Annals of Agricultural Science (2012) 57(2), 183–190

Faculty of Agriculture, Ain Shams University

Annals of Agricultural Science

www.elsevier.com/locate/aoas

Original Article

Usage of treated rice straw with exogenous anaerobic bacterial enzymes (ZAD) for Ossimi sheep

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Received 10 March 2012; accepted 26 March 2012
Available online 25 October 2012

KEYWORDS

Ossimi rams;
Barely;
Exogenous enzymes;
Rice straw

Abstract The objectives of this study were to verify the potential benefits of growing green barley on anaerobic enzyme (ZAD) treated rice straw. In addition, the work intended to investigate the effect of this treatment on digestibility parameters in Ossimi sheep. A complete random design was used to distribute twelve mature male of Ossimi sheep (45.0 + 0.5 kg wt.) on the following treatments: Rice straw with grown barley (RSGB) without either ZAD or orange pulp (control, T1), RSGB plus ZAD (T2), RSGB plus orange pulp (T3) and RSGB + ZAD + orange pulp (T4). The obtained results could be summarized as follow:

1. Significant decreases were observed in %CF from 38.09 for T1 to 32.01 and 30.02 for rations T4 and T2 respectively ($P < 0.05$). Percentage values of NDF were 70.01, 72.10 and 76.01 for rations T4, T2 and T1 respectively ($P < 0.05$); while %ADF values were 50.05, 52.10 and 58.10 for rations T4, T2 and T1 respectively ($P < 0.05$) %ADL was 6.01 for T4 versus 8.01for T1. Significant increases in %CP content to 7.96, 7.10, 7.95 were observed for rations T4, T3, T2 respectively compared to the control ration which was 5.75 ($P < 0.05$).
2. Adding ZAD to RSGB significantly increased ($P < 0.05$) %TDN to 55.02 and 59.02 for treatments T2 and T4 respectively and increased digestibility coefficients of CP to 72.43 and 77.70 respectively.
3. Rams fed rations T2, T3 and T4 had significantly higher values of ruminal ammonia-N 3 h post feeding values were 25.41, 25.03, 25.96 mg/100 ml respectively and total volatile fatty acids 3 h. Post feeding values were 8.20, 8.13 and 8.26 meq/100 ml respectively.
4. Adding either ZAD, orange pulp or both to RSGB significantly increased ($p < .05$) plasma total protein values were 6.43, 6.23, 5.82 g/dl for treatments T4, T3, and T2 respectively, while treating rations with ZAD reflected low level of GPT 6 h. Post feeding values were 20.64 and 20.61 for treatments T2 and T4 respectively versus T1 (20.90 μ/l).

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Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.aoas.2012.09.002

It could be concluded that the anaerobic enzyme matrix (ZAD) improved the nutritive value of soilless green barley and improved their digestibility coefficients in Ossimi sheep.

Introduction

The acute shortage of conventional feedstuffs for livestock feeding resembles a big problem for animal production in Egypt. The big feed gap between the requirements and the available sources forced the planners and nutritionists to look for non-conventional resources where there is no competition with humans. Such agricultural by-products are available around the year but are not efficiently used (Abd El fattah, 2009).

The problems of feeding lignocellulosic materials to farm animals are in general, low protein content, high crude fiber, low digestibility coefficients and the content of some anti-nutrition factors such as tannins and alkaloids (Kholif, 2005).

Thus, to increase the digestibility of these lignocellulosic materials, it is important to breakdown the linkage between cellulose, hemicellulose and lignin or breakdown the compact nature of the tissue (Kholif, 2005).

One of the main shortcomings of rice straw as an animal feed is the low digestibility of its major organic constituents; and the cell wall components. Several treatments and processing methods are being studied around the globe to improve the nutritive value of the lignocellulosic residues; several chemical treatments which aimed at delignifying or disrupting the lignin carbohydrate complex have been tested in attempts to improve the accessibility and exposure of structural carbohydrates to ruminal cellulolytic microorganisms. Some physical treatments such as ball-milling, steaming or irradiation have also been tested. Chemical pretreatment appeared more improvement particularly the digestibility of the hemicellulose fraction (Gado, 1997).

Colombatto et al. (2003) found that, the enzyme product (Liquicell 2500, Specialty Enzymes and Biochemical, Freson, CA) was derived from *Trichoderma reesei* and contained mainly xylanase and cellulase activities. With the addition of these enzymes at concentrations of (0.5, 2.55 and 5.1 mL/g of DM), the absence of ruminal fluid increased ($P < 0.001$) the release of reducing sugars from xylan and the mixture after 20 h of incubation at 20 °C. Incubations with ruminal fluid showed that enzyme (0.5 and 2.55 mL/g of DM) increased ($P < 0.05$) the initial (up to 6 h) xylanase, endoglucanase, and β-D-glucosidase activities in the liquid fraction by an average of 85%. It was concluded that enzymes enhanced the fermentation of cellulose and xylan by a combination of pre- and post-incubation effects (i.e., an increase in the release of reducing sugars during the pretreatment phase and an increase in hydrolytic activity of the liquid and solid fractions of the ruminal fluid) which was reflected in a higher rate of fermentation, whereas the physical treatments had a greater effect on cellulose digestion (Feng et al., 1996; Johnson et al., 1993; Chen et al., 1994), Biological treatments are alternative treatments to modify the digestion of fibrous materials by ruminants. The fungal decay of straw by white-rot fungi improved the *in vitro* dry matter digestibility of the decayed substrate (Dawson and Tricarico, 1999). Besides, Mahrous et al., 2005 had studied the effect of biological treatments (ZAD, fungus and ZAD with fungus) of rice straw on feed intake, digestibility coefficients, nutritive value, nitrogen balance and some rumen liquor and blood parameters. All of the treated rice straw groups did not significantly affect urea, total protein, albumin, globulin, GOT and GPT compared to untreated rice straw groups.

The purpose of this study is to investigate the potentiality of growing barely grains on treated rice straw. This goal will be accomplished through a metabolism trial using Ossimi rams measuring blood parameters, rumen parameters, chemical composition, cell constituents (fiber fraction) and digestibility coefficients of the **RSGB**.

Materials and methods

This study was carried out to investigate the effect of biological treatment (bacterial enzyme treatment) on poor quality roughages (I. e. rice straw and orange pulp) to improve its nutritive value as ruminant feeds.

The experiments were carried out at the laboratory of the Rumen Ecology Center, (Metabolic Unit) and the Experiment Farm of the Meat and Milk Development Center (in Shalakan), Faculty of Agriculture, Ain Shams University, and in the Egyptian company (ECARU).

Preparing of rice straw

This experiment was carried out to avoid rice straw boiling during the preparation process before planting on it, which was the procedure followed in all previous experiments, for the purpose of sterilization and wetting, also to be free from most of its impurities.

The first experiment was performed for the distinction between growing barley on the soaked straw without boiling and boiled straw, in order to save one of the steps that hamper the processing of straw and use it on a large scale; the results showed that there is no significant difference between the two treatments. However, we benefited from reducing the time and effort and employment of users to boil the straw before planting.

Widely adopted experiment

In this experiment, 1.34 tons of rice straw was used and soaked in water with ZAD enzyme, at the same time 152 kg of barely seeds was soaked in the same way as the straw. The concentration of enzyme in soaking water was 3 ml per 1 kg of either rice straw or barely seeds. The soaked straw was placed on plastic sheets, then the soaked seeds were spread and irrigated with the rest of soaking seeds in water, after that it was covered with a plastic sheet for the first 6 days only of 15 days of the summer season; the plants were irrigated daily with 10 ml enzyme (ZAD) per 1 l of water for the first 6 days.

Table 1 Chemical composition of rations.

Sample	T1(Control)	T2	T3	T4	±SE
% Moist.	56.67[b]	60.00[a]	50.10[d]	54.37[c]	±0.55
%CF	38.02[a]	30.02[d]	36.05[b]	32.01[c]	±0.02
%EE	0.99[c]	1.25[b]	1.00[c]	1.57[a]	±0.02
%CP	5.75[c]	7.95[a]	7.10[b]	7.96[a]	±0.04
%Ash	26.10[b]	24.10[c]	34.10[a]	26.01[b]	±0.05
%NDF	76.01[a]	72.10[c]	74.01[b]	70.01[d]	±0.03
%ADF	58.10[a]	52.10[c]	54.10[b]	50.05[d]	±0.05
%ADL	8.01[b]	8.07[a]	6.02[c]	6.01[c]	±0.02
%Silica	18[a]	16[b]	16[b]	16[b]	±0.02

Means with the same letter in the same raw are not significantly different.

Three days before harvest we stopped the irrigation in order to increase the dry matter and to save more water; the trial lasted two weeks until plants reached 18 cm in height. The germination of barely was according to Anwar, 2009.

After that each treatment was put in a mixer separated, then the orange pulp was added for 2 treatments, one with enzyme (ZAD) and the other without enzyme, and then all the treated plants were pressed in bales. Samples were taken for chemical analysis and the chemical composition is shown in Table 1.

The four treatments were as follow:

1. Rice Straw with Grown Barely (**RSGB**) without enzyme (ZAD), without orange pulp (control, T1).
2. **RSGB** with enzyme (ZAD), without orange pulp (T2).
3. **RSGB** without enzyme (ZAD), with orange pulp (T3).
4. **RSGB** with enzyme (ZAD), with orange pulp (T4).

ZAD is a compound of enzymes separated from anaerobic bacteria of the rumen. It contains a mixture of cellulase, hemicellulase, protease and alpha amylase enzymes.

where

T1-**(RSGB)** rice straw with grown barely without enzyme (ZAD), without orange pulp.
T2-**(RSGB)** with enzyme (ZAD), without orange pulp.
T3-**(RSGB)** without enzyme (ZAD), with orange pulp.
T4-**(RSGB)** with enzyme (ZAD), with orange pulp.

The present study was divided into two experiments:

– In the first experiment:

In vitro rate (Merten, 1977) technique was conducted to study the effect of different sources of the experimental material on chemical composition, fiber fraction and *in vitro* dry matter disappearance (IVDMD) of rice straw as poor quality roughage.

– The second experiment:

Metabolism experiments were designed to evaluate the nutrients digestibility, and feeding values of the treated RSGB using 12 male Ossimi rams divided on four randomly treatments of three rams each, fed on rice straw treated with enzyme.

Experiment I

In vitro DM disappearances (IVDMD)

As a primary study, *In vitro* rate technique was used in this trial to determine the rate of DM disappearance for experimental rations.

In vitro disappearance was determined according to the method described by Terry et al. (1969); a total number of 56 samples from treated rice straw were used to determine the rate of DM disappearance, plus 12 tubes as blank.

Experiment II

Metabolism trial (in vivo)

Metabolism trial was carried out to evaluate the nutrients digestibility, rumen and blood parameters; using Ossimi rams fed the experimental rations. The complete random design was used to carry out this experiment. Twelve mature rams (45 ± 0.5 kg wt.) were used in this design. Rams were distributed randomly to four treatments. Each animal was confined in individual metabolic crates for 7 days as an adaptation period followed by 5 days as a collection period.

Animals were fed at maintenance requirements using the allowances of NRC (1985) Samples of feed were taken daily at 8.00 am in the morning and kept in a glass bottle at the laboratory for later analysis. Feces and urine were quantitatively collected daily. Weight of total feces and volume of urine were recorded daily in the morning. The representative samples (10%) were taken from fecal material of each animal during the collection period.

Each sample was sprayed with a solution of 10% formaldehyde in addition to 10% H_2SO_4 solution, and then the samples were dried in a forced air oven at 60–65 °C until constant weight. The dried fecal samples per animal were mixed and kept for laboratory analysis. Urine samples were also collected daily for each goat in glass bottles containing 50 ml diluted sulphuric acid (10%) to avoid ammonia losses. Final DM of feces was determined by drying in an oven at 105 °C until constant weight.

Digestible nutrients (CP, CF, EE and NFE), nitrogen balance was determined for each animal. Samples of feedstuffs used and feces were subjected in duplicate to proximate analysis (DM, CP, CF, EE and Ash) according to AOAC (1995). Nitrogen free extract (NFE) values were calculated by difference.

Feed stuff, ration and feces were analyzed according to the modification of Pakistan Agriculture Research Council (1982) for NDF, ADF and ADL, Cellulose and hemicellulose were determined by difference between ADF and ADL, NDF and ADF, respectively.

$$\text{Digestibility} = 100 - \left[100 \times \frac{\%\text{indicatorin feed}}{\%\text{indicatorin feces}} \times \frac{\%\text{nutrientin feces}}{\%\text{nutrientin feed}}\right]$$

Proximate analysis

Dry matter (DM), crude protein (CP), crude fiber (CF), and ash of the ration and feces samples were analyzed according to AOAC (1995). The nitrogen free extract (NFE) was calculated by the difference.

Fiber fraction

Representative samples of the experimental rations and feces were analyzed for fiber fraction according to the modification of PARC (1982) to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Cellulose and hemicellulose were determined by the difference.

Rumen liquor parameters

Rumen liquor samples
Rumen liquor samples were collected from all experimental rams during metabolism trial pre experimental feeding (0 day) at zero, 3 and 6 h and post experimental feeding (12 days). The samples were collected by rubber stomach tube inserted into the rumen via the esophagus. Rumen liquor was strained through four layers of cheese cloth and immediately used for the determination of rumen pH then the liquor was stored in dried glass bottles in deep freezer at (−20 °C) to measure the other parameters.

Determination of rumen pH
Values of rumen pH were determined immediately using a pH meter (EIL 7010) with a combined electrode.

Ammonia–nitrogen concentration (NH_3–N)
Ammonia–N. concentrations were measured by modified semi-micro kijeldehl digestion method (AOAC, 1995).

NH_3–N concentrations were calculated according to the following equation

$$\mathrm{NH_3\text{–}N, concentration\%} = (\text{ml. of acid titrate} * \mathrm{N.H_2SO_4} * 0.014)/(\text{volume of the rumen fluid(1 ml)}) * 100$$

Total volatile fatty acids
Total volatile fatty acids in the rumen liquor (TVFA's) were measured according to stem distillation procedure as described by Warner (1964).

Total volatile fatty acids were calculated according to this equation

$$\text{Total volatile fatty acids} = \text{ml. Sodium hydroxide titrate} \times \text{N. NaOH} \times 100(\text{M. eq per 100ml rumen fluid})$$

Blood parameters

Blood samples were collected from three animals per treatment during the metabolism trial at zero, 3 and 6 h pre and post experimental feeding. Samples were obtained by allowing blood to flow freely from the jugular vein through heparin tubes. Then, centrifuged for 30 min. at 4000 rpm. Plasma was separated into clean dried glass vials and stored at freeze (−20 °C) till analysis.

Plasma urea
Plasma urea was determined colorimetrically by using commercial kits which were purchased from biomerieux according to method described by Patton and Crouch (1977).

Plasma total protein
Plasma total protein was measured colorimetrically by the biuret reaction method using commercial kits according to method of Peters (1968).

Glutamate oxalo-acetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT)
Plasma glutamic-oxaloacetate transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were determination colorimetrically using commercial kits of biomerieux using the method of Reitman and Frankel (1957).

Statistical analyses

All traits were statistically analyzed by the methods described in SAS (1998). A fixed model was used and to identify significant differences between means, Duncan's multiple range test (Duncan, 1955) was used.

Results and discussion

The present investigation was conducted to study the effect of biological treatments on some poor quality roughages of crop residues. For this purpose, parameters measured were proximate chemical analysis and cell wall constituents of poor quality roughages. Also, some fermentation studies (*in vitro* disappearance), metabolism trial and some nutritive values were determined.

It was observed that treated rations with ZAD had the best estimates of digestibility and fiber fractions compared to the non-treated rations. Adding both orange pulp and ZAD to **RSGB** improved significantly ($P < 0.05$) the rumen parameters, chemical composition, blood parameters, digestibility coefficients, and fiber fraction. These results agreed with those of Pulatov et al. (1983) which concluded that the enzymatic treatment of roughage improved the digestibility of ration and slowing feed passage time throughout the digestive tract that reflected better absorption. These findings were also similar to those of Almquist et al. (1967), Mohamed et al. (1989), Flachowsky and Klnppach (1993) and Fouad et al. (1998).

The highest digestibility coefficients of CP in the rations treated with ZAD compound could be an indicator of increasing the microorganism's biomes, while the improvement of digestibility coefficients of CF may be due to the increase of the activity of enzymes produced by microorganisms.

The digestibility coefficients showed in Table 2 for dry matter (DM), organic matter (OM), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE) were significantly ($P < 0.05$) higher in T4 than T3. These preceding coefficients were also significantly ($P < 0.05$) higher in T2 than T1.

It was also observed from Table 2, that total digestible nutrients (TDN) and digestibility of crude protein were significantly ($P < 0.05$) higher in R4 than in R3. TDN and the digestibility of CP of the two previous rations were significantly ($P < 0.05$) higher than those in T2 and T1. The lowest coefficients of TDN and CP were found in control rations T1.

Treating rations with ZAD increased TDN to 55.02 and 59.02 and increased digestibility of the CP to 72.43 and 77.70 for rations T2 and T4, respectively.

Similar results were observed in fiber fractions which demonstrated the significant efficiency of ZAD in minimizing

Table 2 The digestibility coefficients and nutritive value.

Treat. item	T1 RSGB (control)	T2 RSGB + ZAD	T3 RSGB + orange pulp	T4 RSGB ZAD + orange pulp	±SE
%DM	69.66[b]	70.00[ab]	70.03[ab]	70.33[a]	±0.12
%OM	73.90[d]	76.50[b]	75.43[c]	78.23[a]	±0.20
%CP	58.03[d]	72.43[c]	73.70[b]	77.70[a]	±0.12
%EE	65.17[d]	80.30[c]	83.23[b]	84.83[a]	±0.09
%CF	53.40[d]	60.40[c]	65.47[b]	68.03[a]	±0.16
%NFE	60.63[d]	78.70[c]	82.33[b]	86.47[a]	±0.19
%NDF	60.30[d]	70.27[b]	69.40[c]	71.70[a]	±0.16
%ADF	60.30[b]	67.56[a]	67.40[a]	67.90[a]	±0.15
%TDN	42.76[d]	55.02[b]	48.61[c]	59.02[a]	±0.09

Means with the same letter in the same row are not significantly different.

Table 3 Ruminal pH.

Item	Treatments				±SE
	T1	T2	T3	T4	
Time					
At the beginning (0 day)					
0 h	7.17				±0.05
3 h	6.22				±0.01
6 h	6.70				±0.01
After 12 days					
0 h	6.81[b]	6.84[a]	6.84[a]	6.85[a]	±0.01
3 h	6.27	6.25	6.26	6.25	±0.01
6 h	6.87	6.88	6.86	6.89	±0.01

Means with the same letter in the same row are not significantly different.

Table 4 Ruminal ammonia-*n* concentration.

Item	Treatments				±SE
	T1	T2	T3	T4	
Time					
At the beginning (0 day)					
0 h	8.77				±0.06
3h	12.86				±0.05
6h	10.87				±0.03
After 12 days					
0 h	20.73[b]	20.87[b]	20.84[b]	21.30[a]	±0.11
3h	24.66[d]	25.41[b]	25.03[c]	25.96[a]	±0.08
6h	17.60[d]	18.45[b]	18.01[c]	18.95[a]	±0.06

Means with the same letter in the same row are not significantly different.

the percentages of the neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, hemi-cellulose and lignin. These results were in agreement with those obtained by Han (1974), Mohamed et al. (1989), Stockes (1992) and Gado (1997) that enzymatic treatment reduced the percentage of fiber fraction.

Rumen liquor parameters

Rumen pH values

Table 3 presents the ruminal pH values of rams fed the experimental rations. The results showed almost similar values of pH among the different groups of rams at 0.0 h before feeding. It was clear that pH values started to increase by advancing the time after feeding (up to 6 h) for all rations. These could be explained by the present of TVFA'S in the rumen. However, ram group fed T1 ration showed the lowest ($P < 0.05$) pH value (6.81) before feeding followed by that of T2 (6.84). However, rams group T4 had the highest ($P < 0.05$) value obtained. On the other hand pH values decreased for all rations after 3 h of feeding. There were no significant ($P > 0.05$) differences among the experimental groups in pH values at 3 h after feeding. Contrarily, values of pH increased at 6 h after feeding for all rations under study. Rams group

Table 5 Ruminal total volatile fatty acids (TVFA's).

Item	Treatments				±SE
	T1	T2	T3	T4	
Time					
At the beginning (0 day)					
0 h	5.96				±0.01
3h	7.87				±0.01
6h	6.68				±0.03
After 12 days					
0 h	6.74	6.75	6.75	6.76	±0.01
3h	8.08^{d}	8.20^{b}	8.13^{c}	8.26^{a}	±0.01
6h	7.15^{d}	7.34^{b}	7.30^{c}	7.38^{a}	±0.01

Means with the same letter in the same row are not significantly different.

Table 6 Plasma total protein.

Item	Treatments				±SE
	T1	T2	T3	T4	
Time					
At the beginning (0 day)					
0 h	4.50				±0.18
3h	5.80				±0.06
6h	6.70				±0.08
After 12 days					
0 h	4.53	4.67	4.60	4.83	±0.11
3h	5.14^{c}	5.82^{b}	6.23^{ab}	6.43^{a}	±0.14
6h	6.86^{c}	6.99^{a}	6.52^{b}	6.95^{a}	±0.19

Means with the same letter in the same row are not significantly different.

Table 7 Plasma urea concentration.

Item	Treatments				±SE
	T1	T2	T3	T4	
Time					
At the beginning (0 day)					
0 h	15.45				±0.06
3 h	14.14				±0.07
6 h	11.28				±0.01
After 12 days					
0 h	17.60^{b}	17.80^{a}	17.63^{b}	17.86^{a}	±0.03
3 h	16.48^{a}	16.44^{a}	16.33^{b}	16.26^{b}	±0.02
6 h	15.15	15.13	15.12	15.16	±0.01

Means with the same letter in the same row are not significantly different.

fedT4 had higher ($P < 0.05$) pH values than those groups fed T1, T2 and T3 at 6 h. The recorded values were 6.89, 6.87, 6.88 and 6.86 respectively.

Ruminal ammonia–N concentration

As shown in Table 4, at 0 h, ruminal ammonia–N concentration (mg/100 ml) was significantly ($P < 0.05$) the lower for T1, T2 and T3. These values of ruminal ammonia–N concentration (mg/100 ml) increased after 3-h post feeding and then decreased at 6-h post-feeding. Our results were on line with the results of El ashry et al. (2001) who noticed that these values increased 3 h after feeding. The values of the T3 and T4 rations were significantly ($P < 0.05$) the highest after 3 and 6 h of feeding indicating the beneficial effect of these treating rations.

Volatile fatty acids (VFA'S)

As showed in Table 5, the ruminal fluids of rams of T2 and T4 had significantly ($P < 0.05$) higher rate of VFA's at 0, 3 and

Table 8 Plasma GOT.

Item	Treatments				±SE
	T1	T2	T3	T4	
Time					
At the beginning (0 day)					
0 h	41.57				±0.09
3h	41.77				±0.12
6h	41.43				±0.07
After 12 days					
0 h	41.78	41.75	41.83	41.58	±0.08
3h	41.49	41.52	41.52	41.66	±0.09
6h	41.97[a]	41.68[ab]	41.46[b]	41.47[b]	±0.09

Means with the same letter in the same row are not significantly different.

Table 9 Plasma GPT.

Item	Treatments				±SE
	T1	T2	T3	T4	
Time					
At the beginning (0 day)					
0 h	20.70				±0.04
3 h	20.63				±0.04
6 h	20.93				±0.03
After 12 days					
0 h	20.97	20.62	21.05	20.58	±0.17
3 h	20.45	20.77	20.82	20.51	±0.14
6 h	20.90[a]	20.64[b]	20.84[ab]	20.61[b]	±0.07

Means with the same letter in the same row are not significantly different.

6 h compared to those fed the other rations (T1 and T3). The ruminal fluids of rams fed untreated rations (control groups) had significantly ($P < 0.05$) the lowest VFA's at 0, 3 and 6 h. Our results were in agreement with those of Gupta (1988), Abd El Gawad et al. (1993), Tuen and Dahan (1994) and El madany (1997) who showed that the ruminal VFA'S concentration significantly ($P < 0.05$) increased by the time after feeding.

Plasma total protein (PTP)

The results concerning the effect of feeding lambs on different experimental rations on PTP are shown in Table 6. It was observed that rams fed T2, T3 and T4 had a significantly ($P < 0.05$) higher level of PTP, then those fed control rations (T1).

Plasma urea concentration

Table 7 shows the means of urea concentration of the experimental treatments. No significant ($P > 0.05$) differences among groups were observed in plasma urea concentration after 6 h. Changes in Plasma urea would reflect changes in ruminal ammonia concentration. The results here in were confirmed by those of Lewis (1957) who demonstrated that increasing the concentration of Plasma urea after feeding was caused by the increasing of ruminal ammonia.

Glutamat oxalo-acetate transaminase (GOT) and Glutamat pyruvate transaminase (GPT)

Means of GOT and GPT of rams fed the different experimental treatments are shown in Tables 8 and 9 respectively. GPT of rams seemed to remain stable at the different experimental groups. Rams fed T1, T2, T3 and T4 produced 20.90, 20.64, 20.84 and 20.61 of GPT at 6 h, respectively Table 9, indicating that treating rations with ZAD reflected low level of GPT. Also, the level of GOT of rams had no significant values among experimental treatments (except at 6 h). So it is known that there is no effect of ZAD on kidney function. These results agreed with those of EL marakby (2003) who found that the biological treatment of rice straw had no significant ($P > 0.05$) effect on GOT and GPT. Also these results were in agreement with those obtained by Zewil (2005) who reported that enzymatic treatment of wheat straw had no adverse effect on kidney function.

Conclusion

Treating **RSGB** with ZAD compound improved significantly ($P < 0.05$) the digestibility coefficients, chemical composition and fiber fraction of ration fed to the Ossimi rams under the circumstances of this study. Besides **RSGB** treated with ZAD compound plus orange pulp had significantly ($P < 0.05$) better estimates of the previous nutritional parameters than the other treatments. Treating rations with ZAD compound increased TDN from 42.76% for control to 55.02% and 59.02% for T2 and T4 respectively, and increased digestibility of CP from 58.03% for control to 72.43% and 77.70% for rations T2 and T4 respectively without any abnormal signs on blood and rumen parameters. So we concluded that the best parameters obtained were from the **RSGB** + ZAD + orange pulp (T4).

It can be recommended for sheep breeders to use such treatments to improve their animal performance significantly.

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