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## **EFFECT OF DIFFERENT HCl 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 acid (HCl) treatment on ruminal degradation characteristics of dry matter (DM), crude protein (CP), and effective degradability of groundnut cake (GNC). GNC was either soaked in or sprayed with 0.5N HCl. The treated cake was either air or oven dried at (100°C). Nylon bags technique was employed using three castrated calves. HCl treatment significantly ( $P < 0.05$ ) decreased *in situ* dry matter degradation rate. GNC air dried represented the lowest  $P < 0.05$  CP degradation at all incubation periods except at time interval 36hrs. GNC sprayed had the highest CP degradability at all the incubation periods. Combining heat with HCl was the most effective treatment in protecting GNC from rumen degradation, while spraying resulted in the least protection.

**Key words:** *Groundnut cake/by-pass protein/Insitu DM, CP degradation.*

### **INTRODUCTION**

Ground nut cake (GNC) is the most commonly used protein supplement in the Sudan for both dairy and beef cattle. It is quite palatable and available. GNC contains large amounts of rumen degradable proteins NRC (2001). Lactating dairy cows and rapidly growing ruminants cannot meet their protein requirements with sole microbial protein. So by-pass proteins are important for these animals. Therefore protecting high quality protein sources from ruminal fermentation affects animal performance positively.

Various methods for treating proteins have been used to reduce their degradation in the rumen. These methods are most commonly used in Soya bean 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 (Spears *et al*, 1980), alcohol (Vander Aar *et al*, 1982) and sodium hydroxide, propionic acid and hydrochloric acid (Waltz and Loerch, 1986). In the Sudan, limited data exist in the literature, concerning by-pass proteins.

The objective of this study is to evaluate the effectiveness of acid treatment, applied in different forms, in protecting GNC from microbial degradation in the rumen.

## **MATERIALS and METHODS**

Groundnut seeds were the 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 groundnut cake (GNC) was soaked in excess solution of 0.5N HCl for 15 minutes as described by (Waltz and Loerch, 1986). Half of the amount was allowed to dry by air at room temperature, and it was named GNCHCIA. The other half was dried for 6 hours at 100 C° on a forced air oven and was named GNCHCIH.

Another 500gm were sprayed with 50% water solution of 0.5N HCl. Then it was air dried at room temperature, and named GNCHCIS. The control was 500 gms of GNC soaked in distilled water for 15 minutes, then it was allowed to dry at room temperature, and named UGNCA.

### **Animals and feeding:**

Three castrated calves from a local breed (Kenana) aged 2 - 2½ years, were fitted with rumen cannulae as described by Brown *et al*. (1968). They were maintained with a well balanced ration of concentrates and roughage. They were fed twice daily.

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

According to the polyester bag technique of Mehrez and Qrskov (1977), the bags were prepared from nylon material of length 15.5cm, width 8.5cm and weighing 2 - 3gm. The empty bags were individually weighed and their weights recorded. Three gms of treated or untreated cakes were put in a bag tied with a nylon ribbon, attached to a plastic

tube, of 45.5cm length, 0.8cm diameter, and introduced inside the rumen. The bags (2 bags/animal/period/treatment) were incubated for 6, 12, 24, 36, 48 and 72 hours each.

**Calculation of ruminal degradability:-**

Degraded dry matter percentage was calculated according to 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 CP content determination(AOAC,1980).

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 Orskov 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).

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

**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 determined 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

The proportion of the dry matter disappearance from the nylon bags at different incubation periods for UGNC and all the forms of HCl treatments is shown in Table (1). Significant differences ( $P < 0.05$ ) were

found between the treated GNC and UGNC as well as among treated cakes at all incubation periods. All treatments decreased ( $P < 0.05$ ) DM degradation at all incubation periods except GNCHCI S decreased it at 6hrs and zero time only. Among treatments, GNCHCIH had the lowest ( $P < 0.05$ ) DM degradation rate at all the incubation periods than the other treatments.

Table (2) shows the dry matter disappearance from a fitted model for different HCl treatments. There are significant differences between treated GNC and untreated one, as well as among treatments. With the exception of (b) value of the fitted model, all other values of the control group are higher compared to HCl treated groups. GNCHCIH exhibited the lowest fitted values of DM degradation.

Degraded protein percentage of GNC due to different HCl treatments is shown in Table (3). All treatments significantly decreased ( $P < 0.05$ ) CP degradation. It can be seen that GNCHCIA and GNCHCIH showed similar effect on *in situ* GNC crude protein degradability during the first 24 hrs.

Table (4) shows degraded protein from fitted model for UGNC and different HCl treatments. There are significant ( $P < 0.05$ ) differences between treated and untreated GNC. All treatments significantly reduced values a, b, Pd and effective degradability at different rumen outflow rates. Within treatments GNCHCIS has the lowest effect.

**Table 1:** The effect of different HCl treatments on *in situ* dry matter disappearance (%) of GNC.

| Treatment    | Control Air        | HCl Air            | HCl Spray          | HCl Heat           | SEM  | Significance |
|--------------|--------------------|--------------------|--------------------|--------------------|------|--------------|
| Time (hours) |                    |                    |                    |                    |      |              |
| 0            | 36.76 <sup>a</sup> | 20.10 <sup>c</sup> | 26.40 <sup>b</sup> | 13.06 <sup>d</sup> | 2.63 | *            |
| 6            | 83.80 <sup>a</sup> | 72.33 <sup>c</sup> | 76.40 <sup>b</sup> | 43.43 <sup>d</sup> | 4.64 | *            |
| 12           | 85.93 <sup>a</sup> | 73.63 <sup>d</sup> | 84.93 <sup>a</sup> | 55.03 <sup>c</sup> | 3.78 | *            |
| 24           | 92.80 <sup>a</sup> | 84.20 <sup>b</sup> | 90.43 <sup>a</sup> | 72.93 <sup>c</sup> | 2.40 | *            |
| 36           | 93.03 <sup>a</sup> | 87.20 <sup>b</sup> | 93.13 <sup>a</sup> | 74.83 <sup>c</sup> | 2.25 | *            |
| 48           | 93.96 <sup>a</sup> | 92.50 <sup>a</sup> | 94.43 <sup>a</sup> | 83.76 <sup>b</sup> | 1.33 | *            |
| 72           | 94.20 <sup>a</sup> | 93.56 <sup>a</sup> | 94.33 <sup>a</sup> | 92.33 <sup>b</sup> | 0.25 | *            |

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

a, b,: Means within the same row 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 characteristics from fitted model of different HCl treatments.

| Treatment            | Control Air        | HCl Air            | HCl Spray          | HCl Heat           | SEM  | Significance |
|----------------------|--------------------|--------------------|--------------------|--------------------|------|--------------|
| Fitted Values        |                    |                    |                    |                    |      |              |
| a (%)                | 36.92 <sup>a</sup> | 21.09 <sup>c</sup> | 26.63 <sup>b</sup> | 15.66 <sup>b</sup> | 2.38 | *            |
| b (%)                | 55.82 <sup>c</sup> | 67.71 <sup>b</sup> | 66.19 <sup>b</sup> | 72.42 <sup>a</sup> | 1.84 | *            |
| c (/h)               | 0.28 <sup>a</sup>  | 0.18 <sup>b</sup>  | 0.21 <sup>b</sup>  | 0.06 <sup>c</sup>  | 0.02 | *            |
| Pd (%)               | 92.74 <sup>a</sup> | 88.80 <sup>b</sup> | 92.82 <sup>a</sup> | 88.08 <sup>b</sup> | 0.75 | *            |
| Ed <sub>(0.02)</sub> | 88.98 <sup>a</sup> | 82.24 <sup>c</sup> | 87.21 <sup>b</sup> | 71.02 <sup>d</sup> | 2.11 | *            |
| Ed <sub>(0.05)</sub> | 84.21 <sup>a</sup> | 74.46 <sup>c</sup> | 80.38 <sup>b</sup> | 56.55 <sup>d</sup> | 3.20 | *            |
| Ed <sub>(0.08)</sub> | 80.26 <sup>a</sup> | 68.44 <sup>c</sup> | 74.93 <sup>b</sup> | 48.08 <sup>d</sup> | 3.68 | *            |

\*: significant at (P<0.05).

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

SEM: standard error of the mean.

a: washing loss.

b: degradation of water insoluble.

c: rate constant of b function.

Pd: potential degradability.

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

**Table 3:** Effect of different HCl treatments on *in situ* GNC protein degradability (%).

| Treatment    | Control Air        | HCl Air            | HCl Heat             | HCl Spray          | SEM  | Significance |
|--------------|--------------------|--------------------|----------------------|--------------------|------|--------------|
| Time (hours) |                    |                    |                      |                    |      |              |
| 0            | 9.57 <sup>a</sup>  | 1.54 <sup>c</sup>  | 1.78 <sup>c</sup>    | 5.89 <sup>b</sup>  | 1.00 | *            |
| 6            | 25.33 <sup>a</sup> | 2.62 <sup>c</sup>  | 3.90 <sup>b,c</sup>  | 7.20 <sup>b</sup>  | 2.79 | *            |
| 12           | 32.81 <sup>a</sup> | 19.26 <sup>c</sup> | 19.57 <sup>c</sup>   | 23.74 <sup>b</sup> | 1.66 | *            |
| 24           | 44.77 <sup>a</sup> | 24.53 <sup>c</sup> | 25.40 <sup>c</sup>   | 33.00 <sup>b</sup> | 2.45 | *            |
| 36           | 50.66 <sup>a</sup> | 30.47 <sup>c</sup> | 27.19 <sup>d</sup>   | 33.06 <sup>b</sup> | 2.75 | *            |
| 48           | 51.73 <sup>a</sup> | 32.05 <sup>d</sup> | 33.32 <sup>c</sup>   | 34.98 <sup>b</sup> | 2.40 | *            |
| 72           | 53.42 <sup>a</sup> | 32.43 <sup>c</sup> | 33.96 <sup>b,c</sup> | 35.49 <sup>b</sup> | 2.57 | *            |

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

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

SEM: standard error of the means.

**Table 4:** Effect of different HCl treatments on *insitu* GNC protein degradability characteristics.

| Treatment            | Control Air        | HCl Air            | HCl Heat             | HCl Spray          | SEM   | Significance level |
|----------------------|--------------------|--------------------|----------------------|--------------------|-------|--------------------|
| Fitted Values        |                    |                    |                      |                    |       |                    |
| a (%)                | 9.85 <sup>a</sup>  | -0.97 <sup>c</sup> | 0.02 <sup>c</sup>    | 3.03 <sup>b</sup>  | 1.28  | *                  |
| b (%)                | 44.08 <sup>a</sup> | 35.42 <sup>b</sup> | 35.34 <sup>b</sup>   | 33.76 <sup>b</sup> | 1.26  | *                  |
| c (/h)               | 0.06 <sup>a</sup>  | 0.05 <sup>c</sup>  | 0.04 <sup>b</sup>    | 0.06 <sup>a</sup>  | 0.002 | *                  |
| Pd (%)               | 53.92 <sup>a</sup> | 34.80 <sup>c</sup> | 35.36 <sup>b,c</sup> | 36.83 <sup>b</sup> | 2.40  | *                  |
| Ed <sub>(0.02)</sub> | 43.69 <sup>a</sup> | 24.78 <sup>c</sup> | 25.13 <sup>c</sup>   | 28.86 <sup>b</sup> | 2.32  | *                  |
| Ed <sub>(0.05)</sub> | 34.93 <sup>a</sup> | 17.33 <sup>c</sup> | 17.54 <sup>c</sup>   | 22.12 <sup>b</sup> | 2.16  | *                  |
| Ed <sub>(0.08)</sub> | 29.77 <sup>a</sup> | 13.23 <sup>c</sup> | 13.48 <sup>c</sup>   | 18.17 <sup>b</sup> | 2.02  | *                  |

\*: 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: degradation of water insoluble.

c: rate constant of b function.

Pd: Potential degradability.

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

SEM: standard error of the means.

## DISCUSSION

Treatment of groundnut cake with 0.5N HCl decreased the rate of ruminal degradation of both dry matter and crude protein in the different incubation periods as well as effective degradability. Similar results were found by Waltz and Loerch, (1986) upon treating soybean meal with propionic acid, NaOH, or HCl dried with air. Spraying GNC with 0.5N HCl exhibited the lowest protein protection compared with the other treatment procedures. This agrees with the results of Waltz and Loerch (1986), who found that spraying with acetic acid or propionic acid (2.5 or 5%) caused a higher rate of nitrogen disappearance than when soaked in the same chemicals. This may be attributed to a better distribution of the chemicals into the soaked cake rather than the sprayed cake. Results of the current study revealed that combining heat with HCl produced the best result in reducing ground nut cake DM and CP degradation in the rumen. This is on line with the findings of many researchers who worked in protecting soybean meal from rumen degradation; best results for alkali and acid treatments were achieved by heating at 100C° 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 23C°.

Heat treatment alone was found to be effective in reducing ruminal degradation rate of soybean seeds nitrogen Plegg *et al.* (1982), alfalfa hay nitrogen Yang *et al.* (1993), ground nut cake DM Hussein *et al.* (2005), and of soybean meal proteins Sadeghi *et al.* (2006). Heat treatment results in denaturation of the protein, and probably transforming it into a more resistant structure.

HCl treatment probably produces its protective effect through the alteration of the protein structure, according to Lehninger (1976) exposing proteins to extremes in pH even for short periods of time causes most proteins to undergo denaturation. This is caused by disruption of hydrogen bonding, resulting in an alteration of the tertiary protein structure. A major effect of denaturation is decreased protein solubility in aqueous solutions (Vander Aar *et al.*, 1982).

Sadeghi *et al.* (2006) determined the type of protein, of untreated or treated soybean, which escaped rumen degradation due to different chemical and physical treatments. In this study due to limited facilities this was not done, we hope that further studies will follow.

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