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In vitro and *In vivo* Evaluation of Biological Treated Salt Plants

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Abstract: *Acacia saligna* (A) and *Tamarix mannifera* (T), as salt plants, were biologically treated with two genera of pleurotus (white fruit fungi), Ostreatus (o) and Florida (f) at three weeks as incubation period in order to obtain the following four formulations: R1 (A_f +T_f) R2 (A_o+ T_O), R_3 (A_f + T_o), R_4 (A_o + T_f). The four formulations in addition to two control groups R_5 (untreated plants) and R_6 (berseem hay) were *in vitro* evaluated to study the effect of biological treatments on DM, OM, CP nutrients disappearance and cell wall constituents disappearance in addition to some anti-nutritional factors determination. Based on results of *in vitro* evaluation and anti-nutritional content, the best two mixtures were compared with berseem hay as control in an *in-vivo* feeding trial utilizing 9 Barki rams (3-3.5 years old and 46.3 ± 0.87 average body weight) from the Ras Suder experimental station, belongs to Desert Researsh Center. Animals were randomly assigned in to three equal nutritional groups (3 each). The treatment groups were T1: (A_f + T_o), T_2: (A_O + T_f), T_3: (berseem hay). The roughages were left free choice for the animals in addition to 20 % of the total energy requirements were supplied by barley grains. The preliminary period of the feeding trial lasted 35 days followed by 5 days collection period. Ruminal parameters (ruminal protozoa count, VFA's, ammonia, TP, NPN, true protein) and some blood parameters (RBC,s,WBC,s PCV, Hb) concentrations were determined.The results of *in vitro* study showed that the DM, OM, CP and cell wall constituents disappearance were higher (P<0.05) for *Acacia* and *Tamarix* treated biologically than untreated one however the control still the highest. Likewise, anti-nutritional factors (tannins-alkaloids-saponin-oxalats-flavonoids) percentage was decreased in both *Acacia* and *Tamarix* than untreated ones. The control group (T3) had highercounts ($p < 0.05$) of Isotrachia, *Dasytricha, Epidinum, Eudiplodinum* and *Ophryoscolex* species whereas *Entodinum* and *Polyplastron* species of ruminal protozoa counts were insignificantly higher. The protozoa count insignificantly increased before feeding and then decreased at 3 hr post feeding except Polyplastron species. Values of ammonia nitrogen, total nitrogen and nitrogen fraction (true and NPN) in the rumen liquor as well as blood WBC, s and Zn were higher ($p < 0.05$) in T2 and T1 which contain the biological treated plants. On the other hand, the blood cholesterol and serum Fe significantly ($p < 0.05$) decreased while serum Cu decreased insignificantly with animals treated with fungi. It could be concluded that the utilization of some salt plants could be improved by using white fungi treatments due to decreasing their toxic effect and consequently improve the animal performance.

Key words: sheep · Salt plants · Fungi treatments · Secondary metabolites · Rumen and blood parameters

INTRODUCTION

Livestock production is a major component of the agricultural economy of Egypt. There exists a huge gape between the availability and requirement of feed resources in Egypt. In this context of feed shortage, it is necessary to enrich the available sources like halophytic plants in terms of both digestibility and protein content. The major limitation with halophytic plants like *Acacia* and *Tamarix* includes their poor nutritive value, low palatability [1] and their content of some anti-nutritional factors (ANF's) such as tannins, alkalodis, oxalates and flavonoids [2] and high contents of some minerals [3]. So, various enrichment (treatment) technologies (physical, chemical and biological) were available to improve the nutritive value [4]. Supplementation with other feeds that provide additional protein, minerals and energy is a viable option [5]. The most common one of the alternative supplementation strategies is the use of protein supplements such as oil cake [4]. Devendra [4] and

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Sharma *et al.* [6] evaluated the nutritive value and biodigestibility of many novel feed formulations supplemented with edible mushrooms, oil cakes and legumineous tree leaves. Moreover, Abd El-Hamid *et al.* [7] reported that biological treatments using celluletic fungi are one of the treatments that aimed to improve the utilization of salt plants by increasing hydrolysis of cellulose to glucose and increased its content of crude protein.

The present study is oriented to develop efficient new feed formulations for ruminants by amending the two halophytic plants. *Tamarix mannifera* and *Acacia saligna* inoculated with two genera of pleurotus, which is very popular as an edible mushroom and considered as a single cell protein in soil state fermentations. Also, to investigate the efficacy of pleurotus treated plants on some rumen and blood variables.

MATERIALS AND METHODS

Fungus Used: Two strains of the pleurotus were obtained from the National Center of Agricultural Utilization Research Service, USA and maintained on potato dextrose agar. The two strains were pleurotus Ostreatus (O) and pleurotus Florida (F).

Feed Formulations / Rations: Based on calculation of demand and supply of nutrients for ruminants and in the view of providing balanced diet, different feed formulations with two species of the white fungi were prepared.

Two halophytic plants *Tamarix mannifera* (T) and *Acacia saligna* (A) were collected and chopped into (2-5 cm long), air dried and made ready for use in formulating the experimental diets.

Experiments:

***In vitro* Experiment:** The objective of the *In vitro* experiment was to determine the degradability of OM, DM, CP; the experimental diets were formulated to contain different combinations (Table 1) of the previously prepared plants with two pleurotus spp. of white fungi where plants incubated for three weeks with two spp. Two control diets were formulated as one of them made from untreated plants (A + T) and another one from berseem hay for *in vitro* study. These rations were incubated for 48 hrs with rumen liquor of adult Barki rams fed on berseem hay (the liquor collected 4hr. post feeding), to study DM and OM disappearance according to Norris [8].

Protein disappearance was determined according to AOAC [9]. Cell wall constituents, natural detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignine (ADL) disappearance were measured according to Goering and Van Soest [10]. Hemicellulose and cellulose were calculated by the difference. Other samples were incubated for 48 hours and filtered throw three layers of clothes and divided into three parts to test pH, ammonia and TVFA,s.

***In vivo* Experiment:** As a result of the *in vitro* study, in vivo study was carried out at Ras Suder Research Station (Desert Research Centre) to study the effect of the best two formulation, compared to berseem hay on some rumen and blood parameters of sheep Nine adult Barki rams of 3-3.5 years old and 46.3 ± 0.87 kg were used. Rams were randomly and equally distributed on nutritional groups each contained three rams the treated fodder shrubs were fed ad lib as in Table 2. Barley grains were added to cover 20% of energy maintenance requirements according to Kearl [11].

The preliminary period of the feeding trial lasted about thirty five days followed by five days as a collection period. During the collection days rumen liquor samples were withdrawn before feeding, 3 and 6 hours post feeding using stomach tube to determine pH value immediately, ammonia nitrogen [9], total volatile fatty acids (TVFA,s) according to Warner [12], total nitrogen and non-protein nitrogen (NPN) according to AOAC [13]. True protein nitrogen was calculated by subtracting the non-protein nitrogen from the total nitrogen. Rumen liquor was taken to count and classify the rumen protozoa as described by Naga *et al.* [14].

Blood samples were taken from the jugular vein before feeding to determine cholesterol according to Richmaoand [15], triglycerids according to Trinder [16] and total lipid according to Schmit [17]. Blood film was made to count the blood cells. Trace elements as (Mg), (Cu), (Fe) and (Zn) in blood was determined according to AOAC [9], phytochemical screening was carried out for alcoholic extract of the different feed formulations were tested for the presence of alkaloids, total tannins, saponins and flavonoids as the major ANF's qualitatively using the procedures of Woo *et al.* [18], Balbaa [19], Balbaa *et al* [20] and Karawya and Aboutable [21], respectively.

Anti nutritional factors were analyzed in roughage diets as tannins according to Ali *et al*. [22], oxalates according to Hodgkinson [23], flavonoids according to

Table 1: Feed formulation

Feed formulation or ration	Plants incubated with fungus species	
R_1	*Acacia* and *Tamarix* + *P. florida*	$A_F + T_F$
R_2	*Acacia* and *Tamarix* + *P. ostreatus*	$A_O + T_O$
R_3	*Acacia* and *P. florida* and *Tamarix* with *P. ostreatus*	$A_F + T_O$
R_4	*Acacia* and *ostreatus* and *Tamarix* with *P. florida*	$A_O + T_F$

Table 2: Nutritional groups

Experimental treatments	Roughages	Barley grains
T_1	$A_F + T_O$	20 %
T_2	$A_O + T_F$	20 %
T_3	Berseem hay	20 %

Karawya and Abou [21], alkaloids according to Woo *et al.* [18], saponine according to Balbaa *et al*. [20]. The statistical analysis was tested using analysis of variance procedure described in the SAS [24] and significant differences among means were also tested by multiple range test [25].

RESULTS AND DISCUSSION

Changes in Crude Protein and Cell Wall Contents of Feed Formulations: The increase in crude protein (CP) and natural detergent fiber (NDF) of all the treated plants by pleurotus were analyzed and are presented in Table 3. From these results, it was noted that the crude protein content was improved in feed formulations amended with pleurotus. These data agree with Vijaya *et al.* [5], who reported an increase in CP of all treated feeds by wild and mutant strains of P.ostreatus. Also, they found that, the nutritive value investigated with the rats, pigs and sheep showed that fungal protein is more suitable for ruminants than mono gastric animals. This is probable due to the effect of the fungs on cell wall structure. In this context the increase in CP value by 4-8 folds is considered as suitable and is a desirable sign in improvement of the nutritional status of feed formulation [5]. The digestibility of the fungal protein by sheep is 82% [26]. Belewu and Yahaya [27] recorded the highest CP content of the experimental diet could be due to the addition of microbial protein during the process of biological fermentation.

Changes in Percent of Anti-nutritional Factors (ANF's): A variety of secondary compounds have been present and isolated from different tested feed formulations as indicated in Table 4. Of all the samples evaluated, the berseem hay was free from alkaloids and saponins. The treated *Acacia* (A) and *Tamarix* (T) with pleurotus gave the lowest percent of all anti-nutritional factors. Tannin content was reduced in (R_3) and (R_4).Also, the tabulated data indicating that, the biological treatment with two species of pleuroutes (Florida and Ostreatus) decreased the percent of oxalates by 43.5, 48.60% and 48.4 and 55.9% in *Acacia* and *Tamarix*, respectively, flavonoids, 10, 39%, 12, 40%, Alkaloids by 17.6, 29.5% and 23.8, 38.1%, saponine by 38.7, 43.8% and 34.3, 46.3 %, respectively.

These desirable changes in feed formulations indicating the efficiency of biological treatment on salt plant. Similar to the present investigation by Vijay *et al.* [5], they found that there is improvement of feed formulations quality after incubation with pleurotus ostreatus (wild and mutant) strains. Zadrazil and Kamra [28] also demonstrated the improvement *in vitro* digestibility of wheat straw after fermentation with *Pleurotus sp*. and *Stropharia rugusoannulata*. Similarly, the effect of treatments of cassava waste, rice husk, saw dust and sorghum stover using *Aspergillus niger*, *Trichoderma harzanium* and mushroom on feed intake, apparent digestibility, blood, carcass and organ measurements were reported with encouraging results [29].

Moreover, Belewu and Yahaya [27] reported that, the presence of such anti-nutritional factors like saponin and tannin could prevent the availability of some nutrients like protein to the animal if not properly processed. The present results are in agreement with El-Shaer *et al* [2] and Abd-El Rahman [3], who found that the processing treatment (haylage and silage) on halophytes decreased its secondary metabolites contents. As a result of reducing the anti-nutritional factors content of treated salt plants, Abdelhamid *et al.* [7] found that the intake values of treated *Acacia* and *Tamarix* with pleurotus Florida and Ostreatus were higher than untreated plants. Also they found that the greatest DM intake was observed with sheep fed berseem hay (which had the lowest contents of anti-nutritionl factors) followed by animlas fed (A_o+T_f)-(A_f+T_o).

Table 3: Chemical composition and cell wall constituents of feed stuffs (% on DM basis)

	Proximate analysis							Fiber fraction				
Items	DM	OM	CP	CF	EE	Ash	NFE	NDF	DAF	ADL	Cellulos	Hemicell
Acacia fresh (A)	39.85	87.50	8.38	32.08	2.00	12.5	45.04	61.11	47.60	17.56	13.51	30.04
Acacia + pleurotus Floriad (A_F)	34.95	88.74	16.25	25.90	2.30	11.26	44.29	64.93	55.02	25.79	9.91	29.23
Acacia + pleurotus Ostreatus (A_o)	33.96	88.02	14.44	26.48	2.20	11.98	44.90	65.64	56.75	26.84	8.89	29.91
Tamarix fresh (A)	38.58	74.93	7.94	28.61	2.44	25.07	35.94	63.84	42.90	13.00	13.51	29.9
Tamarix + pleurotus Floriad (T_F)	36.53	76.73	12.44	22.85	2.60	38.84	23.27	53.95	36.98	12.77	16.97	24.21
Tamarix + pleurotus Ostreatus (T_o)	37.09	75.45	12.00	23.42	2.51	37.52	24.55	50.09	26.78	9.18	17.60	23.31
Barley	94.18	95.56	9.94	3.53	1.57	4.44	80.52	53.61	5.26	2.41	12.66	14.41
R_1 ($A_F + T_F$)	91.43	87.53	14.35	24.38	2.40	25.05	33.82	59.44	46.00	19.28	13.44	26.72
R_2 ($A_o + T_o$)	91.56	86.14	13.22	24.95	2.35	24.75	34.73	57.87	41.77	18.01	16.10	23.76
R_3 ($A_F + T_o$)	91.23	88.46	14.13	24.66	2.40	24.39	34.42	57.51	42.44	17.49	15.07	24.95
R_4 ($A_o + T_F$)	92.13	89.12	13.44	24.67	2.40	25.41	34.08	59.80	45.91	19.81	13.89	26.10
R_5 (A + T)	92.04	83.21	8.16	30.35	2.22	16.35	42.92	62.48	42.25	15.77	17.23	29.48
R_6 (berseem hay)	93.96	86.32	12.25	29.22	1.95	13.68	42.90	76.11	41.71	9.50	34.40	32.21

Table 4: Anti-nutritional factors (%) in the experimental roughages

Roughage	Tannins	Oxalats	Flavonoids	Alkaloids	Saponins
Acacia fresh (A)	6.01	3.89	1.44	0.17	3.10
Acacia + pleurotus Florida (A_F)	3.50	2.90	1.30	0.16	1.90
Acacia + pleurotus Ostreatus (A_o)	3.11	2.00	1.27	0.13	1.76
Tamarix (T)	9.30	3.70	3.90	0.21	3.50
Tamarix + pleurotus Florida (T_F)	6.10	1.91	2.40	0.15	2.30
Tamarix + pleurotus Ostreatus (T_o)	5.72	1.63	2.33	0.13	1.88
R_1 ($A_F + T_F$)	5.80	3.10	1.80	0.16	2.20
R_2 ($A_o + T_o$)	5.80	2.10	1.77	0.14	1.86
R_3 ($A_F + T_o$)	4.5	1.56	1.10	0.14	1.07
R_4 ($A_o + T_F$)	4.30	1.20	1.74	0.13	1.53
R_5 (A + T)	7.50	3.20	2.60	0.20	3.80
R_6 (berseem hay)	2.40	3.90	0.47	-	-

Table 5: Nutrients disappearance and fermentation of experimental feed

Items	R1	R2	R3	R4	R5	R6	barley
DMD	45.82^{b}	46.01^{b}	48.91^{ab}	49.13^{ab}	41.79^{c}	54.62^{a}	76.00
OMD	50.36^{b}	50.17^{b}	43.56^{b}	53.77^{b}	43.23^{c}	78.18^{a}	80.60
CPD	47.81^{b}	47.23^{b}	51.92^{ab}	50.84^{ab}	41.60^{c}	57.13^{a}	60.42
NDFD	35.65^{b}	35.98^{b}	40.78^{ab}	39.13^{ab}	25.73^{c}	42.51^{a}	-
ADFD	23.10^{b}	23.63^{b}	28.31^{ba}	26.89^{ab}	18.64^{c}	31.17^{a}	-
ADLD	4.16^{b}	4.36^{b}	5.71^{ba}	4.90^{a}	3.80^{c}	6.44^{a}	-
Fermentation parameters							
pH	7.1^{a}	7.2^{a}	6.56^{b}	6.50^{b}	7.10^{a}	6.37^{c}	6.10
NH_3N mg/100 ml	4.90^{b}	5.10^{b}	5.80^{a}	5.90^{a}	4.60^{c}	6.10^{a}	8.20
TVFA's mg/100 ml	6.20^{b}	6.10^{b}	6.99^{ab}	7.34^{a}	5.70^{c}	7.80^{a}	8.90

a, b, c Means with the different letter are significantly different at ($P < 0.05$)

Changes in IVDMD, IVOMD, IVCPD and Cell Wall Digestibility: During the incubation of the modified feed formulations with two pleurotus species for three weeks, the changes dry matter (IVDMD), organic matter (IVOMD), crude protein (IVCPD) and cell wall constituents (NDFD, ADFD and ADLD) were measured after two days of incubation with rumen liquor and are presented in Table 5. From this table, it was evident that, DMD, OMD, CPD, NDFD, ADFD and ADLD were highest in R_6 (control-berseem hay) group ($P<0.05$) followed by R_4 (A_O+T_F) then R_3 ($A_F + T_O$). The lowest values were regarded in R_5 (A + T).These findings could be attributed to the content of ANf's where the untreated plants contained higher levels of ANf's consistent with this hypothesis Buckley *et al.*[30] and Garrido *et al.* [31] reported that, tannins usually depress *in vitro* digestibilities. Similar to the present data, the investigations of Vijaya *et al.* [5] also demonstrated the increased (lignin decomposition) delignification by mutants pleurotus forms consequently leads to increase in IVDMD which is considered as a positive change in increasing the overall digestibility of feed formulations. Zadrazil and Kamra [28] reported the improvement of *in vitro* digestibility after fermentation with *pleurotus* spp.

Degradation of lignin to water soluble compounds is more important for enhancement of digestibility than its degradation to carbon dioxide [32]. Boguhn *et al.* [33] agree with the present study, while Jung *et al.* [34] assessed the effect of five white-rot basidiomycetes on chemical composition and *in vitro* digestibility to straws for 30 days at 28°C and 90% RH and noticed that cell-wall polysaccharides were improved from the straw, while IVDMD decreased because the fungi removed the most readily fermentable polysaccharides. Tripathi *et al.* [35] found that the three fungi tested (*Phanerochaete chrysosoporium*, *Ganoderma applanatum* and *Coriolus versicolor*); the IVDMD and CP contents were higher in C. versicolor cultured straw with higher delignification between 7 and 28 days of fermentation.

Other *In vitro* digestibility data using sheep rumen fluid (Table 5) indicated that pH values ranged between (6.37 to 7.2) for all studied groups. Rumen ammonia nitrogen (NH_3-N) and total volatie fatty acids (TVFA's) were significantly increased in R_6 berseem hay, followed by R_4 then R_3, the lowest values were determined in the untreated groups. These results may be attributed to the low content of tannin in berseem hay and the treated feeds. Narjisse *et al.* [36] reported that rumen ammonia was depressed ($P < 0.05$) by tannin infusion in sheep Indeed, Kumar and Vaithiyanthan [37] reported that tannins bind to proteins, cell walls and cell solubles and adversely affect rumen microbial and intestinal enzyme activity [38] and consequently reduce ruminal VFA's [39].

Lu and Jorgensen [40] found that reduction of ruminal NH_3-N and total VFA concentration by saponins was due partly to defaunation. Cheeke [41] found that saponins have pronounced antiprotozoal activity. Thus reductions in ruminal protozoa numbers observed when saponins are fed [42] and within in vitro ruminal fermentation systems [43] are caused by reaction of saponins with cholesterol in the protozoal cell membrane, causing break down of the membrane, cell lysis and death.

***In vivo* Changes in Ruminal Fermentation:** Ruminal protozoa count of sheep fed the experimental diets is presented in Table 6. Results showed that rames fed berseem hay (T_3) had significant ($p<0.05$) higher values of *Isotrachia, Epidinum*, *Eudiplodinum and Ophryoscolex* species and insignificant increase of *Entodinum and Polyplastron* species followed by rames fed either (T_2) or (T_1). However T_2 had the higher count of all protozoa species than T_1 except *Isotrachia* specie. Results revealed that T_1showed the lowest count of protozoa species (Table 6). In accordance with this observation the increased protozoa count may be due to low content of tannin, saponin and alkaloids.Ruminal protozoa are unable toadapt to or detoxify saponine [44] similar trends were also reported by Patra [45], who found that saponins are toxic to rumen protozoa which could be beneficial for improved ruminant productivity, in vitro study of Patra *et al.* [46] and Agarwal *et al.* [47] showed that saponins have been shown to inhibit rumen protozoa. Data also indicated that Entodinum species had the highest count of all ruminal protozoa species. Similar trend was observed by Franzolin and Dehority [48] who reported that *Entodinum* species was 90% of total protozoa count. Also, Ivan *et al.* [49] found that *Entodinum* sp. was the most detrimental of ciliate protozoa. Data presented in Table 6 indicated that the protozoa count non-significantly increased at 6 hr post feeding for *Entodinum* and significantly($p<0.05$) increased at 6 hr for *Dasytricha and Epidinum* species. These results agree with those reported by Bhatia *et al.* [50], who found that total protozoa count in rumen of camels increased significantly six hours post feeding. However, Polyplastron reach its peak at 3 hrs post feeding and then decreased at 6 hrs post feeding. The same trend was observed by Fayed *et al.* [51].

Table 6: Rumen protozoa count of sheep fed the experimental diets (1 x 10^6 /ml)

Item	Sampling Time	T1 Mix1	T2 Mix2	T3 Berseem	hay Overall mean
Entodinum	0	7.93 ± 0.570	8.15±1.440	10.71±1.650	8.93±0.791
	3	8.60±2.020	8.91±0.980	9.47±1.110	8.99±0.734
	6	8.10±3.560	11.90±3.436	9.87±0.380	9.96±1.150
Overall mean		8.21±1.640	9.65±1.25	10.02±0.610	9.29±0.510
Isotrachia	0	0.194^{ab}±0.011	0.172^{b}±0.008	0.430^{a}±0.008	0.265±0.006
	3	0.194^{ab}± 0.007	0.108^{b}±0.002	0.323^{a}±0.006	0.208±0.004
	6	0.205^{ab}±0.006	0.129^{b}±0.007	0.409^{a}±0.007	0.248±0.005
Overall mean		0.198^{ab}±0.005	0.136^{b}±0.003	0.387^{a}±0.004	0.240±0.003
Dasytricha	0	0.279^{b}±0.018	0.602^{a}±0.031	0.559^{a}±0.015	0.480^{a}±0.012
	3	0.237^{b}±0.015	0.129^{c}±0.006	0.301^{a}±0.005	0.222^{b}±0.005
	6	0.291^{b}±0.019	0.645^{a}±0.064	0.516^{a}±0.013	0.484^{a}±0.013
Overall mean		0.269^{b}±0.008	0.459^{a}±0.002	0.459^{a}±0.006	0.396±0.008
Epidinum	0	0.645^{b}±0.028	0.645^{b}±0.020	0.968^{a}±0.058	0.753^{a}±0.020
	3	0.473^{b}±0.015	0.624^{a}±0.011	0.258^{c}±0.009	0.451^{b}±0.009
	6	0.468^{c}±0.028	0.731^{b}±0.040	1.011^{a}±0.085	0.737^{a}±0.029
Overall mean		0.529^{b}±0.013	0.667^{ab}±0.014	0.746^{a}±0.032	0.647±0.012
Polyplastron	0	0.151±0.009	0.172±0.018	0.086±0.006	0.136^{b}±0.038
	3	0.172±0.004	0.237±0.0057	0.285±0.019	0.222^{a}±0.061
	6	0.102±0.008	0.086±0.006	0.129±0.011	0.106^{b}±0.031
Overall mean		0.142±0.004	0.156±0.004	0.158±0.007	0.115±0.003
Eudiplodinum	0	0.151^{b}±0.008	0.258^{a}±0.065	0.215^{a}±0.031	0.208^{a}±0.050
	3	0.043^{b}±0.002	0.043^{b}±0.003	0.215^{a}±0.021	0.101^{b}±0.032
	6	0.193^{a}±0.062	0.057^{c}±0.008	0.172^{ab}±0.008	0.158^{ab}±0.050
Overall mean		0.129^{b}±0.005	0.136^{b}±0.041	0.201^{a}±0.045	0.156±0.027
Ohpryoscolex	0	0.065^{c}±0.002	0.172^{bc}±0.004	0.452^{a}±0.037	0.229±0.074
	3	0.194^{b}±0.008	0.065^{c}±0.003	0.323^{a}±0.012	0.194±0.058
	6	0.172^{b}±0.007	0.043^{c}±0.002	0.387^{a}±0.019	0.194±0.083
Overall mean		0.144^{bc}±0.006	0.206^{b}±0.008	0.387^{a}±0.009	0.206±0.040

a, b, c Means with the different letter are significantly different at (P < 0.05)

Table 7: Some rumen parameters of sheep fed the experimental diets

Items	Sampling time	T1	T2	T3	Overall mean
pH	0	6.98^{a}±0.061	6.98^{a}±0.061	7.10±0.086^{a}	7.02^{a}±0.036
	3	6.70^{c}±0.091	6.52^{b}±0.091	6.64^{c}±0.058	6.62^{b}±0.051
	6	6.91^{ab}±0.012	6.83^{ab}±0.012	6.97^{a}±0.100	6.91^{a}±0.035
Overall mean		6.86±0.076	6.79±0.076	6.90±0.080	6.85±0.040
T.V.F.A's m.eq/100 mal	0	6.20±0.046	6.86±0.046	6.58±0.546	6.55±0.382
	3	7.61±0.066	7.35±0.066	6.98±0.479	7.32±0.274
	6	7.23±0.651	6.42±0.651	7.98±0.287	7.21±0.429
Overall mean		6.02±0.232	6.88±0.232	7.18±0.346	7.03±0.226
Ammonia nitrogen					
mg/100 ml	0	30.33^{b}±0.671	32.55^{ab}±0.671	36.94^{a}±0.390	33.27±0.305
	3	36.78^{a}±0.450	38.15^{aa}±0.450	30.70^{b}±0.429	35.21±0.333
	6	32.53^{a}±0.449	36.84^{a}±0.449	36.25^{a}±0.490	35.21±0.305
Overall mean		33.21±1.311	35.85±1.311	34.63±0.245	34.56±0.176
Total nitrogen					
mg/100 ml	0	129.91^{c}±0.455	176.28^{b}±0.306^{a}	193.16^{a}±0.136	166.45±0.202
	3	149.08^{b}±0.0276	101.65^{c}±0.142	176.19^{a}±0.000	140.28±0.132
	6	155.00^{b}±0.100	165.94^{b}±0.188	199.24^{a}±0.126	174.73±0.124
Overall mean		144.64^{b}±0.0160	149.29^{b}±0.163	189.53^{a}±0.526	170.49±0.917
Non-protein nitrogen					
mg/100 ml	0	37.24±0.0110	37.75±0.263	34.57±0.383	36.52±0.408
	3	35.27±0.959	37.62±0.142	31.87±0.142	34.79±0.375
	6	34.08±0.0204	35.09±0.365	35.97±0.128	35.05±0.191
Overall mean		35.53^{ab}±0.429	36.82^{a}±0.225	34.01^{b}±0.140	35.45±0.192
True protein					
mg/100 ml	0	92.67^{b}±0.379	138.53^{ab}±0.280	158.59^{a}±0.123	129.93±0.174
	3	113.73^{b}±0.227	64.03^{c}±0.132	144.72^{a}±0.142	107.49±0.105
	6	120.92^{b}±0.803	134.85^{b}±0.163	163.27^{a}±0.108	139.68±0.107
Overall mean		109.11^{b}±0.000	112.47^{b}±0.143	155.53^{a}±0.454	125.70±0.781

a, b, c Means with the different letter are significantly different at (P < 0.05)

Table 8: Some blood parameters of sheep fed the experimental diets

Item	T1	T2	T3	Overall mean
Triglycerides mg/dl	122.333^{ab}±1.62	142.333^{a}±1.676	135.667^{ab}±1.564	133.444^{a}±0.878
cholesterol mg/dl	163.333^{b}±3.792	210.333^{b}±7.670	266.000^{ab}±5.753	213.222^{b}±3.325
Total lipids mg/dl	11.667±0.299	9.200±0.611	9.400±1.044	10.089±1.013
WBC's x10^{3}	4533.33^{ab}±46.667	5466.67^{ab}±146.600	4133.33^{ab}±26.666	4711.11±49.225
WBC's x10^{6}	3.400±0.321	3.033±0.176	3.633±0.145	3.356±0.143
Hb g/dI	9.667±0.970	8.633±0.570	10.367±0.433	9.556±0.430
PVC	29.000±2.910	26.100±1.800	31.100±1.300	28.733±.1.281
Mg	194.275±1.474	183.100±2.500	260.983±0.515	212.786±1.36
Cu	9.173±0.448	8.625±0.945	9.625±0.429	9.141±0.362
Fe	10.220^{b}±0.556	12.350^{a}±2.758	10.648^{b}±0.168	11.073^{b}±0.894
Zn	5.865^{ab}±0.561	5.813^{ab}±0.557	6.360^{a}±0.754	6.013±0.338

a, b, c Means with the different letter are significantly different at (P < 0.05)

Data in Table 7 showed that time affecting significantly (P<0.01) on ruminal pH values It decreased at 3 hr post feeding and then increased at 6 hr post f zeeding with values being 7.02, 6.62 and 6.91 at 0,3 and 6 h post feeding, respectively. These findings might be related to ruminal fermentation process by rumen microorganisms which took place on the soluble carbohydrates that produce propionate and decrease pH values. These results are in harmony with those reprorted by El Ashry *et al.* [52] and Allam *et al.* [53]. No significant difference among treatments was detected in ruminal pH values (Table 7).

Values of total VFA's did not differ significantly among either treatments or time of sampling. Total ruminal VFA's were similar in both T_2 and T_3 (control) while T_1 was the lowest one. *In vivo* study, barly grains were added as energy source so, Patra [45] reported that gas and total VFA production from barly grain were increased by saponine, whereas those were reduced from alfalfa hay [54]. Patra [45] concluded that various saponins have different responses. The lowest values of VFA'S was obtained before feeding and then increased to the maximum after 3 hr post feeding (Table 7). These increases caused a reduction in pH values. This confirmed well with the results of Khattab *et al*. [55].

Values of ammonia nitrogen in the rumen liquor indicated that T_1 was insignificantly lower than those of both T_2 and T_3.This result may be related to low content of tannin in T_2 and T_3. Similar results were obtained by Salawn *et al.* [56] and Osakwe *et al.* [57].They showed that sheep fed low tannin fodder had a higher ruminal NH_3 concentration. Thalib *et al.* [58] found that administering saponins to sheep every 3 days was effective in suppressing protozoa and reducing ruminal ammonia concentrations.There was a trend of increases in ammonia concentration from 0-up to 6 hrs post feeding. This finding might suggest slow absorption of ammonia from the rumen membrane and consequently increased its availability for microbial protein synthesis. Values of total nitrogen and true protein in rumen liquor showed that T_2 and T_1 (biologically treated roughages) were (p<0.05) lower than the control group. However, the values of non-protein nitrogen (NPN) were higher (p<0.05) with treated plants than berseem hay.

Concerning the effect of sampling time, total nitrogen, true protein and NPN were insignificantly higher at 6 hrs post feeding, whereas the lowest values were recorded at 3hr post feeding.These findings were due to high count of protozoa at 6 hr post feeding which reflected on building true protein inside the protozoa.

Changes in Blood Constituents: It was evident that biological treatments by white fungi affecting blood chemistry of the experimental animals as illustrated in Table 8. Blood cholesterol was reduced significantly in the treated groups (P < 0.05) compared to that of control ones. This finding could be attributed to high tannin and saponin contents. Consistent with results of the *in vivo* experiment Bravo *et al.* [59], who showed that, tannins decreased cholesterol absorption and increased fat excretion. Also, a number of studies have shown that saponins from different sources lower serum cholesterol levels in animals including human subjects [60].

Morehouse *et al.* [61] found that a number of synthetic saponins have been shown to be cholesterol absorption inhibitors causing reduction in plasma non-high-density, lipoprotein cholesterol fraction. Digestibility of fats in ruminants is limited by the lack of emulsifying agents in the rumen [41]. Other suggested mechanisms of action of saponins include delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity [62]. It can be concluded that several dietary saponins do have a hypocholesterolaemic action

[63]. However, levels of triglycerides were elevated significantly ($P<0.01$) among experimental groups but their levels still within normal range. On the other hard, an increase in WBCs count significantly in treated animals can be related to saponin. Johnson *et al.* [64] determined that some saponins increase the permeability of intestinal mucosal cells, facilitating the uptake of substances to which the gut would normally be impermeable.

It should also be acknowledged that this effect of saponins could have negative consequences. Increased gut permeability to large molecules could increase the risk of sensitization to dietary antigens that would not normally be absorbed [41]. Moreover, Haridas *et al.* [65] reported that, saponins have also been shown to be able to prevent some non-specific immune reactions such as inflammation.

The results indicated that there were no significant changes in numbers of RBCs although the present saponins in the treated feed for mulations. Francis *et al.* [63] reported that saponins have long been known to have alytic action on erythrocyte membranes. The haemolytic action of saponin is believed to be the result of the affinity of it to form insoluble complexes with membrane sterols particularly cholesterol. However, Patra [45] determined that various saponins have different responses.

Results of the blood iron showed a significant reduction in treated animals and these findings can be explained by two major phytochemical in experimental materials such as tannins and saponins. Consistent with our hypothesis, Cowieson *et al.* [66] found that tannins increase endogenous losses from animals including minerals losses. These losses are likely to occur by chelation of the minerals within the GIT (gastrointestinal tract) of the animal. Also, Flanagan *et al.* [67] observed that tannins cause a reducation in blood iron. Another explanation applied by saponins where Cheeke [41] reported that saponins may influence the absorption of minerals and vitamins. Southon *et al.* [68] found that saponins reduce iron absoration in rats. They suggested that the mode of action involves an effect on iron transport into or across the mucosal cell, rather than a chemical binding of iron to saponin in the intestinal lumen. Formation of mineral-saponin complexes *in vitro* was reported by West *et al.* [69] including complexes with iron.

As a consequence of iron deficiency in the tested animals zinc (Zn) levels were elevated significantly ($P < 0.05$) (Table 8) by 23% than that of the control ones. Flanagan *et al.* [67] concluded that iron deficing induces an increase in blood Zn.

CONCLUSION AND IMPLICATION

In conclusion, there is a clear indication that nutritional status of salt plants treated with white fungi was improved which more or less make them practical or complete substitutes for the conventional feed sources. Therefore, using of white fungi in feed formulations should be encouraged because of fungi had antitoxic effects. Improve nutritional status of the halophytic plants seems promising as a feasible means of converting such salt plants into viable feed for sheep.

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