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K.Z. Kewan, S. Abo Bakr, M.S. Nassar, Afaf M. El-Shereef, M.A-H. El-Rayes

EDITOR'S NOTE

Researchers often ask, 'If I publish in an Open Access journal, will my work be cited?' James Pingle (nature.com, 07 Dec. 2019), Vice President, Thomson ISI, USA based on a study in respect of papers published in over 190 Open Access journals has concluded that Open Access journals can have similar impact to other journals, and prospective authors should not fear publishing in these journals merely because of their access model. It is duly corroborated by other researchers (Swamy & Kishore, IJLIS, 2017; Kumar & Naveen, IJIDT, 2016; Veena, IJODLS, 2016; Sharma & Kumar, JLIT, 2016; Halloumeh & Jirjees, AJ & C, 2016) who have established the superiority of e-publications for convenience, cost effectiveness, and comprehension. Moreover, a scholar's scientific research output, assessed in terms of h-index (Hirsch, 2005) from Google Scholar is being accepted for research fellowships and holding positions at top universities (Bornmann & Hans-Dieter, Journal of the American Society for Information Science & Technology, July 2007).



**UTILIZATION OF TRIMMING WASTE OF MANDARIN TREES AS FEED
FOR SMALL RUMINANTS: 2- EVALUATION OF BARKI EWES
PERFORMANCE DURING SUCKLING PERIOD**

K.Z. Kewan^{*1}, S. Abo Bakr², M.S. Nassar³, Afaf M. El-Shereef⁴, M.A-H. El-Rayes⁵

ABSTRACT

This investigation was carried out to evaluate the nutritive value of trimming waste of mandarin trees (TWMT) either untreated or treated with two developed additives using rams and ewes in metabolism and feeding trials, respectively, where digestibility, productive performance, rumen functions, blood profile and immune indices were assessed. A feeding trial was conducted using forty-eight pregnant Barki ewes aged 2.0-2.5 years (weighing an average of 34.9±0.5 kg) at the last month of pregnancy. The ewes were selected and randomly distributed into three groups (16 in each). Concentrate feed mixtures (CFM) were offered to cover 65% of the energy requirements of ewes during the pregnancy period and 50% during the lactation one. The experimental roughages were offered ad lib to cover the remaining portion of their daily requirements. Ewes were fed on three experimental diets comprised of untreated TWMT + CFM (Diet C), TWMT treated with liquid feed including Yeast + CFM (Diet Y) and TWMT treated with liquid feed including ZADO + CFM (Diet Z). Data showed that total feed intake was significantly ($P \leq 0.05$) higher by ZADO addition compared to the other two diets, i.e., Y and C. Animals that received the Z diet showed the highest ($P \leq 0.05$) digestibility values for CP, CF, NFE, NDF, and ADF followed by Y and C groups. Feeding values of diets Y and Z were significantly ($P \leq 0.05$) higher by 12% and 18% for TDN, and 27% and 28% for DCP, respectively than that of the control diet (C). Actual daily milk yield and energy corrected milk yield were significantly ($P \leq 0.05$) increased with animals fed on diet Y compared with those fed on Z and C diets. The percentages of milk total solids and fat were significantly ($P \leq 0.05$) improved by both dietary treatments of ZADO and yeast in comparison of control one. The best feed conversion efficiency (kg DM, kg TDN, and Kg DCP required for producing one kg energy corrected milk) was recorded with group fed on Y (3.04, 1.87 and 0.331, respectively). The daily gain of lambs in the Y and Z groups was significantly ($P < 0.05$) higher by 26.73% and 12.87%, respectively than those of C. The relative net return to feed cost was higher in Y group by 25.66% compared to that of C. Also, methane production was significantly ($P \leq 0.001$) decreased in both of treated diets (Y and Z) than that in C diet. Water metabolism, rumen fermentation, some blood parameters and immune indices were also investigated. It could be concluded that trimming waste of mandarin trees (TWMT) could provide an acceptable feed resource after treatment with either ZADO or Yeast which improved digestibility, and caused a positive effects on the lactational performance of Barki ewes and growth of their offspring.

KEYWORDS

Barki ewes, Immunity, Milk, Mandarin trimming waste, Rumen fermentation, Yeast, ZADO

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INTRODUCTION

In Egypt, sheep and goat numbers have increased to become 4 and 5.5 million head, respectively in the year 2017. However, the availability of conventional feed resources is declining as livestock populations increase (FAO, 2018). There was a shortfall in DM (-14.42 million tons) and TDN (-2.21 million tons), but surplus of DCP (+1.11 million tons) in the ruminant feeding balance in 2012 (Kewan and Khattab, 2016).

To overcome this problem, agriculture by-products have received research attention in recent times as new feed materials that do not compete with human food can provide significant amount of feed to covering a great part of the deficit especially in the dry season (Makkar and Ankers, 2014).

The unconventional feed resources in Egypt are characterized by their low crude protein content and dry matter digestibility, but high ash, and crude fiber contents. Mandarin trees are subtropical plants and could grow best in regions with a pronounced change in season. In Egypt, the trimming waste of mandarin trees (TWMT) is disposed as burnt, where such way might cause an environmental pollution as it is inexpensive, available year-round and is not efficiently utilized. Biologically treated trimming waste of mandarin trees could cover the maintenance requirement of nutrients for sheep under the desert conditions and enhance animal immune status, besides reducing feed cost and preventing environmental pollution (Abo Bakr *et al.*, 2020).

Therefore, the aim of the present study was to convert trimming waste of mandarin trees (TWMT) into value-added products by some biological effective treatments and evaluating its impact on ewe's performance during the lactating period, because of massive requirement of nutrients, such as DM, DCP and TDN during lactation.

MATERIAL AND METHODS

This study was conducted at Siwa Research Station that belongs to Desert Research Center (DRC), Ministry of Agriculture and Land Reclamation, Egypt, during 2018. The experimental procedures were approved by the Animal and Poultry Production Division of the DRC committee and followed by the Veterinary and Animal Care Department.

Experimental treatments: Fresh trimming waste of Mandarin trees (TWMT) was purchased from a private factory of oil extraction that is located in Sadat city. It was mechanically chopped (3-5 cm) before extraction of natural oil (Dimethyl anthranilate) by steam treatment technique. The naturally extracted oil has medicinal uses and other biological activities. The required amount of chopped TWMT was air-dried, then packed in layers, and sprayed with different experimental liquid solutions and mixed properly. The experimental roughages were as follows:

- The untreated trimming waste (UTW) was served as control roughage.
- The first tested roughage was prepared by supplementing trimming waste with liquid feed enriched with Bakery yeast (*Saccharomyces cerevisiae*), where each 100 kg trimming waste was treated with liquid feed containing 18 liter water, 6.1 kg molasses, 0.17 kg urea, 0.4 kg mineral mixture, 0.03 kg vitamins and 0.5 kg yeast (YTW).
- The second tested roughage was prepared by supplementing trimming waste with the same previous liquid feed but enriched with 0.25kg ZADO instead of Bakery yeast (ZTW). The ZADO is a compound (patent no: 22155) prepared from a natural, multi/mix of the enzymes such as cellulases (8.2u/g), hemicellulases (6.2u/g), amylase (64.4u/g), and protease (12.3u/g) and also included anaerobic bacteria and *S. cerevisiae* yeast.

Feeding trial: Forty-eighth Barki ewes in their last gestation month, weighing, on an average, 34.9 ± 0.5 kg (aged 2-2.5 years) were kept indoors and allocated to 3 dietary treatment groups in a complete randomized block design and extended to the end of the lactation season. Concentrate feed mixture (CFM) was offered to the animals according to physiological status (NRC, 2007), where CFM was offered to cover 65% of energy requirements during the pregnancy period and 50% during the lactation period. While the experimental roughages were offered ad-lib to cover remain portion according to the physiological status of animals. Concentrate feed mixtures were formulated from 11% soybean meal, 60% ground yellow corn grains, 25% wheat bran, 2.4% limestone, 0.1% premix and minerals, 1.4% salt, and 0.1% Anti-aflatoxin. Ewes were fed one of the following experimental diets:

Group1: untreated TW + CFM (Control diet C).

Group 2: yeast treated trimming waste YTW + CFM (Tested Diet Y).

Group3: ZADO treated trimming waste ZTW + CFM (Tested Diet Z).

Feed intake was measured daily by weighing the offered and the refusal feeds from the previous day. Animals had free access to water throughout the experiment. Lambs were weighed at birth then biweekly till weaning.

Metabolism trials: By the end of the feeding trial, metabolism trials were conducted using twelve adult rams (initial weight of 53.67 ± 0.72 kg, with age 4-5 years) to evaluate the digestibility of nutrients, feeding values of the experimental diets, and the utilization of nitrogen and water intake as well. Animals were fed in groups of 16 for 30 days in shaded pens and then fed individually in metabolic cages for 21 days (14 days as an adaptation period followed by 7 days as a collection period). Total feces were recorded daily and representative samples of about 10% of the total fresh feces weight were taken daily. At the end of the collection period, composited feces samples of each animal were mixed and ground for sampling. Soft water was available for free choice during the experimental period. Urine was allowed to drain into bottles containing 50 ml of 3N HCl as a preservative. The volume of urine was recorded daily and a sample of urine representing 10% of total urine for each animal was taken for proximate analysis. Samples of feed offered, refused, feces, and urine were taken and stored during the collection period for analysis.

Feed, feces, and urine sampling and analysis: Feed and Feces samples were dried at 60°C for 48 h using a forced air oven, ground in a grinder in order to be sieved through a 2-mm screen. Feed and feces were analyzed (AOAC, 2005) in duplicate for dry matter (DM), ash, crude protein (CP), and ether extract (EE). Gross energy (GE) of feed and feces were measured by bomb calorimeter (IKA, model C 200, Staufen, Germany), using benzoic acid as standard. Neutral detergent fiber (Van Soest *et al.*, 1991) and acid detergent fiber (AOAC, 1997) were determined using the ANKOM fiber technology technique. NDF was assayed without the use of an alpha-amylase but with sodium sulfite in the ND. Both NDF and ADF are expressed without residual ash.

Rumen samples and analysis: At the end of the metabolism trial, rumen liquor samples were collected from all animals within each group by a stomach tube at 0, 3, and 6 hours post-feeding. The rumen samples were filtered through four layers of cheesecloth and used as quickly as possible for the measurement of pH by using a digital pH-meter.

Strained rumen liquor was stored in glass bottles (25 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. The rumen fluid samples were preserved for ammonia nitrogen (NH₃-N) determination (Preston, 1995) and total volatile fatty acids (VFA) determination by a Kjeldahl distillation method (AOAC, 1997). The molar concentration of VFA fractions was determined (Soltan *et al.*, 2018) using gas chromatography (model TRACE1300, Thermo Fisher Scientific, Inc., Rodano, Milan, Italy) by the Central Lab for Food and Feed (CLFF), Agricultural Research center, Ministry of Agriculture, Giza, Egypt.

In vitro gas production and dry matter degradability: The experimental diets were used to investigate *in vitro* gas production (Menke and Steingass, 1988). Three ruminally-cannulated Barki sheep were used as a source of rumen liquor inoculum. Sheep were fed a diet containing Berseem hay (60%) and concentrate mixture (40%). Rumen contents were collected before the morning feeding into pre-warmed insulated bottles, pooled among sheep, homogenized in a laboratory blender, filtered through two layers of cheesecloth, and flushed with CO₂. Buffer and mineral solution were prepared and placed in a water bath at 39°C under continuous flushing with CO₂. The well mixed and CO₂ flushed rumen fluid was added to the buffered rumen fluid solution (1:2 v/v), which was maintained in a water bath at 39°C and mixed.

Approximately 200 mg DM of finely ground samples were accurately weighed into glass serum bottles (100 ml). Buffered rumen fluid (30 ml) was pipetted into each bottle, containing the feed samples, and the syringes were immediately placed into the water bath at 39°C (Blummel and Ørskov, 1993). Three Syringes with only buffered rumen fluid were incubated and considered as the blanks. The gas production was recorded after, 3, 6, 12, and 24 h. of incubation. Total gas volume was recorded at each time and the values were corrected for the blank value and gas values are expressed in ml per 200 mg DM. Cumulative gas production (Y) at the time (t) was fitted to the exponential model (Ørskov and McDonald, 1979) as follows:

Gas production (GP) = $a + b(1 - e^{-ct})$, where,
a = the gas production from the immediately soluble fraction,
b = the gas production from the insoluble fraction,
c = the gas production rate constant for the insoluble fraction (*b*),
t = incubation time.

The effective DM degradability (EDMD) for tested rations were estimated from the equation (McDonald, 1981), where $EDMD = a + [bc / (c + k)]$ and k is the out flow rate (0.03). Microbial protein was calculated as 19.3g microbial nitrogen per kg OMD (Czerkawski, 1986).

Milk samples and analysis: Milk production was recorded biweekly starting from the second week of lambing till the 16th week of lactation using the hand-milking procedure after the separation of lambs from their dams. Ewes were milked twice a day with a 6-h interval between milking. The first milking (which was discarded) was performed to empty the mammary gland. The second one was used to access milk yield in 6 h.

Total milk produced after 6 h was weighed and multiplied by four to estimate milk yield per day (Ferreira *et al.*, 2018). To minimize variation in the interval between milking, ewes were milked by one person and at a regular time for each ewe throughout the experiment. Milk samples were collected and frozen immediately at -20 °C until individually analyzed. The frozen milk samples were slowly thawed under refrigeration, homogenized thoroughly, and analyzed for fat, protein, and lactose using infrared Spectrophotometry apparatus (Lactoscan S, Milkotronic Ltd., New Zagora, Bulgaria) calibrated for ewes milk. Energy corrected milk (ECM) was calculated by the following formula (Østergaard *et al.*, 2003):

$$ECM \text{ (g/d)} = \text{Milk production} \times [(0.383 \text{ fat } \%) + (0.242 \text{ protein } \%) + 0.7832] / 3.1138.$$

Blood plasma sampling and analysis: Blood samples were collected monthly from the jugular vein of ewes and their lambs for three consecutive months representing early, mid and late lactating stages. Blood was collected into clean test tubes with anticoagulant (EDTA). Blood samples were centrifuged at 3000 rpm for 20 minutes to obtain plasma that were frozen at -20°C for late biochemical assay. Plasma concentrations of total proteins (TP), albumin (Alb), total lipids (TL), triglycerides (TG), total cholesterol (TC), total antioxidant capacity (TAC), high density lipoproteins (HDL), low density lipoproteins (LDL), liver enzymes activity including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT) and kidney function tests including Creatinine (CR) and Urea (BU), which were assessed calorimetrically using commercial chemical reagent kits (Bio-diagnostic product Kit, Egypt). The concentration of globulin (Glb) and albumin to globulin (A/G) ratio were calculated. Cytokines (interleukins IL-1 β , IL-2 and IL-6) and tumor necrosis factor- α (TNF- α) were determined from undiluted samples using QUANTIKINE commercially available ELISA Kits (R&D Systems, Inc. 614 McKinley Place NE Minneapolis, MN 55413 USA). Complement immune proteins including complement 3 (C3) and complement 4 (C4) and plasma total immunoglobulin subsets (IgG and IgM) were measured by ELISA kits according to Abbott Laboratories instructions (Abbott Park, IL 60064 USA).

Statistical analysis: Experimental byproduct treatments were evaluated using a complete randomized block design, with treatment as the main effect. Statistical data analysis was carried out using the PROC GLIMMIX procedure (SAS, 2004). The statistical model used was as follows:

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

Where, Y_{ij} = experimental observation, μ = overall mean, T_i = effect of treatment, e_{ij} = experimental error. Tukey's range test was used to compare differences among treatments.

RESULTS AND DISCUSSION

Chemical composition of the experimental feeds: Chemical composition of the CFM and the experimental roughages are presented in Table 1.

Table 1: Chemical composition of concentrate feed mixture and expt. roughages.

Items	CFM	Dietary Treatments		
		UTW	YTW	ZTW
DM	91.27	93.13	93.21	93.38
CP	12.57	12.76	13.68	13.61
CF	6.32	23.55	20.17	19.24
EE	2.88	4.44	4.02	3.99
NFE	71.09	45.67	45.88	48.41
Ash	7.14	13.59	15.25	14.75
NDF	53.90	47.15	42.88	41.24
ADF	10.17	33.33	35.95	36.76
NFC ¹	23.51	22.06	24.17	26.41
NDS ²	46.10	52.85	57.12	58.76
GE ³ , MJ/kg DM	16.55	16.90	18.99	18.07

¹NFC (Calsamiglia et al., 1995): Non fibrous carbohydrates% = 100 - (CP% + EE% + Ash% + NDF%). ²NDS: Neutral detergent soluble = 100 – NDF. ³GE: Gross energy.

Data showed that both yeast and ZADO treatments revealed slightly increase in CP, and marked decrease in respect of CF contents as compared with untreated trimming waste, where the values of CP% were 13.68%, 13.61%, and 12.76% for YTW, ZTW, and UTW, respectively and the values of CF% were in the same order, i.e. 20.17%, 19.24% and 23.55%, respectively.

Increment of CP content with yeast or ZADO treatments could be due to one or more of the following reasons: (i) Production of microbial protein in the form of single-cell protein; yeasts and bacteria (Akinfemi and Ladibo, 2013), (ii) As a result of urea that is included in the liquid feed sprayed on trimming waste. This result was in agreement with earlier reports for yeast treatment (Kewan et al., 2019a), for ZADO treatment (Allam et al., 2009), and for both treatments (Abo Bakr et al., 2020).

The percentages of NFC for all roughages were within the lowest range of 20-25% (Wheeler, 2003) and the highest one (40-45%) as recommended for lactation rations (NRC, 2001). In addition, the contents of CF and NDF were decreased and GE was increased because of treatments. This was mainly related to its quality where forages high in fiber had less available energy, since feeds with high fiber are found to be low in energy content and are consumed less by ruminants than the forages with low fiber content (Weiss et al., 1982). Further, both treated diets (YTW and ZTW) showed higher values of ADF and NDS as compared with untreated ones (UTW).

Nutrient Digestibility and feeding values: Mostly the nutrient digestibilities and feeding values appeared to be significantly different ($P \leq 0.05$) among the experimental dietary treatments (Table 2).

Table 2: Nutrients digestibility and feeding values of the experimental rations.

Items	Experimental rations			SE	P value
	C	Y	Z		
Nutrients digestibility (%)					
CP	67.86 ^b	82.57 ^a	85.31 ^a	2.69	0.001
CF	54.85 ^c	62.33 ^b	68.76 ^a	2.05	0.003
EE	59.44 ^b	68.49 ^a	53.11 ^b	2.30	0.004
NFE	56.76 ^b	63.81 ^{ab}	68.77 ^a	1.98	0.029
NDF	50.30 ^c	67.65 ^b	70.34 ^a	2.94	0.003
ADF	20.38 ^b	38.57 ^a	40.42 ^a	3.12	<0.001
Feeding values					
TDN%	54.77 ^b	61.54 ^a	64.61 ^a	1.52	0.007
DE, Mcal/kg	2.41 ^b	2.72 ^a	2.85 ^a	0.06	0.006
ME ¹ , Mcal/kg	1.98 ^b	2.23 ^a	2.34 ^a	0.05	0.005
NEm ² , Mcal/kg	1.13 ^b	1.36 ^a	1.46 ^a	0.04	0.005
DIP ³ , g/d	134.3	134.1	133.5	3.79	0.987
UIP, g/d	49.72	54.23	53.13	6.37	0.875
DCP%	8.60 ^b	10.90 ^a	11.03 ^a	0.38	<0.001

¹ME (Mcal / kg) = 0.82 DE; ²NRC (1985); ³DIP (% on a DM basis) = 0.9 (CP %) - 3.

^{a,b,c} Values in the same row with different letters differ significantly ($p \leq 0.05$).

It seems that animals that received the Z diet showed the highest ($P \leq 0.05$) digestibility values for CP, CF, NFE, NDF, and ADF followed by Y and then C. However, the Y group was superior in EE digestibility.

Improvement of nutrient digestibilities by ZADO treatment may be probably due to the beneficial effects of enzymes on fiber hydrolysis and rumen fermentation activity (Gado *et al.*, 2011). Cellulolytic enzymes in ZADO would be expected to increase fiber digestion by different mechanisms, such as alterations in ruminal fermentation, increasing the rate of ruminal digestion of the potentially digestible fiber, enhance attachment and colonization to the plant cell wall by ruminal microorganisms (Khattab *et al.*, 2011). It also could be explained by the ability of ZADO to lose lignocellulosic bonds in fiber (Salem *et al.*, 2011). It is obvious from the data, the positive effect on the CP content (Table 1) and CP digestibility (Table 2) for trimming waste treated with ZADO might be mainly due to nitrogen content (Urea is about 0.17 kg/100 kg DM) and protease content of the addition ZADO compounds.

Regarding Yeast addition at YTW, some researchers found that yeast addition strategies have measurable effects on nutrient digestibility and rumen metabolism (Habeeb *et al.*, 2017; Mohammed *et al.* 2018; Chaucheyras-Durand *et al.*, 2019). It has been reported earlier that digestibility of CF was increased by 12.3% (Saleh *et al.*, 2004) and 12.8% (Abo Bakr *et al.*, 2020) with yeast supplementation to the diet of Barki sheep based on the control diet that is free from such supplementation, vs. 13.64% in the present study.

The improvement in CF digestibility may be due to that yeast stimulates the cellulolytic bacteria and also attributed to increase in CP digestibility that in consequence of stimulation of proteolytic bacteria.

Data of feeding values (TDN%, DE: Mcal/ kg, ME: Mcal/ kg, NEm: Mcal/ kg and DCP %) in Table (2) differed ($P \leq 0.05$) among the experimental rations where those of both two tested rations were comparable but all its mentioned values were higher ($P \leq 0.05$) than those of control one. The TDN values of Y and Z diets were accounted to be 12% and 18% higher than that of C diets.

Degradable intake protein (DIP) values were not significantly affected by the dietary treatments. Also the experimental diets showed insignificant effect on UIP content while DCP values of Z and Y were significantly ($P \leq 0.05$) higher by 28.26% and 26.74% respectively than those of C. These results might be due to the higher digestion efficiency of most nutrients in tested rations in comparison with the control, particularly in respect of TDN and DCP as reported earlier in Barki sheep (Abo Bakr *et al.*, 2020).

Feed intake and productive performance of ewes during suckling period: The values of feed intake and live body weight changes of Barki ewes are shown in Table 3.

Table 3: Feed intake and performance of Barki ewes during suckling period (112d).

Items	Experimental rations			SE	P value
	C	Y	Z		
Daily feed intake as DM, g/head					
CFM	779 ^b	763 ^b	817 ^a	5.09	<0.001
Roughage	819 ^a	733 ^b	868 ^a	17.27	0.005
Total	1598 ^b	1497 ^c	1685 ^a	14.25	<0.001
TDNI	875 ^c	921 ^b	1089 ^a	9.64	<0.001
DCPI	138 ^c	163 ^b	186 ^a	1.75	<0.001
Ewes body weight change, kg					
Initial BW	35.13	35.32	34.43	0.50	0.784
BW before lambing	40.90	40.45	39.94	0.53	0.789
BW after lambing	35.55	35.60	34.79	0.5	0.795
BW at weaning	36.78	36.45	35.87	0.6	0.780
BW change	1.23 ^a	0.85 ^c	1.08 ^b	0.04	<0.001
Weaned lambs	18	18	18		
Milk yield and composition					
Milk yield, g/d	417 ^b	531 ^a	438 ^b	16.79	0.007
ECM*, g/d	443 ^c	720 ^a	587 ^b	20.03	<0.001
Fat, %	3.67 ^b	5.98 ^a	5.86 ^a	0.21	<0.001
Protein, %	4.63	4.75	4.75	0.07	0.755
Lactose, %	4.63	4.49	4.51	0.06	0.908
Feed conversion					
kg DM/ kg ECM	3.60 ^a	2.08 ^b	2.87 ^a	0.31	0.001
kg TDN/ kg ECM	1.97 ^a	1.28 ^b	1.85 ^a	0.12	0.002
Kg DCP/ kg ECM	0.311 ^a	0.226 ^b	0.317 ^a	19.57	0.006

*ECG=Energy corrected milk

ECG = Milk production \times [(0.383 fat %) + (0.242 protein %) + 0.7832]/ 3.1138.

^{a,b,c} Values in the same row with different letters differ significantly ($P \leq 0.05$).

Roughage and total feed intake were markedly higher with ZADO addition compared to those of other two diets. Furthermore, ZADO and yeast additions significantly increased both TDN and DCP intakes compared to those of control diet (C). It has been confirmed earlier ([Ding et al., 2008](#)) that supplementation of dried yeast into rations of growing goat kids has improved DMI, TDNI and DCPI, although it is contrary to the observation ([Mohammed et al., 2018](#)) that there was no significant difference in DMI for ruminants supplemented with live yeast as compared with control diet.

Regarding the body weight of ewes before and after lambing and after weaning period, its values did not differ significantly ($P \leq 0.05$) among ewe groups. While, body weight change throughout the suckling period (112d) significantly differed and recorded the highest values for ewes fed control diet (C).

The positive body weight change revealed for all ewes in the present study may be due to the reason that these ewes were still in growing stage and had not reached the mature stage for this local breed, which is reported to be 40-45 kg ([Elshazly and Youngs, 2019](#)). However, the difference found herein among groups could also be attributed to other factors like birth weights of lambs and persistence of milk production. It seems that all ewes were in a positive body weight gain but that in Y and Z groups were lower ($P < 0.001$) than that in control one. This finding may be attributed to body weight gain, which was inversely proportional to the ECM. Such trend was not observed in sheep and goats ration supplemented with fibrolytic enzymes ([Salem et al., 2011](#)).

Milk yield, composition and feed conversion: Total milk yield and energy corrected milk yield were significantly higher ($P < 0.05$) with ewes fed diet treated with yeast (Y) than those fed ZADO and the control ones (Table 3).

Also, energy corrected milk yield (ECM) was significantly higher ($P = <0.001$) with Z-ration than those of C-one, but insignificant difference ($P = 0.007$) was observed between them in respect of actual milk yield.

Regarding milk composition, fat contents were significantly ($P \leq 0.05$) improved by both tested treatments (ZADO and yeast), while the other milk constituents (protein, and lactose) were not significantly affected by dietary treatments. Improved milk yield and fat% by yeast addition probably due to its positive effect on nutrient digestibility as seen previously in Table (2) which reflects on improving the feed efficiency to convert nutrients into production.

These results were in accordance with the observation ([Dawson and Tricarico, 2002](#)) who reported that milk yield increased by 7.3% in yeast-supplemented diets based on control diet. The authors suggested that yeast supplements have a significant role in strategies for economically enhancing the performance of ruminant animals.

Concerning to ZADO addition, milk yield (Table 3) tended to increase, while fat % were more ($P \leq 0.05$) affected by such addition compared to those of control diet. These results are in agreement with the findings ([Khattab et al., 2011](#)) which revealed that milk fat, protein and lactose percentages were increased due to treated diets with ZAD and/or ZADO for dairy goats. The authors explained the improvement of milk fat content by the positive effect of enzymes (ZAD and/or ZADO) treatment on fiber digestion.

It was also observed (Abd El-Ghani, 2004) that the values of milk energy and protein were significantly greater in yeast supplemented groups than the un-supplemented one, while, the values of milk lactose (%) were not affected with 3 or 6 g yeast per day compared to un-supplemented one. It has also been reported that, supplementation of enzyme in the form of ZADO powder or ZAD liquid did not affect ($P \leq 0.05$) the total milk yield in Barki ewes (El Hawy *et al.*, 2019).

Concerning feed conversion criteria as kg DM, kg TDN or Kg DCP required for producing one kg of ECM (Table 3), it can be observed that the best feed conversion efficiency for all items were recorded with ewes fed Y (2.08, 1.28 and 0.226, respectively) followed by the other two groups (Z and C). The present findings are in harmony with the report (Mahrous *et al.*, 2019) based on the treatment of Olive trees by-products with EM1 (Product of EMRO Organization in Japan) and EI-mofeed (91% molasses, 2.5% urea and 6.5% mixing minerals and vitamins).

Lamb's performance and feed economic evaluation: Data for newborn lambs' body weight from birth to weaning (112 days) are shown in Table (4).

Table 4: Lamb performance and economic evaluation for the experimental rations.

Items	Experimental rations			SEM	P value
	C	Y	Z		
Lamb performance					
Birth weight of male lambs, kg	4.10	3.68	3.30	-	-
Birth weight of female lambs, kg	3.55	3.53	3.78	-	-
Average birth weight, kg	3.73	3.63	3.62	0.07	0.792
Weaning weight at 112d, kg	26.4 ^c	32.3 ^a	29.1 ^b	0.75	0.024
Average daily gain, g	202 ^c	256 ^a	228 ^b	5.53	0.018
Economic indicators					
CFM intake, g (as fed)/h/d	854	836	895	-	-
Roughage. Intake, g (as fed)/h/d	879	786	930	-	-
CFM cost, LE/day	3.10	3.03	3.25	-	-
Roughage cost, LE/day	0.41	0.68	0.72	-	-
Total feed cost, LE/ewe/day	3.51	3.71	3.97	-	-
Total feed cost, LE/ewe/112day	393	416	445	-	-
Total lambs wt gain, kg/ewe	22.67	28.67	25.48	-	-
Price of total gain, LE*	1587	2007	1784	-	-
Total net return, LE	1194	1591	1339	-	-
Net return/ feed cost, LE	3.04	3.82	3.01	-	-

The assumed 1 Kg live weight price for weaned lambs is 70 LE. The cost of one ton is 3975 LE for concentrate mixture, 500 LE for untreated waste, 930 LE for ZADO trimming waste of mandarin trees; 830 LE for Yeast treated trimming waste. *LE = Egyptian Pound.

^{a,b,c} Values in the same row with different letters differ significantly ($p \leq 0.05$).

The present results showed that yeast treatment (Y) possessed a significant ($P \leq 0.05$) higher effect on lamb weaning weights and average daily gain compared to Z and C groups. Also, Z-diet had significant positive effect respecting these growth parameters compared with those of control one. Average daily gain of lambs in Y and Z groups was higher by 26.73% and 12.87%, respectively than that of control group (C).

Improved ADG of lambs by yeast supplement could be explained by the highest milk production of their mothers, highest total solid and fat contents as compared to control diet. Growth responses to yeast addition in meat production were variable from non-significant increase in average daily gain to more than 20% increase according the prevailing conditions of the study (Habeeb *et al.*, 2017). It has also been reported (Saleh *et al.*, 2004) that adding yeast increased significantly the total gain by 12.1% until 85 days of the experiment or until LBW of lambs reached 42.2 kg; thereafter the differences between control and yeast treatment groups in respecting daily gain were not significant.

These results mean that average daily gain was considerably efficient with lambs from weaning to approximately 42.2 kg LBW i.e. during first growing stage. On the other side, the positive effect on daily gain for lambs in Y (Table 4) agreed with the report (El Hawy *et al.*, 2019) for weaning lambs. The total feed cost (LE/ewe/112d) was higher in groups fed treated diets compared with untreated one (Table 4), this mainly owing to the cost of additive components. The price of total gain was higher in Y and Z groups as relative to C group by 26.49% and 12.47%, respectively. So the total net return per ewe was markedly better in both treated groups compared with control one being values 1590.9, 1338.6 and 1193.9 LE/ewe for Y, Z and C, respectively.

The relative net return to feed cost was increased in Y group by 25.66%, while it decreased in Z group by 0.99% than control. Generally, feed efficiency for ewes fed diet with yeast additive was better than those fed Z and control ration. Comparable results (Kewan *et al.*, 2019a) suggested that treated *Moringa oleifera* tree stalks by different type of probiotic (fungi or yeast) had better economical feed efficiency compared to that of control group. Also, supplementing suckling lambs with yeast increased the net profit by LE 16.8 (Abdel-Mageed and Ali, 2011).

Water metabolism: The data of water metabolism with rams during the digestibility trials are presented in Table (5). The intake of water contained feed and free water as well as water excretion via feces and urine were not different among the experimental groups. However, animals who received Z diet showed the highest ($P \leq 0.01$) IWL value (88.52 g/kg^{0.82}) followed by Y group (80.41 g/kg^{0.82}) and then C group as the lowest one which was 67.46 g/kg^{0.82}. Animals receiving treated diets consumed higher amount of free water compared with C group but without significant differences.

This finding was lower than the report (Kewan *et al.*, 2019a) which stated that biological treatment (fungi or yeast) for *Moringa oleifera* tree stalks caused the increase of free water intake. These findings may be due to higher dry matter intake (Kewan *et al.*, 2011), high ash intake (Kewan *et al.*, 2019b) and/or high crude protein intake (Allam *et al.*, 2009).

The present results are in disagreement with the report (Abo Bakr *et al.*, 2020) which stated that feeding sheep on the same forage of the present study but as sole diets could save water. So the differences might be owing to the high level of CFM intake in the present study. It was also found (Fayed *et al.*, 2009) that IWL values were 3703.67, 3646.33 and 2441.66 ml/h/d for CFM+ Berseem hay (control), CFM+ olive leaves and twigs treated with urea and CFM+ olive leaves and twigs treated with *T. viride* + *S. cerevisiae*, respectively.

Table 5: Water metabolism and utilization efficiency in rams during digestibility trials.

Items	Experimental rations			SEM	P
	C	Y	Z		
BW ^{0.82}	26.25	23.23	26.12	0.29	0.986
Water metabolism					
Feed water, g/kg ^{0.82}	4.07	4.25	4.13	0.04	0.283
Free WI, g/kg ^{0.82}	96.6	113.5	117.3	3.34	0.007
Total WI, g/ kg ^{0.82}	100.6 ^b	117.7 ^a	121.5 ^a	3.36	0.007
Fecal water, g/kg ^{0.82}	16.37	15.24	14.53	0.42	0.253
Urine, g/kg ^{0.82}	16.80	22.09	18.43	1.23	0.265
Total excretion, g/ kg ^{0.82}	33.17	37.33	32.96	1.40	0.458
IWL*, g/ kg ^{0.82}	67.43 ^c	80.37 ^b	88.54 ^a	3.08	0.002
Water utilization efficiency					
g Free WI/ g DMI	2.22	2.42	2.52	0.13	0.716
fecal water/ TWI%	16.27 ^a	12.95 ^b	11.96 ^b	0.67	0.003
Urine/ TWI%	16.70	18.77	15.17	0.90	0.342
Total excretion/TWI%	32.97	31.72	27.13	1.21	0.153
IWL/TWI %	67.03	68.28	72.87	1.21	0.152

*IWL = Insensible water loss.

^{a,b,c} Values in the same row with different superscripts differ significantly (P ≤ .05).

Rumen activity: Ruminal pH values, concentrations of NH₃-N and TVFAs are shown in Table (6). Data showed that both of yeast and ZADO treatments significantly (P ≤ 0.01) decreased ruminal pH values at zero (0), 3 and 6 h post feeding as compared with untreated group, while, ammonia nitrogen concentrations at the most sampling times for the same groups (Y and Z) were higher (P ≤ 0.01) compared to that of untreated one. The TVFAs concentration showed an opposite trend to that of NH₃-N where both treated groups (Y and Z) were significantly (P ≤ 0.01) lower than that of control group only at 3 and 6h post feeding samples, but comparable values were observed among groups at zero time sampling. Acetate percentage at 6hr. post feeding was not affected by the experimental treatments being Z group showed the highest value (71.95%). While, butyrate percentage was higher (P ≤ 0.01) in Z group (6.95%) than both of Y (6.53%) and C (6.45%) groups.

Otherwise, the proportion of propionate decreased (P ≤ 0.01) with yeast and ZADO treatments based on control one. These results are in harmony with the previous reports on supplementation of ZADO (Salem *et al.*, 2015) and yeast (Mohammed *et al.*, 2018). The lower pH values in Y and Z groups may be explained by releasing the yeast extracellular metabolites, including organic acids, in the rumen (Shurson, 2018).

Furthermore, the organic acids and saccharides contained in YC might be beneficial for the growth of microorganisms (*S. ruminatum* and *M. elsdenii*) and its products (organic acids) in the rumen (Nisbet and Martin, 1991). The present results of pH, NH₃-N and VFAs either in total or fractions were found to be different for sheep fed the same roughages as sole diets without concentrate supplement reported earlier (Abo Bakr *et al.*, 2020). Variation in results could be explained due to the associative effect between roughage and concentrate and also to the effect of the present feed treatments with the level of CFM.

Table 6: Rumen fermentation parameters of rams during the digestibility trial.

Items	Experimental rations			SEM	P
	C	Y	Z		
pH					
0	7.25 ^a	6.93 ^b	6.93 ^b	0.09	0.057
3h	6.64 ^a	6.28 ^b	6.42 ^b	0.06	0.008
6h	6.75 ^a	6.41 ^b	6.49 ^b	0.10	0.094
Overall mean ± SE	6.88±0.08	6.54±0.09	6.61±0.09	-	-
NH₃-N, mg/dL					
0	18.21 ^c	34.00 ^a	25.44 ^b	1.04	<0.001
3h	24.92 ^c	38.53 ^a	32.39 ^b	1.52	<0.001
6h	26.45 ^b	35.42 ^a	28.38 ^b	1.40	0.003
Overall mean ± SE	23.19±1.21	35.98±0.84	28.74±1.23	-	-
TVFA, meq/dL					
0	6.76	7.77	7.90	0.44	0.197
3h	13.84 ^a	9.87 ^b	10.46 ^b	0.32	<0.001
6h	11.78 ^a	7.45 ^b	7.87 ^b	1.05	0.032
Overall mean ± SE	10.79±1.05	8.36±0.37	8.74±0.42	-	-
VFA fractions at 6h, %					
Acetate	62.63	61.72	71.95	5.76	0.403
Propionate	20.67 ^a	20.11 ^b	20.15 ^b	0.03	0.003
Butyrate	6.45 ^c	6.53 ^b	6.95 ^a	0.02	<0.001

Values (^{a,b,c}) in the same row with different superscripts differ significantly (P ≤ 0.001)

The current results are in match with the reports on decrease in ruminal pH and increase in NH₃-N concentrations with YC supplementation with high-grain diet fed to lambs (Soren *et al.*, 2013). However, YC supplementation has been found to increase ruminal pH and decrease TVFAs in a low-starch diet (16% corn), which could be related to the interaction between YC and diet composition (Throne *et al.*, 2009).

The highest values of NH₃-N observed in animals of Y group could be explained due to urea and yeast inclusion of Y treatment as found in the previous studies (Fayed *et al.*, 2009; Kewan *et al.*, 2019a). In perspective, the ruminal N-NH₃ concentration is a consequence of the balance between production, absorption and utilization by microorganisms. Definitely all the rumen ammonia concentrations observed in the present study were higher than the value (10 mg N-NH₃/100 mL) which is required for maximum microbial fermentation (Van Soest, 1994). The mean TVFAs concentration values in this study are within the normal range (3.07 to 19.90) of rumen liquor, which has been reported by several researchers (Abdou, 2003; Eid, 2003).

From the overall results on TVFAs concentration, it can be seen that though the TVFAs concentration as well as molar percentage of individual volatile fatty acids appeared to be affected by protein degradability levels, all the values were within the normal range recorded for animals maintained on standard diets. As such, the differences observed may be related to the composition of the fermentable organic matter and its influence on kind of microbes rather than on protein degradability rate, which is also evidenced by the almost normal rumen ammonia nitrogen levels (Kalbande and Thomas 2001).

In vitro gas and methane production: The data of Table (7) illustrate the values of the gas production as results of *in vitro* fermentation of the experimental diets. Results showed that the tested treatments (Y and Z) significantly ($P \leq 0.001$) increased total gas production at 24h of *in vitro* fermentation period. Also, the gas production constants (*a*, *b*, and *c*) were significantly higher than those of untreated diet, being the highest ($P \leq 0.001$) for Z, followed by Y and C. Otherwise, the rate of gas production (*c*, %h⁻¹) was not different among the experimental diets.

Methane production was higher ($P \leq 0.001$) in C diet than those of both treated diets (Y and Z). In fact, gas production, short chain fatty acids, and microbial cells, all resulted mainly from fermentation of carbohydrates more than from protein and fat components of diet. So that, the higher gas production by biological treatments may be due to the increase of available carbohydrates for ruminal fermentation. Moreover, the rate of gas production is associated with the rapid growth phase of microorganism and in mixed cultures, the rate of fermentation will be a result of the interaction between the microorganisms and how they digest the feed particulars (Mauricio *et al.*, 2001). Methane volume represents 24.36%, 15.02% and 16.58% of the total gas volume at 24h. of fermentation, where the highest loss of energy was occurred with control diet as compared with the two tested diets and matched with the ME of diets that mentioned in Table (2).

The trend to higher effective DM degradability observed for Y and Z, as compared with that of untreated diet (C), could be attributed to higher microbial colonization of the tested diets and diet formulated with high levels of fibrous carbohydrates as found earlier (Kleinschmit *et al.*, 2007). Several studies have also focused on improving agricultural byproducts digestibility *in vitro* and increase fiber digestion and utilization by adding the probiotic ZAD as liquid or ZADO in powder form (Gado, 2020).

Table 7: In vitro gas production for the experimental rations.

Items	Experimental rations			SEM	P
	C	Y	Z		
GP _{24h} , ml/ 200mg DM	29.93 ^c	36.63 ^a	33.83 ^b	0.06	<0.001
<i>In vitro</i> gas production constants					
<i>a</i> , ml	2.73 ^c	3.03 ^b	3.27 ^a	0.03	<0.001
<i>b</i> , ml	19.07 ^c	20.43 ^b	21.30 ^a	0.05	<0.001
<i>c</i> , %h ⁻¹	0.08	0.09	0.09	0.00	0.125
Methane, ml	7.29 ^a	5.50 ^c	5.61 ^b	0.02	<0.001
Methane, %	24.36 ^a	15.02 ^c	16.58 ^b	0.03	<0.001
EDMD, % for k=0.03	46.26 ^c	52.77 ^a	50.44 ^b	0.15	<0.001
MP(g/kg DM)	57.8 ^b	65.4 ^a	62.1 ^a	0.73	0.036

^{a,b,c}Values in the same row with different superscripts differ significantly ($P \leq 0.001$).

Studies on the influence of probiotic mixture (ZADO) on *in vitro* gas production and *in vitro* dry matter degradability (DMD) in lambs fed a high concentrate diet has revealed that addition of exogenous enzymes (probiotic) has potential impacts on improving the utilization of high concentrate diet due to improving the ruminal activities (López *et al.*, 2013). Similar results of *in vitro* gas production were also obtained with biological treatments in Barki sheep (Abdou, 2018).

Blood protein indices in Barki ewes and their lambs: Total protein indices were evaluated by the measurements of TP, Alb, and Glb for ewes and their suckling lambs (Table 8). The maximum values as overall means of plasma TP, Alb and Glb parameters were recorded with yeast treatment followed by ZADO treatment and then those of control one that represented the lowest one for both lactating ewes and their lambs. The highest overall means of TP and Alb were recorded during the late lactation stage. However, the lactation stage had no significant effect on the concentration of TP in ewes and lambs. On the other hand, Glb concentration significantly increased at late stage in lambs but it was not affected by the lactation stage in ewes. Suckling lambs in Y group exhibited the maximum values of all measured proteins followed by Z and then control groups. The increase in the concentrations of TP, Alb and Glb in Y group may be due to the increasing of CP digestibility relatively to those of other groups, and also yeast cells are considerable as an additional source of amino acids which are necessary for synthesis of plasma protein (Hooda and Upadhyay, 2014). The present results are in agreement with the report (Bassuny *et al.*, 2003) who found higher significant values of total protein, and albumin with fungi treatment compared to untreated one. However, rams fed fungi treated pruning peach trees by-products were insignificantly increased blood plasma TP compared to those fed control ration (Khir *et al.*, 2015).

Table 8: Plasma protein indices in Barki ewes / lambs in different lactation stages.

Item Para STG	Ewes					Lambs				
	Treatment			Mean	P ≤ F value	Treatment			Mean	P ≤ F value
	C	Y	Z			C	Y	Z		
Total Protein: TP (g/dl)										
Early	5.03	9.64	7.91	7.53	S=0.13	6.33	8.87	6.79	7.33	S=0.25
Mid	6.34	8.98	8.88	8.07	T=0.01	6.32	8.09	6.12	6.84	T=0.01
Late	7.07	9.98	8.32	8.46	Int.=0.2	5.11	8.73	7.04	6.96	Int.=0.16
Mean	6.15 ^c	9.54 ^a	8.37 ^b	-	-	5.92 ^c	8.56 ^a	6.65 ^b	-	-
Albumin: Alb (g/dl)										
Early	2.50	4.74	3.73	3.66 ^b	S=0.01	4.45	5.28	5.53	5.09 ^a	S=0.01
Mid	3.31	4.57	4.10	3.99 ^b	T=0.01	4.83	4.88	3.81	4.51 ^b	T=0.05
Late	3.71	5.43	4.28	4.47 ^a	Int.=0.4	3.12	4.61	4.57	4.10 ^b	Int.=0.11
Mean	3.17 ^c	4.91 ^a	4.04 ^b	-	-	4.14 ^b	4.92 ^a	4.63 ^a	-	-
Globulin: Glb (g/dl)										
Early	2.53	4.90	4.18	3.87	S=0.72	1.88	3.58	1.26	2.24 ^b	S=0.01
Mid	3.03	4.42	4.78	4.07	T=0.01	1.49	3.20	2.32	2.34 ^b	T=0.01
Late	3.37	4.55	4.04	3.99	Int.=0.12	1.99	4.12	2.48	2.86 ^a	Int.=0.03
Mean	2.97 ^b	4.62 ^a	4.33 ^a	-	-	1.79 ^c	3.64 ^A	2.02 ^B	-	-

Int.: interaction between treatment and lactation stage. Means with different letters in each row and column are significantly different ($P \leq 0.05/0.01$).

The others attributed the insignificant increases of blood plasma total protein may to the increase of N-intake and its higher digestibility which reflected on ruminal NH₃-N and finally the blood plasma constituents. Fed untraditional ration as compared to traditional one in lactating ewes and their lambs increased Alb and Glb concentrations while TP values were not affected due to type of feeding and this concluded that untraditional ration increased the immunity.

Liver and kidney function parameters in Barki ewes and their lambs: No significant effect on some liver functions such as AST and GGT activity was observed due to the present dietary treatments (Table 9), while ALT activity was significantly higher with yeast group than that of control one.

Otherwise, ZADO group did not differ significantly with both control and the other tested group respecting ALT item in case of ewes. The concentration of GGT was not significantly affected by the experimental rations in ewes and their lambs. Progressing lactation stage decreased AST and ALT for ewes only.

Both tested diets significantly decreased ALP concentration based on control diet in case of ewes and their lambs. The liver functions of suckling lambs exhibited by ALT, AST and GGT concentrations are elevated with ZADO treated group and decreased with yeast treatments compared to control. There are significant differences among experimental groups in plasma ALT and AST values.

Potential hepatic damage is being considered as a consequence of tannin absorption ([Mahato et al., 2019](#)). Total tannins have been determined to be 17.41 g/kg DM in TWMT in a recent study ([Abo Bakr et al., 2020](#)). The ALP activities are used to detect bile obstruction, i.e. a mild and progressive damage to the liver ([Silanikove and Tiomkin, 1992](#)).

A similar approach demonstrated that feeding growing cattle with *Shorea robusta* did not induce systemic toxicity ([Garg et al., 1984](#)). However, changes in these parameters were found when tannin-related hepatotoxicity occurred ([Zhu and Flippish, 1995](#)). The ALP activity levels in the plasma of animals fed trimming waste were within the physiological range as affected by yeast and ZADO treatments suggesting that no damage to the liver occurred.

Kidney functions in lactating ewes are slightly affected, using the different experimental diets since creatinine showed a significant ($P \leq 0.05$) increase in Y treatment and then Z treatment compared with control. Blood urea concentration was significantly unaffected neither by treatments nor by lactating stage (Table 9).

In suckling lambs, the kidney functions exhibited by plasma creatinine concentration was lower significantly ($P \leq 0.01$) with Y and Z groups than those in C one. While, urea concentration was elevated ($P \leq 0.01$) by Y treatment group as compared to Z and C groups. Previous work ([Frutos et al., 2000](#)) that carried out on tannin compounds reported the lack of toxicity at concentrations equivalent to that found in roughages used in the present study.

Damage to the kidneys would most likely lead to renal failure and to changes in plasma creatinine ([Zhu and Flippish, 1995](#)). The low plasma creatinine concentrations in treated groups suggest that damage to the kidney did not occur to both the experimental animal groups. This was in agreement with the previous data for goats ([Yanez Ruiz et al., 2004](#)).

The present results are in accordance with the report (Bassuny *et al.*, 2003) who found highly significant urea concentration as a result of feeding fungi treated diets as compared to untreated one. However, rams fed fungi treated pruning peach trees by-products were insignificantly increased blood urea compared to those fed control ration (Khir *et al.*, 2015). The others attributed the insignificant increases of blood plasma urea to the increase of N-intake and its higher digestibility which reflected on ruminal NH₃-N and finally the blood plasma constituents. The present findings could be explained by treatments that included molasses, urea, yeast and exogenous enzymes (ZADO) that enhanced the ruminal fermentation otherwise better utilization and metabolism of the nutrients along with the intestinal tract (Chaucheyars-Durand and Fonty, 2001).

Table 9: Liver and kidney function parameters in Barki ewes and their lambs in different lactation stages.

Item Para STG	Ewes					Lambs				
	Treatment			Mean	P ≤ F value	Treatment			Mean	P ≤ F value
	C	Y	Z			C	Y	Z		
Alanine aminotransferase: AST (U/L)										
Early	37.1	66.0	44.4	49.2	S=0.52	56.5	45.4	69.8	57.2	S=0.12
Mid	53.3	51.5	35.8	46.8	T=0.21	50.4	48.6	51.5	50.2	T=0.01
Late	45.3	39.0	40.8	41.7	Int=0.16	59.4	42.2	55.2	52.3	Int=0.09
Mean	45.2	52.1	40.4	-	-	55.4 ^a	45.4 ^b	58.8 ^a	-	-
Aspartate aminotransferase: ALT (U/L)										
Early	39.8	59.8	58.3	52.6	S=0.07	53.6	39.1	65.7	52.8 ^a	S=0.03
Mid	42.8	53.6	35.6	44.0	T=0.02	50.2	34.7	47.1	44.0 ^b	T=0.01
Late	34.1	48.2	39.8	40.7	Int=0.39	49.1	45.5	46.3	47.0 ^a	Int=0.07
Mean	38.9 ^b	53.8 ^a	44.6 ^{ab}	-	-	51.0 ^a	39.8 ^b	53.0 ^a	-	-
Gamma-glutamyltransferase: GGT (IU/L)										
Early	22.8	24.2	30.5	25.8 ^b	S=0.02	25.5	26.1	32.1	27.9 ^b	S=0.01
Mid	33.8	34.1	83.2	50.4 ^a	T=0.24	43.9	41.7	50.5	45.3 ^a	T=0.09
Late	37.6	32.8	20.6	30.3 ^b	Int=0.04	32.5	18.8	33.9	28.4 ^b	Int=0.13
Mean	31.37	30.38	44.73	-	-	33.96	28.85	38.84	-	-
Alkaline phosphatase: ALP (U/L)										
Early	137.9	47.6	88.1	91.2 ^a	S=0.05	17.1	111.7	99.9	76.2 ^a	S=0.01
Mid	89.2	38.5	45.6	57.8 ^b	T=0.01	84.2	45.9	31.9	54.0 ^b	T=0.96
Late	112.1	75.0	66.1	84.4 ^{ab}	Int=0.56	127.3	62.0	94.3	94.3 ^a	Int=0.99
Mean	113.1 ^a	53.7 ^b	66.6 ^b	-	-	76.2 ^a	72.8 ^b	75.3 ^B	-	-
Creatinine (mg/dl)										
Early	0.73	0.96	0.85	0.85	S=0.46	1.04	1.05	1.01	1.03 ^{ab}	S=0.04
Mid	0.66	0.83	0.82	0.77	T=0.02	1.09	0.88	0.86	0.95 ^b	T=0.02
Late	0.74	0.86	0.78	0.79	Int=0.9	1.40	1.00	0.87	1.09 ^a	Int=0.07
Mean	0.71 ^b	0.88 ^a	0.82 ^{ab}	-	-	1.18 ^a	0.98 ^b	0.92 ^b	-	-
Urea (mg/dl)										
Early	26.0	31.5	25.8	27.8	S=0.99	21.9	43.5	39.2	34.9 ^a	S=0.01
Mid	31.0	25.5	27.3	27.9	T=0.28	23.4	27.5	34.1	28.3 ^b	T=0.01
Late	23.3	33.3	26.5	27.7	Int=0.1	28.4	35.5	29.4	31.1 ^a	Int=0.05
Mean	26.8	30.1	26.5	-	-	24.6 ^b	35.5 ^a	24.2 ^b	-	-

Int.: interaction between treatment and lactation stage. Means with different letters with each row and column are significantly different at (P ≤ 0.05/0.01).

Addition of Zado® has been found to increase the ability of lactating ewes to alleviate heat stress in summer (25-44°C) compared to winter (8-22°C) since it caused increase in plasma TP, milk production, milk protein and lactose in the treated groups in comparison to control group under summer conditions (Gado *et al.*, 2014). The present results coincide with the results of the report (Aziz, 2019) who stated that biological treatments had a positive significant effect on blood metabolites of ruminants by using yeast, fungi, or bacteria for a wide range of poor quality by-products. On the other hand, the present results disagree with the observation (Mahrous *et al.*, 2019) who found no significant differences in respect of blood plasma parameters among goat groups fed untreated or EM1 and EI-mofeed treated olive tree by-products.

Lipid profile in Barki ewes and their lambs: Data related to the effect of feeding the experimental rations on plasma lipid profile in lactating ewes and their lambs are presented in Table (10). The TL was not affected by the two tested rations in respect of ewes, while it was significantly higher ($P \leq 0.01$) in Y group than the other two groups in case of lambs.

Table 10: Blood lipid profile in Barki ewes and lambs in different lactation stages

Item Para STG	Ewes					Lambs				
	Treatment			Mean	P ≤ F value	Treatment			Mean	P ≤ F value
	C	Y	Z			C	Y	Z		
Total lipids: TL (mg/dL)										
Early	169.0	228.3	207.8	202 ^a	S=0.01	120.2	207.7	79.0	136 ^b	S=0.01
Mid	170.7	196.4	188.9	185 ^a	T=0.35	148.2	217.8	159.2	175 ^a	T=0.01
Late	164.4	129.1	85.8	126 ^b	Int=0.04	107.2	194.1	139.9	147 ^a	Int=0.07
Mean	168.0	184.6	160.9	-	-	125.2 ^b	206.5 ^a	126.1 ^b	-	-
Triglycerides: TG (mg/dL)										
Early	41.3	51.0	40.0	44.1 ^b	S=0.01	68.5	93.1	32.0	64.5	S=0.86
Mid	70.4	55.8	47.6	57.9 ^a	T=0.01	65.1	61.4	56.1	60.9	T=0.01
Late	53.2	87.6	45.0	61.9 ^a	Int=0.01	70.1	62.4	56.8	63.1	Int=0.48
Mean	55.0 ^{ab}	64.8 ^a	44.2 ^b	-	-	67.9 ^b	72.3 ^a	48.3 ^b	-	-
Total Cholesterol: TC (mg/dL)										
Early	84.4	61.0	62.3	69.3 ^b	S=0.05	31.7	47.3	37.4	38.8 ^c	S=0.01
Mid	126.2	46.9	49.2	74.1 ^a	T=0.05	41.8	47.0	63.1	50.6 ^b	T=0.01
Late	42.6	38.7	25.7	35.7 ^c	Int=0.4	36.2	53.2	54.0	47.8 ^a	Int=0.03
Mean	84.4 ^a	48.9 ^b	45.7 ^b	-	-	36.6 ^b	49.2 ^{ab}	51.5 ^a	-	-
High density lipoproteins: HDL (mg/dL)										
Early	8.33	4.78	7.77	6.96 ^b	S=0.01	1.76	4.53	1.48	2.59 ^b	S=0.01
Mid	20.4	4.40	4.46	9.75 ^a	T=0.01	3.90	5.17	2.72	3.93 ^a	T=0.01
Late	6.06	2.88	2.84	3.93 ^c	Int=0.01	2.91	4.47	2.49	3.29 ^c	Int=0.02
Mean	11.6 ^a	4.02 ^b	5.02 ^b	-	-	2.86 ^b	4.72 ^a	2.23 ^b	-	-
Low density lipoproteins: LDL (mg/dL)										
Early	63.8	44.7	45.4	51.3 ^a	S=0.02	19.4	20.6	13.0	17.6 ^b	S=0.01
Mid	101.3	39.4	33.7	58.1 ^a	T=0.02	25.8	33.6	34.6	31.3 ^a	T=0.05
Late	31.4	16.6	12.2	20.1 ^b	Int=0.5	13.5	30.7	28.2	24.1 ^a	Int=0.3
Mean	65.5 ^a	33.6 ^b	30.4 ^b	-	-	19.6 ^b	28.3 ^a	25.3 ^{ab}	-	-

Int.: interaction between treatment and lactation stage.

Means with different letters within row and column are significantly different ($P \leq 0.05/0.01$)

Regarding TG, its values were markedly superior with Y group than that of the Z group and control one with both ewes and lambs. The significant post-partum increase in TL as a result of increasing free fatty acids uptake by the liver from circulating plasma, and in turn increased TG storage was observed in cattle (Grummer, 1993).

The observed changes in plasma TG concentration between the early and mid-lactation of ewes have also been reported (Gradinski-Urbanac *et al.*, 1986). Also, this result was in accordance with that conducted on goats that showed increases in the values of serum triacylglycerols just before parturition (Hussein and Azab, 1998).

The decreasing pattern of TG and TC in early lactation was also reported in dairy cows which showed the lowest values of these compounds at the onset of lactation for their growing requirement for energy (Marcos *et al.*, 1990). The TC concentration was significantly decreased in two tested groups for ewes, but with lambs it took the reverse trend in comparison with the control group.

Concerning the concentration of HDL and LDL, the values were significantly decreased with ewe groups fed the two tested rations than those fed the control one, while such trends were unclear with lambs.

Ruminant feeding systems based on locally available by-product feedstuffs are often a practical alternative because the rumen microbial ecosystem can utilize such fibrous ingredients, which often contain high levels of structural fiber to meet their nutrient requirements for reasonable level of production (Mirzaei and Sis, 2008).

Immune indices in lactating Barki ewes and their lambs: Feeding treated with TWMT in lactating ewes showed that Immunoglobulin (IgG) values presented a non-significant change versus control (Table 11). On the other hand, feeding both tested diets decreased the values of IgM, C3, C4, IL-2, IL-6 and TNF- α versus control in case of ewes, while the vice versa happened in lambs in respect of the previous measurements. The IL-1 β value was significantly decreased with Z group based on control one, but the value of Y group was significantly higher than that of control with ewes. Such IL-1 β measure was not significantly affected by dietary treatment in lambs. All tested parameters (IgM, C3, C4, IL-2, IL-6 and TNF- α) showed similar values between yeast and ZADO treatments and had statistically similar effects except for C4 value. These parameters act as key immune sentinels with the unique ability to integrate and deliver large quantities of incoming signals to lymphocytes and thereby initiate and regulate an adaptive immune response (Buckwalter and Albert, 2009).

Regarding the lactation stages, Table (11) illustrated the effect of feeding treated diets on the different immune measurements over different lactating periods (early, mid and late). Late lactating stage elevated the values of IgG, IgM, IL-1 β and IL-2. It might be considered that the observed elevation in IgG provide protection from diarrhea that causes death among lambs during neonatal period which will have a positive effect on health and welfare. It is reported (Teleb *et al.*, 2009) that, IgG levels in lamb's serum increased with the increase of TP and Glb. So, determination of serum TP and Glb can be used as an indicator for the passive transfer for IgG. Its values were decreased gradually among the mid and early stages, respectively.

Table 11: Blood immune indices in Barki ewes / lambs in different lactation stages.

Item Para STG	Ewes					Lambs				
	Treatment			Mean	P ≤ F value	Treatment			Mean	P ≤ F value
	C	Y	Z			C	Y	Z		
Immunoglobulin G: IgG (mg/ml)										
Early	2.24	3.60	3.26	3.03 ^c	S=0.01	9.37	6.41	15.47	10.4 ^a	S=0.03
Mid	7.83	1.09	4.78	4.56 ^b	T=0.51	5.63	5.45	11.38	7.48 ^b	T=0.01
Late	5.36	11.99	9.95	9.10 ^a	Int=0.6	9.77	8.59	13.19	10.5 ^a	Int=0.1
Mean	5.14	5.56	5.99	-	-	8.25 ^b	6.81 ^b	13.35 ^a	-	-
Immunoglobulin M: IgM (mg/ml)										
Early	0.77	1.54	1.09	1.13 ^c	S=0.01	4.23	1.91	5.15	3.77 ^a	S=0.03
Mid	6.94	0.78	1.59	3.10 ^b	T=0.01	1.93	1.81	3.79	2.51 ^b	T=0.01
Late	5.79	4.00	3.31	4.36 ^a	Int=0.03	2.59	2.86	4.39	3.28 ^a	Int=0.05
Mean	4.50 ^a	2.10 ^b	2.00 ^b	-	-	2.92 ^b	2.20 ^{ab}	4.45 ^a	-	-
Complement 3: C3 (mg/ml)										
Early	0.89	0.87	0.80	0.85 ^b	S=0.05	1.64	0.77	1.64	1.35	S=0.27
Mid	4.43	0.45	0.80	1.89 ^a	T=0.01	2.30	1.18	1.29	1.59	T=0.01
Late	2.00	1.47	1.24	1.57 ^{ab}	Int=0.1	1.63	1.05	1.50	1.39	Int=0.2
Mean	2.44 ^a	0.93 ^b	0.95 ^b	-	-	1.86 ^a	1.00 ^c	1.48 ^b	-	-
Complement 4: C4 (mg/ml)										
Early	3.66	0.96	2.11	2.24 ^b	S=0.01	7.04	5.02	5.43	5.83	S=0.09
Mid	7.27	1.56	2.86	3.89 ^a	T=0.01	3.65	4.08	5.41	4.38	T=0.32
Late	5.20	3.56	4.69	4.48 ^a	Int=0.01	4.94	4.87	6.53	5.44	Int=0.44
Mean	5.38 ^a	2.02 ^c	3.22 ^b	-	-	5.21	4.65	5.79	-	-
Interleukin 1β: IL-1β (Pg/dl)										
Early	40.3	48.6	48.3	45.7 ^c	S=0.01	116.0	83.3	112.1	104 ^a	S=0.01
Mid	88.6	100.2	47.9	78.9 ^b	T=0.05	66.4	70.6	79.6	72.2 ^b	T=0.15
Late	96.9	107.6	95.1	99.9 ^a	Int=25	86.7	82.6	110.8	93.4 ^a	Int=0.24
Mean	75.3 ^{ab}	85.5 ^a	63.8 ^b	-	-	89.7	78.8	100.9	-	-
Interleukin 2: IL-2 (Pg/dl)										
Early	56.8	36.3	72.9	55.3 ^c	S=0.01	117.4	88.83	124.7	110.3	S=0.07
Mid	100.8	41.4	51.2	64.4 ^b	T=0.01	75.8	98.6	108.9	94.5	T=0.01
Late	85.4	112.7	61.7	86.6 ^a	Int=0.03	99.1	83.7	114.9	99.2	Int=0.15
Mean	81.0 ^a	63.5 ^b	61.9 ^b	-	-	97.5 ^b	90.4 ^{ab}	116.2 ^a	-	-
Interleukin 6: IL-6 (Pg/dl)										
Early	77.5	24.5	36.7	46.2 ^c	S=0.05	60.1	52.7	107.3	73.3	S=0.71
Mid	75.9	54.2	44.8	58.3 ^a	T=0.01	46.7	94.9	84.4	75.4	T=0.01
Late	65.4	40.5	47.7	51.2 ^b	Int=0.2	71.2	43.6	97.6	70.8	Int=0.3
Mean	72.9 ^a	39.7 ^b	43.1 ^b	-	-	59.4 ^b	63.7 ^b	96.4 ^a	-	-
Tumor necrosis factor alpha: TNF-α (Pg/dl)										
Early	40.9	28.9	29.6	33.2 ^b	S=0.01	59.2	40.3	75.3	58.3	S=0.13
Mid	58.9	21.4	31.8	37.4 ^b	T=0.01	55.3	30.7	62.0	49.3	T=0.01
Late	59.1	45.9	48.2	51.1 ^a	Int=0.01	53.1	40.6	67.9	53.9	Int=0.20
Mean	53.0 ^a	32.1 ^b	36.5 ^b	-	-	55.9 ^a	37.2 ^b	68.4 ^a	-	-
Total antioxidant capacity: TAC (nmol/ml)										
Early	0.44	0.97	0.65	0.68 ^b	S=0.01	0.49	0.74	0.78	0.65	S=0.70
Mid	0.31	1.31	0.93	0.85 ^a	T=0.01	0.57	0.70	0.97	0.75	T=0.01
Late	0.43	0.80	0.34	0.52 ^c	Int=0.01	0.45	0.63	0.91	0.66	Int=0.5
Mean	0.39 ^c	1.02 ^a	0.64 ^b	-	-	0.50 ^c	0.69 ^b	0.86 ^a	-	-

Int.: interaction between treatment and lactation stage.

Means with different letters within row and column are significantly different (P ≤ 0.05/0.01)

Progressing the lactation stage enhanced C3, C4 and IL-6 production since their values increased significantly in both late and mid periods when compared to that of early time. Similarly, progressed lactation stage elevated TNF- α values since it recorded the highest value during the late period compared to early and mid-times. Its maximum value appeared in the mid lactating stage.

Concerning TAC values, it was significantly increased by the two tested rations in comparison with that of control one, with either ewes or lambs. The present findings may be attributed to the flavonoids contents in treated TWMT (2.14 g/kg DM) which have antioxidants (Abo Bakr *et al.*, 2020), and immune-stimulating effects (Harborne and Williams, 2000). Generally, the improvement in TAC enzyme and immunity system may be contributed to the ability of flavonoids contained in TWMT to inhibiting or killing many pathogenic bacteria and protozoa and to scavenge free radicals. Also, *S. cerevisiae* yeast (3g and 5g / kg) is being considered as a probiotic which work as preventer of a lot of gastro intestinal diseases and consequently having positive effects on lamb's health (Zanello *et al.*, 2009). These results are in harmony with the present results of yeast group.

CONCLUSION

Based on the findings of this study, it could be concluded that trimming waste of mandarin trees (TWMT) can be used as a beneficial and acceptable fodder for ewes during the period of nursing lambs till weaning. Applying liquid feed enriched with yeast or ZADO treatments with this waste would show positive effects on Barki ewes' performance and their offspring.

Feeding Barki ewes on ZADO treated diet during lactation period elevated the IgG immune index in their lambs which have a positive effect on health and welfare through providing protection from diseases that causes mortality during this period.

Alongside, the present dietary treatments have potentiality to overcome the environmental pollution resulting from the accumulation of Mandarin tree pruning by-products as well as reduced methane emission that warm the environment.

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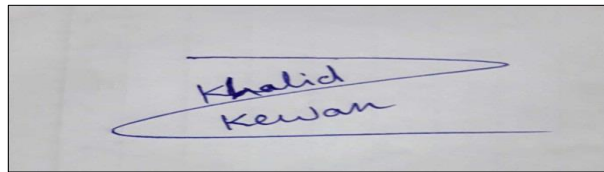
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UNDERTAKING



It is certified that the research paper **UTILIZATION OF TRIMMING WASTE OF MANDARIN TREES AS FEEDFOR SMALL RUMINANTS: 2- EVALUATION OF BARKI EWES PERFORMANCE DURING SUCKLING PERIOD** is an original research work carried out by the authors in Dep. of Animal Nutrition, Division of Animal and Poultry Production, Desert Research Center, Mattaria, Cairo, Egypt. It has neither been published nor contemplated for publication elsewhere.

Signature



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