Howarth, R. E; McArthur, J. M; Hikichi, M. and Sarkar, S. K.(1973). Bloat investigations Denaturation of alfalfa fraction II proteins by foaming. *Can*. *Journal of Animal Science*.**53**:439-443.

Howarth, R. E. (1975). A review of bloat in cattle. *Can. Vet. Jour.* **16**(10):281-294

Howarth, R. E; Majak, W; Waldern, D.E; Brandt, S.A; Fesser, A.C; Goplen, B.P. and Spurr, D.T. (1977). Relationship between ruminant

bloat and the chemical composition of alfalfa herbage. I. Nitrogen and protein fractions. *Can. Journal of Animal Science*. **57**:345-357.

Howarth, R. E; Goplen, B.P; Majak, W; Waldern, D.E. and Chaplin, R. K. (1978). Chloroplast membrane in the etiology of pasture bloat. *Can. Journal of Animal Science*. Abstract. **58**: 820.

Howarth, R. E; Goplen, B.P; Fay, J.P. and Cheng, K.J. (1979). Digestion of bloat-causing and bloat-safe legumes. *Ann. Rech. Vet.* **10** (2/3):332-334.

Howarth, R.E; Chaplin, R.K; Cheng, K.J; Goplen, B.P; Hall, J.W; Hironaka, R; Majak, W and Radostits,O.M.(1991). Bloat in Cattle. *Agriculture Canada Publication* 1858/E. Communications Branch. Agriculture and Agri- Food Canada, Ottawa.

Ismail, Z.B; Al-Majali, A and Al-Qudah, K. (2007). Clinical and Surgical Findings and Outcome Following Rumenotomy in Adult Dairy Cattle Affected with Recurrent Rumen Tympany Associated with Non-Metallic Foreign Bodies. *American Journal of Animal and Veterinary Sciences*,**2** (3): 66-71.

Jain, N.A. (1986). *Shallm's Veterinary haematology.* 4th Ed. Lea and Fibiger. Philadelphia U S A.

Jackson, H.D; Devault, R.L; McNairy, S.A; Hatcher, B.W. and Goetsch, G.D. (1962). Bloat in cattle. Its relationship to a respiratory inhibitor in Ladino clover and to phosphorus fertility of the soil. *Journal of Animal Science*, **21**:235-240.

Johns, A.T. (1954). Bloat in cattle on red clover. *I. N. Z. J. Sci. Tech*, **36A**:289-320.

Jones, W.T. and Elliott, F.C. (1969). Tow rapid assays for saponin in individual alfalfa plants. *Crop . Sci*, **9**:688-691.

Kamal, A.M. (2008). Some Biochemical, Hematological and Clinical Studies of Selected Ruminal and Blood Constituents in Camels Affected by Various Diseases. *Res J Vet Sci*, **1**(1):16-27.

Kelly, W.R. (1984). *Veterinary Clinical Diagnosis*.3rdedition. Baillier Tindal. London.440pp.

King, E.S., and Wooton, J.G.P. (1956). *Microanalysis in: Medical Biochemistry*. 3rd edition. Church hil, J.A. 57-60.

Kleen, J.L. (2004). *Prevalence of sub-acute ruminal acidosis in Deutch dairy herds- A field study,* PhD Thesis, School of Veterinary Medicine Hanover. 93-104.

Kubesy, A.A. (1983). *The Role of Protozoa in Rumen Digestion in Sheep with Special Reference to the Effect of Some Digestive Troubles on Such Organisms*. M. V. Sc. Thesis. Fac. Of Vet. Med., Cairo Univ., 135 P.

Leek, B. (1969). Reticulo-Ruminal Function and Dysfunction. *Veterinary record*, **84**(10):238-243.

Leek, B. (1983). Clinical Diseases of the Rumen: A Physiologist's View. *The Veterinary Record*,**113**(1):10-14.

Lindahl, I. L; Davis, R. E; Tertell, R. T; Whitmore, G. E; Shalkop, W. T; Dougherty, R. W; Thompson, C. R; Van Atta,G. R; Bickoff, E. M; Walter, E. D; Livingston, A. G; Gugolz, J; Wilson, R. H; Sideman, M. B. and DeEds, F.(1957). Alfalfa saponins, studies on their chemical, pharmacological and physiological properties in relation to ruminant bloat. *US Dep. Agric. Tech. Bull. 1161, Agr. Res. Service, Washington, DC.* 83 pp.

Lippke, H; Reaves, J.L. and Jacobson, N.L. (1972). Rumen Pressures associated with the scores of a bloat severity scale. *Journal of Animal Science*,**34**:171-175.

MacAdam, J.W; Hill, J. and Whitesides, R.E. (1995). Frost induced movement of potassium to the apoplast may increase bloat in ruminants grazing alfalfa. *Journal of Crop Science*, **35**:1133-1136.

MacAdam, J. W. and Whitesides, R. E. (1996). Growth at low temperatures increases alfalfa leaf cell constituents related to pasture bloat. *Journal of Crop Science.* **36**: 378-382.

Majak, W; Howarth, R. E;Fesser, A.C; Goplen, B.P. and Pedersen, M W. (1980). Relationships between ruminant bloat and the composition of alfalfa herbage:II. Saponins. *Can. J. Anim. Sci*, **60**:699-708.

Majak, W; Hall, J.W; Rode, L.M. and Kalnin, M.C. (1986). Rumen clearance rates in relation to the occurrence of alfalfa bloat in cattle. 1. Passage of water soluble markers. *J. Dairy Sci*. **69**:1560-1567.

Majak, W. and Hall, J.W. (1990). Sodium and Potassium concentration in ruminal contents after feeding bloat-inducing alfalfa to cattle. *Can. J. Anim. Sci*,**70**: 235-241.

Majak, W; Mcallister, T; Mccartney, D; Stanford, K. and Cheng, K. (2003). Bloat in Cattle, Alberta Agriculture, Food and Rural Development, Information Packaging Centre.

Mangan, J.L. (1959). Bloat in cattle. XI. The foaming properties of proteins, saponins and rumen liquor. *N. Z. J. Agri. Res*, **2**:47-61.

Mathison, G.W; Soofi-Siawash, R; Klits, P.T; Okine, E.K. and Sedgwick, G. (1999). Degradability of alfalfa saponins in the digestive tract of sheep

and their rate of accumulation in rumen fluid. *Can. J. Anim. Sci*,**79**:315-319.

McArthur, J.M; Miltimore, J.E. and Pratt, M.J. (1964). Bloat investigations The foam stabilizing protein of alfalfa. *Can. J. Anim. Sci*,44:200-206.

Melvin, J. (1970). *Duke*. *Physiology of Domestic Animals*, 8th ED Comell University.

Mendel, V.E. and Boda, J.M.(1961). Physiological studies of the rumen with emphasis the animal factors associated with bloat. *Journal of Dairy Science*,**44**: 1881- 1898.

Miltimore, J.E; McArthur, J.M; Mason, J.L. and Ashby, D.L. (1970). Bloat investigations. The threshold fraction 1(18s) protein concentration for bloat and relationships between bloat and lipid, tannin, Ca, Mg, Ni, and Zn concentrations in alfalfa. *Can. J. Anim. Sci*,**50**:61-68.

Mohamed, M.I. (1984). *Study on Some Minerals and Enzymes in Cattle and or Buffaloes in Health and Diseased*. M.V. Sc. Thesis Fac. Of Vet. Med., Alex. University.

Mohamed, H.A and Selim, H.M. (2001). *Hand book of Veterinary Internal Medicine.* 2nd edition**. P**: 12.

Parsad, J. (1977). Further clinic-biochemical studies in secondary rumen dysfunction associated with hypo calcemia, pregnancy and non-specific diarrhea. *Ind. Vet. J*, **5**:352-355.

Petrovski, K.R. (2017).Assessment of the Rumen Fluid of a Bovine Patient. *Journal of Dairy and Veterinary Science*.**2**(3):1-4.

Pressey, R; Synhorst, S.H; Bertram, J; Allen, R.S. and Jacobson, N.L. (1963). Foaming properties of alfalfa and their relationship to bloat. *J. Anim. Sci,* **22**:970- 978.

Radostits, O.M; Mayhew, I.G; Houston, D.M. and Mayhew, I. (2000). *Veterinary Clinical Examination and Diagnosis*, WB Saunders. 770 p.

Radostitis, O. M; Gay, C. C; Blood, D. C. and Hinchcliff, K. W. (2007). *Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats &Horses*,10th Edition, Bailliere Tindall, London.

Robinson, T.(1963). *The organic constituents of higher plants*. Their chemistry and inter relationships Burgess Publishing Company, Minneapolis, Minn.

Romas, A. and Tennessen, T. (1992). Effect of previous grazing experience on the grazing behaviour of lambs. Appl. Anim. Behav. Sci, **33**:43-52.

Saber, M. (2016). Effect of Field and Experimentally-induced Cases of Frothy Bloat on Physical Examination, Rumen Fermentation Pattern and Blood Constituents in Baladi Egyptian Sheep. *International Journal of Livestock Research*, **6** (10): 46 -63.

Smith, R; Underwood, W; Petersen, G. and Harms, R. (1992). Abomasal Dilatation and Impaction in Two Hampshire Rams. *The Veterinary record*, **130**(21):468- 470.

Smith, B.P. (2014). *Large Animal Internal Medicine*. 4th. Ed., Elsevier Health Sciences. 1872. p.

Sollman, T.H. (1957). *A manual of pharmacology and its applications to therapeutics and toxicology*. Ed. 8. W. B. Saunders Co., Philadelphia.

Tanner, G.J; Moate, P; Dailey, L; Laby, R. and Larkin, P.J.(1995). Proanthocyanidins (condensed tannins) destabilise plant protein foams in a dose dependent manner. *Aust. J. Agric. Res*,**46**:1101-1109.

Thompson, D. J; Brooke, B. M; Garland , G.J; Hall, J.W. and Majak, W. (2000).effect of stage of growth of alfalfa on the incidence of bloat in cattle. *Can. J. Anim.sci*. **80**:725-727.

Waghorn, G.C.(1991). Bloat in cattle. Relationship between intra-ruminal pressure, distention and the volume of gas used to simulate bloat in cows. *N. Z. J. Agric. Res*.**34**:213-220.

Woodward, H.E. and Alsberg, C.L. (1916). The relation of the surface tension of saponin solutions to their hemolytic activity. *J. Pharm. Exp. Ther*, **8**:109- 110.

Appendices

Appendix 1 : Physical parameters (Means, minimum, maximum) of experimental goats (n=25) under frothy bloat in South Darfur State.

Parameter	Respiration			Pulse			Temperature		
Time	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
0	25	16	40	54	41	94	39.52	38.9	40.5
2	23	10	40	58	40	96	37.81	36.5	39.2
4	27	16	52	62	40	92	38.76	36.6	40.0
6	32	20	52	65	48	100	39.49	38.8	41.1
8	32	20	68	67	48	120	39.38	36.6	40.2
10	29	16	68	67	42	100	39.37	38.0	40.1
12	24	16	56	65	42	100	38.95	37.5	40.0
14	20	16	36	58	40	100	37.79	36.9	39.5
16	22	16	36	57	42	96	37.90	36.6	39.6
18	25	16	44	57	40	96	38.24	36.7	39.5
20	26	16	40	61	44	100	38.48	37.1	39.5
22	32	16	52	62	40	96	38.89	38.0	39.9
24	31	18	56	63	44	100	38.95	37.0	39.8

Appendix 2: Haematological indices packed cell volume: PCV %, Total white blood cells (WBCs) 10^3 cell/µl, and red blood cell count (RBCs) 10^6 cell/µl(Means, minimum, maximum) of experimental goats(n=25) under frothy bloat in South Darfur State.

Parameter	**PCV**			**TWBCs**			**TRBCs**		
Time	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
0	30	20	38	12.4	9	15	11	7	10
2	28	20	38	12.7	8.6	14.9	13.4	10	20
4	27	18	36	11.7	6.7	15	14.8	10	20
6	28	17	35	10.1	5.1	15	13.1	10	20
8	23	16	33	9.3	5.6	13.8	14.3	10	20
10	24	19	31	11.8	8.4	13.6	10.7	8.2	20
12	29	22	36	9.9	7.5	12.2	12.6	8.2	20
14	23	17	29	10.8	6.6	13	12.6	10	10
16	22	18	31	10.2	8.2	11.5	13.4	10	20
18	27	22	33	10.3	8.4	12.7	10.3	8.4	10
20	24	22	28	8.3	6.5	11.1	13.1	10	10
22	24	22	28	11.3	9.8	14	11	8.3	10
24	25	16	36	11	8.1	13.4	12.6	9.8	20

Appendix 3 : Differential count of the White blood cells %Iymphocytes, Neutrophils, Eosinophils(Means, minimum, maximum) of experimental goats(n=25) under frothy bloat in South Darfur State.

Parameter	**Iymphocytes**			**Neutrophils**			**Eosinophils**		
Time	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
0	62	55	72	29	18	38	4	0	8
2	60	54	66	33	28	38	5	2	8
4	60	52	68	32	26	42	5	2	8
6	57	42	72	36	24	50	4	2	8
8	57	36	70	36	24	54	5	2	8
10	57	40	80	37	18	56	4	0	6
12	56	38	74	38	24	58	4	2	6
14	63	56	74	32	24	40	2	0	6
16	57	48	68	36	26	50	4	2	6
18	59	50	70	36	28	42	2	0	6
20	55	42	72	39	26	50	4	0	6
22	54	44	68	41	28	50	3	0	6
24	55	44	66	39	30	48	3	2	6

Appendix 4: Differential count of the White blood cells % Monocytes, Basophils (Means, minimum, maximum) of experimental goats(n=25) under frothy bloat in South Darfur State.

Parameter	**Monocytes**			**Basophils**		
Time	Mean	Minimum	Maximum	Mean	Minimum	Maximum
0	4	0	10	0	0	2
2	2	0	4	0	0	2
4	2	0	4	0	0	2
6	2	0	6	0	0	2
8	2	0	4	1	0	4
10	2	0	4	1	0	2
12	2	0	4	1	0	2
14	2	0	4	1	0	2
16	2	0	4	0	0	2
18	2	0	4	0	0	2
20	2	0	6	0	0	2
22	2	0	4	1	0	2
24	1	0	4	0	0	2

Appendix 5: **Blood biochemical parameters : Total protein mg/dl, Albumin mg/dl, Urea mg/dl, (Means, minimum, maximum) of experimental goats(n=25) under frothy bloat in South Darfur State.**

Parameter	**T.protein**			**Albumin**			**Urea**		
Time	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
0	8.39	3.9	13.8	3.04	1.7	5.1	37.18	15.6	72.4
2	8.52	3.6	11.5	3.09	2.2	4.4	33.20	15.6	61.2
4	9.38	3.6	16.6	2.87	1.5	3.7	32.08	17.2	55.2
6	8.37	4.5	12.1	2.57	1.9	3.5	33.12	25.2	43.6
8	8.31	4.8	12.4	2.46	1.5	3.3	36.73	16.4	56.4
10	8.18	5.6	12.4	2.63	1.5	4.2	34.34	25.2	45.6
12	6.45	4.6	9.8	2.64	1.7	4.2	39.79	12.3	63.3
14	5.78	3.6	7.3	2.53	1.5	3.5	44.54	25.2	62.3
16	6.49	5.0	11.0	2.60	1.7	3.5	49.96	24.2	79.2
18	6.46	4.8	8.7	2.72	1.9	3.7	45.60	24.7	63.8
20	6.94	0.9	15.5	2.49	1.5	4.2	46.00	14.8	66.8
22	6.84	5.1	9.1	2.53	1.9	3.9	50.56	26.7	69.8
24	7.87	4.8	9.8	2.84	1.7	5.1	39.27	28.2	65.3

Appendix 6: **Blood biochemical parameters : Calcium mg/dl, Sodium mg/dl and Potassium mg/dl (Means, minimum, maximum) of experimental goats(n=25) under frothy bloat in South Darfur State.**

Parameter	Calcium			Sodium			Potassium		
Time	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
0	8.02	4.1	12.5	120.00	103	133	6.20	5	9
2	9.26	5.0	12.5	121.48	115	130	6.52	5	8
4	9.35	4.1	16.6	121.44	105	134	6.28	4	8
6	8.20	3.3	16.6	122.88	110	138	6.68	5	8
8	9.07	5.0	14.0	124.64	110	136	6.28	4	8
10	8.66	5.8	12.5	123.76	112	137	6.40	5	8
12	8.21	4.1	13.3	120.76	109	139	7.32	5	10
14	7.64	5.0	10.8	125.21	112	139	7.48	5	10
16	7.66	4.1	11.6	126.28	109	159	8.04	7	10
18	8.20	4.1	12.5	120.04	106	138	6.79	5	10
20	8.20	5.0	11.6	119.96	103	140	6.20	5	8
22	8.49	5.0	11.6	122.96	111	139	6.04	4	7
24	7.93	5.0	12.5	123.80	95	145	6.52	4	9

Appendix 7: Rumen pH (means, minimum, maximum) of experimental goats(n=25) under frothy bloat in South Darfur State.

Parameter	**PH**		
Time	Mean	Minimum	Maximum
0	6.36	6.1	6.6
2	6.32	6.0	6.8
4	6.25	5.9	6.7
6	6.12	5.7	6.7
8	6.14	5.6	6.4
10	6.13	5.8	6.4
12	6.38	5.9	7.4
14	6.50	6.0	6.8
16	6.55	6.3	6.8
18	6.58	6.1	6.9
20	6.52	6.0	7.0
22	6.57	6.1	6.9
24	6.61	6.1	7.1