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EFFECT OF SOME ANTI-NUTRITIONAL FACTORS ON FIBER CONSTITUENTS DIGESTION KINETICS BY SHEEP FED HALOPHYTIC SHRUBS

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SUMMARY

This study was conducted to demonstrate some problems in fiber determination of a tannin – rich forages and the fate of anti-nutritive factors (ANF's) total tannins, condensed tannins (CT), alkaloids, flavonoids and oxalates and their implications for in vivo studies. Forages from 6 shrub species were used to demonstrate complexation of tannin with fiber and protein through feeding of these shrubs to 24 Barki sheep. Feed and fecal samples were analysed by using a separate sample for neutral detergent fiber (NDF) and acid detergent fiber (ADF) determinations. The acid detergent lignin (ADL) was determined on the ADF residue. All the studied animals showed higher NDF, ADF and ADL concentration in feces than feed. There was a comparative differences in hemicellulose (HC) (NDF – ADF) and cellulose (ADF – ADL) levels among all groups. The digestibility of all fiber fractions (except HC) and protein bound to these fractions was different significantly, among the studied groups. Fecal CT concentration was higher than feed, whereas most of feed alkaloids was excreted in feces. The studied animals showed a negative digestibility for oxalates. It could be recommended that tannins play a major role in impairment of the absorption of nutrients, thus precipitating chronic health changes.

***Keywords**: Anti-nutritional factors, Digestion Kinetics, sheep, Halophytic shrubs*

INTRODUCTION

Halophytes are considered as an important source of nutrients for most classes of wild and domestic ruminants. Palatable halophytic species are generally rare. There are numerous halophytes, some of them are less palatable, which could be used as fodder after appropriate treatments (Gihad *et al.*, 1994). Halophytic plants contain variable amounts of secondary metabolites that affect their palatability and intake. Considerable attention should be given to anti-nutritional factors (Hathcock, 1986), particularly in halophytic plant species to overcome the problems of low palatability and intake. Plants contain a variety of

chemicals that have negative effects on either the ruminant animal, its gastrointestinal microflora, or both (Weimer, 1998). Tannins are able to precipitate proteins and alkaloids. Tannins most can depress fiber – degrading bacteria (McSweeney *et al.*, 1999). Ruminant will gradually adapt to high tannin diets rich in tannins by developing a microflora which is tolerant to high tannins (Kamra, 2005). The same author reported that, tannins do have harmful effects on rumen microbes, but with prolonged feeding some of the tannin-degrading bacteria proliferate and make animals more tolerant to higher levels of tannins in their diet. Tannins interfere in the determination of fiber components, probably by forming complexes with macromolecules such as protein and cellulose which are insoluble in the detergent solutions (Makkar & Singh, 1991).

Kandil & El-Shaer (1990) reported that Tamarix mannifera, Halocnemum strobilaceum, Zygophyllum album and other native range plants containe highe levels of neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and silica which could depress the voluntary intake and nutritive value and they appeared to be unpalatable halophytic species. Forage intake is related to fiber digestibility because intake is reduced when indigestible fiber is increased in the digestive tract (Mertens, 1993). However, only a few studies have reported the fiber digestion characteristics of the native shrubs, even though such information could be used to predict the nutritive value more accurately.

This study was conducted to evaluate some nutritional problems related to the presence of some anti-nutritional factors and fiber constituents in the processed halophytes in sort of silage, haylage and hay fed to sheep. Presence of condensed tannins (CT) and protein as contaminants in fiber fractions of feed and feces of sheep fed these diets has also been demonstrated.

MATERIALS AND METHODS

This study was carried out at Ras Sudr, South Sinai Research Station, Desert Research Center, Egypt.

Forage species selection, collection and preparation:

The forage used in this trial was a blend of cultivated and natural halophytes based on our observations on diet selection. The mixture consisted of: two cultivated plant species: Atriplex nummularia 30%, Acacia saligna 5%, and four naturally growing shrubs : Arthrocnemum glaucum 7.5%, Halocnemum strobilaceum 7.5%, Tamarix mannifera 30%, Zygophyllum album 10% and 10% sugar cane molasses as a source of energy on dry matter (DM) basis as ratio/ton. The mixture was preserved in three forms: ensiled (silage) and made into haylage and sun dried as hay.

The silage was prepared by mixing the green chopped plants with sugar cane molasses and the mixture was placed (ensiled) in a stone built trench (silo) for two months. The ensiled materials were packed in layers, stacked by trampling and finally covered with plastic sheets and a 30 cm layer of sand to ensure anaerobic conditions. The haylage was made by the same procedures as silage except that the plants mixture was wilted in air before ensiled. The objective of ensiling and making haylage was to: 1) reduce the ANF's content of these diets as mention by several workers thus rendering it less harmful to the animals, 2) increase the palatability of these forages then increase their consumption and 3) improve the digestibility of some nutrients, consequently elevate their nutritive value.

Four basal rations were offered to the experimental animals, one in each group. These rations were: berseem hay (*Trifolium alexandrinum*) fed to control group, halophytic mixture silage was offered to silage group, halophytic mixture haylage was offered to haylage group and the last animal group was fed on air-dried halophytic mixture as hay.

The concentrate feed mixture (CFM) was offered to all animal groups as a 50 : 50 CFM : basal rations. The concentrate feed mixture consisting of: 35% undecorticated cotton seed cake, 33% bran, 22% yellow corn grain, 4% rice bran, 3% molasses, 2% limestone and 1.0% salt. It's chemical composition was 89% dry matter (DM), 91.4 organic matter (OM), 15.8 crude protein (CP) and 22.5 crude fiber (CF).

Animal treatments:

A total of 24 healthy male mature Barki sheep (averaged 35.0 Kg live body weight) and 17 months old were divided randomly into four groups(6 animal / group). Each group was offered one of the experimental diets, fed twice daily for 1 month adaptation and were watered *ad libitum*. The forages in each treatment above was fed in equal proportions with the concentrate feed mixture in each treatment group. The experimental periods consisted of 75 days for palatability trial where animals fed their experimental diets *ad libitum* followed by 15 days for the digestibility part of the trial, where animals were placed individually in metabolism cages, each animal was offered 90% of what it consumed during the palatability trial to ensure that the whole amount of diet will be eaten (no feed residues). The feed consumption was recorded for each animal. The first ten days were devoted for an adjustment period and the following five days (a collection period) measurements of 24 hours fecal samples were collected for later anti-nutritional factors (ANF's) and proximate analysis.

Rations and fecal samples were oven dried at 65°c for 48 hour and ground then kept for analysis of DM and organic matter (OM), proximate analysis (A O A C, 1990) and for ANF's such as total tannins (Pharmacopia Europian, 1978), condensed tannins (CT) bound to NDF, ADF and lignin were measured essentially using the Porter *et al.* (1986) procedure and also in forages. In brief, 3 ml of butanol-HCL reagent and 100µl of

the iron reagent were added to 10 mg of the fiber fraction. The tubes were heated at 95°C for 60 min in a heating block and absorbance was measured at 550 nm. Alkaloids (Woo *et al.*, 1977), flavonoids (Karawya & Aboutable, 1982) and oxalates according the method described by Hodgkinson (1971). Also, fiber fractions as neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined as described by Goering & Van Soest (1970) that uses separate samples for NDF and ADF determinations. The difference between cell wall CW (NDF) and ADF was designated as hemicellulose, and between ADF and ADL as cellulose. Nitrogen in rations and feces was analysed by the Kjeldahl method (AOAC, 1990).

Statistical Analysis:

All measurements were carried out in triplicate. Statistical analysis of data were performed using SAS/STAT software (SAS, 1988). Comparisons between variables were determined using analysis of variance (ANOVA), Duncan's multiple range test. Statistical significance was defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition:

The chemical composition of the tested diets (on DM basis) was revealed that , shrub silage, shrub haylage, and shrub hay contained 54.2, 63.7 and 70% for DM respectively, 69.7, 79.8 and 79.1 for OM , 7.5, 7.4 and 6.7 for CP, 28.4, 21.8 and 17.6 for CF respectively. On the other hand berseem hay (third cut) contained 92.0% DM, 85.5 OM, 11.1 CP and 32.2 CF (El-Shaer *et al.*,2005).

Cell wall (cw) constituents in diets and fecal samples:

The results in Table (1) indicated that NDF and ADF contents of berseem hay and their output were the highest ones (67.3% and 53.60%) respectively and (85.4% and 77.84% respectively) compared with other groups. It appears that there are some cell constituents in the aerial parts of the studied shrubs which become precipitated in NDF and don't solubilize upon subsequent acid detergent treatment. However, these cell constituents do become solubilized when samples are subjected directly to acid detergent solution. Similar results have been reported for *Acacia saligna* leaves (Makkar *et al.*, 1995). The same pattern was reported for lignin, cellulose and hemicellulose where the dietary lignin of the diet was 20.05% and it's output was 47.88%, the dietary cellulose and hemicellulose were 33.55% and 13.66% respectively and their output were 29.96% and 7.56% respectively. Among treated groups cell wall or NDF value was uniform where all fecal percent were higher than that of the diet where silage fed animals excrete the highest value (76.09%). Concerning ADF and lignin, hay was the richest forage with both while haylage fed animals excrete the highest value of ADF (67.71%) and hay fed animals excrete the highest percentage of lignin (50.36%).

Table (1): Percentages of fiber constituents and CP in offered rations (R) and excreted in animal feces (F),on DM% basis.

Components	Ration containing			
	Berseem hay	Silage	Haylage	Hay
RDM	92.0	54.2	63.7	70.0
ROM	85.5	69.7	79.8	79.1
RCP	11.1	7.5	7.4	6.7
FCP	12.3	8.4	8.7	8.1
RCF	32.2	28.4	21.8	17.6
FCF	37.7	37.7	31.6	33.3
RNDF	67.3	44.92	48.32	54.05
FNDF	85.4	76.09	72.61	72.08
RADF	53.60	30.89	44.82	49.48
FADF	77.84	66.14	67.71	66.29
RADL	20.05	18.83	21.53	34.39
FADL	47.88	42.10	41.99	50.36
RHC	13.66	14.03	3.50	4.57
FHC	7.56	9.95	5.17	5.78
R Cellulose	33.55	12.06	23.29	15.09
F Cellulose	29.96	24.04	25.71	15.93

* R: ration
* F: fecal

Cellulose is the most widely distributed and abundant polysaccharide in nature (Van Soest, 1994). In the present study cellulose content (%) was variable among treated groups, Silage was the lowest forage in cellulose content (12.06%) where haylage fed animals excrete the highest value (25.71%). These findings could be attributed to the forage content of alkaloids because some alkaloids that are less toxic to ruminant themselves have been shown to inhibit certain ruminal microbial processes (namely cellulose digestion), selective pressure for metabolism of these alkaloids may facilitate development of the ability to degrade them (Wiemer, 1998). Hemicellulose pattern was not uniform among treated diets. Silage forage contain the highest percentage (14.03%) and also it's output (9.95%). In this study, all animals fed the experimental forages excrete more fiber than that in their diets. The same findings were reported by Degen (1995) and (Makkar *et al.*, 1995).

Makkar *et al.* (1995) suggesting a different nature of fibers in feed and in fecal samples. Also the same author explained that the fiber measured in the feed fraction is not the same as that measured in the feces because the increased amounts of CT and protein in the feces show that tannin – protein complexes formed in the digestive tract appeared in the feces of sheep and led to over estimation of fiber and lignin i.e. fecal fiber fractions had a substantial amount of both CT and protein. The extra CT present in the fiber fractions of the feces appear to originate from the extractable CT of the feed. Negative fiber digestibility for fodders high in tannins has been reported previously by several workers Robbins *et al.*, (1987) and Woodward & Reed (1989). Ramirez *et al.* (2000) noted that tannin reduce the utilization of many nutritionally important ruminant feed because CT negatively affected the digestibility and effective degradability of cell wall. In addition to that, the last author regarding that lignification of plant cell walls has long been correlated with decreased digestibility but the responsible mechanism has not been established. Nitrogen metabolism varied with animal species as well as diet (Woodward & Reed, 1997). Sheep of all treated groups fed on comparable values of CP while those of control group fed on the highest value (11.1%). All studied animals excrete higher values of N than the dietary ones as previously recorded by El-Shaer *et al.* (2005). These results are consistent with those obtained by Woodward & Reed (1997). Also Bravo *et al.* (1993) regarding that CT can reduce protein digestibility either by precipitating dietary protein or by inhibiting digestive enzymes which results in increased fecal N excretion. The rise in protein excretion may also be accounted by a stimulation of the excretion of endogenous N (Shahkhalili *et al.*, 1990). This N may come from an increase in mucosal cell turnover and / or digestive secretions (Bravo *et al.*, 1993). On the other hand, tannins induce the production of unique, proline – rich (up to 45%) salivary proteins in the parotid glands (Mehansho *et al.*, 1983). This

proline – rich proteins (PRPs) have a very high affinity for tannins and form strong complexes with them. These PRP-tannin complexes are excreted in feces and may be partially responsible for the increase in fecal N excretion observed in the experimental group (Bravo *et al.*, 1993).

Anti-nutritional factors:

Table (2) shows the concentration of some ANF's (condensed tannins, total tannins, alkaloids, flavonoids and total oxalates) in the tested diets and feces. As can be observed, these total tannins seem to be means that, tannins undergo degradation and hydrolysis by ruminal bacteria causing some of it excreted in feces and some were absorbed. Consistent with our hypothesis, Hagerman *et al.* (1992) reported that non of the ingested tannic acid (HT) is excreted in the feces of sheep because the tannin is hydrolyzed soon after ingestion and the gallic acid that is produced is absorbed and excreted in the urine. The metabolic fate of the CT is more complex. Sheep excrete only about 60% of the ingested CT in feces suggesting that, some of the tannin may be absorbed. Results of CT showed that the fecal excretion of CT was higher than it's intake and this could be attributed to the following fact: Degen *et al.* (1995) showed that tannin-protein complexes formed in the digestive tract appeared in the feces of sheep and to a lesser extent goats and the extra CT present in the fiber fractions of the feces appear to originate from extractable CT of the feed. Moreover, a high level of the extractable CT present in the feed disappeared in the gastrointestinal tract. Robbins *et al.* (1991) demonstrated that 75% of ingested CT were in the feces. The differences in these results could be due to lower binding of CT to proteins (Degen *et al.*, 1995). Makkar *et al.* (1995) proposed that the fiber fractions of fecal samples had a substantial amount of both protein and CT. The present results showed that less CT was present in haylage and hay than in silage and this could be explained by Wina *et al.* (1999) who found that the presence of fiber reduces the toxicity of tannins due to the formation of fiber – tannin complexes, which increase under aerobic conditions and also CP is complexing under aerobic conditions. El-Shaer *et al.* (2005) explained this finding as that, the content of tannins decreased in the haylage due to the anaerobic fermentation during the ensiling process and also due to the effect of air drying during the wilting of the shrub hay.

Forage alkaloids represent a different nutritional situation. A given plant species may contain several classes of alkaloids, with each class represented by a variety of structurally distinct compounds. Each of these compounds are usually present in the plant at fairly low concentration. Total dietary intake of each alkaloid compound may be only a few milligrams per day (Weimer, 1998).

Fecal analysis of the control animals is free from alkaloids because of absence of alkaloids from their diet (berseem hay) while the treated groups excrete around 50% of their intake via feces. These alkaloids in feces may be that complexed with tannins as mentioned by Makkar *et al.* (1996).

Table (2): Concentration of some anti-nutritional factors: total tannins (TT); condensed tannins (CT); Alkaloids; Flavonoids and Oxalates in feed and feces.

Components	Ration containing			
	Berseem hay	Silage	Haylage	Hay
TT (mg/100gDM):				
Ration	5.8	8.8	8.0	10.4
fecal	3.53	2.77	1.63	6.13
CT (mg/100gDM):				
Ration	0.82	11	3.0	0.81
fecal	6.0	4.1	7.2	2.533
Alkaloids (mg/100gDM):				
Ration	00.00	153	197	161
fecal	00.00	110	130	125
Flavonoids (µg/100gDM):				
Ration	0.651	0.68	1.09	1.07
fecal	0.33	0.19	0.13	0.23
Oxalates: (g/100gDM)				
Ration	5.7	4.2	4.2	3.4
Fecal	21.4	28.6	35.2	28.5

The dietary TT, alkaloids, flavonoids and oxalates were derived from the previous study of *El-Shaer et al. (2005).*

In general, animal performance was improved with low – alkaloids cultivars. Reduced animal performance and increased diarrhea incidence become progressively greater with alkaloid levels higher than 0.2% (Cheeke, 1995).

Fecal flavonoids appeared as traces when compared with that of intake. The general mechanism of detoxification of absorbed allelochemicals was in the liver or in the gut, conjugation of plant secondary compounds to sulphate, glucoronic acid or hippuric acid take place. This serves to increase the water solubility of the compound and aids it's excretion via the urine or bile hence decreasing its concentration in the feces. The factor that determines which excretion route is used has been suggested to be the molecular weight of the conjugate (Jessop & Illius, 1997).

Oxalic acid can be metabolized by oxalobacter formigenes, a gastrointestinal bacterium that gains energy by dismutation of oxalic acid to formic acid and Co_2. Because oxalic acid is a highly oxidized compound, it's metabolism generates relatively little energy, and thus considerable amounts of this compound must be metabolized to satisfy the growth requirements of the organism (Weimer, 1998). Data of fecal and dietary oxalate reflex that animals of all studied groups had accumulated dietary oxalates in their feces from the continuous feeding. Coles (1986) and Da Costa *et al.* (1994) were in accordance with the present results, they reported that pre – formed Ca – oxalate was excreted unchanged in the feces whatever the mechanism of action, the available evidence indicates that continued ingestion of excess oxalate inevitably leads to a negative Ca availability. Hodgkinson (1977) reported that Ca in the digestive tract reacting with dietary oxalate, is one of the most important factors reducing oxalate absorption from the gut. The oxalate in the gut is partly destroyed by intestinal bacteria and the remainder is excreted in the feces. Once absorbed, oxalate is poorly metabolized but has a marked effect on mammalian tissue, leading to acute or chronic poisoning.

Implications of fiber values in vivo studies:

Table (3) represents digestibility of fiber fractions and protein and CT bound to fiber fractions from values of fiber obtained using standard method. Digestibilities of fiber fractions were positive in all studied groups with specific variations among them. Silage fed animals had the highest digestibility of NDF, ADF and ADL compared with other experimental groups where it shows 45.7%, 32.8% and 42.8% respectively, while it shows the least cellulose digestibility (2.1%). Also, control animals had the highest hemicellulose digestibility (79.3%) followed by those fed silage (74.4%). Schofield *et al.* (2001) can explain the present findings according to his finding that resulted in inhibition of microbes by tannins leads to reduced fiber digestion in ruminants. Also, Mbatha *et al.* (2002) reported that tannins can bind with intestinal bacterial protein and enzymes, inhibiting bacterial growth and digestion of various dietary components.

Table (3): Digestibilities of cell-wall constituents and protein bound to fiber fractions in sheep fed on experimental shrubs mixture

Item	Ration containing				± SE	P<
	Berseem hay	Silage	Haylage	Hay		
NDF intake(g/d)	956 [a]	497 [c]	700 [b]	738 [b]	50	0.001
NDF output (g/d)	566 [a]	271 [b]	549 [a]	461 [a]	38	0.001
NDF digestibility (%)	40.6 [a]	45.7 [a]	21.6 [b]	37.5 [a]	3.4	0.05
ADF intake (g/d)	727 [a]	345 [c]	579 [b]	611 [b]	42.1	0.001
ADF output (g/d)	514 [a]	232 [b]	509 [a]	426 [a]	37	0.001
ADF digestibility (%)	29.2 [a]	32.8 [a]	12.2 [b]	30.2 [a]	3.2	0.05
ADL intake (g/d)	387 [b]	260 [d]	355 [c]	455 [a]	21	0.001
ADL output (g/d)	321 [a]	149 [b]	317 [a]	324 [a]	26	0.01
ADL digestibility (%)	17.3 [ab]	42.8 [a]	11.0 [b]	28.9 [ab]	4.9	0.05
HC intake (g/d)	229 [a]	152 [b]	120 [c]	126 [c]	13	0.001
HC output (g/d)	47 [a]	40 [a]	42 [a]	35 [a]	11	N.S
HC digestibility (%)	79.3 [a]	74.4 [a]	65.9 [a]	71.9 [a]	8.5	N.S
C intake (g/d)	340 [a]	84 [d]	224 [b]	156 [c]	28	0.001
C output (g/d)	194 [a]	82 [b]	192 [a]	102 [b]	17	0.05
C digestibility (%)	43.0 [a]	2.1 [b]	14.3 [b]	29.3 [ab]	5.8	0.05
NDF-protein intake (g/d)	154.9 [a]	94.9 [b]	104.6 [b]	105.7 [b]	7.2	0.001
NDF-protein output (g/d)	65.0 [a]	22.5 [b]	51.4 [a]	36.3 [b]	5.2	0.001
NDF-protein digestibility (%)	58.2 [cb]	76.4 [a]	50.8 [c]	65.7 [b]	3.1	0.001
ADF-protein intake (g/d)	138 [a]	87 [b]	81 [b]	73 [c]	8	0.001
ADF-protein output (g/d)	72 [a]	22 [b]	56 [a]	34 [b]	6	0.01
ADF-protein digestibility (%)	47.9 [b]	74.3 [a]	31.6 [b]	53.6 [ab]	5.5	0.05
ADL-protein intake (g/d)	129.2 [a]	39.3 [d]	87.8 [b]	48.4 [c]	10.8	0.001
ADL-protein output (g/d)	48.0 [ab]	13.3 [c]	63.1 [a]	38.9 [b]	6.1	0.01
ADL-protein digestibility (%)	62.8 [a]	66.1 [a]	28.3 [b]	19.2 [b]	7.3	0.01
NDF – CT intake (g/d)	0.09 [a]	0.01 [c]	0.02 [c]	0.03 [b]	0.01	0.001
NDF – CT output (g/d)	0.03 [a]	0.02 [a]	0.02 [a]	0.07 [a]	0.02	N.S
NDF– CT digestibility (%)	67.4 [a]	-58.5 [a]	12.6 [a]	- 96.2 [a]	42.8	N.S
ADF – CT intake (g/d)	0.12 [a]	0.01 [c]	0.02 [b]	0.02 [b]	0.01	0.001
ADF – CT output (g/d)	0.02 [a]	0.01 [a]	0.04 [a]	0.02 [a]	0.01	N.S
ADF – CT digestibility (%)	87.7 [a]	59.6 [a]	-158.8 [a]	-31.1 [a]	43.8	N.S
ADL – CT intake (g/d)	0.13 [a]	0.05 [c]	0.03 [d]	0.07 [b]	0.01	0.001
ADL – CT output (g/d)	0.05 [a]	0.02 [b]	0.05 [a]	0.04 [a]	0.01	0.05
ADL–CT digestibility (%)	64.3 [a]	72.0 [a]	- 66.5 [b]	42.0 [a]	17.8	0.001

The digestibilities of protein bound to fiber fractions (NDF, ADF and ADL) were highest for silage fed animals (76.4, 74.3 and 66.1%) in comparison with other groups. In Table (3) the present findings could be attributed to reduced tannin content of silage forage (8.8 mg/100 gDM). These findings are consistent with Makkar (2003) who reported that legume tannins could enhance quality of the silage by preventing excessive degradation of feed proteins. Additionally, the same author regarding that, in light of these observations, the supposed role of tannins as anti-nutritional factors may need to be revised and it is no longer appropriate to refer to them as anti-nutritional.

Moreover, Reichert *et al.* (1980) said that anaerobic storage of moist high tannin sorghum deactivates tannins and El-Shaer *et al.* (1991) concluded that addition of molasses and ensiling process improved the utilization of most nutrients in such halophytes silages. These findings can be explained by Mitaru *et al.* (1984) who reported that during anaerobic storage the tannins either polymerize to higher oligomers which are insoluble and have lost their ability to bind proteins or they bound to protein and other constituents i.e. the high polymerized tannin either are too insoluble, have too few reactive sites or are too large to fit the protein orientation for cross linking.

The present findings agree with several workers and disagree with others as follow: Kamra (2005) reported that tannins are phenolic compounds, able to precipitate proteins, alkaloids and gelatin. Tannins are most effective against the fiber degrading bacteria. CT from the leaves of Onobrychis viciifolia inhibited growth and protease activity in some bacterial strains and was not much affected the others. Also, McSweeney *et al.* (1999) observed that in animals fed tannin rich *Calliandra calothyrsus*, the population of *rumenococcus spp.* and *fibrobacter spp.* was reduced considerably, but fungi, protozoa and proteolytic bacteria were less affected this diet. Sotohy *et al.* (1997) reported that the number of total bacteria in the rumen of goats decreased significantly when the animals were fed tannin-rich plant (*Acacia nilotica*) and the decrease in the numbers was directly proportional to the level of this feed in the diet.

On the other hand, Mbatha *et al.* (2002) found that some bacteria tolerate and grow in CT media and their protein enzymes are not severely affected because binding with tannins does not always represent complete inactivity of bacterial enzymes. It has been reported that there is less interaction between tannin and gram-negative bacteria because their cell walls are rich in lipids and phospholipids that prevent tannin from penetrating the bacterial wall (Sotohy *et al.*, 1997).

Our findings were supported by the previous study of El–Shaer *et al.* (2005) where they found that the hay intake was relatively higher than that of the silage, but the animals showed a slight change in their body weight. Therefore, the DM intake is not the limiting factor for gaining weight but the digestion and utilization of nutrients might be responsible for such

body weight changes. Also, the same authors reported that heating of a fodder aerobically causes a decrease in its protein availability due to the formation of a complex between the tannin and proteins, therefore decreasing it's nutritive value and causing an increase in its consumption to compensate the protein deficiency (Church, 1991 and Wina *et al.*, 1999)

Van Soest *et al.* (1986) indicated that the presence of CT in the cell wall and ADF indicate that tannins are strongly bound with fiber. The results of this study highlight presence of tannins in many nutritionally important shrubs reduces their utilization as ruminant feed. The free CT present in the feed get bound to fiber fractions and protein in the gastrointestinal tract and are present in feces but in the unextractable form (Makkar, 2003).

The data obtained in Table (3) revealed negative digestibilities of CT bound to NDF in silage and hay fed animals but the variations among groups were not significant. The highest digestibility of CT bound to ADF was in control animals (87.7%) followed by those fed on silage (59.6%). Significant variations were detected in the digestibility of CT in ADL where the highest value was in silage fed animals (72.0%) followed by control ones (64.3%) then those fed on hay (42.0%). The present results are explained by Ramirez *et al.* (2000) who found that fiber bound with tannin in leaves of plants containing high levels of CT may resist it's degradation by the rumen microbes and also free tannins would inactivate microorganisms and fiber enzymes, consequently, fermentation would be inhibited in the rumen.

Furthermore, those plants that had high CT or lignin resulted with low effective degradability of cell wall and vice versa. Degen *et al.* (1995) found that the extra CT present in the fiber fractions of the feces appear to originate from the extractable CT of the feed. These extractable tannins bind to feed or endogenous proteins and come out in ADF and ADL fractions as artifact ADF or artifact ADL. Negative lignin and fiber digestibilities for fodders high in tannins have been reported previously and are considered to be due to the presence of artifact lignin. Moreover, Degen *et al.* (1995) reported the results that show that the fiber measured in feed fraction is not the same as that measured in the feces and a high level of the extractable CT present in the feed disappeared in the gastrointestinal tract. In contrast, Robbins *et al.* (1991) demonstrated that 75% of ingested quebracho tannins were in feces. The difference in these results could be due to lower binding of quebracho tannins to proteins.

Also Woodward & Reed (1989) found that in most feeds the true digestibility of lignin is not different from zero. However, tannin-protein complexes formed in the digestive tract were recovered as fecal lignin (Reed, 1986) which led to an apparent negative digestibility of lignin.

Table (4): Fate of the studied ANF's *in vivo*: total tannins (TT); condensed tannins (CT); alkaloids; flavonoids and oxalates.

Item	Ration containing				± SE	P<
	Berseem hay	Silage	Haylage	Hay		
TT:						
intake (mg/d)	62.12 ^{c}	45.68 ^{d}	74.35 ^{b}	93.99 ^{a}	5.36	0.001
output (mg/d)	22.56 ^{ab}	10.11 ^{b}	12.38 ^{b}	39.35 ^{a}	4.17	0.05
digestibility (%)	63.81 ^{a}	52.84 ^{a}	83.35 ^{a}	58.18 ^{a}	7.12	N.S
CT:						
intake (g/d)	0.087 ^{b}	0.150 ^{a}	0.130 ^{ab}	0.107 ^{ab}	0.013	N.S
output (g/d)	0.037 ^{ab}	0.0133 ^{b}	0.057 ^{a}	0.013 ^{b}	0.007	0.05
digestibility (%)	38.12 ^{b}	89.84 ^{a}	56.59 ^{ab}	84.91 ^{ab}	8.69	N.S
Alkaloids:						
intake (g/d)	0.00 ^{d}	0.643 ^{c}	1.620 ^{a}	1.33 ^{b}	0.189	0.001
output (g/d)	0.00 ^{c}	0.393 ^{b}	0.973 ^{a}	0.800 ^{a}	0.118	0.001
digestibility (%)	0.00 ^{b}	21.98 ^{ab}	39.96 ^{a}	39.56 ^{a}	5.96	0.05
Flavonoids:						
intake (µg/d)	5.85 ^{b}	2.850 ^{c}	8.960 ^{a}	8.813 ^{a}	0.758	0.001
output (µg/d)	2.227 ^{a}	0.727 ^{a}	0.963 ^{a}	1.470 ^{a}	0.276	N.S
digestibility (%)	61.62 ^{a}	74.83 ^{a}	89.010 ^{a}	83.47 ^{a}	0.762	N.S
Oxalates:						
intake (g/d)	51.21 ^{a}	17.61 ^{d}	34.51 ^{b}	28.00 ^{c}	3.70	0.001
output (g/d)	140.5 ^{cb}	102.59 ^{c}	263.01 ^{a}	180.24 ^{b}	19.29	0.001
digestibility (%)	- 173.9 ^{a}	- 487.1 ^{b}	- 663.9 ^{b}	- 546.2 ^{b}	65.32	0.05

Finally Makkar (2003) suggested that not all tannin-protein complexes would get dissociated in the post – rumen leading to higher availability of feed protein in the intestine. The reversibility of the tannin – protein complex formation would depend on the binding affinity of tannins to protein and other macromolecules. The reversibility process is dependent on the nature of tannins and its affinity towards macromolecules, and hence the generalization that CT at 4% in diet, irrespective of the source would have beneficial effects, must be avoided; since a unit of tannin measured by gravimetric or spectrophotometric methods could have substantially different biological activity. Furthermore, even if complexation process is completely reversible, the binding of the released tannins to structural proteins and secreted proteins from the intestine could led to loss of endogenous proteins, and the balance of this endogenous protein loss and the availability of the feed protein at the intestine would determine the beneficial effects of tannins (Makkar, 2003).

Data in Table (4) illustrated the fate of the studied ANF's in vivo. The total tannins intake and output were highest in hay fed animals 93.99 and 39.35 mg/d respectively. These findings were expected because hay contained the highest concentration of tannins. 10.4 mg/100mg DM and highest intake as reported by El-Shaer *et al.* (2005). The highest intake of condensed tannins was reported in silage and there is no significant differences were found in the intake and digestibility of CT among the studied groups. Makkar *et al.* (2003) found that , there is no evidence of rumen microorganisms capable of degrading CT. The output of alkaloids in the haylage fed animals was lower than it's intake and the same pattern was reported for flavonoids in the same group. Negative oxalate digestibilities were reported for all studied groups including control animals and these findings explained previously due to oxalates accumulation.

CONCLUSION

It is concluded that the free condensed tannins present in the feed get bound to fiber fractions and protein in the gastrointestinal tract and are present in feces but in the unextractable form. Also, it is suggested that not all tannin-protein complexes would get dissociated in the postrumen leading to higher availability of feed protein in the intestine.

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تأثير بعض مضادات التغذية علي هضم مكونات الألياف عند تغذية الأغنام علي النباتات الملحية

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قد أجريت هذه الدراسة لتوضيح بعض المشاكل المتعلقة بتقدير الألياف في الأعلاف الغنية بالتانينات . وكذلك دراسة مصير بعض مضادات التغذية مثل التانينات الكلية والمكثفة، والقلويدات ، والفلافونات ، والأكسالات وتأثيراتها داخل جسم الحيوان. وقد تم تصنيع أعلاف مكونة من 6 (ستة) نباتات ملحية لتوضيح وملاحظة التركيبات المعقده لمكونات الألياف مع التانين والبروتين وذلك من خلال تغذية أربعة وعشرون من الكباش البرقي عليها.

وقد أجريت تحاليل لعينات من المأكول والروث باستخدام عينات منفصلة لتقدير المكون الطبيعي للألياف (NDF)، والمكون الحامضي (ADF) ، أما اللجنين (ADL) فقد تم تقديره من المتبقي من المكون الحامضي (ADF).

وأظهرت النتائج ايضا أن نسبة مكونات الألياف (NDF, ADF, ADL) في الروث أعلي منها في المأكول. كما وجدت فروق في مستويات الهيميسليلوز والذي يحسب من المعادلة الأتية: (NDF – ADF) والسليلوز من المعادلة (ADF – ADL) بين مجموعات الحيوانات. أيضا هناك فروقا معنوية في هضم مكونات الألياف (فيما عدا الهيميسليولوز) وكذلك البروتين المرتبط بهذه المكونات. كما وجد أن تركيز التانينات المكثفة في الروث أعلي من نظيره في العلف بينما وجد أن أغلب القلويدات في العلف قد تخلص منها الحيوان في الروث. ومن الملاحظ أن هضم الأكسالات جاء سلبيا في جميع حيوانات الدراسة. ومما ترتب علي ذلك أمكن إستنتاج أن هذه التانينات تلعب دورا أساسيا في تقليل إمتصاص المواد الغذائية مما يؤثر علي أكليها مسببا مشاكل صحية مزمنة.