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## **EFFECT OF DIFFERENT NaOH TREATMENTS ON RUMINAL DEGRADATION CHARACTERISTICS OF GROUND NUT CAKE**

(With 4 Tables)

By

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### **SUMMARY**

The study was conducted to determine the effect of chemical (NaOH) treatment on dry matter (DM), crude protein (CP) degradation characteristics, and effective degradability of groundnut cake (GNC). GNC was either soaked in 0.5N NaOH or sprayed with it. The 0.5N NaOH treated cake was either air dried or oven dried at 100C°. Nylon bags technique was employed using three castrated calves. All treatments significantly ( $P < 0.05$ ) decreased *insitu* dry matter and crude protein degradation rate as well as the effective degradability of (GNC). The different treatments had the same CP degradation rate at 0, 6 hrs. Both GNC air dried, or oven dried showed lower CP degradation rate than the GNC sprayed at incubation hours 36, 48 and 72 hours. There were no differences in rumen degradation characteristics of CP among the treatments with the respect to the soluble fraction (a), while the NaOH sprayed GNC showed the highest value for the insoluble degradable fraction (b).

**Key words:** *Ground nut cake/Alkali treatments/Protected proteins*

### **INTRODUCTION**

Groundnut seeds are the most commonly used oilseed in the Sudan for human nutrition, the groundnut cake (GNC) is the major protein supplement in dairy and beef diets. Excessive degradation of GNC protein in the rumen results in reduced efficiency of dietary nitrogen utilization, particularly in high-producing dairy cows.

Various methods of treating proteins have been used to reduce their degradation in the rumen and increase their ruminal by pass protein content. These methods are most commonly used in

Soybean meal (SBM) in the U.S.A feed industry. The methods can be categorized into chemical and physical treatments. Research on chemical methods has concentrated on treatment of SBM with formaldehyde Spear *et al.*, (1980), tannins Hartnell and Satter (1978), alcohols Vander Aar *et al.*, (1982), and sodium hydroxide, propionic acid and hydrochloric acid Walts and Loerch (1986). While Sadeghi *et al.* (2006) studied the protein degradation kinetics of protected and unprotected soybean meal.

In the Sudan data concerning treatments of feed, to increase their bypass proteins, seems to be limited. Therefore, the current study was conducted to evaluate the effectiveness of chemical treatment (NaOH), applied in different methods, in protecting GNC from microbial degradation in the rumen.

## **MATERIALS and METHODS**

Ground nut seeds were the fresh crop of the year (2006), from Kordofan State West of the Sudan. The cake was obtained by mechanical extraction of the oil at an oil mill in Omdurman.

### **Treatment of the cake:-**

One kg of GNC was soaked in excess solution of 0.5N NaOH for 15 minutes as described by Waltz and Loerch, (1986). Half of it was allowed to dry by air at room temperature and it was named GNC NaOHA. The other half was dried for 6 hours at 100C° in an air forced oven and was named GNC NaOHH.

Another 500gms were sprayed with 50% solution of 0.5N NaOH, water was added to aid in the distribution of the alkali in the cake, then it was air dried at room temperature, and was named GNCNaOHS. 1kg of GNC was soaked in distilled water for 15 minutes, then it was air dried at room temperature, to be used as a control and was named UGNC. Treated and untreated GNC were processed through a hammer mill with a screen of 3mm.

### **Animals and feeding:-**

Three castrated calves from local breed (Kenana) aged 3 - 3½ years, were fitted with rumen cannulae as described by Brown *et al.*, (1968). They were maintained on a well balanced ration of concentrates and roughage, and were fed twice daily. Water and salt were available all the time.

**Ruminal dry matter (DM) and crude protein (CP) degradability:**

According to the polyester bag technique of (Mehrez and Ørskov, 1977), the bags were prepared from nylon material of 35-40 µm pore size and weighing 2 - 3g. The empty bags were individually weighed and their weights were recorded. 5gms of treated or untreated cakes were put in the bag tied with a nylon ribbon, attached to a plastic tube of 45.5cm length, 0.8cm diameter, and were introduced inside the rumen. The bags (2 bags/animal/period/ treatment) were incubated for 6, 12, 24, 36, 48 and 72 hours each. The DM and CP contents of the treated and untreated cakes, before and after incubation were determined as described by AOAC, (1980).

**Calculation of the ruminal (DM, CP) degradability:-**

Degraded dry matter percentage was calculated by the formula:

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

Residual samples after incubation for each period were separately mixed, pooled and made ready for analysis.

Degraded protein was calculated according to the formula:-

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

The degradation kinetics of the incubated cake (treated or untreated) was described by curve-linear regression of DM or CP loss from the bags with time by the equation of Ørskov and McDonald (1979).

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

Where:

P = potential degradability (percentage)

a = the soluble fraction (percentage).

b = the potentially degradable fraction (percentage).

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

t = time (hour).

The effective degradability of samples was calculated using the equation of Ørskov and McDonald (1979), at three rumen fractional outflow rates, of 0.03, 0.05 and 0.08 h<sup>-1</sup>.

**Statistical analysis:-**

The data obtained were subjected to one way analysis of variance to examine the effect of the treatment on DM and CP degradation kinetics. Significant differences among the treatments were assessed

using Least Significant Differences (LSD) test according to Gomez and Gomez, (1984). The Statistical Package for Social Sciences Program (SPSS) was used for the analysis.

## RESULTS

Ruminal DM degradation of untreated and 0.5 NaOH treated GNC at the different incubation times is displayed in Table (1). NaOHS treatment has no effect on the dry matter disappearance rate at all the incubation periods, while GNCNaOHH represented the lowest proportion of dry matter degradation rate at all the incubation periods followed by GNCNaOHA.

Table (2) shows *insitu* dry matter rumen degradation from fitted model for different NaOH treatments. Significant differences ( $p < 0.05$ ) were found between treated and untreated GNC as well as among treatments for all the fitted values.

GNC NaOH H had the lowest constant (a, c), Pd and effective degradability at three different rumen outflow rates. GNCNaOHS did not cause any change in DM effective degradability at all the rumen outflow rates.

**Table 1:** *In situ* dry matter disappearance rate (%) for NaOH treated and untreated GNC.

Treatment	UGNC	GNCNaOHA	GNCNaOHS	GNCNaOHH	SEM	Significance level
Time (hours)						
0	36.76 <sup>b</sup>	56.53 <sup>a</sup>	56.90 <sup>a</sup>	28.06 <sup>c</sup>	3.87	*
6	83.80 <sup>a</sup>	75.16 <sup>b</sup>	84.46 <sup>a</sup>	65.33 <sup>c</sup>	3.73	*
12	85.93 <sup>a</sup>	82.63 <sup>b</sup>	86.30 <sup>a</sup>	62.96 <sup>c</sup>	2.91	*
24	92.80 <sup>a</sup>	87.03 <sup>b</sup>	92.93 <sup>a</sup>	74.26 <sup>c</sup>	2.31	*
36	93.03 <sup>a</sup>	87.86 <sup>b</sup>	93.06 <sup>a</sup>	77.00 <sup>c</sup>	1.98	*
48	93.96 <sup>a</sup>	93.13 <sup>b</sup>	94.03 <sup>a</sup>	87.66 <sup>c</sup>	0.80	*
72	94.20 <sup>a,b</sup>	93.70 <sup>b</sup>	95.00 <sup>a</sup>	92.40 <sup>c</sup>	0.32	*

\* : Significant at ( $P < 0.05$ )

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

SEM : Standard Error of the Means.

**Table 2:** *In situ* GNC dry matter rumen degradability kinetics for NaOH untreated and treated GNC.

Treatment	Control	GNC NaOH Air	GNC NaOH Spray	GNC NaOH Heat	SEM	Significance level
Fitted Values						
A	36.92 <sup>b</sup>	57.12 <sup>a</sup>	57.25 <sup>a</sup>	31.70 <sup>c</sup>	3.55	*
b	55.82 <sup>b</sup>	34.48 <sup>c</sup>	36.07 <sup>c</sup>	58.36 <sup>a</sup>	3.31	*
c	0.28 <sup>a</sup>	0.11 <sup>c</sup>	0.19 <sup>b</sup>	0.06 <sup>c</sup>	0.02	*
Pd	92.74 <sup>a,b</sup>	91.60 <sup>a,b</sup>	93.32 <sup>a</sup>	90.06 <sup>b</sup>	0.49	*
Ed <sub>(0.02)</sub>	88.98 <sup>a</sup>	86.21 <sup>b</sup>	89.95 <sup>a</sup>	75.24 <sup>c</sup>	1.77	*
Ed <sub>(0.05)</sub>	84.21 <sup>a</sup>	80.75 <sup>b</sup>	85.99 <sup>a</sup>	63.42 <sup>c</sup>	2.71	*
Ed <sub>(0.08)</sub>	80.26 <sup>a</sup>	77.03 <sup>b</sup>	82.88 <sup>a</sup>	56.61 <sup>c</sup>	3.14	*

\*: significant at (P<0.05).

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

a: washing loss.

b: water insoluble nutrients which is potentially degradable by microorganisms.

c: degradation rate of b/hour.

Pd: Potential degradability.

Ed: Effective degradability at three rumen fractional outflow rates (0.02, 0.05, and 0.08).

SEM: Standard Error of the Mean.

The effect of the various 0.5N NaOH treatments on *insitu* crude protein degradation is displayed in Table (3). All treatments significantly (P<0.05) decreased CP degradation percentage at all the incubation periods. Table (4) shows CP protein degradation kinetics of treated and untreated GNC. All alkali treatments lowered washing loss (a) value; potentially degradable fraction (b), potential degradability (Pd) and effective degradability (Ed) at three rumen outflow rates. There were no differences among the treatments with respect to fraction (a). GNC NaOHS showed the highest (b) value. The highest decrease in constant (c) value, (Pd) and effective degradability at three different rumen outflow rates was observed with GNCNaOHA.

**Table 3:** Effect of different NaOH treatments on *insitu* GNC protein degradability (%).

Treatment	Control	NaOH Air	NaOH Heat	NaOH Spray	SEM	Significance level
Time (hours)						
0	9.57 <sup>a</sup>	3.78 <sup>b</sup>	2.81 <sup>b</sup>	4.94 <sup>b</sup>	8.55	*
6	25.33 <sup>a</sup>	6.21 <sup>b</sup>	6.26 <sup>b</sup>	7.13 <sup>b</sup>	2.50	*
12	32.81 <sup>a</sup>	12.53 <sup>c</sup>	17.24 <sup>b</sup>	17.94 <sup>b</sup>	2.31	*
24	44.77 <sup>a</sup>	17.24 <sup>c</sup>	19.82 <sup>b</sup>	20.47 <sup>b</sup>	3.36	*
36	50.66 <sup>a</sup>	21.26 <sup>c</sup>	23.21 <sup>b,c</sup>	27.13 <sup>b</sup>	3.59	*
48	51.73 <sup>a</sup>	24.52 <sup>c</sup>	25.64 <sup>c</sup>	29.36 <sup>b</sup>	3.34	*
72	53.42 <sup>a</sup>	26.51 <sup>c</sup>	28.60 <sup>c</sup>	31.61 <sup>b</sup>	3.26	*

\*\* : Significant at (P<0.05)

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

SEM : Standard Error Mean

**Table 4:** Effect of different NaOH treatments on *insitu* GNC protein degradability characteristics from fitted values.

Treatment	Control	NaOH Air	NaOH Heat	NaOH Spray	SEM	Significance level
Fitted Values						
a(%)	9.85 <sup>a</sup>	3.00 <sup>b</sup>	2.35 <sup>b</sup>	3.93 <sup>b</sup>	0.94	*
b (%)	44.08 <sup>a</sup>	26.41 <sup>c</sup>	26.55 <sup>c</sup>	29.87 <sup>b</sup>	2.19	*
c/hour	0.07 <sup>a</sup>	0.03 <sup>c</sup>	0.05 <sup>b</sup>	0.04 <sup>b</sup>	0.003	*
Pd (%)	53.93 <sup>a</sup>	29.41 <sup>c</sup>	28.90 <sup>c</sup>	33.80 <sup>b</sup>	3.1	*
Ed <sub>(0.02)</sub>	43.69 <sup>a</sup>	19.41 <sup>d</sup>	20.99 <sup>c</sup>	23.64 <sup>b</sup>	2.95	*
Ed <sub>(0.05)</sub>	34.93 <sup>a</sup>	13.51 <sup>d</sup>	15.26 <sup>c</sup>	16.99 <sup>b</sup>	2.60	*
Ed <sub>(0.08)</sub>	29.77 <sup>a</sup>	10.73 <sup>c</sup>	12.23 <sup>b</sup>	13.70 <sup>b</sup>	2.32	*

\*\* : Significant at (P<0.05)

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

a: washing loss.

b: water insoluble nutrients which are potentially degradable by microorganisms.

c: degradation rate of b.

Pd: Potential degradability.

Ed: Effective degradability at rumen outflow rates (0.02, 0.05, 0.08).

SEM: Standard Error of the Mean.

## DISCUSSION

Treatment of groundnut cake with 0.5N NaOH decreased the rate of ruminal degradation of both dry matter and crude protein at all the incubation periods as well as the effective degradability. This agrees with the finding of Waltz and Loerch, (1986), who treated soybean meal with propionic acid, NaOH and HCl dried with air. Spraying GNC with 0.5N NaOH exhibited the lowest protein protection compared with the other treatments. This result is in line with the findings of Waltz and Loerch (1986), who found that soaking soybean meal with 2.5 or 5% acetic acid or propionic acid reduced the rates of nitrogen disappearance at higher rates than when sprayed with these chemical. This could be attributed to a better distribution of the alkali into the soaked cake rather than the sprayed cake. GNCNaOH decreased dry matter disappearance more than the GNCNaOH and GNCNaOHs respectively. Heat treatment alone was found to be effective in reducing ruminal degradation rate of soybean seeds nitrogen Plegg *et al.* (1982), groundnut cake (DM) Hussein *et al.* (2005), and of soybean meal proteins Sadeghi *et al.* (2006). In the current study, the heat treatment did not add any extra effect to NaOH treatment. This is contradictory to previous reports of many researchers, who worked in protecting soybean meal from rumen degradation; that best results for alkali and acid treatments were achieved by using heat at 100°C Waltz and Loerch (1986), Cleale *et al.* (1987) applied reducing sugars with heat, while, Lynch *et al.* (1987) found that 70% ethanol heated at 78°C improved nitrogen utilization more than heating at 23°C, Plegg *et al.* (1982) found that roasting soybean seeds at 159°C and 185°C increased the percentage of N recovered as acid detergent insoluble nitrogen. The variation in the response to combination of heat and chemical treatment could have resulted from a difference in the protein source and how it had been processed before the treatment, the moisture content of the cake and the evaporation rate, duration of heating and the type of chemical used (Cleale *et al.*, 1987)

Heat treatment causes denaturation of the protein through thermal agitation, and probably transforming it into a more resistant structure to microbial action. NaOH treatment may have produced the protective effect through the hydroxy group altering the protein structure, thus decreasing the number of protease specific bond that can be cleaved. In summary the best NaOH treatment of groundnut cake is achieved by soaking the cake and after dry in air.

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