Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

Determination of Dry matter and Crude Protein degradability of Roselle Seed (*Hibiscus sabdariffa*) Using Nylon Bags Technique

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ARTICLE INFO

ARTICLE HISTORY
Received: 15th December 2015
Accepted: 14 March 2016
Available online:1st June 2016

KEYWORDS:

Degradability,
Nylon bags,
Roselle seed and
Oil seed coke.

ABSTRACT

This study was carried out to determine the rumen degradability of dry matter (DM) and crude protein (CP) degradation of Roselle Seed (CRS), and three oilseed cakes, Groundnut cake (GNC), Cotton seed cake (CSC) and Sunflower cake (SFC). In situ trial three fistulated bulls were employed; nylon bags were used, and incubated in the rumen of each animal for 3, 6, 9, 12, 24, 36, 48 and 72hrs. Significant differences ($P<0.05$) were obtained among the oilseed cakes for (DM) and (CP) degradability according to feed samples and incubation times. Dry matter and crude protein of GNC were found higher than those of other cakes at different incubation time and CSC had the lowest (DM) and (CP) disappearance percentage than SFC and CRS. While SFC and CRS recorded medium degradation of dry matter in all incubation period intervals. Moreover CSC revealed the highest content of the nutrient detergent fibre NDF, acid detergent fibre ADF and acid detergent lignin ADL. Followed by SFC, GNC and CRS. The result of the present study compared well with other similar degradation studies.

INTRODUCTION

Nylon bags technique is a simple means of obtaining estimates of the potential degradability of supplements and feedstuff for ruminants. Inclusion of values for the fractional clearance of undigested feed residues from the rumen degradability provide estimates for the rate of

degradation of the various components of the material which more closely approximate the true degradability of the material in the rumen. Roselle seed **(***Hibiscus sabdariffa*), malvacea family, known commonly as *Karkadeh*. It is known under different names in different countries. Jamaica sorrel, India sorrel, Guinea sorrel, sour and green land jelly plant. (Mahadeven *et al.*, 2009; Morton, 1987). In Sudan locally known as (Karkadeh), it is an important annual crop grown in tropical climate (Coply, 1975). The plant is originated in tropical Africa (Mcclean, 1973). In Sudan the plant is mainly grown under rain fed condition in the western part. Two red cultivars are grown in Sudan; El Fashir and El Rahad varieties. The large thick calyces; not the seeds; are mostly used in the preparation of cold and hot beverages. *Karkadeh* as an oilseed; attracted attention as potential which provide a good quality protein. The economical part of the plant is the fleshly calyx (sepals) surrounding the fruit (capsules). In Sudan fully developed fleshy (calyx) is peeled off from the fruit by hand and dried naturally under shade to give the dry (calyx), which is the consumable product. The plant is 0.5 to 2 meters in height. The colour of calyx plays an important role in determining the quality of the crop. (El Naim and Ahmed, 2010).The crop is produced in traditional grown conditions by small farmers, depending on rain fed and natural soil fertility without using chemical fertilizers or insecticides. Apportion of the crop produced is used locally; however the large portion is exported. It has many industrials and domestic uses. In Sudan dried calyx is soaked in water to prepare a colourful cold drink, traditionally, the product has been used for medicinal purposes importance in the manufacture of many small industries, e.g cosmetics, sweets, jams, and jellies substitute for tea and also used as medicine, especially with the problems related to the digestive tract. The total cultivated area in Sudan during 1999 - 2000 seasons was estimated as 140.000 ha (El Awad, 2001). The average yield of farmers was estimated at 227kg.\fed (Department of Statistic and Agriculture Economic, 1984**)**.Roselle in Sudan facing many problems resulted in unstable total production. The main yield limiting factor area. The amount distribution of rain fall, labour requirement for harvesting which amount to about half the total cost of production.

The present study was conducted with objective of testing the degradation of characteristic of some oilseed cakes according to nutritive value. Okasha *et al.*, (2008). reported that alkaloids, cardiac glycoside, deoxy sugar Flavonoids steroidal ring and tannin) concentration were moderate, low and high respectively when they study the effect of aqueous seed extract on serum production level of lactating female albino rats.

The objectives of this study were to determine chemical composition, macro element and nutrient detergent fibre and acid of oilseed cakes. As well as to examine dry matter and crude protein disappearance percentage and effective degradability of oilseed cakes.

MATERIALS AND METHODS

The experiment was carried out at College of Veterinary Medicine, Sudan University of Science and Technology, Department of Animal physiology. All oilseeds cakes bought from kuku market, except Roselle seeds were collected from Abu Gaud area western of Kordofan state, prepared by crushing to small particle easy to digest.

Chemical analysis (approximate analysis): The chemical composition and

approximate analysis were carried out at Faculty of Animal Production University of Khartoum, crude protein (CP), crude Fibre (CF), ether extracts (EE), moisture, ash, total carbohydrates and total nitrogen were determined according to (AOAC 2003). Protein was calculated as N%×6.25, moisture content was calculated by drying samples at 105^0c over night (AOAC 2003), and then dry matter was calculated. Crude fibre content was determined by acid/alkali digestion method of Southgate.

Dry matter determination: The AOAC (2003) Methods for determination of moisture in animal feed has been adapted for the working routine in the laboratory procedure (Zaklouta, *et al;* 2011). In ICARDA' s

Calculation**:**

% DM = $(W_\circ - W_1) / W_s) \times 100$ (AOAC 2003).

Determination of ash: Dry matter samples were ignited for an overnight at 550C° in a muffle furnace and Cooled in desiccators to room temperature. (Zaklouta *et al.*, 2011).

Weight ignited crucible + sample (W_a).

Calculation:

% Ash = $(W_a – W_t) / (W_\circ – W_t) \times 100$ (AOAC 2003)

Determination of fat: The dried feed samples were dissolved with petroleum ether and then ether was evaporated in soxhlet apparatus. The residues are crude fat. A Soxtherm apparatus from Gerhardt GmbH is used after termination of program. In the animal nutrition laboratory at ICARDA. Dry beakers at 105C° overnight, cool beakers down to room temperature in dissectors and weight.

Calculation:

% crude fat = $(W_e - W_0) / (W_s \times$ % DM) $\times 100$

Crude protein determination: Determination of the total nitrogen (crude protein) was conducted by using Kjeldahl methods. The samples digestion in sulfuric acid using K_2So_4/ $CuSo_4$ / Tio_4 as catalyst. N is converted in to NH_3 then distilled trapped in boric acid and titrated with H_2SO_4. (Zaklouta, *et al* 2011).

Calculation**:**

%N = $(1.4007 \times (V_a - V_b) \times$ N/W

V_a: volume of acid used for sample titration.

V_b: volume of acid used for blank.

N: normality of acid.

W: sample weight in gram.

1.4007 conversion factor mill equivalent weight of nitrogen and N percentage. % C.P = F = 6.25 for all forage.

F = 5.70 for grains. F = 6.38 for milk

Detergent fibre analysis: Important fractions of feed are fibres that affect digestibility. They consist of cellulose, hemicelluloses and polysaccharides that are bound to protein and phenols, especially to lignin. Detergent fibre is reducing of plant cells after fractionation using detergent solution for the volatilization of protein and starch (Zaklouta *et al.*, 2011).

Determination of neutral detergent fibre: Insoluble fibre in feed was determined as nutrient detergent fibre (NDF) nutrient detergent solution recovers its main component cellulose, hemicelluloses and lignin (Zaklouta, *et al.*, 2011).

Total Minerals: Minerals determination in samples extract was prepared by the drying ash method as described by Pearson *et al.*, (2010). The amount of ferrous was determined according to the analytical method of atomic absorption spectroscopy (Perkin- Elmer 750). Phosphor was determined by the ammonium molybdate/ammonium and magnesium was determined by titration method of Chapman and Pratt (1961).

ISSN (Print): 1605 427x e-ISSN (Online): 1858-6716

Sodium and potassium were determined according to the AOAC (2003), using flame photometer (Corning EEL).

Statistical analysis: The data obtained to one way ANOVA analysis of variance to examine the effect of the disappearance % of DM and CP degradation kinetics. Significant differences among different sources of cakes were assessed using LEAST significant difference test (LSD) according to Gomez and Gomez (1984). SPSS fit curve was used in raw data to determine significant differences (Sendecor and Chochran, 1980) followed by Duncan s multiple range test ($P< 0.05$) at out flow rate (fraction per hour) 0.02, 0.05 and 0.08 respectively.

Degradability trials: The experiment was carried out at College of Veterinary Medicine, Department of Animal Physiology in Sudan University of Science and Technology. Three fistulated bulls from local breed (Kenana) at age of 3- 3.5 years. Were fitted with a rumen cannula described by Brown *et al.,* (1968). All cakes were collected from Kuku local market while Roselle seeds were collected from Abu Gaude area western of Kordofan State 1. Kg from each sample. The nylon bags technique used in this study to determine the effective degradability and disappearance percentage of dry matter and crude protein the bags were incubated in the rumen at the different periods (3, 6, 9, 12, 24, 36, 48, and 72hrs). The bags were removed at the end of each incubation period, washed under tap water dried in oven (70°C) for an overnight and cooled in desiccators and weighted immediately to determine dry matter and crude protein disappearance percentage. Approximate analysis to determine the chemical composition of experimental oilseed cakes was performed according to AOAC (2003). Samples from fresh residuals were taken regularly for dry matter and crude protein calculation. Fit curve program was used to determine the disappearance of crude protein and dry matter, and for evaluation of effective degradability of dry matter and crude protein estimated at out flow rate K= 0.02, 0.05, and 0.08 respectively. Data for all response variable were subjected to analysis of variance (ANOVA) using the SPSS, (2008). And significant difference between treatment means was determined by using least significant difference (LSD) at $P < 0.05$ according to the Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

Chemical composition of oilseed cakes Sunflower cake (SFC), Cottonseed cake (CSC), Groundnut cake (GNC) and Crush Roselle seed are shown in Table (1). Dry matter and crude protein percentage of CRS, CSC, GNC and SFC were (97.91-26.38), (97.91- 25.16), (96.33 - 42.33) and (97.34 - 26.91) respectively. The results of this study are in range of the finding obtained by Afaf (2002).

Table 1: Dry matter disappearance % of oil seed cakes

Time	0	3	6	9	12	24	36	48	72
CRS	33.00±1.22^{b}	34.00±1.00^{b}	35.11±1.05^{a}	44.33±2.12^{c}	47.44±0.72^{c}	73.11±1.46^{c}	74.22±2.96^{c}	75.87±2.03^{c}	76.07±0.81^{c}
CSC	32.44±1.51^{b}	33.11±1.17^{b}	34.00±0.71^{ba}	38.33±1.22^{d}	41.56±1.23^{d}	71.00±0.70^{d}	73.55±1.34^{d}	74.43±.672^{d}	74.55±1.04^{d}
SFC	34.22±1.39^{ab}	34.78±1.30^{a}	35.61±1.11^{a}	47.22±1.20^{b}	51.11±1.76^{b}	75.41±2.04^{b}	77.34±2.20^{b}	78.22±1.30^{b}	78.54±1.47^{b}
GNC	34.89±1.27^{a}	35.56±1.67^{a}	36.22±1.92^{a}	51.11±2.47^{a}	55.00±2.00^{a}	84.11±1.70^{a}	86.33±0.50^{a}	87.78±1.39^{a}	88.22±0.83^{a}
SIG	**	**	**	**	**	**	**	**	**

CRS: Crushed Roselle seed, **GNC:** groundnut cake, **SFC:** sunflower cake, **CSC:** cottonseed cake.

****:** Highly significant difference at ($P< 0.05$).

ISSN (Print): 1605 427x e-ISSN (Online): 1858-6716

Significant differences (P<0.05) were obtained among the oilseed cakes for dry matter and crude protein degradability in term of feed samples and incubation time Table (2). In the present study (GNC) had significant (P<0.01) high dry matter disappearance percentage after 72hrs there were (88.22), (78.54), (76.07) and (74.55) for GNC, SFC, CRS and CSC respectively. This result is in agreement with the results obtained by Turki, (2002); Adnan *et al.*, (2010); and Turki and Actham (2011) who investigated degradability of some oilseed cakes using nylon bags technique. They reported that groundnut cake had the highest dry matter disappearance percentage and singed (88.2, 88.77, and 88.6) respectively at 48hrs. These results did not include CRS, while in the present study *(Hibiscuss abdariffa*) recorded medium dry matter disappearance and signed (76.07) at 72hrs.

Table 2: Dry matter Kinetics Characteristics of experimental oil seed cakes

Fitted value	GNC±SD	SFC±SD	CRS±SD	CSC±SD	Sig
A	27.20±0.96	27.95±0.88	27.31±1.36	25.91±0.63	**
B	65.37±2.09	55.46±1.94	53.68±1.03	55.43±1.07	**
C	0.53±0.00	0.51±0.00	0.05±0.01	0.43±0.00	**
a+b	93.1±2.93	83.92±1.51	81.04±1.26	81.38±1.33	**
Pd	93.18^{A}2.93	84.03^{AB}±3.94	81.11^{C}±1.65	80.7^{C}±0.91	**
Ed (0.02)	$74.50±2.70^{A}$	$67.59±3.94^{AB}$	$63.31±1.65^{B}$	$63.88±0.91^{C}$	**
Ed (0.05)	$60.68±1.74^{A}$	$55.77±0.82^{AB}$	$54.04±1.77^{B}$	$51.71±0.92^{B}$	**
Ed (0.08)	$53.11±1.35^{A}$	$49.63±0.60^{AB}$	$47.98±1.71^{B}$	$46.45±3.16^{C}$	**

****:** Highly significant difference at (P < 0.001).

A-B: Means within the same raw followed by different superscripts are significantly p(0.05) different.

a: Washing loss, **Pd:** Potential degradability. **b:** Water insoluble nutrients which is potentially degradable by microorganisms. **c:** Degradation rate of b/hours.

CRS: Crushed Roselle seed. **GNC:** groundnut cake. **SFC:** sunflower cake. **CSC:** cottonseed cake.

Many factors may affect degradability of DM and CP in the rumen such as variation in the degradation values which may refer to the Season, Samples washing procedures, animal digestive system performance…etc. (Nocek and Russell 1988). In the present study the result indicated that both of SFC and CRS had medium values for dry matter and crude protein disappearance percentage, CRS its rich in (K), and (P) minerals than SFC Table (4). Potential degradability (Pd) of CRS at out flow rate K=0.02 was recorded (63.31, 67.59, 63.88 and 74.50) for CRS, SFC, CSC and GNC respectively. While Crude protein disappearance percentage of experimental samples found in Table (3).

Table 3: Crude protein disappearance %

Samples	0	3	6	9	12	24	36	48	72
SFC	11.90±1.25	11.41±2.05	15.35±1.85	18.95±1.22	22.49±1.08	34.40±1.03	44.10±1.08	51.80±1.30	62.86±1.94
CSC	9.01±1.06	9.60±1.32	11.60±1.17	13.69±1.00	15.56±1.33	22.54±1.75	28.33±1.17	33.39±1.01	41.17±1.59
CRS	12.03±2.01	12.55±1.51	16.55±1.60	20.53±1.90	24.26±1.82	36.44±1.77	45.62±1.99	52.47±1.321.	61.88±1.74
GNC	19.07±1.13	19.78±1.09	24.43±1.03	32.70±1.14	43.22±1.11	53.32±2.03	62.38±1.55	70.74±1.54	77.39±1.53
Sign	**	**	**	**	**	**	**	**	**

CRS: Crushed Roselle seed. **GNC:** Groundnut cake. **SFC:** Sunflower cake. **CSC:** Cottonseed cake.

****:** Highly significant difference at (P < 0.001).

Cottonseed cake signed the lowest crude protein disappearance percentage (41.17) followed by CRS and SFC singed medium crude protein disappearance percentage (61.88), (62.86) respectively at incubation period 72hrs. GNC recorded the highest

ISSN (Print): 1605 427x e-ISSN (Online): 1858-6716

crude protein disappearance percentage (77.74), than other oilseed cakes in crude protein disappearance percentage at incubation period 72hrs, these results are similar to the results obtained by Turki, (2002), and Turki and Actham, (2011) who recorded GNC had a highest degradable crude protein disappearance percentage in the rumen. Moreover determine the amino acids concentration and molecular weight of some oilseed cakes available in Sudanese markets.

Table 5: Chemical Composition of Experimental Four Plant Protein Source

INGREDIENT	SFC	CSC	CRS	GNC	±SE
D.M	97.34^{a}	97.91^{ab}	96.49^{c}	96.33^{c}	0.14
C.P	26.91^{b}	25.16^{d}	27.38^{b}	42.33^{a}	0.04
C.F	17.25^{c}	22.62^{a}	18.09^{b}	9.21^{d}	0.09
E.E	10.94^{a}	8.10^{b}	5.73^{d}	7.30^{c}	0.17
N.F.E	36.99^{b}	35.20^{c}	39.18^{a}	32.07^{d}	0.13
ASH	5.25^{d}	5.82^{b}	6.11^{a}	5.14^{c}	0.05
MOISTURE	2.66^{d}	3.09^{c}	3.51^{bc}	3.67^{ab}	0.35

DM: Dry matter, **CP:** Crude protein, **CF:** Crude fiber, **EE:** Ether Extract, **NFE:** Nitrogen free extract.
SE: Standard Error of means.

Table 6: Macro Minerals of Experimental oilseed Cakes

MINERALS	SFC	CSC	CRS	GNC	±SE
Ca	0.370^{a}	0.250^{b}	0.320^{b}	0.210^{c}	0.09
Na	0.050^{b}	0.085^{a}	0.033^{c}	0.028^{d}	0.09
Mg	0.360^{a}	0260^{a}	0.305^{b}	0.180c	0.06
P	0.491^{c}	0.527^{b}	0.532^{a}	0.036^{d}	0.01
K	0.886^{d}	1.240^{b}	1.330^{a}	0.984^{c}	0.07
Mc	1.770^{d}	2.545^{b}	2.720^{a}	0.233^{d}	0.01

Ca: Calcium **P:** Phosphorus **Na:** Sodium **K:** Potassium **Mg:** Magnesium **Mc:** Moisture content
****:** Highly significant difference at (P <0.05).

Table 7: Detergent fibre analysis of Experimental oilseed Cakes:

INGREDIENT	SFC	CSC	CRS	GNC	±SE
NDF	51.19^{c}	60.17^{a}	37.50^{d}	52.20^{b}	0.17
ADF	25.40^{b}	30.07^{a}	19.90^{c}	25.12^{b}	0.49
ADL	16.36^{b}	16.32^{a}	15.32^{c}	17.12^{a}	0.15
CELLULOSE	25.85^{b}	30.35^{a}	17.62^{c}	26.05^{b}	0.21
HMICELLULOSE	9.06^{b}	13.75^{a}	4.42^{c}	9.46^{b}	0.30
LIGNIN	16.35^{b}	16.32^{b}	15.32^{c}	17.12^{a}	0.14

NDF: Nutrient detergent fiber. **ADF:** Acid detergent fiber. **ADL:** Acid detergent lignin.
****:** Highly significant difference at (P <0.05).

Table 8: Crude protein Kinetics Characteristics of experimental oilseed cakes

Fitted value	GNC	SFC	CRS	CSC	Sign
A	12.11±0.90	7.23±0.89	8.01±0.67	7.40±0.63	**
B	70.20±2.08	70.00±1.92	66.04±1.66	54.10±1.07	**
C	0.41±0.01	0.19±0.01	0.02±0.02	0.14±0.00	**
a+b	82.72±2.93	77.42±1.53	74.07±1.45	61.64±1.33	**
Pd	82.75^{A}±1.05	77.65^{B}±1.43	74.44^{B}±1.42	61.70^{C}±0.93	**
Ed (0.02)	57.71^{A}±2.07	43.60^{B}±2.01	34.61^{B}±1.90	29.30^{C}±0.92	**
Ed (0.05)	42.60^{A}±1.88	27.70^{B}±1.78	29.10^{B}±1.70	18.90^{C}±1.44	**
Ed (0.08)	34.90^{A}±1.35	21.50^{B}±1.15	23.01^{B}±1.09	15.22^{C}±1.01	**

****:** Highly significant difference at (P < 0.001).
A-B: Means within the same raw followed by different superscripts are significantly P (<0.05) different.
a: Washing loss. **Pd:** Potential degradability. **b:** Water insoluble nutrients which is potentially degradable by microorganisms **c:** Degradation rate of b/hours. **Ed:** Effective degradability.

ACKNOWLEDGEMENTS

The Authors would like to acknowledge the Sudan University of Science and Technology (SUST), Department of Animal Physiology. Africa City of Technology (ACT) Department of Biotechnology and National Institute for Medicinal and Aromatic Plant (NIMAP) for help and co-operation.

RECOMMENDATIONS

This study has shown that (*Hibiscus sabdariffa L*.,) Seed growing in western Sudan have a good quality protein source just as good as that (NDF) and (ADF), also high energy was found in Roselle seed which give the protein some quality characteristics and increase the nutritive value of the protein. Moreover Roselle seed have the potential of becoming an economical protein source of animal feeding. However further studies are required to investigate the genetics improvement and raise their productivity.

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