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Influence of *Trichoderma reesei* or *Saccharomyces cerevisiae* on performance, ruminal fermentation, carcass characteristics and blood biochemistry of lambs fed *Atriplex nummularia* and *Acacia saligna* mixture

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ABSTRACT

The aim of this study was to evaluate whole substitution of Egyptian berseem hay (*Trifolium alexandrinum*) with a mixture of *Atriplex nummularia* and *Acacia saligna* (1:1 DM) in the diet of Barki lambs for 70 days. Thirty six lambs (27.0 ± 0.89 kg initial BW) were divided into four treatment groups of nine lambs each and fed: (1) the Control group with no substitution (70% concentrate mixture and 30% berseem hay, DM basis), (2) *A. nummularia* and *A. saligna* mixture without fungal treatment (treatment group AU), or (3) *Trichoderma reesei* treated *A. nummularia* and *A. saligna* mixture (treatment group AF), or (4) *A. nummularia* and *A. saligna* mixture supplemented with *Saccharomyces cerevisiae* at 0.5 g/kg DM of feed (treatment group AS) replaced 100% of berseem hay in the diet. Live-weight change, rumen fermentation parameters, blood chemistry, carcass characteristics and intestinal histology were investigated. Significant ($P < 0.05$) interactions occurred between diet and period for feed conversion efficiency and blood serum urea. Lambs in the AS treatment consumed less ($P < 0.05$) feed than lambs in the AF treatment, with no difference between the other treatments ($P > 0.05$). Lambs fed AF and AU diets had lower ($P < 0.05$) feed conversion efficiency than lambs fed the AS and Control diets. Lambs fed AF and AS had increased ($P < 0.05$) volatile fatty acid production compared to Controls. Blood albumin and urea concentrations increased ($P < 0.05$) with lambs in AS treatment compared to lambs in the other treatments, while lambs fed AF had lower ($P < 0.05$) cholesterol and glucose concentrations compared to the Controls. The AS lambs had the highest ($P < 0.05$) dressing percentage. Decreased intramuscular fat weights were obtained with lambs fed halophytes compared to Control lambs. Histology of the ileum, sub mucosa and Peyer's patches were normal in all lambs. In conclusion, untreated halophyte mixtures of *A. nummularia* and *A. saligna* (at 1:1 DM) can be substituted for berseem hay without negative effects on performance while treatment with *S. cerevisiae* may improve performance and, like *T. reesei*, change certain biochemical responses.

1. Introduction

There is an increased awareness of the value of halophytic or saltbush forage shrubs as animal feeds in arid and semi-arid regions (Alsersy et al., 2015; Salem et al., 2015). Halophytes are widely distributed, and at high density, in many areas under conditions of water shortage and high soil salinity. Feeding halophytes to ruminants is a possible solution to feed shortages in these areas (Ahmed et al., 2015; Alsersy et al., 2015; Salem et al., 2015).

Saltbush (*Atriplex* spp.) and shrubs including wattles (*Acacia* spp.) have received increased interest as livestock feed, especially in arid zones of North Africa and the northern region of Egypt (Ahmed et al., 2015; Alsersy et al., 2015; Salem et al., 2015). However, these species accumulate high concentrations of salt, oxalates and secondary plant compounds in their leaves

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(Papanastasis et al., 2008) making them less palatable and of lower nutritive value (Ben Salem et al., 2010). The degree of impact that this has will depend on the level of inclusion in the diet. Saltbushes and shrubs, in general, contain high levels of crude protein (CP), which is reasonably digestible, soluble carbohydrates and a relatively high mineral content (Al-Owaimer et al., 2008). A deficiency of available metabolisable energy and the rapid fermentation of CP in the rumen may be reasons for poor utilisation by ruminants. Most of the CP in saltbushes and shrubs is associated with non-protein nitrogen (N) compounds (Le Houérou, 1992) which can be converted into microbial protein or ammonia in the rumen dependant on the availability of metabolisable energy (Pearce et al., 2010). Therefore, supplementation with energy sources such as, barley grains, corn grains or molasses have been suggested as strategies to stimulate intake and improve the utilization of ruminal ammonia–N by rumen microorganisms when these shrubs are fed (Ahmed et al., 2015).

Acacia saligna is a successful species of colonising *Acacia,* due to its tolerance of dry environmental conditions, and its ability to produce large amounts of biomass with a relatively high CP content and nutritive value (Degan et al., 1997). However, it cannot be used as a sole nutrient source due to its high content of condensed tannins (Degan et al., 1997), which precipitate proteins and form indigestible tannin–protein complexes (Degan et al., 1995). It also complexes with soluble carbohydrates, cellulose, hemicelluloses and amino acids resulting in reduced digestibility of these substrates (Salem, 2005). *Atriplex nummularia* is a perennial halophyte shrub which is palatable; it remains green even during prolonged drought and maintains a relatively high CP content throughout the year. Ben Salem et al. (2010) in their review showed that *Atriplex* spp. contain a balanced amino acid profile. Khalil et al. (1986) stated that the essential amino acids, especially methionine and lysine are higher in *Atriplex* than in cereal proteins.

Mixing more than one type of halophyte may improve their utilization as animal feed. Abd El-Rahman et al. (2014) mixed *A. saligna* and *Brassica nigra* hay in the diet of sheep and goats, and stated that supplementation of both plants with barley grain can enhance performance of both sheep and goats. In addition, Shaker (2014) mixed *A. nummularia*, *Sorghum bicolour* and Pearl millet in the diet of Barki sheep and concluded that feeding a mixture of salt tolerant plants improved lamb performance (live-weight gain). Appropriate mixing of different halophyte species, based on their complementary nutritive profiles, could reduce the negative consequences of anti-nutritional factors, and thus improve animal performance (Shaker, 2014). Gihad and El Shaer (1994) stated that feeding ruminants on saltbushes (*Atriplex* spp.) combined with low salt forage (*A. saligna*) is desirable to dilute the high salt content of *Atriplex* spp.

The yeast *Saccharomyces cerevisiae* can be used as a probiotic, and has been shown to specifically alter the rumen environment, and enhance microbial activity (Elghandour et al., 2014, 2015). Yeast appears to play a role in removing traces of oxygen that may be toxic to rumen bacteria thereby increasing the number of total anaerobic and cellulolytic bacteria (Jouany, 2001). The treatment of feeds with fungi, through enzymatic action, can remove anti-nutritional factors from feed, and improve the nutritive value of the feed (Fayed, 2009; Khattab et al., 2013; Kholif et al., 2014). Fungal lignocellulolytic enzymes break the polysaccharide–lignin complex resulting in enhanced digestibility and improved animal performance (Kholif et al., 2014). Therefore, the current study aimed to evaluate the impact of replacing berseem hay, in a complete diet, with a mixture of equal parts *Atriplex* and *Acacia* that had been either left untreated or supplemented with fungal probiotic treatments.

2. Materials and methods

Animals were cared and handled in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

2.1. Plant forage preparation

Leaves and stems of fresh nursery plants of saltbush *A. nummularia* and wattle *A. saligna* were collected from the north-western desert region of Borg El-Arab, Alexandria (Egypt), dried and chopped into 3–5 cm lengths and stored in a dry environment.

2.2. Fungal treatment of forage

Trichoderma reesei was obtained from the Animal Production Research Institute, Cairo, Egypt. The fungus was maintained on potato dextrose agar in petri dishes at ambient temperature (22–25 °C).

The mixture of *A. nummularia* and *A. saligna* (1:1 DM) was autoclaved (Tuttnauer USA Co. Ltd., NY, USA) at 121 °C and 1.5 psi for 15 min to destroy any microbes. The content was allowed to cool and later inoculated with the spores of *T. reesei* at a rate of 40 mL of the spore suspension containing 10^7 spores per mL/kg DM of autoclaved *A. nummularia* and *A. saligna* mixture. The inoculated substrates were then incubated at ambient temperature for 10 days. By the end of the incubation period, the forages were fully covered with the fungus. They were then oven dried at 70 °C in a forced air drying oven (Cascade TEK's Model TFO-10, OR, USA) for 24 h so as to stop fungal growth and prevent further denaturation of proteins.

2.3. Diets

Lambs were fed one of four diets. The Control diet consisted of a mixed concentrates mixture based on barley, wheat bran and soybean meal mixed together and then combined with Egyptian berseem hay (*Trifolium alexandrinum*) at a ratio of 70:30 on a DM basis. The treatment diets were the same as the Control, but the berseem hay was totally replaced with either *A. nummularia* and *A. saligna* mixture without fungal treatment (treatment group AU), or *A. nummularia* and *A. saligna* mixture treated with *T. reesei* (treatment group AF), or *A. nummularia* and *A. saligna* mixture supplemented with *S. cerevisiae* (Brookside Agra, USA; contained 8×10^8 CFU/g) at 0.5 g/kg DM of feed (treatment group AS). Ingredients and chemical composition of the experimental diets are presented in Tables 1 and 2, respectively.

2.4. Animals and feeding

Thirty six Barki lambs with initial BW 27.0 ± 0.89 kg and final BW 37.2 ± 0.43 kg and at approximately 13 months of age were used. Prior to the experiment, the lambs were treated for internal and external parasites. Lambs were divided into four treatment groups of nine lambs each and fed diets as detailed previously. These diets were formulated to meet their maintenance requirements (NRC, 1985). Before the experiment, lambs were fed on a diet of concentrates and berseem hay at a ratio of 1:1 on a DM basis. Lambs were adapted to the experimental diets for two weeks and fed for a total of 70 days. Lambs were fed twice daily at 08:00 and 16:00 h. Berseem hay or *A. nummularia* and *A. saligna* hays were offered first followed by concentrates.

2.5. Animal performance

Lambs were individually weighed every two weeks prior to

Table 1
Ingredients (g/kg DM) of the experimental diets[a] containing *Atriplex nummularia* and *Acacia saligna* (1:1 DM) treated with *Trichoderma reesei* or *Saccharomyces cerevisiae*.

	Control	AU	AF	AS
Forages				
Berseem (*Trifolium alexandrinum*) hay	300	0	0	0
Atriplex nummularia	0	150	150	150
Acacia saligna	0	150	150	150
Concentrates				
Barley	265	265	265	265
Wheat bran	250	250	250	250
Soybean meal	100	100	100	100
Molasses	55	55	55	55
Limestone ($NaHCO_3$)	20	20	20	20
Salt	5	5	5	5
Mineral and vitamin mixture[b]	5	5	5	5

[a] The diets contained 30% berseem hay+70% concentrate mixture (Control), AU=15% *Atriplex*+15% *Acacia*+70% concentrate mixture (negative control); AF=AU treated with *Trichoderma reesei*, and AS=AU supplemented with *Saccharomyces cerevisiae* as a probiotic at 0.5 g/ kg DM of feed.
[b] Mineral and vitamin mixture (/kg): Cu, 8 mg; Fe, 35 mg; Mn, 80 mg; Se, 0.6 mg; Zn, 60 mg; vitamin A, 12,000 IU; vitamin D_3, 2500 ICU; vitamin E, 20 IU; menadione, 1.3 mg; riboflavin, 5.5 mg; vitamin B_{12}, 10 μg; vitamin B_6, 3 mg; thiamine, 3 mg; folic acid, 1.0 mg; D-biotin, 50 μg; Ca-pantothenate, 1 mg; nicotinic acid, 50 mg; choline chloride, 600 mg.

morning feeding. The feed consumption (DM basis) was calculated daily by the difference between offered and refused feed before morning feeding. Feed conversion efficiency was calculated as kilograms of body weight gain to kilograms of feed consumed (DM basis). Water consumption was measured daily and a bucket with the same amount of water offered was placed in the animal shed to estimate water evaporation for the calibration of water consumption.

2.6. Blood chemistry

Four lambs per treatment were randomly chosen for blood sample collection from each treatment group at day 35 and day 70. About 10 mL of blood from each lamb was collected into a clean dry tube (BD Vacutainer® Tubes, Franklin Lakes, NJ, USA) from the jugular vein immediately before morning feeding. Heparin was used as an anticoagulant. Heparinized blood samples were centrifuged at 4000*g* at 4 °C for 20 min. Plasma was separated into clean dried glass vials and frozen at −20 °C for later analysis.

2.7. Slaughter procedure

All lambs were slaughtered after being on the experimental diets for 70 days. Prior to slaughter, lambs were fasted for 18 h and then slaughtered at a commercial slaughterhouse. Slaughter body weight was obtained for all lambs immediately after death. Also, the hot carcass weight including tail fat and kidney fat was determined following dressing. Heads, legs, lungs, hides, hearts, livers, and full digestive tract were weighed. Tail and kidney fat were left on the carcass. Weight of gut content was subtracted from slaughter weight to obtain the empty body weight; dressing was expressed as a percent of slaughter weight. The best ribs (9th–11th ribs) were separated from the right side of each carcass and analysed for meat, bone and fat proportions. Meat samples were ground through a 4 mm sieve. From this, 30–40 g of a mixed sample from all lambs in each treatment was placed in a plastic bag and stored at −20 °C for chemical analysis.

2.8. Ruminal fermentation parameters

Rumen samples were collected at slaughter, according to the method described in Ahmed et al. (2015) and Kholif et al. (2015). About 100 mL of rumen contents was collected and strained through four layers of cheesecloth. Ruminal pH was immediately determined using a digital pH metre (GLP 22, Crison Instruments, Barcelona, Spain). Strained rumen samples were placed into 45-mL glass bottles with a few drops of toluene and paraffin oil added to just cover the surface, prior to storage at −18 °C for ammonia–N and volatile fatty acid (VFA) analyses.

2.9. Histological parameters

According to the method of Culling (1983), immediately after necropsy, tissue specimens from the small intestine (ileum) were rapidly fixed in 10% neutral buffered formalin solution for at least 24 h. The fixed specimens were processed through the conventional paraffin embedding technique of dehydration through ascending grades of ethanol, cleared in chloroform and embedded in paraffin wax at 60 °C. Paraffin blocks were prepared, from which 7 μm thick sections were cut on a freeze microtome (Thermo

Table 2
Chemical composition (g/kg DM) of the experimental diets containing *Atriplex nummularia* and *Acacia saligna* (1:1 DM) treated with *Trichoderma reesei* or *Saccharomyces cerevisiae*.

	Forages source			Experimental diets[a]			
	Berseem hay	*Atriplex nummularia*	*Acacia saligna*	Control	AU	AF	AS
Organic matter	902	754	863	890	872	804	872
Crude protein	167	189	176	155	161	170	161
Ether extract	20	11	12	17	18	18	18
Non-structural carbohydrates[b]	294	89	236	439	390	245	390
Neutral detergent fibre	421	465	439	126	145	371	145
Acid detergent fibre	281	265	238	232	229	215	229
Secondary compounds							
Total phenolics		113	61				
Saponins		124	24				
Alkaloids		2.3	3.2				
Aqueous fraction[c]		475	68				

[a] The diets contained 30% berseem hay+70% concentrate mixture (Control), AU=15% *Atriplex*+15% *Acacia*+70% concentrate mixture (negative control); AF=AU treated with *Trichoderma reesei*, and AS=AU supplemented with *Saccharomyces cerevisiae* as a probiotic at 0.5 g/ kg DM of feed.
[b] Non-structural carbohydrates calculated by difference [100−(%NDF+%CP+%EE+%ash)].
[c] Aqueous fraction (lectins, polypeptides and starch).

Scientific™ HM 325 Rotary Microtome, Waltham, MA, USA). These sections were stained with Haematoxylin and Eosin before microscopic examination.

2.10. Chemical analyses

Conventional analysis of feed samples was carried out according to AOAC (1997) for DM, ash, N and ether extract (EE). The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed by the method of Van Soest et al. (1991) using ANKOM^{200} Fibre Analyser unit (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed without use of an alpha amylase but with sodium sulphite. Both NDF and ADF are expressed without residual ash. Proximate analysis of meat samples was according to AOAC (1997).

The secondary metabolite concentrations of both plants were determined as previously described by Salem et al. (2014).

Ruminal ammonia–N concentration was determined according to the method of Gips and Wibbens-Alberts (1968). Ruminal VFA concentration was determined as described by Warner (1964).

Blood plasma samples were analysed calorimetrically for total protein, albumin, urea–N, glucose, total cholesterol, high density lipoproteins (HDL) and low density lipoproteins (LDL), using specific kits obtained from Stanbio Laboratory (Boerne, Texas, USA) according to the procedure outlined by the manufacturer. Globulin concentration was calculated by subtracting the values of albumin from those of total proteins.

2.11. Statistical analysis

Dry matter intake, water consumption, daily weight gain and feed conversion efficiency were analysed with diet type, period (as repeated measurements) and their interaction (diet × period) as the experimental factors having fixed effects using PROC MIXED of SAS (SAS Inst. Inc. Cary, NC, USA, 2002) in two-way ANOVAs according to the following statistical model:

$$Y_{ijk} = \mu + D_i + T_j + (D \times T)_{ij} + A_k + e_{ijk}$$

where Y_{ijk} is the dry matter intake, water consumption, daily weight gain or feed conversion efficiency, respectively, μ is the overall mean, D_i is the fixed effect of diet ($i=4$), T_j is the fixed effect of period ($j=5$), $(D \times T)_{ij}$ is the fixed effect of interaction between diet and period, A_k is the random effect of animal ($k=9$), and e_{ijk} is the random residual error. Significance was declared at a level of $P<0.05$ and trend at $P \leq 0.10$.

The remaining data obtained were analysed using one-way ANOVAs according to the following statistical model:

$$Y_{ij} = \mu + D_i + A_j + e_{ij}$$

where Y_{ij} is the observation, μ is the overall mean, D_i is the fixed effect of diet, A_j is the random effect of animal, and e_{ij} is the random residual error. The comparisons among treatments were performed with Duncan's multiple range test.

3. Results

3.1. Feed intake, water consumption and body weight gain

No interaction occurred ($P>0.05$) between period × diet for DM intake, water consumption and daily weight gain; however, significant interaction in feed conversion efficiency did occur. Lambs in the AS group consumed less feed than those in the AF group ($P<0.05$) with no differences ($P>0.05$) between AF, AU and Control lambs (Table 3).

Lambs fed different diets consumed the same amount of water and achieved the same daily weight gain ($P>0.05$) during the whole experiment (Table 3).

Significantly decreased feed conversion efficiency was observed ($P<0.05$) in lambs from the AF and AU treatment groups compared to the Control and AS groups. There was no significant difference ($P>0.05$) between the AS and Control treatment groups (Table 3 and Fig. 1).

3.2. Ruminal fermentation and blood parameters

Lambs in the AF and AS treatment groups had higher VFA concentrations than the Controls. There were no differences in ruminal ammonia–N between groups (Table 4).

As shown in Table 4, interactions between diet × period occurred ($P<0.01$) for urea with no significant interactions ($P>0.05$) with other serum parameters. Lambs in the AS treatment had more serum albumin (after 10 weeks) and urea (after 5 weeks) compared to Control lambs. Increased globulin (after 5 weeks) with decreased cholesterol (after 5 weeks) and glucose concentrations (after 10 weeks) being observed in the AF group lambs as compared to Control lambs. No effect was observed ($P>0.05$) between any group of lambs in terms of blood total protein, LDL and HDL (data not shown).

3.3. Carcass characteristics

No significant differences were observed ($P>0.05$) between treatments in slaughter body weight, empty and full alimentary tract, gut content, empty body weight and carcass weights. Moreover, organ weights of tail, trachea, feet, head, mesentery weights, pelt, spleen, liver, heart and heart fat were the same in all lambs. Lambs in the AS and AF groups had a significantly higher dressing percentage compared to the Control lambs. In addition, no differences were observed ($P>0.05$) between different

Table 3
Intake, daily body weight gain and feed conversion efficiency of growing Barki lambs fed experimental diets containing *Atriplex nummularia* and *Acacia saligna* (1:1 DM) treated with *Trichoderma reesei* or *Saccharomyces cerevisiae*.

	Experimental diets[1]				SEM	*P* value		
	Control	AU	AF	AS		Diet	Period	Diet × period
DM intake (g/lamb/day)	1043^{ab}	1019^{ab}	1070^{a}	943^{b}	33.6	0.037	< 0.001	0.408
Water consumption (L/lamb/day)	3.4	3.9	3.8	3.8	0.15	0.075	0.954	1.000
Daily weight gain (g/lamb/day)	150	139	144	149	5.6	0.470	0.005	0.661
Feed conversion efficiency (kg live weight gain/kg feed)	0.134^{a}	0.117^{b}	0.111^{b}	0.137^{a}	0.0042	0.001	< 0.001	< 0.001

^{a,b}Means within the same row with different superscripts differ significantly among treatments ($P<0.05$).

[1] The diets contained 30% berseem hay + 70% concentrate mixture (Control), AU = 15% *Atriplex* + 15% *Acacia* + 70% concentrate mixture (negative control); AF = AU treated with *Trichoderma reesei*, and AS = AU supplemented with *Saccharomyces cerevisiae* as a probiotic at 0.5 g/ kg DM of feed.

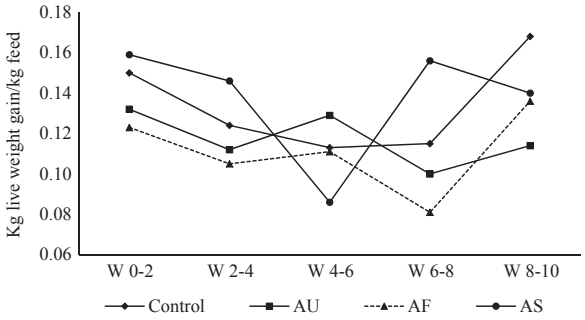

Fig. 1. Feed conversion efficiency of Barki lambs fed on the experimental diets across the experimental period from week 0 to week 10. SEM=0.004; $P<0.001$. Control=30% berseem hay+70% concentrate mixture, AU=15% *Atriplex*+15% *Acacia*+70% concentrate mixture (negative control); AF=AU treated with *Trichoderma reesei*, and AS=AU supplemented with *Saccharomyces cerevisiae* as a probiotic at 0.5 g/kg DM feed.

treatment groups in the chemical composition of the meat or for physical composition, with the exception of intramuscular fat where lambs in the AF, AS and AU groups had significantly less intramuscular fat compared to the Control lambs (Table 5).

3.4. Intestinal histopathology

Lambs fed the halophytic diets (i.e. in treatments AU, AF and AS) showed a normal intestinal histology when compared to Control lambs. All lambs showed normal histology of the ileum, sub mucosa and Peyer's patches. In comparison to the Control lambs (Fig. 2), microscopic examination of the ileum of AU group lambs (Fig. 3), AF group lambs (Fig. 4), AS group lambs (Fig. 5), did show some minor differences. The main difference being a reduction in the height of the ileal villi.

4. Discussion

4.1. Feed intake and water consumption

Throughout the experiment lambs fed the AS diet consumed less feed than lambs in the AF treatment. However, the AF diet had a lower (about 8%) OM content than the AS diet. This level of ash may be acceptable for ruminal microbial activity and is a physiologically acceptable concentration. It was expected that feeding halophytes would decrease intake as a result of high concentrations of secondary plant metabolites, such as tannins, phenolics, steroids, cyanogenic substances and alkaloids that are known to decrease palatability (Moujahed et al., 2005). However, this was not observed in the current study, suggesting that palatability was not an issue. The fungal treatment is the most likely reason for the increased intake by lambs in the AF group compared to those receiving the AS diet. Improved feed intake and nutritive value from the halophytic plants may be a result of reduced anti-nutritional factors, through the removal or degradation of secondary plant compounds by the fungus (Fayed, 2009). Fungal treatment could, therefore, improve the potential for using halophytic plants as substitutes for more conventional feed sources.

Table 4
Ruminal fermentation parameters and blood chemistry of growing Barki lambs fed experimental diets containing *Atriplex nummularia* and *Acacia saligna* (1:1 DM) treated with *Trichoderma reesei* or *Saccharomyces cerevisiae*.

	Experimental diets[1]				SEM	*P* value		
	Control	AU	AF	AS		Diet	Period	Diet × period
Ruminal fermentation parameters								
Volatile fatty acids (mmol /100 mL)	13[b]	14[ab]	15[a]	15[a]	0.8	0.010		
Ammonia–N (mg /100 mL)	114	115	136	138	10.1	0.249		
Blood chemistry (mg/dL)								
Albumin								
After 5 weeks	2.8	3.0	3.1	2.8	0.18	0.598		
After 10 weeks	2.7[b]	2.9[b]	2.8[b]	3.2[a]	0.11	0.012		
SEM	0.10	0.12	0.18	0.17				
P value	0.542	0.445	0.322	0.103		0.309	0.893	0.109
Globulin								
After 5 weeks	3.2[ab]	2.6[b]	3.8[a]	3.3[ab]	0.23	0.011		
After 10 weeks	3.4	2.9	2.9	3.2	0.21	0.417		
SEM	0.13	0.21	0.28	0.23				
P value	0.447	0.340	0.046	0.766		0.042	0.396	0.052
Cholesterol								
After 5 weeks	90[a]	80[a]	77[b]	79[a]	3.1	0.031		
After 10 weeks	97[a]	95[a]	94[a]	85[b]	2.7	0.017		
SEM	3.2	3.4	2.5	2.4				
P value	0.116	0.008	0.004	0.084		0.002	<0.001	0.217
Urea								
After 5 weeks	33[c]	39[b]	45[b]	59[a]	1.5	<0.001		
After 10 weeks	32[c]	53[b]	75[a]	52[b]	2.3	<0.001		
SEM	1.1	1.9	2.2	2.3				
P value	0.459	0.001	<0.001	0.068		<0.001	<0.001	<0.001
Glucose								
After 5 weeks	101	94	88	88	4.2	0.108		
After 10 weeks	65[a]	61[ab]	53[b]	70[a]	3.1	0.003		
SEM	3.7	4.3	3.4	3.1				
P value	<0.001	<0.001	<0.001	0.007		0.009	<0.001	0.053

[a,b,c]Means within in the same row with different superscripts differ significantly among treatments ($P<0.05$).

[1] Diets contained 30% berseem hay+70% concentrate mixture (Control), AU=15% *Atriplex*+15% *Acacia*+70% concentrate mixture (negative control); AF=AU treated with *Trichoderma reesei*, and AS=AU supplemented with *Saccharomyces cerevisiae* as a probiotic at 0.5 g/kg DM of feed.

Table 5
Carcass characteristics, physical and chemical composition of the best ribs of growing Barki lambs fed experimental diets containing *Atriplex nummularia* and *Acacia saligna* (1:1 DM) treated with *Trichoderma reesei* or *Saccharomyces cerevisiae*.

	Experimental diets[1]				SEM	*P* value
	Control	AU	AF	AS		
Carcass characteristics						
Slaughter body weight (kg)	37	37	37	37	1.2	0.352
Alimentary tract full (kg)	6.2	6.5	7.4	6.3	1.49	0.165
Alimentary tract empty (kg)	2.4	2.3	2.3	1.9	0.16	0.624
Gut content (kg)	4.0	4.2	5.1	4.4	0.40	0.524
Empty body weight (kg)	27	27	27	27	1.3	0.851
Carcass weight (kg)	15	15	15	16	0.6	0.163
Dressing percentage						
Based on slaughter weight	40[b]	40b	42[a]	42[a]	0.4	0.045
Based on empty body weight	54[c]	55[b]	57[b]	58[a]	1.6	0.010
Physical composition of the 9th–11th ribs (kg)						
Best ribs weight	0.41	0.35	0.35	0.41	0.025	0.234
Meat weight	0.21	0.18	0.19	0.23	0.020	0.356
Intramuscular fat weight	0.10[a]	0.08[b]	0.05[c]	0.06[b]	0.001	0.010
Bone weight	0.10	0.09	0.11	0.12	0.080	0.452
Best ribs area highest (mm)	37	35	35	39	2.4	0.245
Best ribs area width (mm)	55	47	47	53	2.3	0.345
Chemical composition of the 9th-11th ribs (g/kg)						
Dry matter	516	486	484	502	29.1	0.423
Protein	194	189	185	183	8.4	0.358
Ether extract	282	317	324	307	25.7	0.426
Ash	7.8	7.9	7.6	8.2	0.65	0.125

[a,b,c]Means within in the same row with different superscripts differ significantly among treatments ($P < 0.05$).

[1] The diets contained 30% berseem hay+70% concentrate mixture (Control), AU=15% *Atriplex*+15% *Acacia*+70% concentrate mixture (negative control); AF=AU treated with *Trichoderma reesei*, and AS=AU supplemented with *Saccharomyces cerevisiae* as a probiotic at 0.5 g/ kg DM of feed.

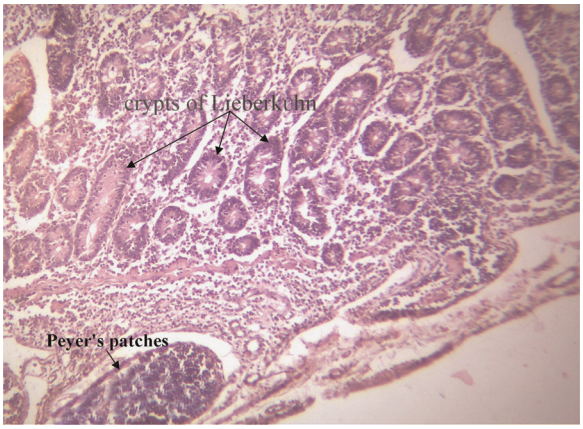

Fig. 2. Photomicrograph of ileum of Control Barki lambs stained with hematoxylin and eosin (160 ×): Normal histology.

In addition, feeding lambs diets that contain *Atriplex* or *Acacia* was expected to increase water consumption of the lambs due to the high content of salt in *Atriplex* and *Acacia* compared to berseem hay (Ahmed et al., 2015). However, there was no significant difference between groups, which may indicate an ability of lambs to adapt to the concentrations of salt in the feed on offer, or that the salt concentration was at an acceptable concentration.

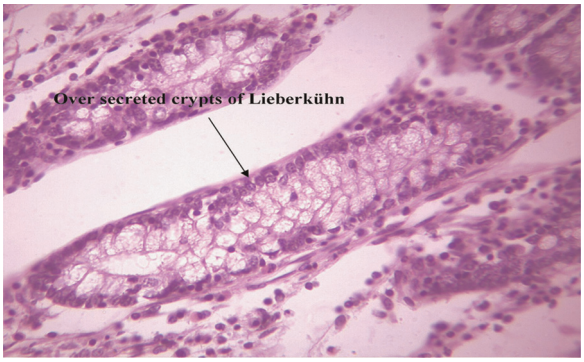

Fig. 3. Photomicrograph of ileum of AU Barki lambs stained with hematoxylin and eosin (160 ×): Normal histology.

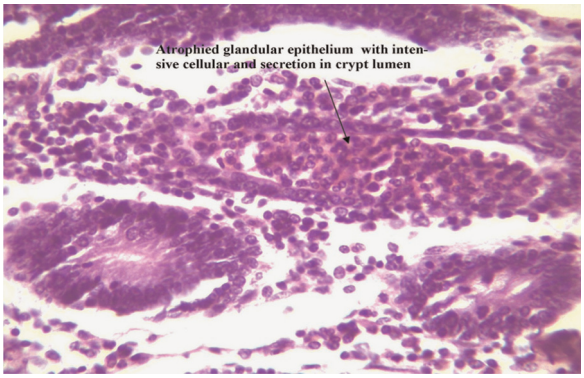

Fig. 4. Photomicrograph of ileum of AF Barki lambs stained with hematoxylin and eosin (160 ×): Normal histology.

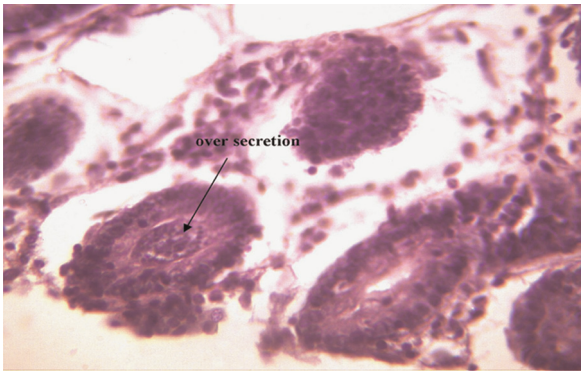

Fig. 5. Photomicrograph of ileum of AS Barki lambs stained with hematoxylin and eosin (160 ×): Normal histology.

4.2. Rumen fermentation

Improved ruminal fermentation in terms of VFA production was observed with the AF and AS treatments compared to Control. This suggests that the fermentation of diets with *T. reesei* or supplemented with *S. cerevisiae* was more efficient and produced more VFA than in the Control diet. Fayed (2009) showed that the treatment of *A. saligna* and *Tamarix mannifera* mixture with white rot fungi (*Pleurotus ostreatus* and *Pleurotus florida*) improved utilization and improved VFA concentrations of Barki rams.

4.3. Blood chemistry

The examination of blood parameters is recommended as a good way of assessing the health status of animals and the physiological, nutritional and pathological status of farm animals

(Etim et al., 2013). Lambs in the AS treatment group had increased serum albumin and urea concentrations compared to Control lambs which reflects better protein digestion and utilization in these lambs. This is likely a result of higher protein content in these diets (halophytes *versus* berseem hay; Ahmed et al., 2015) and also a result of yeast addition (Elghandour et al., 2015). Increased plasma urea concentrations with diets containing halophytes (i.e., AS, AF, AU) possibly reflects the biodegradation of protein within the rumen (Kholif et al., 2014); however, ruminal ammonia–N concentration did not differ between treatments. Lambs in the AF group had lower cholesterol concentrations. Fayed et al. (2010) showed that blood cholesterol was reduced significantly with lambs fed an *Atriplex* and *Acacia* mixture treated with white rot fungus. These findings were attributed to the high tannin and saponins content of *Atriplex* and *Acacia* (Bravo et al., 1993). Saponins have been shown to be cholesterol absorption inhibitors causing a reduction in plasma cholesterol (Morehouse et al., 1999).

4.4. Growth performance and feed conversion efficiency

There were no differences between treatment groups in terms of liveweight change. An expected negative outcome of feeding *Atriplex* and *Acacia* on body weight gain, due to the presence of anti-nutritional factors, did not materialise (Ahmed et al., 2015; Alsersy et al., 2015).

Decreased feed conversion efficiency was observed with AU and AF lambs compared to AS and Control lambs. This is because AS and Control lambs consumed less feed while achieving almost the same daily gain as the other lambs. Improved feed conversion efficiency reflects better feed utilization with lambs in AS and Control groups compared to the other lambs. Increased feed conversion efficiency has also been observed in lambs fed *A. nummularia* at 15% of the diet and replacing the same amount of berseem hay (Ahmed et al., 2015).

4.5. Carcass characteristics

Increased dressing weight percentages (based on empty body weight) were obtained with the lambs fed halophytes. Mousa (2011) found that the dressing weight percentage of lambs fed experimental diets containing *Atriplex* and/or *Acacia* was higher than that of lambs fed diets without halophytes. Lambs fed the AS diet had an increased dressing percentage compared with lambs fed the AU diet. This result indicates that yeast may have a positive role in improving dressing percentage (Elghandour et al., 2015).

The physical characteristics of the best ribs (the 9th–11th ribs), with the exception of intramuscular fat, was not affected by the treatments imposed. The most likely reason for the reduction in fat deposition in lambs fed halophytes is the high protein:energy ratio, but may also be associated with secondary compounds in halophytes (Pearce et al., 2010). A high dietary protein:energy ratio will result in less fat deposition (Campbell, 1988). Al-Owaimer et al. (2008) found that lambs fed a complete diet containing *Atriplex leucoclada* had a significantly lower muscle fat percentage than those fed a control diet of alfalfa hay.

4.6. Intestinal histopathology

The main difference observed between diets was a reduction in the height of the ileal villi in lambs fed AU, AF and AS diets. Robins and Brooker (2005) stated that a marked increase in villous height and crypt depth were recorded for abomasal and intestinal samples from sheep fed *Acacia aneura*. It was clear that the feeding of untreated or treated *Atriplex* and *Acacia* led to minimal changes associated with over secretion of the intestinal glands, which may reduce the absorptive surface in the digestive tract. In this respect, Ahmed et al. (2015) observed that lambs fed halophytes (*A. nummularia* and/or *A. saligna*) instead of 50% of berseem hay (15% of the diet) showed a normal histology of ileum, sub-mucosa and Peyer's patches.

5. Conclusion

The untreated halophyte mixture of *A. nummularia* and *A. saligna* (at 1:1 DM) can be substituted for berseem hay without loss of performance, while treatment with *S. cerevisiae* may improve performance and, like *T. reesei*, change certain biochemical responses. Treatment with *T. reesei* was more effective than *S. cerevisiae* supplementation for treating the halophyte mixture. However, a contribution to the improvement in the AF group could be attributed to autoclaving and drying the feed as the high temperatures and pressures may have altered some feed components.

Conflict of interest

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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