

Effect of macroalgae and yeast culture on body performance, blood metabolites, ruminal fermentation and digestibility coefficients of Ossimi lambs

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Abstract

This study was carried out to evaluate the effect of macroalgae (*Halimeda opuntia*) and yeast culture (*saccharomyces cerevisiae*) as feed additives on body performance, some blood plasma constituents, some rumen parameters and nutrients digestibility of growing lambs. Fifteen Ossimi lambs 5-6 months old and 25.51 ± 2 kg live body weight were randomly divided into three equal groups (5 animals each). Control group (CON) was fed the basal diet and other two treatment groups were fed the same basal diet, supplemented with either 1% macroalgae powder (ALG) or 1% yeast culture (YC). Lambs were weighed to determine performance of growing lambs and adjusted the requirement. Blood samples were collected monthly from all animals before morning feeding. Rumen samples were collected to determine ruminal pH values, ammonia-N concentration and total volatile fatty acids (TVFAs) concentration. The digestibility trials were carried out to evaluate nutrients digestibility of the different experimental rations. Final body weight and total gain not affected by treatments. Lambs of ALG group had the worst feed conversion ratio compared with YC and CON group. Ruminal pH, ammonia-N concentration and TVFAs were not affected by treatments. Dietary yeast or macroalgae increased ($P < 0.05$) blood plasma total protein and albumin concentrations, while decreased ($P < 0.05$) plasma urea -N concentration compared with those of control animals. Dietary macroalgae increased ($P < 0.05$) DM, OM, CP, CF and NFE digestibility compared with YC and CON groups. In conclusion, macroalgae and yeast culture as feed additives may have a beneficial effect on nutrients digestibility and blood metabolites of Ossimi sheep male.

Keywords: macroalgae, *Halimeda opuntia*, *Saccharomyces cerevisiae*, rumen fermentation.

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1. Introduction

Algae are organisms that can range in size, from microscopic microalgae to large macroalgae. The most common algae are red, brown and green algae. Macroalgae do not require fertilizers, pesticides, or freshwater (Chojnacka *et al.*, 2012). Macroalgae can be used to improve basal feed quality because they are rich in primary metabolites essential to metabolic function as minerals, vitamins, proteins, lipids and polysaccharides that (MacArtain *et al.*, 2007; Marin *et al.*, 2009; Rjiba Ktita *et al.*, 2019). Macroalgae are one of the richest sources of calcium (7 % of the dry weight, Singh *et al.*, 2016). They are also rich source of other minerals like sodium, potassium, magnesium, chlorine, sulfur, phosphorus, iodine, iron, zinc, copper, selenium and molybdenum (Archer *et al.*, 2008; Holdt and Kraan, 2011; Rey-Crespo *et al.*, 2014; Ventura and Castañón, 1998). High mineral content makes it a potential additive to animal feedstuffs for replacing a part or whole of the mineral supplementation (Singh *et al.*, 2017). Macroalgae have anti-bacterial, anti-viral, antioxidant, and anti-inflammatory properties that enhance animal health and function (Bach *et al.*, 2008) because, they contain many biologically active compounds such as fucoidan, betaine, and glucans (Archer *et al.*, 2008; Holdt and Kraan, 2011), which enhance animal's immunity and carcass quality (Singh *et al.*, 2017). Yeast culture (*Saccharomyces cerevisiae*) one of the most common probiotics in ruminants (Raghebian *et al.*, 2016). Yeast culture has displayed positive impact on the growth and viability of rumen microflora through encouraging

cellulolytic bacterial growth within the ruminal environment (Ovinge *et al.*, 2018; Swyers *et al.*, 2014). Live yeast consumes free oxygen in the rumen with respiration, so provides an anaerobic environment that proper for rumen metabolic function (Newbold *et al.*, 1996). Inclusion of yeast culture to ruminant diets has improved fiber digestibility (Dawson *et al.*, 1990), increased protozoal count (Singh *et al.*, 2008). The aim of this study to evaluate the effect of macroalgae (*Halimeda opuntia*) as natural mineral source and yeast culture (*Saccharomyces cerevisiae*) as feed additives on body performance, ruminal fermentation, and digestibility of Ossimi lambs.

2. Materials and methods

This study was conducted at the Research Farm of Animal Production Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. The laboratory measurements conducted at the Animal Production Department laboratory at the same faculty to evaluate the influence of macroalgae (*Halimeda opuntia*) and yeast culture (*Saccharomyces cerevisiae*) as feed additives on body performance, ruminal fermentation, and digestibility of Ossimi lambs. The study was divided into two experiments. The first experiment was growing trial while, the second one was digestibility trial.

2.1 Growing trial

2.1.1 Animals management and rations

Growing trial was carried out using fifteen

Ossimi lambs 5-6 months old and about 25.51 ± 2 kg body weight, animals were randomly distributed into three groups, each with five lambs, the trial lasted for 120 days. The lambs in group one was considered as control (CON) which fed total mixed ration which consisting of 65 % concentrate mixture and 35% wheat straw. Concentrate mixture contain 42% yellow corn, 15% soybean meal, 30%

wheat bran, 10% undecorticated cotton-seeds meal, 2% limestone and 1% salt. The second (ALG) and third (YC) groups received the same basal diet supplemented with either 1% macroalgae (*Halimeda opuntia*) or 1% yeast culture (*Saccharomyces cerevisiae*) of total mixed ration, respectively. Ingredients and chemical composition of control and supplemented rations are presented in Table (1).

Table (1): Ingredients and chemical composition of control and macroalgae and yeast culture-treated rations.

Item	Treatment*		
	CON	ALG	YC
Yellow corn	27.3	27.3	27.3
Soybean meal	9.75	9.75	9.75
Wheat bran	19.5	19.5	19.5
Undecorticated Cottonseeds meal	6.5	6.5	6.5
Wheat straw	35	35	35
Macroalge powder (<i>Halimeda opuntia</i>)	—	1	—
Yeast culture (<i>Saccharomyces cerevisiae</i>)	—	—	1
Limestone	1.3	1.3	1.3
Salt	0.325	0.325	0.325
Minerals	0.325	0.325	0.325
Chemical composition			
Dry Matter %	92.12	92.12	92.12
Organic matter %	90.97	90.58	90.81
Ash %	9.03	9.42	9.19
Crude protein %	14.55	14.73	14.85
Crude fiber %	18.08	17.80	17.72
Ether extract %	2.99	3.26	3.18
NFE %	55.34	54.79	55.06

*CON=basal diet, consisted of 65% concentrate mixture and 35 % wheat straw; ALG = basal diet supplemented with 1% macroalgae (*Halimeda opuntia*) powder and YC = basal diet supplemented with 1% yeast culture (*Saccharomyces cerevisiae*) of total mixed ration.

Ration was fed to the treated group as follow: First group, (CON) lambs fed basal diet (total mixed ration). Second group, (ALG). Lambs fed basal diet supplemented by 1% of macroalgae (*Halimeda opuntia*). Third group, (YC). lambs fed basal diet supplemented by 1% of yeast culture. The rations were

formulated to satisfy sheep requirements according to NRC omitted (1985). Daily ration (total mixed ration) was divided into two equal parts and offered at 8.00 a.m. and 3.00 p.m. daily. Fresh water was available all the day. The chemical analysis of feed samples and residuals was carried out according to methods of

AOAC (1990). Nitrogen-free extract and organic matter were calculated by difference.

2.1.2 Blood sampling and analysis

At the beginning of the experiment and every month during the experimental period about 10 ml of blood were collected from the jugular vein from each animal within each group before morning feeding. Blood samples were directly collected into clean dried glass culture tubes with EDTA (Ethylene Diamine Tetra Acetic Acid) which work as anticoagulant then centrifuged at 4000 rpm for 20 minutes; blood plasma was separated into a clean dried glass vial and stored at -20°C till chemical analysis. Plasma metabolites: total protein, albumin, (globulin calculation was obtained as the difference between total protein and albumin concentration), cholesterol, and urea were done according to the methods described by Gornall *et al.* (1949). Webster (1974), Allain *et al.* (1974) and Tabacco *et al.* (1979), respectively.

2.1.3 Rumen liquor

At the end of growing trial, rumen samples were collected from lambs using a stomach tube. Samples were taken 2 hrs after morning feeding. Rumen liquor samples were filtrated through 3 layers of cheese cloth. Rumen pH values were immediately determined after collection of rumen liquor using digital pH meter (Beckman, model 45, USA). Strained

rumen liquor was stored in blastic bottles (100 ml) with few drops of toluene and paraffin oil just to cover the surface and stored at a deep freeze (-18°C) till chemical analysis. Ruminant ammonia-N concentration was determined according to AOAC (1990) and total VFA's concentration was determined according to Warner (1964).

2.2 Digestibility trials

The digestibility trials were carried out to evaluate nutrients digestibility of the different experimental rations.

2.2.1 Animals and rations

Three mature rams about 50 ± 2.5 kg body weight from each group were randomly selected for the digestion trial. Animals were individually placed in metabolic cages for 21 days, the first 14 days as a preliminary period followed by 7 days as a collection period. Daily rations offered and residuals were recorded every day. Faeces were collected daily and 10% of total weight was dried at 60°C for 24 hrs then it was mixed and ground for the chemical analysis.

2.2.2 Chemical Analysis and digestion coefficients calculated

Samples of feed ingredient and faeces were analyzed for dry matter, ash, crude protein, crude fiber and ether extract according to methods of AOAC (1990). Nitrogen-free extract and organic matter were calculated by difference. The

apparent digestion coefficients of nutrients were calculated by expressing the difference between the content of nutrients in both consumed feed and faeces as a percentage of its intake.

2.3 Statistical analysis

Data were statistically analyzed using general linear model (G.L.M) procedure of S.A.S (2002). For body weight change, nutrients digestibility, rumen liquor parameters and feed conversion ratio the following model was used, $Y_{ij} = \mu + T_i + E_{ij}$, Where, Y_{ij} = experimental observation, μ = general mean, T_i = the effect of treatment, $i = T_1, T_2$ and T_3 and E_{ij} = the errors related to individual observation. The blood parameters were analyzed according to the following statistical model:

$$Y_{ijk} = \mu + T_i + B_j + (BA)_{ij} + e_{ijk}$$

Where, Y_{ijk} = experimental observations, μ = general mean, T_i = the effect of treatment, $i = T_1, T_2$ and T_3 , B_j = the effect

of time blood sampling, $(BA)_{ij}$ = interaction between time and treatments and e_{ijk} = the errors related to individual observation. The significance differences among treatment means were tested by (Duncan`s multiple range test 1995).

3. Results and Discussion

3.1 Growing trial

3.1 Performance of growing lambs

The effect of treatments on performance of growing lambs is presented in Table (2). The data revealed that the final body weight and total gain were similar in all groups, that means there was no significant difference among groups. However, dietary yeast tended to increase daily gain compared to other treatment groups. Feed conversion ratio was significantly ($P < 0.05$) improved in favor of YC and control groups as compared with ALG group.

Table (2): Effect of dietary dietary macroalgae and yeast culture on performance of growing lambs.

Item	Treatment*			
	CON	ALG	YC	SEM
Initial body weight (kg)	25.88	25.51	25.15	0.303
Final body weight (kg)	48.01	47.54	48.21	0.317
Total feed intake (kg)	151.74	148.15	156.58	
Total gain kg	22.13	22.03	23.06	0.243
Duration of the experiment	120 days			
Daily gain (g/d)	184.38	183.58	192.14	1.012
TDN	66.56 ^b	70.25 ^a	67.11 ^b	0.426
Feed conversion (kg TDN /kg gain)	4.575 ^b	4.726 ^a	4.571 ^b	0.065

^{a,b,c} Means with the same letter within rows are not significantly different. SEM= standard error of means, *CON=basal diet, consisted of 65% concentrate mixture and 35 % wheat straw; ALG = basal diet supplemented with 1% macroalgae (*Halimeda opuntia*) powder and YC = basal diet supplemented with 1% yeast culture (*Saccharomyces cerevisiae*) of total mixed ration.

These results are in agreement with the results obtained by Bach *et al.* (2008), found that dietary macroalgae (*Ascophyllum nodosum*) at level of 10 gm/kg diet had no effect on body weight and average daily gain in steers. Also, Samara *et al.* (2013) and Abdoun *et al.* (2014) found that dietary macroalgae had no effect on body weight and average daily gain of growing lambs fed diet supplemented with green macroalgae *Ulva lactuca* at levels of 3% and 5 %. Also, Macedo *et al.* (2006) found that yeast culture had no significant effect on body weight and average daily gain of growing lambs. Also, Soren *et al.* (2012), Pienaar *et al.* (2012), and Hamdon and Farghaly (2016) found that yeast culture at levels of 1.5%, 0.5% and 1% had no effect on body weight and average daily gain in growing lambs.

3.1.2 Rumen fermentation

The effect of treatments on rumen liquor pH, Ammonia-N concentrations and total volatile fatty acids concentration are presented in Table (3). Results indicated

that there were no significant differences among groups in rumen liquor pH values, ammonia-N and total volatile fatty acids concentration. These results are in agreement with many author's results *i.e.* Tripathi and Karim (2011) indicated that rumen ammonia-N concentration and total volatile fatty acids concentration were not affected by yeast culture supplementation at level 1gm/kg live weight of growing lamb. Also, Soren *et al.* (2012) found yeast culture (at level 1.5%) had no effect on rumen fluid pH values and total volatile fatty acids concentration. These results are in agreement with the result obtained by Zhou *et al.* (2018) they found that brown macroalgae (*Ascophyllum nodosum*) supplementation at levels of 1% and 3% had no effect on total VFA, NH₃-N concentrations and pH of rumen liquor. Similarly, Ead and Maklad (2011) found that ammonia-N concentration and total volatile fatty Acids of rumen liquor were not affected by supplementing fattening Friesian steers` ration by 0.4% and 0.9% of macroalgae.

Table (3): Effect of dietary algae and yeast culture on rumen fermentation of Ossimi lambs.

Item	Treatment*			SEM
	CON	ALG	YC	
pH values	6.770	6.783	6.500	0.052
Total volatile fatty acids (meq/100ml)	11.00	11.33	11.67	0.179
Ammonia-N (mg/dl)	17.46	18.66	17.58	0.361

*CON=basal diet, consisted of 65% concentrate mixture and 35 % wheat straw; ALG = basal diet supplemented with 1% macroalgae (*Halimeda opuntia*) powder and YC = basal diet supplemented with 1% yeast culture (*Saccharomyces cerevisiae*) of total mixed ration.

3.1.3 Blood constituents

The effect of treatments on blood plasma constituents are presented in Table (4). The data revealed that plasma total protein and albumin concentrations increased significantly in treated groups compared with control one with no significant difference between treated groups (ALG

and YC) whereas, there were no significant difference among groups in plasma globulin, albumin: globulin ratio and cholesterol concentrations. On the other hand, plasma urea concentration decreased significantly in treated groups as compared with control one with no significant difference between ALG and YC groups.

Table (4): Effect of dietary macroalgae and yeast culture on some blood plasma constituents of lambs.

Item	Treatments*				Sampling time						Significance		
	CON	ALG	YC	SEM	0 month	1 st month	2 nd month	3 rd month	4 th month	SEM	Diets	Time	D×T
Total proteins	5.88 ^B	6.18 ^A	6.13 ^A	0.039	5.70 ^d	5.96 ^c	6.17 ^b	6.31 ^a	6.19 ^{ab}	0.034	**	***	**
Albumin	2.88 ^B	2.99 ^A	3.00 ^A	0.034	2.75 ^d	2.68 ^d	2.92 ^c	3.07 ^b	3.37 ^a	0.034	**	***	**
Globulin	3.00 ^A	3.19 ^A	3.13 ^A	0.036	2.96 ^b	3.27 ^a	3.25 ^a	3.25 ^a	2.82 ^b	0.039	**	***	**
AL/GL	0.97	0.95	0.98	0.019	0.93 ^b	0.83 ^c	0.90 ^b	0.96 ^b	1.20 ^a	0.022	Ns	***	**
Cholesterol	85.85	85.51	85.74	0.583	92.12 ^a	79.95 ^e	82.16 ^d	84.12 ^c	90.15 ^b	0.483	Ns	***	Ns
Urea	42.99 ^A	38.23 ^B	37.45 ^B	0.574	45.03 ^a	41.55 ^b	39.43 ^c	35.20 ^d	36.56 ^d	0.506	***	***	**

A,B,C,a,b,c,d,e Means with the same letter within rows are not significantly different. SEM= standard error of means **= $P < 0.01$, ***= $P < 0.0001$, NS= non-significant D × T= interaction between diets (D) and sampling times (T), *CON=basal diet, consisted of 65% concentrate mixture and 35% wheat straw; ALG = basal diet supplemented with 1% macroalgae (*Halimeda opuntia*) powder and YC = basal diet supplemented with 1% yeast culture (*Saccharomyces cerevisiae*) of total mixed ration.

The sampling time affected significantly ($p > 0.01$) plasma total protein albumin, globulin, albumin: globulin ratio, cholesterol and urea concentration. The increase of blood total protein and albumin in treated groups may be due to the addition of macroalgae and yeast culture stimulate the development of intestinal microflora resulting in improved feed digestion and utilization of feed nutrients (Karatzia et al., 2012) or may be due to macroalgae supplementation can enhance immune function and overall animal health in lambs (Saker et al., 2004).

3.2 Digestibility trial

Inclusion of macroalgae powder by 1% in

ALG group improved ($P < 0.05$) nutrients digestibility as compared with CON and YC groups (table 5). It could be noticed that, there were improved ($P < 0.05$) in DM, OM, CP, CF and NFE digestibility of ALG group compared to those of other two groups. The improvement in nutrients digestibility in ALG group may be due to that macroalgae are rich in primary metabolites essential to metabolic function as minerals, vitamins, proteins, lipids and polysaccharides that can be used to improve basal feed quality (MacArtain et al., 2007; Marín et al., 2009). Also, such improvement, may be due to that macroalgae increased bacterial number in the rumen which resulted in improvement of nutrients digestibility.

Table (5): Effect of dietary macroalgae and yeast culture on nutrients digestibility coefficients.

Item	Treatment*			SEM
	CON	ALG	YC	
Dry matter %	63.69 ^b	67.72 ^a	64.87 ^b	0.440
Organic matter %	68.37 ^b	72.44 ^a	68.86 ^b	0.485
Crude protein %	65.31 ^c	71.66 ^a	69.22 ^b	0.652
Crude fiber %	54.05 ^b	56.91 ^a	52.40 ^b	0.615
Either extract %	78.36	80.12	78.14	0.581
Nitrogen free Extract %	73.29 ^b	77.07 ^a	73.35 ^b	0.480

^{a,b,c} Means with the same letter within rows are not significantly different. SEM= standard error of means, *CON=basal diet, consisted of 65% concentrate mixture and 35 % wheat straw; ALG = basal diet supplemented with 1% macroalgae (*Halimeda opuntia*) powder and YC = basal diet supplemented with 1% yeast culture (*saccharomyces cerevisiae*) of total mixed ration.

These results are in agreement with the results obtained by Rjiba-Ktita *et al.* (2019), they found that using of seaweeds (*Ruppia* sp.) in the concentrate ration of Barbarine male lambs increased ($P < 0.05$) crude fiber digestibility. Similarly, Ead and Maklad (2011) found that the digestion coefficients of OM, NFE, ADF and cellulose were slightly higher when fattening Friesian steers fed ration supplemented with seaweeds. In contrast our results, some studies found no effect by adding seaweeds on any digestion coefficient *i.e.* Singh *et al.* (2017) found no effect of macroalgae (*Sargassum wightii*) on digestion coefficient of Sahiwal cows. Also, Zhou *et al.* (2018) found that brown seaweed had no effect on the digestibility coefficients of various nutrients in ram. This difference may be due to the difference in the percentage used for macroalgae.

4. Conclusion

It could be concluded that macroalgae and yeast culture as feed additives may have a

beneficial effect on blood metabolites and digestion coefficients of Ossimi lambs.

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