EFFECT OF FEEDING KOCHIA AND PEARL MILLET AS HAYLAGE OR HAY TO SHEEP ON THE FATE OF ANTI-NUTRITIONAL FACTORS OF THE DIET

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SUMMARY

Plant secondary metabolites (PSM) which are include a wide variety of phytochemicals, have always been constituents of the diets of some animals. Although these phytochemicals have been present in very low concentrations in plants, they have adverse effects on animals when they are ingested. A study was conducted to determine forage quality and feeding patterns in sheep. Chemical composition, plant secondary metabolites content and digestibility of forages were determined using proximate procedures and *in vitro* techniques of PSM estimation respectively. Forage quality was studied in sheep using different treatments for the same mixture of plants. Berseem hay (D1), and haylage (D2) and hay (D3) made from mixture of *Kochia indica* and *Pearl millet* (1 : 1) with 7% molasses were offered to nine mature Barki sheep (3 animals in each group) as basal roughage diets. Fecal and urinary samples were used to determine the major route of PSM excretion. The results of the study indicated that the PSM levels were affected by forages processing especially making haylage. The results showed also that although haylage contained higher crude protein (CP) and lower PSM content, higher values of feed intake and body weight gain were recorded in hay fed group (D3) as compared to haylage (D2) one. Percentage of total PSM excretion revealed that alkaloids and nitrate–N were transported mainly via urine while total tannins (TT), condensed tannins (CT), saponins and oxalic acid were excreted mainly through the feces. Among the blood chemistry parameters blood calcium (Ca) and blood urea–N values were reduced significantly in animals of haylage and hay fed groups compared to those of berseem hay group. It was concluded that different method of processing for the same forage species for sheep was adequate to affect feed quality and consequently its intake and toxicity. Moreover, the processed forages may continue to serve as an important supply of nutrients for maintenance of livestock during drought or when feed supply is limited.

***Keywords**: Plant secondary metabolites (PSM), Excretion, sheep.*

INTRODUCTION

Plant secondary metabolites (PSM), the so-called phytochemicals or anti-nutritional factors (ANF) are a large group of compounds, represent a diverse group of natural products (Wink, 2004). PSM are generally thought to be present in plants primarily for self-defense purposes (Ralphs *et al.*, 2004); they may also have a possible nutritional role, particularly the N-containing PSM. The anti-nutritional factors (ANF) are defined as those substances generated in natural feeding stuffs by the primary metabolism of plants or by different, so-called secondary, mechanisms. PSM have been extensively studied because of the adverse effects that they have when ingested by animals (Acamovic *et al.*, 2004). Some

of these PSM were initially considered as problematic when consumed by animals, nevertheless, the beneficial effects of PSM in animals have also been investigated recently by Bento *et al.* (2005). However, Acamovic and Brooker (2005) reported that some of PSM have no nutritional value or anti-nutritional properties.

The study was aimed to evaluate the main PSM in a mixture of *Kochia indica* and *Pearl millet* in the forms of hay and haylage as was fed to sheep. A comparison between PSM excretion via urinary and digestive systems and their clearness in relation to the nutritive values of the tested diets were also detected.

MATERIALS AND METHODS

The experiment was carried out at Ras Suder Research Station, Desert Research Center, South Sinai Governorate where the experimental forages diets Kochia, Burningbush, *(Kochia indica)* and Pearl millet grass (*Pennisetum americanum*) were cultivated in salt affected soils of the Research Farm and irrigated with underground saline water with an average of 6000 mg/kg total dissolved salts. *Kochia indica* shrubs and pearl millet were harvested at early bloom stage according to Hanafy *et al.* (2007) and Fahmy (2001) respectively.

1 Materials

1.1. Animals

Nine mature Barki rams weighing 47.8 ±1.62 kg and 4 years old were used. Sheep were divided into three groups (3 animals in each) and allocated to one of three dietary treatments. Animals received the experimental diets for 30 days as a preliminary period then digestibility trial was followed for 15 days where animals were kept in individual metabolic cages. The first ten days of the trial were devoted as an adjustment period. Weighed forages and water were offered ad libitum every day and the remains were weighed and recorded during the preliminary period. In the following five days (collection period) measurements of 24 hours urine and fecal samples were collected for chemical analysis. At the end of collection period, blood samples were taken before the morning feeding. Body weight and forage intake were recorded at the beginning and at the end of the experiment.

1.2. Diets

Kochia indica and Pearl millet grass were collected separately and chopped into small pieces and kept for processing. The experimental diets were made from a mixture of the chopped *Kochia indica* and Pearl millet grass (1:1) with 7% molasses in sort of haylage (D2) and hay (D3) as basal diets compared to berseem hay (D1) as a good quality roughage. Haylage was made from the experimental plants in dry form, 7% molasses and a quantity of water were added to maintain moisture content to be about 50% then they were ensiled in a silo for 45 days. All animals were offered concentrate feed mixture (CFM) to cover 25% of TDN maintenance requirements according to Kearl (1982). The CFM consists of 25% cotton seed cake, 30% yellow corn, 35% wheat bran, 3% rice bran, 3% molasses, 1% urea, 2% limestone and 1% common salt.

2 Sample preparation

2.1. Forages

Composite samples from forage hay, haylage and berseem hay were dried at 65 °C till constant weight, then kept for further analysis (proximate composition and PSM determination).

2.2. Feces

Fecal samples (without urine or hair contamination) were collected and oven dried at 65 °C for 48 hours then ground and kept for PSM determination.

2.3. Urine

Urine sample were collected once daily in bottles containing 50% (v/v) sulphuric acid to fix and kept nitrogen, in presence of thymol as antibacterial and antifungal then kept in refrigerator for PSM analysis.

2.4. Serum

Blood samples were collected at the end of the feces and urine collection period (20 ml) with jugular vein puncture into sterile serum separator tubes, allowed to clot at room temperature for 35 minutes and centrifuged at 3000 r.p.m. for 10 minutes. Serum was decanted and stored at -20 °C until analyses for blood chemistry parameters.

3. Laboratory analysis

3.1. Proximate analysis

Proximate chemical analysis for all feed ingredients were determined (DM: dry matter, CF: crude fiber, CP: crude protein, EE: ether extract, NFE: nitrogen free extract) according to the method of A.O.A.C. (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970).

3.2. Anti-nutritional factors analysis

a) Phytochemical screening

The alcoholic extract of berseem hay, haylage and hay were tested for presence of alkaloids (Alk), total tannins (TT), condensed tannins (CT) and saponins (Sap) as the main ANF qualitatively using the procedures of Woo *et al.* (1977), Balbaa (1986), Porter *et al.* (1986) and Balbaa *et al.* (1981) , respectively.

b) Quantitative estimation

Quantitative estimation for alkaloids in feed, feces and urine samples was carried out by gravimetric method (Woo *et al.*, 1977). Total tannins (Balbaa, 1969), condensed tannins (Porter *et al.*, 1986), saponins (Balbaa *et al.*, 1981) and total oxalates (Hodgkinson, 1971) were estimated in feed and fecal samples. Nitrate (NO_3) content was evaluated by colorimetric method of Cataldo *et al.* (1975) in feed, feces and urine. Oxalates (OX) in urine were determined by the method described by Menache (1974). All serum samples were analyzed for calcium (Gitelman, 1967), inorganic phosphorus (Goodwin, 1970), triglycerides (Trinder, 1969), cholesterol (Roeschlau *et al.*, 1974), total protein (Reinhold, 1953), albumin (Rodkey, 1965), urea –N (Berthelot, 1959), creatinine (Seelig and Wust,

1969), alkaline phosphatase (Bowers and McComb, 1966), alanine amino transferase (ALT) and aspartate amino transferase (AST) (Wilkison *et al.*, 1972).

4. Statistical analysis

Analysis of variance (ANOVA) was used to analyze the obtained data using the general linear modeling procedure (SAS, 2000) and the least significant difference test was used for mean comparisons at probability 5%.

RESULTS AND DISCUSSION

1. Proximate composition and plant secondary metabolites

Results of Table (1) indicated that haylage has higher CP , ash , ADF and ADL and lower CF, EE and NDF content as compared to hay. Both haylage and hay had the same level of carbohydrates. The high CP and low CF content of haylage typified as a high quality diet.

Table (1) : Proximate analysis of the studied forages on dry matter basis (DM%)

Parameter	Berseem hay	Haylage	Hay	CFM
DM	83.0	70.0	68.0	88.5
OM	84.58	79.94	82.97	92.34
Ash	15.42	20.06	17.03	7.66
EE	1.66	1.43	2.29	2.45
CP	14.88	10.95	9.32	15.66
CF	33.92	20.16	24.02	10.48
NDF	64.14	51.38	59.23	33.25
ADF	42.49	32.07	28.92	14.81
ADL	14.03	11.40	9.99	9.61
NFE	34.12	47.40	47.34	63.75

Phytochemical screening as shown in Table (2) of the experimental plants and berseem hay (as control diet) revealed that all of them contained the studied ANF: alkaloids, tannins, condensed tannins, and saponins except the berseem hay which was free from saponins. Therefore, the quantitative determination for such undesirable compounds was necessary before feeding.

Table (2): Preliminary phytochemical screening of the different forages fed to sheep

Forages	Phytochemicals			
	Alkaloids	Total tannins	Condensed tannins	Saponins
Berseem hay	+	+	+	-
Kochia hay	+	+	+	+
Kochia haylage	+	+	+	+

+ = present, - = absent

The ANF concentrations in the experimental diets are summarized in Table (3). It was obvious that the concentrations of all ANF in haylage were less than those in hay due to

the effect of anaerobic fermentation during the ensiling process and the effect of air drying during the wilting of haylage (El-Shaer *et al.*, 2005). Similar findings were observed by Ulloa Rojas (1997) who reported that different treatments may cause reduction in the ANF content. Gihad *et* al. (2003) reported that ensiling of the less palatable and unpalatable plants decreased the detection of alkaloids and saponins.

Table (3): Concentrations of the anti-nutritional factors (ANF) in the experimental feed materials

Group	Anti-nutritional factors					
	Alk g/100gDM	TT mg%	CT g/100gDM	Sap g/100gDM	No_3 ppm	OX mm/g
Berseem hay	0.71	5.76	0.9	0.0	176	0.75
Haylage	1.24	5.04	0.8	3.6	80.1	1.13
Hay	1.854	6.73	1.0	3.8	290.4	1.25

Alk : alkaloids ; T.T : total tannins; CT : condensed tannins; Sap : saponins; NO_3 : nitrates; Ox : oxalic acid

On the other hand fresh forage and silage materials that are high in nitrate will have lower nitrate levels after being ensiled due to the microbial activity in the fermentation process. Ensiling nitrate accumulating plants will decrease the nitrate present and allow safe consumption (Goelz, 2002). The berseem hay contained the lowest alkaloids and oxalate contents and it was free from saponins. Carboline alkaloids (harmane and harmine) were found in *Kochia scoparia* studied by others (Drost –Karbowska *et al.*, 1978), but alkaloids in *Kochia indica* in the present study have not yet been identified. Thilsted *et al.* (1989) reported that, alkaloids of unknown structure and toxicity have been reported for kochia, and their concentrations changing with maturity of the plant. It seems that haylage typified as a good quality basal diet for sheep under the arid conditions of Sinai since it contained comparable levels of some ANF compared to those of berseem hay and also it has crude protein in amounts seem sufficient to sustain moderate growth.

2. Voluntary feed intake (VFI) and body weight changes

2.1. Voluntary feed intake (VFI)

Anti-nutritional factors (ANF) have been associated with reduction of digestibility nutrients and decrease in nutrient bioavailability (Medoua *et al.*, 2007).

Data in Table (4) showed that the maximum values of voluntary feed intake (VFI) was recorded for animals fed hay followed by those fed haylage as compared to control diet. El-Shaer *et al.* (2005) concluded that secondary metabolites could limit the utilization of these shrubs as animal feed and its reduction rendering it less harmful to the animals. The present results were contradicting to the above cited authors because the highest intake was recorded in hay fed animals although, hay diet contained the highest concentrations of alkaloids, tannins, saponins, nitrates and oxalates. Hay diet also contained higher amount of CF, EE and lower CP and ash than haylage. These results could be explained by Wina *et al.* (1999) who reported that presence of fiber reduces the toxicity of tannins due to the formation of fiber – tannin complexes which are increase under aerobic conditions. The

same authors found an inverse correlation between the crude protein content and its intake and they attributed it to to form complex of CP with tannins under aerobic conditions causing a lowering in the available protein intake. This forces the animal to eat more to compensate this deficiency so excess of ANF is also consumed leading to a certain degree of toxicity as shown by the slight increase in body weight. Moreover, Potter *et al.* (1993) showed that saponins reduce protein digestibility by the formation of less digestible saponin – protein complexes affecting the nutritive value of the diet. The presence of higher amount of saponins and tannins in the diet could be also affect on the intake as revised by Rogosic *et al.* (2006) who hypothesized that the complementarity's of tannin – rich shrubs and a saponin-rich shrub positively influenced biomass intake. Simultaneous presence of tannins and saponins might alleviate the adverse effect of each other (Makkar, 2003).

Table (4): Average values of voluntary feed intake and body weight changes of the sheep were fed with the tested rations during the palatability trial

Item	Berseem hay	Haylage	Hay
Initial live body weight, kg	47.9	48.0	47.8
Final live body weight , kg	49.4	49.70	49.7
Body weight change, kg	1.50	1.70	1.90
Dry matter intake, g/head/day:			
Roughage	852	1086	1223
Concentrate	165	169	164
Total	1017	1255	1387
Dry matter intake, g/kg bw:			
Roughage	18.6	23.5	26.7
Concentrate	3.6	3.7	3.6
Total	22.2	27.2	30.3

2.2. Body weight changes

Data in Table (4) revealed that live body weight changes were positive for all animal groups. The highest weight gain for sheep was recorded for the animals fed on hay diet. This increase in body weight was matching with the corresponding intake of the animal groups. The amount of concentrate feed mixture (CFM) was similar as it was offered to all animals as a feed supplement to cover 25% of TDN maintenance requirements according to Kearl (1982). The observed increase in the body weight of animals could be attributed to saponin content of hay diet. Patra (2007) observed that saponins (*Yucca schidigera* or *Quillaja saponaria*) or saponin containing forage and fruits are toxic to rumen protozoa which could be beneficial for improved ruminant productivity depending on the diets and the saponin involved. Generally, Patra (2007) agreed that removing or suppressing protozoa would result in increased ruminant performance, particularly on low–protein diet. Kamra (2005) studied the effect of saponins (saponin containing fruit) on rumen microbes of sheep and reported that its extract caused a 57% reduction in the protozoa number and 69% increase in bacterial population which resulted in improved feed conversion efficiency and better gain in body weight of the animals. Agarwal *et al.* (2006) concluded similar results.

Gee *et al.* (1996) also supporting the present results according to their findings where they found that some saponins increase the permeability of intestinal mucosal cells *in vitro*, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed. Lu and Jorgensen (1987) found that saponin increase intestinal motility and reduce microbial degradation of various nutrients through its inhibitory effect on the microorganisms.

3 Excretory routes of PSM from sheep

Urinary and fecal PSM excretion was illustrated in Tables (5) and (6). The present study revealed that a considerable proportion of the plant secondary metabolites were excreted via feces and urine. There is considerable interaction between ingested PSM and tissues, enzymes and other compounds within the animal. The interaction during absorption, deposition, metabolism and excretion are highly dependent on the physico–chemical attributes of the compounds such as molecular size and architecture; pH of the environment; hydrophyl or lipophyl character; charge and polarity; ability to form micelles and solubility (Acamovic and Brooker, 2005). Table (5) shows the concentration of some of the ANF, such as alkaloids (Alk), total tannins (TT), condensed tannins (CT), saponins, nitrates (NO_3) and oxalates as oxalic acid in feces and urine samples of the experimental animals.

Table (5): Concentration of the PSM in fecal and urinary samples of the experimental animals during the digestibility trial

Group PSM	Berseem hay	Haylage	Hay	± SE	Significance
Feces:					
Alk g/100gDM	0.92	0.42	0.56	0.241	N.S.
TT g/100gDM	4.03^{b}	4.94^{a}	4.23^{ab}	0.358	*
CT g/100gDM	0.57^{a}	0.23^{b}	0.22^{b}	0.062	***
Saponine g/100gDM	0.0^{c}	0.56^{b}	1.17^{a}	0.064	***
No_3 ppm	0.0	0.0	1.33	1.088	N.S
Oxalic acid mm/g	0.83^{b}	1.25^{a}	1.33^{a}	0.096	**
Urine:					
Alk g/24h	0.21^{b}	0.39^{a}	0.21^{b}	0.079	**
TT mg/24h	n.ev	n.ev	n.ev	-	-
CT g/24h	n. ev.	n. ev.	n. ev.	-	-
Saponin g/24h	n. ev.	n. ev.	n. ev.	-	-
No_3 ppm	618.1^{a}	348.73^{c}	458.57^{b}	24.64	***
Oxalic acid mg/24h	0.0017	0.0013	0.0025	.00003	N.S

N.S.: not significant; *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$, n.ev : not evaluated
Alk : alkaloids; T.T : total tannins; CT : condensed tannins; Sap : saponins; NO_3 : nitrates; Ox : oxalic acid
^{a,b,c} Means values in the same row having different superscripts are differed significantly

3.1.Excretion of alkaloids

Results of Table (5) revealed that most of alkaloids were excreted via feces and only small proportions were excreted via urine. As can be observed control animals excrete higher levels of alkaloids through feces followed by hay fed animals then haylage ones. On the other hand, haylage fed animals eliminate large proportion of alkaloids via urine.

Control and hay fed animals excrete equal levels of alkaloids through urine. These findings could be explained by the results of Acamovic and Brooker (2005) who reported that alkaloids tend to have high acid dissociation constants (pH: >7) and their solubility and thus toxicity (or otherwise) is therefore highly dependent on the pH within the GIT. Acamovic *et al.* (2004) found that pyrizolidine and other alkaloids are also metabolized to more toxic compounds within the animal. These fecal alkaloids may be that complexes with tannins as mentioned by Makkar *et al.* (1996). In general, animal performance was improved with low – alkaloids cultivars.

3.2. Excretion of tannins

Dealing with excretion of total tannins (TT) and condensed tannins (CT), tannins are classified into two groups: hydrolysable and condensed tannins. It is clear that most of dietary TT was excreted via feces (Table 5). This mean that, tannins undergo degradation and hydrolysis by rumen bacteria causing some of them were excreted in feces and some were absorbed. Fecal analysis for total tannins revealed that all animals of different groups excrete comparable levels of (TT) ($p<0.05$) and they differ in CT significantly ($p< 0.001$). The haylage fed group excrete higher levels of TT followed by hay fed one then control animals. Acamovic and Brokker (2005) explained their hypothesis by the fact that hydrolysable tannins is hydrolysed and degraded readily because of the ester linkage to the glucose moiety and the degradation products are absorbed from the GIT and cause toxicity (Cheeke, 1998).

Condensed tannins or proanthocyanidins, although hydrophilic and water soluble, are not absorbed from the GIT and with other tannins, they alter microflora populations, reduce attachment of fungi and bacteria to substrates, increase endogenous losses and damage the gastrointestinal tract (GIT) in animals (Bento *et al.*, 2005). The metabolic fate of the CT is more complex. The result of fecal excretion of CT was higher in control animals followed by haylage and hay fed animals, respectively. These variations in excreted levels were highly significant ($p<0.001$). Hagerman *et al.* (1992) reported that sheep excrete only about 60% of the ingested CT in feces suggesting that some of tannin may be absorbed, while Robbins *et al.* (1991) demonstrated that 75% of ingested CT were in the feces, Makkar *et al.* (1995) proposed that the fiber fractions of fecal samples had a substantial amount of both protein and CT. Rumen microbes are capable of degrading hydrolysable tannins. The toxicity therefore, appears to be due to absorption of degraded products of hydrolysable tannins and higher load of phenols in the blood stream which is beyond the capability of liver to detoxify them (Makkar, 2003). Obviously, TT and CT were not evaluated in urine samples of the present animals. The CT compounds are not absorbed into the blood stream (Terrill *et al.*, 1994), these therefore, under normal physiological conditions, are not likely to damage organs such as liver, kidney, spleen etc., as has been observed for hydrolysable tannins (Garg *et al.*, 1992). Makkar (2003) concluded that, under situation of intestinal damage due to consumption of high levels of tannins or of other intestinal membrane irritants, CTs may get absorbed to blood and may cause organ damages similar to those observed for hydrolysable tannins. Moreover the same author reported that the free CTs present in feed get bound to fiber fractions and protein in the GIT and are present in feces but in the non-extractable form.

3.3. Excretion of saponins

Fecal analysis of the control animals is free from saponins because of absence of saponins from their diet (berseem hay) while the treated groups excrete a considerable amount via feces with a significant variations ($p<0.0001$) as illustrated in Table (5). Hay-fed animals excreted higher values of saponins than haylage fed ones. It is known that saponins are steroidal sapogenin covalently linked to oligosaccharide moiety (Price *et al.*, 1987). Thus, the intact saponin molecules have both fat-soluble and water-soluble nuclei. The hydrophobic portion of the saponin (sapogenin) associated with the hydrophobic sterol nucleus of cholesterol in a stacked micellar aggregation (Oakenfull and Sidhu, 1989). In the present study, we were not evaluate saponins in urine because it is not absorbed and saponin itself has a high molecular weight (Patra, 2007) in addition to that, when saponins taken by mouth, saponins are comparatively harmless being not absorbed from the intestinal tract (Balbaa *et al.*, 1981). Kim *et al.* (2003) found that when saponin degraded, the fat soluble nucleus may be form complexes with cholesterol and excreted in bile, thus inhibiting enterohepatic cholesterol recycling. Lu and Jorgensen (1987) considered that alfalfa saponins were degraded by microorganisms in the gastrointestinal tract and/or excreted in feces. The hydrophilic nucleus may be excreted in urine and it is not specific for saponins. It is previously noted that hay forage was higher in tannins and saponins compared to haylage one so, tannins are known to bind saponins (Freeland *et al.*, 1985) and also to proteins and may even decrease the absorption of tannins and amino acids into the blood. The factors leading to higher or lower absorption of tannins or nutrients in presence of saponins or as a matter of fact of any other phytochemical are not known (Makkar, 2003).

3.4. Excretion of nitrates – nitrogen (NO_3-N)

Regarding to nitrate excretion, it is clear that nitrates were mainly eliminated in the urine in the present study and excretion in feces was negligible as demonstrated in Table (5). The same results were reported by Wang *et al.* (1981) in rats.

3.5. Excretion of oxalic acid

Oxalic acid is broken down in the rumen by *oxalobacter formigenes* following the period of adaptation (Allison *et al.*, 1985). Rumen breakdown of oxalic acid will reduce the amount of oxalic acid absorbed (Duncan *et al.*, 1998). Significant variations were reported between animals of treated groups (haylage and hay) and those of the control one ($p<0.01$). Slightly elevated levels of oxalic acids were found in the feces of berseem fed animals compared to that in their feeds while haylage fed animals excrete about similar values as dietary one. It should be noted that traces of oxalic acid were eliminated via urine in all animal groups. It could be explained by Jaeger and Robertson (2004) who found that the amount of oxalate available for absorption throughout the intestine is highly dependent on the state of oxalate (a) in the food ingested and (b) in the intestinal contents at each section of the intestinal tract since only the soluble form of oxalate can be absorbed. The main factors that control how much oxalate is in the soluble form are pH and the concentrations of Ca, Mg, and indirectly phosphate. Also the amount of free oxalate in the colon is also controlled by the presence or absence of *Oxalobacter formigenes*, an anaerobe that has an obligate requirement for oxalate as a source of energy and cellular carbon. The slight increase of fecal oxalate than dietary ones reflex that animals had accumulated dietary oxalate in their feces from the continuous feeding. Results of Da Costa *et al.* (1994) were in accordance with the present results. They reported that

preformed 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. Another approach was carried out to demonstrate the average of PSM excretion levels per each group at the end of the experiment and to illustrate the primary excretory rout of every metabolite from sheep.

4 Excreted levels of PSM from sheep

Table (6) summarized the total mean daily PSM excretion for the experimental treatments for both urinary and fecal transports and the proportion of the total PSM excretion for each treatment. It was obvious that a greater proportion of alkaloids and nitrates was excreted through the urinary transport in three groups especially haylage fed animals than the fecal transport. Eckert *et al.* (1978) concluded that the route of excretion of the ergot alkaloids was found to be dependent on the molecular weight of the compound investigated. Generally, Smith (1992) suggested that ruminant animals often tolerate poisonous plants better than non ruminants owing to microbial detoxification of poisonous compounds. On the other hand, appearance and disappearance of urinary alkaloids is most likely to occur if the alkaloids are soluble in rumen fluid (Hill *et al.*, 2001). According to the studied results in Table (4) the hay fed animals gain the higher weight than haylage fed animal and according to Table (6). It was obviously that haylage fed animals excrete a greater proportion of alkaloids via urine than hay fed ones so, it may be another reason for less weight gain of haylage fed animals. According to the results of Hill *et al.* (2000) urinary alkaloids excretion of ergot alkaloids is inversely proportion to average daily gain of cattle.

Concerning to the nitrate excretion, negligible amounts of nitrate–N were found in feces of the animals. A highly significant variations in urinary values of nitrate were reported among the experimental animals ($p<0.0001$). The control animals excrete higher levels of nitrates followed by hay fed animals then haylage (Table 5). These results were illustrated in Table (6) and it was clear that hay fed animals were excrete a very small portion in feces. It is well known that the nitrate entering the rumen gets metabolized into nitrite which is absorbed into the blood rapidly and oxidizes oxy-hemoglobin to met-hemoglobin, thus reducing the oxygen–carrying capacity of red blood cells. Though some nitrite is metabolized further into ammonia, depending upon its level toxicity may occur (Kamra, 2005). Vough *et al.* (2006) also found that the toxicity level depends on both how much and how fast nitrate is consumed. They reported that even forage with lower levels of nitrate may become toxic if animals are nutritionally stressed or ill and they suddenly consume large quantities of the forage. The present data revealed that, oxalate was excreted mainly in feces of animals as reported previously.

The total excretion of the studied PSM as percentage of total output for each metabolite were expressed to evaluate the main excretory rout for each metabolite per group. It was obvious that, alkaloids and nitrates were mainly eliminated via urine while CT, TT, saponins and oxalates would be excreted through fecal transport.

In general, the smaller the molecule and the greater the hydrophylicity, the greater is likelihood of absorption of the compound from the GIT when ingested (Harborne, 2001).

Table (6): Mean plant secondary metabolites (PSM) excretion levels per animal group for the experimental period

Group	Mean Daily PSM Excretion / Animal						Percent (%) of total PSM excretion transported by					
PSM	Control		hay		haylage		Urine			Feces		
	urine	feces	urine	feces	urine	feces	control	hay	haylage	control	hay	Haylage
Alk	4.04	3.46	6.54	3.97	12.46	2.97	**53.85**	**62.21**	**80.76**	46.15	37.79	19.24
T.T	n. ev	15.09	n. ev	29.14	n. ev	26.2	n. ev	n. ev	n. ev	**100**	**100**	**100**
CT	n. ev	2.15	n. ev	1.83	n. ev	0.94	n. ev	n. ev	n. ev	**100**	**100**	**100**
Sap	n. ev	0.0	n. ev	15.13	n. ev	1.91	n. ev	n. ev	n. ev	**0.0**	**100**	**100**
No_3	10833.1	0.0	13970.4	47.167	9590.67	0.0	**100**	**99.92**	**100**	**0.0**	0.085	**0.0**
OX	0.0319	3.126	0.0808	9.124	0.0364	6.671	1.01	0.88	0.54	**98.99**	**99.12**	**99.46**

n.ev : not evaluated
The number had written in **bold** means the highest percentage
Alk : alkaloids T.T : total tannins CT : condensed tannins Sap : saponins
No_3 : nitrates Ox : oxalic acid

5 Effects of the experimental diets on blood serum constituents

Table (7) shows the effect of feeding the experimental diets on blood serum constituents at the end of the experiment. It was observed that a significant decrease in Ca level in treated groups compared to the control one ($P< 0.001$). Similar to the previous study of Rankins *et al.* (1991) who found a slight drop in Ca level in sheep fed kochia hay. This hypocalcemia in the treated groups could be attributed for many reasons where Da Costa *et al.* (1994) suggested that ingested soluble oxalates were degraded to alkali carbonates before reaching the true stomach. These compounds were believed to be responsible for a sever alkalosis interfering with calcium absorption. Hypocalcemia was coincidental with rather than a cause of presence of tannins because tannins can also disturb the absorption of minerals by chelation of them within the GIT of the animal (Cowieson *et al.*, 2004) and/or increase the endogenous losses of the minerals such as calcium (Mansoori and Acamovic, 1997). In addition to the previous findings, dietary saponins may share in the present hypocalcemia, because saponins impair the absorption of some micronutrients. For instance Jenkins and Atwal (1994) found that, the dietary saponins reduce plasma Ca and zinc concentration in pigs. Normal physiological values of calcium in sheep were (9 – 13 mg/dl) according to Puls (1988). However, blood plasma phosphorus and triglycerides were unchanged.

Cholesterol levels were not significantly elevated in haylage and hay fed animals compared to control one. Rankins *et al.* (1991) showed an increased levels of cholesterol and explained these findings as lowered plane of nutrition. Normal physiological range of cholesterol according to Puls (1988) was (52-76 mg/dl). Nearly, total protein, albumin and globulin were in normal level. Normal physiological range of total proteins and albumin according to Puls (1988) were (6-7.9g/dl) and (2.4-3 g/dl), respectively.

Urea –N was decreased ($P<0.001$) in treated animals compared to the control animals reflecting low protein intake by sheep fed the experimental diets. Rankins *et al.* (1991) were in agreement with the present results and explain their findings to low protein intake by steers fed kochia although serum total protein, albumin and globulin were elevated but protein intake was not low enough to compromise protein synthesis by liver. Romero *et al.* (2000) also showed that sheep fed with tanniferous diet had reduced blood urea-N level than sheep fed lower dietary tannin. Normal physiological values of serum urea were (8-20 mg/dl) according to Puls (1988).

Creatinine levels were slightly increased ($p<0.05$) in haylage fed animals indicating impairment of renal functions (Brenner *et al.* 1987). Normal physiological levels of creatinine according to Puls (1988) were (1.0-2.9 mg/dl).

Serum enzymes were not elevated, indicating that no hepatotoxicosis occur within the experimental period. Only AST was elevated ($p<0.05$) in hay fed animals as compared to haylage fed animals. This elevation could be attributed to tannin related toxicity, since hay fodder was rich in tannin and poor in CP. Silanikove *et al.* (1996) also reported elevated AST in goats and cattles fed on tanniferous forages.

Overall, serum chemistry profiles indicated inadequate nutrient intakes. Wall *et al.* (2005) concluded that kochia affects digestive tract kinetics in a low quality diet by increasing the rate of passage and decreasing retention time as the level of kochia increases in the diet.

Table (7): Blood serum constituents in male sheep fed haylage and hay from *Kochia indica* as 50% mixed with pearl milt as 50%

Constituents	Diets			± SE	Significance
	Berseem hay	Haylage	Hay		
Calcium (mg/dl)	10.63[a]	8.22[b]	8.45[b]	0.44	***
Phosphorus (mg/ dl)	12.79	13.97	14.00	0.816	N. S
Triglycerides (mg/ dl)	21.67	23.65	18.48	3.03	N. S
Cholesterol (mg/ dl)	56.09	68.28	65.52	13.16	N. S
Total protein (g/dl)	6.74	6.63	7.01	0.23	N. S
Albumin (g/dl)	3.51	3.48	3.46	0.114	N. S
Globulin (g/dl)	3.23[a]	3.15[a]	3.55[a]	0.45	N. S
Blood urea – N (mg/dl)	55.37[a]	26.46[b]	34.15[b]	21.49	***
Creatinine (mg/dl)	1.08[b]	1.47[a]	1.24[b]	1.45	*
Alkaline phosphatase(U/l)	198.33	173.67	230.0	24.41	N. S
Aspartate aminotransferase(U/l)	45.33[ab]	37.33[b]	52.0[a]	3.64	*
Alanine amino transferase(U/l)	13.33	12.0	14.33	2.61	N. S

N. S : non significant; *= $p<0.05$, **= $p<0.01$; ***= $p<0.001$

[a,b]Means values in the same row having different superscripts are differed significantly

CONCLUSION AND RECOMMENDATION

In the light of these observations, the treatments evaluated had slight adverse effects toward animals although most of ANF present in these forages were excreted through normal excretory pathways of animals. Greater understanding of the role of plant phytochemicals (ANF) and their interactions will lead to reach to a balanced ration. A balanced ration that provides adequate amount of nutrients will tend to reduce problems from ANF in the ration. Finally, the treated forages may continue to serve as an important supply of nutrients for maintenance of livestock during drought or when feed supply is limited.

Efforts to improve animal tolerance of toxicants become more important than efforts to reduce plant content of toxicants because some of them are beneficial for ruminants.

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تأثير تغذية الأغنام علي هيلاج و دريس الكوخيا و الدخن علـي مـصير مـضادات التغذيـة الموجودة في العليقة

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أجريت هذه الدراسة علي عدد ٩ من ذكور الأغنام البرقي قسمت إلي ثلاث مجموعات (٣ حيوانات بكل مجموعة) المجموعة الأولي (المجموعة الضابطة) إعتمدت في تغذيتها علي دريس البرسيم ، والمجموعة الثانية (مجموعة ٢) تمت تغذيتها علي هيلاج خليط من نباتات الكوخيا والدخن بنسبة (١:١) أما المجموعة الثالثة (مجموعة ٣) تم تغذيتها علي دريس مخلوط الكوخيا والدخن وأضيف للمجموعة الثانية والثالثة المولاس بنسبة ٧% وتم الإستعانة بعينات من الروث والبول لمعرفة المسار الرئيسي للتخلص من وإخراج هذه المركبات الأيضية الثانوية .

أظهرت نتائج الدراسة أن المعاملات المختلفة (المعاملة البيولوجية – التجفيف الهوائي) للأعلاف قد أثرت علي محتواها من المركبات الأيضية وخاصة الهيلاج. كما أظهرت النتائج أنه علي الرغم من ان الهيلاج يحتوي علي أعلي نسبة من البروتين الخام وأقل نسبة من المركبات الأيضية الثانوية ومع ذلك فقد سجلت الحيوانات المغذاه علي دريس النباتات المجففة (مجموعة ٣) أعلي نسبة مأكول وكذلك زيادة في وزن الحيوان.

ثبت أن البول هو المسار الرئيسي لإخراج القلويدات والنترات بينما الروث هو المسار الرئيسي لإخراج التانينات الكلية والمكثفة والصابونينات والأكسالات. سجلت الحيوانات التجريبية إنخفاضا معنويا في مستوي الكالسيوم والبوليـا بالـدم. كما سجلت حيوانات مجموعة ٢ ارتفاعا معنويا فـي الكريـاتينين و حيوانـات مجموعة ٣ ارتفاعا معنويا في انزيم الناقل الركيزي للأسبرتات

ومما سبق أمكن إستنتاج ان المعاملات المختلفة لنفس العلف تؤثر بشكل كبير علي جودته وكذلك تركيز بعض المركبات الأيضيه الثانوية (مدي سميته) وبالتالي المأكول منه.