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RESEARCH ARTICLE

UTILIZATION OF TRIMMING WASTE OF MANDARIN TREES AS FEED FOR SMALL RUMINANTS 1: PALATABILITY AND NUTRITIVE VALUE FOR TREATED VS. UNTREATED WASTES AND THE EFFECT ON ANIMAL IMMUNE STATUS

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Abstract

In Egypt, a tremendous amount of trimming byproducts of Mandarin is usually produced without benefits usage. This experiment was conducted at Siwa Research Station to assess the nutritive value and utilization of Mandarin trimming waste (TW) after treating with certain additives to be used as untraditional roughage. Two trials were conducted for this purpose. The first was a palatability trial using twenty-four Barki ewes (with 36.13 ± 0.175 kg as average body weight). The second was a digestibility trial using sixteen Barki rams (with an average body weight of 43.94 ± 0.157 kg). The main objectives were to determine nutrient digestibility, nutritive value, water utilization as well as rumen fermentation and blood profile. In addition, the effect of these roughages on animals' blood chemical components and immune status were evaluated. Animals of both trials were divided into four groups and fed four roughages as follows. The first group fed Berssem hay (BH) as control; the second roughage is un-treated trimming waste without any additives (UTW); the third roughage is the waste treated with liquid feed enriched with yeast (*Sacchromyces cerevisiae*) (YTW); and the fourth roughage is the waste treated with liquid feed enriched with ZADO (ZTW). The results showed that silica, saponin and total tannin contents were higher in untraditional roughage (UTW) than traditional roughage (BH). The treated wastes (YTW and ZTW) were higher in CP, Ash, EE, and GE contents than either untreated one (UTW) or BH. However, the opposite trend was found relating CF content. At the end of palatability trial, DMI improved by 31.5, 70.8, 70.9 and 133.5% for hay, UTW, YTW and ZTW groups, respectively. The experimental treatments resulted in higher ($P < 0.05$) DM, OM, CP, NDF and ADF digestibility compared with UTW or BH (Table 5). In addition YTW increased CF digestibility by 12.8% more than ZTW. Both of two treated waste (YTW and ZTW) and BH diets were almost similar ($P < 0.05$) in nutritive values as TDN% but UTW showed the lowest value. Furthermore, DCP% were higher ($P < 0.05$) in treated waste (YTW and ZTW) than in both of UTW and BH roughages. All sheep were in a positive nitrogen balance (NB). Animals of BH group tended to drink

much more ($P < 0.05$) water than the other three groups. Meanwhile, animals fed ZTW ration showed the highest ($P < 0.05$) value of insensible water loss. The rumen fermentation indicators (pH, $\text{NH}_3\text{-N}$ and TVFA's) were also investigated. Serum total proteins and Albumins exhibited almost similar values for the experimental groups. Animals fed treated trimming waste (YTW and ZTW) expressed higher values of Kidney function indicators (creatinine, urea, and uric acid) as compared with the other two groups (UTW and BH). The experimental diets has no effect ($P < 0.01$) on blood concentration of total antioxidant capacity (TAC) and liver enzymes (aspartate amino transferase, alanine amino transferase, gamma glutamyl transferase and alkaline phosphatase). Values of RBC's and Hb were similar in both YTW and ZTW groups compared to UTW. The values of serum immunoglobulins (IgG and IgM) in rams were higher ($P < 0.05$) in those fed trimming waste than that fed BH. So, it can be concluded that using biologically treated trimming waste of mandarin trees could cover the nutrients required for maintenance of sheep under the desert conditions and enhanced animal immune status as well as reduction of feed cost and preventing environmental pollution.

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Introduction:-

Animal nutrition is one of the most critical factors that limits the development and profitability of animal production. Feed accounts for about 70 to 80% of the cost of the livestock production. In Egypt, large number of animals are raised in the desert areas that suffer of insufficiency of feed stuffs. [Alnaimy et al. \(2017\)](#) found a negative feed balance in terms of total digestible nutrients (TDN) for ruminants in Egypt; especially under desert conditions; so that there is need to about 3.4 million tons of TDN over the total actual amount produced (9.6 million tons) to equal that required (13.0 million tons). The use of locally available by-product feedstuffs were found to cover some of the requirements and reduce feed cost ([Kour et al., 2016](#)). Therefore, nutritionists suggest resorting to the use of available organic waste as animal feed including the use of pruning fruits trees as un-traditional feeds for ruminants ([Fayed et al., 2009](#); [Phillip et al., 2014](#) and [Galal et al., 2016](#)). In addition, utilization of by-products in farm animal nutrition reduces the environmental impact of the food industry and improves profitability and valorization of the agricultural by-products ([El Ferink et al 2008](#)).

Egypt and other countries (Spain, Italy, Greece) contribute with 24% of world production of citrus ([Bampidis and Robinson, 2006](#)). Mandarin was one of the original citrus species. It can be grown in tropical and subtropical areas ([Morton 1987](#)), and is one of the most important citrus fruit crops in Egypt. Areas which cultivated by Mandarin in Egypt estimated by 47646 hectare ([Abobatta 2018](#)). As a result of farm operation, there is a great quantity of trimming byproduct of citrus (almost 3.1 million tons) produced annually ([AOAD, 2006](#)) without beneficial usage and are considered as wastes.

Attempts were made to improve nutrients utilization of by-products by various methods such as physical, chemical, biological treatment and mixing with certain additives ([Abdel-Aziz et al., 2015](#)). The objective of this study was to assess the nutritive value and utilization of mandarin byproduct as roughage in feeding small ruminants with or without using yeast (*Sacchromyces cerevisiae*) or ZADO as additives. In addition, the influence of these roughages on serum biochemical and immunological indices of Barki sheep were investigated.

Materials And Methods:-

Experimental location:

The present study was conducted at Siwa Research Station (Tegzerty Experimental Farm for Animal Production), which belongs to Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Geographically, Siwa Oasis lies between longitudes $25^{\circ} 16'$ and $26^{\circ} 12'$ E and latitudes $29^{\circ} 06'$ and $29^{\circ} 24'$ N. It locates at 330 Km Southwest of the Mediterranean shoreline and at 65 Km East of the Libyan borders. Siwa Oasis is characterized by desert climate ([Zidane, 2010](#)).

Preparation of roughage diets:

Fresh trimming waste of Mandarin trees (TWMT) was purchased from private factory for oil extraction and located in Sadat city. It was mechanically chopped (3-5 cm) before extraction of natural oil (Dimethyl anthranilate) by steam treatment. Amount of chopped TWMT were air dried for reduced moisture, then packed till using it. After that, TWMT was filled in layers, then sprayed with different liquid solution according to proposal and will have good mixing.

The experimental roughages involved control and three treatments as follows:

1. The first which saved as control, Berssem hay (BH) is the main roughage.
2. The second roughage is un-treated trimming waste without any additives (UTW).
3. The third roughage is yeast (*Sacchromyces cerevisiae*) treated trimming waste (YTW), where trimming waste supplemented with liquid feed enriched with Bakery yeast (for each 100 kg trimming waste treated with liquid feed containing 18 litter water, 6.1 kg molasses, 0.17 kg urea, 0.4 kg mixture of minerals, 0.03 kg vitamins and 0.5 kg yeast).
4. The fourth roughage is ZADO treated trimming waste (ZTW). Trimming waste supplemented with the same liquid feed but enriched with 0.25kg ZADO instead of Bakery yeast. The ZADO is a compound (patent no: 22155) prepared from natural, multi/mix of the enzymes cellulases (8.2u/gm), hemicellulases (6.2u/gm), amylase (64.4u/gm), and protease (12.3u/gm) and also included anaerobic bacteria and *S. cerevisiae* yeast.

The palatability trial:

Twenty-four Barki dry ewes with 36.13 ± 0.175 kg as average body weight and about 3-4 years of age were used in palatability trial. Animals were divided into four groups, each group was housed in three stables (two ewes in each) to determine palatability of the experimental trimming waste of mandarin trees as compared with Berseem hay. All the roughages were offered ad libitum and the refusal was daily collected and weighed to assess intake before any new feed was offered. Palatability trial lasted for two months.

Digestibility and nitrogen balance trials:

Sixteen Barki rams with an average body weight of 43.94 ± 0.157 kg and about 2-3 year of age were used in a complete randomized design experiment. Rams were divided into four groups (four lambs in each) and placed in metabolic cages (1.6 m x 0.53 m), and fed roughage ad lib. Rams' refusal, feces and urine were collected for 5 consecutive days after an adaptation period of 14 days. Water was offered twice daily with free choice to record daily free water intake. Samples of feces (10% of wet weight) were dried in a forced air oven at 70 °C, composited by 5-d collection, and ground in Wiley mill (1-mm screen) before laboratory analysis. Urine was collected in containers containing 50 ml of 6N H₂SO₄ to maintain pH.

Sampling of rumen liquor:

Rumen liquor samples were taken by stomach tube from all animals on the day following the digestibility trials. The samples were taken before morning feeding (zero-time) and at 3 and 6 hr. post feeding. The rumen samples were filtered through two layers of cheese-cloth and used quickly as possible for measurement of pH by using pH meter. Rumen liquor was stored in plastic bottles with a few drops of 0.1 N HCl and stored at a deep freeze (-20°C) till analysis.

Sampling of blood:

Blood samples were collected weekly from the jugular vein of each ewe of palatability trial, throughout two consecutive months (8 samples). Blood was collected into clean test tubes with anticoagulant (EDTA). Blood samples were divided into two portions. In the 1st portion, hematological parameters including count of red (RBC's) and white (WBC's) blood cells, hematocrit value (Ht) and hemoglobin (Hg) concentration in the whole blood were immediately measured after collection. The 2nd portion was centrifuged at 3000 rpm for 20 minutes to obtain plasma that were frozen at -20 °C for late biochemical assay.

Laboratory analysis:

The samples of feed, feces and urine were analyzed for proximate analysis according to AOAC (2000), dry matter (DM; method 930.15), ether extract (EE; method 954.02), crude protein (CP; method (Kjeldhal) 955.04) and ash (method 942.05). Cell wall constituents (neutral detergent fiber; NDF and acid detergent fiber; ADF) were determined according to Van Soest et al. (1991) using the automated ANKOM fiber analyzer. Gross energy (GE) of feeds and feces were determined by using Calorimeter (KIKA® WERKE C5001). Metabolizable energy (ME) was

estimated according to the equations of [Abate and Meyer \(1997\)](#) where $ME (MJ\ kg^{-1}\ DM) = 20.27 - 0.1431CF - 0.111\ NFE - 0.2200\ Ash$. Metabolic water was calculated as one g TDN intake yields for 0.6 g of water.

Silica was determined as method described by [Thonney et al. \(1985\)](#). Saponin content of the sample was determined by double solvent extraction gravimetric method ([Harbone, 1973](#)) and ([Obadoni and Ochuko, 2001](#)). Nitrate, Total oxalates and Total tannins were determined according to [Makkar et al. \(2007\)](#) and the Total Flavonoids as [Chandra et al. \(2014\)](#).

The pH of filtered rumen liquor samples were recorded using Beckman pH meter just after collection. The stored strained rumen liquor samples were thawed at room temperature and then analyzed for ammonia nitrogen (NH_3-N) using Markham micro-distillation apparatus ([Markham, 1942](#)), total volatile fatty acids (TVFA) according to [El-Shazly et al. \(1963\)](#) and VFA fractions by gas chromatography (GC) using methyl valerate as internal standard ([Jouany, 1982](#)) in an Agilent 6890 series GC equipped with a capillary column (HP-FFAP19095F-123, 30 m, 0.53 mm diameter).

Hemoglobin concentration (Hb g/dl) was assayed by the method of [Zijlstra \(1960\)](#), using kits provided by Pasteur Lab. Diagnostic (Egypt). Haematocrit (Ht%) was determined according to [Hodgetts \(1959\)](#) using Wintrobe tubes. The numbers of red blood cells (RBC's 10^6 cells/ml) and white blood cells (WBC's 10^3 cells/ml) were counted using Neubauer's hemocytometer cited by [Bauer \(1970\)](#). Packed cell volume (PCV%) was estimated using micro-hematocrit tubes by Wintrob methods. Wintrob indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) were calculated by the following equations:

$MCH, \text{ pictogram } \mu g = \text{Hb content} \times 10 / \text{RBCs}$

$MCHC, \% = \text{Hb content} \times 10 / \text{PCV}$

$MCV, \text{ fl} = \text{PCV} \times 10 / \text{RBCs in million}$

Plasma concentrations of total proteins, albumin, total lipids, triglycerides, total cholesterol, total antioxidant capacity (TAC), high density lipoprotein (HDL), low density lipoprotein (LDL), Very low density lipoprotein (VLDL), liver activity enzymes and kidney functions were estimated calorimetrically using commercial chemical reagent kits (Bio-diagnostic product Kit, Egypt). However, globulin concentration and albumin/globulin ratio were calculated.

Cytokines (interleukins 1 & 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, IgM) were measured by ELISA kits according to Abbott Laboratories instructions (Abbott Park, IL 60064 USA).

Statistical analysis:

Data obtained in this study was statistically analyzed by one way of variances according to [SAS \(2004\)](#) using the following model; $Y_{ij} = \mu + T_i + e_{ij}$, Where; Y_{ij} = experimental observation, μ = overall mean, T_i = effect of treatment, e_{ij} = experimental error. Differences among means were compared by Duncan's multiple range Test ([Duncan, 1955](#)).

Results And Discussion:-

Climate conditions:

The meteorological data of the experimental region of Siwa Oasis throughout July and August of year 2018 are presented in table (1). The mean ambient temperature was higher at post meridian than that of ante meridian since it recorded $38.6 \pm 0.27^\circ\text{C}$ (ranged from 38.10 - 39.0°C) and 29.7 ± 0.27 (ranged from 29.3 - 30.2°C), respectively.

The relative humidity was inversely related to ambient temperature and fluctuated between 59.3 and 56.0% with overall mean 58.1% during ante meridian. This value decreased during post meridian to 30.0% with a range from 29.3-30.3%.

The temperature of solar radiation was found to augment the environmental heat since it recorded $35.5 \pm 0.36^\circ\text{C}$, at ante meridian versus $42.1 \pm 0.36^\circ\text{C}$, at post meridian.

Table (1):- Average values of meteorological data in the experimental range throughout the experiment period.

Items	am				Overall men ± SE	pm				Overall men ±SE
	06:00	07:00	08:00	09:00		12:00	01:00	02:00	03:00	
AT, °C	29.3	29.3	29.8	30.2	29.7± 0.27	38.1	39.0	38.7	38.5	38.6±0.27
RH, %	59.3	59.3	57.7	56.0	58.1± 0.66	30.3	29.3	30.0	30.3	30.0±0.66
SR, °C	35.0	35.0	35.5	36.4	35.5± 0.36	41.7	42.3	42.3	42.0	42.1±0.36

AT: Ambient temperature °C, RH: Relative Humidity, SR: Solar radiation

Anti-nutritional factors:

Table (2) presents anti-nutritional factors (secondary metabolites) found in untreated trimming Mandarin trees waste compared with those in Berseem hay. The data showed that, Silica content was higher in untraditional roughage UTW (5.97%) than traditional roughage BH (1.74%). This finding may be due to Mandarin trees is planted in sand soil otherwise conditions of transportation and handling of trimming wastes.

Saponin content in UTW was higher than that BH, being vales were 6.41 vs 0.17 g/kg DM, respectively. [El Shreef \(2012\)](#) reported that BH was saponine free compared with untraditional roughage. In General, Saponins are among several plant compounds which have beneficial effects, as antibacterial and anti-protozoal properties. ([Ramteke et al. 2019](#)). However [Makkar et al \(2007\)](#) reported that saponins have been shown to inhibit various digestive enzymes, including trypsin and chymotrypsin, and are also known to inhibit protein degradation by forming saponin-protein complexes.

The total tannin content was high in UTW by 79.85% when compared with BH, so, feeding ruminants on UTW as a sole diet may cause reducing the digestibility of dry matter, protein and other nutrients as reported by [Ramteke et al. \(2019\)](#).

Table (2):- Composition of Anti-nutritional factors and total flavonoids in un-treated waste (UTW) compared with Berseem hay (BH).

Items	Anti-nutritional factors					T. Flavonoids (g/kg DM)
	Silica (%)	Saponin (g/kg DM)	Oxalate (g/kg DM)	T. Tannins (g/kg DM)	Nitrate (µg/kg DM)	
BH	1.74	0.17	4.46	9.68	21.15	1.01
UTW	5.97	6.41	3.17	17.41	19.36	2.14

The amount of nitrate (µg/kg DM) in BH and UTW was almost the same and also lower than the safe limit (<2500 ppm) recorded by [Ball et al. \(2002\)](#). In ruminants, nitrate is normally converted to nitrite - ammonia - amino acid to protein in the rumen in presence of microorganisms.

UTW showed double fold content of total flavonoids when compared with BH. Flavonoids has different benefits for ruminants. It is considered as promoter of the growth and enhancer of the products quality of animal due to its anti-microbial and antioxidative properties. Also, it has positive effect on the productivity and health of animals as well as rumen fermentation and control of nutritional stress such as bloat and acidosis ([Kalantar, 2018](#)). Furthermore, [Seradj et al. \(2014\)](#) observed higher rumen pH values in high grain heifers diets supplemented with flavonoids compared to the control group, which likely due to the beneficial effect of flavonoid in enhancing lactate-consuming microorganisms i.e. *M. elsdenii*.

Nutrients composition:

Nutrients composition of the experimental roughages are shown in Table (3). Data revealed that CP, Ash, EE, and GE contents in treated waste (YTW and ZTW) were higher than either untreated one (UTW) or BH. Increment of CP content with yeast or ZADO treatment could be due to one or more of the following reasons: i) the 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 included in the liquid feed sprayed on trimming waste. This result was agreement with [Sabbah et al. \(2009\)](#). Also, increased the ash content when applied the experimental treatments mainly due to the mineral mixture included in the liquid feed.

On the other hand CF content in treated waste roughages were lower than untreated one and BH. This decline in CF content may be due to the effect of cellulase enzymes in ZADO treatment or the effect of yeast and also, urea addition. These results were agreed with [Abo Bakr \(2012\)](#) and [Kewan et. al. \(2019\)](#).

ZADO treatment (ZTW) showed lower NDF and ADF content values compared with the other treatment (YTW) and untreated one (UTW) but still higher than those in BH. These findings agree with those found by [Gado et al. \(2006\)](#). Higher NDF value recorded by YTW compatible with results reported by [Kewan et al. \(2019\)](#) who found higher NDF for Moringa stalks treated with *Saccharomyces cerevisiae* compared with untreated one. The same trend was observed with GE which increased in YTW compared with the other roughages. Generally treated roughages (YTW and ZTW) recorded higher values of GE than either untreated one or BH, which may resulted from higher content of energetic nutrients like EE.

The market price showed that untreated trimming waste has the lowest price as it still not familiar for the animal breeders and its nutritive values for ruminants is unknown Therefore it was not included in the forage analysis tables. The treated waste (YTW and ZTW) were lower in price than BH by 69.4% and 75.7%, respectively.

Table (3):- Chemical composition and market price of the experimental roughages.

Items	Experimental roughages ¹			
	BH	UTW	YTW	ZTW
DM	90.88	93.65	92.66	92.02
Chemical Composition (% as DM)				
OM	87.20	86.61	86.57	85.09
CP	13.36	14.37	15.57	15.90
CF	32.30	27.00	24.90	18.47
EE	3.82	3.93	4.31	4.39
NFE	37.71	41.31	41.79	46.33
Ash	12.80	13.39	13.43	14.91
NDF	48.06	62.63	64.68	58.01
ADF	36.44	42.74	47.17	39.82
GE, MJ/Kg DM	17.65	18.57	20.49	20.15
Egyptian Market Price²				
LE/Ton	3500	600	1070	1200

¹BH: Berseem hay; UTW: untreated waste; YTW: yeast treated waste; ZTW: ZADO treated waste

²Including transportation cost.

Palatability data:

The Initial body weight of ewes in palatability trial were almost the same. Data in Table (4) indicates that, minimum value of DMI was lower for ewes fed either treated or untreated trimming waste than that fed BH. This finding may be due to ewes other than BH group were not adapted on untraditional roughage (TW). While, Maximum value of DMI was almost the same among the experimental groups. Regardless of BH groups and in relation to UTW, Maximum DMI was higher by 16.9% and 16.2% for YTW and ZTW, respectively. At the end of palatability trial, DMI improved by 31.5, 70.8, 70.9 and 133.5% for hay, UTW, YTW and ZTW groups, respectively. These results were agreement with those findings by [Phillip et al. \(2014\)](#).

Differences among groups related on average DMI expressed as g/d, and percentages either of maintenance DMI requirement recommended by [Kearl \(1982\)](#) or body weight showed the same trend of significance ($p < 0.5$), where UTW group recorded the lowest values (586.5 g/d, 67.28% and 1.64%, respectively) but BH group recorded the highest ones (849.7 g/d, 96.13 and 2.33, respectively), in addition, both of two groups fed treated waste diets (YTW and ZTW) showed comparable values and medium between UTW and BH. The same trend was found concerning TDNI when expressed either as g/d or as a percentage of maintenance TDN requirement. These results matches also with MEI expressed as Kcal/d or as a percentage of maintenance ME requirement which followed the same trend OF TDNI.

On the other hand, both of YTW and ZTW groups showed comparable DCPI values and at the time they were higher ($P < 0.05$) than the other two groups (UTW and BH). These finding may be due to the highest CP content resulted from liquid feed included urea and consequently increased CP digestibility, and more than that the highly

nitrogenous yeast cells in YTW diet (Kewan et al. 2019) and the positive effect of ZADO in ZTW diet which led to better palatability and utilization by the host animal (Gado et al. 2006) and Abo Bakr (2012).

Table (4):- Palatability of the experimental roughages fed to Barki ewes.

Items ¹	Experimental groups				SEM
	BH	UTW	YTW	ZTW	
Ewes LBW, kg	36.41	35.89	35.99	36.22	0.28
Minimum DMI, g/d	682	415	485	353	-
Maximum DMI, g/d	897	709	829	824	-
Improved DMI %	31.5	70.8	70.9	133.5	-
Average DMI, g/d	849.7 ^a	586.5 ^c	681.0 ^b	704.1 ^b	26.86
% of maintenance DMR	96.13 ^a	67.28 ^c	77.92 ^b	80.03 ^b	3.06
DMI/ BW%	2.33 ^a	1.64 ^c	1.89 ^b	1.95 ^b	0.07
TDNI, g/d	478.3 ^a	281.0 ^c	394.7 ^b	398.9 ^b	14.75
% of maintenance TDNR	124.2 ^a	74.1 ^c	103.9 ^b	104.1 ^b	3.86
MEI, Kcal/d	1731 ^a	1017 ^c	1429 ^b	1444 ^b	53.37
% of maintenance MER	125.6 ^a	74.59 ^c	104.6 ^b	105.2 ^b	3.90
DCPI, g/d	66.37 ^b	47.58 ^c	76.67 ^a	83.79 ^a	2.81
% of maintenance DCPR	199.3 ^b	145.1 ^c	233.1 ^a	253.2 ^a	8.52

LBW: live body weight; DMI: Dry matter intake; DMR: Dry matter requirement; TDNI: Total digestible nutrients; MEI: Metabolizable energy intake; MER: Metabolizable energy requirement; DCPI: Digestible crude protein intake; DCPR: Digestible crude protein requirement

Means with different letters within each row are significantly different ($P < 0.05$).

Comparing with BH diet, both of YTW and ZTW diets were consumed less as DM but at the same time covered energy and protein required for maintenance, these emphasize that the supplemented diets were more bulk density and highly nutritive value. In a general view, the experimental liquid feed supplementation enhanced intakes of DM, TDN, ME and DCP from treated trimming wastes as compared with untreated one, and become suitable for covering both of energy and nitrogen required for maintenance.

Nutrients digestibility and nutritive values:

Nutrients intake, Digestion coefficients and feeding values of the experimental roughages are presented in Table (5). Data revealed that, DMI was significantly higher ($P < 0.05$) for ZTW (1127 g/d) followed by YTW (977 g/d) while UTW recorded the lowest DMI (726 g/d). These finding was agreement with that obtained by Phillip et al. (2014), who reported that higher DMI for lambs fed ration containing treated pruning grape by-product with biological treatment than untreated one. The same trend was observed for AshI, that may be as a result of high Ash content and DMI for both groups. Liquid feed including ZADO or yeast treatments increased DMI as a percentage of animal BW by 54.5% and 34.5%, respectively when related to UTW group. In this concern we can notice that these values were higher than those found by ewes mentioned in Table (4) and we might explain that by rams have a higher basic metabolic rate than ewes otherwise age also has an effect (Abbott, 2018).

The experimental treatments resulted in higher ($P < 0.05$) DM, OM, CP, NDF and ADF digestibility compared with UTW or BH (Table 5). In addition YTW increased CF digestibility by 12.8% more than ZTW. The improvement of nutrients digestibility of rations supplemented with yeast or ZADO may attributed to increased feed intake, enhance availability of nutrients for digestion and absorption and also slowing feed passage time out of the digestive tract which was reflected in better absorption. These results are in agreement with those obtained by Sabbah et al. (2009) for ZADO treatment and Kewan et al. (2017) for yeast treatment. It is noted that UTW recoded the lowest digestion coefficient for most nutrient. This may be due to high tannin content. This result was agreed with those obtained by Ramteke et al. (2019), who reported that high tannin content reduce the digestibility of dry matter, protein and other nutrients, and also depresses cellulase activity and thus affects digestion of crude fiber.

Two of treated waste (UTW and ZTW) and BH diets were almost similar ($P < 0.05$) in nutritive values as TDN% but UTW showed the lowest value (Table 5). Furthermore, DCP % was affected by treatment and showed higher ($P < 0.05$) values for YTW and ZTW compared with both of UTW and BH diets. The increase in TDN% and DCP% by the present treatments may be attributed to increasing nutrients digestibility and also to the urea included in liquid

feed treatments (Salman et al., 2011). Similar results were obtained by Khir, et al. (2015) who reported that TDN and DCP of pruning peach trees by-products were improved by chemical or biological treatments compared with untreated roughage.

Table (5):- Nutrients intake, digestibility and nutritive values of the experimental roughages fed to Barki rams.

Item	Experimental groups				SEM
	BH	UTW	YTW	ZTW	
	Body weight and nutrients intake				
Number of Rams	4	4	4	4	-
Average LBW, kg	43.63	43.93	44	44.18	0.36
DMI, g/d	1048 ^{ab}	726 ^c	977 ^b	1127 ^a	31.3
DMI/ BW%	2.40 ^a	1.65 ^b	2.22 ^a	2.55 ^a	0.08
GE intake, MJ/d	18.50 ^b	13.49 ^c	20.02 ^b	22.72 ^a	0.63
AshI, g/d	134.3 ^b	97.0 ^c	131.3 ^b	138.0 ^a	4.31
	Nutrients Digestibility, %				
DM	58.72 ^a	49.43 ^b	55.65 ^a	58.76 ^a	1.27
OM	61.14 ^a	52.51 ^b	62.83 ^a	63.63 ^a	2.15
CP	58.42 ^b	56.44 ^b	72.31 ^a	74.97 ^a	1.30
CF	66.40 ^a	59.26 ^b	68.57 ^a	60.76 ^b	1.13
EE	62.15 ^a	49.08 ^c	65.94 ^a	57.9 ^b	1.21
NFE	57.50	47.07	55.56	61.42	4.18
NDF	56.28 ^b	53.54 ^b	61.68 ^a	64.39 ^a	1.22
ADF	50.31 ^c	60.52 ^b	66.29 ^a	68.35 ^a	1.55
	Nutritive Values				
TDN%	56.28 ^a	47.89 ^b	57.95 ^a	57.32 ^a	1.88
TDNI, g/d	591.0 ^a	347.9 ^b	566.3 ^a	645.6 ^a	27.8
DCP%	7.80 ^c	8.11 ^c	11.25 ^b	11.92 ^a	0.19
DCPI, g/d	81.9 ^c	58.9 ^d	110.0 ^b	134.4 ^a	4.16
ME, MJ/d	7.97 ^b	5.27 ^c	8.58 ^{ab}	9.78 ^a	0.43
ME, Kcal/ kg ^{0.75}	112.2 ^b	73.8 ^c	120.2 ^{ab}	136.4 ^a	6.18
% of maint. ME*	120.6 ^b	79.3 ^c	129.2 ^{ab}	146.6 ^a	6.65

*Maintenance ME = 93 Kcal/ kg^{0.75} (Kearl 1982).

Means with different letters within each row are significantly different (P < 0.05).

Data in Table (5) revealed that, UTW was underestimate of ME required for maintenance that recommended by Kearl (1982), but both of YTW and ZTW were overestimate and they are considered save for maintenance requirements for rams under desert conations.

Nitrogen utilization:

Nitrogen intake, N-excreted and N-balance by sheep fed different experimental roughage are presented in Table (6). Data showed significant (P<0.05) differences among groups for all nitrogen values (g/d) as affected by the experimental feed liquid supplements. The highest (P<0.05) mean value of N intake (NI) obtained for rams fed ZTW diet (28.70) followed by YTW group (24.33), while the lowest value was recorded for UTW group (16.67). The results of the present study are in agreement with Khir et al (2015) reported that feeding biological or chemical treatment of pruning peach trees by-products had higher NI than untreated one.

It seems that, either treated or untreated trimming waste decreased (P<0.05) excretion of fecal N compared with BH. However, treatments (YTW and ZTW) enhanced (P<0.05) N absorbed as a percentage of body weight compared with UTW or BH groups. Animal group fed ZTW diet excreted more than double amount of urea N (3.25 g/d) compared with UTW group (1.22 g/d). on the other hand, YTW group showed the least total nitrogen loss as a percentage of total nitrogen intake (32.70%) followed by ZTW group (36.35%) and then UTW group (51.31%). Similar results were obtained by Fayed et al. (2009).

All sheep were in a positive nitrogen balance (NB), however; owing to sharing wool process for animals three months before conducting the experiment, the retained nitrogen values were higher than that reported in literature.

So that fractionation of nitrogen to N for maintenance and that for growth of either body or wool, showed superiority of ZTW (15.58 g/d) and YTW (13.70 g/d) in N retained for growth. These finding may be due to enhanced of N utilization as a result of ZADO (Salem et al., 2015) or yeast cell N and urea included liquid feed in both treatments. These results were agreed with those obtained by Farghaly (2009) and Salman et al.(2011), who indicated that small ruminants fed ration contained biologically (yeast, bacteria and mixture of them) treated low quality roughage had higher positive N-balance. Furthermore, Sabbah et al.(2009) reported that the improvement in nitrogen balance with treated roughage may be due to added of yeast which leads to increase DM, CP and hemicellulose digestibility and increased ruminal bacteria numbers. Generally, the highest value of NB could be attributed to higher nitrogen intake and improvement of CP digestibility. Similar trend was obtained by Abo Bakr (2012). On the other hand, might be the lower digestion of crude protein in UTW (Table 4) resulted in low N retention. The same trend was observed by Dada et al.(1999) that the lower nitrogen retention could be ascribed to the lower protein digestibility and reduced utilization of nitrogen for microbial protein synthesis.

In agreement, Macedo et al. (2007) found that fresh citrus pulp not only improved protein fermentation and digestion, but also promoted more efficient use by the sheep, because the greater percentage of retained nitrogen compared to absorbed nitrogen indicated better protein metabolism of the diets.

Table (6):- Nitrogen balance and utilization in Barki rams fed different experimental roughages

Item	Experimental groups				SEM
	BH	UTW	YTW	ZTW	
NI, g/d	22.40 ^b	16.67 ^c	24.33 ^b	28.70 ^a	0.76
FN, g/d	9.30 ^a	7.27 ^b	6.74 ^b	7.17 ^b	0.27
% of NI	41.57 ^a	43.56 ^a	27.69 ^b	25.03 ^b	1.30
N absorbed, g/d	13.10 ^c	9.40 ^d	17.60 ^b	21.47 ^a	0.67
% of NI	58.43 ^b	56.44 ^b	72.31 ^a	74.97 ^a	1.29
UN, g/d	3.72 ^a	1.30 ^b	1.22 ^b	3.25 ^a	0.20
% of NI	16.56 ^a	7.75 ^c	5.01 ^d	11.32 ^b	0.71
TNEx, g/d	13.02 ^a	8.57 ^c	7.85 ^c	10.42 ^b	0.31
% of NI	58.13 ^a	51.31 ^b	32.70 ^d	36.35 ^c	0.98
NR, g/d	9.38 ^c	8.13 ^c	16.38 ^b	18.26 ^a	0.55
N for Maintenance ¹	2.67	2.69	2.67	2.68	0.01
Remaining N ² , g	6.72 ^c	5.44 ^c	13.70 ^b	15.58 ^a	0.56
NR/ NI %	41.87 ^d	48.69 ^c	67.30 ^a	63.65 ^b	0.98
NR/ NA %	71.74 ^c	86.26 ^b	93.07 ^a	84.91 ^b	0.89
g NR/ DCPI%	11.48 ^c	13.80 ^b	14.89 ^a	13.59 ^b	0.14
g NR/GEI (MJ/d)%	50.71 ^c	60.28 ^b	81.80 ^a	80.36 ^a	1.22
g NR: MJ MEI	1.19 ^c	1.54 ^b	1.91 ^a	1.87 ^a	0.06
mg NR/ g TDNI	6.08	2.33	2.90	2.83	2.22
mg NR/g DMI	8.95 ^c	11.19 ^b	16.77 ^a	16.19 ^a	0.23
mg NR/ g OMI	10.27 ^c	12.92 ^b	19.36 ^a	19.03 ^a	0.26
mg NR/ g CFI	27.71 ^d	41.45 ^c	67.34 ^b	87.67 ^a	0.91
mg NR/ g NFEI	23.74 ^d	27.09 ^c	40.11 ^a	34.96 ^b	0.55

¹NRC (1985). ²The remaining nitrogen for body and wool growth.

Means with different letters within each row are significantly different (P< 0.05).

Water utilization:

Table (7) presents the effect of feeding treated trimming wastes on various water utilization parameters compared to positive (UTW) or negative controls (BH). The combined feed water was mainly affected (P<0.05) by feed intake of the experimental diets and showed the same trend. Animals BH group tended to drink much more (P< 0.05) water than the other three groups, which may be as a result of higher CF content and its water holding capacity compared with that of trimming wastes. Fortunately, it can be say that feeding sheep on trimming waste could be save water under the same condition of present experiment. The present results are disagreement of that reported by Kewan et al (2019) who found that, biological treatment (fungi or yeast) for Moringa oleifera tree stalks caused the increase of free water intake. These might be owing to the type of roughage used. Furthermore, higher free water intake might be due to higher dry matter intake (El Shreef 2012), high ash intake (Abo Bakr 2006) as previously illustrated in Table 5, and/or high crude protein intake (Sabbah et al 2009).

Metabolic water varied significantly ($p < 0.05$) among groups. Rams fed UTW ration recorded the lowest value of metabolic water ($9.39 \text{ g/kg}^{0.82}$) as a result of lower TDN intake.

Water excreted via faces and urine (ml/h/d or ml/kg BW) showed the trend of significant differences ($p < 0.05$) among groups. Regardless BH, the higher urinary water in ZTW may be due to higher mineral intake of ash. These results were in accordance with those obtained by Eid (2003) who reported that the high content of ash lead to push animals to increase excretion of urine as a natural channel to excrete minerals.

Table (7):- Water metabolism as affected by the experimental roughages fed to Barki rams.

Items*	Experimental groups				SEM
	BH	UTW	YTW	ZTW	
MBW, $\text{kg}^{0.82}$	22.1	22.23	22.27	22.33	0.16
Feed WI, $\text{g/kg}^{0.82}$	4.76 ^a	2.22 ^c	3.48 ^b	4.38 ^a	0.12
Free WI, $\text{g/kg}^{0.82}$	219.4 ^a	124.8 ^d	134.5 ^c	191.7 ^b	2.62
Free WI/ DMI, g/g	4.63 ^a	3.83 ^b	3.08 ^c	3.80 ^b	0.11
Free WI/ AshI, g/g	36.18 ^a	28.57 ^b	22.93 ^c	25.49 ^c	0.83
MW, $\text{g/kg}^{0.82}$	16.02 ^a	9.39 ^b	15.29 ^a	17.34 ^a	0.77
TWI, $\text{g/kg}^{0.82}$	240.2 ^a	136.4 ^d	153.3 ^c	213.4 ^b	2.91
FW, $\text{g/kg}^{0.82}$	29.12 ^a	5.48 ^d	8.48 ^c	13.36 ^b	0.79
UW, $\text{g/kg}^{0.82}$	67.57 ^a	19.83 ^d	11.37 ^c	34.30 ^b	0.72
TWEx, $\text{g/kg}^{0.82}$	96.7 ^a	25.33 ^c	19.83 ^d	47.67 ^b	1.07
IWL, $\text{g/kg}^{0.82}$	143.7 ^b	111.3 ^d	133.3 ^c	165.3 ^a	2.15
IWL/ TWI %	59.74 ^d	81.44 ^b	87.05 ^a	77.67 ^c	0.39
TWL, $\text{g/kg}^{0.82}$	240.3 ^a	136.7 ^d	153.3 ^c	213.3 ^b	2.98

MBW: metabolic body weight; WI: water intake; MW: metabolic water; TWI: total water intake; FW: fecal water; UW: urine water; TWEx.: Total water excretion; IWL: Insensible water loss or unmeasured water; TWL: Total water loss.

Means with different letters within each row are significantly different ($P < 0.05$).

Meanwhile, animals fed ZTW ration showed the highest ($P < 0.05$) value of insensible water loss ($165.3 \text{ g/kg}^{0.82}$). However, calculated values of total water loss illustrated that BH ration caused the highest value of loss ($240.3 \text{ g/kg}^{0.82}$) followed by ZTW ($213.3 \text{ g/kg}^{0.82}$), YTW ($153.3 \text{ g/kg}^{0.82}$) and then UTW ration ($136.7 \text{ g/kg}^{0.82}$). It seems that total water loss affected mainly by total water intake as they both showed the same trend.

Rumen fermentation parameters:

Rumen fermentation parameters; pH, $\text{NH}_3\text{-N}$ and TVFA's are shown in Table (8). Rumen fermentation activity in animals depends on nutrient digestibility, rate of absorption, rate of digestive passage from rumen, and the activities of microbial population in the rumen. No significant ($P > 0.05$) differences were found among the experimental groups for ruminal pH values at zero time after feeding. It's obviously that, YTW showed the highest ($P < 0.05$) values of pH at both 3 (6.30) and 6hrs (6.38) post-feeding. However, the lowest ($P < 0.05$) values recorded for ZTW at 3hrs (5.92) and BH at 6hrs (5.86). The overall mean of ruminal pH values were not differed among groups and ranged between 6.18 and 6.50. Generally, the values of ruminal pH in this study are within the range reported by Rakha (1988), who reviewed from several studies that the normal value of ruminal pH of sheep is ranging between 4.96 and 7.92, and also. It was within normal range for roughage diets as reported by Ørskov and Ryle, (1990).

Concentrations of $\text{NH}_3\text{-N}$ were significantly ($P < 0.05$) differed among groups at 0, 3 and 6hrs as well as the overall means. Generally, animal fed ZTW ration showed the highest values followed by YTW group. These findings mainly attributed to feed treatment under the present investigation and also supported by those of Abo-Eid et al. (2007) who reported that ruminal ammonia was significantly ($P < 0.05$) higher for rams fed rations containing biologically (T. reesei) treated roughages (rice straw and corn stalks) than those fed untreated ration. In addition, Bassuny et al. (2003) and Fayed et al. (2009) found that rams fed ration contained urea treated roughage had the highest $\text{NH}_3\text{-N}$ concentration followed by those fed ration contained biologically treated roughage. In general, the maximum concentration of total $\text{NH}_3\text{-N}$ were observed at 3 hrs. post feeding then tended to decrease at 6hrs post feeding. This result may be related to degradation of dietary degradable protein and hydrolysis of NPN substances

(Reddy et al., 1989). However, the increase in $\text{NH}_3\text{-N}$ with the present treatments could be a result of breakdown of protein and other nitrogenous compound to $\text{NH}_3\text{-N}$ (Fayed et al., 2009).

Table (8):- Rumen fermentation parameters in Barki rams fed the experimental roughages.

Item	Experimental groups				SEM
	BH	UTW	YTW	ZTW	
pH:					
0hr	6.71	6.89	6.83	6.81	0.08
3hr	5.98 ^{ab}	6.23 ^{ab}	6.30 ^a	5.92 ^b	0.11
6hr	5.86 ^c	6.15 ^{ab}	6.38 ^a	6.01 ^{bc}	0.07
Overall mean	6.18	6.42	6.50	6.24	0.13
$\text{NH}_3\text{-N}$ (mg/dl):					
0hr	5.87 ^b	6.55 ^b	6.82 ^{ab}	7.64 ^a	0.31
3hr	8.46 ^{bc}	7.51 ^c	9.83 ^b	14.56 ^a	0.62
6hr	8.19 ^{bc}	7.31 ^c	9.01 ^{ab}	9.98 ^a	0.47
Overall mean	7.51 ^b	7.12 ^b	8.55 ^b	10.73 ^a	0.64
TVFA (meq/dl):					
0hr	11.10 ^a	6.13 ^b	5.85 ^b	5.08 ^b	0.52
3hr	8.19 ^b	8.31 ^b	8.60 ^b	10.96 ^a	0.54
6hr	10.57 ^a	9.02 ^{ab}	8.42 ^b	10.57 ^a	0.59
Overall mean	9.95 ^a	7.82 ^b	7.62 ^b	8.87 ^{ab}	0.67
VFA fractions% at 6hr:					
Acetic acid	73.83 ^a	52.43 ^b	72.77 ^a	75.03 ^a	2.70
Propionic acid	15.63 ^b	16.67 ^b	19.7 ^b	39.57 ^a	3.14
Butyric acid	10.53	8.30	7.50	8.03	1.34

Means with different letters within each row are significantly different ($P < 0.05$).

Data in Table (8) indicated that, TVFA's concentration at zero time post feeding was significantly higher ($P < 0.05$) for BH group (11.10 meq/dl) than the other three comparable groups. This might due to the low level of tannin (Table 1) and polyphenol compounds in BH versus higher level of tannin and polyphenol compounds in trimming waste leads to reduce TVFA concentration in rumen liquor of animals (Ahmed and Shaarawy, 2019). At 3 and 6hrs post feeding, animals fed ZTW showed the highest ($P < 0.05$) values (10.96 AND 10.57, respectively). These finding may be due to ZADO included enzymes and yeast which lead to increase the rate of rumen fermentation due to its increased the total and viable count of bacteria (Newbold et al., 1996) and cellulolytic bacteria (Kumar et al., 1997). TVFA's concentration in YTW and ZTW groups increased post feeding to reach the highest value at 3hrs. post feeding then decreased with progressed time of feeding, this trend of TVFA's concentration might be related to the fermentation of unstructured carbohydrates of the ration as reported by Aziz (2004) and Fouad (1991). The mean values of TVFA,s concentration in this study are within the normal range (3.07 to 19.90) of rumen liquor which were reported by several researchers, (Abdou, 2003 and Eid, 2003).

Ruminal volatile fatty acid fractions (%) recorded at 6hrs. post feeding are shown in Table (8). The data cleared that, ZTW and YTW were significantly higher molar percentage of acetic and propionic than UTW and BH groups. On the other hand, there were no significant differences among different groups for Butyric acid although the highest value was recorded for BH group (10.53%). Increment of propionate production is favorable as that propionate acts a very important role as a major precursor of hepatic gluconeogenesis also propionate is a major precursor of meat which in turn help in during growth period or pregnancy period. The data in the present study were similar with those obtained by Aziz (2019).

Blood hematological parameters:

Using untreated mandarin waste (UTW) resulted in lower ($P < 0.01$) values of red blood cell count (RBCs) haemoglobin (Hb) and haematocrit percentage (Ht %) (Table 9). Treating mandarin waste by either Yeast or ZADO recovered the values of these parameters. Adding Yeast increased ($P < 0.05$) values of mean cell volume (MCV) and mean cell haemoglobin (MCH), while treating with ZADO slightly reduced it.

The high tannin content in UTW compared with BH (Table 2) might be the reason for low values of RBC's, Hb and Hct. These findings were in agreement with [El-Bassiony \(2013\)](#) and [El-Hawey \(2013\)](#) who reported significant decrease in RBC's and WBC's in different animals species (sheep, goats, camels and cattle) that fed tanniniferous plants when compared to control group which fed Berseem hay. However, [Abelhameed et al. \(2006\)](#) found insignificant effect to feeding tanniniferous plants shrubs on Hb and Hct%.

The increased RBCs, Hb and Ht% in ewes of ZTW and YTW groups might be attributed to haemoconcentration due to the significant low total water intake that decreased from 96.7 in BH group to 19.83 and 47.67 g/kg0.82 in YTW and ZTW groups, respectively (Table 7).

White blood cells (WBCs) decreased ($P < 0.05$) in ewes at feeding UTW, but adding Yeast and ZADO significantly increased it than control (BH). The increase in WBCs was through the increase in granulocytes% on the account of lymphocytes and monocytes percentages.

The increased lymphocytes% at feeding UTW could be attributed to the ability of this type of feed to activate lymphocytes and accessory cell types. This lead to enhance production of antibodies thereby increasing cell mediated immunity ([Banji, et al., 2012](#)). This could mean that even feeding untreated waste did not harm animal immune system.

Table (9):- Average values of complete blood components in Barki ewes fed different experimental roughages.

Parameter	Experimental groups				SEM
	BH	UTW	YTW	ZTW	
	Erythrocyte indices				
RBC (10 ⁶ /mm ³)	10.33 ^a	08.21 ^b	10.24 ^a	10.96 ^a	0.38
Hb (g/dl)	11.73 ^a	10.00 ^b	12.20 ^a	11.33 ^a	0.33
Hct (%)	38.10 ^a	30.50 ^c	40.57 ^a	36.93 ^b	0.61
MCV (Fl=liter×10 ⁻¹⁵)	37.10 ^{ab}	37.20 ^{ab}	40.17 ^a	34.00 ^b	1.62
MCH (pg=10 ⁻¹² g)	11.40 ^{ab}	12.23 ^a	12.00 ^a	10.43 ^b	0.43
MCHC (%)	30.87	32.90	30.20	30.83	1.29
	Leucocyte indices				
WBCs (10 ⁻³ Cells/mm ³)	6.31 ^b	3.27 ^c	9.47 ^a	7.51 ^{ab}	0.67
Lymphocytes (%)	48.28 ^b	51.40 ^a	43.05 ^c	42.73 ^c	0.74
Granulocytes (%)	47.33 ^b	43.05 ^c	53.83 ^a	53.23 ^a	0.73
Monocytes (%)	04.39 ^b	05.55 ^a	03.12 ^c	4.04 ^b	0.29

RCs: red blood cell counts, Hb: haemoglobin, Ht%: haematocrit percentage, MCV: mean cell volume, MCH: mean cell haemoglobin, WBCs: white blood cells

Means with different letters within each row are significantly different ($P < 0.05$).

Blood biochemical parameters:

Serum total proteins and albumins exhibited almost similar values for the animals in the four experimental groups (Table 10). Ewes fed UTW and ZTW had the higher ($P < 0.05$) values of globulins (3.10 and 2.87 g/dl, respectively). Meanwhile, ewes fed BH and YTW showed insignificantly the highest albumin values, and significantly the lowest values of globulins, as a result they had the highest values of A/G ratio.

Plasma proteins are part of the immune response through making antibodies. Ewes fed UTW showed the lowest values of albumin and A/G ratio. These two parameters increased in ewes fed treated mandarin waste by either Yeast or ZADO. These two additives help animals to get more digestible protein in the diet. In the present study, treating mandarin waste by either Yeast or ZADO increased the digestibility percent of CP (from 56.44 to 72.31 and 74.97%, respectively and the content of DCP (from 8.11 to 11.25 and 11.92%, respectively, Table 5). The protein levels in the diet can influence the resistance to infection ([Louvandini et al., 2006](#)) and provide the supply necessary to replace the blood lost and the damaged tissues, as well as enhancing the immunological system's response ([Nnadi et al., 2007](#)). Treating mandarin waste is necessary to obtain good overall health status of the ewes.

Kidney function indicators expressed as creatinine, urea, and uric acid concentrations revealed that both of two animal groups fed treated trimming wastes (YTW and ZTW) showed higher ($P < 0.05$) concentrations of these criteria compared with the other two groups (UTW and BH). High protein intake exerts some stress on kidney function. These findings were in harmony with the results of CP% content and its digestion (Table 5), and absorbed N as well as retained N (Table 6). However, all Kidney function indicators were within the normal ranges.

The experimental diets had no effect on liver function as the enzymes under investigation (aspartate amino transferase; AST, alanine amino transferase; ALT, gamma glutamyl transferase; GGT and alkaline phosphatase; Alk Ph) did not differ significantly and all were located within the normal range that were reported by [Daramola et al. \(2005\)](#). Alkaline phosphatase is insignificantly higher in ewes fed treated mandarin waste. Alkaline phosphatase is a hydrolase that helps to improve the immunization of organisms ([Ming et al., 2012](#)). Again, treating mandarin wastes improve healthful effects on ewes.

Ewes of all groups exhibited similar results of total lipids, total cholesterol and LDL, that indicated the absence of hypocholesterolemia. The same was found in goats ([Olafadehan, 2011](#)). Serum concentrations of triglyceride and VLDL were higher in ewes of UTW and ZTW groups than their counterparts in BH and YTW groups. On the other hand, ewes of BH group exhibited the highest level of blood HDL followed by ewes in UTW group. In general, lipid profile was not impaired by feeding mandarin waste.

No significant differences were observed among the experimental groups concerning blood concentration of total antioxidant capacity (TAC). It was found that trimming waste under investigation contains flavonoids with concentration of 2.14 g/kg DM (two fold of BH) as shown in Table (2). These polyphenolic compounds could significantly act as free radical terminator as stated by [Oyedemi et al. \(2010\)](#) and [Satish et al. \(2013\)](#) and might enhance phagocytic activity and increase stimulation of immune and antioxidant activity [Venkatesan et al. \(2012\)](#), as well as increased ferric reduction antioxidant power ([Zhu et al., 2002](#) and [Santos et al. 2014](#)). Moreover, [De Whalley et al. \(1990\)](#) demonstrated that flavonoids were potent inhibitors of macrophage modification of low density lipoproteins (LDL) through inhibition of generation of lipid hydro-peroxides. Interestingly, the flavonoid compounds were also very active in conserving the α -tocopherol content of LDL, and they delayed the onset of measurable lipid peroxidation. Accordingly, trimming waste of mandarin trees is of valuable nutritional quality in ruminants feeding.

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complexation and radical scavenging. Accordingly, trimming waste of mandarin trees is of valuable nutritional quality in ruminants feeding.

Table (10):- Average values of blood plasma biochemical parameters in Barki ewes fed the experimental roughages.

Parameter	Experimental groups ¹				SE
	BH	UTW	YTW	ZTW	
	Blood Plasma Proteins				
T. Proteins (g/dl)	5.41	5.69	4.80	5.68	0.26
Albumin (g/dl)	2.84	2.59	2.64	2.81	0.15
Globulins (g/dl)	2.57 ^{ab}	3.10 ^a	2.16 ^b	2.87 ^a	0.15
A/G ratio (%)	1.14 ^{ab}	0.84 ^c	1.22 ^a	0.97 ^{bc}	0.05
	Kidney Functions				
Creatinine (mg/dl)	0.66 ^b	0.66 ^b	1.05 ^a	1.06 ^a	0.06
Urea (mg/dl)	26.33 ^b	24.67 ^b	55.00 ^a	40.67 ^a	4.52
Uric acid (mg/dl)	2.50 ^b	2.99 ^{ab}	3.23 ^{ab}	3.65 ^a	0.26
	Liver Functions				
AST (IU/L)	26.99	25.55	30.86	19.02	5.62
ALT (IU/L)	30.12	28.88	35.70	31.84	5.56
GGT (IU/L)	29.91	40.12	30.12	42.78	12.22
Alk Ph (IU/L)	27.20	20.00	67.00	40.10	24.44
	Lipid Profiles				
T. Lipids (mg/dl)	133.50	127.20	121.11	118.15	11.70
Triglycerides (mg/dl)	26.20 ^b	36.74 ^a	25.37 ^b	30.84 ^{ab}	3.14
T. Cholesterols (mg/dl)	40.04	38.15	36.33	35.44	3.51
HDL (mg/dl)	4.87 ^a	3.90 ^b	3.53 ^b	3.50 ^b	0.26
LDL (mg/dl)	28.21	26.19	27.31	24.03	3.58
VLDL (mg/dl)	5.24 ^b	7.35 ^a	5.08 ^b	6.18 ^{ab}	0.63
	Plasma Antioxidant				
TAC ² (nmol/ml)	0.448	0.450	0.418	0.411	0.03

¹BH: berseem hay, UTW: untreated waste, YTW: yeast treated waste, ZTW: waste treated with ZADO,

²TAC: Total Antioxidant Capacity.

Means with different letters within each row are significantly different ($P < 0.05$).

Immune Proteins and cytokines indices:

Values of serum immunoglobulins (IgG and IgM) in ewes were higher ($P < 0.05$) in animals fed trimming waste than that fed BH (Table 11). Ewes in UTW group recorded the highest ($P < 0.05$) values (2.38 and 0.80 mg/ml) compared with the other two treated groups (2.16 and 0.72 mg/ml for ZTW and 1.44 and 0.48 mg/ml for YTW). [Pourhossein et al. \(2015\)](#) found that, citrus by-products extract significantly improves the serum levels of IgM, and IgG in broiler chickens. [Eissa et al. \(2017\)](#) concluded that, IgG levels in lamb's serum were closely related to the concentration of total protein and globulin, as IgG levels increased with the increase of total protein and globulin. So, determination of serum protein and globulin can be used as an indicator for the passive transfer of IgG. Plasma globulins increased by feeding UTW and ZTW (Table 10).

In addition, feeding trimming waste significantly increased complement 3 (C3), but reduced ($P < 0.05$) complement 4 (C4). The proteins which constitute the complement system are normal components in the fluid portion of blood and other fluids. Both complement components (C3) and (C4) perform important role in enhancing the ability of antibodies and phagocytic cells to eliminate both pathogens and pathogen damaged cells ([Guéguinou et al., 2014](#)). [Caroprese et al. \(2012\)](#) working on lactating sheep suggested that dietary protein enhance C3. In the present study, the two additives; yeast and ZADO, helped animals to get more digestible protein in the diet (Table 5). Moreover, mandarin trimming waste have high content of flavonoids (Table 2). [Harborne and Williams \(2000\)](#) stated that, Flavonoids have antioxidant, anti-inflammatory, antibacterial and immune-stimulating effects. Cytokines (IL-1, IL-2 and IL-6) levels attained their maximum values regarding trimming waste groups (Table 11). Cytokines are proteins secreted by specific cells of immune system responsible for modulation of the immune response to infection or inflammation and regulate inflammation via a complex network of interactions ([Spaulding et al., 1997](#)).

Table (11):- Average values of blood plasma immune indices in Barki sheep fed different experimental roughages.

Parameter	Experimental groups				SEM
	BH	UTW	YTW	ZTW	
	Immune Proteins				
IgG (mg/ml)	1.08 ^b	2.38 ^a	1.44 ^{ab}	2.16 ^{ab}	0.39
IgM (mg/ml)	0.36 ^b	0.80 ^a	0.48 ^{ab}	0.72 ^{ab}	0.13
Complement 3 (mg/ml)	0.43 ^c	0.74 ^{ab}	0.60 ^b	0.79 ^a	0.07
Complement 4 (mg/ml)	2.71 ^a	1.08 ^b	0.82 ^b	1.35 ^b	0.37
	Cytokines Indices				
IL-1 β (pg/ml)	29.42 ^b	48.61 ^a	36.27 ^{ab}	45.17 ^a	4.84
IL-2 (pg/ml)	39.51 ^b	67.96 ^a	50.76 ^{ab}	61.44 ^a	5.74
IL-6 (pg/ml)	27.79 ^b	51.26 ^a	38.35 ^{ab}	44.21 ^{ab}	5.60
TNF- α (pg/ml)	18.02	29.18	24.98	27.67	5.00

Ig: immunoglobulin, IL: interlocking,

Means with different letters within each row are significantly different ($P < 0.05$).

It is widely accepted that IL-1 cytokine plays a key role in several kinds of defense responses. The increased levels of IL-1 and IL-6 observed in the present study may be attributed to the feed consumption which enhanced cellular and humoral immunity in animals (Rebecca et al., 2000). Immune system can be also affected by dietary supplementation through altering immunological and inflammatory processes such increased in plasma secretion of pro-inflammatory cytokines, including IL-6 and TNF- α (Caroprese et al., 2010). However, feeding by-products resulted in an increase in TNF- α among the different groups compared to the control.

Polyphenols also affect various mechanisms within different immunological responses, and thus, targeted immunotherapy requires an understanding of these mechanisms of action. Regarding immune protection, polyphenols can not only regulate the host immune system but also directly target the pathogen. To increase the efficacy of polyphenols, researchers must not only understand the immunological effects of different types of polyphenols but also determine the appropriate mechanism.

Conclusion:-

Summing up, full replacing of Barseem hay by trimming waste of Mandarin trees especially after processing by liquid feed enriched with either yeast or ZADO achieved the following advantages: 1) enhance the nutritive values, 2) resulted in higher retained nitrogen, 3) make the animal drink a little and lose a little, 4) leads to high concentration of rumen acetic and propionic acids so consequently used as feeds for meat and milk production, 5) Increased concentration of immune markers in the blood, 6) decrease feeding cost, and 7) avoids damage to the environment.

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