



Sudan University of Science and Technology

## **College of Graduate Studies**



Effect of Sodium Hydroxide and Calcium Oxide treatments on the Nutritive Value of Bagasse as ruminant feed

تاثير المعاملة بهيدر وكسيد الصوديوم واكسيد الكالسيوم على القيمة الغذائية للبقاس غذاء للمجتر ات

BY:

## **Mohamed Mergany Basher Mohamed**

B.Sc. in Animal Production Science and Technology

Sudan University of Science and Technology, (SUST) 2013

A Thesis Submitted for partial fulfillment of the requirement for the degree of Master of Science (M.Sc.) in Tropical Animal Production

## Supervisor : Prof . Shadia Abdelatti Omer

## الاستهلال

بسم الله الرحمن الرحيم

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ)

صدق الله العظيم

سورة العلق الاية (1)

## **Dedication**

I dedicate this research with love and respect to:

The soul of my Father...

The soul of my Mother...

My Colleagues and Friends...

My Supervisor...

## Acknowledgment

First and heart fully thanks to ALLAH who had it not been for his help, we could not have done anything .

Iwould like to express my deepest and sincere appreciation to my supervisor, Prof. Shadia Abdo ALattee for the constant support, valuable guidance and encouragement during the study.

My deep thanks are extended to the staff-members of the Nutrition Laboratory of Khartoum University .

Also thanks and appreciations are expressed to my family members, friends, colleagues who encouraged me during the study.

## List of content:-

Item	Page No
Opening	Ι
Dedication	II
Acknowledgement	III
List of contents	IV
List of tables	VIII
List of figure	IX
Abstract	Х
Arabic abstract	XI

Titles No	titles	Page No						
Chapter one : Introduction								
1- Introduction								
1-2	Objective							
	Chapter two : Literature Review							
2-	Literature Review	2						
2-1	Livestock feed in Sudan	2						
2-2	Agorindustrial by products used in livestock feed	2						
2-2-1	Oil seeds by products							
2-2-2	By products from the milling industry	3						
2-2-3	By product from sugarcane industry	3						
2-2-3-1	Molasses	3						
2-2-3-2	Sugar cane bagasse	4						
2-2-3-2-1	Chemical composition of sugar cane bagasse	4						
2-3	Treatment of low quality roughages	8						

2-3-1	2-3-1 Chemical treatment of crop residues						
2-3-2	2-3-2 Chemical treatment of sugar cane bagasse						
2-4	Digestion of carbohydrates in ruminants	12					
	Chapter three : Materials and methods						
3-	Materials and methods	16					
3-1	Collection of samples						
3-2	Chemical treatment						
3-2-1	Calcium oxide (CaO) treatment						
3-2-2	Sodium hydroxide (NaOH) treatment						
3-3	Chemical analysis						
3-4	Digestibility of treated and untreated sugar cane bagasse						
3-4-1	Experimental animals						
3-4-2	-2 In vitro dry matter digestibility (IVDMD)						
3-4-3	In vitro gas-production technique						

3-5	Statistical analysis						
Chapter four : Results							
4-	Results						
4-1	The effect of the NaOH and CaO treatments on the chemical composition of sugar cane bagasse						
4-2	The effect of NaOH and CaO treatments on in vitro dry matter digestibility (IVDMD) of sugar cane bagasse	22					
4-3	The effect of NaOH and CaO treatments on organic matter digestibility (OMD)						
4-4	-4 The effect of NaOH and CaO treatments on in vitro gas production						
	Chapter five: Discussion						
5-	Discussion	31					
	Chapter six : Conclusions and Recommendations						
6-1	Conclusions	35					
6-2	Recommendations	36					
	References	37					

## Lists of tables:-

Tables no	Titles of tables						
2-1	Chemical composition and fiber fractions of sugar cane bagasse from different authors in Egypt						
2-2	Mean and range of laboratory analyses of 66 sugarcane varieties grown on organic soil plots in south Florida	6					
2-3	Chemical composition of sugar cane bagasse in Sudan	7					
4-1	Proximate analysis of treated and untreated sugar cane bagasse	24					
4-2	Fiber fraction of treated and untreated sugar cane bagasse	25					
4-3	Cell wall content of treated and untreated sugar cane bagasse	26					
4-4	<i>In vitro</i> dry matter digestibility of treated and un treated sugar cane bagasse	27					
4-5	Organic matter digestibility of treated and un treated sugar cane bagasse	28					
4-6	Fermentation gas parameter characteristics of treated and un treated sugar cane bagasse	29					

## List of figure:-

Figure No	figure	Page No
2-1	First stage the digestion of carbohydrates	14
2-2	Second stage the digestion of carbohydrates	15
4-1	Gas production in treated and un treated sugar cane bagasse	30

#### Abstract

The study objective was to investigate the effect of chemical treatments on the nutritive value of sugar cane bagasse (SCB) as ruminants feed and compare it with the untreated SCB(USCB).SCB was treated with sodium hydroxide (SCBNa) at 4% and calcium oxide (SCBCa) at 8%.The nutritive value of the treated SCB and the USCB was assessed by comparing their chemical composition, gas production characteristics , *in vitro* dry matter digestibility and organic matter digestibility.The standard analytical methods were used . Both NaOH and CaO treatments caused significant reductions (P<0.01) in crude fiber ,neutral detergent fiber, acid detergent fiber, acid detergent lignin, hemicellulose content of SCB and a significant increase in its cellulose content .

Both chemical treatments caused significantly (P $\leq$ 0.01) decrease in the gas production from the immediately soluble fraction (a) and Significantly(P $\leq$ 0.01) increase gas production from the insoluble fraction (b) compared with the untreated bagasse *.In vitro* dry matter digestibility was increased (P $\leq$ 0.01) from 42.56% in USCB to 65.72% and 68.33% in SCBNa and SCBCa respectively. Organic matter digestibility was increased from 22.44% in USCB to 47.13% and 29.82% with SCBNa and SCBCa respectively.

It is concluded that alkali treatments improved *In vitro* dry matter digestibility and organic matter digestibility of sugar cane bagasse through changes in its chemical composition.

Key words: Sugar cane bagasse, Alkali treatment, Ruminants feed.

الملخص بالعربي

هدفت هذة الدراسة الي معرفة تاثير المعاملات الكيمائية علي القيمة الغزائية للبقاس كغزاء للمجترات مقارنة مع البقاس الغير معامل كيميائيا ،تمت معاملة البقاس ب4% هيدروكسيد صوديوم و8% اوكسيد كالسيوم ،القيمة الغزائية للبقاس المعامل كيميائيا والبقاس الغير معامل كيميائيا تقدر بالتركيب الكيميائي ،الغاز الناتج ، قابلية الهضم للمادة الجافة و قابلية الهضم للمادة العضوية وهي من طرق التحليل القياسية ، اوضحت النتائج ان معاملة البقاس بهيدروكسيد الصوديوم واكسيد الكالسيوم ادت الي انخفاض عند مستوي معنوية (P<0.01) في الالياف الخام، ADF ، NDF، اللجنين ، الهيميسللوز والي زيادة في السللوز مقارنة مع البقاس الغير معامل كيمائيا .

المعاملات الكيمائية ادت الي انخفاض عند مستوي معنوية ( P<0.01) في الغاز الناتج من الجزء الزائب (a) و زيادة في الغاز الناتج من الجزء الصعب الزوبان (b) مقارنة مع البقاس الغير معامل كيميائيا ، حدثت زيادة في قابلية الهضم للمادة الجافة من 42.56% في البقاس الغير معامل كيميائيا الي 65.72% و 68.33% في البقاس المعامل بهيدروكسيد الصوديوم واوكسيد الكالسيوم علي التوالي وزيادة في قابلية الهضم للمادة العضوية من 22.44% في البقاس الغير معامل كيميائيا الي 1.55% و 68.33% في البقاس المعامل بهيدروكسيد الصوديوم واوكسيد الكالسيوم علي التوالي وزيادة في قابلية الهضم للمادة العضوية من 1.54% في البقاس الغير معامل كيميائيا الي 1.55% و 29.28% في البقاس المعامل بهيدروكسيد الصوديوم واكسيد

خلصت الدراسة الي ان المعاملات الكيمائية ادت الي تحسن في قابلية الهضم للمادة الجافة وقابلية الهضم للمادة العضوية من خلال التغير في التركيب الكيميائي للبقاس .

# Chapter one

## **Chapter one**

## **1. Introduction**

According to the Ministry of Animal Resource Fisheries and Range (2015). the livestock population of Sudan amount to 106.622 million head, Feeding these animals really problem in Sudan because animals are owned mainly by nomadic groups who depend on poor range land for their feeding, concentrates are very expensive and not available in great amount in production areas, so there are many difficulties that made concentrates out of reach for more farmers. In Sudan there are many sugar cane companies producing more than one million tons of sugar cane bagasse which can be use for cattle feeding .These byproducts characterized by low content of protein, minerals and vitamins as well as high content of indigestible fiber due to lignification of cellulose. Mohammed and Salih (2015).

It has been known for almost 100 years that the digestibility of highly lignified materials may be improved by physical, microbiological and chemical treatment method for improvement of poor quality forages, roughages and by product . Sundst (1988) .

## 1-2 Objective:-

The objective of the study is to investigated on the improvement of the nutritive value of sugar cane bagasse by chemical treatments as ruminant feeds.

## Chapter two

## **Chapter two**

## 2. Literature Review

#### 2.1. Livestock feed in Sudan :-

Livestock population in the Sudan amounts to 106.622 million heads of which the Cattle are around 30.376 million heads, sheep 40.210 million heads, goats 31.227 million heads and camels 4.809 million heads ( Ministry of Animal Resource Fisheries and Range (2015). Sudan as one of the developing countries is fighting to satisfy the nutrient requirements of this large number of livestock, because of inadequate production of concentrates in addition to the competition between humans and animals (Aregheore, 2000). Another problem which challenges animal production in Sudan is that most of the animals are owned by nomads who depend on poor range land for feeding their animals as concentrates are out of their reach. The range land is decreasing due to the expansion of agriculture and war.

## 2.2. Agorindustrial by products used in livestock feed:-

#### 2.2.1. Oil seeds by products:-

These are the byproduct obtained after oil had been extracted the from the seeds of cotton, sunflower, sesame and groundnut and the final product is called cake. Oil seeds cakes are good source of protein for ruminants and poultry but these feeds contain approximately 20 percent ether extract (EE)

or fat and their use should be limited based on the fat content of the ration. The cakes can be used to provide an additional 2 to 3 percent fat above that provided by the basal ingredients in the ration with no more than 5 to 6 percent total fat in the dry matter. (John 2012).

#### 2.2.2. By products from the milling industry:-

These come from the milling industry such as bran, rice polishing, wheat millings, maize gluten, sorghum gluten these by products are good sources of energy and protein for animals. (Preston 2007).

### 2-2-3 By products from sugar cane industry:-

These are useful by products from the sugar industry, such as molasses and sugar cane bagasse :-

#### 2-2-3-1 Molasses :-

This by product is produced from manufactory of sugar, molasses are use in rations of cattle being a good source of energy, which can be substitute for grain but molasses is a poor source of protein, and needs to be supplemented with urea as a non-protein source of nitrogen for sustaining higher levels of production. The constraint in utilizing high levels of molasses is its toxicity, experience indicates that molasses may be toxic when fed in large quantities, so the recommended inclusion rate that does not usually exceed 15% for cattle and 8% for sheep.(McDonald *et.al.* 2010).

#### 2-2-3-2 Sugar cane bagasse :-

Sugar cane bagasse is the residue that is obtained from crushing sugarcane in the sugar industry. Sugar cane bagasse is the most abundant material in tropical countries such as South Africa. In Sudan there are many sugar cane factories producing more than one million tons of sugar cane bagasse which can be used for cattle feed (Mohamme and Salih 2015).Sugar cane bagasse can be used in feeding livestock, for power generation and manufacturing cellulosic ethanol.

#### 2-2-3-2-1Chemical composition of sugar cane bagasse:-

Sugar cane bagasse contains low protein, low soluble carbohydrates ( nitrogen free extract), low fat and high crude fiber(Mohammed and Salih 2015). Fibre contents of sugar cane bagasse is in the form of cellulose, hemicelluloses and lignin . Cellulose in bagasse is lignified as it associates with lignin by a bond which makes it more or less unavailable for the microbes in the ruminants digestive tract and makes sugar cane bagasse dry matter digestibility less than 50%, however sugar cane bagasse nutritional value can be sufficiently increased by physical, chemical or biological treatments (Fouda 2008).In table 2-1 , 2-2 and 2-3 chemical composition of sugar cane bagasse in Egypt , South Florida and Sudan :- **Table 2-1:-**

Chemical composition and fiber fractions of sugar cane bagasse from different authors in Egypt :-

Chemical composition						Fil	ber fra	ction				
Authors	DM	СР	EE	CF	NFE	OM	ash	ADF	NDF	ADL	Hemic	cellul
1	-	3.0	3.1	42.6	-	92.3	-	64.4	78.6	10.4	54.0	14.2
2	93.9	1.7	2.4	45.2	46.0	95.3	4.7	57.7	84.5	18.5	26.8	39.2
3	92.1	1.8	1.2	56.7	37.3	97.0	3.0	63.7	91.9	15.6	28.2	48.1
4	75.0	4.1	1.1	37.2	54.5	96.8	3.2	-	-	-	-	-

Source: Fouda (2008)

## **Table 2-2:-**

## Mean and range of laboratory analyses of 66 sugarcane varieties grown on organic soil plots in south Florida:-

analysis	Mean%	Low%	High%
Dry matter	25.8	17.0	30.5
Crude protein	2.3	1.1	3.1
Crude fiber	28.1	22.7	35.9
NDF	52.7	42.6	67.7
ADF	35.4	28.3	41.5
Cellulose	27.0	21.9	32.0
Lignin	6.3	4.6	8.4
ash	4.3	2.7	7.1
Calcium	0.20	0.06	0.35
phosphorus	0.05	0.02	0.09

Source: Pate et.al (1984)

## Table2-3:-

## Chemical composition of sugar cane bagasse in Sudan:-

Analysis	DM%	CP%	EE%	CF%	Ash%	NFE%	Me/mj/kg
Sugar cane bagasse	96.2	1.13	0.3	45.94	1.79	46.86	5.46

Source: Yosif and Afaf (1999)

## 2-3 Treatment of low quality roughages:-

Agricultural by-products like cereal straws and sugarcane bagasse are high in lingo-cellulose. It has been recognized that in order to improve the nutritive value of lingo-cellulosic materials for livestock, some form of pretreatment or processing of the plant material is required (Helmling *et.al.* 1989). Hence a pretreatment of the substrate is required to alter significantly the structural characteristics of the lingo-cellulosic matrix. Such a pretreatment must enhance the close contact between microbe and fibers to provide an efficient enzyme action (Rolz *et.al.* 1987).

A great effort was done to improve the quality of straw, crop residues ,and agro industrial byproducts. Physical ,chemical or biological treatments were studied and to used upgrade these stuffs .

#### 2.3.1.Chemical treatment of crop residues:-

History search proven chemical treatments was made improvement the nutritive value of straw and sugar cane bagasse by break down the ester bond linkages between lignin , cellulose and hemicelluloses, resulted the carbohydrates to become more available to the microorganisms in the rumen lead improve the benefit of feed by animal .Fouda (2008) .

Orskov and Grubb (1978) investigated a mixture of NaOH with other chemicals like urea to increase the intake and digestibility of roughages and found a limited interaction between the two chemicals .

Chaudhry and Miller (1995) treated wheat straw with sodium hydroxide and alkaline hydrogen peroxide and investigated their effect on the chemical composition of wheat straw and they found increase in cellulose, crude protein ,acid detergent fiber, decrease in neutral detergent fiber, acid detergent lignin , ash , hemicellulose and improvement on *in vitro* dry matter digestibility .

Chaudhry(1998). Treated wheat straw by calcium oxide, sodium hydroxide and alkaline hydrogen peroxide .Observed an improvement on metabolisable energy, organic matter digestibility and increase in gas production parameter.

Munever(1998) Investigated the effect of sodium hydroxide treatment on chemical composition and digestibility of straw, he found that sodium hydroxide treatment reduced the hemicellulose, protein and crude fiber content of the straw and increased the cell wall content of cellulose and lignin as well as the organic matter digestibility.

Wang *et al* (2006) .Treated rice straw with a combination of sodium hydroxide and ammonia bicarbonate. They found significant changes in the physical properties of the treated straw. They concluded that the increase in ash and changes in the physical properties is an indication of improvement in the digestibility of the straw.

Calcium hydroxide was recommended for the treatment of low quality roughage by many researchers as it is less caustic, easier to handle than NaOH and provides CaO. The effect of calcium hydroxide and urea treatment of barley straw with calcium hydroxide and urea decreased their content of hemicellulose and neutral detergent fiber and increased *in vitro* organic matter digestibility, ash and dry matter (Zaman and Owen ,1995). Metha *et.al.*(2009) studied the effects of treating rice straw with urea (5.5%) or urea and calcium hydroxide(2.2+2.2%), on feed intake, digestibility, rumen fermentation and milk yield of dairy cows. Their study revealed a decrease in neutral detergent fiber, acid detergent fiber and dry matter and significant improvements in dry matter intake and digestibility.

Mohammad*et.al.*(2010) investigated the effects of calcium oxide and calcium hydroxide treatments on the chemical composition and *in vitro dry* matter digestibility of soybean straw. They found that treatment by calcium oxide and calcium hydroxide caused changes in the chemical composition as decline in neutral detergent fiber, acid detergent lignin, acid detergent fiber, crude protein, cellulose and hemicellulose, but there was an increase in ash and *in vitro dry* matter digestibility .The reduction in the cell wall composition implies that the intake of soybean straw may be improved by chemical treatment.

#### 2.3.2. Chemical treatment of sugar cane bagasse:-

The Effect of delignification on the *in vitro* rumen digestion of polysaccharides of sugar cane bagasse made by(Robert*et.al*.1973) observed reduce the lignin content and increased the cellulose content. Improvement dry matter digestibility.

A simplified method for alkali treatment of low quality roughages for use by small holders in developing countries, was adopted by Kategile *et.al* (1981),they observed an improvement on the dry matter digestibility of roughages by the different levels of sodium hydroxide . Molina *et.al.* (1983) investigated the effect of treating sugar cane bagasse with different levels of NaOH, before ensiling, on its cell wall composition and digestibility of both dry and organic matter. They found that the cell wall content of neutral detergent fiber (NDF) decreased with increasing NaOH level but the acid detergent fiber (lignocelluloses fraction) was not changed. *In vitro* digestibility of dry matter, organic matter and cell wall contents increased with the increase in NaOH level.

Tudor and Inkerman (1989) sprayed fresh sugar cane bagasse with a 30% solution of NaOH and fed them to weaned cattle. They did not observe any health problems and found an increase in the sugar cane bagasse palatability in addition to an increase *in vitro* digestibility from 30% to 55%.

Fouda (2008) treated sugar cane bagasse with different chemical treatments (urea,CaOH and HCl) at different concentrations and incubation periods. He found changes in the chemical composition of the sugar cane bagasse, linear reduction in crude fiber content and nitrogen free extract He concluded that treatment increased in dry matter, ash and improved the organic matter digestibility of sugar cane bagasse.

Chaji *et.al.* (2010) processed sugar cane bagasse by NaOH, high steam or a combination of high steam and sodium hydroxide and assessed their nutritive value by *in vitro* gas production technique, result obtained show improvement in metabolisable energy and organic matter digestibility. All the treatments resulted in increase in gas production from the insoluble fraction (b) and the gas production rate for the insoluble fraction (c).

An increase in crude protein and hemicellulose and a decrease in neutral detergent fiber and acid detergent fiber and cellulose were the changes observed in the chemical composition of sugar cane bagasse treated with different levels of urea and moisture (Tiwari, 2013).

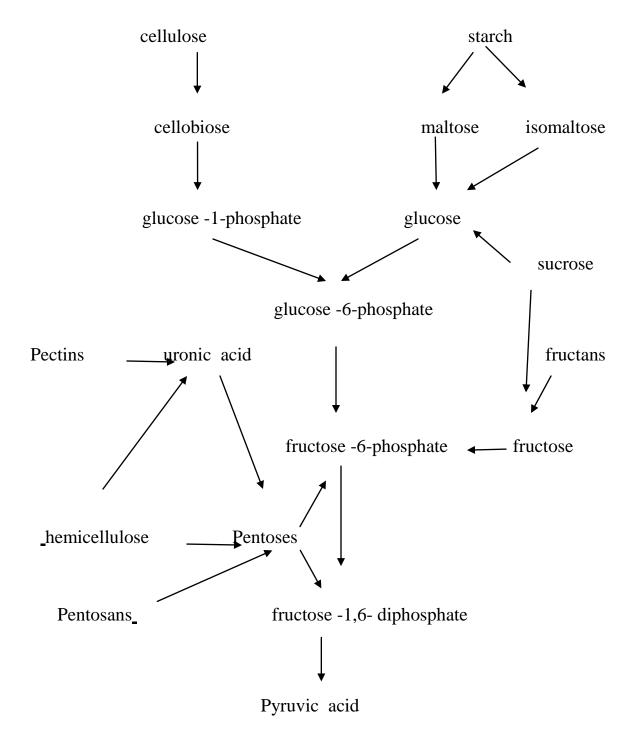
Gas production and ruminal degradability of sugar cane bagasse treated by calcium oxide were investigated by Nirawan *et.al.*(2014). Their results revealed that ruminal degradability, gas production from the insoluble fraction (b), potential extent of gas production (a+b) and cumulative gas production was increased by calcium oxide treatment.

Mohammed and Salih (2015) investigated the effect of feeding by sugar cane bagasse treated with urea on the properties and quality of fresh meat of Sudan Baggara Zebu Bulls . They found that treatment of sugar cane bagasse with urea increased the sugar cane bagasse crude protein and reduced its crude fiber content without any effect on the meat quality attributes and meat chemical composition of logissimus dorsi .

Shreck*et.al* (2013) treated crop residues with calcium oxide and sodium hydroxide and fed them to steers partially replacing corn. He observed that calcium oxide treatment improved *in vitro* organic matter digestibility and performance of steers .

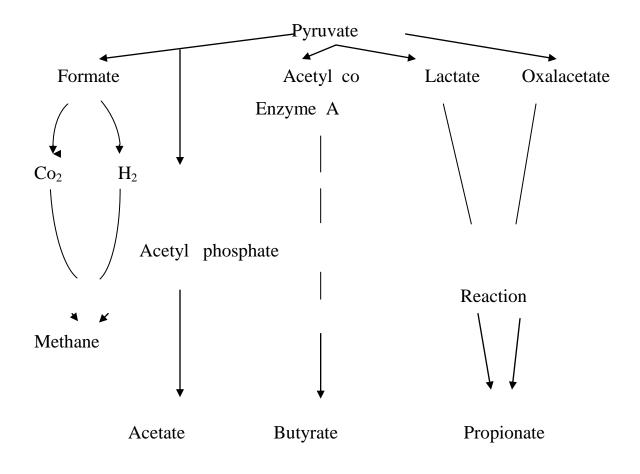
### 2-4 Digestion of carbohydrates in the ruminants:-

Chemically carbohydrates are composed of carbon, hydrogen, and oxygen. Carbohydrates of plant origin are the primary component in livestock feed such as roughages, hay, straw and sugar cane bagasse the proportion of cellulose and hemicelluloses is much higher while the water soluble carbohydrate is much lower .The end products of carbohydrate breakdown in the rumen are known as volatile fatty acid (acetic acid, propionic acid and butyric acid) .They are very important being major source of energy for the ruminant .Volatile fatty acids are absorbed through the walls of the rumen then transported in the blood stream to the liver ,in the liver they are converted to other sources of energy . From the liver energy produced is used for various functions (i.e. milk production, maintenance of body systems, pregnancy and growth) .Gases are also an end product of breakdown the carbohydrates such as Carbon dioxide and methane are produced during the fermentation of carbohydrates and the lost by eructation (McDonald *et. al.* 2010) .



## Figure No 2-1 :-

First stage the digestion of carbohydrates. McDonald et.al(2010)



## Figure No 2-2:-

Second stage the digestion of carbohydrates. McDonald et.al (2010)

## Chapter three

## **Chapter three**

## **3.** Materials and methods

### **3.1. samples preparation :-**

Sugar cane bagasse was purchased from kuku market and chopped manually by a knife to about 3cm<sup>3</sup>.Two thirds of the sugar cane bagasse were subjected to one of the following chemical treatments and the third portion was untreated and served as a control.

## 3.2 Chemical treatment:-

#### 3.2.1.Calcium oxide (CaO) treatment:-

Eight hundreds grams of CaO were dissolved in ten liters of water. The chopped sugar cane bagasse was soaked in the solution, at a concentration of 80 gm CaO : Kg dry matter sugar cane bagasse, for 45 minutes. After that the mixture was drained through a sieve and the liquid sugar cane bagasse ratio was 2:1, initial temperature of the solution was 48°C and a PH of more than 13.The mixture was put into well sealed and air evacuated plastic bags and after fifteen days were removed and air dried at room temperature .

#### 3.2.2.Sodium hydroxide (NaOH) treatment:-

Four hundreds grams of NaOH were dissolved in 15 liters of water. The chopped sugar cane bagasse was soaked in the solution , at a concentration of 40 gm NaOH : Kg dry matter sugar cane bagasse, for 45 minutes. After that the mixture was drained through a sieve and the liquid sugar cane bagasse ratio was 3:1 , initial temperature of the solution was 50°C and more

than 13 initial PH. The mixture was put into well sealed and air evacuated plastic bags and after fifteen days were opened and the sugar cane bagasse was removed and air dried at room temperature.

## 3.3. Chemical analysis:-

Proximate analysis of the untreated and chemically treated of sugar cane bagasse samples was performed according to the Association Official of Analytical Chemist (AOAC, 1990). Crude fiber (CF) was determined according to the method of (Van Soest *et al*, 1991;) . Neutral detergent fiber (NDF) ,Acid detergent fiber (ADF) and Acid detergent lignin (ADL) content was determined by the method of Goering and Van Soest (1970). Cell wall content that is cellulose , hemicellulose and lignin were calculated by the following equations according to Van Soest *et.al.* (1991) :

Cellulose % = ADF% - ADL%.

Hemicellulose % = NDF% - ADF%.

Lignin% = ADL%.

NFE was calculated by the following formula:-

NFE% = 100 - (Moisture% + CF% + CP% + EE% + Ash%).

## **3.4.** Digestibility of treated and un treated sugar cane bagasse:-

#### 3.4.1. Experimental animals:-

Study was conducted at the Farm of Sudan University of Science and Technology, Kuku area. Three Kenana steers aged 4-5 and were fitted with rumen cannula as described by Brown, (1968); for collection of the rumen fluid that was used for *in vitro* digestibility and gas production study techniques. They were fed a balanced ration of concentrate and roughage to maintenance level with free access to water and salt lick.

#### 3.4.2. In vitro dry matter digestibility (IVDMD):-

The two stage IVDMD procedure of Tilley and Terry, (1963) was adopted. The method includes two consecutive digestion phases.

During the first digestion phase, the control and the treated sugar cane bagasse were incubated under anaerobic conditions with rumen microorganisms for 48 hours at 39°C. This was followed by a 48 hour acidpepsin digestion phase at 39°C, under anaerobic conditions. Following this 96 hour incubation, the residues were collected and oven dried (105°C for 12 hours). Calculation of IVDMD was calculated by the following equation:

%IVDMD =  $(1 - wd - wb/ws) \times 100$ 

wd = weight of dry plant residue, wb = weight of dry residues from blank, and <math>ws = dry weight of original plant sample.

#### 3.4.3. In vitro gas-production technique:-

The Menke *in vitro* gas-production technique (Menke *et.al.* 1979) was adopted. 200mg of dry matter from each sample, in triplicate, were put into precision glass syringes then the rumen fluid buffer mixture was added to them, and they were incubated in a water bath with a stirrer .The blank was syringes containing only rumen fluid-buffer mixture. All the samples and the blank were run into replicates

The gas produced was read at a series of incubation times (sequential incubation), mainly 3, 6, 12, 24, 48, 72 and 96 hours. The net gas production of the samples was determined by subtracting the volume of gas produced in

the blanks (Menke and Steingass, 1988). Gas production data were fitted to the model of Ørskov and McDonald (1979)

Y=a+b (1-e<sup>-ct</sup>)

a = the gas production from the immediate soluble fraction (ml).

b = the gas production from the immediately insoluble fraction (ml).

c = the gas production rate for the insoluble fraction (ml).

a + b = the potential gas production (ml/h).

t = the incubation time (h).

Y = the gas production at time t.

The ME (MJ/ kg DM) contents of the samples were calculated using equation of (Menke and Steingass 1988) as follows:

ME (MJ/ kg DM) = 2.20 + 0.136 GP + 0.057 CP

Where, GP is the 24 h net gas production (ml/ 200 mg) and CP is the crude protein (%).

Organic matter digestibility (OMD) (%) of samples were calculated using equation of (Menke and Steingass 1988) as follows:

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA

Where, GP is the 24 h net gas production (ml/ 200 mg DM)

CP = Crude protein (%)

XA=Ash content (%)

## 3.5. Statistical analysis:-

The data obtained were subjected to one way analysis of variance to determine the variation among the sugar cane bagasse samples with regard to DM digestibility and fermentation kinetics. Significant differences among the samples were assessed using Least Significant Differences (LSD) test according to Gomez and Gomez, (1984). The Statistical Package for Social Sciences Program (SPSS version16) was used for the analysis.

# Chapter four

### **Chapter four**

#### 4. Results

# 4.1. The effect of NaOH and CaO treatments on the chemical composition of sugar cane bagasse :-

The effect of the treatments on the chemical composition of sugar cane bagasse is shown in table (4-1) .The lipid content of the sugar cane bagasse was not affected by any of the two treatments. The treated sugar cane bagasse showed a significant increase in ash and dry matter (P<0.01), a significant decrease in nitrogen free extract, crude protein and crude fiber (P<0.01).The effect of treatments on the metabolizeable energy was increase (P<0.01) from 3.30 Mj/Kg DM in control to 7.12 Mj/Kg DM and 4.46 Mj/Kg DM in treated by sodium hydroxide and calcium oxide respectively.

Table (4-2) shows the fiber fractions of treated and untreated sugar cane bagasse there was a decreased (P<0.01) NDF from 86.85% in untreated sugar cane bagasse to 66.45% and 63.1% with sodium hydroxide and calcium oxide treatments respectively, also ADF was significantly reduced (P<0.01) from 72.22% in the control to 59.02% and 59.91%% with sodium hydroxide and calcium oxide treatments respectively, ADL was significantly decreased (P<0.01) from 39.83% in control to 19.40% and 22.45% with sodium hydroxide and calcium oxide treatments respectively.

Cell wall content of treated and untreated sugar cane bagasse is displayed in table (4-3) .cellulose was increased (P<0.05) from 32.37% in the control to 39.62 % and 37.46% with sodium hydroxide and calcium

oxide treatments respectively, hemicellulose was decreased (P<0.01) from 14.62% in the control to 7.42% and 3.18% by sodium hydroxide and calcium oxide treatments respectively, lignin was decreased (P<0.01) from 39.85% in the control to 19.40 and 22.45% with sodium hydroxide and calcium oxide treatments respectively.

# 4.2. The effect of NaOH and CaO treatments on *in vitro* dry matter digestibility (IVDMD) of sugar cane bagasse :-

Table (4-4) shows the *in vitro* dry matter digestibility (IVDMD) of treated and untreated sugar cane bagasse. (IVDMD) was increased from 42.56% in the control to 65.72% and 68.33 % with sodium hydroxide and calcium oxide treatments respectively (P<0.01).

# **4.3.The effect of NaOH and CaO treatments on organic matter digestibility (OMD) :-**

Organic matter digestibility of treated and untreated sugar cane bagasse is show in table (4-5) there was increase (P<0.01) in organic matter digestibility from 22.44% in control to 47.13% and 29.82% in sodium hydroxide and calcium oxide treatment .

# 4.4 The effect of NaOH and CaO treatments on *in vitro* gas production:-

Cumulative gas production data are shown in table (4-6) the gas production from the immediately soluble fraction (a) was decreased (P<0.01) from 1.06 ml in untreated sugar cane bagasse to -1.23 ml and 1.02 ml with sodium hydroxide and calcium oxide treatments respectively. The gas production from the insoluble fraction (b) was increased (P<0.01) from

13.35 ml in untreated sugar cane bagasse to 62.58 ml and 44.92 ml with sodium hydroxide and calcium oxide treatments respectively. Potential gas production (a+b) was increased (P<0.01) from 14.42ml in untreated bagasse to 61.34ml and 45.94ml with sodium hydroxide and calcium oxide treatments respectively. The gas production rate (c) was reduced (P<0.05) from 0.03ml/hour in the control to 0.02ml/hour and 0.01ml/hour with sodium hydroxide and calcium oxide treatments respectively. In figure (4-1) there are increase in curve of gas production in treated sugar cane bagasse compared the untreated sugar cane bagasse on different time .

#### Table 4-1 :-

#### Proximate analysis of treated and untreated sugar cane bagasse :-

Treatments	Untreated sugar cane	Treated sugar cane	Treated sugar cane	Significance
Parameters	Bagasse (control) M±SD	bagasse (NaOH) M±SD	bagasse (CaO) M±SD	level
Dry matter %	$82.77\pm0.07^{\rm c}$	$83.70 \pm 0.16^{b}$	$84.78\pm0.01^a$	**
Crude protein %	3.24±0.04 <sup>a</sup>	$1.77{\pm}0.01^{b}$	1.83±0.01 <sup>b</sup>	**
Either extract %	$0.03 \pm 0.00$	$0.03 \pm 0.00$	$0.03 \pm 0.00$	NS
Crude fiber %	$43 \pm 0.00^{a}$	34.30± 0.01°	$37.87 \pm 0.02^{b}$	**
Ash %	4.19±0.28 <sup>c</sup>	17.74±0.17 <sup>a</sup>	16.10±0.13 <sup>b</sup>	**
Nitrogen free extract %	32.32±0.31ª	29.86± 0.33 <sup>b</sup>	29± 0.17 <sup>b</sup>	**
metabolizeable energy MJ/kg calculated by gas test study $3.30 \pm 0.14^{\circ}$		7.12±0.11 <sup>a</sup>	$4.46 \pm 0.20^{b}$	**

Means with different superscript within the same row are significantly different at (P < 0.05).

\*\*: Significance level P<0.01 .

\*: Significance level P<0.05 .

#### **Table 4-2:-**

#### Fiber fraction of treated and untreated sugar cane bagasse:-

Treatments Parameters	Untreated sugar cane Bagasse (control) M±SD	Treated sugar cane bagasse (NaOH) M±SD	Treated sugar cane bagasse (CaO) M±SD	Significance level
NDF%	86.85 ±0.03 <sup>a</sup>	$66.45 \pm 1.34^{b}$	$63.1 \pm 1.12^{\circ}$	**
ADF%	72.22±1.01 <sup>a</sup>	59.02 ±0.09 <sup>b</sup>	59.91 ±0.58 <sup>b</sup>	**
ADL%	$39.83 \pm 0.17^{a}$	19.40± 1.21°	$22.45\pm0.28^{b}$	**

Means with different superscript within the same row are significantly different at (P < 0.01).

- \*\*: Significance level P<0.01 .
- \*: Significance level P<0.05 .
- NS: Not significant .
- NDF : Neutral detergent fiber .
- ADF: Acid detergent fiber .
- ADL: Acid detergent lignin.

#### **Table 4-3:-**

#### Cell wall content of treated and untreated sugar cane bagasse:-

Treatments Parameters	Untreated sugar cane Bagasse (control) M±SD	Treated sugar cane bagasse (NaOH) M±SD	Treated sugar cane bagasse (CaO) M±SD	Significance level
Cellulose %	32.37±1.17 <sup>b</sup>	39.62±1.3 <sup>a</sup>	37.46±0.86 <sup>a</sup>	*
Hemicellulose %	$14.62 \pm 1.06^{a}$	7.42± 1.43 <sup>b</sup>	$3.18 \pm 0.54^{\circ}$	**
Lignin %	$39.85 \pm 0.16^{a}$	19.40±1.21 <sup>c</sup>	22.45 ±0 .28 <sup>b</sup>	**

Means with different superscript within the same row are significantly different at (P < 0.05).

- \*\*: Significance level P<0.01 .
- \*: Significance level P<0.05 .

#### **Table 4-4:-**

## *In vitro* dry matter digestibility of treated and un treated sugar cane bagasse:-

Treatments Parameter	Untreated sugar cane Bagasse (control) M±SD	Treated sugar cane Bagasse (NaOH) M±SD	Treated sugar cane Bagasse (CaO) M±SD	Significance level
<i>In vitro</i> dry matter digestibility %	$42.56 \pm 2.01^{b}$	$65.72 \pm 1.31^{a}$	$68.33 \pm 1.85^{a}$	**

Means with different superscript within the same row are significantly different at (P < 0.01).

- \*\*: Significance level P<0.01 .
- \*: Significance level P<0.05 .

Table 4-5 :-

# Organic matter digestibility of treated and un treated sugar cane bagasse :-

Treatments Parameter	Untreated sugar cane Bagasse (control) M±SD	Treated sugar cane Bagasse (NaOH) M±SD	Treated sugar cane Bagasse (CaO) M±SD	Significance level
Organic matter digestibility %	$22.71 \pm 1.25^{\circ}$	$48.30\pm0.62^{a}$	31.30± 1.94 <sup>b</sup>	**

Means with different superscript within the same row are significantly different at (P < 0.01).

- \*\*: Significance level P<0.01 .
- \*: Significance level P<0.05 .

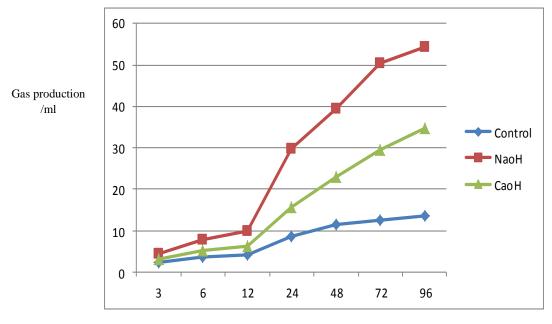
#### **Table 4-6 :-**

## Fermentation gas parameter characteristics of treated and un treated sugar cane bagasse :-

Treatments gas parameter	Untreated sugar cane bagasse(control) M±SD	Treated sugar cane bagasse (NaOH) M±SD	Treated sugar cane bagasse (CaO) M±SD	Significance Level
a (ml)	1.06±0.30 <sup>a</sup>	-1.23±0.92 <sup>b</sup>	1.02±1.14 <sup>a</sup>	**
b (ml)	13.35±0.90°	62.58±4.12 <sup>a</sup>	44.92±1.52 <sup>b</sup>	**
C (ml/hour)	0.03±0.01 <sup>a</sup>	$0.02{\pm}0.01^{a}$	$0.01 \pm 0.00^{b}$	*
a+ b (ml)	14.42±1.20 <sup>c</sup>	61.34±4.41 <sup>a</sup>	45.94±1.43 <sup>b</sup>	**

Means with different superscript within the same row are significantly different at (P < 0.05).

- \*\*: Significance level P<0.01 .
- \*: Significance level P<0.05 .
- NS: Not significant .
- a = the gas production from the immediately soluble fraction (ml).
- b = the gas production from the insoluble fraction (ml).
- c = the gas production rate constant for the insoluble fraction (ml/hour).
- (a+b) = the potential extent of gas production (ml) .



Incubation time / hours

## Figure 4-1:-

#### Gas production in treated and un treated sugar cane bagasse

# Chapter five

### **Chapter five**

#### **5.** Discussion

Chemical treatments of sugar cane bagasse caused an increase in dry matter content from 82.77% in untreated sugar cane bagasse to 83.70% and 84.78% with sodium hydroxide and calcium oxide treatments respectively, the results are on line with Zaman and Owen (1995) and Fouda(2008), these result were due to increase in ash content, Crude protein decrease from 3.24% in control to 1.77% and 1.83% with sodium hydroxide and calcium oxide treatments respectively. These results agree with Münever (1998) and Mohammad *et al* (2010) but disagree with Chaudhry (1998), Wange (2006), Fouda (2008) and Methaet.al. (2009), Crude fiber content decreased from 43% in control to 34.30% and 37.87% with sodium hydroxide and calcium oxide treatments respectively .These result similar Münever (1998) and Fouda (2008), the significant decrease in crude fiber content due to chemical treatments may be attributed to the breakdown of the lingocellulosic bonds due to chemical hydrolysis which resulted later in the release of available fermentable carbohydrate Abd elbaki et.al.(1984), Chemical treatments of sugar cane bagasse increase ash content from 4.19% in untreated bagasse to 17.74% and 16.10% with sodium hydroxide and calcium oxide treatments respectively, this result accord Zaman and Owen (1995), Wange *et.al.*(2006), Fuoda (2008) and Mohammad *et.al.* (2010), ash content was increased in treated sugar cane bagasse. This is might be due to the addition of basal minerals (NaOH and CaOH) Bakshiet.al. (1985), nitrogen free extract was decreased from 32.32% in control to 29.86 % and 29% with sodium hydroxide and calcium oxide treatments respectively. This results is on line Fuoda (2008), Changes occurred in nitrogen free extract values may be due to the increase in ash content. Fuoda (2008), metabolisable energy increased from 3.30 Mj/Kg DM in control to 7.12 Mj/Kg DM and 4.46 Mj/Kg DM in treated by sodium hydroxide and calcium oxide respectively. This result on line with Chaudhry (1998) and Chaji *et.al.*(2010). Improvement in metabolisable energy due to decrease in hemicellulose and lignin surrounding cellulose. Lead increase in soluble part of cell wall content (cellulose). Singh *et.al.*(1998).

The two different chemical treatments caused a decrease in neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). These results agree with the findings of (Chaudhry and Miller, 1995; Wange *et.al.* 2006; Metha*et.al.* 2009, Mohammad *et.al*, 2010 and Nirawan *et.al.* 2014). The changes in the sugar cane bagasse fiber content with the alkaline agent can be attributed to the chemical break of the ester bonds between lignin and hemicellulose and cellulose in the sugar cane bagasse (Fadel *et.al.* 2003).

The sugar cane bagasse cellulose content increased from 32.37% in the control to 39.19% and 37.36% with sodium hydroxide and calcium oxide treatments respectively. This supports the findings of Münever(1998), Robert*et.al.*(1973)and Fouda(2008).This result due to decrease in hemicellulose and lignin surrounding the cellulose, lead increase in cellulose content . Singh *et.al.*(1998).

Hemicellulose content decreased from14.62% in the control to 7.42% and 3.18% with sodium hydroxide and calcium oxide treatments respectively. This result agree with the findings of Zaman (1995) ,Chaudhry and Miller (1995), Münever (1998) and Mohammad *et.al.*(2010),lignin content was decreased from 39.85% to 19.40% and 22.45% in treaded sugar cane bagasse by sodium hydroxide and calcium oxide respectively. This result agrees with Robert *et.al.* (1973), but disagree with Münever (1997) and Chaudhry (1998) .This reduction is most probably due to solublization of the hemicellulose and lignin with the alkali treatment .

Chemical treatment improved *in vitro* dry matter digestibility from 42.56% in control to 65.72% and 68.33% with sodium hydroxide and calcium oxide treatments respectively. These result are on line with the findings of Fouda (2008), Metha *et al* (2009) and Mohammad *et al* (2010), Shreck (2013) and Nirawan *et.al.*(2014). When the sugar cane bagasse is exposed to an alkali, the ester linkages between lignin, cellulose and hemicelluloses are hydrolyzed, so that the carbohydrates becomes more available to the microorganisms in the rumen for improvement in digestibility McDonald *et.al.* (2010).

*In vitro* organic matter digestibility was improved from 22.44% in the control to 47.13% and 29.82% with sodium hydroxide and calcium oxide treatments respectively .These findings are on line with the findings of Molina *et.al.*(1983), Münever(1998), Wange *et.al.*(2006), Chaji *et.al.* (2010) , and Shreck (2013). These improvement in organic matter digestibility due to decrease in crude fiber content in treated sugar cane bagasse .

Gas production from the insoluble fraction (b) was increased from 13.35ml in untreated sugar cane bagasse to 62.58 ml and 44.92 ml with sodium hydroxide and calcium oxide treatments respectively. Potential extent of gas production (a+b) was increased from 14.42ml in untreated sugar cane bagasse to 61.34ml and 45.94 ml with sodium hydroxide and calcium oxide treatments respectively. These results agree with Chaji et.al. (2010) and Nirawan et.al. (2014). This result due to decrease in crude fiber content in treated sugar cane bagasse, lead increase the digestibility and gas production compared untreated sugar cane bagasse. McDonaldet.al. (2010). Gas production rate constant for the insoluble fraction (c) was decreased from 0.03ml in control to 0.02 ml and 0.01ml with sodium hydroxide and calcium oxide treatments respectively. Gas production from the immediately soluble fraction (a) decreased from 1.06 ml in untreated sugar cane bagasse to -1.23 ml and 1.02 ml with sodium hydroxide and calcium oxide treatments respectively. This result without agree Chaji et.al.(2010) and Nirawan *et.al* (2014). There are increase in curve of gas production in treated sugar cane bagasse compared the untreated sugar cane bagasse on different times. These results agree with Chaji et.al. (2010). Increase of gas production due to improve the digestibility of sugar cane bagasse.

# Chapter six

## **Chapter six**

## 6. Conclusions and Recommendations

## 6-1Conclusions:-

Alkali treatment improved the nutritive value of sugar cane bagasse as shown by :-

The cell wall cellulose was increased .

The cell wall hemicellulose was decreased .

*In vitro* dry matter digestibility, Organic matter digestibility, metabolizeable energy was improved .

## 6-2 Recommendations:-

More studies are needed to support alkali treatment of sugar cane bagasse as ruminant feeds more so on palatability, intake, performance and economical feasibility.

#### **References:-**

Abdelbaki, S.M; El Shobokshy, A.S; Swedan, F.Z; Abel Rahman, G.A and Hassona, E.M (1984). Chemical treatments of some poor quality roughages. First Egyptian British conference on Animal and Poultry Production. Zagazig.

AOAC. (1990). Official methods of analysis of the association of official analytical chemists . 15<sup>th</sup> edition . Arlington , Virginia ; AOAC .

Aregheore, E.M. (2000). Chemical composition and nutritive value of some tropical by product feedstuffs for small ruminant *in vivo* and *in vitro* digestibility. Animal Feed Science . Volume 85. Page 99-109.

Bakshi,M.P.S ; GuptaV.K and LangarP.N. (1985) . Acceptability and nutritive evaluation of pleurouts harvested spent wheat straw in buffaloes . Life , Earth and Health science. Volume13 . Page 51-57 .

BrownG.F;Armestrong D.G; and MacRae J.C. (1968). The establishment in one operation of a cannula into the rumen and reentrant cannula into the duodenum and ileum of the sheep. British Veterinary Journal,124:78.

Chaji,M;Mohammada,T; Mamouei,M and Tabatabaei,S (2010) The effect of processing with high steam and sodium hydroxide on nutritive value of sugar cane bagasse by *in vitro gas* production. Journal of Animal and Veterinary advances. Volume 9. Page 1015 - 1018.

Chaudhry, A.S and Miller, E.L. (1995). The effect of sodium hydroxide and alkaline hydrogen peroxide on chemical composition of wheat straw voluntary intake, growth and digest kinetics in store lambs, Animal Feed Science and Technology. Volume 60. Page 69-86. Chaudhry,A.S.(1998) Nutrient composition, digestion and rumen fermentation in sheep of wheat straw treated with calcium oxide, sodium hydroxide and alkaline hydrogen peroxide. Animal Feed Science and Technology. Volume74. Page 315 - 328.

Dukes,H.H.(1993). Physiology of domestic animal . 7<sup>th</sup> . person Ithaca, New york . page 352-355 .

Fadel,A.M.A; Sekine,J; Hishinuma,M and Hamana,K (2003). Effects of ammonia, urea plus calcium hydroxide and animal urine treatments on chemical composition and *in sacco* degradability of rice straw. <u>Asian-Australasian Journal of Animal Sciences</u>. Volum16. page 368-373.

Fouda.S,M.(2008).Nutrient study of chemical and biological treatment of sugar cane bagasse . PhD theses . Alazhar university .

Goering,H.KandVanSoest,P.J. (1970) . Forage fiber analyses(Apparatus, Reagents, Procedures, and Some Applications) . 1<sup>th</sup> edition . Pearson: United states page 8-11 .

Gomez,K.AandGomez,A.G (1984). Statistical procedures for agricultural research . 2<sup>th</sup> edition . Pearson: John Wiley and Sons , New York .

Helmling,O; Arnold,G; Rzehak,H; Fahey,J.r; Berger,L.L and Merchen, N. R.(1989).Improving the nutritive value of lignocellulosics: the synergistic effects between alkaline hydrogen peroxide and extrusion treatments. Biotechnology and Bioengineering . volume 2 page 237-241 .

John,K.B(2012).Considerations for using by Product Feeds . *Cooperative Extension Bulletin*. page 1-11 .

Kategile, J.A; Urio, N.A; Sundstøl, F and Mzihirwa, Y.G. (1981). Simplified method for alkali treatment of low quality roughages for use by small holders in developing countries. Animal feed Science and Technology Volume 6, Pages 133–143.

McDonald,P ; Edwards,R.A ;Greenhalgh ,J.F.D ; Morgan,C.A ; Sinclair, L.A and Wilkinson,R.G (2010).Animal Nutrition .Seventh edition . Pearson: England , London , New york , Paris, page 555 , 171-176 .

Menke,K.H;Raab,L;Salewski,A; Steingass,H ; Fritz,D and Schneider,W. (1979) . The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. Journal of Agricultural Science Cambridge . page 217-222 .

Menke,K.H and Steingass,H.(1988).Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid .Animal Research Development.Volume 28 .page 7-55 .

Metha,W; Sineenart,P; Kitsada,B; Chaowarit,M and Anusorn,C (2009) . Effects of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows . Livestock Science . volume 125 . Page 238–243 .

Ministry of Animal Resource Fisheries and Range (2015). Information center. Khartoum. Sudan .

Mohammad,K ; Shahryar,K; Taher,Y; and Gholam,R. (2010) .Effects of calcium oxide and calcium hydroxide on the chemical composition and *in* 

*vitro* digestibility of soybean straw. Journal of Food Agricultures and Environment. Volume 8. Page 356-359.

Mohammed,H and Salih, A . (2015) . Effect of feeding urea treated sugar cane bagasse on properties and quality of fresh meat of Sudan Baggara Zebu bulls . International Journal of Animal Biology .Volume 1 page 45-49 .

Molina,E ; Boza,J andAguilera,J.F.(1983) .Nutritive value for ruminants of sugar cane bagasse ensiled after spray treatment with different levels of sodium hydroxide .Veterinary and Animal Science Journal.Volume 9 . Pages 1–17 .

Münever, A. (1998). The effect of sodium hydroxide treatment on chemical composition and digestibility of straw. Turkish Journal of Veterinary and Animal Sciences . Volume 22 . Page 165-170 .

Nirawan,A;Metha,W;Pongsatorn,G;Anusorn,CandWalailuck,K.(2014). Effect of sugarcane bagasse treatment on gas production and ruminal degradability by using *in vitro* gas production technique .Khon kaen Agriculture Journal . Volume 4 Page 35- 40 .

Pate,F.M; Alvarez,J; Phillips, J.D ; and Eiland,B.R.(1984). Sugar cane as a Cattle Feed( Production and Utilization).Institute of Food and Agricultural Sciences . page 1-21.

Preston, T.R .(2007). Better utilization of crop residues and by products in animal feeding. Food and Agriculture Organization of the United Nations concerning the legal status of any country. Originated by Agriculture and Consumer Protection. Page 35-36. Qrskov,E.R; and Grubb,D.A. (1978). The effect of urea on digestion and voluntary intake by sheep of diets supplemented with fat.Animal Production. volume 27 .page 241-245 .

Qrskov,E.RandMcDonald.I.(1979). Estimation of protein degradability in the rumen from incubation measurements weighed according to rate passage. Journal of Agricultural Science . Volume 92 . page, 499-503 .

Robert,P.F ; Dekker,H and Geoffrey,N.(1973) . Effect of delignification on the *in vitro* rumen digestion of polysaccharides of bagasse. Journal of the Science of Food and Agriculture . Volume 24, pages 375–379 .

Rolz,C ; Leon,R ; Arriola,MC and Cabera,S.(1987)White rot fungal growth on sugar cane lingocellulosic residue. Application Microbiological Biotechnology . Volume 25 . Page 535-541 .

Shreck,A.L ; Buckner,C.D ; Erickson,G.E ; Klopfenstein,T.G and Cecava ,M.J . (2013) . Factors affecting treatment success of calcium oxide and sodium hydroxide treated crop residues , PhD Dissertation (published) University of Nebraska . http:// digitalcommons.unl.edu/animalscidiss/74 . Animal Science , page 55-70 .

Shreck,A.L; Nuttelman,B.L; Harding,J.L; Griffin,W.A; Erickson,G.E; Klopfenstein,T.J and Cecava,M.J (2013), Digestibility and performance of steers fed low quality crop residues treated with calcium oxide to partially replace corn in distillers grains finishing diets. PhD Dissertation (published) University of Nebraska . http:// digitalcommons.unl.edu/animalscidiss/74 . Animal Science, Page 71-104.

Singh,S.K. and Devi,A.A.(1998). Effect of grasses fed to pigs by different methods on their growth rate and feed conversion efficiency. Indian Journal Animal Science . Volume 68 page 693-695 .

Sundst,F (1988) Improvement of poor quality forages and Roughages. Feed Science. Volume 10. Page 257-277.

Tiwaril,R.K ; Garg,A.K and Singh,P. (2013) . Changes in the chemical composition of sugar cane crop residues treated with different levels of urea and moisture . Veterinary World .Volume 6 . Page 1004-1007 .

Tilley, J.M.A and Terry, R.A. 1963). Two stage technique for the *in vitro* digestion of forage crops . Journal of the British Grass land . Volume 18 . page 104-111 .

Tudor,G.D and Inkerman,P.A. (1989). Alkali treated bagass potential as feed for ruminant. Animal Research Institute . page 53-66.

Van Soest,P.J ; Robertson,J.B. and Lewis,B.A.(1991).Methods for dietary fiber , neutral detergent fiber and non starch polysaccharides in relation to animal nutrition . Journal of Dairy Science . Volume 74 . page 3583-3597 .

Wange ,J.K ; LiuI,J.X ; Wu,Y.M and Ye,J.A . (2006) . Improvement of organic matter digestibility along with changes of physical properties of rice straw by chemical treatments. Journal of Animal and Feed Sciences .Volume 15 . Page 147–157 .

Yosif, R and Afaf, A. (1999). The nutrient composition of Sudanese animal feed bulletin (3) . Central Animal Nutrition Research Laboratory ., Animal Production Research Center . Khartoum , north Sudan .

Zaman,M.S and Owen,E. (1995) .The effect of calcium hydroxide and urea treatment of barley straw on chemical composition and *in vitro* digestibility . Animal Feed Science and Technology .Volume 51 . Page 165-171 .