

UTILIZATION OF FIBEROLYTIC ENZYMES FOR IMPROVING DIGESTIBILITY IN ASSAF LAMBS FEED

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SUMMARY

The three sets of 12 Assaf lambs (4 each) feed the following diets: Control, which comprised of 15% alfalfa hay and 85% concentrate feed mixture, (CFM) (on DM basis); (T1): Control + 2 g fibrolytic enzymes/h/day; and (T2): Control + 4 g fibrolytic enzymes/h/day. The growth experiment went on for 28 weeks. Lambs were fed diets at levels of 3.5 % DM of body weight. The weight of animals was measured weekly before feeding at 8:00 a.m. to ascertain the average daily gain (ADG). Lambs were fasted for 12 hours before butcher, weighed just before slaughter (SW) and after complete lose blood from the body. Weights of cadaver separable fat, inward and outer offals and body fats were weighted and computed as percentage of body weight at slaughtering (SW). Blood serum specimen and rumin liquor were taken at the last day of treatment immediately before butchering from jugular vein from all lambs. The fundamental outcomes outlined as follows: Feed intake and body weight: There were no significant impact between lambs regarding feed intake, primary LBW and last LBW. Carcass traits: Dressing percentages A or B were not significantly influenced by fibrolytic enzymes treatment. Cadaver cuts: Lambs fed portions with low (T1) or high level of fibrolytic enzymes (T2) significantly raised ($P<0.05$) weights of shoulder, loin and neck than control in the diet, but did not significantly ($P>0.05$) influence weights of legs, rack, brisket and flank. Low and high level of fibrolytic enzymes reduced the percent of shoulder and rack than control, but raised the percent of loin, flank and neck than control. Edible offals: Lambs fed portions supplemented with fibrolytic enzymes (low level) (T1) significantly ($P<0.05$) raised the weights of liver, kidney, tests and heart than control. Anyway, high level of fibrolytic enzymes significantly ($P<0.05$) reduced kidney, testes and heart weight than control. Total edible offals weight was unrivalled with T1 followed by control, then T2. Non-edible parts: Lambs fed diets with high fibrolytic enzyme (T2) had higher pelt and empty gastro-intestinal part (GIP) than control group with significantly ($P<0.05$) differences. Notwithstanding, there were no significant differences ($P>0.05$) between the diverse sets relating to head, feets and GIP full. Rumen liquor: pH demonstrated insignificant ($P>0.05$) values by Assaf lambs fed on control, T1 and T2 diets. All ruminal pH values were above 6.0. Time of sampling had a significant impact ($P<0.05$) on rumen pH values. Total volatile fatty acids: TVFA's concentration demonstrated higher ($P<0.05$) values by Assaf lambs fed fibrolytic enzymes supplemented-diets compared with Assaf lambs fed control. $\text{NH}_3\text{-N}$ demonstrated significant ($P<0.05$) raise by Assaf lambs fed fibrolytic enzymes supplemented-diets compared with Assaf lambs fed control. Time of sampling had a significant impact ($P<0.05$) on rumen $\text{NH}_3\text{-N}$ with an opposite trend of pH values. Blood components: Total protein and its fractions: Supplementing lambs diets with low level of fibrolytic enzymes (T1) significantly ($P<0.05$) raised total protein and globulin contents than control or high fibrolytic enzymes level (T2). Albumin content reduced with high (T2) enzymes (T2) than the control or (T1) diets. Kidney functions (urea or creatinine), Liver functions (AST or ALT) or Lipids (triglycerides and cholesterol) and glucose metabolism: Treated rations did not significantly ($P>0.05$) affect its levels. Economical efficiency: was raised to be the highest with high fibrolytic enzymes level and was the lowest with control. From the previous results, it could be concluded that supplementation of fibrolytic enzymes as 2 or 4g/h/d enhanced productive performance of Assaf lambs, but it was the highest with high level of fibrolytic enzymes supplementation than low fibrolytic enzymes level.

Key words: *fibrolytic enzymes, growing Assaf lambs, rumin, blood serum and economical efficiency.*

INTRODUCTION

There is an intense lack of traditional feed materials for animal nutrition in Palestine. The huge feed hole between the necessities and the accessible sources constrained the organizers and nutritionists to search for non-traditional resources where there is no vying with persons, such as agricultural by-

products which accessible around the year however are not effectively utilized or others is one of this agro-industrial by products.

Titi and Tabbaa (2004) have studied the efficiency of immediate feeding a cellulase enzyme on lamb diets digestibility. The researchers came to conclude that cellulase enzyme raised dry matter and organic matter digestibilities of treated lambs compared to those of control. A comparable pattern was watched for the crude fiber, NDF and ADF digestibility coefficients. Furthermore, Abdel-Gawad *et al.* (2007) assessed the impacts of fibrolytic enzymes on the *in vivo* nutrient digestibilities by sheep for corn stalks, wheat straw, rice straw and sugarcane bagasse. The researchers found that fibrolytic enzymes supplementation significantly raised digestibilities of DM, OM, CP, NFE, CF and hemicellulose of all roughage compared with control.

Pinos-Rodríguez *et al.* (2002) have researched the impact of an immediately fed exogenous fibrolytic enzyme on intake and digestion by sheep. The diets were alfalfa hay, alfalfa hay in addition to exogenous fibrolytic enzymes, ryegrass hay and ryegrass hay in addition to enzymes. The enzymes raised clear digestibility of CP, hemicellulose and NDF for alfalfa diet compared with the other diets.

Titi and Tabbaa (2004) explored the efficiency of immediate feeding of a cellulase enzyme on lamb diets digestibility. Outcomes demonstrated that cellulase enzyme raised ($P < 0.05$) dry matter and organic matter digestibilities of treated lambs compared to those of control. A comparative pattern was watched for the crude fiber, NDF and ADF digestibility coefficients. Also, no distinctions were seen in crude protein digestibility between treated and control lambs.

In 2007, Abdel-Gawad *et al.* estimated the impacts of fibrolytic enzymes consisting of xylanase and cellulase on the *in vivo* nutrient digestibilities for corn stalks, wheat straw, rice straw and sugarcane bagasse. Also, the researchers observed that fibrolytic enzymes supplementation increased ($P < 0.05$) digestibilities of DM, OM, CP, NFE, CF and hemicellulose of wheat straw compared with control (berseem hay), while ADL was significantly ($P < 0.05$) higher for wheat straw than the control and corn stalks.

Gado *et al.* (2007) examined the impact of biological treatments (cellulase; rumen liquor and *Cellulomonas cellulasea*) of bagasse to enhance the performance of Baladi lambs. The researchers showed that treated bagasse with various treatments had a significant positive impact on DM and CP digestibilities. Moreover, cellulase enzyme raised ($P < 0.05$) the percent of DM digestibility coefficient when compared with the other treatments. Digestibility of OM, EE, NFE positively influenced by cellulase treatment.

Muwalla *et al.* (2007) investigated the impact of fibrolytic enzyme inclusion on nutrient digestibility of Awassi lamb fed on a high concentrate diet. They noted that DM, OM, CP, and NDF digestibilities were unaffected by the enzyme inclusion.

The current research intends to study the impact of adding cellulolytic and pectolytic enzymes to diets of Assaf lambs on its productive performance.

MATERIAL AND METHODS

The present experiment was conducted in a private sheep farm in Khan Younes at Jabalia, South governorate, Gaza sector, Palestine, during the period of 1st March 2013 to the end of October 2013. This work aimed to study the effect of adding cellulolytic and pectolytic enzymes to diets of Assaf lambs on its productive performance.

Growth trial

Twelve male Assaf lambs (5/8 Awassi x 3/8 East Frisian) aging about 6 months with average weight of 26.61 kg were used in this experiment. Lambs were randomly divided into three experimental groups (4 lambs of each). Average initial live body weights of animals were 26.775 ± 1.25 kg, 26.675 ± 1.23 kg, and 26.376 ± 1.23 kg for control, T1 and T2 groups, respectively. The dietary treatments were control; control + 2.0 g fibrolytic enzyme/h/day (T1); and control + 4.0 g fibrolytic enzyme /h/day (T2). The control diet consisted of 15% alfalfa hay and 85% concentrate feed mixture, on DM basis. Fibrolytic enzyme which imported from Japan (Tomoko® is a commercial enzymes source of Biogenkoji Research Institute – Japan) was used. The enzyme product was made from *Aspergillus awamori* (3×10^6 cells/g) including 1000 unit/g of acidic protease, 30 unit/g of pectinase, 25 unit/g of xylanase, 20 unit/g of α -

amylase, 10 unit/g of phytase, 5 unit/g of glucoamylase and 4 unit/g of cellulase as provided from the manufacture) was obtained from Egypt. Fibrolytic enzyme was added to a portion of wheat bran then mixed thoroughly with the other concentrate ingredients. The growth trial lasted for 24 weeks. Lambs were fed at levels of 3.5 % DM of body weight. Chemical compositions of the ingredients are shown in Table (1). Animals were fed individually twice a day at 8:00 a.m. and 4:00 p.m. Fresh water was available all the time round. Feed intake and body weight changes of the lambs were recorded every two weeks during the experimental period.

Table (1): Chemical composition of feed ingredients and control diet.

| Item | DM** | DM basis % | | | | | |
|---------------|------|------------|------|------|-----|------|------|
| | | OM | CF | CP | EE | NFE | Ash |
| CFM | 90.3 | 93.5 | 11.1 | 16.0 | 4.3 | 62.1 | 6.5 |
| Alfalfa hay | 88.3 | 89.6 | 30.0 | 12.6 | 2.7 | 44.3 | 10.4 |
| Control diet* | 90.0 | 92.9 | 13.9 | 15.5 | 4.1 | 59.3 | 7.1 |

CFM: Concentrate feed mixture consisted of 49% maize, 24% wheat bran, 12% barley, 12% soybean meal, 2% limestone and 1% sodium chloride. * Calculated. ** (DM) dry matter, (OM) organic matter, (CF) crude fiber, (CP) crude protein, (EE) ether extract, (NFE) nitrogen free extract.

Slaughter technique

Animals were fasted for 12 hours before slaughter, which was performed according to the Islamic rules. Animals were weighed just before slaughter and after complete bleeding. Head, skin and feet were separated and weighed. Internal organs and offals (heart, lungs, liver, testes, spleen, kidneys and digestive tract) were removed and individually weighed. Gastro-intestinal tract full and empty weights were recorded. Fat tail of sheep was removed, weighed along with omentum, intestinal and kidney fats. Weights of carcass knife separable fat, internal and external offals and body fats were calculated as percentage of body weight at slaughtering (SW).

Rumen liquor sampling

At the last day of each experimental period, rumen liquor samples were collected by stomach tube from each animal at zero, 3 and 6 hrs post-feeding of the ration. Samples were strained through two layers of cheese cloth and immediately used for determination of ruminal pH using digital pH-meter. Rumen liquor samples were stored in glass bottles with drops of toluene and thin layer of paraffin oil and stored in a deep freeze (-18°C) for analysis of ammonia nitrogen (NH₃-N), and total volatile fatty acids (TVFA's).

Blood sampling

Blood samples were taken at the final day of treatment directly before slaughtering from jugular vein from all animals. Collected blood samples were centrifuged at 4000 r.p.m. for 20 min. and the supernatant was stored in glass vials at -18°C till analysis.

Methods of analysis

Dry matter (DM), crude protein (CP), ether extracts (EE), crude fiber (CF) and ash of feed ingredient samples were analyzed according to A.O.A.C. (1995). Nitrogen free extract (NFE) was calculated by the difference. Values of rumen pH were determined using Hanna digital pH meter. The concentration of ammonia-nitrogen in the rumen liquor was determined by Kjeldahl distillation method (A.O.A.C., 1995). While rumen total volatile fatty acids were determined by steam distillation method as described by Warner (1964). Serum total protein was measured calorimetrically by the biuret reaction according to Gornal *et al.* (1949). Serum albumin was determined by calorimetric method as described by Doumas *et al.* (1971). Serum globulin was calculated by subtracting the values of albumin from corresponding value of total protein for each sample. The A/G ratio was calculated by dividing the albumin value of each sample by its corresponding globulin value. Serum glucose was determined calorimetrically according to Trinder (1969). Serum alanine transferase (ALT) and aspartate transfrase (AST) were determined calorimetrically according to the method of Reitman and Frankel (1957). Serum urea was determined calorimetrically according to Fawcett and Scott (1960). Serum total cholesterol was determined calorimetrically according to Allain *et al.* (1974).

Economical evaluation

The relation between feed costs and live body gain was calculated for the different experimental animal groups. The general equation by which the costs of each one kg of live body weight gain was calculated as follow: The cost for one kg gain = Total costs of feed intake US\$ / Total gain (kg).

$$\text{Economic Efficiency} = \text{Total gain price} / \text{Total gain cost.}$$

Statistical analysis

Data were statistically analyzed according to SAS (2000). The differences among groups were estimated using the general linear model (GLM) procedures. One way ANOVA procedure used to analyze the data of the main effects on growth, carcass traits, meat quality and blood serum constituents according the following model:

$$Y_{ij} = \mu + Z_i + E_{ij}$$

Where: μ = general mean, Z_i = effect of treatment, E_{ij} = experimental error. Significance was tested at ($P < 0.05$) for all means separation according to Duncan (1955).

RESULTS AND DISCUSSION

Growth performance

The impact of dietary fibrolytic enzyme levels on live body weight (LBW) can be discovered via comparing the outcomes of control diet (zero fibrolytic enzyme added) with those of low fibrolytic enzyme (T1, 2.0 g/h/d) or high fibrolytic enzyme (T2, 4.0 g/h/d). Table (2) demonstrated that there was no significant impact between lambs relating to feed intake. Also, the high level of fibrolytic enzyme supplementation insignificantly ($P > 0.05$) increased feed intake. Additionally, Table (2) demonstrated that there was no significant impact between lambs relating to initial LBW and last LBW. Moreover, treatments with fibrolytic enzymes indicated insignificantly raises ($P > 0.05$) in total LBW gain. Daily LBW gain insignificantly raised ($P > 0.05$) only with high level (T2) of fibrolytic enzymes supplementation.

Table (2): Effect of fibrolytic enzymes level on live body weight (LBW) gain and feed intake of Assaf lambs.

Dissimilar superscripts at the same row mean significant differences ($P < 0.05$).

| Parameter | Treatments | | | | | |
|---------------------|---------------------|------|---------------------|------|---------------------|------|
| | Control | | T1 | | T2 | |
| | Mean | SD | Mean | SD | Mean | SD |
| Initial LBW (kg) | 26.775 ^a | 1.33 | 26.675 ^a | 0.76 | 26.375 ^a | 1.26 |
| Final LBW (kg) | 70.00 ^a | 2.97 | 70.25 ^a | 3.25 | 72.70 ^a | 5.19 |
| Total LBW gain (kg) | 43.225 ^a | 3.12 | 43.575 ^a | 2.02 | 46.325 ^a | 1.88 |
| Daily LBW gain (g) | 237.5 ^a | 21.4 | 239.4 ^a | 17.0 | 254.5 ^a | 19.8 |
| Total intake (kg) | 342.5 | - | 345.9 | - | 352.1 | - |

- T1= 2 g fibolytic enzyme/h/day, T2=4 g fibolytic enzyme /h/day

- Data was calculated on 182 treatment-days basis.

Bhat (2000); Saber *et al.* (2010) pointed that three types of cellulase enzymes are associated with the cellulase hydrolysis operation including cellobiohydrolase, endoglucanase or carboxy methylcellulase (CMC) and β -glucosidases.

Lynd *et al.* (2002); and Zhang and Lynd (2004) indicated that the widely acceptable mechanism for enzymatic cellulose hydrolysis includes synergistic actions by endoglucanase, exoglucanase or cellobiohydrolase and β -glucosidase.

Carcass traits

Data relating with the carcass traits of the growing Assaf lambs fed the experimental rations condifferent levels of fibrolytic enzymes are illustrated in Tables (3-6).

Dressing percentage:

Table (3) provides a fasting weight, empty body weight, dressing (A) and (B) percent (based on fasting weight and empty body weight, respectively). Lambs fed rations with fibrolytic enzymes supplementation (T1 and T2) had higher fasting and empty body weight than those fed control ration, but the differences were not significant ($P>0.05$). These outcomes showed that fibrolytic enzymes supplementation to rations raised fasting weight by about 0.35 and 3.85%, and raised empty body weight by 0.04 and 3.73% for T1 and T2, respectively. Dressing percentages, A or B were not significantly influenced ($P>0.05$) by the dietary treatment

Table (3): Effect of dietary fibrolytic enzymes levels on fasting weight, empty body weight and dressing percentages of Assaf lambs.

| Parameter | Treatments | | | | | |
|--------------------------|------------|------|-------|------|-------|------|
| | Control | | T1 | | T2 | |
| | Mean | SD | Mean | SD | Mean | SD |
| Fasting weight (kg) | 70.00 | 5.64 | 70.25 | 3.96 | 72.70 | 3.24 |
| Digestive tract wt. (kg) | 14.55 | 0.95 | 14.76 | 0.82 | 15.18 | 0.65 |
| Empty body weight* (kg) | 55.45 | | 55.47 | | 57.52 | |
| Hot carcass weight (kg) | 35.44 | 3.08 | 35.68 | 1.88 | 36.10 | 1.87 |
| Dressing A (%)** | 50.63 | | 50.08 | | 49.66 | |
| Dressing B (%)*** | 63.91 | | 64.68 | | 62.76 | |

Dissimilar superscripts at the same row mean significant differences ($P<0.05$).

T1= 2 g fibrolytic enzymes/h/day, T2=4 g fibrolytic enzymes/h/day

** Empty body weight = Fasting weight – Digestive tract weight*

***Dressing % =Hot cacass weight relative to body weight*

**** Dressing % =Hot cacass weight relative to empty body weight*

Researches on goats (Atti, *et al.* 2004) and lambs (Rocha, *et al.* 2004; and Woolley *et al.* 2005) utilizing different levels of crude protein in diets showed the similar pattern. Moreover, the outcomes of Malcolm-Callis, *et al* (2000) and Spears and Kegley (2002) on steers; and McBeth, *et al.* (2002) and Shaeffer (2006) on cattle, utilizing 20-30 mg Zn / kg DM in rations did not influence cadaver weight or dressing percentage. Furthermore, outcomes. of El-Shanti *et al.* (2012) on Assaf lambs confirm these outcomes.

Carcass cuts:

Shoulder, legs, loin, neck, rack, brisket and flank weights of carcass weight are illustrated in Table (4).

Outcomes in Table (4) presented that supplementing rations with low (T1) or high level of fibrolytic enzymes (T2) significantly raised ($P<0.05$) weights of shoulder, loin and neck than control in the diet, but did not significantly ($P>0.05$) influence weights of legs, rack, brisket and flank. The outcomes of El-Shanti *et al.* (2012) on Assaf lambs agreed with these results.

Table (4): Effect of dietary fibrolytic enzymes levels on carcass cuts of Assaf lambs.

| Parameter | Treatments | | | | | |
|--------------------------|-------------------|------|--------------------|------|--------------------|------|
| | Control | | T1 | | T2 | |
| | Mean | SD | Mean | SD | Mean | SD |
| Hot carcass weight (kg) | 35.44 | 3.08 | 35.68 | 1.88 | 36.10 | 1.87 |
| Carcass cuts weight (kg) | | | | | | |
| Shoulder | 7.75 ^b | 0.39 | 8.15 ^a | 0.36 | 7.95 ^{ab} | 0.44 |
| Legs | 1.22 ^a | 0.13 | 1.34 ^a | 0.06 | 1.38 ^a | 0.08 |
| Loin | 8.00 ^b | 0.55 | 8.74 ^{ab} | .052 | 8.89 ^a | 0.68 |
| Neck | 2.66 ^b | 0.33 | 3.30 ^a | 0.26 | 3.11 ^a | 0.49 |
| Rack | 8.35 ^a | 0.52 | 8.15 ^a | 0.87 | 8.22 ^a | 0.97 |
| Brisket | 6.24 ^b | 0.42 | 6.33 ^b | 0.39 | 6.80 ^a | 0.88 |
| Flank | 2.05 ^a | 0.11 | 2.16 ^a | 0.23 | 2.31 ^a | 0.29 |

* Empty body weight = Fasting weight – Digestive tract weight

- Dissimilar superscripts at the same row mean significant differences ($P < 0.05$).

T1= 2 g fibrolytic enzymes/h/day, T2=4 g fibrolytic enzymes/h/day

Edible offals:

Table (5) shows liver, kidney, testes, spleen, heart, lungs and trachea weights and percentages of carcass weight. The outcomes demonstrated that low level of fibrolytic enzymes supplementation (T1) significantly ($P < 0.05$) raised the weights of liver, kidney, tests and heart than control. Also, high level of fibrolytic enzymes significantly ($P < 0.05$) reduced kidney testes and heart weight than control. Total edible offals weight was superior with T1 followed by control, then T2. A similar pattern was noted with edible offals percentage (relative to empty body weight), except with testes, which demonstrated higher percent with low level of fibrolytic enzymes supplementation followed by control then high level of fibrolytic enzymes supplementation. It is important to take note of that the total edible offals percentage was most elevated with low level of fibrolytic enzymes supplementation (T1), followed by control, then high level of fibrolytic enzymes (T2). The causes of this pattern are not clear.

Table (5): Effect of dietary fibrolytic enzymes levels on edible offals (weight) of Assaf lambs.

| Parameter | Treatments | | | | | |
|----------------------------|--------------------|-------|-------------------|-------|--------------------|-------|
| | Control | | T1 | | T2 | |
| | Mean | SD | Mean | SD | Mean | SD |
| Empty body weight* (kg) | 55.45 | -- | 55.47 | -- | 57.52 | -- |
| Edible offals weights (kg) | | | | | | |
| Liver | 1.16 ^b | 0.08 | 1.29 ^a | 0.12 | 1.24 ^{ab} | 0.11 |
| Kidney | 0.19 ^a | 0.042 | 0.19 ^a | 0.005 | 0.16 ^b | 0.025 |
| Testes | 0.86 ^b | 0.11 | 1.15 ^a | 0.023 | 0.71 ^c | 0.06 |
| Spleen | 0.08 ^b | 0.014 | 0.08 ^b | 0.002 | 0.10 ^a | 0.02 |
| Heart | 0.40 ^{ab} | 0.026 | 0.43 ^a | 0.05 | 0.33 ^b | 0.08 |
| Lungs and Trachea | 0.84 ^a | 0.13 | 0.84 ^a | 0.11 | 0.84 ^a | 0.10 |
| Total edible offal weights | 3.53 | -- | 3.98 | -- | 3.38 | -- |

* Empty body weight = Fasting weight – Digestive tract weight

- Dissimilar superscripts at the same row mean significant differences ($P < 0.05$).

T1= 2 g fibrolytic enzymes/h/day, T2=4 g fibrolytic enzymes/h/day

Non-edible parts:

Non-edible parts (weight) of butcher weight are appeared in Table (6). Lambs fed diets with high fibrolytic enzyme (T2) had higher pelt and empty gastro-intestinal part (GIP) than control group with significantly ($P < 0.05$) differences. Also, there were no significant differences ($P > 0.05$) between the different groups concerning head, feet and GIP full. These outcomes concurred with those prominent by El-Shanti et al. (2012) on Assaf lambs affirm these outcomes. These results, likewise, concurred with

those prominent by Atti, *et al.* (2004) that there were no significant differences between groups treated with various proportions of crude protein or zinc concentrations. The weight of pelt and the other components, which are rich in bone and had a low metabolic activity (head and four feet) were differed somewhat with ration. Because these organs are early maturing parts (Wallace, 1948) and thus they are less influenced by dietary in growing animals (Kamalzadch, *et al.* 1998).

Table (6): Effect of dietary fibrolytic enzymes levels on non-edible offals (weight) of Assaf lambs.

| Parameter | Treatments | | | | | |
|------------------------------|-------------------|------|-------------------|------|-------------------|------|
| | Control | | T1 | | T2 | |
| | Mean | SD | Mean | SD | Mean | SD |
| Empty body weight* (kg) | 55.45 | -- | 55.47 | -- | 57.52 | -- |
| Fasting weight (kg) | 70.00 | 5.64 | 70.25 | 3.96 | 72.70 | 3.24 |
| Non-edible cuts weights (kg) | | | | | | |
| Pelt | 6.09 ^b | 0.31 | 6.01 ^b | 0.38 | 6.82 ^a | 0.67 |
| Head | 3.11 | 0.37 | 2.98 | 0.23 | 3.38 | 0.09 |
| Four feet (legs) | 1.22 | 0.13 | 1.34 | 0.06 | 1.38 | 0.08 |
| GIP full | 14.55 | 0.95 | 14.76 | 0.82 | 15.18 | 0.65 |
| GIP empty | 3.23 ^b | 0.13 | 3.56 ^a | 0.14 | 3.63 ^a | 0.42 |

* Empty body weight = Fasting weight – Digestive tract weight

- Dissimilar superscripts at the same row mean significant differences ($P < 0.05$).

T1= 2 g fibrolytic enzymes/h/day, T2=4 g fibrolytic enzymes/h/day

Rumen liquor

Rumen liquor parameters are imperative indicators of rumen environment, microbial activity and subsequently rumen metabolism. The most indicative parameters decided in this research were rumen pH, rumen total volatile fatty acids (TVFA's) and rumen ammonia nitrogen ($\text{NH}_3\text{-N}$).

Ruminal pH

Ruminal pH showed insignificant ($P > 0.05$) values by Assaf lambs fed on control, T1 and T2 diets (see Table, 7). All ruminal pH values were above 6.0 which indicate a better digestion of cellulolytic materials (Mertens, 1978). These findings might be due to the intensive fermentation operation of both nonstructural and structural carbohydrates and the production of volatile fatty acids.

Table (7): Ruminal pH of the experimental Assaf lambs (Mean±SD).

| Experimental diets | Sampling Time | | | Overall Mean | ± SD |
|--------------------|-------------------|-------------------|-------------------|-------------------|------|
| | Pre feeding | Post feeding | | | |
| | Zero | 3 hrs | 6 hrs | | |
| Control | 7.05 | 6.58 | 6.83 | 6.82 ^a | 0.06 |
| T ₁ | 7.15 | 6.55 | 6.88 | 6.86 ^a | 0.09 |
| T ₂ | 7.05 | 6.50 | 6.85 | 6.80 ^a | 0.08 |
| Overall Mean | 7.08 ^a | 6.54 ^c | 6.85 ^b | | |
| ± SD | 0.085 | 0.086 | 0.087 | | |

^{a,b,c}: Means designated with the same letter within the same row (column) are not significantly different at ($P < 0.0$

Khattab *et al.* (1996) and Azzaz (2009) reported that fibrolytic enzymes treatment significantly reduced ruminal pH.

Table (7) shows the mean values of ruminal pH in the different experimental diets during the different sampling times of Assaf lambs. The average pH values at zero time (before feeding) were 7.05, 7.15 and 7.05 for control diet, T₁ and T₂, respectively. Then, the pH values were progressively reduced to reach minimal values at 3 hrs post feeding, being 6.58, 6.55 and 6.50 for the expected diets orderly. At 6 hrs post feeding, the pH values were raised to reach 6.83, 6.88 and 6.85 in the same respective order. These outcomes demonstrated that time of sampling had a significant impact (P<0.05) on rumen pH values.

Mean values of ruminal pH in the different experimental diets through the different sampling times of lactating Assaf lambs are shown in Table (7). The average pH values at zero time (before feeding) were 7.05, 7.15 and 7.05 for control diet, T₁ and T₂, respectively. Then, the pH values were gradually decreased to reach minimum values at 3 hrs post feeding, being 6.58, 6.55 and 6.50 for the expected diets orderly. At 6 hrs post feeding, the pH values were increased to reach 6.83, 6.88 and 6.85 in the same respective order. These results indicated that time of sampling had a significant effect (P<0.05) on rumen pH values.

Ruminal total volatile fatty acids

Table (8) presents ruminal total volatile fatty acids (TVFA's) concentration demonstrated higher (P<0.05) values by Assaf lambs fed (T₂) and (T₁) diets being 11.68 and 11.02 meq/dl, respectively than those fed control diet (9.42 meq/dl).

Shafie and Ashour (1997) observed that the raise of TVFA's value were 23.99 and 16.98 % for T₂ and T₁, respectively compared with control. The pattern of TVFA's values reflects the pattern of fermentation activity in the rumen).

According to Lewis *et al.* (1996) and Azzaz (2009) fibrolytic enzymes treatment significantly reduced ruminal pH and raised TVFA's concentration in the rumen.

Table (8) illustrates mean values of ruminal total volatile fatty acids (TVFA's) in the Assaf lambs fed the different experimental diets during the different sampling times. The results demonstrate the mean values of total volatile fatty acids at zero time were 7.24, 8.45 and 9.05 meq/dl for control, T₁ and T₂, respectively. Maximum values of TVFA's were recorded at 3 hrs post feeding, being 11.23, 13.18 and 14.49 meq/dl for control, T₁ and T₂, respectively. while, at 6 hours post feeding, it was reduced to reach 9.78, 10.81 and 11.50 in the same respective order.

By and large, the data got from this research showed that TVFA's concentration was conversely related with pH value. Comparable results were noted by Giacomini *et al.* (1985); Burrin and Britton (1986); Taie (1993) and Baraghit *et al.* (1995). The researchers pointed out that the progress of raising ruminal TVFA's concentration paralleled with a decrease in ruminal pH.

Hume *et al.* (1970); Walker and Nader (1970) and Mathison and Milligan (1971) indicated that the energetic of rumen microbial production is related to the fermentable materials degraded to TVFA's by the rumen microorganisms through digestion.

Table (8):Ruminal total volatile fatty acids concentration (meq/dl) of the experimental Assaf lambs.

| Experimental diets | Sampling Time | | | Overall Mean | ± SE |
|--------------------|-------------------|--------------------|--------------------|--------------------|------|
| | Pre feeding | Post feeding | | | |
| | Zero | 3 hrs | 6 hrs | | |
| Control | 7.24 | 11.23 | 9.78 | 9.42 ^b | 0.46 |
| T ₁ | 8.45 | 13.18 | 10.81 | 11.02 ^a | 0.53 |
| T ₂ | 9.05 | 14.49 | 11.50 | 11.68 ^a | 0.51 |
| Overall Mean | 8.25 ^c | 13.18 ^a | 10.70 ^b | | |
| ± SE | 0.22 | 0.52 | 0.27 | | |

^{a,b,c}:Means designated with the same letter within the same row (column) are not significantly different at (P<0.05).

Ruminal ammonia nitrogen (NH₃-N) concentration

According to Table (9), ruminal ammonia nitrogen (NH₃-N) showed significant (P<0.05) increment by Assaf lambs fed (T₂) and (T₁) diets compared with Assaf lambs fed control diet. The increment of rumen ammonia nitrogen concentration with the fibrolytic enzymes treatments may be due to higher CP digestibility and higher fermentation rate in fibrolytic enzymes treated diets. Khorshed (2000) watched that ruminal ammonia-N increment in rumen of sheep and lambs when fed on rations treated with biological treatments.

Table (9) provides the mean values of ruminal NH₃-N in the different experimental diets during the different sampling times of Assaf lambs. The means of NH₃-N concentration at zero time among the different treatment were 13.01, 16.64 and 16.99 mg/dl for control, T₁, and T₂, respectively. The NH₃-N concentrations were raised at 3 hrs post feeding to be 22.38, 25.02 and 25.71 mg/dl, then it was reduced after 6 hours to reach 18.72, 20.93 and 21.06 in the same respective order.

The differences in the mean values of NH₃-N among the different sampling times were significant (P<0.05). The lowest values (P<0.05) was recorded at zero time (before feeding) (being 15.54 mg/dl) and then values raised (P<0.05) to reach the highest value at 3 h post feeding (being 24.37 mg/dl) and then reduced (P<0.05) to reach 20.24 mg/dl at 6 h post feeding. This pattern concurred with the pattern on goats got by El-Ashry *et al.* (1988).

Table (9): Ruminal ammonia nitrogen concentration (mg/dl) of the experimental Assaf lambs.

| Experimental diets | Sampling Time | | | Overall Mean | ± SE |
|--------------------|--------------------|--------------------|--------------------|--------------------|------|
| | Pre feeding | Post feeding | | | |
| | Zero | 3 hrs | 6 hrs | | |
| Control | 13.01 | 22.38 | 18.72 | 18.04 ^b | 1.07 |
| T ₁ | 16.64 | 25.02 | 20.93 | 20.86 ^a | 0.92 |
| T ₂ | 16.99 | 25.71 | 21.06 | 21.25 ^a | 1.38 |
| Overall Mean | 15.54 ^c | 24.37 ^a | 20.24 ^b | | |
| ± SE | 0.79 | 1.06 | 0.93 | | |

^{a,b,c}: Means designated with the same letter within the same row (column) are not significantly different at (P<0.05).

Blood components

Biochemical parameters of blood serum have been used as indicators of the nutritional and physiological status of growing lambs. The results of blood serum constituents in Assaf lambs fed the tested diets are presented in Table (10).

Total protein and its fractions:

Table (10) shows the overall means of serum total protein, albumin and globulin concentrations, and albumin/globulin ratio. The results indicate that the supplementing lambs diets with low level of fibrolytic enzymes (T1) significantly raised (P<0.05) total protein content than control or high fibrolytic enzymes level (T2).

In any case, albumin, globulin and albumin/globulin ratio demonstrated an insignificant pattern (P>0.05). At the same time, albumin content reduced with high level of fibrolytic enzymes (T2) than the control or low level (T1) diets. This pattern could be attributed to the change in blood serum globulin content.

Omole (1982) indicated that the increment in serum total protein content may be associated with the increment in blood albumin concentration. Moreover, Habeeb, *et al.* (1989) indicated that the increment of total protein may be associated with the increment of thyroxin production, which stimulates the protein synthesis. Moreover, Freeman (1983) observed that zinc may play essential role in activation of some enzymes that are responsible of use of amino acids in protein synthesis and other physiological functions associated with metabolic rate. In addition, it is realized that the change in albumin level reflects the change in liver function. The diminishment in albumin synthesis when rations supplemented with fibrolytic enzymes (T1 and T2) could be associated with depression in anabolic hormonal secretion such

as thyroxin (El-Masry, 1987) and insulin (Habeeb, 1987) or to the raising in catabolic hormones such as glucocorticoids (Alvarez and Johnson, 1970).

Despite the changes in serum total protein and its fractions are significant between treatments, the data were within the normal range.

Table (10): Blood serum parameters of Assaf lambs fed rations supplemented with low or high level of fibrolytic enzymes.

| Parameter | Treatments | | | | | |
|----------------------|-------------------|-------|-------------------|-------|-------------------|-------|
| | Control | | T1 | | T2 | |
| | Mean | SD | Mean | SD | Mean | SD |
| Total protein (g/dl) | 6.30 ^b | ±0.47 | 7.20 ^a | ±0.56 | 6.30 ^b | ±0.49 |
| Albumin (g/dl) | 3.80 | ±0.44 | 3.80 | ±0.38 | 3.30 | ±0.41 |
| Globulin (g/dl) | 2.50 | ±0.28 | 3.50 | ±0.32 | 3.00 | ±0.38 |
| A / G ratio | 1.52 | ±0.85 | 1.09 | ±0.60 | 1.10 | ±0.75 |
| Glucose (mg/dl) | 68.33 | ±4.48 | 63.33 | ±3.28 | 65.67 | ±4.37 |
| Urea (mg/dl) | 35.00 | ±1.07 | 37.00 | ±1.22 | 37.33 | ±1.28 |
| Creatinine (mg/dl) | 0.78 | ±0.26 | 0.87 | ±0.35 | 0.88 | ±0.21 |
| AST (u/l) | 111.0 | ±1.26 | 141.0 | ±1.45 | 116.0 | ±1.32 |
| ALT (u/l) | 15.33 | ±0.62 | 16.00 | ±0.73 | 19.67 | ±0.87 |
| TG (mg/dl) | 110.0 | ±2.53 | 117.0 | ±2.59 | 114.0 | ±2.50 |
| Cholesterol (mg/dl) | 87.67 | ±1.46 | 125.00 | ±1.54 | 90.00 | ±1.22 |

Dissimilar superscripts at the same row mean significant differences (P<0.05).

T1= 2 g fibrolytic enzymes/h/day, T2=4 g fibrolytic enzymes/h/day

Kidney functions:

The impact of supplementing rations with different levels of fibrolytic enzymes on blood serum urea and creatinine as indicators of kidney functions of growing Assaf lambs are shown in Table (10). The results indicate that treated rations did not significantly (P>0.05) affect urea level in the blood serum of lambs.

Thomas et al. (1988) reported that the serum creatinine gradually raised (P>0.05) with raising fibrolytic enzymes in lambs rations. Unaffected of serum urea due to fibrolytic enzymes supplementation to lambs' rations reflect the fact that the raise in blood urea nitrogen comes from raising dietary crude protein level. A raise in rumen ammonia absorption resulting in greater amounts of ammonia being utilized in liver to synthesize urea. Thus, urea concentration in blood reflects the ingestion of dietary protein.

Liver is the main site for urea formation, which contains all enzymes involved in urea production.

According to Murray *et al.* (1991), creatinine, the anhydride of creatine, is formed largely in muscles by irreversible non enzymatic dehydration of creatine phosphate, which is now known to be concerned with the energy mechanism of these tissues and serves primarily as a temporary store of energy. At the same time, blood serum creatinine concentration is a better indicator of glomerular filtration rate (Enger and Blegen, 1964).

In spite of the changes in serum creatinine is significant between treatments, the data were within the normal range.

Liver functions:

The results in Table (10) illustrate the influence of supplementing rations with different levels of fibrolytic enzymes on blood serum transaminases (aspartate aminotransferase, AST and alanine aminotransferase, ALT) as indicators of liver functions of growing Assaf lambs. Treated rations did not significantly (P>0.05) impact AST or ALT levels in the blood serum of lambs. furthermore, it could be detecting a somewhat increment in AST and ALT (P>0.05) with raising fibrolytic enzymes level in lambs rations. Raising the metabolism operation and growth performance of fibrolytic enzymes supplemented groups may be a response to incremented ALT activity of lambs fed diets supplemented with fibrolytic

enzymes as noted by Davidson (1994), who reported that the function of ALT enzymes is the transfer of amino group from amino acid to synthesis another one and play an essential role in gluconeogenesis. Moreover, a raise of ALT activity is a response to the raise required for gluconeogenesis.

Lipids and glucose metabolism:

Data in Table (10) illustrated that the impact of supplementing rations with different levels of fibrolytic enzymes on blood serum triglycerides and cholesterol as indicators of lipid metabolism of growing Assaf lambs. Treated rations did not significantly ($P>0.05$) impact triglycerides and cholesterol levels in the blood serum of lambs. Also, glucose content gradually reduced ($P>0.05$) with raising fibrolytic enzymes in lambs rations. It is realized that lipids virtually have an essential role in all aspects of biological life, serving as hormones or hormones precursor, aiding in digestion, providing energy storage and metabolic fuel and acting as functional and structural components in biomembranes (Tietz, 1990).

In 1981, Noble pointed out that the dietary lipids are contributed as a source of plasma lipids as they are absorbed as non-esterified fatty acids, rapidly esterified to triglycerol then packaged into chylomicrons and very low density lipoproteins within the intestinal mucosa, from here lipids enter the lymph and finally the venous blood.

Also, King *et al.* (1985) indicated that 17-29% of fatty acids synthesis attributed to acetate. Passage of dietary lipids through the rumen resulted in significant fatty acids biohydrogenation, which is reflected in a relatively high proportion of saturated fatty acids in the circulating triglycerol and non-esterified fatty acids (Pethick and Dunshia, 1993).

From the information above it could be expected that raise metabolism and growth performance of fibrolytic enzymes supplemented groups might be considered as a response to reduced cholesterol content in blood serum of lambs supplemented with fibrolytic enzymes.

Economical efficiency

Data in Table (11) presented the findings of economical assessment of Assaf lambs as influenced by dietary fibrolytic enzymes supplementations.

These findings demonstrated that the total feed intake costed 193.60, 191.45 and 190.70 US\$ (involving the price of supplements), while the cost of total body weight gain was 259.38, 261.48 and 277.98 US\$ for control, T1 and T2, respectively (on the basis that the cost of every kg gain equals 6.00 US\$, the cost of one kg mixed feed equals 0.50 US\$ and the cost of one kg fibrolytic enzymes equals 3.50 US\$). It is of important to take note that feed price per kg weight gain were 4.478, 4.393 and 4.116 US\$ for control, T1 and T2, respectively. Clearly, feed price/kg weight gain diminished with supplementing ration with fibrolytic enzymes from 4.478 to 4.393 with low fibrolytic enzymes level (about 98.1% of control) and to 4.116 with high fibrolytic enzymes level (about 91.9% of control).

So the net income was the highest with T2 (high fibrolytic enzymes level) and was the lowest with control group. At the same time the economical efficacy was raised to be the highest with T2 (high fibrolytic enzymes level) and was the lowest with control group. The relative economical efficacy raised by 2.24% and 8.96% for groups T1 and T2 above control, respectively, (Table 11) as a result of feed supplements.

Shahriarinnour *et al.* (2011) mentioned the great interest in using cellulose wastes as feedstock during fermentation processes thereby converting low price starting materials into products of great value.

From the previous outcomes, it could be concluded that supplementation of fibrolytic enzymes as 4 g/h/d could be enhanced the economical efficacy than the low level of fibrolytic enzymes (2 g/h/d) or control diets.

Table (11): Effect of dietary fibrolytic enzymes levels on economical efficiency of Assaf lambs fed tested rations.

| Parameter | Treatments | | |
|--|------------|--------|--------|
| | Control | T1 | T2 |
| Total body weight gain, BWG, (kg) | 43.23 | 43.58 | 46.33 |
| Total revenue (US \$) (A) | 259.38 | 261.48 | 277.98 |
| DMI kg/h/d | 1.7962 | 1.7648 | 1.7472 |
| Total DMI kg/h | 352.05 | 345.90 | 342.45 |
| Total feed intake, as fed (kg/head) | 387.2 | 380.49 | 376.70 |
| Price/kg feed (US \$) | 193.60 | 190.25 | 188.35 |
| Total of fibrolytic enzymes added (g) | 0.00 | 336 | 672 |
| Price of fibrolytic enzymes added US\$ | 0.00 | 1.176 | 2.352 |
| Total feed cost (US \$) (B) | 193.60 | 191.45 | 190.70 |
| Feed cost / kg gain (US \$) | 4.478 | 4.393 | 4.116 |
| Net revenue (US \$) (A-B) | 65.78 | 70.03 | 87.28 |
| Economic efficiency (A/B)* | 1.34 | 1.37 | 1.46 |

* Economical efficiency expressed as the ratio between the price of total live weight gain and the price of feed consumed.

- Price of one kg of mixed of concentrate feed mixture and hay (85:15) as fed was 0.50 US\$, one kg fibrolytic enzymes was 3.50 US\$ and one kg live body weight was 6.0 US\$

-T1= 2 g fibrolytic enzymes/h/day, T2=4 g fibrolytic enzymes/h/day.

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استخدام الإنزيمات المحللة للسليولوز في تغذية الحملان العساف لتحسين العملية الهضمية

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تقسم الإنتاج الحيواني، كلية الزراعة، جامعة الأزهر، غزة، فلسطين.

صممت هذه التجربة لدراسة تأثير : استخدام الإنزيمات المحللة للألياف (بمستويين: منخفض أو عالي) لتحسين الهضم في غذاء الحملان العساف على مواصفات النمو و صفات الذبيحة و سائل الكرش و بعض مكونات الدم و الكفاءة الاقتصادية للحملان. استخدم في هذه الدراسة 12 حمل من نوع العساف عمر حوالي 8 شهور (متوسط أوزانها 30.68 كجم) قسمت إلى ثلاث مجموعات متساوية (4 حمل / مجموعة). المجموعة الأولى (المجموعة الضابطة) غذيت على عليقة مركزة (بنسبة 85% من العليقة) بالإضافة إلى دريس البرسيم الحجازي (بنسبة 15% من العليقة) أما المجموعة الثانية فقد تم إضافة 2 جرام من الانزيم المحلل للسليولوز إلى العليقة لكل رأس يوميا، بينما تم إضافة 4 جرام من الانزيم المحلل للسليولوز إلى العليقة لكل رأس يوميا في المجموعة الثالثة. و قد استمرت التجربة لمدة 28 أسبوعا، و تم تغذية الحملان بنسبة 3.5 % كمادة جافة من الوزن الحي، و تم وزن الحملان مرة كل أسبوعين لحساب معدل النمو اليومي كما تم سحب عينات سائل الكرش قبل الاكل و بعده ب 3 و 6 ساعات من التغذية الصباحية و تم عمل تجارب الذبح و قياسات الذبيحة و تقديرات الدم. ويمكن تلخيص النتائج فيما يلي:

- 1- المأكول ووزن الجسم: لم يتأثر معنويا بالمعاملات.
- 2- صفات الذبيحة: نسبة التصافي: لم تتأثر نسبة التصافي بشكل معنوي في المجموعات المختلفة.

قطيعات الذبيحة: أظهرت الحملان المغذاة على علائق محتوية على انزيمات محللة للسليولوز سواء المستوى العالي او المستوى المنخفض زيادة معنوية (على مستوى 5%) في أوزان الكتف و الخاصرة و الرقبة عنها في المغذاة على عليقة المقارنة،

الأجزاء الصالحة للأكل: أظهرت الحملان المغذاة على علائق محتوية على الانزيمات المحللة للسليولوز (المستوى المنخفض) زيادات معنوية في أوزان الكبد والكلى والخصية والقلب عنها في عليقة المقارنة، بينما اظهر المستوى العالي من الانزيمات المحللة للسليولوز انخفاضا معنويا في أوزان الخصيتين و الكلى و القلب عنها في عليقة المقارنة. وكان مجموع وزن الأجزاء الصالحة للأكل أعلى في المعاملة الأولى يليها المقارنة و أخيرا المعاملة الثانية.

الأجزاء غير الصالحة للأكل: أظهرت الحملان المغذاة على علائق محتوية على الانزيمات المحللة للسليولوز (المستوى العالي) أعلى اوزان في القناة الهضمية الخالية عنها مع عليقة المقارنة معنويا.

سائل الكرش: لوحظ عدم وجود فروق معنوية بين المعاملات بالنسبة لدرجة الـ pH ، حيث كانت اعلى من 6.0، و مع ذلك كانت هناك فروق معنوية بالنسبة لوقت سحب العينة على مستوى 5%. و اظهرت الاحماض الدهنية الطيارة فروق معنوية بين المعاملات، حيث كانت اعلى مع الحملان المغذاة على علائق مدعمة بالانزيمات المحللة للسليولوز. نيتروجين امونيا الكرش اخذ نفس اتجاه الاحماض الدهنية الطيارة و عكس اتجاه درجة الـ pH ، بمعنوية (على مستوى 5%)

3. مكونات الدم: أظهرت الحملان المغذاة على علائق محتوية على الانزيمات المحللة للسليولوز (المستوى المنخفض) أعلى بروتين كلى و جلوبيولين بصورة معنوية ($P < 0.05$) عنها مع عليقة المقارنة و المستوى العالي من الانزيمات المحللة للسليولوز.. و لم تؤثر المعاملات على وظائف الكلى او الكبد او تمثيل الدهون و الجلوكوز

الكفاءة الاقتصادية: كان صافي الإيرادات أعلى مع المستوى العالي من الانزيمات المحللة للسليولوز وكان أدنى مع المجموعة المقارنة، في نفس الوقت تم زيادة الكفاءة الاقتصادية أيضا.

من النتائج السابقة، يمكن أن نخلص إلى أن تناول العلائق المحتوية على الإنزيمات المحللة للسليولوز في علائق الحملان ينسب 2 او 4 جرام/رأس /يوم أدى إلى تحسين الأداء الإنتاجي للحملان العساف، و كان أعلى مع زيادة مستوى الإنزيمات المحللة للسليولوز في علائق الحملان حتى مستوى 4 جرام/رأس/يوم.

الكلمات الدالة: انزيمات محللة للسليولوز، حملان عساف، نمو- مواصفات ذبيحة، سائل كرش، مكونات دم، جدوى اقتصادية.