International Journal of Agriculture Innovations and Research
Volume 4, Issue 6, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 30/05/2016 | Accepted on : 07/06/2016 | Published : 13/06/2016

Effect of Using Different Roughage and Enzymes Sources on Rumen Fermentations and Blood Parameters in Awassi Lams

Shaker A. Hassan
Department of Animal production /College of Agriculture / University of Baghdad/Iraq

Yasseen A. Almaamory
Department of Animal production /College of Agriculture / University of Baghdad/Iraq

Abstract – **This study was conducted in the animal farm of animal production department / college of Agriculture / University of Baghdad to study the effect of treatment of two source of roughages which are alfalfa hay (AH) and wheat straw (WS) with two source of exogenous fibrolytic enzymes (EFE) which are local enzyme product (LEP) and commercial enzyme product (CEP) on rumen fermentations and some parameters of the blood. Twenty-four Awassi lambs with average initial weight of 29.84 ± 1.37 kg and 9 months were individually fed in a 2×3 factorial experiment. The lambs were randomly divided into six groups according to the type of diet. The diets were: concentrated diet + 8 ml LEP pre-treated WS (T1), concentrated diet + 8 gm CEP pre-treated WS (T2), concentrated diet + untreated WS (T3), concentrated diet + 8 ml LEP pre-treated AH (T4), concentrated diet + 8 gm CEP pre-treated AH (T5) and concentrated diet + untreated AH (T6). The results showed that NH3-N and pH were not affected by the source of roughages, source of enzyme and them interaction. But, the molar proportions of acetic acid, probionic acid, butyric acid and TVFA were affected by the source of enzyme and the source of roughages and them interaction. Results showed that the blood parameters were not affected by the source of enzyme and the source of roughages and them interaction except the serum glucose was increased (P<0.01) when used LEP, AH and them interaction. It could be concluded from this study; treatment roughages with EFE decreased the molar proportions of acetic acid and increased the molar proportions of probionic acid, butyric acid compared with control.**

Keywords – **Blood Parameters, Exogenous Fibrolytic Enzymes, Rumen Fermentations.**

I. INTRODUCTION

There are limited areas for planting foreges and Grazing in most third world countries including Iraq. This limitation leads to scarcity of green feed which causes the use of residues and wastes from agricultural and industrial crops such as wild reeds, Hays, palm fronds, date pulp and other alternatives forage with low nutritional value [1]. In Iraq, the agricultural wastes and by-products industry are considered as a stable source of feed for ruminant animals [2]. The primary focus of the specialists in livestock field is to find strategies to improve the productivity of animals. Most researchers focus on reduce the economic cost especially the cost of feed because of the high cost of feed materials in developing countries [3]. Over the years, nutritionists have developed physical, chemical and biological methods to overcome problems related to the feed. With the come into view concerns of food safety issues related to animal products, application of biological treatment methods is in the center of attention. As a biological treatment method utilization of exogenous enzymes, it has interested a growing attention of researchers and it becomes a widely discussed theme among animal nutritionists [4]. The idea of supplementation the exogenous enzymes in ruminant diets is not new, though a great number of research that interest in this field has been appeared in 1990s. The exogenous enzymes that used in ruminant diets can be divided into main categories such as fibrolytic, amylolytic and proteolytic based on the specific substrate on which their enzyme activity can execute [3].

The exogenous enzymes used in ruminants are from fungal sources (largely *Trichoderma longibrachiatum*, *Aspergillus niger*, and *A. oryzae*) and bacterial sources (*Bacillus spp.* and *Penicillium funiculosum*) with high cellulosic and hemicellulosic activity [5]. Rumen is a rich source of fibrolytic enzymes such as cellulase, xylanase and β-glucanases. A big number of anaerobic fungi, bacteria and protozoa has a very efficient cellulolytic method which helps to increase the capacity of feed conversion ratio [6]. The rumen microbial ecosystem includes a various population of microorganisms, and the microbial variety can be affected by many factors such as pH of treatment, level of feeding and roughages to concentrate ratio. Rumen bacteria are the major components of the rumen microorganisms, which account for more than 95 % of the population of the whole rumen microbial population [6]. Ruminants and ruminal microorganisms have a coexistence relationship, while microbial fermentation of intake feed is a main function of providing energy for metabolic actions in cattle. In the rumen, microorganisms metabolize feeds to volatile fatty acids (VFAs), microbial biomass, vitamins, and other materials for the host's nutritional needs. The rumen ecosystem includes a various cooperative population of anaerobic bacteria, ciliated protozoa, archaea, and fungi [7]. Varlyakov, et al [8] supplemented the ration containing 60% concentrate mixture and 40% meadow hay with EFE (Hostazym C100 has a predominant endo-1,4-α-glucanase and secondary cellulase, α-amylase, protease and hemicellulase activity in a dose of 1 g/kg of concentrate mixture). They found that enzymatic treatment had no significant effect on blood parameters (sugar, alkaline reserves, urea, total protein, albumins, globulins, albumins/globulins, total lipids and cholesterol). Hristov, et al [9] reported that when fed cannulated heifers a diet containing 86% barley grain and 14% barley silage (DM basis), and once daily there were given direct into the

rumen through cannula with EFE (dose 0, 100, 200, or 400 g) containing cellulase, xylanase, β-glucanase and amylase activity. They found that glucose and urea concentrations in blood were not affected by addition of EFE.

Therefore, the objective of this study is to improve the nutritional value of low quality roughages by using different types of enzymes and to know their effectiveness on rumen fermentation and some blood parameters of Awassi lambs.

II. Materials and Methods

This study was conducted in the animal farm of the college of Agriculture / University of Baghdad to study the effect of treatment of feed with exogenous fibrolytic enzymes (EFE) on rumen fermentations and some parameters of the blood.

A. Animal and Housing

Twenty-four Awassi lambs with average initial weight of 29.84 ± 1.37 kg and average 9 months of age at the start of the experiment were taken for 10 weeks (2 August 2015 to 15 October 2015). The objective was designed to investigate the effect of two types of EFE (Local enzyme product (LEP) named HAMU extracted from *Streptomyces MS* bacteria as a lignin peroxidase crude enzyme and commercial enzyme product(CEP) named ZY 1050-I is a specific enzyme which prepared by Lohmann company is a mixture of enzymes containing: ß-glucanase (IUB 3.2.1.6) (50 U) and xylanase (IUB 3.2.1.8) (1000 U) activities per gram of enzyme preparation and two types of roughages (alfalfa hay and wheat straw) on rumen fermentation (ruminal pH, ammonia-N and volatile fatty acids), and some blood parameters [serum urea nitrogen (SUN), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (S GPT), serum glucose (SG), serum cholesterol (SCH)] . A 2×3 factorial experiment using completely randomized design was used in this experiment. The lambs were separately and randomly allocated to the treatment according to live weight and they housed in each treatment inside pens (4× 4 m). All animals in pens were supplied with a plastic container used to offer concentrated and roughage diets. Pens were also supplied by clean fresh water.

B. Roughages Treatments with Enzymes

Wheat straw (WS) and alfalfa hay(AH) were pre-treated with 8 ml/kg of LEP and 8 gm/kg of CEP, and then each enzyme dissolved in 50 liters of water in a large Plastic container, separately. The WS (4 kg) and AH (4 kg) soaked in enzyme solution for 24 h. At the end of treatment period, the treated wheat straw and alfalfa hay were transferred to plastic sheets to be dried by the sun (3-5 days) as described by Al-Wazeer [10] with stirring once a day until given to animals.

C. Dietary Treatments

Awassi lambs were divided into six groups according to the type of diet. The diets were: concentrated diet + 8 ml LEP pre-treated WS (T1), concentrated diet + 8 gm CEP pre-treated WS (T2), concentrated diet + untreated WS (T3), concentrated diet + 8 ml LEP pre-treated AH (T4), concentrated diet + 8 gm CEP pre-treated AH (T5) and concentrated diet + untreated AH (T6).

The concentrated diet was composed of barley grain (40%), wheat bran (35%), yellow corn (13%), soybean meal (7.5%), sun flower oil (2.5%), Salts and lime (2%). The roughage was as WS (either treated or untreated with enzymes) and AH (either treated or untreated with enzymes). The chemical composition of concentrated diets and roughages are presented in tables 1.

Table 1: Chemical composition of concentrated diet and roughages (on DM% basis)

Ingredients	Conc.	T1 (WS+LEP)	T2 (WS+CEP)	T3 (WS untreated)	T4 (AH+LEP)	T5 (AH+CEP)	T6 (AH untreated)
DM % of fresh	91.18	91.38	92.29	90.92	91.20	90.79	91.96
OM	89.19	80.35	89.66	90.64	91.11	90.02	87.93
CP	13.21	3.53	3.55	3.36	13.89	13.30	13.65
EE	4.89	1.53	1.13	1.84	3.72	3.90	2.23
CF	11.85	47.48	58.02	42.78	47.32	48.53	32.9
NFE	50.42	19.49	20.26	22.62	19.37	21.08	39.61
TDN	58.91	28.11	30.42	40.09	44.31	45.95	44.64
NDF	38.38	70.97	74.24	52.60	58.43	68.30	66.84
Hemicelluloses	31.87	28.84	21.97	15.16	16.45	25.11	39.26
ADF	6.51	42.13	52.28	37.44	41.98	43.19	27.58
Cellulose	3.15	24.94	16.41	23.14	24.38	33.61	16.14
ADL	3.36	17.19	35.87	14.30	17.61	9.58	11.44
Ash	10.81	19.65	10.34	9.36	8.89	9.98	12.07
ME*(MJ/Kg DM)	9.09	3.35	3.78	5.59	6.37	6.68	6.43

WS= Wheat straw, LEP = local enzyme product (HAMU), CEP = The commercial enzyme product (ZY 1050-I).
* Metabolizable ene rgy (ME) values are estimated according to following equation:
ME (MJ/kg DM) = [- 0.45 + (0.04453×% TDN)] × 4.184
TDN is estimated according to equations of Kearl [11] as follows:
TDN (% of DM) = -17.2649+1.2120(%CP) + 0.8352 % NFE+2.4637 % EE+0.4475 %CF

D. Feeding Trails

The concentrated diet was given to animals gradually for two weeks (preliminary period) before the beginning of the experiment. Roughages and concentrated diet were given at the same time at 8.00 am. Concentrated diets were given to the animals at a rate of 2.5% of body weight. The WS and AH (either pre-treated or untreated) were given *ad libitium*.

E. Sampling of Rumen Liquor

Rumen liquor samples were collected from lambs during the last day of the experiment. They were withdrawn to study rumen fermentation characteristics through the determination of the ruminal pH, NH_3-N and TVFA concentrations and a molar portion of individual VFA. Samples were withdrawn by using a smooth rubber stomach tube which connected to Hand Operated Siphon Pump (SI-60) and inserted into the rumen via the esophagus as described by Saeed [12] with some modifications. Rumen liquor was strained through four layers of cheesecloth to discard the solid unfermented particles and immediately measured for pH using Portable digital pH meter (ph-80) after adjusting with standard pH buffer solutions (pH=7). After that, approximately of 10 ml of the rumen liquor was preserved with 2-3 drops of toluene to prevent fermentation and then the samples stored at -20 °C until analysis [13].

F. Determination of Rumen Liquor Parameters

Frozen strained rumen liquor samples were thawed at room temperature and were shaken. The contents were transferred into glass tubes and centrifuged at 4000 rpm for 10 minutes. The supernatant was analyzed for ruminal ammonia-N (NH_3-N) by the method of steam distillation with MgO using a Kjeltec (Gerhardt-Germany) distillate unit as method ID.954.01 [14] . The NH_3-N concentrations were calculated according to the following equation:

NH_3-N (mg/100ml) = [ml. of HCl titrate ×normality of HCl × 0.014 / volume of rumen liquor (1ml)] ×100 [14]

Total volatile fatty acids (TVFAs) in the rumen liquor were estimated according to the steam distillation method [15]. The TVFAs were calculated according to the following equation:

TVFA (mmol/L) = (ml. NaOH titrate ×normality of NaOH/volume of rumen liquor (1ml) ×1000) [15]

The molar proportions of individual volatile fatty acid (VFA) were determined by chromatography technique using high-performance liquid chromatography (HPLC) model 10AV-LC, LC-10A, UV-Vis 10A-SPD Shimadzu, Japan, according to the method described by [13, 16]. The rumen fluids were sent to the Ministry of science & Technology/ Environment & water Research & Technology Directorate (EWRTD)/ pollutants processing center to measure the individual volatile fatty acid, acetic acid, propionic acid and butyric acid.

G. Blood sampling and preservation

Blood biochemical changes were studied during the last day of the experiment. Blood samples (10 ml) were withdrawn via jugular vein puncture into vacationer tubes without anticoagulant which were immediately centrifuged at 4000 rpm for 10 min and the serum samples were harvested to determine SG, SGOT,SGPT,SCH and SUN concentrations. The blood samples were sent to BASHAEER AL-HARTHYIA laboratory in Baghdad.

H. Determination of Blood Parameters

All blood parameters were measured spectrophotometrically. Methods of determination were corresponded with commercial kits, and the manufactured companies are mentioned below. The following parameters were measured using biochemical analyzer system (ACCENT 200) to determine SG according to Trinder [17], SUN according to Crocker [18], SGOT and Serum SGPT according to Richmond [19].

I. Statistical Analysis

Data were statistically analyzed separately for Awassi lambs with 2×3 factorial experiment in completely randomized design (CRD) using ANOVA procedure of the SAS [20] to study effect of two Types of roughages (AH and WS) with two type of EFE (LEP And CEP) and without enzymes (Control) on rumen fermentation characteristics and blood parameters in Awassi lambs. Duncan's multiple range tests were used to determine the significance of differences between treatments means [21]. Analysis of variance was carried out on all data separately. The treatments were partitioned into main effects and their interaction using the following model:

$$Y_{ijk} = \mu + R_i + E_j + RE_{ij} + e_{ijk}$$

Where: Y_{ijk} = the response; μ = the overall mean; R_i = the effect of Source of roughage (i=1,2); E_j = the effect of Source of enzyme (j=1,2,3); RE_{ij} = the interaction Source of roughage $_i$ × Source of enzyme $_j$; e_{ijk} = the experimental error $_{ijk}$

III. Results and Discussion

A. Main effect of source of roughages on rumen fermentation characteristics

The main effects of source of roughages on rumen fermentation characteristics are present in table 3.

Results showed that increased ($p<0.01$) in acetic acid from 68.47 mmol/l to 72.16 mmol/l for AH and WS respectively. Probionic acid and TVFA were increased ($p<0.01$) with AH compared with WS. Statistical analysis showed there was no significant effect($p<0.01$) due to source of roughages on butyric acid, NH3-N and rumenal pH as shown in table 3.

Similar results showed by Bueno, et al [22] who found that addition of high doses of EFE (5 or 10 g of EFE kg^{-1} DM) to oat strow in lambs fed was not affected ($P>0.05$) in the pH and NH3-N.

B. Main effect of source of enzyme on rumen fermentation characteristics

The main effects of source of enzyme on rumen fermentation characteristics are present in table 2. Results showed that decreased ($p<0.01$) in acetic acid with LEP and CEP groupe (65.42 and 70.75 mmol/l respectively) compared with control groups (74.76 mmol/l) as shown in table 2. Dean, et al [23] suggested that the ruminal molar proportion of acetic acid was lower in cows fed TMR trated with Promote enzyme 4 g/head/d ($P<0.05$) and bermudagrass forage trated with Promote enzyme 4

g/head/d (P<0.01) than in those fed the control diet. This lower of acetic acid similarity the effects of supplementation with ionophores on ruminal energetic efficiency, and implies that less energy was wasted as CO2 and methane, and more net energy was available from the TMR trated with Promote enzyme diet. That's similar to the results in this study.

Regarding to Probionic acid and Butyric acid were increased(p<0.01) with LEP group (30.59 and 11.94 mmol/l respectively) and CEP group (29.19 and 14.66 mmol/l respectively) compared with control group (27.57 and 10.95 mmol/l respectively) as shown in table 4.7. the TVFA was increased (p<0.01) with CEP compared with LEP and control.While, the NH3-N and rumenal pH were not affected (p<0.01) with source of enzyme (Table 2).

This results similar to concluded of Dean, et al [23] who found that addition the EFE to bermudagrass forage was decreased the TVFA compared with control diet.similiar result reported by Arce-Cervantes, et al [24] who found the pH and NH3-N concentrations in the ruminal fluid were not affected by the enzymatic extracts(from the heat tolerant *basidiomycete sp.*).

C. Interaction effect between source of roughages and source of enzyme on rumen fermentation characteristics

The interaction effect between source of roughages and source of enzyme on rumen fermentation characteristics are present in table 2. Statistical analysis showed that acetic acid was decreased (p<0.01) with interaction between AH with LEP (R1E1) and AH with CEP (R1E2) comparid with them control (R1E3) . also acid was decreased (p<0.01) with interaction between WS with LEP (R2E1) and WS with CEP (R2E2) comparid with them control (R2E3) as shown in table 2. Regarding to Probionic acid, Butyric acid and TVFA were decreased (p<0.01) with interaction between AH with LEP (R1E1) and WS with LEP (R2E1) compared with them control (R1E3 and R2E3 respectively) . The NH3-N and rumenal pH were not affected (p<0.01) with interaction between source of roughages and source of enzyme for all treatments (Table 2).the butyric acid was increased (p<0.01) with intraction between CEP and AH (R1E2) compared with control group (R1E3) as showen in table 2. Similar results showed by Torres, et al [25] who found that addition extracts from C. flavigena and T. longibrachiatum increased (P<0.05) the percentage of butyric acid compared with the control group, While NH3-N and ruminal pH were not affected by exogenous enzymes. The EFE promote the populations of protozoa that produce butyrate [26], especially, *Dasytricha ruminantium* [27]. Considering that an improve in fiber digestibility, but not the associated production of butyric acid, improved the activity of the bacteria *Butyrivibrio fibrisolvens* [28, 29], this suggest another major micro-organisms that can improve butyric acid in the rumen [25].Similar result to this study, Mendoza, et al [30] concluded that the ruminal pH in sheep treated with the EFE was only 0.3 lower than the control group, which conclude that, the clinical acidosis were not affected by the addition of EFE to the feed.

Table 2: Main effect of source of roughages, source of enzyme and interaction between them on rumen fermentation characteristics

Factors	Acetic acid mmol/l	Probionic acid mmol/l	Butyric acid mmol/l	TVFA mmol/l	NH3-N Mg/dl	pH
Source of roughage (R)						
AH (R1)	**68.47±3.32b**	**30.12±1.98a**	**12.64±0.79a**	**125.64±7.24a**	**28.79±0.44a**	**6.71±0.04a**
WS (R2)	**72.16±1.19a**	**28.11±2.86b**	**12.38±0.88a**	**122.56±3.33b**	**28.50±0.77a**	**6.78±0.08a**
Sign.(N=6)	**	**	**NS**	**	**NS**	**NS**
Source of enzyme (E)						
LEP (E1)	**65.42±4.28c**	**30.59±3.79a**	**11.94±0.70b**	**117.94±8.73c**	**29.54±0.69a**	**6.76±0.09a**
CEP (E2)	**70.75±1.24b**	**29.19±3.06b**	**14.66±0.20a**	**127.77±6.36a**	**28.08±0.98a**	**6.83±0.09a**
control (E3)	**74.76±0.16a**	**27.57±2.46c**	**10.95±0.75c**	**126.57±5.04b**	**28.32±0.39a**	**6.64±0.02a**
Sign.(N=4)	**	**	**	**	**NS**	**NS**
Interaction between source of roughage and source of enzyme (RE)						
R_1E_1	**58.00±0.17e**	**24.03±0.01d**	**10.73±0.12e**	**102.81±0.31f**	**29.46±1.35a**	**6.83±0.03a**
R_1E_2	**72.91±0.21c**	**34.50±0.00b**	**14.98±0.16a**	**138.79±0.10a**	**28.19±0.50a**	**6.70±0.07a**
R_1E_3	**74.50±0.10b**	**31.83±0.17c**	**12.23±0.27d**	**135.31±0.37b**	**28.74±0.21a**	**6.61±0.01a**
R_2E_1	**72.85±0.05c**	**37.16±0.27a**	**13.15±0.14c**	**133.07±0.29c**	**29.63±1.03a**	**6.70±0.20a**
R_2E_2	**68.60±0.03d**	**23.87±0.13ed**	**14.34±0.12b**	**116.76±0.42e**	**27.98±2.35a**	**6.96±0.13a**
R_2E_3	**75.02±0.14a**	**23.31±0.30e**	**9.66±0.06f**	**117.84±0.24d**	**27.90±0.75a**	**6.68±0.05a**
Sign. (N=2)	**	**	**	**	**NS**	**NS**

a,b,c Column means for each item with unlike subscript letters different ($P < 0.01$), *: ($P<0.01$), NS: not significant,, R_1 and R_2 represent Source of roughage WS= wheat straw and AH=Alfalfa Hay. E_1, E_2 and E_3 represent Source of enzyme LEP= local enzyme product, CEP = commercial enzyme product and control= without enzyme.

D. Main effect of source of roughages on some blood parameters

The main effects of source of roughages on SUN, SGOT, SGPT, SG and SCH are present in table 3. Statistical analysis showed that there was no significant effect due to source of roughages on all blood parameters as shown in table 3. Peters, et al [31] reported that the supplementation of exogenous fibrolytic enzymes

(Roxazyme) to dairy cows diets not affected on blood parameters. Holtshausen, et al [32] reported that the exogenous fibrolytic enzymes treatment reduced plasma Blood hemoglobin concentration, indicating a positive effect of exogenous fibrolytic enzymes on body fat mobilization in early and mid-lactation cows.

E. Main effect of source of enzyme some blood parameters

The main effects of source of enzyme on SUN, SGOT, SGPT, SG and SCH are present in table 4. Results showed that SUN, SGOT, SGPT and SCH were not affected ($p<0.05$) by source of enzyme. But, The SG was increased ($p<0.05$) with LEP compared with CEP and control, while not significant effect ($p<0.05$) between CEP and control in SG as shown in table 3. Azzaz, et al [33] reported that the GOT activity was not affected in goats consuming diets treated with EFE (Asperozym and Bacillozym®), but there was a significant increase in GPT activity with goats consuming diets treated with EFE (Bacillozym®). Wahyuni, et al [34] explained that the increasing levels of EFE (0, 2, 4 and 6 g/kg) addition to goats ration was not affected in SUN concentration.

F. Interaction effect between source of roughages and source of enzyme on some blood parameters

The interaction effect between source of roughages and source of enzyme on SUN, SGOT, SGPT, SG and SCH are present in table 4. Statistical analysis showed that there was no significant effect due to the interaction between source of roughages and source of enzyme on SUN, SGOT, SGPT and SCH as shown in table 4.8. But the SG was increased ($p<0.01$) with interaction between AH with LEP (R1E1) compared with other treatments were not significant difference ($p<0.01$) among them as shown in table 3. Mahrous, et al [35] reported that the addition of EFE (ZAD) on corn cobs not affected on blood parameters (urea, total protein, albumin, globulin, GOT and GPT) compared to untreated corn cobs group.similar results reported by Gomaa, et al [36] who found the addition of EFE (ZAD) on Rice Straw with Grown Barely(RSGB) was not affected on blood parameters (urea,GOT and GPT) compared wit control group (untrated RSGB). Dean, et al [23] reported that addition of EFE (Promote enzyme 4 g/head/d) on bermudagrass forage had lower ($P<0.05$) SUN concentrations than those fed the control diet. They suggests that these treatments increased the efficiency of N utilization by ruminal microbes since N intake was similar among treatments.

Table 3 : Main effect of source of roughages, source of enzyme and interaction between them on some blood parameters

Factors	SUN (mg/dl)	SGOT (U/L)	SGPT (U/L)	SG (mg/dl)	SCH (mg/dl)
Source of roughage (R)					
AH (R1)	**41.94±1.75a**	**133.66±11.26a**	**24.94±2.01a**	**73.50±4.21a**	**48.93±4.21a**
WS (R2)	**36.83±2.28a**	**125.77±14.38a**	**28.88±2.11a**	**72.11±0.81a**	**42.51±4.79a**
Sign.(N=6)	**NS**	**NS**	**NS**	**NS**	**NS**
Source of enzyme (E)					
LEP (E1)	**36.41±3.19a**	**131.87±16.99a**	**29.25±2.74a**	**78.25±4.93a**	**47.54±7.31a**
CEP (E2)	**41.58±1.92a**	**146.57±17.66a**	**24.00±2.26a**	**70.16±1.97b**	**45.55±2.94a**
control (E3)	**40.16±2.93a**	**110.71±5.72a**	**27.50±2.79a**	**70.00±1.84b**	**44.07±6.92a**
Sign.(N=4)	**NS**	**NS**	**NS**	*****	**NS**
Interaction between source of roughage and source of enzyme (RE)					
R_1E_1	**40.00±3.00a**	**141.20±39.43a**	**27.16±3.83a**	**85.67±6.00a**	**59.02±6.95a**
R_1E_2	**41.83±4.50a**	**139.96±5.96a**	**21.16±3.16a**	**67.00±1.67b**	**43.18±5.28a**
R_1E_3	**44.00±3.00a**	**119.83±4.93a**	**26.50±3.83a**	**67.83±2.16b**	**44.58±6.01a**
R_2E_1	**32.83±5.16a**	**122.55±1.95a**	**31.33±4.66a**	**70.83±0.50b**	**36.06±3.03a**
R_2E_2	**41.33±1.33a**	**153.18±41.81a**	**26.83±2.16a**	**73.33±0.66b**	**47.91±3.61a**
R_2E_3	**36.33±3.66a**	**101.60±2.40a**	**28.50±5.50a**	**72.17±2.50b**	**43.56±15.83a**
Sign. (N=2)	**NS**	**NS**	**NS**	******	**NS**

a,b,c Column means for each item with unlike subscript letters different ($P < 0.05$), *: ($P<0.05$), ** ($P<0.01$) NS: not significant, R_1 and R_2 represent Source of roughage WS = wheat straw and AH = Alfalfa Hay. E_1, E_2 and E_3 represent Source of enzyme LEP = local enzyme product, CEP = commercial enzyme product and control= without enzyme

IV. Conclusions

It could be concluded from this study; treatment roughages with EFE decreased the molar proportions of acetic acid and increased the molar proportions of probionic acid, butyric acid compared with control.

References

[1] Hassan SA, Hashim AJ, Al-Samaraae WH. Effect of Physical and Chemical Treatment of Frond and Barley Straw on Chemical Coposition, Phenolic Compound Concentration and Anaerobic Bacteria. Jordan Journal of Agricultural Sciences. 2010;4(3).

[2] Hassan SA, Tawffek JA. Effect of washing and physical form of chemical treated barley straw on nutritive value,phenolic compound and activity of rumen bacteria. 1-Sodium hydroxide treatment. Iraqi Jof AgricSci. 2009;40(1):138-47.

[3] Sujani S, Seresinhe R. Exogenous Enzymes in Ruminant Nutrition: A Review. Asian Journal of Animal Sciences. 2015;9(3):85-99.
[4] McAllister T, Hristov A, Beauchemin K, Rode L, Cheng K. Enzymes in ruminant diets. Enzymes in farm animal nutrition Oxon: Cab International. 2001:273-97.
[5] Mendoza GD, Loera-Corral O, Plata-Pérez FX, Hernández-García PA, Ramírez-Mella M. Considerations on the use of exogenous fibrolytic enzymes to improve forage utilization. The Scientific World Journal. 2014;2014.
[6] Puniya AK, Singh R, Kamra DN. Rumen Microbiology: From Evolution to Revolution: Springer; 2015.
[7] Krause DO, Russell JB. An rRNA approach for assessing the role of obligate amino acid-fermenting bacteria in ruminal amino acid deamination. Applied and Environmental Microbiology. 1996;62(3):815-21.
[8] Varlyakov I, Grigorova N, Slavov T. EFFECT OF HOSTAZYM C 100 ON GROWTH PERFORMANCE AND SOME HEMATOLOGICAL AND ETHOLOGICAL INDEXES OF YEARLING RAMS. Bulgarian Journal of Agricultural Science. 2010;16(5):659-64.
[9] Hristov A, McAllister T, Cheng K. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: Effects on nutrient digestion in cattle fed a barley grain diet. Journal of Animal Science. 2000;78(2):477.
[10] Al-Wazeer AAM. Application of exogenous fibrolytic enzymes on the performance of Awassi lambs and Shami goats College of Agriculture,University of Baghdad (Ph.D. Dissertation); 2015.
[11] Kearl L. Nutrient requirements of ruminant in development contries. Logan: Utah State University. 1982.
[12] Saeed AA. Effect of level and degradability of dietary protein fed without bakers yeast (saccharomyces cerevisiae) on Turkish Awassi lamb's performance: College of Agriculture,University of Baghdad (Ph.D. Dissertation); 2011.
[13] Filípek J, Dvořák R. Determination of the volatile fatty acid content in the rumen liquid: comparison of gas chromatography and capillary isotachophoresis. Acta Veterinaria Brno. 2009;78(4):627-33.
[14] AOAC. (Association of Official Analytical Chemists).Official methods of analysis. 18th ed: AOAC International, Gaithersburg, Maryland, USA.; 2005.
[15] Warner A, editor Production of volatile fatty acids in the rumen: methods of measurement. Nutrition abstracts and reviews; 1964.
[16] Mathew S, Sagathevan S, Thomas J, Mathen G. AN HPLC METHOD FOR ESTIMATION OFVOLATILE FATTY ACIDS IN RUMINAL FLUID. Indian journal of animal sciences. 1997;67(9):805-7.
[17] Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry: An international journal of biochemistry in medicine. 1969;6(1):24-7.
[18] Crocker C. Rapid determination of urea nitrogen in serum or plasma without deproteinization. The American journal of medical technology. 1966;33(5):361-5.
[19] Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clinical chemistry. 1973;19(12):1350-6.
[20] SAS. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA. 2012.
[21] Duncan D. Multiple range and multiple F-tests. Biometrics 11, l-42. JMF Abreu, AM Bruno-Soares/Animal Feed Science Technology 70 (1998) 49-57 Sl. 1955.
[22] Bueno AL, Martínez G, García P, García J, Pérez F. Evaluation of High Doses of Exogenous Fibrolytic Enzymes in Lambs Fed an Oat Straw Based Ration#. Animal Nutrition and Feed Technology. 2013;13(3):355-62.
[23] Dean D, Staples C, Littell R, Kim S, Adesogan A. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. Animal Nutrition and Feed Technology. 2013;13(3):337-57.
[24] Arce-Cervantes O, Mendoza G, Hernández P, Meneses M, Torres-Salado N, Loera O. The effects of a lignocellulolytic extract of Fomes sp. EUM1 on the intake, digestibility, feed efficiency and growth of lambs. Animal Nutrition and Feed Technology. 2013;13(3):363-72.
[25] Torres N, Mendoza G, Bárcena R, Loera O, González S, Aranda E, et al. Effects of Various Fibrolytic Enzyme Extracts on Digestibility and Productive Performance of Lambs Fed a Forage-Based Diet#. Animal Nutrition and Feed Technology. 2013;13(3):381-9.
[26] Williams AG, Ellis AB, Coleman GS. Subcellular distribution of polysaccharide depolymerase and glycoside hydrolase enzymes in rumen ciliate protozoa. Current Microbiology. 1986;13(3):139-47.
[27] Yarlett N, Lloyd D, Williams A. Butyrate formation from glucose by the rumen protozoon Dasytricha ruminantium. Biochemical journal. 1985;228(1):187-92.
[28] Marounek M, Dušková D. Metabolism of pectin in rumen bacteria Butyrivibrio fibrisolvens and Prevotella ruminicola. Letters in applied microbiology. 1999;29 (6):429-33.
[29] Diez-Gonzalez F, Bond DR, Jennings E, Russell JB. Alternative schemes of butyrate production in Butyrivibrio fibrisolvens and their relationship to acetate utilization, lactate production, and phylogeny. Archives of microbiology. 1999;171(5):324-30.
[30] Mendoza G, Mota N, Plata F, Martínez J, Hernández P. Effects of exogenous glucoamylase from aspergillus niger and grain level on performance of lambs. Anim Nutr Feed Techn. 2013;13:391-8.
[31] Peters A, Meyer U, Dänicke S. Effect of exogenous fibrolytic enzymes on performance and blood profile in early and mid-lactation Holstein cows. Animal Nutrition. 2015;1(3):229-38.
[32] Holtshausen L, Chung Y-H, Gerardo-Cuervo H, Oba M, Beauchemin K. Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. Journal of dairy science. 2011;94(2):899-907.
[33] Azzaz H, Kholif A, Murad H, Hanfy M, Gawad M. Utilization of cellulolytic enzymes to improve the nutritive value of banana wastes and performance of lactating goats. Asian Journal of Animal and Veterinary Advances. 2012;7(8):664-73.
[34] Wahyuni RD, Ngampongsai W, Wattanachant C, Visessanguan W, Boonpayung S. Effects of enzyme levels in total mixed ration containing oil palm frond silage on intake, rumen fermentation, and growth performance of male goat. Songklanakarin Journal of Science & Technology. 2012;34(4):353-60.
[35] Mahrous M, El-Shafie M, Abd E-K, editors. TM, 2005. Effect of biological, chemical and chemic-biological treatments on the nutritive value of corn cubes. Animal Prod Res Inst, Second Conference; 2005.
[36] Gomaa R, Gado H, El-Sayed H, El Mawla SA. Usage of treated rice straw with exogenous anaerobic bacterial enzymes (ZAD) for Ossimi sheep. Annals of Agricultural Sciences. 2012;57(2):183-90.

AUTHORS' PROFILES

Shaker A. Hassan
Department of Animal production /College of Agriculture / University of Baghdad/Iraq.
Email: Shakeratar@yahoo.com

Yasseen A. Almaamory
Department of Animal production /College of Agriculture / University of Baghdad/Iraq.
Email: Yasalmaamory@yahoo.com