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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 $8 \times 10^8$ 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 $27.0 \pm 0.89$ kg and final BW $37.2 \pm 0.43$ 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