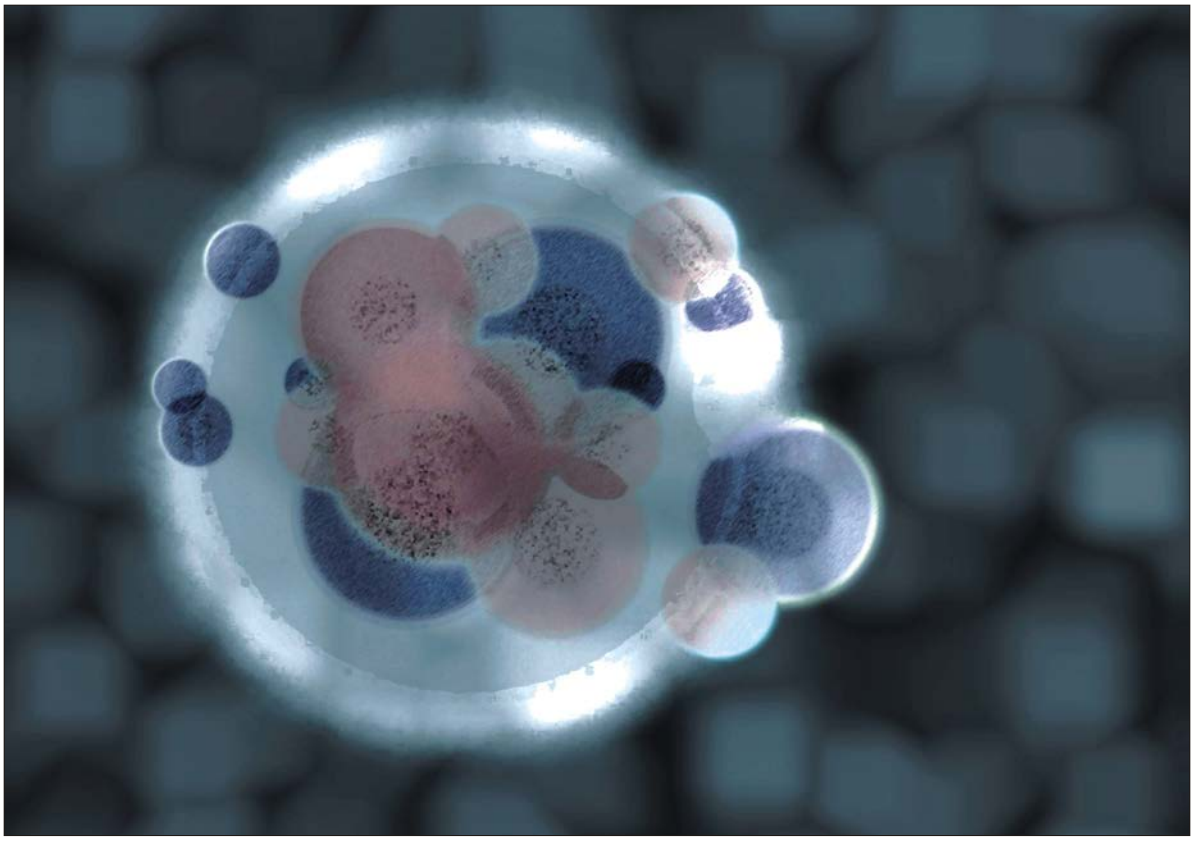

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Research Article
Yeast and *Trichoderma viride* Don't Synergistically Work to Improve Olive Trees by Products Digestibility and Lactating Barki Ewe's Productivity

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Abstract

Background and Objective: Olive trees by products (OTB) as agro-waste not efficiently used and left it without treatment may cause serious economical, social and environmental problems. Biological treatments for such wastes can upgrade their nutritive values to be used as alternative feeds for ruminants. Investigate if their synergism between *T. viride* and *S. cerevisiae* and impact of each of them or their mixture on OTB digestibility and lactating Barki ewe's productivity are the main objectives of this study. **Materials and Methods:** Early lactating Barki ewes were randomly assigned into four groups of seven animals each using complete random design. Ewes were fed (4% of their body weight DM), 70% concentrate feed mixture (CFM)+30% untreated OTB (control group), 70% CFM+30% OTB treated with *Trichoderma viride* (R_1), 70% CFM+30% OTB treated with *Saccharomyces cerevisiae* (R_2) and 70% CFM+30% OTB treated with *T. viride*+*S. cerevisiae* (R3). **Results:** No synergism was noted between *T. viride* and *S. cerevisiae* on the tested parameters. Ruminal total volatile fatty acids and NH_3-N concentrations, microbial protein synthesis and total protozoa count were higher in treated groups than control. Biological treatments increased ($p<0.05$) all nutrients digestibility, fiber fractions digestibility, milk production and milk components yields. Blood serum globulin, urea, ALT and AST concentrations were not change among all ewes groups, while biologically treated ewes had higher ($p<0.05$) serum total protein and albumin than those of control. **Conclusion:** Inclusion of biologically treated olive tree by products (OTB) in lactating ewe's rations improved their productive performance with no deleterious effects on the treated animal's health.

Key words: *T. viride*, *S. cerevisiae*, biological treatments, olive trees by products, lactating ewes

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There is a big gap between available and required feed ingredients for livestock animal's feeding in Egypt. This shortage in feedstuffs estimated to be around 3.1 million tons of total digestible nutrients/year[1]. In addition, roughage feed resources represent the backbone of small ruminant's nutrition in Egypt[2]. The continuous increase of traditional roughages prices and low productivity of local animals encouraged the nutritionists for searching for alternative feed resources[3]. In this concern, agricultural and agro industrial activities generate around 30 million tons of by- products, only 15% of it used for livestock feeding[4].

Olive trees by products (OTB) (leaves and twigs) are not used efficiently and left it without treatment may causes serious problems from the economical, social and environmental point of view. Fayed *et al.*[1] reported that each olive tree produce annually around 22 kg of leaves. The olive trees fruitful area reached 97000 feddan as reported by the Egyptian ministry of agriculture and land reclamation[5]. These huge amounts of olive trees by products (OTB) can be considered as alternative roughage feeds for ruminants[1]. The chemical composition of OTB varies depending on its origin, climatic and storage conditions, moisture content and degree of lignification[6]. The air dried OTB contain 50-60% dry matter, 7-11% crude protein, 5-7% ether extract, 13-23% crude fiber, 53-59% nitrogen free extract, 40-45% neutral detergent fiber, 28-35% acid detergent fiber and 18-20% acid detergent lignin[1-6,7]. Also, the leaves contain high levels of glutamic, aspartic, arginine, leucine and valine amino acids, but are low in tyrosine and cysteine. Moreover, palmitic and linolenic acids are the major fatty acids in olive leave's crude fat[8].

As obvious from the chemical composition, the main obstructions for enlargement in incorporation of OTB in ruminants diets are low protein and high fiber contents and presence of the anti-nutritional factors (e.g., tannins) which may led to OTB poor palatability and digestibility. Thus, to upgrade the OTB nutritive value as lignocellulolytic material it must breaks down the chemical bonds between lignin, cellulose and hemicellulose[9,10]. Several methods have been suggested to do that like mechanical, chemical and biological treatments[6]. However, the chemical treatments are tedious, costly and need further process to eliminate their side effects. In contrast, the microbiological methods improved nutritional quality of the agro-wastes with no side effects on the animal's health[11]. The microorganisms which used for conducting the biological treatments must be: (1) Able to grow in a wide range of the fermentation media and (2) Able to convert the treated substrate to biomass with high protein content.

Trichoderma viride is a potent fungus capable to hydrolyze plant's cell wall to fermentable sugars by owns fibrolytic enzymes, while yeast (*Saccharomyces cerevisiae*) has the ability to provide the treated substrate with high content of proteins and vitamins[9,12]. The effect of each *Trichoderma viride* and *Saccharomyces cerevisiae* on nutrients utilization and animal's performance has been extensively studied[12-15]. But, the synergistic effect of the both (*T. viride* and *S. cerevisiae*) on ration's digestibility and dairy animal's productivity has been rarely studied. So the main object of this study was to investigate the effects of treated OTB with *T. viride* and/or *S. cerevisiae* on lactating Barki ewe's milk productivity, nutrients digestibility and some of rumen and blood parameters.

MATERIALS AND METHODS

This study was carried out at a private farm (Alsttar farm for animal production), Khatatba city, Monofia governorate, Egypt. The entire experimental period was extended from July 23, 2017 to October 21, 2017. The olive trees by products (OTB) were collected from olive farms along Cairo-Alexandria Saharan Road, then chopped and dried naturally for 2 weeks and packed till use. *Trichoderma viride* and *Saccharomyces cerevisiae* were obtained from microbiology LAB-Diary Sciences Department, National Research Centre. The microbiological and chemical analyses were performed at the Laboratories of Diary Sciences Department, National Research Centre, Dokki, Giza, Egypt and Animal Nutrition Department, Desert Research Center, Cairo, Egypt.

Olive trees by products (OTB) biological treatments: *Trichoderma viride* and *Saccharomyces cerevisiae* were cultivated and maintained on slants of potato dextrose agar medium (PDA). Ten sterilized milliliter of malt medium [(g L^{-1}) malt extract 30, peptone 5.0 and sucrose 20] were added to each slant of *T. viride* and *S. cerevisiae* and scratched with a sterile needle. This suspension was transferred to 250 mL erlenmeyer flasks containing 40 mL of malt medium. After incubation on shaking incubator (140 rpm) at 28+1°C for 48 h, the grown cultures were employed as inocula for a sterilized liquid medium containing (g L^{-1}) 4% molasses, 0.4% urea, 0.2% KH_2PO_4 and 0.03 Mg $SO_4.7H_2O$ at rate of 10% (V/V). After 7 days of incubation *T. viride* and *S. cerevisiae* cultures were used for conducting the biological treatments for olive trees by products as follow:- T_1: Olive trees by products (1000 kg)+*T. viride* culture (2L), T_2: Olive trees by products (1000 kg)+*S. cerevisiae* culture (2L) and T_3: Olive trees by products (1000 kg)+*S. cerevisiae* culture (1L)+*T. viride* culture

(1L). The treated olive trees by products (OTB) were put on plastic sheets (150×225 cm) and incubated in a 4×4 m room maintained at 25-30°C for 21 days. The moisture was kept at 60% by moistened the bulk with 10% molasses solution using sprayer' tank with complete and continuous stirring every 2 days. After the incubation period the fermented OTB were air dried to 6-8% moisture then packed and stored for feeding purpose.

Ewes feeding and experimental design: Twenty eight lactating Barki ewes (about 2 years old and weighting on average 33.5±0.75 kg) after 7 days of parturition randomly assigned into four groups of seven animals each using complete random design. The entire experimental period was 90 days. Ewes were fed dry matter according to 4% of their body weight twice daily at 8:00 am and 4:00 pm, water was offered freely. Ewes first group was fed control ration consisted of 70% concentrate feed mixture (CFM: 60% corn, 22% soybean meal, 15% wheat bran,1% limestone, 1% minerals and vitamins mixture and 1% NaCl)+30% untreated OTB. The second group was fed R_1 ration (70% CFM+30% OTB treated with *Trichoderma viride*). The third group was fed R_2 ration (70% CFM+30% OTB treated with yeast *Saccharomyces cerevisiae*), while the last group was fed R_3 ration (70% CFM+30% OTB treated with *T. viride*+*S. cerevisiae*). The feed ingredients and the chemical composition of the experimental rations are shown in Table 1.

Apparent digestibility: A grab sample method was applied at which acid insoluble ash was used as an internal marker for determination of nutrient digestion coefficients. During the last seven days of each month of the experimental period, fecal grab samples were collected in cloth bag connected to the animal back at 1 pm, from four animals of each group. About 10% from the collected faeces were taken as representative samples then dried in an oven at 70°C for 48 h. The dried feces from each animal were mixed and ground to pass a 1 mm sieve in a feed mill (FZ102, Shanghai Hong Ji instrument Co., Ltd., Shanghai, China) for chemical analysis. The digestibility coefficient of nutrient was calculated according to the following equation[16]:

$$\text{Digestion co-efficient} = 100 - \left[\frac{\text{Indicator in feed (\%)}}{\text{Indicator in feces (\%)}} \times \frac{\text{Nutrient in feces (\%)}}{\text{Nutrient in feed (\%)}}\right]$$

Feed and fecal analysis: Feedstuffs and fecal samples were analyzed according to the AOAC[17] methods to determine crude protein (CP), ether extract (EE), crude fiber (CF) and ash contents. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin contents were determined using the methods described by Van Soest *et al.*[18]. Non-fiber carbohydrates (NFC) was calculated according to the following equation:

$$\text{NFC} = 1000\text{-}[\text{NDF}+\text{CP}+\text{EE}+\text{ash}]$$

Sampling and analysis of rumen liquor: At the last day of each month of experimental period, rumen liquor samples were collected by stomach tube from two animals each group at 12 pm. Samples were strained through two layers of gauze cloth to remove feed particles and immediately used for determination of ruminal pH using digital pH-meter. Rumen liquor samples were stored in glass bottles with drops of toluene and thin layer of paraffin oil and stored in a deep freeze (-20°C) until analysis. Ammonia nitrogen concentration (NH3-N) was determined by the modified semi-micro-kjeldahl digestion method according to AOAC[17]. The total volatile fatty acids (TVFA's) were determined according to method of Warner[19]. The ruminal microbial protein was estimated as

Table 1: Chemical composition of feed ingredients and experimental rations (g kg^{-1}) on dry matter basis

Items	CFM	OTB	OTBT	OTBY	OTBTY	Control	R_1	R_2	R_3
Dry matter	935.60	939.50	940.20	944.20	937.70	936.77	936.98	938.18	936.23
Organic matter	920.60	865.00	852.90	852.50	850.50	903.92	900.29	900.17	899.57
Crude protein	155.10	64.80	97.10	77.80	88.30	128.01	137.70	131.91	135.06
Ether extract	31.00	58.80	60.40	58.40	59.60	39.34	39.82	39.22	39.58
Ash	79.40	135.00	147.10	147.50	149.50	96.08	99.71	99.83	100.43
NDF	306.50	645.00	553.20	632.30	595.20	408.05	380.51	404.24	393.11
ADF	175.60	374.50	324.60	363.20	343.50	235.27	220.30	231.88	225.97
ADL	8.50	117.00	91.50	109.50	96.80	41.05	33.40	38.80	34.99
Cellulose	167.10	257.50	233.10	253.70	246.70	194.22	186.90	193.08	190.98
Hemi-cellulose	130.90	270.50	228.60	269.10	251.70	172.78	160.21	172.36	167.14
NFC	428.00	96.40	137.20	89.00	107.40	328.52	340.76	326.30	331.82

CFM: Concentrate feed mixture, OTB: Olive trees by products, OTBT: Olive trees by products treated with *Trichoderma viride*, OTBY: Olive trees by products treated with yeast, OLTY: Olive leaves treated with *T. viride*+yeast. Control ration: 70% CFM+30% olive trees by products, R (1): 70% CFM+30% olive trees by products treated with *Trichoderma viride*, R (2): 70% CFM+30% olive trees by products treated with yeast, R (3): 70% CFM+30% olive trees by products treated with *T. viride*+yeast. NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, NFC: Non fiber carbohydrate

described by Makkar *et al.*[20]. For classification and determination of ruminal ciliate protozoal count, the filtered rumen liquor were fixed and stained with 4 times volume of methyl-green formalin saline solution as described by Ogimoto and Imai[21], then stoked in dark place until examination. After gentle mixing of fixed rumen liquor sample, one drop was poured on hemocytometer slide, covered with a cover slip and examined under a light microscope for identification of protozoal genera and species according to the description published by Dehority[22].

Sampling and analysis of blood serum: Blood samples were taken from jugular vein of two animals each group through the last 3 days of each month of the experimental period. At about 4 h after morning feeding the blood samples were collected in glass tubes and left to coagulate at room temperature. Serum was separated by centrifugation at 4000×g/20 min and kept frozen at -20°C for later analysis. Serum total protein was determined according to method of Armstrong and Carr[23], albumin[24], globulin was calculated by subtracting the albumin from total protein, urea[25], aspartate aminotransferase (AST) and alanin aminotransferase (ALT)[26].

Sampling and analysis of milk: The ewes were milked twice a day at 8.00 am and 4.00 pm during the last 3 days of each month of experimental period. Milk samples were immediately collected from each animal after morning and evening milking and milk yield was recorded. The sample of each animal represented a mixed sample of constant percentage of the morning and evening yield. Milk samples were analysed for total solids, fat, protein and lactose by Bentley150 infrared milk analyzer (Bentley Instruments, Chaska, MN, USA) according to AOAC[17] procedures. Solids-not-fat (SNF) was calculated by subtracting fat from total solids percentage. Fat corrected milk (4% fat) was calculated by using the following equation according to Gaines[27]:

$$FCM = 0.4\ M+15\ F$$

Where:
M = Milk yield (g)
F = Fat yield (g)

Statistical analysis: Data obtained from this study were statistically analysed by IBM[28] SPSS Statistics for Windows using the following general model procedure:

$$Y_{ij} = \mu+T_i+e_{ij}$$

where, Y_{ij} is the parameter under analysis of the ij bottles of rumen liquor trails or ewes of digestibility and lactation trails, μ is the overall mean, T_i is the effect due to treatment on the parameter under analysis, e_{ij} is the experimental error for ij on the observation, the Duncan's multiple range tests was used to test the significance among means[29].

RESULTS

Biological treatments effects on rumenal fermentation characteristics: Ewes fed control ration recorded the highest ($p<0.05$) value for ruminal pH followed by ewes fed on (R_2) ration, while, ewes fed (R_1) ration recorded the lowest ($p<0.05$) value for ruminal pH then ewes fed on (R_3) ration (Table 2). In contrast, ruminal total volatile fatty acids (TVFA's), ammonia nitrogen (NH_3-N) and microbial protein synthesis recorded the highest ($p<0.05$) concentrations by ewes fed R_1 ration and the lowest ($p<0.05$) concentrations by ewes fed ration of control. No significant differences were detected between ewes fed R_2 and R_3 rations in ruminal TVFA's, NH_3-N and microbial protein synthesis concentrations, but ruminal TVF's, NH3-N and microbial protein synthesis concentrations significantly differ in ewes fed R_2 and R_3 rations compared with those of control.

The biologically treated OTB effects on ewe's ruminal protozoa count ($\times 10^4$ cell mL^{-1} rumen liquor) is shown in Table 3. The untreated OTB decreased ($p<0.05$) total count of protozoa, while the biologically treated olive trees by products increased ($p<0.05$) total count of protozoa in rumen of the tested ewes. Among biologically treated ewe's groups, ewes fed *T. viride* treated ration (R_1) had the highest ($p<0.05$) ruminal protozoa count followed by ewes fed the treated ration with the mixture of *T. viride* and *S. cerevisiae* (R_3), while ewes fed on *S. cerevisiae* treated ration give the lowest ($p<0.05$) count of ruminal protozoa. Concerning with rumen protozoa species, *Entodinium* recorded the largest count among all different protozoa species in rumen of all ewes groups. Moreover, *Entodinium*, *Ophryoscolox* and *Dasytrachia* count tended to be greater ($p<0.05$) in rumen fluid of ewes fed R_1 ration than those fed R_2, R_3 and control rations. Furthermore, *Epidinium* gave the highest ($p<0.05$) count in rumen fluid of ewes fed R_3 ration, while *Entodinium, Epidinium*, *Ophryoscolox*, *Polyolastron* and *Dasytrachia* gave the lowest ($p<0.05$) count in rumen fluid of ewes fed control ration. No significant difference were detected in count of *Polyolastron* and *Isotrchia* among ewes fed R_1 and R_3 rations, while no change was found in *Diplodinum* count among all ewes groups.

Table 2: Effect of biological treatments on nutrient digestibility and nutritive value of the experimental rations

	Experimental groups				
Items	Control	R_1	R_2	R_3	±SE
Live body weight	33.30	34.25	33.80	33.65	0.269
Feed intake (g h^{-1}/day)	1332.00	1370.00	1352.00	1346.00	3.492
Digestibility (%)					
Dry matter	68.04^{c}	75.81^{a}	72.59^{b}	73.26^{b}	0.377
Organic matter	70.97^{c}	78.79^{a}	74.10^{b}	75.29^{b}	0.289
Ether extract	79.34^{c}	83.29^{a}	80.66^{bc}	81.85^{b}	0.451
Crude protein	67.23^{c}	74.64^{a}	71.68^{b}	73.19^{b}	0.980
Crude fiber	61.10^{c}	65.91^{a}	61.86^{bc}	63.72^{b}	0.529
Nitrogen free extract	71.41^{c}	77.06^{a}	73.52^{b}	74.38^{b}	0.571
Fiber fractions (%)					
Neutral detergent fiber	59.41^{c}	64.43^{a}	60.89^{bc}	62.17^{b}	0.590
Acid detergent fiber	51.03^{c}	59.07^{a}	54.88^{b}	56.09^{b}	0.489
Cellulose	58.55^{c}	64.29^{a}	60.18^{bc}	61.52^{b}	0.382
Hemicellulose	58.72^{c}	68.74^{a}	63.16^{b}	64.51^{b}	0.594
Nutritive value (%)					
Total digestible nutrients (TDN)	68.84^{c}	73.06^{a}	69.43^{b}	70.48^{b}	0.294
Digestible crude protein (DCP)	8.61^{c}	10.28^{a}	9.45^{b}	9.88^{b}	0.867

Means with different litters with each row are significantly different ($p<0.05$). Control: Ewes fed 70% CFM+30% olive trees by products, R (1): Ewes fed 70% CFM+30% olive trees by products treated with *Trichoderma viride*, R (2): Ewes fed 70% CFM+30% olive trees by products treated with yeast, R (3): Ewes fed 70% CFM+30% olive trees by products treated with *T. viride*+yeast

Table 3: Effect of biological treatments on ewe's rumenal fermentation characteristics

	Experimental groups				
Items	Control	R_1	R_2	R_3	±SE
pH	6.69^{a}	6.62^{c}	6.68^{a}	6.65^{b}	0.022
TVFA's (mL equiv/100 mL)	8.38^{c}	9.33^{a}	8.95^{b}	9.09^{ab}	0.085
Ammonia nitrogen (mg/100 mL)	30.53^{c}	38.54^{a}	33.76^{b}	34.40^{b}	0.229
Microbial protein (mg/100 mL)	84.77^{c}	95.75^{a}	90.39^{b}	91.62^{b}	0.134

Means with different litters with each row are significantly different ($p<0.05$), Control: ewes fed 70% CFM+30% olive trees by products, R (2): Ewes fed 70% CFM+30% olive trees by products treated with *Trichoderma viride*, R (3): Ewes fed 70% CFM+30% olive trees by products treated with yeast, R (4): Ewes fed 70% CFM+30% olive leaves treated with *T. viride*+yeast

Table 4: Effect of biological treatments on ruminal ciliate protozoa ($\times 10^4$ cell mL^{-1} rumen liquor)

	Experimental groups				
Items	Control	R_1	R_2	R_3	±SE
Entodinum spp.	5.255^{d}	6.127^{a}	5.529^{c}	5.725^{b}	0.045
Epidinium spp.	0.180^{c}	0.199^{b}	0.194^{b}	0.206^{a}	0.001
Diplodinum spp.	0.148	0.158	0.148	0.154	0.024
Ophryoscolox spp.	0.176^{c}	0.198^{a}	0.189^{ab}	0.187^{b}	0.003
Polyolastron spp.	0.337^{c}	0.378^{a}	0.359^{b}	0.385^{a}	0.005
Isotrchia spp.	0.224^{b}	0.234^{a}	0.224^{b}	0.230^{a}	0.001
Dasytrachia spp.	0.435^{c}	0.474^{a}	0.447^{bc}	0.453^{b}	0.004
Total protozoa count	6.755^{d}	7.768^{a}	7.090^{c}	7.340^{b}	0.055

Means with different litters with each row are significantly different ($p<0.05$). Control: Ewes fed 70% CFM+30% olive trees by products, R (2): Ewes fed 70% CFM+30% olive trees by products treated with *Trichoderma viride*, R (3): Ewes fed 70% CFM+30% olive trees by products treated with yeast, R (4): Ewes fed 70% CFM+30% olive trees by products treated with *T. viride*+yeast

Biological treatments effects on ration's nutrient digestibility and nutritive value: Rations containing treated OTB with *T. viride* (R_1) or *T. viride* and *S. cerevisiae* combination (R_3) increased ($p<0.05$) all nutrients digestibility and NDF, ADF, cellulose and hemicellulose digestibility compared with the control ration (Table 4). The ewes fed ration containing treated OTB with *S. cerevisiae* (R_2) showed increase ($p<0.05$) DM, OM, CP, nitrogen free extract (NFE), ADF and hemicellulose digestibility than those fed the control ration. The ewes fed (R_1) ration showed superiority in nutrients and fiber fraction digestibility over those fed (R_3) ration, while no significant differences were found between ewes fed (R_2) and control rations in digestion of ether extract (EE), crude fiber (CF), NDF and cellulose.

Table 5: Effect of biological treatments on ewe's blood parameters

	Experimental groups				
Items	Control	R_1	R2	R_3	±SE
Total proteins (g dL^{-1})	6.02[c]	6.66[a]	6.43[b]	6.55[b]	0.084
Albumin (g dL^{-1})	3.65[c]	4.25[a]	4.05[b]	4.15[a]	0.092
Globulin (g dL^{-1})	2.37	2.41	2.38	2.40	0.108
Urea mg (dL^{-1})	27.43	29.56	30.69	30.08	0.704
ALT (U L^{-1})	24.71	22.88	23.52	24.75	1.131
AST (U L^{-1})	16.31	14.56	16.06	15.68	0.242

Means with different litters with each row are significantly different (p<0.05). Control: Ewes fed 70% CFM+30% olive trees by products, R (2): Ewes fed 70% CFM+30% olive trees by products treated with *Trichoderma viride*, R (3): Ewes fed 70% CFM+30% olive trees by products treated with yeast, R (4): Ewes fed 70% CFM+30% olive trees by products treated with *T. viride*+yeast

Table 6: Effect of biological treatments on ewe's milk yield and milk composition

	Experimental groups				
Items	Control	R_1	R_2	R_3	±SE
Milk yield					
Milk yield (g/day)	874.44[c]	1013.88[a]	957.5[b]	963.88[b]	3.93
4% FCM (g/day)	797.05[c]	945.44[a]	879.94[b]	888.70[b]	3.97
Protein yield (g/day)	31.57[c]	38.32[a]	35.62[b]	36.15[ab]	0.94
Fat yield (g/day)	29.82[c]	35.99[a]	33.13[b]	33.54[b]	0.77
Lactose yield (g/day)	37.25[c]	44.21[a]	41.27[b]	41.83[b]	0.91
Ash yield (g/day)	8.31[c]	10.04[a]	9.48[b]	9.45[b]	0.16
Total solids yield (g/day)	106.94[c]	128.56[a]	119.50[b]	120.97[b]	1.49
Solids not fat yield (g/day)	77.13[c]	92.57[a]	86.37[b]	87.42[b]	1.37
Milk composition (%)					
Protein	3.61[c]	3.78[a]	3.72[b]	3.75[ab]	0.005
Fat	3.41[c]	3.55[a]	3.46[b]	3.48[b]	0.008
Lactose	4.26[c]	4.36[a]	4.31[b]	4.34[ab]	0.014
Ash	0.95	0.99	0.99	0.98	0.002
Total solids	12.23[c]	12.68[a]	12.48[b]	12.55[ab]	0.006
Solids not fat	8.82[c]	9.13[a]	9.02[b]	9.07[ab]	0.010

Means with different litters with each row are significantly different (p<0.05). Control: Ewes fed 70% CFM+30% olive trees by products, R (1): Ewes fed 70% CFM+30% olive trees by products treated with *Trichoderma viride*, R (2): Ewes fed 70% CFM+30% olive trees by products treated with yeast, R (3): Ewes fed 70% CFM+30% olive trees by products treated with *T. viride*+yeast

The ewes fed rations containing biologically treated olive trees by products (R_1, R_2 and R_3) showed higher (p<0.05) digestible crude protein (DCP) and total digestible nutrients (TDN) than those fed control ration (Table 4). However, the ewes fed R_1 showed significant (p<0.05) increase in (DCP) and (TDN) values compared with those fed R_2 and R_3 rations.

Biological treatments effects on ewe's blood parameters: The ewes fed biologically treated rations (R_1, R_2 and R_3) had higher (p<0.05) serum total protein and albumin than those fed the control ration (Table 5). In addition, serum globulin, urea alanin aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were not change among all ewes groups. Among biologically treated ewe's groups, ewes fed *T. viride* treated OTB (R_1) had the highest (p<0.05) serum total protein and albumin concentrations followed by ewes fed the treated OTB with the mixture of *T. viride* and *S. cerevisiae* (R_3). While, ewes fed on *S. cerevisiae* treated OTB recorded the lowest (p<0.05) blood serum total protein and albumin concentrations.

Biological treatments effects on ewe's milk yield and milk composition: Ewes fed on biologically treated OTB (R_1, R_2 and R_3) produce more (p<0.05) milk, milk constituents and fat corrected milk than those fed the control ration (Table 6). Among biologically treated animal's groups, ewes fed R_1 had the highest (p<0.05) milk, milk constituents and fat corrected milk yields followed by ewes fed R_3 then ewes fed on R_2 ration. Also, milk composition (%) matches the trend of milk constituent's yields (g/day). In addition, there is no significant difference between all ewes' groups in milk ash percentage.

DISCUSSION

The utilization of agricultural residues in ruminants feeding is determined by its content of protein and complex carbohydrate (cellulose, hemicellulose and lignin). The biological treatments for such residues by numerous microorganisms have been studied and its efficiency for improving animals feed quality has been proved. But, the

synergistic action between most of microbes used in agricultural residue's biological treatments has not been studied extensively. Therefore, the focus of this study was to answer the following question, Does *T. viride* and yeast (*S. cerevisiae*) will work synergistically to enrich the productivity and digestion efficiency of the lactating ewes fed rations containing olive trees by products or not?

All the results obtained from this study suggest that the answer for the previous question is NO. The lack of synergism between *T. viride* and *S. cerevisiae* may be due to their competition for nutrients or their inoculum size may not be enough to induce the desired synergy. Accordingly, the effects of *T. viride* and/or *S. cerevisiae* on the productive performance of the tested animals will be generally discussed.

Biological treatments effects on rumenal fermentation characteristics: The efficiency of the fermentation process in the rumen is largely limited by the extent of change in pH values and TVFA's and NH3-N concentrations. The reduction of pH values and increase microbial protein synthesis, NH3-N and TVFA's concentrations in ewe's rumen liquor fed biologically treated OTB can be attributed to: (1) The hydrolytic effect of *T. viride* on NDF, ADF, ADL, cellulose and hemicellulose of OTB (Table 1), (2) Improve protein quantity and quality for the biologically treated OTB due to the microbial biomass of *T. viride* and/or *S. cerevisiae*, (3) Enhancement of anaerobic nutrients fermentation due to the positive action of *S. cerevisiae* on the rumen environment and (4) Alteration of rumen microbial populations especially protozoa species (Table 3).

Current data support by the previous findings of Khorshed[30] and El-Sayed *et al.*[31] on goats and Allam et al.[32] on growing lambs. They reported that rumen liquor pH was significantly decreased and NH3-N and TVFA's concentration increased for animals fed diets containing cotton stalks or sugar beet pulp treated with *T. viride* and *S. cervevisiae* or their combinations than those of control. Also, Kholif *et al.*[33] showed that values of ruminal TVFA's increased significantly by treatment of goat's diets with *T. viride* followed by *S. cerevisiae* compared to the control. In addition, Sharma *et al.*[34] reported that ruminal microbial protein synthesis increased significantly by treatment of animal's rations with *S. cervevisiae* culture. In contrast, El-Shafie *et al.*[14] found that ruminal TVFA' and NH_3-N concentrations for sheep fed *T. viride* treated wheat straw was significantly decreased compared with the control.

Ruminal protozoa have potent effects on digestive capacity in the rumen, specific growth rate of ruminal bacteria beside their general effects on the rumen environment[35,36]. Several factors seem to influence the count and composition of the protozoal fauna in the rumen, these include, feeding level, ration composition, frequency of feeding, ruminal pH and ruminal turnover rate[37]. In the present study, the increase of protozoa numbers in rumen liquor of biological treated animals than control group may be related to more utilization of the dietary energy and positive fermentation in the rumen[33]. Also it may be due to increased digestibility of organic matter[13]. It has been reported that, yeast treatment increased the rate of rumen fermentation through increase the total and viable count of rumen microbes[38]. The previous findings of Hend[6] are supporting our data, she found that feeding Barki lambs on olive tree by-products treated with mixture of *T. viride* and *S. cervevisiae* led to increase ruminal protozoa count ($\times 10^6$ cell mL^{-1} rumen liquor) significantly than those in untreated lamb's group. The dominance of *Entodinium* spp., in rumen of all tested animals in the current study is in line with findings of Franzolin and Dehority[39], they stated that *Entodinium* spp., represent 90% of the ruminal total protozoal count. Changeless of *Diplodinum* count among all ewes groups may be due to its resistance to changes in the ruminal pH.

Biological treatments effects on ration's nutrient digestibility and nutritive value: Relatively low digestibility of nutrients in the control ration could be explained by higher cell wall constituents of crude OTB (Table 1). High lignin (ADL) content of untreated OTB and the fact that most of OTB total nitrogen is linked to lignocellulose[40] may be the reason for control ration's TDN and DCP depression. On the other hand, the biological treatments positive effects on ration's nutrient digestibility and nutritive value can be attributed to (1) Better palatability of treated rations than those of control, (2) Solubilize fibers of OTB due to fibrolytic enzyme's action of *T. viride*, (3) Satisfy the requirements of nitrogen for ruminal microbes which may have been partially or completely met due to protenic biomass of *S. cervevisiae* and/or *T. viride* and (4) More utilization of dietary energy and protein in rumen of the treated animals. These results are in agreement with those of El-Shafie *et al.*[14], who reported that treatment of wheat straw by *T. viride* led to improve all nutrients digestibility by treated small ruminants than those of control. Also, inclusion of treated olive tree by-products with mixture of *S. cerevisiae* and *T. viride* in lamb's diets increased the CF, CP, NFE, OM and DM digestibility by treated group than control group[1,6].

Biological treatments effects on ewe's blood parameters: Higher blood serum total protein and albumin values for biologically treated ewes than those of control are reasonable depending on higher digestibility of all nutrients (especially OM and CP) of the biologically treated rations than control (Table 4). These results indicated that ewes fed biologically treated rations had adequate amounts of protein for maintenance and milk production. It is important to note that values of A/G ratio were higher than 1.0 which indicated that animals did not suffer from any healthy problems that might affect the performance of the experimental animals. Current results were parallel with that obtained by Khorshed[30] and El-Sayed *et al*.[31], they indicated that serum total protein and albumin for goats fed on treated cotton stalks with *T. viride* and/or *S. cervevisiae* were significantly higher than those of control. Kholif *et al*.[33] showed that total proteins and albumin were increased significantly with yeast treatment compared with other treatments (*Penicillium funiculisums* and *Trichoderma viride*), however serum globulin and A/G ratio were not affected by treatments. The use of biological treatments (specially combined *T. viride* and *Saccharomyces cerevisiae*) in goat's rations is useful and did not cause any abnormal conditions in kidney and liver functions[13]. In addition, treated olive tree by-products with *S. cerevisiae*+ *T. viride* increased lamb's blood serum total proteins and albumin compared with the control[1,6]. On the other hand, Khorshed[30] and El-Sayed *et al*.[31] found that treatment of cotton stalks with *T. viride* and *S. cervevisiae* or their combination increased goats blood serum urea-nitrogen GOT and GPT concentrations than those of control.

Biological treatments effects on ewe's milk yield and milk composition: Higher ewe's milk production by 16, 9.5 and 10.3% and fat corrected milk (FCM) by 18.6, 10.4 and 11.5% with *T. viride*, *S. cerevisiae* and *T. viride*+*S. cerevisiae* treatments than the control is logical. High milk productivity and milk components by the treated animals can be explained by occurring sequence improvements in the nutritive value of the treated rations (Table 1), rumen environment including enhancement of protozoa total counts, digestibility and metabolism of feed nutrients which led to provide animals with adequate and continuous amount of energy and protein. Similar results are reported by Azzaz *et al*.[12] and Kholif *et al*.[15], they found that animals treated with *S. cerevisiae* produced more milk and milk components. Also, Fazaeli *et al*.[41] stated that feeding late lactating Holstein cows on total mixed ration contains fungal treated straw up to 30% led to improve the nutrients digestibility and increase yield of fat corrected milk by 13%.

CONCLUSION

Feeding lactating ewes on rations contains olive trees by products biologically treated with *T. viride*, *S. cerevisiae* and their mixture increased milk and milk components yields, increased all nutrients digestibility, improved rumen environment with development of protozoa total counts with no deleterious effect on treated animal's health. No synergism was noted between *Trichoderma viride* and *Saccharomyces cerevisiae* on the tested parameters. Further studies are needed to determine what factors affect the microbial synergism in agricultural residues biological treatment process.

SIGNIFICANCE STATEMENT

Use of the biologically treated olive trees by products (OTB) as alternative feed resource may help to meet the feed requirements for the animals in the Saharan, arid and semi arid areas in economic way. *Trichoderma viride* and yeast (*S. cerevisiae*) don't work synergistically to enrich the nutritive value of OTB. In the light of results of this study it can speculate that use of live cells of S. cerevisiae as feed additive in dry form may be make it more effective. More studies are needed to get deep knowledge about the relations between microbes which used for biological treatments for crops residues and also discover the relations between rumen microbes.

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