



Sudan University of Science and Technology
College of Graduate Studies



**Effect of Ethanol Treatment on Cottonseed Cake Ruminant
Degradability**

تأثير المعامله بالايثانول لكسب بذرة القطن على تكسيره بالكروش

**A Thesis Submitted in Partial Fulfillment for Requirement of M.Sc.
Degree in Animal Production in Tropics**

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الآية

قال الله تعالى:

وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً لُّسُقِيكُمْ مِمَّا فِي بُطُونِهَا وَلَكُمْ فِيهَا مَنَافِعُ كَثِيرَةٌ
وَمِنْهَا تَأْكُلُونَ ﴿٢١﴾ وَعَلَيْهَا وَعَلَى الْفُلْكِ تُحْمَلُونَ ﴿٢٢﴾

صدق الله العظيم

سورة المؤمنون - الآية (21 - 22)

Dedication

To

My Father

Mother

Sisters

Brothers

Friends

Acknowledgement

First and finally I thank my God ALLA who gave me the patience to conduct and finish study, I **thank Prof. Shadia Abdalati Omer** for her Supervision and advice during this study.

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Abstract

The study was conducted to determine the effect of treating cotton seed cake (CSC) with three different ethanol concentrations on its ruminal degradation characteristics of dry matter (DM) and crude protein (CP).

Three ethanol water solutions were prepared (v/v) at 50%, 70% and 90% concentrations, then 500gm of CSC were soaked in excess of each solution for an hour at 78°C. Each mixture was drained through cheese cloth and the treated cakes (ETCSC) were air dried at room temperature. The control was untreated CSC (UCSC). Nylon bags technique was employed using two castrated calves.

All ethanol treated CSC showed significantly ($P \leq 0.05$) lower values of the water soluble DM fraction (a) than UCSC with no variation among the treatments. Degradation of the water insoluble fraction (b) was significantly ($P \leq 0.05$) reduced by ethanol treatments the highest protection was in 90% ETCSC and no variation was observed between 50% and 70% ETCSC. Treated CSC with 70% and 90% ethanol showed significantly ($P \leq 0.05$) lower CP washing loss and degradation of the water insoluble fraction (b) than that of 50% ETCSC and UCSC. The rate constant (c) for (b) function was not affected by any of the three treatments for both DM and CP. All the treatments significantly ($P \leq 0.05$) reduced the effective degradability at three different rumen outflow rates. Within treatments 90% ETCSC showed the strongest effect and no variation was found between the other two treatments.

الملخص

أجريت هذه الدراسة لتحديد تأثير معاملة كسب بذرة القطن بثلاث تركيزات مختلفة من الإيثانول على خصائص التكسر بالكرش لمحتواه من المادة الجافة والبروتين الخام.

أعدت ثلاثة محاليل من الماء والإيثانول (V/V) بتركيزات 50% و70% و90%، ثم غمرت 500 جم من كسب بذرة القطن بفائض من كل محلول لمدة ساعة عند 78° درجة مئوية. صفي الخليط من خلال قطعة شاش ومن ثم جفف الكسب المعالج في درجة حرارة الغرفة. والتحكم كان بكسب غير معالج. واستخدمت تقنية أكياس النايلون بعجلين مخصيين.

أظهر كل كسب بذرة القطن المعالج بالإيثانول قيماً أقل بدلالة معنوية ($p \leq 0.05$) بكثير لجزء المادة الجافة القابل للذوبان في الماء (أ) من الكسب الغير معالج و بدون أي تباين بين المعالجات. تم تقليل تكسر الجزء غير القابل للذوبان في الماء (ب) بشكل كبير بدلالة معنوية ($p \leq 0.05$) عن طريق معالجة الإيثانول، وكانت أعلى نسبة حماية في المعالجة بتركيز الإيثانول 90% ولم يلاحظ أي اختلاف بين المعالجة بتركيزي الإيثانول 50% و70% لكسب بذرة القطن.

أظهر كسب بذرة القطن المعالج بـ 70% و90% من الإيثانول انخفاضاً كبيراً في معدل غسل البروتين الخام وتكسر الجزء غير القابل للذوبان في الماء (ب) بدلالة معنوية ($p \leq 0.05$) عن المعالج بتركيز 50% من الإيثانول والغير معالج. لم يتأثر معدل ثابت (ج) لدالة (ب) بأي من المعالجات الثلاث لكل من المادة الجافة والبروتين الخام. جميع المعالجات خفضت بشكل كبير من التكسر الفعال عند ثلاث معدلات مختلفة لسريان الكرش. بين المعالجات، أظهرت المعالجة بتركيز الإيثانول 90% التأثير الأقوى ولم يوجد أي تباين بين المعالجتين الأخريين.

List of Content

| Items | Page |
|--|------|
| الآية | I |
| Dedication | II |
| Acknowledgement | III |
| Abstract | IV |
| Abstract Arabic | V |
| List of Contents | VI |
| List of Table and Figures | VIII |
| Introduction | |
| Introduction | 1 |
| Chapter One | |
| 1. Literature Review | |
| 1.1 livestock | 3 |
| 1.2 Oilseeds | 4 |
| 1.2.2. Cottonseed: (Gossypium) | 5 |
| 1.2.3 Soya Been Meal | 7 |
| 1.2.4 Groundnut Cake | 7 |
| 1.2.5 Sesame Seed Meal | 8 |
| 1.2.6 Sun Flower Seed Meal | 9 |
| 1.3 Digestion in Ruminants | 9 |
| 1.4 Proteins | 10 |
| 1.4.1 Protein Digestion in Ruminants | 11 |
| 1.4.2 Utilisation of non—protein compounds by the ruminant | 13 |
| 1.4.3 Metabolizable protein | 14 |
| 1.5 Degradation of cake Proteins In the rumen | 15 |
| 1.5.1 Quickly Degradable Protein, (Q D P) | 15 |
| 1.5.2 Slowly Degradable Protein (SDP) | 15 |
| 1.6 Microbial Protein Synthesis in the rumen | 16 |
| 1.6.1 Estimation of Digestible True protein supply | 17 |
| 1.7 Biological value of protein | 17 |
| 1.8 Undegradable or by pass protein | 18 |
| 1.9 Factors influencing protein degradation in rumen | 18 |
| 1.10 Rumen PH | 19 |

| | |
|---|----|
| 1.11 Heat treatment | 19 |
| 1.12 Formaldehyde treatment | 20 |
| 1.13 Alcohol treatment | 21 |
| Chapter Two | |
| 2. Materials And Methods | |
| 2.1 Chemical Treatment | 22 |
| 2.2 Animal preparation and surgery | 22 |
| 2.3 In Situ Study | 22 |
| 2.4 Statistical Analysis | 24 |
| Chapter Three | |
| 3. Result | |
| 3.1 The proximate analysis of untreated and treated cotton seed cake | 25 |
| 3.2 The effect of ethanol treatment on <i>in situ</i> dry matter degradability (%) | 26 |
| 3.3. The effect of ethanol treatment on <i>in situ</i> CSC dry matter degradation kinetics | 26 |
| 3.4. The effect of ethanol treatment on <i>in situ</i> CSC crude protein degradability (%) | 29 |
| 3.5. The effect of ethanol treatment on <i>in situ</i> CSC crude protein degradation kinetics | 29 |
| Chapter Four | |
| 4. Discussion and Conclusion | |
| 4.1 Discussion | 32 |
| 4.2 Conclusion | 34 |
| 4.3 Recommendations | 35 |
| References | 38 |

List of Tables

| Table | Page |
|--|-------------|
| Table (1) In situ chemical analysis of cotton seed cake with different alcohol treatments. | 25 |
| Table (2) In situ dry matter disappearance (%) of cotton seed cake treated with different alcohol concentration | 27 |
| Table (3) In situ cotton seed cake dry matter rumen degradability characteristics (%) from model of different alcohol treatment. | 28 |
| Table (4) In situ crude protein disappearance (%) of cotton seed cake treated with different alcohol concentration. | 30 |
| Table (5) In situ cotton seed cake crude protein rumen degradability characteristics (%) from model of different alcohol treatment. | 31 |

Figure

| Page | Table |
|--|-------|
| Figure (1) The effect of ethanol treatment on <i>in situ</i> dry matter degradability (%) 40 | 36 |
| Figure (2) The effect of ethanol treatment on <i>in situ</i> crude protein degradability (%) 41 | 37 |

Introduction

Introduction

Cottonseed meal is the byproduct of oil extraction from cotton seed a protein rich feed cottonseed meal is a common source of protein for ruminants notably in cotton producing areas such as India, china and USA where it is used as partial substitute for soybean meal. Sudan possesses a huge animal wealth, pasture and range that provide about 85% of the national feed resources, agro-industrial by products and crop residues contribute about 11% and cereal grains and forages about 4%.

Agriculture is the major source of income in the Sudan, the major crops in Sudan are cotton, sesame, groundnuts, cereals (mainly sorghum, wheat and millets) and sugar-cane, which are raised under both irrigated and dry farming systems. Whole cottonseed is the unprocessed and unadulterated oilseed which has been separated. From the cotton fiber. Cottonseed is fed to high producing dairy cows as source of fat and highly-digestible fiber. They are also used as a forage replacer. Declined cottonseed contains slightly more protein, fat and energy, but less fiber than whole cottonseed. There are both mechanically and acid declined cottonseed products the mechanically declined cottonseed is more palatable than acid declined Cottonseed and is the preferred declined product for dairy cows. Little difference in animal performance between whole cottonseed and mechanically declined cottonseed has been reported. **(Coppock and Ianham and Horner 1987).**

Upper feeding limits on cottonseed are 6—7 Ib of dry matter per cow per day, the inclusion rate of cottonseeds is often restricted because of their high fat content and the use of other high-fat ingredients in the diet. Precautions are generally taken to not supplement dietary fat from high fat plant sources above 1.5 Ib per cow per day. Cottonseed is often used as a grain replacer.

Cottonseeds should be monitored for gossypol contamination. This is especially true for gin-run cottonseed that may be high in moisture content causing mold problems in storage. Cottonseed may be offered at a lower price, but may not be a good buy when potential storage problems and the higher moisture content are considered. In UK the free gossypol content of foods is strictly controlled by law. The introduction of cottonseed in the central Sudan (Gezira, 1925) was preceded by the establishment of cotton based Agriculture Research (Shambat, 1904 and Madani, 1918), where basic scientific information had been available on agricultural environment, varieties, cultural practices and crop protection.

Cotton, the king of natural fiber is mainly cultivated for its lint which is the most sought after textile fiber till date due to its inherent eco friendly and comfort characteristics. It is also one of the important cash crops of many of the Afro-Asian countries like India, Iran, Egypt, Sudan, Uzbekistan, Tanzania, etc. and plays a major role in their economic development. However, of late, cotton cultivation in general and especially in these countries is becoming non-remunerative on account of higher cost of inputs by way of plant protection measures, low productivity in rain fed cultivation, etc.

As a result, the cultivators are not able to get adequate returns commensurate with their inputs. Hence, there is an urgent need to explore alternative means of increasing the returns from cotton farming. While efficient use of available resources, good quality seeds, organic cultivation, transgenic cotton etc.

The objective of this study was to evaluate and determine the effect of chemical and physical treatments on the rate of degradation material crude protein and effective degradation of cottonseed cake.

Chapter One

Literature Review

Chapter One

Literature Review

1.1 Livestock:

Sudan has the second largest livestock. Inventories in Africa, next to Ethiopia Good natural pastures cover almost 24 million hectares and the nomadic pastoral sector accounts for more than 90% of the huge animal population. Cattle and sheep and goats provide an important capital asset and a risk management tool for pastoralists and farmers in time of drought, and they are increasingly important in agricultural irrigated areas as well. (FAO, 2005a).

In Sudan there are three farming systems characterize Sudan: irrigated (21.1% of agricultural GDP), rain fed semi mechanized like hand driven threshers, 6.3% and rain fed traditional agriculture 12.5% . Rain fed is the dominant farming system in terms of rural population and includes transhumance, nomadic and sedentary agriculture comprising over 90% of animal population .This system exists to some extent in very state, but it is most prevalent in the three Kordofn states the three Darfur states. Sinnar and Blue and White Nile states. Some commercial animal companies have been establish in the vicinity of Khartoum. (FAO, (2005a). But over three quarters of poultry are raised in rual villages FAO, (2005b).

The credit or financing benefits of livestock owners to dispose of their Animal for particular purposes at a time that they choose –their ability to cash in on the value of the animals as needed this flexibility give livestock owners access to money without the need to borrow and confers an additional financial beyond the sale, Slaughter or transfer value of their livestock This additional financial can be estimated as the opportunity cost of rural credit.

What it would otherwise cost a livestock owner to obtain funds comparable to those produced by liquidating apart of herd. (Bosman et, al 1997).

1.2 Oilseeds:

Oilseeds, such as soybean, cotton seed, sesame seed, sunflower seed and groundnut are annual plant. (O,Brien et, al 2000).

They are the largest source of vegetable oils even though most oil –bearing tree fruits provide the highest oil yield like olive, coconut and palm tree. (Gunstone, 2002).

Oilseed are also used in animal feed because of their high protein content. Their seed contain energy for the sprouting embryo mainly as oil, compared with cereals, which contain the energy in the form of starch (Lucas, 2000).

Oilseeds are grown in a range of countries and world oilseed stock have been estimated at 39.8 million tons for 2003/2004 (USDA, 2004). They is an increase in a small number of crops, including soybean, sunflower and rape seed account for the increasing in world production of oil. However, according to the Food and Agriculture Organization (FAO), more traditional oil crops like groundnut and sesame seeds continue to be important in the food supply and food security of many countries like Sudan and Myanmar. (Bruinsma, 2003).

Oilseeds can be stored for long time before being processed. Although oilseeds can be eaten whole the majority are crushed to produce oil, and about one sixth of the production is retained as seeds for planting and for food (Animal and Human). (Gunstone, 2002).

Oilseeds meal are important in animal nutrition as they are used in feed compounds. Oilseeds meals are high in protein. With most being over 40%. (They also contain about 10% carbohydrate and some fat. (Yunus, *et.al.*, 2004)

1.2.2 Cottonseeds (Gossypium):

Although cotton was grown primarily for its use in the textile industry, cottonseed dominated the world oil market prior to world when soybean oil took over, it still contributes 4% to the world's vegetable oil production but its production is linked to the demand for cotton fiber. (Bruinsma, 2003).

Cottonseed oil meal can also be used as nitrogen source for ruminal organism, in study CSM had no significant effect on concentration of ammonia in rumen fluid which on average of 3.5 mg per 100 ml of fluid, but when 2.5 and 5 percent of urea was added to molasses, rumen ammonia increased to 7.6 and 22.3 mg per 100 ml respectively. (Sambrook and Rowe,1982).

According to Tashev and Todorov, (1981) when CSM was incorporated in dairy animal diet, there were no difference intake of feed units per kg milk corrected for weight gain during the 90 days in milk protein, sugars and minerals among groups. However, butter fat was of cow given cottonseeds meal especially 40 to 50 days after calving, also the study indicate that feeding was depressed the protein percentage of milk (Coppock and Ianham and Horner, 1987).

Cottonseed meal is a good protein source for ruminants it is palatable with a nutritive value (for dehulled meals). Slightly low (85 – 90%).Than that of soybean meal. It is among the least expensive sources of protein in some regions. (NDDB.2012). Cottonseed meal is a good protein supplement for poor quality and fibrous by products because of its high protein digestibility. Association with a source of degradable energy increase the efficiency of cottonseeds meal supplementation. Since it decreases the urinary nitrogen. Indeed most of cottonseed meal energy comes from its fat content. (for cottonseed meals with a high amount of residual oil (Mc Gregor, 2000).

That high levels, does not contribute to development of rumen microbial population (Bonsi *et al.*, 1997). In the USA under typical conditions, even high production dairy cows can be fed cottonseed meal without adverse effects. Cottonseed meal is a good protein source for dairy cow feed fiber by –products (straws) or forages of low nutritive value (Mc Gregor, 2000).

Generally, cottonseed meal can replace other oilseed meal (Soybean, Sunflower, sesame and Groundnut). Without affecting milk yield and composition. However due to the variability of the fat, protein and gossypol content, results are sometimes contradictory. When supplementing highly digestible forages such as maize silage. Cottonseed meal can replace negative when diet protein is only 13% (Coppock and Inham and Horner 1987).

Calves are susceptible to gossypol toxicity because of their incomplete rumen development. It is recommended that concentrates for calves under 5 months old contain no more than 10-15% cottonseed. Results obtained with growing calves are variable. In diets for pre and post weaning calves, cottonseed meal gave the same weight gains as repealed meal or soybean meal (Coppock and Ianham and Horner 1987) or slightly lower gains than soybean meal in buffalo calves cottonseed meal gave higher weight gains when compared with sunflower meal. It is probable that those results are influenced by interactions in the diet (level of under gradable protein in the rumen or lignin content. In growing heifers, steers and bulls, cottonseed meal is a valuable protein supplement and can replace other oil meals. That is (soybean or sunflower), (Yunus *et al.*, 2004).

Cottonseed meal can replace sesame or groundnut meal as the protein source in diets for rams with a similar daily weight gain of 76.3 g/d and better feed conversion ratio of 0.83 (Ahmed *et al.*, 2005).

Cottonseed meal used in diets for growing animal (lambs), gave the same performance probably due to a reduction of gossypol combined with increased rumen under gradable protein (Nagalakshmi *et al.*, 2003). While less susceptible than mono gastric, ruminants are not immune to the toxic effect of gossypol. Its effect on erythrocyte fragility increase with duration and dose and is age dependent: younger animals are more susceptible to gossypol toxicity than older ones (Yunus *et al.*, 2004).

1.2.3 Soybean Meal:

Soybeans and soya products have played an important parts. However, there was little use of soybean oil prior to world because of problems with flavor reversion. (O'Brien *et al.*, 2000).

Soybean contain up to 40% of crude protein and about 20% of fat, and soybean meal is characterized with higher content of crude protein about (40–49%). Soybean meal standardized on (44 and 49%). Of protein there is on feed market. The protein of soybean contains a considerable quantity of lysine (6.2 g /1b g n), but value of protein is limited by methionine and cystine content (2.9 g/1b g n), with regard on high protein content soybean meal can approximate to 40%. Generally soybean seeds contain 5.6 -11.5% of water, range for crude protein is from 32 to 43 % for fat from 15.5 to 24.7% for crude ash from 4.5 to 6.4% for neutral detergent fiber (N D F) from 10 to 14.9% (Ensminger *et al.*, 1990).

1.2.4 Groundnut Cake:

Groundnut cake with crude protein content of 40–45 % is a good supplement. It promotes growth and is palatable to the animal. Groundnut cake protein is known to be deficient in lysine and methionine and also a limited amount of tryptophan and threonine but amino acid quality improves in artificial diets when reinforced with lysine methionine and tryptophan (NRC.

2001). Groundnut is a valuable source of vitamins E, K and B. It is the richest plant source of thiamine (B1) and also rich in niacin, which is low in cereal FAO, (2003). Groundnut meal is a good source of protein for ruminants and there are no restriction on the use of groundnut cake, and it is little exported and not longer much used for ruminant in developed countries but is widely used as protein source in tropical countries where it is an indigenous crop (Blair, 2011). Aflatoxin contamination has been shown to be lethal or at least very detrimental to cattle particularly to young ones administration of 1mg /kg of aflatoxin B1 in the diets of calves also reduced live weight gains. Dairy cows fed diet containing 13 –20% aflatoxin contaminated groundnut meal showed significant reductions in milk yield (Mc Donald *et.al.*, 2010).

Groundnut meal is highly digestible in ruminants, with Om digestibility values above 80%.

Its energy value is about 89 –92 % that soybean meal. Groundnut meal protein is very degradable (N effective degradability comprised between 72 and 90%) (Ensminger,1990).

1.2.5 Sesame Cake:

Sesame is primarily grown for its edible seeds and oil used 56% of sesame seeds are used for oil extraction and 35% for food. Sesame seeds have an outstanding amount of oil and desirable nutty flavor after cooking for these reasons. Sesame seeds are much appreciated in industry and other food specialties (Hansen, 2011). Sesame oil meal (or sesame oil cake). Is the prorein - rich by product obtained after oil extraction. Depending on the way oil has been extracted. The hulls resulting from the dehulling of sesame seeds are discarded (Mohmoud *et al* 2015; Abdullah *et al* 2011). Sesame seeds have good viability can be stored about 5 years at room temperature it is important to dry there down to 8 - 6 % in order to prevent moist heating and rancidity frost might hamper seed quality. Sesame meal can be food grade or used as a feed for livestock. It is a valuable source of protein for animals .Sesame oil

meal is a valuable protein and energy source for ruminants. Reported in vitro digestibility is high 83% in (Ch and Rasekharaiah *et.al.*, 2002). Several processes have been tested to improve nutritional value for ruminants treatment with 1.5–2% formaldehyde decreased ruminal protein degradability with no effect or apposite effect nutrient intake (Bugalia *et.al.*, 2008).

Heat treatment of sesame oil meal at 140 C, 150 C or 160 C during 1h, 2h, or 3h increased by pass protein and most efficient heat treatment was at 150 C (Mahala *et.al.*, 2007).

1.2.6 Sunflower Cake:

The cultivated sunflower (*Helianthus annuus* L) is one of 67 species in genus *Helianthus*. It is a dicotyledonous plant and a member of the compositas (*Asteraceae*) family and has atypical composite flower. The inflorescence or sun head, consists of 700 to 8000 flower depending on the cultivar. (Lucas, 2000). Sunflower was common crop in world and the oil represents about 9% of the total oilseed world production. Sunflower meal (S F M) IS obtained as a by-product of oil extraction process and has a high protein content makes S.f.M an attractive source for the isolation of protein. The suitability for food applications of the S F M protein depends mainly on the oil extraction method. Due to this process the protein may be denatured to large extent, resulting in S F M with high content of insoluble protein denaturation may occur during seed conditioning expelling (up to 140 C and desolventising toasting (FAO 2003).

1.3 Digestion in Ruminants:

The ruminant digestive tract includes the mouth, tongue salivary glands. (producing saliva for buffering rumen PH), four compartment stomach (rumen - reticulum – omasum – abomasum), pancrease gall bladder, small intestine duodenum – jejunum and ileum). And large intestine (cecum – colon and rectum).

The rumen is the largest compartment, and it contains billions of bacteria, protozoa, molds and yeasts. These microorganisms live with the cow and they are the reason, cattle can eat and digest large amounts of roughage. The rumen microorganisms are adaptable enough that cattle can digest a large variety of feeds from grass. (Ensminger, 1990). Although rumen microbes can digest a great variety of different feeds. The reticulum with its honeycomb - like lining, is a compartment of the stomach that is involved with rumination. It also act as trap for foreign objects ingested by the cow. The omasum is also known as “the book“ or many piles because of its many leaf like folds. It functions as the gateway to the abomasum, filtering large particles back to the reticulorumen and allowing fine particles and fluid to be passed to the abomasum. The abomasum is also known as the “True stomach“. It function much like human stomach producing acids and some enzymes to start protein digestion. Animals that go off feed or have acidosis can develop a displaced abomasum or twisted stomach. The abomasum will actually float out of place and become torsioned stopping the flow of digesta. (Ensminger, 1990).

1.4 Proteins:

Protein are complex organic compounds of high molecular weight, built up of numerous amino acids (AA). The amino acids are linked together to peptides by peptide bonds, which form when the amino group (-NH₂) of one AA reacts with the carboxyl group (-COOH) of a second AA release a water molecule and form a covalent bond (Horton *et.al.*, 2002).

The structure of protein is divided into four different levels: the primary structure is the AA sequence of polypeptide chain, the secondary structure refers to the conformation of the AA chain, the tertiary structure refers to the overall, three dimensional shape of a single protein molecule, and the

quaternary structure describes a protein consisting of more than one polypeptide chain (Horton *et.al.*, 2002).

Dietary protein fed to ruminants generally refers to crude protein (CP) defined as the N content x 6.25. This definition is based on the assumption that all N in the feed is present as protein and that the average feed protein contains 16% N the N content of feeds is usually determined by a modified version of the kjeldahl technique (AOAC. 1984) .

Amino acids composition of protein contain nutritive value. The concentration of essential amino acids lysine - methionine theonine and tryptophan (Sharma *et.al.*, 2012).

Protein are very complex compounds which form the greater part of the body tissue, for this reason young animal require protein in considerable quantities for growth while adult need a certain amount for the replacement of worn out tissues. Twenty four amino acids being needed to maintain the animal body in health about ten of them. Known as non essential amino acid can be formed in the animal body, but the remainder, termed essential must be provided in the diet. The uncertainly about the precise number in that this varies according to the species of animal and the rate of growth. (King. 1978).

1.4.1 Protein Digestion in Ruminants:

The digestion of protein in rumen, food protein are hydrolysed to peptide and amino acid by rumen microorganisms but some amino acid are degraded further, to organic acids, ammonia and carbon dioxide. An example of deamination of amino acids in is provided valine, which as mentioned above, is converted to is butyric acid found in rumen liquor are derived from amino acids. (Mc Donald, 2010). The main protcolytic organisms are peptostreptococci species and the protozoa. The ammonia produced, together with some

small peptides and free amino acids, is utilised by the rumen organisms to synthesis microbial protein.

Some of the microbial protein is broken down in rumen and its nitrogen is recycled (like. Taken up by microorganism). (McDonald, 2010).

For their synthetic activities the microorganism require a source of energy, and ammonia is most effectively incorporated in to bacterial protein when the diet is rich in soluble carbohydrates, particularly starch. (Ensminger *et.al.* 1990).

When the organisms are carried through to the abomasums and small intestine their cell protein are digested and absorbed. An important feature of the formation of microbial protein is that bacteria are capable of synthesizing essential as well as non – essential amino acids, thus rendering their host independent of dietary supplies of the former. (Mc Donald, 2010).

Dietary protein degradation in rumen un valves attachment of bacteria to feed particles, followed by activity of cell bound microbial proteases. (Brock *et.al.*, 1983).

A large number of different microbes species form a consortium that attaches to a feed particle acting symbiotically to degrade and ferment nutrients. including protein. Products resulting from this process are peptide and amino acids because the number of different bonds within a single protein is large, the synergistic action of different products is necessary for complete protein degradation. (Wallace *et.al.*, 1997).

The rate and extent at which protein degradation occurs will depend on proteolytic activity of ruminal microflora and the type of protein. (susceptibility and accessibility of peptide bonds). Peptide and AA resulting from the extracellular rumen proteolytic activity are transported inside

microbial cell. Peptidases into AA and the latter can be incorporated into microbial protein or further deaminated to VFA, CO₂ and ammonia the fate of absorbed peptide and AA once inside the microbial cell will be transaminated or used directly for microbial protein synthesis. However if energy is limiting AA will be deaminated and their carbon skeleton fermented into VFA. Some ruminal bacteria lack mechanisms of AA transport from the cytoplasm to the extra cellular environment and AA absorbed in excess must be excreted from cytoplasm as ammonia. (Taminga, 1979).

1.4.2 Utilisation of Non –Protein Compounds by the Ruminant:

Dietary protein is not the only contributor to the ammonia pool in the rumen. As much as 30 per cent of the nitrogen in ruminant food may be in the form of simple organic compounds such as amino acids, amides and amines or of inorganic compounds such nitrates. Most of these are readily degraded in the rumen, their nitrogen entering the ammonia pool. In practice it is possible to capitalize on the ability of rumen microorganisms to convert non –protein nitrogenous compounds to protein by adding such compounds to the diet. The substance most commonly employed is urea, but various derivatives of urea and even ammonium salts may also be used it is to avoid accidental overconsumption of urea, since the subsequent rapid absorption of ammonia from the rumen can overtax the ability of the liver to reconvert it to urea hence causing the ammonia concentration of peripheral blood to reach toxic levels. (Harper and Yosnimura 1995).

An additional non –protein nitrogenous compound that can be utilised by rumen bacteria and hence by the ruminant, is uric acid. This is present in high concentration and these are sometimes dried for inclusion in diet for ruminants, although in some countries the use of excreta as food is restricted or prohibited the practical significance of these non – protein nitrogenous substance as potential protein source. (Mc Donald, 2010).

1.4.3 Metabolizable Protein:

It is the AA, and not the protein per se, that are the required nutrient for the host animal and that use as building blocks for the synthesis of protein required for maintenance, growth reproduction and lactation. In ruminant CP feeding consideration must be taken of both the N requirements of rumen microbes and the AA requirements of the host animal. Lack of supply to either of these could adversely affect animal performance. A schematic representation of the fate of dietary CP. Protein metabolism in the rumen can be divided into two separate actions: protein degradation, which provides N for the bacteria; and microbial protein synthesis. Bacteria, the most abundant microorganisms in the rumen, are the major organisms involved in protein degradation. (Schwab *et.al.*, 2005).

The amount of protein that is degraded in rumen depends on several factors of which the chemistry of the feed CP is the single most important. (N R C,2001). Another important factor is the predominant microbial population, which in turn depends on the type of ration, ruminal passage rate and PH. (Bach *et.al.*, 2005).

1.5 Degradation of Cake Protein in the Rumen:

The key protein parameters in the proposed MP system, quickly degradable protein, slowly degradable protein and digestible undegraded feed protein, are derived from measurements of the rates of degradation of feed protein (dg) Suspended in a Dacron bag in the rumen for various periods of time, normally up to 48 hours of concentration and 72 hours for forages. (Orskov and Mehrez, 1977).

The proportion of total nitrogen lost from the dacron bag with time zero is then plotted against time (T). The value at time zero is obtained by washing the

Dacron and contents in a washing machine modified to give a suitable cold rinse cycle similar data plots for typical concentration (Ensiminger, 1990)

1.5.1 Quickly Degradable Protein (Q D P):

The cold water extracted fraction of the feed total crude protein (CP), defined by the constant, a , is called quickly degradable protein (Q D P), and for any feed is calculated as: $(Q D P) (DMg/ Kg) = a \times ((CP)(DMg/Kg))$.

This fraction of the total crude protein, comprising considerable amounts of non-protein N in the case of silage, but also water soluble small protein molecules, is released rapidly when the feed enter the rumen, resulting in an efficiency of capture by the rumen microbes of less than one.

Any urea added to the feed is to be included in the QDP fraction of the diet since. (A R C, 1980).

1.5.2 Slowly Degradable Prtein (S D P):

The slowly degradable or bypass nutrients may occur in feed in their natural form, but feeds can also be manipulated to restrict their degradation in the rumen. Nutrients should be made resistant to microbial enzyme to such an extent so that rumen microorganisms get, sufficient nutrients for efficient rumen functioning with respect to fiber digestion and microbial protein synthesis. (N R C, 2001).

The amount of protein slowly degradable during the residence of the feed in the rumen is determined by time spent in rumen with the feed exposed to rumen bacterial digestion which is a function of level of feeding and outflow rate. (Ensiminger *et.al.*, 1990).

1.6 Microbial Protein Synthesis in the Rumen:

The microbial processes of the rumen confer the ability to convert fibrous feeds and low –quality protein even non –protein -nitrogen into valuable nutrients for the ruminal animal (Wilkins and Jone 2000). or concentrations (Beerman *et.al.*, 2000). As protein resources for ruminants. So that microbial protein must be considered as an important protein resource the metabolisable protein supply from microbial protein is similar to that from un degraded dietary protein from grass silage 64%. (Agricultural and food Research council, 1992).

And rumen microbes have a variable, but generally good amino acids profile. (Storm and Orskov, 1983): (Clark *et.al.*, 1992).

The synchronization of energy and nitrogen sources in rumen can improve microbial protein synthesis and efficiency of the utilization can improve ruminant productivity. For instance urea can be degraded much faster than other nitrogen source such amino acids peptide, and feed protein. Therefore it would be hard to match with the volatile fatty acids (F V C), production rate, which is an indicator of ATP synthesis from the carbohydrate degradation. VFA production rate can vary in the different carbohydrate source and the microbial protein production rate from feed nitrogen can be dependent on the ruminal degradation. In addition digestion rate of feed nitrogen can affect the microbial protein synthesis. (Crooker, *et al* 1978). And can be associated with some energy suppl. (Russell *et.al.*, 1981).

In contrast, the high degradation rate of energy source cannot permit ATP produced to be recruited for microbial protein synthesis, instead of the accumulation of carbohydrate in body. protein digestibility in rumen may affect on the flux of amino acid into small intestine. According to the national Research council (NRC, 1994). Microbial protein synthesis in rumen is

important for the demand of the protein synthesis in small intestine is the key for the demand and it will be decided by un degradable protein (UDP) contents of feed protein. (Koeln and Paterson, 1986).

1.6.1 Estimation of Digestible True Protein Supply:

The principle of estimation of microbial protein supply. Nucleic acids (NA), leaving the rumen are essentially of microbial origin, quantifying microbial protein synthesis in the rumen. Is important for ruminant nutrition for a number of reasons. Correction are will be absorbed in the lower digestive tract of the animal. Requires estimates of two fracas (Broderick and Merchen 1992).

1-True protein content of MCP (MTP), is mated to be 0.75 of MCP by AFRC (1992) compared to 0.8 suggested by (ARC,1980).

2-Digestibility of MTP (DMTP), is estimated to be a constant 0.85 as recommended by (Brooderick, *et.al.*, 1988) .

1.7 Biological Value of Protein:

The Biological value (BV) of protein is a measure of how efficiently food protein. Once absorbed from the gastrointestinal tract, can be turned into body tissue. The Biological value of a food then depends on how closely its amino acids pattern reflects the amino acids pattern in the body tissue. (Harper and Yoshimura, 1993).

Most chemical and microbiological test for nutrient substance give information about the total amount of a nutrient present in a particular feedstuff or ration. The biological value of protein the percentage of the digestible protein of feed or mixture which is usable as protein by the animal. It can be determined by a balance experiment a measured intake of protein is compared undigested protein in the feces of animal.

There are 2 Types of Biological value of protein foods:

1- High biological value, contain all essential amino acids complete protein, animal source s.

2- Low biological value lack some essential amino acids incomplete protein plant source. (Ensminger, *et.al.*, 1990).

1.8 Un Degradable or By Pass Protein:

The purpose of feeding bypass protein is that a large proportion of the protein is available directly at the lower part of gastrointestinal tract. Where it is digested and then absorbed as amino acids for utilization at tissue level. Feeding of bypass starch reduce excess production of lactic acids in the rumen which would otherwise result in low rumen PH (acidosis). The fats are thus digested mostly in the small intestine and absorbed as unsaturated fatty acids without affecting the fermentation of fibrous feeds in rumen. (Satter *et.al.*,1977).

Dietary protein that escape from rumen unchanged are available for digestion, these are termed bypass protein in lower digestive tract where it is digested and absorbed. (Ensminger, *et al.*,1990).

1.9 Factors Influencing Protein degradation in Rumen:

Protein solubility and differences in protein structure (resulting from disulphide bridges and cross linking), appear to be important factors in the ruminal degradation of protein tends to be higher than it is with feeds containing mainly prolamins and glutelins the solubility of protein in feedstuffs is affected by the PH of the feedstuffs concerned. Drying of forages in the field allows proteases to become active, which increase protein solubility. Some carbohydrates and protein are degraded during silage making due to fermentation. Which result in nitrogen contain end – products of the fermented

protein occurring in the soluble fraction concerned. Essentially all of the soluble nitrogen as well 40% to 50% of insoluble nitrogen is degraded in rumen. (Ensminger *et.al.*, 1990).

The extent of protein breakdown is a function of the rate of proteolysis and the retention in the rumen. Retention time is influenced by the particle size of the diet components and the level of feed intake. (Tamminga, 1979).

1.10 Rumen pH:

Kolver and de Veth (2002), also reported that lactating cows fed diets containing a mean of 80% pasture had a mean daily ruminal PH of 6.2.

(Range 5.6 to 6.7). Rumen PH fluctuates throughout the day depending on diet, time of feeding of concentrates and the. Supplementation of fiber source such as hay, physical from the diet like reduction in the forage particle size or the processing of grain decreases ruminal PH (Krause *et.al.*, 2002).

Feeds high in pre formed acids such as some silage, will also reduce rumen PH. Rumen PH starts to decline immediately after feeding concentrates concentrates cause more rapid decline in rumen pH than silage (Krause, *et.al.*, 2002).

1.11 Heat Treatment:

Heat treatment protects dietary protein for ruminants. But its important appropriate temperature and heating times are employed for particular feeds the temperature affect soluble N content and N digestibility. (Kempton *et.al.*, 1988).

Soybean meal is frequently fed to ruminants as protein sources. Heat treatment of oilseed meals decreases rumen degradability of protein and

increases the supply of dietary protein to the lower gut improving body weight gain, feed efficiency and nitrogen retention in calves (Reddy *et.al.*, 1993).

Therefore, rumen degradation of phytate in these oilseed meals is possibly suppressed by heat treatment with lower protein degradability in the rumen. The objective of this experiment was to determine the effect of heat treatment of soybean meal (Orskov and Mc Donald, 1979).

High heat treatment developed in the expeller process in removing the oil-bearing seed also has been found to cause injury (Broderick *et.al.*, 1988).

Found that heating decreased the degradation rate or the more slowly degraded fraction and reduce protein solubility. However heat treatment such as flame roasting (Mcninen *et.al.*, 1995) .

The most successful physical treatment has been heat. Heat facilitates the millard or non –enzymatic browning reaction between suger aldehyde group and free amino acids group of protein to yield an amino suger complex (Grffin *et.al.*, 1993).

Heat treatment of feed stuffs can decrease proteolysis by blocking reactive sites for microbial proteolysis enzymes. Heat has been used to decrease the supply of dietary protein the duodenum (Shezana *et.al.*, 2007).

The temperature may affect the seed not just chemically influenced (Reboller and Bals 2001).

1.12 Formaldehyde Treatment:

Formaldehyde reduce protein degradability by forming cross links between protein chins and has antimicrobial properties that may out the bacteria population and fermentation pattern (Woolford, *et al.*, 1975).

Formaldehyde (1g/100g crude protein). Treatment reduced protein degradability of groundnut cake (GNK). Gingelly sesame cake (GSK) and

rubber seed cake (RK) by 69 – 48 and 35%, respectively, at an outflow rate of 0.04 /h.(Sampath and Sivaraman, 1987).

Formaldehyde treated sesame meal as a main source of protected protein it was concluded that treated sesame meal decreased organic matter and protein degradability as well body weight losses and blood urea concentration. Moreover there were slight responses in milk production when treated sesame meal was added to supplements. (Woolford,1975).

1.13 Alcohol Treatment:

Diet of high producing ruminant are based on inexpensive and abundant supplies of cereal grains and oilseeds meals. Soybean meal (SBM) is widely used as a protein supplement in ruminant diets utilization is inefficient 60 to 80% of soybean protein is degraded in rumen (Satter, *et.al.*, 1977).

Treatment of oilseed protein with an aqueous alcohol solution produce a permanent change in their dimensional structure of protein molecule.

Treatment of (SBM) with ethanol or propanol at room temperature reduced in situ N disappearance (Van der *et. al.*, 1982).

The values recorded for dry matter degradability, the degradation rate soluble nitrogen and effective protein degradation of sesame cake are 0.20+0.05, 31.79+11.34, 66.06+11.66 respectively concurred with (Wall, *et.al.*, 2000).

Chapter Two

Materials and Methods

Chapter Two

2. Materials and Methods

This study was conducted at to study the rumen degradability and degradation kinetics on cotton seed cake (CSC).

2.1 Chemical Treatment of CSM:

Two kilograms of CSC were brought from Aljazeera State local market. CSC was finely milled with a laboratory hammer mill. Then the cake was divided into four equal parts .Three portions were treated by soaking them separately in one of the following three different ethanol water solution (v/v) concentrations that is 50%,70% or 90% for one hour at 78°Cand they were left to dry at room temperature. The treated and untreated cakes were named (50% ETCSC), (70% ETCSC) and (90% E TCSC).The control was untreated CSC (UCSC).

2.2 Animal Preparation and Surgery:

TWO castrated Kenana calves which were fitted with rumen cannulae as described by (Brown *et. al.*, 1968). They were fed a mixture of concentrates and roughage to satisfy their maintenance needs and were provided with water and salt lick all the time.

2.3 In Situ Study:

This was done according to the polyester bag technique of Mahrez and Qrskov, (1977). The bags were 15.5x8.5 cm and weighing 1-2 gms .Five grams from each sample were weighed in a bag and tied with a nylon ribbon and then attached to a thin plastic tube (45.5 cm length and 0.8cmdiam eter). Eight bags were attached to tube (two bags/sample/period/animal).The plastic

tubes with the bags were introduced into the rumen above the fistula level to ease the movement of the bags inside the rumen. The incubation periods were 3, 6, 12, 24 and 48 hours.

The bags were immediately removed at the end of each incubation period and thoroughly washed under tap water and dried in a forced air oven at 72°C overnight. After drying the bags were cooled in a desiccators and after that weighed. Dry matter content of the residues for each bag was calculated as follows:

$$\frac{\text{Weight of sample incubated} - \text{weight of residue after incubation}}{\text{Weight of sample incubated}} \times 100$$

Weight of sample incubated

The dry matter disappearance at zero time (soluble fraction) was estimated as the washing loss from each sample. Five grams from each sample were weighed in a nylon bag then rinsed under running tap water. Residual sample from incubation for every period and for each animal were separately mixed pooled and made ready for analysis.

Degraded protein was calculated with the following equation:

$$\frac{\text{C.P of sample incubated} - \text{C.P of residue after incubated}}{\text{C.P of sample incubated}} \times 100$$

C.P of sample incubated

The degradation kinetic of the incubated cakes (treated or untreated) were described by a curve linear regression of dry matter or crude protein loss from the bags with the time by the equation of Orskov and Mc Donald (1979)

$$P = a + b(1 - \exp^{-ct})$$

Where:

P = potential degradability (%)

a = the water soluble fraction.

b = potentially degradable water insoluble fraction.

c = degradation rate constant of fraction b (percentage /hour) .

t =time(hour).

Effective degradability (Ed) of DM and CP was determined, at 0.02, 0.05, and 0.08 ruminal outflow rates, using the equation of Oraskov and McDonald (1979) stated above.

2.4. Statistical Analysis:

The data were then subjected to one way analysis of variance test (ANOVA) according to Gomez and Gomez(1984) to examine the effects of the different ethanol treatments on ruminal dry matter (DM) and crude protein (CP) degradability and degradation kinetics. Statistical Package for Social Sciences (SPSS version 10) was used.

Chapter Three

Results

Chapter Three

3. Results

3.1 The proximate analysis of untreated and treated cotton seed cake:

Chemical composition of CSC was not affected by any of the ethanol treatments

Table (3/1) The proximal analysis of treated and untreated cotton seed cake with different alcohol.

| Treatment Analysis | Untreated C.S.C | 50% | 70% | 90% |
|--------------------|--------------------|--------------------|--------------------|--------------------|
| DM | 92.41 ^a | 90.81 ^a | 92.11 ^b | 91.21 ^c |
| C.P | 24.40 ^a | 23.11 ^a | 24.00 ^b | 23.75 ^c |
| C.F | 21.2 ^a | 20.1 ^a | 21.11 ^a | 21.00 ^a |
| E.E | 8.70 ^a | 8.11 ^b | 8.61 ^b | 8.23 ^a |
| ASH | 5.4 ^a | 4.77 ^a | 4.99 ^a | 4.81 ^a |

Means with different superscripts ($p < 0.05$)

Dame row are significantly different ($p < 0.05$).

3.2 The effect of ethanol treatment on in situ dry matter degradability (%):

Figure (1) shows the proportion of the dry matter which disappeared from the nylon bags at different incubation periods. The dry matter disappearance increased with the length of the period. All treatments decreased DM degradation at all the incubation periods, except of 50% ETCSC at 6hrs incubation period.

3.3 The effect of ethanol treatment on in situ CSC dry matter degradation kinetics:

All ethanol treated CSC showed significantly lower values of the water soluble DM fraction (a) and no variation was found among the three treatments. Ethanol treatment significantly reduced the degradation of the water insoluble fraction (b); the lowest value was found in 90% ETCSC and no significant variation was found between 50% ETCSC and 70% ETCSC .The rate constant (c) for (b) function was not affected by any of the three treatments. The potential degradability (Pd) was significantly reduced by ethanol treatment. All the treatments significantly reduced the effective degradability at three different rumen outflow rates; within the treatments 90% ETCSC showed the strongest effect and no variation was found between the other two treatments.

Table (3/2)

In situ dry matter disappearance (%) of cotton seed cake treated with different alcohol concentration.

| Treatment Time | Untreated C.S.C | 50% | 70% | 90% | Significance Level |
|-------------------|---------------------------|----------------------------|---------------------------|---------------------------|-----------------------|
| Zero | 10.52 ± 0.77 ^a | 9.74 ± 0.49 ^b | 9.8 ± 0.71 ^b | 9.69 ± 0.73 ^b | * |
| 3 | 22.53 ± 0.19 ^a | 21.42 ± 0.43 ^b | 21.02 ± 0.12 ^b | 20.61 ± 0.28 ^c | * |
| 6 | 25.69 ± 0.82 ^a | 25.53 ± 0.59 ^a | 24.19 ± 1.24 ^b | 23.72 ± 1.08 ^b | * |
| 12 | 40.99 ± 0.47 ^a | 40.52 ± 0.91 ^{ab} | 40.36 ± 0.5 ^b | 38.82 ± 0.88 ^c | ** |
| 24 | 43.56 ± 0.39 ^a | 42.71 ± 0.64 ^b | 42.74 ± 0.43 ^b | 41.46 ± 0.51 ^c | ** |
| 48 | 55.45 ± 1.13 ^a | 54.44 ± 0.61 ^b | 54.14 ± 0.03 ^b | 49.31 ± 0.45 ^c | ** |

N.S: non significant

* : significant at ($P < 0.05$)

** : significant at ($P < 0.01$)

a,b,c,: means within the same raw followed by different superscripts are significantly ($P < 0.05$) different.

Cont: untreated cake

50%, 70%, 90% CSC treated with alcohol concentration.

Table (3/3)

In situ cotton seed cake dry matter rumen degradability characteristics (%) from model of different alcohol treatment.

| Characteristics | Untreated (Cont) | 50% | 70% | 90% | Significance Level |
|-----------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------|
| a | 10.62 ± 0.77 ^a | 9.73 ± 0.49 ^b | 9.8 ± 0.34 ^b | 9.69 ± 0.73 ^b | * |
| b | 55.45 ± 1.13 ^a | 54.17 ± 1.04 ^b | 53.98 ± 2.3 ^b | 49.31 ± 1.00 ^c | ** |
| c | 0.05 ± 0.00 ^a | 0.05 ± 0.00 ^a | 0.05 ± 0.00 ^a | 0.05 ± 0.00 ^a | NS |
| Pd | 66.07 ± 1.43 ^a | 63.86 ± 1.23 ^b | 63.78 ± 1.66 ^b | 59.04 ± 1.35 ^c | ** |
| Ed (o.o2) % | 65.0 ± 4.6 ^a | 61.13 ± 1.31 ^b | 61.15 ± 2.41 ^b | 55.28 ± 2.92 ^c | ** |
| Ed (o.o5) % | 62.0 ± 3.14 ^a | 59.03 ± 1.4 ^b | 59.05 ± 2.28 ^b | 54.2 ± 1.12 ^c | ** |
| Ed (o.o8) % | 60.4 ± 2.5 ^a | 57.93 ± 1.44 ^b | 57.93 ± 2.26 ^b | 53.05 ± 1.06 ^c | ** |

N.S: non significant

* : significant at ($P < 0.05$)

** : significant at ($P < 0.01$)

a,b,c,: means within the same raw followed by different superscripts are significantly ($P < 0.05$) different.

a : washing loss

b : degradation of water insoluble fraction

c : rate constant of b function

pd: potential degradability

Ed: Effective degradability at rumen outflow (0.02), (0.05), (0,08)

3.4 The effect of ethanol treatment on *in situ* CSC crude protein degradability (%):

Table (2) shows the proportion of CP which disappeared from the nylon bags at different incubation periods. The CP disappearance (%) increased with the length of the incubation period. All the treatments decreased CP degradation at all the incubation periods. The lowest CP disappearance (%) was found in 90% ETCSC.

3.5 The effect of ethanol treatment on *in situ* CSC crude protein degradation kinetics:

Treated CSC with 70% and 90% ethanol showed lower CP washing loss and degradation of the water insoluble fraction (b) than that of 50% ETCSC and UCSC. The rate constant (c) for (b) function was not affected by any of the three treatments. All the treatments significantly reduced the effective degradability at three different rumen outflow rates. Within treatments 90% ETCSC showed the strongest effect and no variation was found between the other two treatments except at the fastest flow rate.

Table (3/4)

In situ crude protein disappearance (%) of cotton seed cake treated with different alcohol concentration.

| Treatment Time | Untreated C.S.C | 50% | 70% | 90% | Significance Level |
|-------------------|---------------------------|---------------------------|---------------------------|---------------------------|-----------------------|
| Zero | 9.88 ± 0.15 ^a | 9.78 ± 0.04 ^b | 9.59 ± 0.43 ^b | 9.18 ± 0.37 ^c | * |
| 3 | 32.46 ± 0.33 ^a | 32.40 ± 0.7 ^b | 32.29 ± 0.28 ^b | 31.11 ± 0.48 ^c | ** |
| 6 | 41.61 ± 1.63 ^a | 41.51 ± 0.31 ^b | 40.08 ± 0.01 ^b | 38.29 ± 1.59 ^c | ** |
| 12 | 61.79 ± 0.81 ^a | 61.65 ± 0.73 ^b | 59.53 ± 0.29 ^b | 55.54 ± 0.69 ^c | ** |
| 24 | 76.16 ± 0.16 ^a | 75.73 ± 0.42 ^b | 70.45 ± 0.72 ^b | 66.83 ± 0.87 ^c | ** |
| 48 | 87.2 ± 0.23 ^a | 86.91 ± 0.62 ^a | 84.22 ± 0.45 ^b | 80.93 ± 0.33 ^c | ** |

N.S: non significant

* : significant at ($P < 0.05$)

** : significant at ($P < 0.01$)

a,b,c,: means within the same raw followed by different superscripts are significantly ($P < 0.05$) different.

Table (3/5)

In situ cotton seed cake crude protein rumen degradability characteristics (%) from model of different alcohol treatment

| Treatment | Untreated C.S.C | 50% | 70% | 90% | Significance Level |
|-----------|---------------------------|----------------------------|---------------------------|---------------------------|-----------------------|
| a | 9.88 ± 0.15 ^a | 9.78 ± 0.04 ^a | 9.59 ± 0.43 ^b | 9.18 ± 0.37 ^b | * |
| b | 76.97 ± 0.74 ^a | 75.33 ± 0.8 ^{ab} | 72.30 ± 0.48 ^b | 70.87 ± 0.79 ^c | ** |
| c | 0.9 ± 0.01 ^a | 0.89 ± 0.03 ^a | 0.88 ± 0.04 ^a | 0.88 ± 0.06 ^a | NS |
| Pd | 86.85 ± 0.43 ^a | 85.11 ± 0.04 ^{ab} | 81.89 ± 0.07 ^b | 80.05 ± 0.05 ^c | ** |
| Ed (o.o2) | 75.23 ± 0.23 ^a | 74.02 ± 0.36 ^b | 73.80 ± 0.36 ^b | 73.1 ± 0.18 ^c | ** |
| Ed (o.o5) | 62.6 ± 0.14 ^a | 61.28 ± 1.15 ^b | 60.55 ± 0.06 ^b | 59.9 ± 0.43 ^c | ** |
| Ed (o.o8) | 58.35 ± 0.30 ^a | 56.70 ± 1.08 ^b | 55.7 ± 0.07 ^c | 54.7 ± 0.07 ^c | ** |

N.S: non significant

* : significant at ($P < 0.05$)

** : significant at ($P < 0.01$)

a,b,c,: means within the same raw followed by different superscripts are significantly ($P < 0.05$) different.

a : washing loss

b : degradation of water insoluble fraction

c : rate constant of b function

pd: potential degradability

Ed: Effective degradability at rumen out flow rat (0.02)%, (0.05)%, (0,08)%.

Chapter Four

Discussion and conclusion

Chapter Four

4. Discussion and conclusion

4.1 Discussion:

All the treatments significantly reduced both the potential and the effective degradability of CSC this accords with the findings of many researchers. Vander *et. Al* .(1982) treated soya bean meal (SBM) with 70% alcohol aqueous solution for thirty minutes, and found that it reduced nitrogen solubility by 33% and degradability by 10%. Lynch *et.al.*,(1987) found that treating SBM with 70% ethanol for one hour decreased both the N solubility and the degradability by 41% and 33%respectively and they suggested that Alcohol denature the protein. Corley *et. al.*, (1999) found that the optimal nitrogen solubility of SBM will be achieved by 70% ethanol treatment for 12 hours and any longer application of the same treatment than 12hours is not beneficial on DM and N solubility. These researchers found that 90% ethanol treatment is less effective than 70% ethanol treatment which contradicts the finding of this work. This variation may be due to the fact that SBM protein is rapidly degradable than CSC or the application method of the treatment.

Aqueous solution of alcohol denature the protein as 100% alcohol does not protect the protein from microbial degradation .It was found that water breaks down the outer hydrophilic portion of the protein which allows the alcohol to disrupt the hydrophobic portion, thus reducing the protein solubility in water (Fukushima 1969). Alcohol does not denature all the protein subunits with the same degree (Sadeghi, 2006) .

Hameed and Pasha (2000) treated CSC with three different levels of formaldehyde (0.5%, 1.0% and 1.5%) and autoclaving at15 pound steam pressure for different periods (30,45 and 60 minutes). They observed the

maximum rumen degradable protein was at 50.59% at 1% formaldehyde treatment and they did not find any variation among the three different formaldehyde concentrations and they suggested that 0.5% formaldehyde treatment can be used effectively. Formaldehyde is more economic than alcohol in protecting CSC from ruminal degradation. VanSoest (1982) suggested that formaldehyde alters the protein structure rendering it resistant to microbial degradation. This resistant structure was achieved by formation of acid irreversible linkages between the amino acids.

This may be due to a variation in the processing like Schroeder *et. al.*, (1995) were observed that the temperature and time of processing increase the protein portion that bypass the rumen to the small intestine. This may explain the variation between the results of this work and previous studies

The rate constant (c) for (b) function was not affected by any of the three treatments for both DM and CP. This shows that alcohol works on the extent of CP degradation rather than the rate.

Pena *et.al.*, (1986) reported 15.3% bypass protein values of cotton seed cake which are lower than the values of this work. Weakley *et.al.*,(1983) found that diet of the animal, substrate particle size, material and porosity of the bags are factors which may affect *in situ* digestion of feed stuff in the rumen.

4.2 Conclusion:

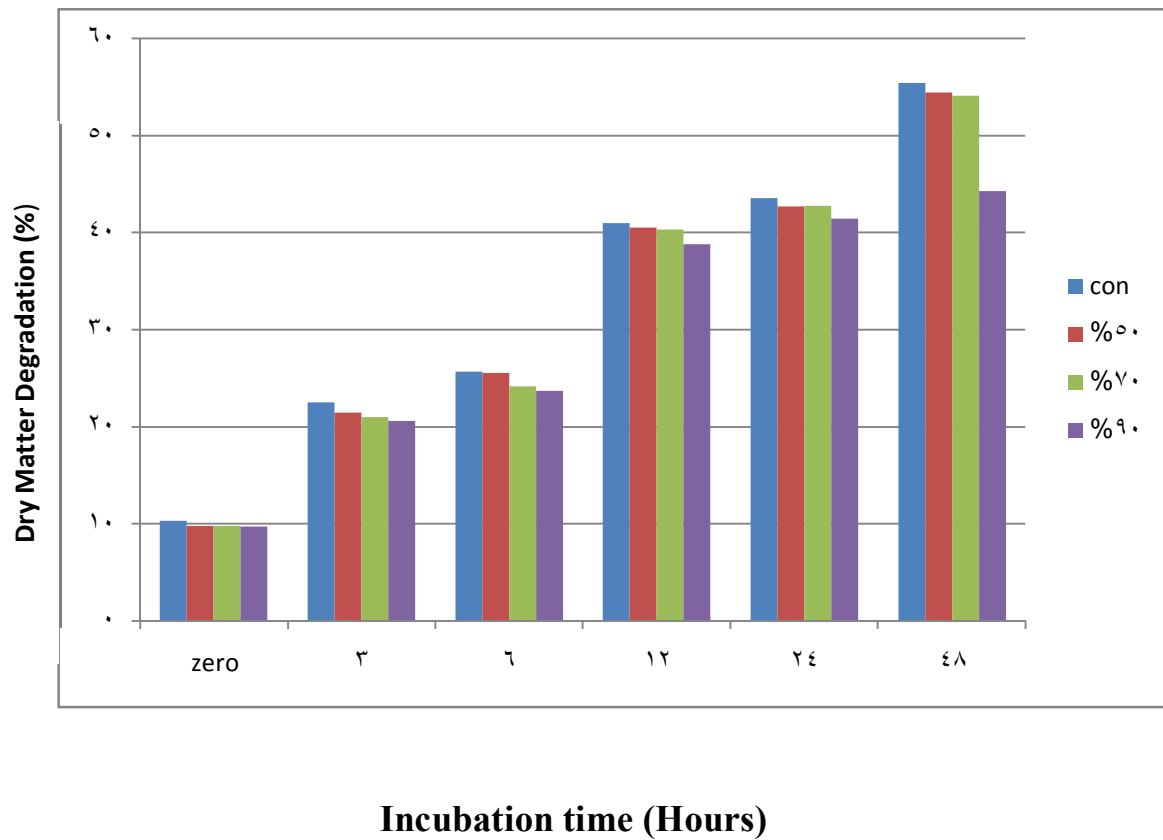
- Treatments with 70% and 90% alcohol significantly reduced CSC solubility and potentially degradable fraction (b).
- The degradation rate of fraction (b) was not affected by ethanol treatment.
- All the three ethanol treatments significantly reduced the effective ruminal degradability at three different ruminal outflow rates.
- The best protection of CSC was achieved by 90% ethanol treatment followed by 70% treatment and the lowest was found in 50% treatment.

4.3 Recommendations:

More studies should be done to determine:

- Ruminal degradability of ethanol treated CSC amino acid.
- The intestinal digestibility of the protected CSC.
- The efficiency of absorption and utilization of ECSC through feedlot trials.

Figure (1) The effect of ethanol treatment on in situ dry matter degradability (%):



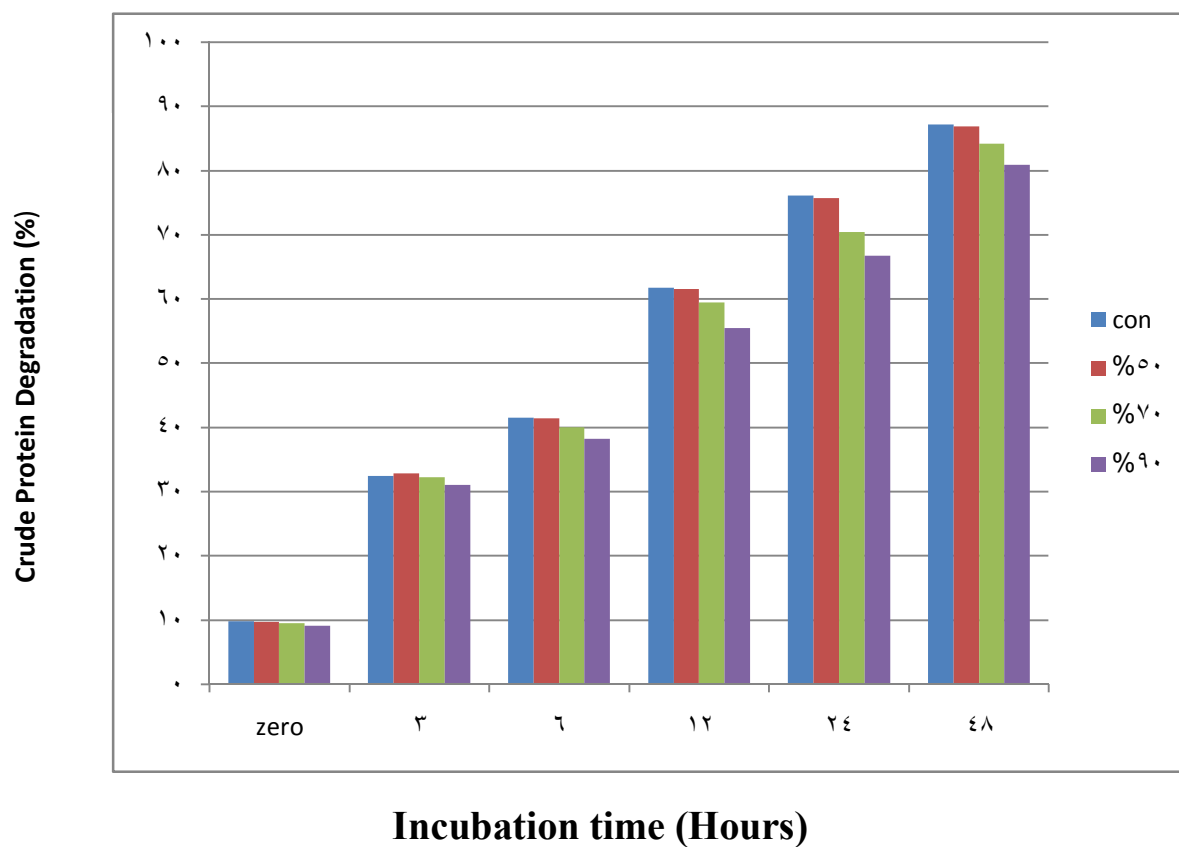
CON: untreated cottonseed cake

Treatment with 50% alcohol concentration

Treatment with 70% alcohol concentration

Treatment with 90% alcohol concentration

Figure (2) The effect of ethanol treatment on *in sit* crude protein degradability (%):



CON: untreated cottonseed cake

Treatment with 50% alcohol concentration

Treatment with 70% alcohol concentration

Treatment with 90% alcohol concentration

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