POSSIBILITY OF TREATING AGRICULTURAL BY-PRODUCTS BY EXOGENOUS ENZYMES AND ANEAROBIC BACTERIA TO PRODUCE RUMINANT FEEDS

By

RAAFAT MAHMOUD MOHAMED GOMAA

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2005

A thesis submitted in partial fulfillment
of
the requirements for the degree of

MASTER OF SCIENCE

in
Agricultural Science
(Animal Nutrition)

Department of Animal Production
Faculty of Agriculture
Ain Shams University

2012

POSSIBILITY OF TREATING AGRICULTURAL BY-PRODUCTS BY EXOGENOUS ENZYMES AND ANEAROBIC BACTERIA TO PRODUCE RUMINANT FEEDS

By

RAAFAT MAHMOUD MOHAMED GOMAA

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2005

This thesis for M.Sc. degree has been approved by:

Dr. Salwa Hamdy Mahmoud ……..…..
Prof. of Animal Nutrition, Faculty of Agriculture, Ain Shams University.

Dr. Mohamed Sayed Farghaly ……..
Prof. of Animal Nutrition, Faculty of Agriculture, Cairo University.

Dr. Hany Mahmoud Ahmed Gado ..……..
Prof. of Animal Nutrition, Faculty of Agriculture, Ain Shams University.

Date of Examination: / / 2012

POSSIBILITY OF TREATING AGRICULTURAL BY-PRODUCTS BY EXOGENOUS ENZYMES AND ANEAROBIC BACTERIA TO PRODUCE RUMINANT FEEDS

By

RAAFAT MAHMOUD MOHAMED GOMAA

B.Sc. Agric. Sc. (Animal Production), Ain Shams University, 2005

Under the supervision of:

Dr. Hany Mohamed Gado

Prof. of Animal Nutrition, Department of Animal Production, Faculty of Agriculture. Ain Shams University (Principal Supervisor).

Dr. Hamdy Mahmoud El sayed

Prof. of Animal Nutrition, Department of Animal Production, Faculty of Agriculture. Ain Shams University.

Dr. Soliman Mohamed Abd el mawla

Prof. of Animal Nutrition, Department of Animal Production, Faculty of Agriculture. Ain Shams University.

ACKNOWLEDGEMENT

I thank Allah, the most gracious, most beneficent and merciful for the help and guidance to achieve goals and make them possible.

I wish to express my sincere appreciation and deepest gratitude to my advisor **Professor Dr. Hany M. Gado**, professor of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Ain Shams University for suggesting the problem, continuous supervision support, valuable helps, guidance and patient throughout the course of this work.

I'm sincerely thankful to **Professor Dr. Hamdy M. El sayed**, professor of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Ain Shams University, for that every possible help and guidance kindly offered during the investigation.

Deep thanks are due to **Professor Dr. Soliman M. Abd El mawla**, professor of Animal Nutrition, Animal Production Department, Faculty of Agriculture, Ain Shams University for his continuous help during the experimental work of the research.

Many thanks are also due to my family father, mother and sister. I can't forget my wife and my daughters Maryam and Eman.

Grateful acknowledgment should be also extend to the staff members of the Department of Animal Production, Faculty of Agriculture, Ain Shams University for all facilities they offered to make this work possible.

ABSTRACT

Raafat Mahmoud Mohamed Gomaa. Possibility of Treating Agricultural by-products by Exogenous Enzymes and Anaerobic Bacteria to Produce Ruminant Feeds. Unpublished M.Sc. Thesis, Department of Animal Production, Faculty of Agriculture, Ain Shams University, 2012.

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 ($P< 0.05$) and %ADL was 6.01for T4 versus 8.01for T1. And significant increases in %CP content to 7.96, 7.10, 7.95 for ration T4, T3, T2 respectively compared to the control ration 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 hrs. post feeding values were 25.41, 25.03, 25.96 mg/100ml respectively and total volatile fatty acids 3 hrs. post feeding were 8.20, 8.13 and 8.26 m.eq/100ml 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, T2 respectively , while treating rations with ZAD reflected low level of GPT 6 hrs. post feeding values were 20.64 and 20.61 for treatments T2 and T4 respectively versus T1 (20.90 µ/l).

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

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

Keywords: Ossimi sheep, Barely, Exogenous enzymes, Rice straw, Ruminant feed, digestibility, nutritive value, rumen liquor parameters, blood serum parameters.

Contents

Page

1. INTRODUCTION

There is a wide gap between the available feeds and animals requirements in Egypt. It was estimated as a shortage of 4.79 million tons of TDN per year **(El ashry, 2007).** By-products can play an important role to minimize this gap. The annual agriculture by-products estimated to be around 30 million tons of dry material per year.

By-products can be utilized by many ways included; sources of plant nutrients, feed ingredients for farm animals, substrates for methane generation and substrates for microbial and protein synthesis. The wastes have the most economic value for use as animal feed. The utilization of by-products can not only be used in favor of solving feed shortage problems but also to prevent environmental pollution. Incorporating by-products in animal rations in general or after up-grading their nutritive value by mechanical, chemical or biological treatments can also reduce the cost and increase the profit of animal production projects.

Biotechnology methods

The main limitations to the use of by-products in diets for farm animals are the uncertainty of the likely response in terms of animal production and their need for some treatments to provide a diet adequate for the needs of production. These problems are found at all levels of animal production from subsistence systems to commercial farming.

Biological treatments of some by-products are very essential in order to degrade lignocellulosic into lignin, cellulose, hemicellulose and improve crude protein content. Enzyme treatment methods for improvement of poor quality forages and roughages have not been used in practice to date, but it may prove to be one of the most promising in the future. The main problem in the biological upgrading of poor quality materials, is to find suitable microorganisms which decompose lignin without using too much of the hemicellulose and cellulose. Attempts have been made to decompose lignin by microbial and enzymatic means to increase digestibility of lignocellulosic

material. Organisms that degrade cellulose and hemicelluloses are of no use, since they deplete the straw of valuable nutrients that the animal itself can digest.

Biological treatments such as ZAD compound **(Gad, *et al.*, 2005; Gado *et al.*, 2006, Gado 2011 and 2007)** and ZADO compound were used to improve the nutritive value and digestibility of poor quality roughages.

The objectives of this study were:

1- To study the effects of feeding RSGB.

2- To study the effect of biological treatments by using ZAD on the chemical composition, nutritive value, rumen fermentation and some blood parameters of Ossimi sheep.

2. Review of literature

2.1.1. Lignocellulosic agricultural by-products and its manufacturing in Egypt

Agricultural and agro-industrial activities produce thousands of tons of by-products in Egypt **Table (1)**.These abundant wastes are mostly left in the field, causing environmental problems. Although these residues are nutritious, a small portion is being used directly as feed or as components for industrially formulated farm animal diets **(Yang et al., 2001).**

Table1. Quantity of main crops by-products produced in Egypt.

By products	Quantity (million tone)	By products	Quantity (million tone)
Wheat straw	8.280	Bean straw	0.281
Com stalks	5.762	Barley straw	0.163
Rice straw	3.912	Berseem straw	0.143
Cotton stalks	0.612	Soy straw	0.098

Source: Ministry of Agriculture and Land Reclamation Report (2008).

On the other hand, the bioconversion of agro-industrial residues has been receiving attention in recent years **(Pandey et al., 1999** and **Rosales et al., 2002)** Several processes have been developed based on these materials as substrates in bioprocesses for production of single cell protein, organic acids, ethanol, mushrooms, enzymes and biologically important secondary metabolites **(Pandey et al., 1999** and **Massadeh et al., 2001)**.Use of these agricultural wastes in bioprocesses may provide alternative substrates and furthermore, helps to solve environmental problems, which are otherwise caused by their disposal.

2.1.2. Biological treatments for agricultural by-products

The main shortcoming of straws and other by-products as animal feed lies in their high crude fiber, low protein content, low digestible energy and containing of anti-nutritional compounds (tannins).Thus, to increase their digestibility and improve crude protein content, it is important to destroy the linkage between cellulose, hemicellulose and lignin or destroy the compact nature of the tissue. There have been attempts to do that by mechanical, chemical or biological treatments **(McHan, 1986a; Lyo and Antai, 1988; Hunt et al., 1992 and Singh et al., 1993)**.

The microbiological methods for improving the nutritional quality of plant residues were reported by **Han (1974)**, **Han** and **Anderson (1975)**, **Zadrazil (1977 and 1978**), **Kahlon), Nikhat et al. (1983)and Gado (1997).** They found that the feed produced by this way was better than that produced by chemical methods and no side effects were noticed on the cattle.

It is well known that biological treatments could be conducted by administration of the microbial cells, microbial extracts or microbial enzymes such as cellulase enzyme. **(McHan, 1986b** and **Morrison, 1988).**

2.1.3. How cellulase effect on agricultural by-products

Cellulose present in renewable lignocellulosic material is considered to be the most abundant organic substrate on the earth. Cellulose is a long chain polymer polysaccharide, of beta-glucose. It forms the primary structural and principal constituent of the cell wall of plants. Cellulase (a complex multi enzyme system) acts collectively to hydrolyze cellulose from agriculture wastes to produce simple glucose units (Smith, 1996). Cellulase refers to a family of enzymes (Fiberolytic enzymes) which act in concert to hydrolyze fiber of plant cell wall to glucose; cellobiose or cello-oligosaccharides

The widely accepted mechanism for enzymatic cellulose hydrolysis involves synergistic actions by endoglucanase, exoglucanase or cellobiohydrolase and β-glucosidase (**Knowles et al., 1987; Wood** and **Garica Campayo, 1990; Henrissat, 1994; Teeri, 1997; Lynd et al., 2002; Zhang** and **Lynd, 2004)**. Endoglucanases hydrolyze accessible intramolecular [3-1, 4- glucosidic bonds of cellulose chains randomly to produce new chain ends; exoglucanases processively cleave cellulose chains at the ends to release soluble cellobiose or glucose; and β-glucosidases hydrolyze cellobiose to glucose in order to eliminate cellobiose inhibition.

2.1.4. Treating with ZAD® as a liquid enzyme product

2.1.4.1. ZAD® with rice straw

There is a big mount of rice straw in Egypt and this mount is useless, have no nutritive value almost for animal nutrition, we did more than five trials to rise the nutritive value of rice straw, **(Gado., 1997)** had a feeding trial for 12 weeks to evaluate the effect of enzymatic treatment on rice straw silage using forty Baladi yearling male goats.

Cellulatic enzymes suspensions were added at (100, 200, 300 ml/1000 kg roughage), and he found that the concentration of acetic acid and pH value were lower ($P<0.01$) and concentration of lactic acid and reduce sugars were higher ($P<0.01$) in the cellulase treating silage.

In vitro NDF degradation was higher ($P<0.05$) for cellulase silage than control.

Increasing cellulase concentration positively correlated with NDF digestibility ($r = 0.95$). And the apparent N retention and weight gain were improved ($P<0.01$) in goats fed cellulase treated silage.

2.1.4.2. ZAD® with sugar cane bagasse

For the sugar cane bagasse we had more than six experiments first was on **(Gado., 1997)** treated bagasse with cellulatic enzymes added at (100, 200, 300 ml/1000 kg roughage), the result was that the concentration of acetic acid and pH value were lower ($P<0.01$) and concentration of lactic acid and reduce sugars were higher ($P<0.01$) in the cellulase treating ensiled bagasse.

In vitro NDF degradation was higher ($P<0.05$) for cellulase ensiled bagasse than control. Increasing cellulase concentration positively correlated with NDF digestibility ($r = 0.95$). The apparent N retention and weight gain were improved ($P<0.01$) in goats fed cellulase treated ensiled bagasse.

Also in the same trial found that the Bagasse had higher ($P<0.05$) NDF degradation value than rice straw in the cellulase treated groups.

In another experiment trial (**Gado et al., 2007**) used the in vitro procedure to compare the effect of six treatments on dry matter disappearance in bagasse silage.

Bagasse silage was treated as follow: (1) untreated control (2) treated with additive mixture solution (3) cellulase enzyme 15%, (4) cellulase enzyme 25%, (5) Rumen liquor, (6) cellulomonas sp. bacteria. The results indicated that 15% cellulase caused the highest DMD and OMD while the untreated bagasse silage had the lowest value.

In this study it was concluded that the ZAD treatment improved digestibility and palatability of fiber source which resulted in improving animal performance and feed efficiency. It appears from the results that ensilage process and adding suitable source of readily available CHO in the ration of ruminants play an important role with ZAD addition in obtaining such positive results.

2.1.4.3. ZAD® with corn stalks

In a study on ZAD (**Gad et al., 2005**) treated and ensiled bagasse and corn stalk, the percentage of CP increased in treated ensiled fiber sources ($P<0.05$) and %CF, %NDF, %ADF decreased. In this study were two trials the first using 40 male goats averaged 23 kg, and the second using 56 male Baladi lambs averaged about 20 kg, and it was concluded that the ZAD treatment improved digestibility and palatability of fiber source which resulted in improving animal performance and feed efficiency.

2.1.4.4. ZAD® with bean straw

In a study(**Fatma et al.,2006**) six rations were formed as wheat straw (control ration) R1, wheat straw treated with 1 litter ZAD (R2), wheat straw treated with 2 litters ZAD (R3), bean straw as a control ration (R4), bean straw treated with 1 litter ZAD(R5), bean straw treated with 2 litter ZAD(R6). The period of the study was 180 days, the performance of animals was better in treated groups than the control.

2.1.4.5. ZAD® with Orange pulp

(**Omima 2006**) was conducted this experiment to evaluate the effects of replacing 15% of the total mixed ration (TMR) with ensiled orange pulp (EOP) in presence or absence of ZAD as an exogenous enzyme mixture on lamb growth performance, digestibility, ruminal fermentation and some blood metabolites. And the conclusion was that partially replacement of TMR with orange pulp silage treated with 0.5% ZAD improved the nutritive value, animal performance and immunity.

2.2. Biological treatment for agriculture by products

2.2.1. Biological treatments for silage and roughages on goats

(**Etab et al., 2006**) had reported this study to investigate the effect of using four strains and one species of cellulolytic bacteria (*Cellulomonas cellulasea, Acetobacter xylinum, Thermonospora fusca, Ruminococcus albus* and *Bacillus sp*.) on three roughages treated in anaerobic condition. Four experimental trials were conducted; experiment (1): Four strains and one species of cellulolytic bacteria (*Cellulomonas cellulasea, Acetobacter xylinum, Thermonospora fusca, Ruminococcus albus* and *Bacillus sp.*) were separated from rumen liquor of Baladi goats and evaluated by electrophoresis method. Experiment (2): Bacteria were used as biological treatments of silage to study the changes that occur in chemical composition and cell wall constituents of three roughages.

Silages were made from three roughages (corn stalks, bagasse and rice straw). Experiment (3): investigated effect of treatments by 4 strains and one species of bacteria on WDM and IVOM disappearance of silage. Experiment (4): in complete randomized design with periods of 21 days, sheep about 34:0.5 kg body weights were fed on three different roughages untreated and treated with two strains of cellulolytic bacteria to study effect of treatments on digestibility, nitrogen balance, rumen parameters and blood parameters.

Results were concluded that the four strains and one species of bacteria were different significant and all strains secretes cellulase enzymes according to the electrophoresis method. It was indicated that using cellulolytic bacteria caused marked increase in crude protein from average 1.98% to 15.16% and decrease crude fiber from average 52.6% to 38.1% in all roughages compared with the untreated roughages. All treatments significantly decreased NDF, ADF and ADL.

2.2.2. Effect of biological treatments on sugarcane bagasse digestibility and performance of Baladi goats

(**Gado et al., 2007**) reported that work to study the effect of biological treatments on poor quality roughage (bagasse) to improve the performance of small ruminants. Bagasse ensiled or not using one of the following treatments was: (1) untreated control, (2) with additive mixture solution, (3) cellulase enzyme 15%, (4) cellulase enzyme 25%, (5) rumen liquor, and (6) Cellulomonas cellulase. Two experimental trials were conducted, experiment (1): in a 5x5 Latin square design with periods of 21 days, goats weighing about 27 kg (0.5kg) were fed one of the treated bagasse silage groups plus corn (1, 2,3,4,5 and 6).

The chemical composition, nutrient intake, digestibility and nitrogen balance were determined. Experiment (2): Twenty male goats (18kg average initial weight) were used in this experiment. Animal performance (ADG, feed intake and feed efficiency) was calculated each week through-out the experimental period (90 days).

Results indicated that treatments with rumen liquor or cellulase enzyme (15%) resulted in higher digestibility ($p< 0.05$) compared with the other treatments. It was concluded that treatment of bagasse silage improved its digestibility, ADG, feed intake and feed efficiency.

2.2.3. Effect of chemical and biological treatments of some crop-residues on their nutritive value on goats

(**El Ashry et al., 2002**) reported that experiment doing four digestibility and nitrogen balance trials were conducted on Baladi goats fed rations containing 25% of biologically treated cotton stalks. 25% wheat straw and 50% concentrate teed mixture, some rumen liquor and blood parameters were determined.

Results indicated that. Use of biological treatments (specially combined *Trichoderma viride* and *Saccharomyces cerevisiae*) in goats' rations is useful and did not cause any abnormal condition on rumen activity, Liver and kidney functions and animal performance as well.

2.2.4. Biological treatment on rice straw on sheep

(**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.

Four treatments were tested, the first treatment (T1) was the control (rice straw untreated), second treatment (T2) was rice straw treated with ZAD ad. Libitum. Third treatment (T3) was rice straw treated with fungus (*Pleurotus osteratus*) and fourth treatment (T4) was rice straw treated with ZAD and fungus. T2, T3 and T4 increased ($P<0.05$) crude protein of rice straw and decreased ($P<0.05$) dry matter, crude fiber, NFE, NDF, ADF, ADL and cellulose contents than the control group.

Dry matter intake (DMI) increased ($P<0.05$) in the groups fed rice straw treated with ZAD, fungus and ZAD+ fungus than the control group. ZAD, fungus and ZAD + fungus treatments increased ($P<0.05$) digestibility coefficients of CF, ADF, NDF, ADL, cellulose and hemicellulose than the untreated rice straw. Total digestible nutrients (TDN) and digestibility of crude protein (DCP) for T2, T3 and T4 were higher ($P<0.05$) than untreated rice straw.

All of treated rice straw groups had no significant differences ($P<0.05$) urea, total protein, albumin, globulin, GOT and GPT than untreated rice straw. Twenty four Ossimi rams weighed 20.05:0.3 kg were used in feeding trial lasted 120 days to evaluate the effect of biological treatments on the nutritive value of rice straw. Animals were divided into four groups.

In addition **(Gado et al., 2006)** reported that study to demonstrate the effect of biological treatments (ZAD, fungus and ZAD with fungus) on feed intake, digestibility coefficients, nutritive value and nitrogen balance of rice straw and some rumen liquor and blood parameters.

Twelve adult Ossimi rams were divided into four similar groups and used to carried out four metabolic trials using three animals for each group (all groups were fed the rice straw ad. Libitum) the first (T1) was the control (untreated rice straw), the second (T2) was rice straw treated with ZAD the third (T3) was rice straw treated with fungus (*Pleurotus orteratus*) and the fourth (T4) was rice straw treated with ZAD and fungus.

T2, T3 and T4 increased ($P<0.05$) crude protein of rice straw and decreased ($P<0.05$) dry matter, Crude fiber, NFB, NDF, ADF, ADL and cellulose contents than the control group. Dry matter intake (DMI) increased ($P<0.05$) in the groups fed rice straw treated with ZAD, fungus and ZAD+ fungus than the control group, ZAD, fungus and ZAD + fungus treatments increased ($P<0.05$) digestibility coefficients of CF, ADF, NDF, ADL, cellulose and hemicellulose titan the untreated rice straw.

Total digestible nutrients (TDN) and digestibility of crude protein (DCP) for T2, T3 and T4 were higher (P<0.0S) than untreated rice straw. The rumen liquor parameters (NH,-N) and TVFA's concentrations were highest value alter 3 hours of feeding in all groups. All of treated rice straw groups had no significant differences (P<0.0S) for urea, total protein, albumin, globulin, GOT and GPT than untreated rice straw.

Twenty-four Ossimi lambs averages (20.0 2 0.3 kg) were used in feeding trial lasted 120 days. Feeding trail results showed all biological treatments had higher dry matter intake (1037, 990. 896 and 7863/d) and average daily gain (169.2, 163.3, 134.3 and 92.5g) for T2, T3, T4 and T1,

respectively. Biological treatments indicated that it they could be good method to improve the nutritive value of rice straw.

2.2.5. Treatment of agricultural by-products to manufacture silage

(**Gad Al rab et al., 2005**) reported that the cost of industrial enzymes is very high. One of these enzymes is the industrial cellulase. They represent study is an attempt to produce an industrial compound from anaerobic bacteria in the rumen to introduce cellulase function.

The effect of this compound which termed as ZAD was investigated as a nutritional additive when added to each of two materials of agricultural by products representing crude fiber sources (corn straw and bagasse) in the rations of ruminants .This study was divided into four experimental trials.

The first trial was ensiling two fiber sources (corn straw and bagasse). Percentage of CP in treated ensiled fiber sources increased ($p<0.05$) and CF%, NDF%, ADF% decreased.

In the second trial, IN Vitro DM and OM disappearances were carried out by incorporating the obtained silage in eight diet formulas containing the same DCP and TDN with two sources of readily available CHO (corn grain and potatoes roots). The treatments with ZAD increased IVDMD and IVOMD ($p< 0.05$).

In the third trial a metabolic trial was carried out in which 40 male goats averaged 23 kg were divided randomly and equally into eight experimental groups fed the previous indicated eight diets based on maintenance allowances of NRC (1975).Digestibility coefficients were improved and concentration of plasma protein in treated groups increased ($p< 0.05$). Also, (NH3-N) and TVF‘S in the rumen of treated groups increased.

In the fourth trial , a feeding trial was carried out for six months in which (56) Male Baladi lambs averaged about 20 kg in the beginning of the

experiment were used and divided into the same eight groups. Results indicated that feed intake, growth rate and feed efficiency improved with treated animals ($p< 0.05$).

It was concluded that the ZAD treatment improved digestibility and palatability of fiber sources which resulted in improving animal performance and feed efficiency. It appears from the previous results, ensiling process and adding suitable source of readily available CHO in the ration of ruminants play an important role with the ZAD addition in obtaining such positive results.

2.2.6. Effect of biological treatments by cellulolytic bacteria on chemical composition and cell wall constituents of some roughages

(**Etab et al., 2005**) reported that experiments to separate five strains of cellulolytic bacteria (*Cellulomonas sp., Acetobacter sp., Thermonospora sp., Ruminococcus sp.* and *Bacillus sp*.) from rumen liquor of Baladi goats and evaluation of these bacteria by electrophoresis method.

Then these bacteria were used as biological treatments of silage to study the changes that occur on chemical composition and cell wall constituents of three roughages. Silages were made from three roughages (corn stalks, bagasse and rice straw) and incubation for two months.

The additive (additive DM, w/w) contained water 200%, urea 3%, molasses 4%, formic acid 0.03%, Acetic acid 0.5% and one of the cellulolytic bacteria at the rate of 2 liter/ Ton.

Results indicated that using cellulolytic bacteria caused marked increase in crude protein from average 1.98%to 15.16% and decrease crude fiber from average 52.6% to 38.1% in all roughages compared with the untreated roughages. All treatments significantly decreased NDF, ADF and ADL. It was concluded that the five strains of bacteria were different

significant and all strains secretes cellulase enzymes according to the electrophoresis method.

In addition, the biological treatments by *Cellulomonas sp*. and *Ruminococcus sp.* was succeeded more with bagasse and corn stalks while *Thermonospora sp*. and Bacillus sp. succeeded more with rice straw. However, the biological treatments of corn stalks, bagasse and rice straw silage improved its chemical composition and cell wall structure.

2.2.7 Effect of different cellulolytic rumen bacteria on fiber digestion

(**Gado, 1999**) reported that *Ruminococcus flavefaciens* & *R. albus* were obtained before feeding from different ruminant species: goats, sheep, cattle and water buffalo.

The goat source showed the highest (P<.01) ability for xylan, flax hemicellulose, corn hull hemicellulose and berseem hemicellulose digestion. The results of rate of digestion showed similar results with statistical differences (P<.05). That was reflected on first order kinetics with highest (P<.01) b values

The *R. flavefaciens* from goat origin, these predominant ruminal cellulolytic bacteria were grown in different binary combinations to determine the outcome of cellulose digestion. *R. flavefaciens* from goat origin showed its domination (P<.01) in cellulose limitation co culture. The retention of *R. albus* in the cellulose limited co culture may result from its ability to utilize glucose.

2.3. Fibrolytic enzymes

2.3.1. Fibrolytic enzymes and ruminants

Incomplete digestion of fibrous substrates often limits the overall digestive process in the rumen and can significantly influence animal performance in livestock production systems that use forages as a major component of the diet. As a result, many strategies have been developed to stimulate the digestion of the fibrous components in ruminant feeds. These have included the use of specific nutrients which stimulate fiber digestion and processing feeds to increase the rate and extent of fiber digestion.

Recent advances in fermentation technology and biotechnology have allowed for the economic production of large quantities of biologically active enzymes that can also be used as livestock feed supplements. These technologies provide new possibilities for altering digestive processes in a wide variety of animals. Fibrolytic enzymes preparations can be used to drive specific metabolic and digestive processes in the gastrointestinal tract and may augment natural digestive processes to increase nutrient availability and feed intake (**McAllister et al., 2001**).

In the last decade, specific enzyme preparations have become valuable tools for economically manipulating digestive processes in monogastric animals and poultry (**Annison and Choct, 1993 and Johnson et al., 1993**) but there has also been considerable) interest in using enzymes as supplements for ruminant diets (**Feng et al. 1996; Lewis et al., 1996; Annison, 1997 and Howes et al., 1998**). Strategies that use supplemental cellulase enzyme activity in the rumen may be important since the digestibility of organic matter in the rumen dose not reaches 100% and even small changes in digestibility can influence the efficiency of ruminal fermentations.

2.3.2. Treating with fungi enzymes

(**Abd El Fattah et al., 2009**) they started a study aimed to producing of cellulases to be involved in animal feeding trials. Five fungal strains (*Asperigillus niger*, *Fusarium oxysporum*, *Fusarium avenaceum*, *Cephalosporium acremonium* and *Asperigillus flavus* NRRL 5521) were grown as stand cultures in 100ml conical flasks containing Cellulose Powder Medium (CPM). *A .niger* was chosen on the basis of the best mean cellulase activity for production of laboratory produced cellulase (Asperozym).

The maximum production of cellulase by *A. niger* was achieved at inoculum ratio of 4%, 48 hr. of incubation period, initial pH 6.0, meat extract as a nitrogen sources at a concentration of 0.33 g N/l and wheat straw as a carbon source at a concentration of 1% (W/V). Two experiments were carried out to evaluate the effects of cellulases supplementation on in vitro degradation of banana waste and in vivo nutrients digestibility, milk yield and composition by lactating Zaraibi goats. In the in vitro experiment, dry matter and organic matter disappearance (IVDMD and IVOMD) were determined for banana waste supplemented separately with (Asperozym) and commercial cellulolytic enzyme source (Bacillozym®) at 4 levels (0, 0.77, 1.54, 2.31and 3.08 Unit / kg DM).

Increasing the Asperozym supplementation levels up to 3.08 U/kg DM exhibited the highest ($P<0.05$) values of IVDMD and IVOMD, while Bacillozym® recorded the highest ($P<0.05$) IVDMD and IVOMD values at 1.54 U/kg DM compared with the untreated banana waste (Control). In the in vivo experiment, six lactating Zaraibi goats after 7 days of parturition were divided into three groups, two animals each, using 3x3 Latin square designs. The first group was fed 50% concentrate feed mixture (CFM), 25% banana waste and 25% berseem straw (Control diet).

The second group was fed control diet supplemented with Asperozym at level of 3.08 U/kg DM (T,). The third group was fed control diet supplemented with Bacillozym® at level of 1.54 U/kg DM. (T2).

Asperozym and Bacillozym® supplementation significantly ($P<0.05$) increased nutrients digestibility, nutritive values, ruminal total volatile fatty acids (TVFA's) and ruminal total nitrogen for treated groups compared with the control group. Milk yield was significantly ($P<0.05$) increased for Asperozym and Bacillozym® treated groups compared with the control group. However milk composition did not significantly ($P>0.05$) change among all groups. Asperozym was superior over Bacillozym® for improving feed digestion and milk production by Zaraibi goats.

(**El Ashry et al., 2002**) used rice straw in a biological treatments using solid state fermentation technique (SSF) to upgrade its nutritive value to be serve as a part of ruminant ration.

The residues were moistened as solid: liquid ration 1:2 with solution composed of 4%molases, 0.4% urea, and 0.2% K2H PO4 and 0.03% MgSO4.7H2O

The moistened residues were inoculated with 10% (v/m) inoculum of *Tricoderma viride,* or *Saccharomyces cerevisiae* and 5% (v/m) of both when co culture was applied. The inoculated residues were incubated at room temperature ($30^{o}C\pm2$) for 21 days.

The result was high increase in crude protein contents for rice straw was achieved by co culture of *T. virid* and *S. cerevisiae* treatment. The losses in dry matter (DM) as a result to co culture treatments were 9.0% in rice straw.

The Biological treatments significantly decreased neutral detergent fiber (NDF) and acid detergent fiber (ADF) especially for treatment by

fungus or fungus followed by yeast. Nutritive values were enhanced for treated residues as IVOMD for rice straw was 62.45 to be 72.4.

Using Corn stalk in a biological treatments using solid state fermentation technique (SSF) to upgrade its nutritive value to be serve as a part of ruminant ration. The highest increase in crude protein content for cotton stalk was achieved by co culture of *T. virid* and *S. cerevisiae* treatment.

The lowest losses in dry matter (DM) as a result to co culture treatments were showed for mid ripe of date palm it was 2.55.

The Biological treatments significantly decreased neutral detergent fiber (NDF) and acid detergent fiber (ADF) especially for treatment by fungus or fungus followed by yeast. Nutritive values were enhanced for treated residues as IVOMD for mid ripe of date palm was 72.35 to be 76.02.

The Biological treatments significantly decreased neutral detergent fiber (NDF) and acid detergent fiber (ADF) especially for treatment by fungus or fungus followed by yeast. Nutritive values were enhanced for treated residues as IVOMD for Pinnae of date palm was 72.65 to be 78.89.

In another experiment on goats using 25% wheat straw (H. El-Sayed 2002) the result was indicate that use of biological treatments (specially combined *T. virid* and *S. cerevisiae*) in goats rations is useful and did not cause any abnormal condition on rumen activity. Liver and kidney functions and animal performance as well.

2.3.3. Fibrolytic enzymes and the digestibility

2.3.3.1. The effect on In-vitro and in-situ digestibility

Lewis et al. (1996) studied the effect of a solution containing cellulases and xylanases on the digestion of a forage-based diet, and they found that the rate of (IVDMD) of enzyme treated grass hay was improved compared with the untreated grass hay.

Mohamed et al. (2005) studied the effect of an enzymatic mixture with cellulase, xylanase and protease activities on the fermentation of substrate composed of 65% forage (Berseem hay and rice straw) and 35% concentrates by using batch culture of mixed ruminal microorganisms. Who found that after 24 hr. of incubation, all enzymatic treatment decrease final pH and increased dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility of substrate. Also, acetate and propionate productions were increased by all enzymatic treatments.

Colombatto et al. (2006) examined (in vitro) the impact of fiberolytic enzymes on the rate and extent of fermentation of alfalfa stems. A commercial enzyme product was added to alfalfa stems at six levels: 0, 0.51, 1.02, 2.55, 5.1 and 25.5 g/kg DM. They found that addition of enzyme linearly increased in vitro OM, DM, NDF, ADF, and hemicellulose degradation.

Eun et al. (2006) evaluated the use of exogenous enzymes as a potential means of improving the cell wall degradation of rice straw, Two developmental cellulases, two developmental xylanases, and two commercial enzyme products (combination of endoglucanases and xylanases) were used. The results indicated that adding enzymes increased degradability of rice straw.

Abd El Gawad et al. (2007) evaluated effects 0f (Fibrozyme) commercial fibrolytic enzymes composed of xylanase and cellulase activities on (IVDMD and IVOMD) of com stalks, wheat straw, rice straw and sugarcane bagasse. These four low quality roughages were used at three roughage: concentrate ratio (100:0, 40:60 and 30:70%). Enzyme was supplemented at 4 levels (0, 2, 2.5 and 3 gm/kg DM).

Results indicated that using 2 gm/kg DM of Fibrozyme supplementation increased in vitro dry matter and organic matter

disappearance of ration contained 30% roughage. Com stalks followed by wheat straw showed better response for digestion pattern than rice straw) and sugarcane bagasse.

Giraldo et al. (2007) investigated the effects of exogenous pure cellulases on ruminal microbial growth and fermentation of 70:30 grass hay: concentrate (DM basis) substrate in Rusitec fermenters. The results indicated that adding cellulases enhanced in vitro fermentation by increasing substrate fiber degradation, Volatile Fatty Acids (VFA) production, and ruminal microbial growth.

Pinos-Rodriguez et al. (2007) studied the effects of exogenous fibrolytic enzymes on in situ degradability of Total Mixed Ration (TMR) with three different forage concentrate ratios (400:600, 500:500, 600:400 g/g) and two (0 or 2 g) levels of enzymes/kg TMR DM. It was indicated that enzymes improved ruminal disappearance rates of DM and NDF in situ.

Ranilla et al. (2007) investigated whether a fibrolytic enzyme preparation with xylanase and cellulase activities could stimulate in vitro rumen fermentation of alfalfa hay, grass hay and barley straw. The enzyme preparation was added at levels of: 0 (control), 50 mg/g substrate DM and 100 mg/ g substrate DM. Results indicated that this fibrolytic enzyme preparation stimulated in vitro fermentation of substrates at short (5 and 10 h), but not at long (24 h) incubation times. **Krueger and Adesogan (2008)** studied effect of combination of ferulic acid esterase (FAE), cellulase (CEL), and xylanase (XYL) on hydrolyzing mature bahia grass in the absence and Bermuda grass in the presence of rumen fluid.

This study identified certain mixtures of XYL, CEL, and FAE that increased 24-h DM disappearance of bahia grass in the absence of rumen fluid. Application of these multi enzyme cocktails reduced the lag phase and

improved efficiency of fermentation of mature Bermuda grass in the rumen fluid.

On the other hand, **Colombatto et al. (2004)** examined the influence of fibrolytic enzymes applied at maize silage. The results demonstrated that enzymes did not change the total amount of fermentable substrate.

Dean et al; (2008) measured the effects of applying NH3 or a fibrolytic enzyme on the fiber concentration, and DM and fiber digestibility of two tropical grass hays. The enzymes were applied at 0 (Control) 0.5, 1, and 2 times the rates recommended by the respective manufacturers. The results indicated that, ammunition was more effective than any enzyme treatment at improving in situ degradability.

2.3.3.2. In-vivo digestibility

2.3.3.2.1. Effect on nutrients digestibility

Feng et al. (1996) tested in-vivo responses of enzyme treatments contained cellulase and xylanase. In these treatments enzymes were applied to fresh forage, wilted forage, then dried; applied to dry forage immediately before feeding (E-dry) compared with untreated forage. Data from this study suggested that the rate of total tract DM and NDF digestibility were greater in case of E-dry treatment than the other treatments.

Lewis et al. (1996) found that digestibility of DM, NDF, and ADF were greater ($P < 0.1$) for steers received cellulase and xylanases enzymes applied to grass forage 24 hrs. before feeding (F-24), and enzyme applied to grass forage at feeding (F-0), than for control (water applied to grass forage at feeding time). Digestibility of DM ($P = 0.15$), NDF ($P = 0.13$) and ADF ($P = 0.10$) tended to be greater for steers received all enzyme treatments than control, and the digestibility of DM ($P = 0.17$), NDF ($P = 0.18$), and ADF ($P = 0.24$) tended to be 10wer_ in steers received the enzyme ruminal infused 2 hrs. after feeding (R1) than (F-24) and (F-0). Starch digestibility was quite

extensive and was not affected ($P > 0.10$) by the enzyme treatment. They found that DMI was numerically greater ($P = 0.27$) for cows fed the enzyme treatment (22.4 vs. 20.5 kg/d).

Krause et al. (1998) conducted a study to determine the effects of treating barley grain with a fibrolytic enzyme mixture on digestibility. Steers were given ad libitum access to one of four diets that consisted of 95% barley-based concentrate and 5% forage (DM basis). The concentrate was either control or enzyme-treated, and the forage was either barley silage or barley straw. They stated that enzyme treatment of barley increased total tract dietary ADF digestibility by 28%.

Beauchemin et al. (1999) investigated the effects of grain source and fibrolytic enzyme supplementation on nutrient digestion. Two grains were combined with and without enzyme which contained primarily cellulase and xylanase activities. It was observed that enzyme supplementation increased nutrient digestibility in the total tract.

Rode et al. (1999) investigated the effects of exogenous fibrolytic enzyme supplementation on digestibility by dairy cows. The enzyme preparation contained mainly cellulase and xylanase was added to the concentrate to supply 1.3 g/kg of total mixed ration (dry matter basis). They reported that total digestibility of nutrients, determined was dramatically increased by enzyme treatment.

Yang et al. (1999) used Holstein cows to investigate fibrolytic enzyme supplementation on nutrient digestion. The four diets consisted of 45% concentrate, 10% barley silage, and 45% cubed alfalfa hay (dry matter basis) and differed in enzyme supplementation: control cubes, control plus 1 g of enzyme per /kg of cubes, control plus 2 g of enzyme per /kg of cubes and both concentrate and cubes treated with 1 g of enzyme mixture per/kg of dry matter] The enzyme supplement contained primarily cellulase and xylanase.

They indicated that digestion of OM and NDF in the total tract was higher for cows fed the high dosage of enzyme than for those fed the control ration.

Yang et al. (2000) investigated the effects of method of adding fibrolytic enzymes to diets of dairy cows on digestibility. The treatments were control, enzymes applied to the total mixed ration and enzymes added to the barley-based concentrate. They found that total tract digestibility of dry matter was higher for enzyme supplemented ration than for the control one.

Bowman et al. (2002) investigated a fibrolytic enzyme product contained cellulase and xylanase. Treatments were included enzyme supplemented to concentrate (45% of TMR), enzyme applied to supplement (4% of TMR), and enzyme applied to premix (0.2% of TMR) compared with enzyme free diet (control). It was indicated that digestibility of OM, NDF and ADF in the total tract was increased in comparison to the control when enzymes were added to the entire concentrate.

Pinos-Rodriguez et al. (2002) studied the effect of a directly fed exogenous fibrolytic enzyme on intake and digestion by sheep. The diets were alfalfa hay, alfalfa hay plus exogenous fibrolytic enzymes, ryegrass hay and ryegrass hay plus enzyme. The enzyme increased apparent digestibility of CP, hemicellulose and NDF for alfalfa.

Titi and Tabbaa (2004) investigate the efficacy of direct feeding a cellulase enzyme on lamb diets digestibility. Result indicated that cellulase enzyme increased ($P < 0.05$) dry matter and organic matter digestibility of treated lambs compared to those of control. A similar trend was observed for the crude fiber, NDF and ADF digestibility coefficients. However, no differences were observed in crude protein digestibility between treated and control lambs. .

Abd El Gawad et al. (2007) evaluated the effects of fibrolytic enzymes composed of xylanase and cellulase on the in vivo nutrient

digestibility for corn stalks, wheat straw, rice straw and sugarcane bagasse. They found that fiberolytic enzymes supplementation significantly ($P<0.05$) increased digestibility 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 com stalks.

Gado et al. (2007) studied the effect of biological treatment (cellulase; rumen liquor and *Cellumonas cellulasea*) on bagasse to improve the performance of Baladi goats. They indicated that treated bagasse with different treatments had a significant positive effect on DM, CP digestibility. However, cellulase enzyme increased ($P<0.05$) the percent of DM digestibility coefficient when compared with the other treatments digestibility of OM, EE, NFE positively affected by cellulase treatment.

Knowlton et al. (2007) studied the effect of an exogenous phytase and cellulase containing enzyme formulation on nutrient digestibility and excretion in Holstein cows. They found that cows fed the enzyme formulation had lower fecal dry matter, neutral detergent fiber, and acid detergent fiber excretion and lower fecal excretion of nitrogen and Phosphorus. Apparent digestibility of DM, ADF, and N tended to increase with the enzyme formulation.

In contrast, **Nadeau et al. (2000)** determined the effect of a cellulase alone or combined with bacterial inoculants on digestibility of orchard grass and alfalfa silages. They stated that cellulase application decreased silage NDF digestibility 18%.

Dhiman et al. (2002) evaluated production responses of Holstein dairy cows to cellulase and xylanase enzyme application on the forage portion of the diet. They concluded that feed intake, milk yield, milk energy output, milk components and body weight (BW) gain of cows were not affected by enzyme treatment

Knowlton et al. (2002) found that digestibility of DM was similar for control and fibrolytic enzyme-supplemented diets in early lactation cows, but in late lactation cows, DM digestibility was numerically greater with the enzyme addition compared to control. Apparent NDF digestibility was not affected by enzyme treatment. Apparent protein (P) digestibility, milk P, and P retained in body tissue were not significantly affected by enzyme treatment or by the interaction of stage of lactation and treatment. **Sutton et al. (2003)** investigated the effect of a fibrolytic enzyme application method on digestive processes. They indicated that rumen digestibility of dry matter and organic matter was unaffected by the enzyme. Digestibility of NDF was lowest on enzyme supplemented T diet in the rumen but highest post ruminally.

Muwalla et al. (2007) studied the effect of fibrolytic enzyme (FE) inclusion on nutrient digestibility of Awassi lamb fed on a high concentrate diet (with or without the addition of fibrolytic enzyme). They mentioned that Dry matter, OM, CP, and NDF digestibility were all unaffected by the enzyme inclusion. The effects of exogenous fibrolytic enzymes on digestibility of TMR with different forage: concentrate ratios were studied, using three treatments including (forage: concentrate) ratios (400:600, 500:500, 600:400 kg/kg) and two (0 or 2 g) levels of enzymes per/kg TMR DM. The study indicated that, there were no differences among treatments in DM and NDF digestibility due to enzyme addition to the diet.

(Pinos-Rodriguez et al., 2007) Effects of applying NH3 or a fibrolytic enzyme on the fiber concentration, and DM and fiber digestibility of two tropical grass bays were studied by **Dean et al. (2008)**. The enzymes were applied at (Control) 0.5, 1, and 2 times the rates recommended by the respective manufacturers. The authors indicated that fibrolytic enzymes had negligible effects on the extent of DM and fiber digestion of the hays.

2.4. Effect of treated agricultural by product on rumen and blood serum parameters

2.4.1. Effect on rumen parameters

The effect of fungal enzyme preparation on ruminal fermentation was studied on Wither Lamps. Ruminal pH, NH3 concentrations, TVFA and proportion of individual acids were not influenced by the addition of enzyme preparation. **(Judkins and Stobart, 1988).**

Feng et al. (1996) evaluated In vivo responses of enzyme treatments including enzyme applied to fresh forage, wilted forage, then dried; enzyme applied to dry forage immediately before feeding and untreated forage. The authors mentioned that ruminal fluid ammonia nitrogen concentration, total VFA concentration, and pH were not altered by dietary treatments.

Lewis et al. (1996) compared the delivery method of a solution containing cellulases and xylanases on the digestion of a forage-based diet using beef steers randomly assigned to a control or enzyme treatments. This study indicated that ruminal pH was lower and total VFA concentration at 6 h after feeding was greater for steers fed enzyme treatments compared with the control.

Broderick et al. (1997) reported that ruminal pH and ammonia concentration for Holstein cows were not influenced by the solutions of xylanase and cellulase enzymes supplemented to cow's diets. **Krause et al. (1998)** determined the effects of treating barley grain with a fibrolytic enzyme mixture on ruminal fermentation in cattle. They found no effect of diet on ruminal pH.

Beauchemin et al. (1999) studied effect of cellulase and xylanase enzymes on rumen parameters of cows fed barley hull-less. They found that ruminal pH and TVFA's concentrations were unaffected by cellulase and

xylanase treatments, while ruminal NH3-N concentration was significantly reduced for cows fed the hull-less barley treated or not treated by enzymes.

Yang et al. (1999) found that ruminal fermentation characteristics were not affected by enzymatic treatments. Ruminal VFA concentration was numerically higher for cows fed diets containing enzymes (cellulase and xylanase) than for cows fed the control diet

Nadeau et al. (2000) determined the effect of a cellulase alone or combined with bacterial inoculants on orchard grass and alfalfa silages. It was indicated that silage treated with cellulase had lower pH and NH3-N concentrations than untreated silage of both plant species.

Pinos-Rodriguez et al. (2002) demonstrated the effect of a directly fed exogenous fibrolytic enzyme on alfalfa and ryegrass hay digestibility by sheep. Treatments were alfalfa hay; alfalfa hay plus exogenous fibrolytic enzyme, ryegrass hay and ryegrass hay plus enzyme. The enzyme increased TVFA concentration (3 and 6 h) for both hays.

Gado et al. (2007) studied the effect of biological treatment (cellulase; rumen liquor and Cellumonas cellulasea) of bagasse to improve the performance of Baladi goats. They indicated that rumen liquor pH values did not differ significantly among treatments. Total VFA's values for treated bagasse by cellulase enzyme, rumen liquor and Cellumonas cellulasea were higher than that for untreated bagasse.

2.4.2. Effect on some blood serum parameters

Gado et al. (2007) reported that biological treatment (cellulase; rumen liquor and cellumonas cellulasea) of bagasse increased plasma total protein and urea concentrations. Also, **Kholif (2006)** found that animals fed on fibrolytic enzymes or fungi treated silage had higher values of serum total protein ($P<0.05$), albumin ($P>0.05$), and glucose. Serum globulin, urea total lipids GOT and GPT concentrations were not affected by treatments.

Bader (1993) fed different levels of treated fungal wheat straw to rams and reported that, the values of serum total protein were 6.94, 6.67 and 6.28 (g/dl) for group one (as a control group), group two (50 % fungal treated wheat straw) and group three (75% fungal treated wheat straw), respectively. Whereas for the different values at different intervals after feeding were 6.7, 6.25, 6.8 and 6.77 (g/d) for 0, 2, 4 and 6 hrs. post feeding, respectively. Concerning serum albumin, the mean values were 2.94, 3.13 and 2.59 (g/d) for groups 1, 2 and 3 ,respectively, while for different intervals the mean values were 3.09, 2.96, 2.74 and 3.02 (g/d) for 0, 2, 4 and 6 hrs. post feeding, respectively. The values of total serum globulin ranged from 2.77 to 4.2 (g/d) in blood serum. For albumin / globulin ratio (A/G ratio), the deference between such groups were not significant. Also, for serum urea, it was noticed to be the highest in the control group (0.41 g/dl) at all measuring times compared to the other treatments (0.298, 0.335 g/l for G2 and G3, respectively).

Zewil (2005) found that the biological treatment increased the level of total serum protein, albumin, globulin, urea concentration and serum creatinine values than untreated rice straw group (control group).

2.4.3. Effect on Sheep

In an experiment **(Mahrous et al., 2006)** testing four treatments, the first (T1) the control (rice straw untreated), (T2) was rice straw treated with ZAD ad. Libitum. (T3) was rice straw treated with fungs (Pleurotus osteratus) and the(T4) was rice straw treated with ZAD and fungus. Using twenty four Ossimi rams weighted 20.0± 0.3 Kg were used in feeding trial lasted 120 days to evaluate the effect of biological treatments on the nutritive value of rice straw and the animals had a bitter performance in treated groups than the control.

In a study(**Fatma et al.,2006**) using thirty cross bred (finnish x Rahmani) ram-lambs of about 22 kg were divided to six groups, six rations were formed as wheat straw (control ration) R1, wheat straw treated with 1 litter ZAD (R2), wheat straw treated with 2 litters ZAD (R3), bean straw as a control ration (R4), bean straw treated with 1 litter ZAD(R5), bean straw treated with 2 litter ZAD(R6). The period of the study was 180 days.

It was found that ration contained wheat straw treated with 2 litters of ZAD (R3) compound had significantly ($P<0.05$) the highest digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), nitrogen free extract (NFE). It had also the best ($P<0.05$) growth performance, followed by the ration contained bean straw treated with 2 litters of ZAD (R6), then rations contained wheat or bean straw with only one litter of ZAD compound (R2 and R5) and control rations(R1 and R4). It seems that the best ration for rumen metabolism.

2.4.4. Effect on Goats

In an experiment (**Gado 2007**) the design was 5 x 5 Latin square and the period was 21 days, goats about 20 Kg weight, were fed one of treated bagasse silage group pulse corn.

The chemical composition, nutritive intake and digestibility, nitrogen balance, rumen and blood parameters were measured.

Results indicate that treatment with rumen liquor enzyme caused higher digestibility ($P<0.05$) than the other treatments.

Anther experiment with Baladi goats **(El sayed et al., 2002)**Doing four digestibility and nitrogen balance trials on fed rations containing 25% of biologically treated cotton stallks.25%wheat straw and 50% concentrate feed mixture .Some rumen liquor and blood parameters were determined.

Result indicates that use of biological treatments (specially combined *T. virid* and *S. cerevisiae*) in goats rations is useful and did not cause any

abnormal condition on rumen activity. Liver and kidney functions, also the animal performance as well.

2.4.5. Evaluation differences of some ruminal bacteria by In vitro dry matter, cellulose and hemicellulose disappearance rate and extent of bagasse

(Gado et al., 2009) reported that study to evaluate the differences among five cellulolytic bacteria isolated from the rumen regarding in vitro dry matter, cellulose and hemicellulose disappearance. Cellulolytic bacteria such as Cellulomonas cellulasea, *Acetobacter xylinum*, *Thermonospora fusca*, *Ruminococcus albus and Bacillus sp.* had been isolated from cow, sheep, buffalo and camel.

Sugarcane bagasse was incubated with each strain for 48 hrs. incubation (extent) and the regression coefficient of in vitro was rate of disappearance per hour. The increase recorded of in vitro dry matter disappearance values. Five strains isolated from sheep were more effective than that isolated from buffalo and cow.

The results revealed that *Cellulomonas* and *Ruminococcus* isolated Born camel showed the highest ($p<0.05$) value of in vitro cellulose disappearance of bagasse (65% and 62%). While Bacillus isolated from sheep showed the highest ($p<0.05$) value of in vitro cellulose and hemicellulose digestion (53% and 47%, respectively).

Thermonospora and *Acetobacter* isolated from cow showed higher of in vitro cellulose digestion (56% and 46%) more than those isolated from camel, sheep and buffalo. The results recorded that *Thermonospora* isolated from cow had the highest value of in vitro hemicellulose disappearance (52%), while Cellulomonas isolated from buffalo showed the highest value (64%).

Acetobacter and *Ruminococcus* isolated from camel showed the highest value of hemicellulose (44% and 62%). it was concluded that the live strains isolated from four ruminant animals significant differences on in vitro dry matter, cellulose and hemicellulose disappearance of sugarcane bagasse with the same strain.

2.4.6. Use of biotechnology to improve the utilization of rumen contents in ruminant rations

(Kholif, 2006) reported that study using twelve lactating Baladi goats weighing 24-27 kg live weight in the first week of lactation were randomly assigned among four experimental treatments (three animals each) using 4x4 Latin square design.

The period of this trial extended for 120 days divided to four experimental periods each of 30 days. Animals were fed the following treatments: 60% concentrate feed mixture (CFM) and 40% berseem clover (Control), 60% CFM + 20% berseem clover + 20% dried rumen contents (T1), 60% CFM‘+ 20% berseem clover + 20% dried rumen contents treated with biological compound ZAD (T2) and 60% CFM + 20% berseem clover + 20% dried rumen contents treated with ZAD compound + 20g biological compound ZADO /h/d (T3). Results showed that (T3) and (T2) groups recorded higher values of digestibility coefficients compared with the control and (T,) groups.

Total digestible nutrients (TDN) and digestible crude protein (DCP) increased with all biological treated rumen contents (T3 and T2) and control group in comparison of (T1) group. The groups contained rumen contents (T1, T2 and T3) recorded higher values ($P>0.05$) of ruminal pH and NPN than control group. The biologically treated groups (T 2 and T2) showed higher increase ($P<0.05$) for rumen liquor ammonia, NPN and TVFA's ($P>0.05$) compared with (T1) group.

However, (T2), (T2) and control groups increased (P>0.05) ruminal TN and True-PN (P<0.05) compared with T1 group. Moreover, results showed that inclusion of rumen contents in ration decreased (P>0.05) blood serum total proteins, globulin, urea, creatinine and AST compared with control ration.

While, it increased (P>0.05) ALT compared with control. The treated group (T3) increased (P<0.05) blood serum albumin and total lipids compared with (T1) group.

Also, (T3) and (T2) increased (P>0.05) blood serum total proteins, A/G ratio, ALT and glucose compared with (T1) group. However, (T1) group increased globulin, creatinine and AST compared with other groups.

(T3) and (T2) groups increased (P>0.05) milk yield, 4% FCM, fat yield, TS yield, SNF yield and lactose yield compared with (T1) group. All the groups contained rumen contents slightly increased (P>0.05) milk pH compared with control group. However, control group increased (P>0.05) SNF yield, lactose yield, ash yield and milk acidity compared with the groups contained rumen contents.

Feed efficiency was insignificantly (P>0.05) improved with biological and control treatments it could be concluded that feeding animals on ration containing sun dried rumen contents treated with ZADO or/and ZAD compounds improved the performance of lactating goats without any adverse effect on animals health.

2.4.7. Effect of some biological treatments on the nutritive value of agricultural by-products

(El-Mahy, 2009) reported that study in order to evaluate the effect of biological treatments on chemical composition, in vitro and in vivo digestibility, nutritive value and N- balance of some low quality roughages i.e., wheat straw, rice straw, com stalk and sugarcane bagasse.

The effect of the biological treatments on rumen fermentation and some blood parameters were also determined. Control samples (untreated) were sun dried to about 90% DM. The experimental treatments were either 1 or 3 liters of ZAD compound (a biotechnical product made from natural sources of cellulase enzyme from anaerobic bacteria) added to 1000 litter water + 50 kg molasses and 20 kg urea for l ton of the feedstuff The samples were treated with the ZAD compound and pressed in plastic bags (holding 1 kg) and closed for either one, two or four weeks. Two 3 x 3 Latin square designs were applied in the in vivo trial using three Ossimi rams. The results obtained reveal that:

1- The CP content was significantly increased in all treated materials with all levels of ZAD. Values of CP were linearly increased as the time of ensiling increased.

2- The biological treatment with ZAD caused a decrease in most fiber traction especially with sugarcane bagasse.

3- The biological treatments improved (P<0.0l) IVDMD and IVOMD for all the tested roughages. The highest values of IVDMD and IVOMD were reported with 3 litters ZAD for four weeks.

4- The incubation media (positive control) of sugarcane bagasse improved DM digestibility from 29.86% to 37.29% while ZAD increased (P<0.0l) DM digestibility up to 54.61%.

5- Digestibility of CP was equal for RS- and RS+ (19.21 and 21.31%) and for SC- and SC+ (37.11 and 37.0%) and higher for the ZAD treated ones (60.19 and 65.07%).

6- No differences were found between negative and positive controls being 27.26 and 30.14 for RS- and RS+ and 31.19 and 30.46% for SC- and SC+ ,While ZAD treatment improved (P<0.0l) CF digestibility by about 30 unit in RS (60.12%) and 15 unit in SC (45.13%).

7- In general, treatment of both rice straw and sugarcane bagasse with BL of ZAD improved most of the call wall constituent's digestibility.

8- Feeding values (TDN and DCP) were improved due to the biological treatment of both rice straw and sugarcane bagasse.

9- Nitrogen balance and biological value improved only with RS and SC treated with ZAD. The N output positively correlated with N intake.

10- Treated RS and SC with ZAD caused an increase in VFA and NH3- N in the rumen of sheep; it reached the peak at 4h post feeding; pH followed the opposite trend.

11- The levels of all blood parameters were within the normal ranges.

It could be concluded that treating the available roughage sources with ZAD improved the chemical composition, digestibility, feeding value, N balance and rumen fermentation without any adverse effect on the functions of neither liver nor kidney. Therefore, it could be recommended that the best level of ZAD treatment is 3 liter and ensiling time of 4 weeks. The best effect was reported for sugarcane bagasse.

2.4.8. Effect of anaerobic enzyme matrix on fiber digestibility

(Gado et al., 2010) reported that study to verify the effect of nutritive value aerobic constancy of rice straw.

Although, if it could be enhanced by addition of exogenous cellulases, hemicellulase, protease and α amylase enzymes (ZAD) preparations at ensiling. Rice straw was chopped to 5 cm without treatment (control) or after treatment with ZAD (1 or 3 L/ 1 ton of DM of rice straw) including 30 kg of sugar cane molasses and 20 kg of DDGS. The enzymes were sprayed on the rice straw at ensiling (50% of water was added). Ten 500-kg replicates of chopped (5 cm) rice straw were ensiled for 30 d in plastic bales.

Five plastic bales per treatment were used for chemical analysis and 5 for aerobic constancy monitoring. The silage juice was analyzed for organic

acids, pH, water-soluble carbohydrates (WSC), ammonia-N, and soluble N. Samples were analyzed for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). In vitro digestibility of DM (IVDMD), NDF (IVNDFD), and ADF (IVADFD) were determined.

Materials treated with ZAD had lower ($P < 0.05$) DM losses, and lower ($P < 0.05$) pH and ammonia-N concentration than control silages. Residual WSC concentration was greater ($P < 0.01$) in ZAD treated silages either 1 or 3 L than in control silages. Compared with control silages, NDF concentration was lower ($P < 0.01$) in silages treated with 3 L followed 1 L of ZAD.

Aerobic constancy was increased ($P < 0.05$) by ZAD. ZAD at 3L increased the IVDMD and IVNDFD at 6 and 48 h. The 48-h IVADFD was also increased ($P < 0.01$) by treatment with 3L ZAD. These results show that 3L ZAD applied at ensiling can improve the digestibility, fermentation, and aerobic constancy of rice straw silage.

2.4.9. Enzymatic treatments of sugarcane bagasse by different sources of cellulase enzymes

(Gado et al., 2009) reported that work to study the effect of using three sources of cellulase enzyme for sugarcane bagasse treatment under anaerobic conditions. Two experimental trials were conducted; Experiment 1: was designed to study the effect of biological treatments on chemical composition and structure of cell wall of bagasse. Experiment 2: Rate and extent technique using in vitro procedure to compare among the effect of six treatments on dry and organic matter disappearances (IVDMD and IVOMD) of bagasse silage.

Bagasse was ensiled or not using one of the following treatments: (1) untreated control, (2) additive mixture solution, (3) cellulase enzyme 15%, (4) cellulase enzyme 25%, (5) rumen liquor, and (6) *Cellulomonas*

cellulasea. Results indicated that addition of 15% cellulase enzyme to bagasse caused the highest DMD and OMD while the untreated bagasse silage had the lowest value.

It was concluded that enzymatic treatments of bagasse silage improved its chemical composition, DMD and OMD through its effect on cell wall structure.

2.5. Feeding values of rations

El-Marakby (2003) noted that TDN and DCP of rations containing wheat straw were significantly (P<0.01) lower than the control ration. The values of TDN and DCP decreased by 15.26, 11.84%, 31.65 and 23.51 in rations containing (50% CFM +SWS ad lib.) than ration containing (25% CFM +SWS ad lib.), respectively.

Zewil (2005) found that the mean values of DM intake expressed as g/h/d was significantly (P<0.01) affected by feeding T1 (25% CFM +ad lib biological treated rice straw), while the control ration (T2) recorded the lowest values. Average daily gain was 122 and 65 gm. for lambs fed T1 and T2, respectively, during the whole period of the trail.

The same author reported that the average feed efficiency values expressed as Kg TDN /Kg gain were 5.62 and 4.87 for lambs fed T1 and T2 ,respectively, while the corresponding average of feed efficiency as Kg DCP /kg gain were 0.84 and 0.69 ,respectively .

Economical cost expressed as feed cost per one kg weight gain for T1 showing better (4.86 L.E. /kg weight gain) than control group. (10.65 L.E. /kg weight gain).

Ward and Perry, (1982) compared ground corn cobs treated with cellulose from *T. Viridi* and untreated in a diet of lamb digestion of DM (4.8%), NFE (9%) TDN (18%) and decreased in digestibility of CF (41%) and ether extract (8.8%).

Deraz, (1996) indicated that TDN values of control and biological treated rice straw were 63.96 and 72.31, respectively. Also, the biological treated rice straw was higher in terms of DCP (11.74%), compared to control ration (8.08%).

El- Ashry et al., (1997) found that both chemical and biochemical treatments of crop residues improved significantly ($P<0.05$) the nutritive value of rations as total digestible nutrients (TDN) and digestible crude protein (DCP) compared to control rations.

Felton et al., (2001) reported that the ruminally degradable protein RDP requirement of nonstructural carbohydrate NSC fermenting bacteria was lower than that currently fed in many diets.

2.6. Fibrolytic Enzyme Treatment of Barley Grain

(Krause et al., 1998) they conducted a study to determine the effects of treating barley grain with a fibrolytic enzyme mixture on chewing activities, ruminal fermentation, and total tract digestibility in cattle. We also investigated the potential benefits of using barley straw rather than barley silage as a roughage source in high-grain diets for feedlot cattle. Steers were given ad libitum access to one of four diets that consisted of 95% barley-based concentrate and 5% forage (DM basis).

The concentrate was either control or enzyme treated, and the forage was either barley silage or barley straw. Applying the enzyme mixture onto the barley lowered the concentrations of dietary ADF and NDF. However, it is not certain when this fiber hydrolysis occurred relative to feed consumption because the fiber analyses were conducted after the study was completed. Enzyme treatment of barley increased total tract dietary ADF digestibility by 28% ($P < .05$). Acetate-to-propionate ratio tended to decrease, which suggests that enzymes may have increased ruminal starch digestion as a result of enhanced digestion of barley hulls.

Replacing silage with straw increased ADF intake ($P < .05$) and resulted in 1-h/d increase in rumination time ($P < .05$). Even though there was no effect of diet on ruminal pH, replacing silage with straw increased ruminal acetate, as a percentage of total VFA, and total tract ADF digestion ($P < .01$).

This study demonstrates that using a fibrolytic enzyme mixture in high-grain diets that contain mainly barley grain can improve fiber digestion and grain utilization, but the mode of action is unclear. Straw can be used rather than silage to increase the effective fiber content of a high-grain feedlot diet.

2.7. Green fodder production under hydroponic conditions

(Ghazi et al 2011) they conducted a study to reduce agricultural water use while maintaining or improving economic productivity of the agricultural sector is a major challenge in arid and semiarid regions

Hydroponic green fodder production affords advantages in direct natural resource use (less water required) and higher productivity (i.e., much higher product yield per unit of resources used) relative to the conventional field cultivation.

The objectives of this study were to evaluate five forage crops alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), cowpea (*Vigna unguiculata*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*) for green fodder production and water use efficiency under hydroponic conditions. The experiment has been conducted under temperature controlled conditions (24±1ºC) and natural window illumination at growth room of Soilless Culture Laboratory, Sultan Qaboos Center for Developed and Soilless Agriculture, Arabian Gulf University, Manama, Bahrain.

The results showed that green forage can be produced in 8 days from planting to harvest using hydroponic technique. Highest values for green

fresh yields were recorded for the crops cowpea, barley, and alfalfa which gave 217, 200, and 194 tons/ha, respectively. However, only cowpea and barley crops gave the highest green dry yield, but not alfalfa. Barely crop used water more efficiently than the other four tested crops when produced about 654 kg fresh matter/m3 water in comparison to 633, 585, 552 and 521 kg fresh matter/m3 water for cowpea, sorghum, wheat and alfalfa, respectively.

No significant differences between barley and cowpea for water use efficiency were noted. From results of this study, it can be concluded that barley crop is considered the best choice that can be used for production of hydroponic green fodder with less water consumption, especially seeds of this crop are mostly available in the market at lower price than others which reduce the cost of hydroponic fodder production.

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.**

3- 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 Meat and Milk Development Center (in Shalakan), Faculty of Agriculture, Ain Shams University, and in the Egyptian company (ECARU).

3.1. Preparing of rice straw

This experiment was carried out to avoid rice straw boiling during preparation process before plantation, which was the procedure practiced in all previous experiments, for the purpose of sterilization and to free materials from most of their impurities.

The first experiment was performed to growing barley on the soaked straw without boiling and to be compared with boiled straw.

3.2. Seed Selection

This experiment was carried out in order to choose the best type of seeds in terms of growth speed and endurance of thirst on the hay and the strength of Plant growth using some annual winter and summer seeds (such as Cow Peas (*Vigna Sinensis,L*) and Sudan grass (*Sorghum Vulgare Var Sudanese*), Peart Millet (*Pennisetum Culuceum,L*), Sweet Sorghum (*Sorghum Valgare Var Saccharatum*), Fenugreek (*Trigonella Foenum-graecum*), Barley (*Hordeum Vulgare*) and Canary grass seeds (*Phalaris Canariensis*) and a mixture of 1/3 Fenugreek with 2 / 3 Barley) . the comparison was depended on chemical analysis of the final output, taking into account the economical evaluation where the seed price of along with the rest of the specifications.

3.3. The quantity of straw and of barely

The amount of rice straw in 0.1 square meters was 0.5 Kg. with this amount the chemical composition and the growth of shoots was very good.

The next experiment was designed to find out the optimum amount of seeds to spread on the straw. It was chosen 50% and 25% and 30%, this amount calculated according to **Anwar 2009.**

3.4. Mixing between rice straw and orange pulp

The aim of this trial is to use orange pulp in order to provide more energy to the animals and to improve the palatability.

We mixed the orange pulp to rice straw at the following percentages: 50:50, 75:25 and 90:10.

3.5. The optimum quantity of water

This experiment carried out in order to reduce the amount of water lost during the irrigation. This was done using a plastic sheets placed under the straw. It was started with 500 ml water per day in 0.1 square meters and reached to the amount of 200 ml water in the same area per day but we must cover the seeds with plastic sheets for the first 6 days of 15 days in summer.

3.6. The optimum quantity of enzyme

In experiment (1) is to reduce the amount of enzyme used to reduce the economic cost. We start with 30,20, and 10 ml of the enzyme (ZAD) the result shown that is 20 ml is the best for changing the chemical composition and economically.

Experiment (2)l was done through the use of soaking in the enzyme with water once for 12 hr. at the beginning of the experiment - instead of irrigation it with water throughout the experiment (with amount of 20 ml enzyme per liter of water) - and that for straw once, and the seeds once or the two together with a permutation of them.

Experiment (3) was carried out mixing between soaking in enzyme (3 ml enzyme /Kg of both seeds and straw) and irrigation with enzyme of

amount 10 ml/1 litter of water for the first 6 days only from plantation, and that was the best.

3.7. Ensiling experiment

In this experiment, 1.34 tons of rice straw were used and sprayed with water and ZAD enzyme at the same time 152 kg of barely seeds were socked in water and enzyme as according to our procedure which explained earlier. The concentration of enzyme in soaking water was 3 ml per 1 kilo of either rice straw or barely seeds. The socked straw was placed on plastic sheets, then spread the socked seeds and irrigated with the rest of socking seeds water, after that it was covered with a plastic sheet for the first 6 days only of the 15 days of the experiment; the plants were irrigated daily with 10 ml enzyme (ZAD) per 1 litter of water daily for the first 6 days.

Three days before harvest, we stopped the irrigation in order to increase the dry mater and to save more water; the trial lasted two weeks until plants reached 18 cm height.

After that each treatment was put in a mixer wagon separately, and then the orange pulp were added for (treatment T2 and T4)2 treatments, one with enzyme (ZAD) and the other without enzyme, and then all treatments were pressed in bales. Samples were taken for chemical analysis and the chemical composition is shown in Table (28).

The 4 treatments were as follow

-Treatment1 (T1), CONTROL: Rice Straw with Grown Barely (RSGB) without enzyme (ZAD), without orange pulp.

- Treatment1 (T2): RSGB with enzyme (ZAD), without orange pulp.
- Treatment1 (T3): RSGB without enzyme (ZAD), with orange pulp.
- Treatment1 (T4): RSGB with enzyme (ZAD), with orange pulp.

3.8. Proximate analysis

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

3.9. 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 difference.

The present study was divided into three experiments:

- The first experiment:

- *In vitro* rate (**Merten, 1977**) technique was conducted to study the effect of different sources of the experiment 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 for the experimental treatments, and feeding values of treated RSGB using 12 male of Ossimi sheep divided on 4 randomly treatments 3 rams each.

- The third experiment (Feeding trial)

This experiment was done to study the effect of biological treatments on feed intake, growth rate and feed efficiency of Ossimi sheep fed rice straw treated with ZAD® enzyme and rumen Liquor.

3.10. *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.

Two tubes as a replicate of each sample were used at different incubation times (0, 2, 4, 6, 8, 24, 48 hrs.). Two blank tubes were prepared for each incubating time. Rumen fluids were collected by stomach tube from the rumen of three mature Ossimi sheep which were fed only on berseem hay as a basal diet. Fluids from different sheep were mixed together to have one representative sample of rumen fluid.

The fluids were squeezed through 4 layers of cheese cloths and placed in pre warmed thermos.

The ruminal fluid was immediately flushed with carbon dioxide (CO2) and placed in water bath (39°C) until particulate matter rises to the top, dark material at bottom which was discarded and the clear yellow portion was mixed with pre warmed buffer. The ruminal fluid and buffer were mixed in a ratio of 1: 4, respectively. The components of the buffer used are shown in **Table (2).**

Approximately 0.5 gm. of the sample was added into 50 ml in vitro tube. The buffer and rumen liquor were placed in 39°C water bath. Carbon dioxide was bubbled through the buffer and rumen liquor 5 ml of the rumen liquor and 20 ml of the buffer were dispensed into the *in vitro* tube dispersing the sample and swirling to break up all clumps.

Table (2): The components of the buffer

Compound	gm / Littre
$NaHCO_3$	9.8
Na_2HPO_4	3.71
KCI	0.57
NaCl	0.47
$MgSO_4.7H_2O$	0.12
Urea	0.5
$CaCl_2 2H_2O$	0.05
PH value	6.9 - 7.0

The tube then was stopper and placed in 39^o C water bath. The samples were incubated for 2, 4, 6, 8, 24, and 48 hrs.

All the tubes were swirled gently several times during the incubation period.

The calculations

$$\%DM\ disappearance = \frac{1-(R-F)-Blank}{Sample\ DM} \times 100$$

Where:

R= Weight of residue and filter paper.
F=Weight of filter paper.
Blank=(R-F) when substrate is not added medium.

3.11. Metabolism trial (in vivo)

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

in 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 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 the forced air oven at 60-65°C until constant weight. The dried fecal samples per each animal were mixed and kept for laboratory analysis. Urine samples were also collected daily for each goat in glass bottles contained fifty mal diluted sulphoric 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 were 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 **A.O.A.C., (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.

$$\text{Digestibility} = 100 - \left[100\, x \frac{\%\ indicator\ in\ \ feed}{\%\ indicator\ in\ \ feces}\, x \frac{\%\ nutrient\ in\ \ feces}{\%\ nutrient\ in\ \ feed} \right]$$

3.12.1. Rumen liquor parameters

3.12.1.1. Rumen liquor samples

Rumen liquor samples were collected from all experimental sheep during metabolism trial pre experimental feeding (0 day) at zero, 3 and 6 hrs. 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 4 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.

3.12.1.2. Determination of rumen pH

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

3.12.1.3. Ammonia nitrogen concentration (NH, - N)

Ammonia - N. concentrations were measured by modified semi-micro kijeldehl digestion method **(A.O.A.C., 1995).**

Calculation of NH3 – N

NH_3 - N, concentration % =

$$\frac{ml.acid\ to\ titrate \times N.\mathrm{H2SO4} \times 0.014}{Volume\ of\ rumen\ fluid(1ml)} \times 100$$

3.12.1.4. Total volatile fatty acids (TVFA's)

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

Calculations of total volatile fatty acids:
Total volatile fatty acids = ml. Sodium hydroxide titrate x N. NaOH x 100 (m.eq per 100 ml rumen fluid).

3.12.2. Blood parameters

3.12.2.1. Blood plasma sampling

Blood samples were collected from 3 animals per treatment during the metabolism trial at zero, 3 and 6 hrs. 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 r.p.m.,

plasma was separated into clean dried glass vials and stored at freeze (-20°C) till analysis.

3.12.2.2. 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).**

3.12.2.3. Plasma total protein

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

3.12.2.4. Plasma total lipids

It was determination colorimetrically using commercial kits of Diamond Biodignostic using the method of **Zollner and Kirsch (1962).**

3.12.2.5. Plasma creatinine

It was determination colorimetrically using commercial kits of biomerireux using the method of **Henary (1974).**

3.12.2.6. and 7. Glutamate oxalo – acetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT)

Plasma glutmic-oxaloacetat transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were determination colorimetrically using commercial kits of biomerireux using the method of **Reitman and Frankel (1957).**

3.13. Experiment III (Feeding Experiment)

3.13.1. Animal and rations

The objective of this trial was to study the effect of biological treatments of rice straw silage on feed intake, growth rate and feed efficiency of Ossimi sheep.

Twenty (20) female Ossimi sheep were used in this study; sheep were bought from the local markets with an average weight of 22.8 kg, and 4:6 months old.

All sheep were well examined clinically for parasitic infestation during the preliminary period of three days (out of the experimental period) and they were dosed against internal and external parasites.

Sheep were also vaccinated against infectious diseases before starting the experimental periods.

All groups were fed at maintenance and growth requirements according to **NRC, (1985);** since the treatments of the enzyme free solution and bacteria showed the lowest digestibility in experiment Ill, therefore these treatments were not included in the feeding experiment.

3.13.2. Management

Animals were randomly distributed into four experimental groups of three animals each group.

The average initial live body weights (LBW) of the different groups were 22.20, 22.60, 25.80 and 20.60 kg for groups T1, T2, T3, and T4, respectively, Each group (five animals) was housed in a semi shaded pen and were groups fed. Sheep were fed according **NRC, (1985)** requirements.

Daily **RSGB**'S were offered ad libitum; the animals were received their daily rations in addition to the water. Feed residuals (if any) were collected and weighted to calculate actually daily feed intake. The animals were weighed weekly in the early morning before feeding (fasted).

Animal performance was taken through the entire experimental period for each experimental group.

3.14. Statistical analyses

All traits were statistically analyzed by 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.

Data of rumen liquor were analyzed according to repeated measures design, where the model was; $Y_{ijkl} = \mu + T_i + A_j (T_i) + S_k + (T*S)_{ik} + E_{ijkl}$

Where, Y expressed the every observation of the j^{th} animal in the k^{th} sampling time given i^{th} treatment, T (1-4) expressed the treatments effect, A (T) expressed the animal within treatments, S (1-3) expressed the sampling time effect, T*S expressed the interaction between the treatments and sampling times effect and E expressed the experimental error.

4- Results and discussion

PART I

The present investigation was conducted to study the effect of biological treatments on some poor quality roughages 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 done.

4.1. Effect of boiling water and soaked water and ZAD on the Chemical composition of rice straw

In this trial the rice straw was irrigated with water, with and without ZAD enzyme. And that after boiling or after soaking in water. The data is presented in **Table (3).**

Table (3): Effect of boiling and soaked water and ZAD on the Chemical composition of rice straw

	Boiled rice straw		Soaked rice straw		**±SE**	**P value**
	- ZAD	+ ZAD	- ZAD	+ ZAD		
%Moist.	82.80[b]	83.37[a]	82.53[c]	82.50[c]	±0.07	<.0001
%CF	40.88[a]	30.55[d]	40.76[b]	30.71[c]	±0.01	<.0001
%EE	1.26[b]	1.26[b]	1.53[a]	1.03[c]	±0.02	<.0001
%CP	3.31[c]	5.31[a]	3.31[c]	5.11[b]	±0.01	<.0001

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

From **Table (3)** it can be observed that adding ZAD increased CP but decreases the CF. Also, the results showed that there is no significant difference between the two treatments. However, we benefited from this trial in the reducing of the time and effort and workers users to prepare the straw before planting.

4.2. Effect of growing some seeds using ZAD

4.2.1. Using of Cow Peas, Sudan grass, Peart Millet and Sweet Sorghum (50% cover percentage)

In this experiment every type of seed was planted separately on a soaked rice straw and irrigated with water plus ZAD enzyme. The concentration of enzyme was 20 ml per litter of water for irrigation; the plants were irrigated twice a day for 10 days.

The amount of water was 1 litter per day. The chemical composition results lasted in **Table (4).**

Table (4): Chemical composition whole plant +rice straw media

	Cow Peas	Sudan grass	Peart Millet	Sweet Sorghum	± SE	P value
% Moist.	83.48[a]	82.78[b]	82.48[c]	82.50[c]	±0.08	<.0001
% CF	28.14[c]	32.95[a]	26.61[d]	31.67[b]	±0.05	<.0001
% EE	7.83[d]	8.24[b]	8.12[c]	8.90[a]	±0.01	<.0001
% CP	10.10[b]	5.02[d]	9.75[c]	11.43[a]	±0.02	<.0001
% Ash	17.01[b]	16.22[c]	17.16[a]	13.91[d]	±0.02	<.0001

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

In Table (4) it was found that the CP and EE of Sweet Sorghum was the highest among the other plants in this trial. On the other hand the lowest CP in the trial was Sudan grass.

The highest CF was for the Sudan grass but the lowest was for Peart Millet.

After this trial it found that:

The shoots were weak in the Cow Peas and relatively weak in the Sudan grass and sweet sorghum but in the case of peart millet plants were strong in growth but it was feeble to tolerate excess or shortage of irrigation or fall of water drops on it.

Also found that the price of alfalfa seeds is very expensive in non-economic way, which led to their exclusion.

So it shown that the Barley was the best seed in both growth and economic.

4.2.2. Using of Fenugreek 50% cover percentage

In this trial the fenugreek was grown on soaked previously treated rice straw (the rice was treated with ZAD enzyme before use) and irrigated with water, with and without ZAD enzyme separately.

The concentration of enzyme was 20 ml per litter of water for irrigation; the plants were irrigated twice a day for 8 days. The amount of water was 1 litter per day. The chemical composition results of this trial using fenugreek seed with50% percentage lasted in **Tables (5, 6, and 7).**

Table (5): Chemical composition of steam and the leaves of the plants

	Fenugreek only **green** part of plant		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	88.67	88.37	±0.18	<.0001
% CF	21.60[a]	20.50[b]	±0.06	<.0001
% EE	3.05[a]	1.01[b]	±0.02	<.0001
% CP	46.39[a]	45.30[b]	±0.10	<.0001
% Ash	17.05	17.01	±0.02	<.0001
%NDF	19.05[b]	33.17[a]	±0.07	<.0001
%ADF	22.23[b]	25.10[a]	±0.05	<.0001
%ADL	4.05[b]	7.10[a]	±0.05	<.0001

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

In **Table(5)** it can be observed the access of CP and the EE results was 46.39 and 3.05 respectively, for the treated plants and on the other hand the decrease of CF, NDF,ADF and ADL .

It can be say that the irrigation with ZAD affected the chemical composition of the green shots of the plants.

Table (6): Chemical composition of the root + rice straw media.

	Fenugreek only **root** part of plants + media		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	82.60[a]	77.63[b]	±0.10	<.0001
% CF	31.46[b]	39.53[a]	±0.06	<.0001
% EE	5.03[a]	4.01[b]	±0.02	<.0001
% CP	13.36[a]	11.26[b]	±0.07	<.0001
% Ash	22.16[b]	23.10[a]	±0.07	<.0001
%NDF	37.01[b]	76.61[a]	±0.01	<.0001
%ADF	51.11[b]	60.51[a]	±0.01	<.0001
%ADL	5.01[b]	8.01[a]	±0.01	<.0001

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

In Table (6) it can be observed the effect of ZAD on the access of CP to 13.36 from 11.26, also adding ZAD decreases the CF, Ash, NDF, ADF and ADL.

It can say that ZAD improved the chemical composition of rice straw as poor quality roughage to the way that the animal can consume it perfectly.

Table (7): Chemical composition of whole plants +rice straw media

	Fenugreek **whole** plant		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	82.60	82.63	±0.11	<.0001
% CF	31.50[b]	38.79[a]	±0.04	<.0001
% EE	2.05[a]	1.01[b]	±0.02	<.0001
% CP	14.84[a]	12.34[b]	±0.02	<.0001
% Ash	20.05	20.23	±0.10	<.0001
%NDF	34.43[b]	74.43[a]	±0.13	<.0001
%ADF	50.10[b]	58.71[a]	±0.04	<.0001
%ADL	3.50[b]	7.01[a]	±0.04	<.0001

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

In **Table** (7) it can be observed the decreases in CF to 31.5 for the treated with ZAD from 38.79 for the untreated one, also the increasing of CP with 14.84 with ZAD, and the increase of EE to 2.05 from 1.01.

It can be notice the decreases in NDF, ADF and ADL for the treated with ZAD.

From this result it can say that treating with ZAD made a good addition to the poor quality roughage of rice straw and improve it to become useful to the ruminant animals.

4.2.3. Effect of ZAD on the chemical composition of Barley (50% cover percentage)

In this trial the Barley was grown on soaked treated rice straw (the rice was treated with ZAD enzyme before use) and irrigated with water, with and without ZAD enzyme separately.

The concentration of enzyme was 20 ml per litter of water for irrigation; the plants were irrigated twice a day for 8 days in season and for 15 days out of season. The amount of water was 1 litter per day. The chemical composition of this trial lasted in **Table (8, 9 and 10).**

Table (8): Chemical composition of steam and the leaves of the plants

	Barely only **green** part of plant		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	89.60[a]	81.40[b]	±0.12	<.0001
%CF	27.47	27.60	±0.06	<.0001
%EE	6.16[a]	3.63[b]	±0.09	<.0001
% CP	35.30[a]	32.40[b]	±0.18	<.0001
%Ash	24.0[a]	19.8[b]	±0.58	<.0001
%NDF	26.59[b]	44.01[a]	±0.04	<.0001
%ADF	25.10[b]	28.00[a]	±0.04	<.0001
%ADL	2.02	3.0	±0.01	<.0001

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

In **Table(8)** it can be observed the access of CP and the EE results was 35.30 and 6.16 respectively, for the treated plants and on the other hand the decrease of CF, NDF,ADF and ADL .

It can be say that the irrigation with ZAD affected the chemical composition of the green shots of the plants.

Table (9): Chemical composition of the root + rice straw media.

	Barely only **root** part of plants + media		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	80.1[b]	82.2[a]	±0.06	<.0001
%CF	33.05[b]	39.10[a]	±0.05	<.0001
%EE	0.70[a]	0.43[b]	±0.05	<.0001
% CP	13.40[a]	11.60[b]	±0.06	<.0001
%Ash	20.0[a]	16.01[b]	±0.41	<.0001
%NDF	36.01[b]	76.17[a]	±0.12	<.0001
%ADF	49.30[b]	55.41[a]	±0.11	<.0001
%ADL	5.03[b]	7.02[a]	±0.02	<.0001

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

In Table (9) it can be observed the effect of ZAD on the access of CP to 13.40 from 11.60, also adding ZAD decreases the CF, NDF, ADF and ADL.

It can say that ZAD improved the chemical composition of rice straw as poor quality roughage to the way that the animal can consume it perfectly.

Table (10). Chemical composition of whole plants +rice straw media

	Barely **whole** plant		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	81.05[b]	82.36[a]	±0.03	<.0001
%CF	32.46[b]	38.10[a]	±0.05	<.0001
%EE	1.30[a]	0.54[b]	±0.05	<.0001
% CP	15.90[a]	14.99[b]	±0.04	<.0001
%Ash	19.0	19.60	±0.58	<.0001
%NDF	33.0[b]	73.46[a]	±0.02	<.0001
%ADF	44.10[b]	54.86[a]	±0.05	<.0001
%ADL	4.03[b]	6.02[a]	±0.02	<.0001

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

In **Table** (10) it can be observed the decreases in CF to 32.46 for the treated with ZAD from 38.10 for the untreated one, also the increasing of CP with 15.90 with ZAD, and the increase of EE to 1.30 from 0.54.

It can be notice the decreases in NDF, ADF and ADL for the treated with ZAD.

From this result it can say that treating with ZAD made a good addition to the poor quality roughage of rice straw and improve it to become useful to the ruminant animals.

4.2.4. Using of Canary grass seeds 50% cover percentage

In this trial the Canary grass was grown on soaked treated rice straw (the rice was treated with ZAD enzyme before use) and irrigated with water, with and without ZAD enzyme separately.

The concentration of enzyme was 20 ml per litter of water for irrigation; the plants were irrigated twice a day for 8 days. The amount of

water was 1 litter per day. The chemical composition of this trial lasted in **Table (11).**

Table (11): Chemical composition of whole plant +rice straw media

	Canary grass **whole** plant		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	76.82[a]	76.34[b]	±0.03	<.0001
%CF	25.43[b]	35.50[a]	±0.05	<.0001
%EE	3.21[a]	1.31[b]	±0.003	<.0001
% CP	14.93[a]	13.63[b]	±0.02	<.0001
%Ash	21.0[a]	19.0[b]	±0.46	<.0001
%NDF	64.00[b]	73.11[a]	±0.004	<.0001
%ADF	43.02[b]	55.06[a]	±0.003	<.0001
%ADL	3.0[b]	4.0[a]	±0.003	<.0001

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

In **Table** (11) it can be observed the decreases in CF to 25.43 for the treated with ZAD from 35.50 for the untreated one, also the increasing of CP with 14.93 with ZAD, and the increase of EE to 3.21 from 1.31.

It can be notice the decreases in NDF, ADF and ADL for the treated with ZAD.

From this result it can say that treating with ZAD made a good addition to the poor quality roughage of rice straw and improve it to become useful to the ruminant animals.

4.2.5. Using of 1/3 of fenugreek seeds with 2/3 of Barley seeds (50% cover percentage)

In this trial the mixing seeds of 1/3 fenugreek and 2/3 Barley was grown on soaked treated rice straw (the rice was treated with ZAD enzyme before use) and irrigated with water, with and without ZAD enzyme separately.

The concentration of enzyme was 20 ml per litter of water for irrigation; the plants were irrigated twice a day for 8 days. The amount of water was 1 litter per day. The chemical composition of this trial lasted in **Table (12, 13and 14).**

Table (12): Chemical composition of steam and the leaves of the plants

	1/3 of fenugreek seeds with 2/3 of Barley seeds only **green** part of plants		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	76.73[a]	76.27[a]	±0.13	<.0001
%CF	22.03[b]	24.02[a]	±0.02	<.0001
%EE	5.50[b]	6.13[a]	±0.06	<.0001
% CP	35.12[a]	31.63[b]	±0.07	<.0001
%Ash	8.02	8.05	±0.02	<.0001

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

In **Table (12)** it can be observed the access of CP was 35.12, for the treated plants and on the other hand the decrease of CF and the EE results were 22.03 and 5.50 respectively.

It can be say that the irrigation with ZAD affected the chemical composition of the green shots of the plants. Also, it was observed that the tow type of plants effect on each other in the strangeness of grown shoots

Table (13): Chemical composition of the root + rice straw media.

	1/3 of fenugreek seeds with 2/3 of Barley seeds only **root** part of plants + media		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	76.37[a]	76.58[a]	±0.09	<.0001
%CF	35.04[b]	39.01[a]	±0.02	<.0001
%EE	3.03[a]	2.80[b]	±0.04	<.0001
% CP	8.96[a]	7.96[b]	±0.03	<.0001
%Ash	14.66[a]	15.03[a]	±0.24	<.0001

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

In Table (13) it can be observed the effect of ZAD on the access of CP to 8.96 from 7.96, also adding ZAD decreases the CF.

It can say that ZAD improved the chemical composition of rice straw as poor quality roughage to the way that the animal can consume it perfectly.

Table (14): Chemical composition of whole plants +rice straw media

	1/3 of fenugreek with 2/3 of Barley **whole** plant		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	76.75[a]	76.40[b]	±0.08	<.0001
%CF	30.04[b]	36.01[a]	±0.02	<.0001
%EE	4.13[a]	2.86[b]	±0.03	<.0001
% CP	14.98[a]	8.24[b]	±0.02	<.0001
%Ash	14.2[a]	14.07[a]	±0.12	<.0001

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

4.2.6. The chemical analysis of some seeds

The chemical composition of this seeds lasted in **Table (15).**

Table (15): Chemical composition of the seeds

	fenugreek seeds	Barley seeds	Canary grass seeds	**±SE**	**P value**
%CF	10.06[a]	7.1[c]	8.04[b]	±0.04	<.0001
%EE	5.87[a]	3.70[c]	4.71[b]	±0.02	<.0001
% CP	31.2[a]	17.54[c]	21.94[b]	±0.08	<.0001
%Ash	5.10[b]	3.50[c]	6.11[a]	±0.05	<.0001
%NDF	32.28[c]	66.60[a]	63.18[b]	±0.04	<.0001
%ADF	10.10[b]	5.66[c]	11.10[a]	±0.05	<.0001
%ADL	1.10[c]	1.23[b]	3.07[a]	±0.09	<.0001

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

In **Table** (15) it can be notice the chemical composition of some type of seeds without adding any type of treatments.

It can notice also that the biggest CP among this seeds is for the fenugreek seeds, and the lowest in CF was the barley seeds. And the smallest number of NDF and ADL was for fenugreek seeds.

4.2.7. The chemical analysis of the rice straw that has been used in all above trials

This rice straw was previously treated with ZAD. The chemical composition of this type of rice straw lasted in **Table (16).**

Table (16): Chemical composition of rice straw

	Soaked rice Straw irrigated with water		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	81.14[a]	81.30[a]	±0.04	<.0001
%CF	30.48[b]	32.53[a]	±0.03	<.0001
%EE	1.13[a]	0.56[b]	±0.03	<.0001
% CP	11.50[a]	7.43[b]	±0.06	<.0001
%Ash	20.66[b]	25.50[a]	±0.32	<.0001
%NDF	35.10[b]	70.03[a]	±0.05	<.0001
%ADF	36.35[b]	48.26[a]	±0.01	<.0001
%ADL	3.10[b]	5.10[a]	±0.06	<.0001

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

The result in **Table** (16) can show the effect of ZAD without planting any type of seeds. It can be notice the access of CP, EE, Ash, NDF, ADF and ADL. This previously treated rice straw with ZAD it's CP is 7.43 becomes 11.5 after irrigation with water + ZAD. This type of rice straw was used as a media for planting the seeds.

4.2.8. The chemical analysis of the rice straw that has been used in all below trials

The chemical composition of this type of rice straw lasted in **Table (17).**

Table (17): Chemical composition

	Soaked rice Straw irrigated with water		**±SE**	**P value**
	+ ZAD	- ZAD		
% Moist.	81.20[a]	81.30[a]	±0.06	<.0001
%CF	32.06[b]	34.03[a]	±0.05	<.0001
%EE	1.2[a]	0.9[a]	±0.09	<.0001
% CP	4.50[a]	2.55[b]	±0.05	<.0001
%Ash	20.07[b]	34.07[a]	±0.04	<.0001

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

In **Table (17)** it can notice the effect of ZAD on the natural rice straw and the rising of CP to 4.50 from 2.55 only by irrigation with ZAD. Also the decrease of Ash to 20.07 from 34.07.

4.3. The trial of reducing the amount of seeds used

In this trial it was used the amount of 25%of seeds coverage in the unit area against of 50% coverage. In this trial the seeds was grown on soaked rice straw and irrigated with water, with and without ZAD enzyme separately.

The concentration of enzyme was 3 ml per kg of rice straw for irrigation; the plants were irrigated once a day for 8 days in season and for 15 days out of season. The chemical composition of this trial lasted in **Table (18, 2019and 20).**

Table (18): Effect of the cover percentage on the leaves Chemical composition

	Barely only **green** part of plant				**±SE**	**P value**
	Cover conc. 25%		Cover conc. 50%			
	+ ZAD	- ZAD	+ ZAD	- ZAD		
% Moist.	76.73	73.55	76.53	76.41	±1.45	<.0001
%CF	15.03^{d}	28.05^{b}	16.03^{c}	30.01^{a}	±0.03	<.0001
%EE	3.05^{b}	2.66^{c}	3.20^{a}	3.09^{b}	±0.03	<.0001
% CP	20.10^{a}	15.10^{c}	19.83^{b}	15.26^{c}	±0.06	<.0001
%Ash	19.30^{a}	19.35^{a}	18.54^{b}	16.65^{c}	±0.09	<.0001

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

In **Table (18)** it can be observed that the CP in 25% +ZAD are more than the CP in 50%+ZAD, in my opinion that because the plants in 50% was crowded in the area so the plants could not do the photo senses in the perfect way. On the other hand the decrease of CF and EE explain that.

Table (19): Effect of the coverage percentage on the root Chemical composition

	Barely only **root** part of plants + media				**±SE**	**P value**
	Cover conc. 25%		Cover conc. 50%			
	+ ZAD	- ZAD	+ ZAD	- ZAD		
% Moist.	76.36^{b}	76.53^{b}	76.84^{a}	76.44^{b}	±0.08	<.0001
%CF	24.03^{c}	35.05^{b}	22.03^{d}	37.02^{a}	±0.03	<.0001
%EE	1.13^{ab}	0.90^{c}	1.21^{a}	1.00^{bc}	±0.04	<.0001
% CP	4.70^{a}	4.54^{b}	4.73^{a}	4.54^{b}	±0.03	<.0001
%Ash	21.70^{c}	23.22^{b}	13.85^{d}	34.27^{a}	±0.03	<.0001

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

In Table (19) it can be observed that there are no significant differences between CP of cover concentrate 25% + ZAD and the other concentration with ZAD.

It can say that cover concentrate 25% is better in this trial in the economically way, tacking in mind the differences in the chemical composition between the two concentrations of the seed cover

Table (20): Chemical composition whole plants +rice straw media

	Barely **whole** plant				**±SE**	**P value**
	Cover conc. 25%		Cover conc. 50%			
	+ ZAD	- ZAD	+ ZAD	- ZAD		
% Moist.	76.71[a]	76.41[b]	76.44[ab]	76.68[ab]	±0.08	<.0001
%CF	22.05[c]	31.03[b]	20.01[d]	33.01[a]	±0.02	<.0001
%EE	2.10[b]	1.76[c]	2.51[a]	1.30[d]	±0.04	<.0001
% CP	7.95[a]	4.10[b]	7.73[a]	4.21[b]	±0.07	<.0001
%Ash	17.15[c]	18.27[b]	12.51[d]	20.85[a]	±0.01	<.0001

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

In **Table** (20) it can be observed that there are no significant differences between CP of cover concentrate 25% + ZAD and the other concentration with ZAD.

It can say that cover concentrate 25% is better in this trial in the economically way, tacking in mind the differences in the chemical composition between the two concentrations of the seed cover

From this result it can say that the best percentage of cover concentration economically without significant influence was 25%.

4.4. ZAD Effect on orange pulp - rice straw mixture

In this trial it has been use 10% of orange pulp with 90% of soaked rice straw in the media of agriculture.

The concentration of enzyme was 3 ml per kg of rice straw for irrigation; the plants were irrigated once a day for 8 days. The chemical composition of this trial lasted in **Table (21, 22 and 23).**

Table (21): ZAD Effect on orange pulp - rice straw mixture ON THE Chemical composition of steam and the leaves of the plants

	Barely only **green** part of plant		**±SE**	**P value**
	+ orang pulp + ZAD	- orang pulp+ ZAD		
% Moist.	89.27[a]	89.37[a]	±0.16	<.0001
%CF	17.80[b]	27.43[a]	±0.02	<.0001
%EE	4.50[b]	6.16[a]	±0.08	<.0001
% CP	18.30[b]	35.20[a]	±0.09	<.0001
%Ash	15.43[b]	24.0[a]	±0.42	<.0001

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

In **Table (21)** it can be observed the decrease of CP, EE, CF and Ash results was 18.30, 4.50, 17.80 and 15.43 respectively.

It can be saying that the adding of orange pulp to the media of plantation has a bad effect on the plants, and made molds in the media.

Table (22): ZAD Effect on orange pulp - rice straw mixture on the chemical composition of the of the root + rice straw media.

	Barely only **root** part of plants + media		**±SE**	**P value**
	+ orang pulp + ZAD	- orang pulp+ ZAD		
% Moist.	80.33[a]	80.23[a]	±0.16	<.0001
%CF	25.10[b]	33.05[a]	±0.02	<.0001
%EE	2.06[a]	0.70[b]	±0.04	<.0001
% CP	5.06[b]	8.37[a]	±0.15	<.0001
%Ash	18.3[b]	20.0[a]	±0.41	<.0001

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

In **Table** (22) it can be observed the effect of orange pulp on the decreases of CP, CF and Ash.

It can say that orange pulp has a bad effect to the media of rice straw that appear on the chemical composition of rice straw.

Table (23): ZAD Effect on orange pulp - rice straw mixture ON THE Chemical composition OF THE whole plants +rice straw media

	Barely **whole** plant		**±SE**	**P value**
	+ orang pulp + ZAD	- orang pulp+ ZAD		
% Moist.	81.31[a]	81.08[a]	±0.11	<.0001
%CF	20.31[b]	32.47[a]	±0.02	<.0001
%EE	3.30[a]	1.30[b]	±0.04	<.0001
% CP	7.83[b]	15.88[a]	±0.02	<.0001
%Ash	18.06[a]	19.03[a]	±0.39	<.0001

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

In **Table** (23) it can be observed the decreases in CF when adding orange pulp, also the increasing of CP without orange pulp, and increased EE.

From this result it can be notice that the %CP decreased when use orange pulp in the media because the orange pulp reducing the growth of plant

The results of adding orange pulp to the media of rice straw was bad because the mold appeared in all types of media; except the last one, the chemical composition was taken in it because the mold was very little; so the decision was to put the orange pulp after the planting and mix it with the final product

4.5. Effect of the Reduction of enzyme and water amounts

In this trial it was used soaking in water plus enzyme for seeds and for rice straw separately.

The enzyme concentration was 3 ml per kg of rice straw on dry matter basis for soaking, and the same concentration for irrigation in the first 3 days of 8 days of growing in season, and for the first 7 days in 15 days of growing out of season.

The water amount were reduced into 200 ml per day in 0.1 m^2 , and that was by putting a plastic sheet under the soaked rice straw and also covered by another plastic sheet in the first 3 days of 8 days in season and for the first 5 days in 15 days of growing out of season. The chemical composition showed in **Tables (24, 25 and 26).**

Table (24): Chemical composition of steam and the leaves of the plants

	Barely only **green** part of plant				**±SE**	**P value**
	enzyme soaked **straw**+ **enzyme** soaked **seeds**	**enzyme** soaked **straw**+ **water** soaked **seeds**	**water** soaked **straw**+ **enzyme** soaked **seeds**	**water** soaked **straw**+ **water** soaked **seeds**		
%Moist.	76.80^{a}	76.41^{c}	76.66^{ab}	76.55^{bc}	±0.06	<.0001
%CF	20.00^{d}	26.16^{c}	31.10^{a}	29.02^{b}	±0.08	<.0001
%EE	4.39^{a}	4.11^{b}	3.90^{c}	2.20^{d}	±0.01	<.0001
% CP	14.45^{c}	14.10^{d}	18.37^{a}	15.22^{b}	±0.07	<.0001
%Ash	17.87^{d}	20.97^{a}	19.15^{b}	18.18^{c}	±0.01	<.0001

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

In **Table (24)** it can be observed the access of CP for the seeds which soaked in enzyme and grown on rice straw soaked in water.

It can be say that soaking with ZAD affected the chemical composition of the green shots of the plants.

Table (25): Chemical composition of the root + rice straw media.

	Barely only **root** part of plants + media				**±SE**	**P value**
	enzyme soaked **straw**+ **enzyme** soaked **seeds**	**enzyme** soaked **straw**+ **water** soaked **seeds**	**water** soaked **straw**+ **enzyme** soaked **seeds**	**water** soaked **straw**+ **water** soaked **seeds**		
%Moist.	76.35^{b}	76.53^{b}	76.82^{a}	76.44^{b}	±0.08	<.0001
%CF	30.00^{d}	35.03^{c}	37.03^{b}	46.03^{a}	±0.02	<.0001
%EE	0.40^{a}	0.31^{b}	0.30^{b}	0.43^{a}	±0.02	<.0001
% CP	5.44^{a}	4.38^{c}	4.65^{b}	3.72^{d}	±0.02	<.0001
%Ash	20.74^{b}	12.02^{d}	17.17^{c}	30.03^{a}	±0.02	<.0001

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

In Table (25) it can be observed the effect of soaking both rice straw and seeds in water + ZAD on the access of CP and EE, also soaking in ZAD decreases the CF.

It can say that ZAD improved the chemical composition of rice straw as poor quality roughage to the way that the animal can consume it perfectly.

Table (26): Chemical composition whole plants +rice straw media

	Barely **whole** plant				**±SE**	**P value**
	enzyme soaked **straw**+ **enzyme** soaked **seeds**	**enzyme** soaked **straw**+ **water** soaked **seeds**	**water** soaked **straw**+ **enzyme** soaked **seeds**	**water** soaked **straw**+ **water** soaked **seeds**		
%Moist.	76.70[a]	76.40[b]	76.50[ab]	76.50[ab]	±0.06	<.0001
%CF	29.07[d]	34.01[c]	36.10[b]	40.01[a]	±0.03	<.0001
%EE	1.51[a]	1.30[b]	1.20[c]	1.06[d]	±0.02	<.0001
% CP	7.43[b]	8.25[a]	5.92[c]	4.10[d]	±0.03	<.0001
%Ash	17.68[b]	13.54[c]	13.20[d]	25.06[a]	±0.02	<.0001

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

In **Table** (26) it can be observed the effect of soaking both rice straw and seeds in water + ZAD on the access of CP and EE, also soaking in ZAD decreases the CF.

The best percentage of CP in this table is 8.25 and this from soaking the rice straw in ZAD but with soaking seeds in water only.

From this result it can say that treating with ZAD made a good addition to the poor quality roughage of rice straw and improve it to become useful to the ruminant animals.

PART II

4.6. Widely adopted experiment (Chemical composition of feeding rations)

The average values of DM, OM, CP, EE, CF and NFE of the different experimental rations are illustrated in **Table (27).** The control ration (T1) had the lowest value of EE and CP, but had the highest values of CF, Ash, NDF and ADF, the values were 0.99, 5.75, 38.02, 26.10, 76.01and 58.10%, respectively. On the other hand, the ration of (T4) had the lowest values of CF, Ash, NDF and ADF, but also, recorded the highest values of EE and CP. The corresponding values were, 32.01, 26.01, 70.01, 50.05, 1.57 and 7.96%. While, the chemical composition of treatment (T2) had this values 30.02, 24.10, 72.10, 52.10, 1.25 and 7.95% for CF, Ash, NDF, ADF, EE and CP respectively. It is of interest to notice that T_1 had the highest values of CF, Ash, NDF and ADF while it had the lowest values of EE and CP. Also, (T3) had higher values of chemical composition than T2. The biological treatments with ZAD increased EE and CP while decreased CF, Ash, NDF and ADF compared with untreated rations (T1 &T2).

Table 27. Chemical composition of rations

sample	T1(Control)	T2	T3	T4	±SE	P value
% Moist.	56.67[b]	60.00[a]	50.10[d]	54.37[c]	±0.55	<.0001
%CF	38.02[a]	30.02[d]	36.05[b]	32.01[c]	±0.02	<.0001
%EE	0.99[c]	1.25[b]	1.00[c]	1.57[a]	±0.02	<.0001
%CP	5.75[c]	7.95[a]	7.10[b]	7.96[a]	±0.04	<.0001
%Ash	26.10[b]	24.10[c]	34.10[a]	26.01[b]	±0.05	<.0001
%NDF	76.01[a]	72.10[c]	74.01[b]	70.01[d]	±0.03	<.0001
%ADF	58.10[a]	52.10[c]	54.10[b]	50.05[d]	±0.05	<.0001
%ADL	8.01[b]	8.07[a]	6.02[c]	6.01[c]	±0.02	<.0001
%Silica	18[a]	16[b]	16[b]	16[b]	±0.02	<.0001

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

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

4.7. Rumen parameters

4.7.1. Ruminal pH

Table (28) 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 hr. before feeding. It was clear that pH values started to increase by advancing the time after feeding (up to 6 hrs.)

for all rations. These could be explained by the present of TVFA'S in the rumen. However, rams 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 hrs. of feeding. There were no significant (P> 0.05) differences among the experimental groups in pH values at 3 hrs. after feeding. Contrarily, values of pH increased at 6 hrs. after feeding for all rations under the study. Rams group fedT4 had higher (P<0.05) pH values than those groups fed T1, T2 and T3 at 6 hrs. The recorded values were 6.89, 6.87, 6.88 and 6.86 respectively.

Table (28). Ruminal pH

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			7.17		±0.05	<.0001
3hrs.			6.22		±0.01	<.0001
6hrs.			6.70		±0.01	<.0001
After 12 days						
0 hr.	6.81[b]	6.84	6.84	6.85[a]	±0.01	<.0001
3hrs.	6.27	6.25	6.26	6.25	±0.01	<.0001
6hrs.	6.87	6.88	6.86	6.89[a]	±0.01	<.0001

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

4.7.2. Ruminal ammonia-n concentration

As shown in **Table (29).** At 0 hr., Ruminal ammonia–N concentration (mg /100 ml) was significantly (P< 0.05) the lowest for T1, T2 and T3. These values of Ruminal ammonia – N concentration (mg /100 ml) increased after 3-hrs post feeding and then decreased at 6-hrs post-feeding. Our results were on line with the results of **El ashry (2001)** that these values increased 3 hrs. after feeding. The values of the T3 and T4 rations were significantly (P<

0.05) the highest after 3 and 6 hrs. of feeding indicating the good effect of treating these rations.

Table (29). Ruminal ammonia-n concentration

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.	8.77				±0.06	<.0001
3hrs.	12.86				± 0.05	<.0001
6hrs.	10.87				±0.03	<.0001
After 12 days						
0 hr.	20.73^{b}	20.87^{b}	20.84^{b}	21.30^{a}	±0.11	<.0001
3hrs.	24.66^{d}	25.41^{b}	25.03^{c}	25.96^{a}	±0.08	<.0001
6hrs.	17.60^{d}	18.45^{b}	18.01^{c}	18.95^{a}	±0.06	<.0001

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

4.7.3. Ruminal total volatile fatty acids (TVFA's)

As shown in **Table (30)** the ruminal fluids of sheep of T2 and T4 had significantly (P< 0.05) higher rate of VFA's (m.eq/100ml) at 0, 3 and 6 hrs. compared to the other fed rations (T1 and T3). The ruminal fluids of sheep fed untreated rations (control groups) had significantly (P< 0.05) the lowest VFA's at 0, 3 and 6 hrs. Our results were in agreement with those of **Puri** and **Gupta (1989), 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.

Table (30). Ruminal total volatile fatty acids

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			5.96		±0.01	<.0001
3hrs.			7.87		±0.01	<.0001
6hrs.			6.68		±0.03	<.0001
After 12 days						
0 hr.	6.74	6.75	6.75	6.76	±0.01	<.0001
3hrs.	8.08^{d}	8.20^{b}	8.13^{c}	8.26^{a}	±0.01	<.0001
6hrs.	7.15^{d}	7.34^{b}	7.30^{c}	7.38^{a}	±0.01	<.0001

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

4.8. Blood plasma parameters

4.8.1. Plasma total protein (PTP)

The results concerning the effect of feeding lambs on different experimental rations on PTP are shown in **Table (31).** It was observed that rams fed T2, T3 and T4 had significantly (P< 0.05) higher level of PTP (g/dl), then those fed control rations (T1).

Table (31). Plasma total protein

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			4.50		±0. 18	<.0001
3hrs.			5.80		±0.06	<.0001
6hrs.			6.70		±0.08	<.0001
After 12 days						
0 hr.	4.53	4.67	4.60	4.83^{a}	±0.11	<.0001
3hrs.	5.14^{c}	5.82^{b}	6.23^{ab}	6.43^{a}	±0.14	<.0001
6hrs.	6.86^{c}	6.99^{a}	6.52^{b}	6.95^{a}	±0.19	<.0001

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

4.8.2. Plasma urea concentration

Table (32) shows the means of urea concentration (mg/dl) of the experimental treatments. No significant (P> 0.05) differences between groups were observed in plasma urea concentration after 6 hrs. Changes in Plasma urea would reflect changes in ruminal ammonia concentration. Our results 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.

Table (32). Plasma urea concentration

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			15.45		±0.06	<.0001
3hrs.			14.14		±0.07	<.0001
6hrs.			11.28		±0.01	<.0001
After 12 days						
0 hr.	17.60[b]	17.80[a]	17.63[b]	17.86[a]	±0.03	<.0001
3hrs.	16.48[a]	16.44[a]	16.33[b]	16.26[b]	±0.02	<.0001
6hrs.	15.15[a]	15.13[a]	15.12[a]	15.16[a]	±0.01	<.0001

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

4.8.3. Plasma creatinine

Effect of the experimental treatments on serum creatinine (mg/dl) of sheep received the different experimental treatments are presented in **Table (33)**. The serum creatinine was 0.81, 0.89 and 0.90 (mg/dl) for T2 & T3 & T4 vs. the control group (T1) was 0.82. There were insignificant differences (P>0.05) among treatments in the serum creatinine **Table (33).** Serum creatinine content was almost the same in (T1 & T2). These results are on line with those obtained by **Gado et al. (2006)** who found that serum creatinine was not affected by enzymatic treatments.

Generally, serum creatinine level is a useful indicator of glomerular filtration in the kidney. From the previous data, it was found that the values of serum creatinine for the experimental sheep were within the normal levels.

Regarding to the results of serum urea and serum creatinine concentrations, it is clear that experimental animals were not in a catabolism situation and kidney function was not adversely affected by the biological treatments.

Table (33). Plasma creatinine

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			0.82		±0. 01	<.0001
3hrs.			0.83		±0.01	<.0001
6hrs.			0.84		±0.01	<.0001
After 12 days						
0 hr.	0.82	0.85	0.88	0.86	±0.03	<.0001
3hrs.	0.89	0.88	0.90	0.92	±0.01	<.0001
6hrs.	0.82^{bc}	0.81^{c}	0.89^{ab}	0.90^{a}	±0.02	<.0001

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

4.8.4. Plasma GPT and plasma GOT

Means of GOT (µ/l) and GPT (µ/l) of sheep fed the experimental treatments are shown in **Table (34)** and **Table (35)** respectively. GPT of sheep seemed to remain stable at the different experimental groups. Sheep fed T1, T2, T3 and T4 produced 20.90, 20.64, 20.84 and 20.61 of GPT, respectively, indicating that treating rations with ZAD reflected low level of GPT. Also, the level of GOT of sheep had no significant values between experimental rations. So it says 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)** that enzymatic treatment of wheat straw had no adverse effect on kidney function.

Table (34). Plasma GPT

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			20.70		±0.04	<.0001
3hrs.			20.63		±0.04	<.0001
6hrs.			20.93		±0.03	<.0001
After 12 days						
0 hr.	20.97[a]	20.62[a]	21.05[a]	20.58[a]	±0.17	<.0001
3hrs.	20.45[a]	20.77[a]	20.82[a]	20.51[a]	±0.14	<.0001
6hrs.	20.90[a]	20.64[b]	20.84[ab]	20.61[b]	±0.07	<.0001

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

Table (35). Plasma GOT

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			41.57		±0. 09	<.0001
3hrs.			41.77		± 0.12	<.0001
6hrs.			41.43		±0.07	<.0001
After 12 days						
0 hr.	41.78[a]	41.75[a]	41.83[a]	41.58[a]	±0.08	<.0001
3hrs.	41.49[a]	41.52[a]	41.52[a]	41.66[a]	±0.09	<.0001
6hrs.	41.97[a]	41.68[ab]	41.46[b]	41.47[b]	±0.09	<.0001

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

4.8.5. Plasma total lipids

Effect of the experimental treatments on serum total lipids of sheep received the different experimental treatments are shown in **Table (36).** Values of plasma total lipids concentration (mg/dl) were; 201.66, 201.67, 201.33 and 201.00 (mg/dl) for (control T1), T2, T3 and T4 respectively after 6 hrs. of feeding. There were insignificant differences (P>0.05) among control ration and ration contained ZAD. **Yildiz et al. (1995)** found that serum total lipids no differ between ration contained rumen contents or

control in Merino rams. However, **Khaled (1995)** noticed that group fed control ration had lower serum total lipids compared those fed ration contained rumen contents.

Table (36). Plasma total lipids

Item	Treatments					
	T1	T2	T3	T4	±SE	P value
Time:						
At the beginning (0 day)						
0 hr.			191.67		±0.33	<.0001
3hrs.			195.33		±0.40	<.0001
6hrs.			202.33		±0.40	<.0001
After 12 days						
0 hr.	190.6	190.33	191.33	190.33	±0.33	<.0001
3hrs.	195.00	195.10	195.04	195.09	±0.57	<.0001
6hrs.	201.66	201.67	201.33	201.00	±0.58	<.0001

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

4.9. IN VIVO and In Vitro DMD (effect of treatments on nutrients digestibility)

The average values of DM, OM, CP, EE, CF and NFE digestibility coefficients of different experimental treatments are given in **Table (37).** The control ration (T1) significantly (P<0.05) decreased all nutrients digestibility compared with the other ration.

Significant (P< 0.05) differences were shown between rations contained untreated or treated rice straw for OM, NFE, CP and EE digestibility coefficients. The OM, NFE, CP and EE of T4 (RSGB with ZAD and orang pulp) were significantly (P < 0.05) higher than those of T3 (RSGB with orang pulp). Also, significant difference (P < 0.05) was observed in T2 (RSGB with ZAD) higher than T1 (RSGB), indicating the effect of ZAD on the preceding elements.

Higher (P < 0.05) digestibility coefficients of CP were obtained in rations contained ZAD, to those not contained ZAD including (control

group). Treating RSGB with ZAD significantly ($P < 0.05$) improved DM, OM, CP, EE and TDN of rations.

The same trend was observed for CP and CF digestibility coefficients where the CP and CF of T4 were significantly ($P<0.05$) higher than those of the other groups of RSGB. It was observed that the CP and CF of T2 was also significantly ($P<0.05$) higher than (T1).

These results of increasing digestibility coefficients of CP and CF agreed with those obtained by **Mohamed et al. (1989)** and **Fouad et al. (1998)**, However, the increase in CP digestibility coefficients could be related to the increase of microorganism's biomass while the digestibility coefficients of CF may be due to the increase of activity of enzymes produced by microorganisms.

Table (37). Nutrients digestibility and IVDMD

Treatment	T1	T2	T3	T4	±SE	P value
%DM	69.66^{b}	70.00^{ab}	70.03^{ab}	70.33^{a}	±0.12	<.0001
%OM	73.90^{d}	76.50^{b}	75.43^{c}	78.23^{a}	±0.20	<.0001
%CP	58.03^{d}	72.43^{c}	73.70^{b}	77.70^{a}	±0.12	<.0001
%EE	65.17^{d}	80.30^{c}	83.23^{b}	84.83^{a}	±0.09	<.0001
%CF	53.40^{d}	60.40^{c}	65.47^{b}	68.03^{a}	±0.16	<.0001
%NFE	60.63^{d}	78.70^{c}	82.33^{b}	86.47^{a}	±0.19	<.0001
%NDF	60.30^{d}	70.27^{b}	69.40^{c}	71.70^{a}	±0.16	<.0001
%ADF	60.30^{b}	67.56^{a}	67.40^{a}	67.90^{a}	±0.15	<.0001
%TDN	42.76^{d}	55.02^{b}	48.61^{c}	59.02^{a}	±0.09	<.0001
%IVDMD	31.00^{d}	53.67^{b}	47.00^{c}	58.00^{a}	±0.53	<.0001

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

The improvement of digestibility of ration supplemented by ZAD attributed to many factors:

1- Improvement of the absorption during digestion, by increasing the reactive surface areas of nutrients.
2. Improving feed utilization by slowing feed passage time throughout the digestive tract which reflected on better absorption.

The data of nutritive values are summarized in **Table (37).** Higher ($P<0.05$) feeding values as TDN scored significant differences among different experimental rations. The feeding values were (55.02, 48.61 and 59.02%) for TDN of treatments (2), (3) and (4), respectively.

Higher ($P<0.05$) feeding values as Digestibility of CP scored significant differences between different experimental rations. The feeding values were (72.43, 73.70 and 77.70 %) for Digestibility of CP of treatments (2), (3) and (4) respectively. These finding could be explained by the increase of digestibility coefficients for nutrients of RSGB treated with ZAD. These results coincided with those obtained by **Gupta et al.,(1988)** who reported that the nutritive values as TDN increased when they used wheat straw treated with fungal for feeding goats.

In this study, it was observed that treated rations with ZAD produced the best estimates of digestibility, feeding values and fiber fraction compared to the untreated rations. These results agreed with these of **Fouad et al. (1998), Mahrous et al. (2005) and Zewil (2005).**

4.9.1. Effect of observed treatments on In vitro DM disappearance (IVDMD)

Data of IVDMD of the experimental treatment at difference incubation time are shown in **Table (37)**. These results indicated that dry matter disappearance (IVDMD) value of T4 (58.00) was significantly ($P< 0.05$) the highest. While that of T1 (31.00) of RSGB as a control group was significantly ($P< 0.05$) the lowest. While groups (2), and (3) had 53.76 and 47.00 respectively, indicating the good effect of using ZAD.

Stockes (1992) reported that in vitro DM digestibility increased when alfalfa was ensiled with an enzymatic cellulose additive. **Ali et al. (1987)**

concluded that treating rice straw with *P. osteatus* was more rapid and complete as compared to raw wheat straw which needed longer incubation period for improving digestibility. Also, our results were confirmed by those of **Zadrazil (1977)**. In addition**, Ibrahim** and **Pearce (1980)** who observed an increase in digestibility, which were 10 units when using biological treatment (*Penio phora Gigantea* on barley straw). In our results, T4 value 58.00) was significantly (P< 0.05) the best as a digestibility coefficient compared to the other groups.

4.10. Feeding trial

Means of increased live weights of the sheep according to the four rations studied are shown in **Table (38).** Sheep fed rations treated with ZAD (T2 and T4) were significantly (P<0.05) heavier, grew faster and had higher weight gain than sheep fed control rations (T1). It means that the enzymatic treatment with ZAD, improved the ruminal media which became more suitable for increasing anaerobic bacteria and micro-organisms. These findings agreed with those of **Erwin et al. (1957)**, **Nowar at al. (1993)** and **Ali (1995)** in case of sheep and **Gado (1997)** in case of kids. Sheep fed RSGB with ZAD (T2), or with orang pulp (T3) and with ZAD and orang pulp (T4) were significantly (P< 0.05) heavier, grew faster, gained more weight. It was observed that ZAD had a significant (P< 0.05) effect on growth performance of the sheep under the study.

Table (38). Increased live weights of sheep

Treatment	**T1**	**T2**	**T3**	**T4**
0 (initial weight)	23	21	22	24
W2 (kg/15d)	6	7	7	8
W3 (kg/15d)	3	3	3	3
W4 (kg/15d)	2	3	3	2
W5 (kg/15d)	2	2	2	2
sum(Σ)	13	15	15	15

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

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.

In addition Salem et al. (2011) reported, in the growth performance trial, DMI was increased ($P<0.05$) by addition the daily addition of ENZ to sheep and goats diets during the 65 days of the experimental. Total DMI was increased ($P<0.01$) in sheep than goats, while average daily gain (ADG) was increased ($P<0.01$) goats than sheep with the addition of ENZ (2.2 and 6.3 g/kg $BW^{0.75}$, respectively). Feed efficiency was increased in goats than sheep with ENZ addition. An increased ($P<0.01$) in metabolizable protein intake and net energy for growth occurred by ENZ addition sheep and goats diets and the highest ($P<0.01$) values were observed in goats received ENZ.

5. SUMMARY AND CONCLUSIONS

Twelve Ossimi sheep of about 45±0.5 kg live weight were used in the present study. Animals were randomly assigned among four experimental treatments. The experimental period extended to 12 days which divided to 7 days as an adaptation period followed by 5 days as a collection period. Animals were fed four experimental treatments:

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

The objective of this study was to study the possibility of feeding (RSGB) and the effect of biological treatments by using ZAD on the chemical composition, nutritive value, rumen fermentation and some blood parameters of Ossimi sheep. The results and conclusions are summarized as follow:

1. Feed intake and nutrient digestibility coefficients

1.1. The biological treatments (T4 & T2) increased all nutrients digestibilities compared with control and T1.

1.2. There were no differences between experimental groups and control ration in DMI.

2. Nutritive value of the experimental rations

The biological treatments increased rations nutritive values compared with control and untreated group (T1).

3. Rumen parameters:

3.1. The groups fed on treated and untreated RSGB (T2, T3, T4) recorded slightly higher ($P>0.05$) rumen pH compared with control, however, differences among treatments were insignificant.

3.2. The untreated group (T3) and the biologically treated groups(T2 & T4) led recorded slightly higher ($P>0.05$) value of rumen total volatile fatty acids compared with T1 group. Differences among treatments were insignificant.

3.3. Groups T4, T3 and T2 recorded higher (P>0.05) values of rumen liquor Ammonia-nitrogen compared with T1 group. Differences among treatments were insignificant.

3.4. All rumen parameters were significantly affected by sampling times.

4. Results of blood serum parameters.

4.1. Rations T2, T4 and T3 slightly increased (P>0.05) serum total protein compared with T1 ration.

4.5. The treated groups T2 and T4 recorded slightly higher (P>0.05) serum urea compared with T1 group.

4.6. T3 and T2 groups recorded slightly (P>0.05) higher serum creatinine compared with T1 and T4 groups.

4.7. All values of serum GPT for all groups were in normal range; Where T1 ration increased (P>0.05) serum GPT compared with other rations.

4.8. All values of serum GOT for all groups were in normal range. Where T2 and control rations increased (P>0.05) serum GOT compared with other rations.

4.10. Rations T2 & T3 increased (P>0.05) serum total lipids compared with T1 ration.

CONCLUSION

Using of RSGB as a ruminant feed increased all nutrients digestibility, animal performance of Ossimi sheep. Feeding ration treated ZAD improved the performance of sheep without any adverse effect on animal's health.

6-Reference

Abd El fattah H. (2009). Effect of Cellulytic Enzymes Addition to Diets on the Productive Performance of Lactating Goats. pp. 5-20. M.Sc. Thesis, Fac. Agric., Cairo Univ., Egypt.

Almquist H.J., Christensen H.L. and Maurer S. (1967). The effect of bentonite on nutrient retention by turkey. **J. of Feed Stuffs, 39: 20-54.**

Abdel-Gawad, M. H.; Gad, S.M.; El Sabaawy, E. H.; Ali, H. M.and El-Bedawy, T.M. (2007). In vitro and in vivo digestability of some low quality roughages supplemented with fibrolytic enzyme for sheep. Egyptian J. Nutrition and Feeds., 10 (2)(Special Issue): 663-677.

Abd El-Gawad, A.M.; K.M.A. Zahran and N.S.A. Khadr (1993). Use of ammonited wheat or rice straw in goat rations. J. Agric, Sci Maansoura Univ. 18(12). 3466 – 3480.

Ali, M.E.A. (1995). **Improvement of utilization of chemical treated poor quality roughages by ruminant.** M.Sc., Thesis. Fac. Agric., Zagazig Univ., Egypt.

Ali, A.F.; A. Azim; F.A. Mir and M.H. Bhatti. (1987). Treatment of Cereal straw with white rote fungi. Pak. J. Agric. Res. 8(4). 428 – 432.

Annison, G. (1997). The use of enzymes in ruminant diets. ln:"Biotechnology in the Feed Industry", Proceedings of the 13thAnnual Symposium. (Eds. Lyons, T. P and Jacques, K.A)Nottingham University Press, Nottingham, Leics.UK, pp. 115-155.

Annison, G. and Choct, M. (1993). Enzymes in poultry diets. ln:"Enzymes in Animal Nutrition" Proceedings, of the 1st Symposium. lnstitut fur Nutzienwissenschaften, Zurich. (Eds;. Wenk, C and Boessinger, M.), pp.61-104.

Anwar, D. A. 2009. Effect of Germination Methods on Characters and active Ingredient Contents of Sprouts of some Medicinal Plant Seeds. M.Sc. Thesis, Ain Shams Univ., Cairo, Egypt.

A.O.A.C. (1995). Official methods of Analysis of AOAC international, 16th Ed. Vol. 1, "Agricultural, Chemicals, Contaminants, Drugs". Washington, D.C., USA, 521p.

Beauchmin, K. A.; Yang, W. Z. and Rode, L. M. (1999). Effect of grain source and enzyme additive on site and extent of nutrients digestion in dairy cows. J. Dairy Sci., 82:378-390.

Broderick, G.A.; Derosa, R. and Reyna, S. (1997). Value of Treating Alfalfa Silage with Fibrolytic Enzymes Prior to Feeding the Silage to Lactating Dairy Cows, US Dairy Forage · Research Center, 74p.

Bowman, G. R.; Beauchemin, K. A. and Shelford, J. A. (2002). The proportion of the ration to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. J. Dairy Sci., 85:3420.

Chen J., Stokes M.R. and Wallace C.R., (1994). Crop and Corn silages J. Dairy Sci. 77: 501 - 512.

Colombatto D., Hervas G., Yang W. Z. and Beauchemin K.A. (2003). Fermentation in continuous culture, maintained at high and low pH. **J. Anim. Sci. 81: 2617-2627.**

Colombatto, D.; Mould, F.L.; Bhat, M.K. and Owen, E. (2006). Influence of exogenous fibrolytic enzyme level and incubation pH on the in vitro ruminal fermentation of alfalfa stems. Anim. Feed Sci. Technol., 137: 150.

Colombatto, D.; Mould, F. L.; Bhat, M. K, ; Phipps, R. H. and Owen, E. (2004). *In vitro* evaluation of fibrolytic enzymes as additives for maize (Zea mays L.) silage. III: Comparison of enzymes derived from psychrophilic, mesophilic or thermophilic sources. Anim. Feed Sci. Technol., 111:145.

Dawson K. A. and Tricarico J.M. (1999). The use of exogenous fibrolytic enzymes to enhance microbial activities in the rumen and the performance of ruminant animal. In: "Biotechnology in the Feed Industry", **pp. 303-312** (Eds. Lyons T.P. and Jacques K.A.). **Proc. 15th Annual Symp.**Nottingham Univ**.** Press. Loghborough, Leics, UK**.**

Dean, D. B.; Adesogan, A.T.; Krueger, N.A. and Littell, R.C. (2008). Effects of treatment with ammonia or fibrolytic enzymes on chemical composition and ruminal degradability of hays produced from tropical grasses. Anim. Feed Sci., 145: 68-83

Deraz, T.A. (1996). The production of microbial protein from some agricultural wastes and its utilization in ruminant. Ph.D. Thesis, faculty of agriculture. Ain Shams University.

Dhiman, T. R.; M. S. Zaman.; Gimenez, R. R.; Walters, J. L. and Treacher, R. (2002). Performance of dairy cows ·fed forage treated with fibrolytic enzymes prior to feeding. Anim. Feed Sci. Technol., 101:115-125.

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11:1.Effects of enzymes inoculant systems on preservation and nutritive value of hay

El ashry M.; M.E. Ahmed; S.A. EL saadany: M.E.S. Youssef; I.A. Gomaa and T.A.A. Deniz (1997). Effect or mechanical VS. chemical or bio chemical treatments of crop on their use in ruminant rations: Digestibility, nitrogen balance and some blood and rumen liquor parameters of sheep. Egyptian .T. Nutrition and feeds .1: (special 1 issue) : 173 -I76 .

El-Ashry, M. A.; Kholif, A. M.; Fadel, M.; El-Alamy, H.A.; El-Sayed, H.M. and Kholif, S.M. (2002). Effect of biological treatments on chemical composition and in vitro and in vivo digestibilities of poor quality roughages. Egyptian J. Nutrition and Feeds., 6:113-126 .

El ashry M.A.; A.M. Kholif; H.A. EI-Alamy; H.M. El-Sayed and EI-Hamamsy, T.A. (2001). Effect of different yeast cultures supplementation to diet on the productive performance of lactating buffaloes. Egyptian .T. Nutrition feeds 4(1): 21.33.

El ashry, M. A. (2007). Animal recourses in the frame of the Egyptian agriculture development. Horizons of animal's resource development – ruminants. Ministry of agriculture. Agricultural Research Center, Giza, Egypt. P37-42

El madany, l. A. (1997). Nutritional Using Treated Rice Straw in Goat Rations. pp.40-45. M.Sc. Thesis, Fac. of Agric., Zagazig University, Egypt.

El mahy M.(2009). Effect of some biological treatments on the nutritive value of agricultural by-products.pp.30-50. M.Sc. Thesis, Fac. of Agric., Monofia University, Egypt.

El marakby K.M.A. (2003) Biological Treatments of Poor Quality Rough and Its Effect on Productive Performance of Ruminants. pp.42-47. M.Sc. thesis, Fac. Agric., Zagazig University, Egypt.

El sayed H. ; El ashry M.A.; Metwally H.M.; Fadel M. and Khorshed M.M.(2002). Effect of chemical and biological treatments of some crop-residues on their nutritive value: 3-digestion coefficient, rumen and blood serum parameters of goats. Egyptian J. Nutrition and Feeds, 5 (1): 55-69.

Erwin, K.S.; C.J. Elam and I.A. Dyer (1957). The influence of sodium bicarbonate in vitro and in the ration of steers. J. Anim. Sci., 63: 16-858.

Etab R. (2000). Some Nutritional Factors Affecting the Performance of small Ruminants.pp.30-50. M.Sc. thesis, Fac. Agric., Ain Shams University, Egypt.

Etab R. (2005). Effect of Biological Treatments on Silage and Feeding Value of Roughages in Ruminants.pp40-55. PhD. thesis, Fac. Agric., Ain Shams University, Egypt.

Etab R. (2006). Effect of biological treatments by cellulolytic bacteria on chemical composition and cell wall constituents of some roughage. Egyptian J. Nutrition and Feeds, 9 (2): 100-112.

Eun, J.-S.; Beauchemin, K.A.; Hongh, S.H. and Bauer, M.W. (2006). Exogenous enzymes added to untreated or ammoniated rice straw: Effects on in vitro fermentation characteristics and degradability. Anim. Feed Sci. Technol., 131:86-101.

Fatma R. (2006). Effect of Biological Treatments on Silage and Feeding Value of Roughages in Ruminants.PP.45-60. M.Sc. thesis, Fac. Agric., Ain Shams University, Egypt.

Felton, C.J.D; J.W. Lehmkuhler and M.S. Kerley (2001). Ruminal peptide concentration required to optimize microbial growth and efficiency. J. Anim. Sci. 2001. 79: 1305 – 1312.

Feng P., Hunt C.W., Pritchard G.T., and Julian W.E. (1996).

Effect of enzyme preparations on in situ and in vitro degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci., 74: 1349-1357.

Flachowsky G. and G. Klnppach (1993).

In Sacco degradability of straw treated with cellulolytic enzymes containing fermented substrates**. Archives of Animal Nutrition (43)4:381-385**.

Fouad, R.T.; T.A. Deniz, and S.A.A. Ismail (1998). Biological versus urea treatment of roughages for sheep .J. Agric. Sci Mansoura Univ. 23(1): 103-116.

Gad, A. M.; H. Gado and H. M. Metwally (2005). Effect of ZAD as a biological additive on the performance of small ruminantes fed rations based on agriculture by-products. Res. Bult., Ain Shams Univ., 2005-1

Gado H. (1997). Effect of enzymatic treatments for poor quality roughage on fiber digestibility and nitrogen metabolism in Baladi goats. Egyptian. J. of Nutrition and feeds, 1: (Special Issue), 49 – 56.

Gado H. (1999). Effect of different cellulolytic rumen bacteria on fiber digestion. Egyptian J. of nutrition and feeds , 2: (Special Issue), 487-493.

Gado, H.; Sohair A. Nasr; Bahira K. Mohamed and A. A. Mahrous (2006). Effect of biological treatments on the nutritive value of rice straw. Egyptian J. Nutrition and Feeds, 9 (2): 207-219.

Gado, H. M.; Metwally, H. M.; EL-Basiony, A. Z.; Soliman, H. S. and Etab R. I. Abd El Galil. (2007). Effect of biological treatments on sugarcane bagasse digestibility and performance of baldi goats. Egyptian J. Nutrition and Feeds, 10 (Special Issue.2): 535-551

Gado, H. ; Salem A.Z.M. ; Robinson P.H. And Hassan M. (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim. Feed Sci. Technol., 1248: P 1-11.

Gado H. M. And Borhami B. E. (2010). Effect of anaerobic enzyme matrix on fiber digestibility. ADSA.ASAS, 895

Gado, H. ; Salem A.Z.M. ; Odongo N.E. And Borhami B. E. (2011). Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. Anim. Feed Sci. Technol., 1248: P 1-6.

Ghazi N. Al-Karaki and M. Al-Hashimi (2011). Green fodder production and water use efficiency of some forage crops under hydroponic conditions. Jordan J. Agric. Sci. 9: P11-25.

Giraldo, L.A.; Tejido, M.L.; Ranilla, M.J. and Carro, M.D. (2007). Effects of exogenous fibrolytic enzymes on in vitro ruminal fermentation of substrates with different forage: concentrate ratios. Anim. Feed Sci. Technol. 141: 306-325

Gupta V.K. (1988). Fibrous crop residues as animal feed. Eddied by Kiran Siagh , .T.B.Schiere proceeding of an international workshop held on 27and 28 October. 1988.

Han Y.W. (1974).
Microbial fermentation of rice straw: Nutritive composition and in vitro digestibility of fermentation product. **App. Microbial., 29:510-514.**

Han, Y. W. and Anderson, A .W. (1975). Semi solid fermentation of ryegrass straw. App. Microbial., 30:930.

Henary, R. J. (1974). Clinical Chemistry, Principles and Techniques. Second Edition, Harper and row, p 525.

Howes, D.; Tricarico J. M.; Dawson, K. A. and Karnezos, P. (1998). Biotechnology in The Feed Industry, (Eds. Lyons, T.P. and Jacques, K.A.) . Proceedings of the 14th Annual Symposium Nottingham University Press, Nottingham, UK, 393 p.

Hunt C.W., Kexar W. and Vinande R. (1992). Yield, chemical composition and ruminal ferment ability of corn whole plant, ear and Stover as affected by maturity. J. Prod. Agric., 5:286-294

Ibrahim, M.N.M. and G.R. Pearce (1980). Effect of white-rot fungi on the composition and In-vitro digestibility of crop by-product Agric. Wastes, 2(3): 199-205.

Johnson R., Williams P. and Campbell R. (1993). Enzymes in pig production. In "**Enzymes in Animal Nutrition**" (Eds. Wenk C., and Boessinger M.). **Proceedings of the 1st Symposium. Institut fur Nutzien issenschaften, Zurich. pp.150-199**.

Judkins, M.B. and Stobart, R.H. (1988). Influence of two levels of enzyme preparation on ruminal fermentation, particulate and fluid passage and cell wall digestion in weather lamps consuming either a 10% or 25% grain diet. J. Anim. Sci., 66:1101.

Kahlon, S. S. and Nikhat, P. (1983). Protein errichment of wheat straw with non-toxic fungus *Pleurotus ostreatus*. J. Res. Punjab Agric. Univ., 20:327.

Khaled, N. F. (1995). Response of sheep to feeding on different levels of dried rumen contents. Thesis of M. Sc. Fac. of Vet. Cairo Univ.

Kholif S. M. (2005).
Use of Biotechnology to Improve the Utilization of Rumen Contents in Ruminant Rations. 20-40p. M.Sc. Thesis, Fac. Agric., Ain Shams Univ., Egypt

Kholif S. M. (2006). Effect of improving the nutritional value poor quality roughages on the yield and composition of goat's milk. Egyptian J. Dairy Sci., 34:197-205.
.

Knowles, J.; Lethtovaara, P. and Reeri, T.T. (1987). Cellulase families and their genes. Trends Biotechnol., 5:255-261.

Knowlton, K. Taylor, M. S.; Hill, S.; Cobb, R. C. and Wilson, K. F. (2007). Manure nutrient excretion by lactating cows fed exogenous phytase and cellulase. J. Dairy Sci., 90:4356-4360.

Knowlton, K. F.; McKinney, J. M. and Cobb, C. (2002). Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation Holstein cows- J. Dairy Sci., 85:3328.

Krause M., Beauchemin K. A., Rode L. M., Farr B. I. and Norgaard P. (1998).

Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle**. J. Anim. Sci., 76: 2912-2920**

Krueger, N.A. and Adesogan, A.T. (2008). Effect of different mixtures of fibrolytic enzymes on the digestion and fermentation of bahiagrass hay. Anim. Feed Sci. Technol., 145: 84-94.

Lewis, D. (1957). Blood urea concentration in relation to prevent utilization in the ruminant. J. Agric. Sci. 48: 438.

Lewis, G. E.; Hunt C. W.; Sanchez, W. K.; Treacher, R.; Pritchard, G. and Feng, P. (1996). Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage- based diet fed to beef steers. J. Anim. Sci., 74:3020-3028.

Lynd, L.R.; Weimer, P.J.; van Zyl, W.H. and Pretorius, I.S. (2002). Microbial cellulose utilization: fundamentals and biotechnology. Microbiol; Mol Biol. Rev., 66:506-577.

Lyo A. H. and Antai S.P. (1988). Effect of different nitrogen source on lignocellulose degradation. Mol. Biol. Rev., 66:506-577.

Mahrous M.H, El-Shafie M. A. and Abd El-Khalek T.M. (2005). Effect of biological, chemical and chemic-biological treatments on the nutritive value of corn cubes. Animal Prod. Res. Inst., **pp. 30-40. Second Conference 27 – 29 September 2005. Sakha, Kafer El-Sheakh, Egypt.**

Mahrous M.H. (2006). Treatment of agricultural by-products to manufacture silage and its effects on performance of small ruminants. Egyptian J. Nutrition and Feeds, 9 (2): 150-160.

Massadeh M., Yusoff W.M.W., Omar O. and Kader J. (2001). Synergism of cellulase enzymes in mixed culture solid substrate fermentation. Biotechnol Lett., 23:1771.

McAllister, T.A.; Hristov, A.N.; Beauchemin, KA.; Rode, L.M. and Cheng, KJ. (2001). Enzymes in Ruminant Diets. In: "Enzymes in Farm Animal Nutrition". (Eds. Bedford, M. R. and. Partridge, G. G.). CABI Publishing, Finn feeds Marlborough Wiltshire, UK, pp. 273-298.

McHan F. (1986a). Cellulase treated coastal bermudagrass silage and production of soluble carbohydrates, silage acids and digestibility. J. Dairy Sci., 69:431-438.

McHan, F. (1986b). Pretreatment of coastal bermudagrass with sodium hydroxide and cellulase before ensiling. J. Dairy Sci.,69:1837-1846.

Merten D.R. (1977). Dietary fiber components: Relationship to the rate and extent of ruminal digestion. Federation Proceeding 36:187-192.

Mohamed, A.M.; El-Saidy, B.E.; Ibrahim, K.; Tejido, M.L. and Carro-, M.D. (2005). Effect of exogenous enzymes on in vitro ruminal fermentation of a high forage diet and productive response of lactating ewes. Egyptian J. Nutrition and feeds, 8 (Special Issue): 591-602

Mohamed J.S., Fwad M., Amira A.I., Basima A.A., Siaham S.A. and Raya S.B. (1989). Cellulose production from actiomycetes isolated from soils: III. Detection and quantitative assay of cellulose production by actinomycetes and singcongo red staining of substarte. J. Biol. Sci. Res. Vol. 20 (2) 1989.

Morrison, I.M. (1988). Influence of chemical and biological pretreatments on the degradation of lignocellulosic material by biological systems. J. Sci. Food .Agric., 42:295-304

Muwalla, M.M.; Haddad, S.G. and Hijazeen, M.A. (2007). Effect of fibrolytic enzyme inclusion in high concentrate fattening diets on nutrient digestibility and. growth performance of Awassi lambs. Livestock Science. 1:255.

Nadeau, E. M.; Russell, J. R. and Buxton, D. R. (2000). Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. J. Anim. Sci., 78:-2980.

Nikhat, P.; Kahlon, S. S.; Sethi, R. P. and Chopra, A. K. (1983). Solid state fermentation of wheat straw into animal feed. Indian J. Anim. Sci., 5: 1193.

N.R.C., National Research Council, (1985). Nutrition requirements of sheep 6^{th} revised ED. National Academy Press. Washington.D.C., USA.

Nowar, ll. S.K. AI-Shinawy and H.N. Khoury (1993). Effect on fattening lambs performance with special reference to blood hematology liver and kidney functions. J. Biol. Sci. Res. Vol. 21 (2) 1989.

Pakistan Agriculture Research Council, (P.A.R.C.) 1982. Manual for feed Analytical laboratory. Karl Heinz Merke and Herbert Steingass. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. **Institute for Animal Nutrition, Univ. of Hobenbeim.**

Pandey A., Selvakumar P., Soccol C.R. and Nigam P. (1999). Solid-state fermentation for production of industrial enzymes. Curr Sci., 77:149-162.

Patton, C.J. and S.R. Crouch. 1977. Determination of urea (urease modified Berthelot reaction). Anal. Chem., 49: 464-469.

Peters, F.G. (1968). Determination of blood total protein. Clin. Chem., 14:1147.

Pinos, R. J.; Moreno, R.; Gonzales, S.S.; Robinson, P.H. Mendoza, G. and Álvarez, G. (2007). Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. Anim. Feed Sci. Technol., 10: 1016

Pinos, R. J.; Gonzalez, S. S.; Mendoza, G. D. ; Barcena, R.; Cobos, M. A. ; Hernandez, A. and Ortega, M. E. (2002). Effect of exogenous fibrolytic enzyme on ruminal fermentation and digestibility of alfalfa and rye-grass hay fed to lambs. J. Anim. Sci., 80:3016.

Pulatov G.S., Lgnotov A.D. and Nelyubin V.P. (1983). Biological properties of zeolites. **Trudy skogol Veterinarnogo institute**, **35: 30-33**

Ranilla, M.J.;Tejido, M.L.; Giraldo, L.A.; Tricarico, J.M. and Carro, M.D. (2007). Effects of an exogenous fibrolytic enzyme preparation on in vitro ruminal fermentation of three forages and their isolated cell walls. **Feed Sci. Technol., 10:05- 46**

Reitman A. and Frankel S. (1957). Colorimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvate transaminases. **Amer. J. Clin. Path., 28 : 56-58.**

Rode, L.M.; Yang, M.Z. and Beauchmen, K.A. (1999). Fibrolytic enzyme supplements for dairy cows in early lactation. **J. Dairy Sci., 82:2121**.

Rosales, E.; Couto, r. and Sanroman, A. (2002). New uses of food wastes: application to laccase production by *Trametes hirsute.* Biotechnology Lett., 24:701. Cited by Maulin et al. (2005).

SAS (1998). Statistical Analysis System. SAS User's Guide Statistics. SAS Institute Inc. Editors, Cary, NC., USA.

Salem, A. Z. M.; El- Adawy, M.; Gado, H.; Camacho, L. M.; Ronquillo M.; Alsersy, H.; Borhami, B. (2011). Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs**. Tropical and Subtropical Agroecosystems, 14 (2011): 867-874**

Singh M., Amrit-Kumar M.N., Rai S.N. and Paradhan P.K. (1993). Urea and Ammonia Treatment of Straw under Village Conditions, Reasons for Success and Failure. ICAR, New Delhi and Agric. Univ. Wageningen, pp. 289-296.

Smith, E. J. (1996). An industrial application of cellulases, J Biotech. Bioeng. 73: 68-83.

Stockes, M.R. (1992). Effects of an enzyme mixture, an inoculant, and their interaction on silage fermentation and dairy production. J. Dairy Sci., 75: 764 -773.

Sutton, J. D.; Phipps, RQH.; Beever, D. E.; Humphries, D. J.; Hartnell, G. F.; Vicini, J. L. and Hard, D. L. (2003). Effect of method of application of a fibrolytic enzyme product on digestive processes and milk production in Holstein-Friesian cows. J. Dairy Sci., 86:546.

Teeri, T.T. (1997). Crystalline cellulose degradation: new insights into the function of cellobiohydrolase. Trends Biotechnol., 15:160-167.

Terry, R.A.; .J.M.A. Tilley and G.E. Outen (1969). Effect of pH on cellulose digestion under in vitro conditions. J. Sci. Fd. Agric., 20: 317.

Titi, H.H. and Tabbaa, M.J. (2004). Efficacy-of exogenous cellulase on digestibility in lambs and growth of dairy calves. Liv. Prod. Sci., 87:207-214.

Tuen, A.A. and M.M. Dahan (1994). Chemical composition and utilization of rice straw by goats in Malasia. Nut. Agric. & Res.(series B). 64: p3538-3560.

Ward and Perry (1982). Enzymatic conversion of corn cobs to glucose with Trichoderma viride fungus and the effect on nutritional value of the corn cobs. J. of Anim. Sci., Vol. 54, No. 3:609 – 617.

Warner, A. C. J. (1964). Production of volatile fatty acids in the rumen. Methods of Measurements. Nut. Abst., and Rev., 34: 339.

Wood, T. M. and Garica Campayo V. (1990). Enzymology of cellulose degradation. Biodegradation., 1: 147-161.

Yang, X.; Chen, H.; Gao, H. and Li, Z. (2001). Bioconversion of corn straw by coupling ensiling and solid-state fermentation. Bioresour. Technol., 78: 277-280.

Yang, W. Z.; Beauchemin, K. A. and Rode, L. M. (2000). A comparison of methods of adding Hydrolytic enzymes to lactating cow diets. J Dairy Sci., 83:2512.

Yang, W.Z.; Beauchemin, K.A. and Rode, L.M. (1999). Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci., 82: 391-403.

Yildiz, G; O. H. Muglali and T. Dikicioglu (1995). Uses of rumen contents in lamb diets. Lalahan Hayvancilik Arastirma Enstitusu Dergisi., 35: 3 4, 71 91.

Zadrazil, F. (1977). The conversation of straw into feed by basidiomycetes. Eur. J. Appl. Microbial 4, 237 - 281.

Zadrazil, F. (1978). The Biology and Cultivation of Mushrooms, Academic Press, Ine., New York, 521p.

Zewil, M.G. (2005). Evaluation of some Treatment for Rice Straw. pp.45-58. M.Sc. Thesis, Fac. Agric. Al azhar University, Cairo, Egypt.

Zhang, Y, H.P. and Lynd, L.R. (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: non complexed cellulase systems. Biotechnol Bioeng., 88:797-824.

Zollner, N. and Kirsch, K. (1962). Determination of total lipids by colorimetric methods. Z.ges.exp.Med., 135-545.

الملخص العربي

أجريت هذه الدراسة في مزرعة كلية الزراعة جامعة عين شمس وشركة ايكارو لتدوير المخلفات الزراعية ومعامل تغذية الحيوان بقسم الإنتاج الحيواني بكلية الزراعة جامعة عين شمس. وكان الهدف من الدراسة هو بحث مدى قدرة بعض المعاملات البيولوجية باستخدام إنزيمات طبيعية على تحسين القيمة الغذائية لقش الارز المنمى عليه حبوب الشعير وتأثير ذلك على أداء أغنام الاوسيمى من خلال دراسة تأثير تلك المعاملات على معامل الهضم للمواد الغذائية المختلفة و نشاط بيئة الكرش و بعض قياسات الدم.

تم استخدام اثني عشر ذكر اوسيمى مكتمل النضج بمتوسط أوزان (0.5±45) كجم. وقد قسمت الحيوانات عشوائيا إلى أربع مجموعات لتلقى أربع معاملات، حيث استمرت التجربة لمدة 12 يوم(7 أيام تمهيدية ، 5 أيام تجريبية), وكانت العلائق في المعاملات المختلفة تتكون من الآتي:-

1- مجموعة المقارنة(المعاملة الاولى): قش الارز المنمى علية شعير (RSGB) بدون اضافة انزيم زاد وبدون اضافة قشر البرتقال(T1).

2- المعاملة الثانية: (RSGB) مع اضافة انزيم زاد وبدون اضافة قشر البرتقال (T2).

3- المعاملة الثالثة: (RSGB) بدون اضافة انزيم زاد ومع اضافة قشر البرتقال (T3).

4- المعاملة الرابعة: (RSGB) مع اضافة انزيم زاد و باضافة قشر البرتقال (T4).

وقد أوضحت النتائج ما يلي

1. معاملات الهضم الظاهرية للمركبات الغذائية المختلفة.

1.1. لم يلاحظ وجود فروق معنوية في المأكول من المادة الجافة بين المعاملات المختلفة.

2.1. سجلت المجموعات المعاملة بيولوجيا (الثانية والرابعة) قيما أعلى لمعاملات الهضم لكل العناصر الغذائية المختلفة بالمقارنة بالمجموعة المقارنة، كما سجلت المجموعة الثالثة قيما أعلى لمعاملات الهضم بالمقارنة بالمجموعة الأولى غير المعاملة.

2. القيمة الغذائية للعلائق المستخدمة.

ارتفعت القيمة الغذائية للعلائق المستخدمة مقدرة كمركبات غذائية كليه مهضومة (TDN) وبروتين مهضوم (DCP) بالمعاملات البيولوجية (المعاملة الثانية والرابعة) والمجموعة الثالثة مقارنة بالمجموعة الأولى (غير المعاملة).

3. نتائج قياسات سائل الكرش.

تم أخذ عينات سائل الكرش من كل الحيوانات داخل كل مجموعه قبل التغذية و بعد 3 و 6 ساعات من التغذية وقد أوضحت النتائج ما يلي:-

1.3 لوحظ ارتفاع بسيط في قيم pH سائل الكرش في المجموعات (الثانية والرابعة) أو غير المعاملة (الثالثة) مقارنة بالمجموعة المقارنة.

2.3. أدت المعاملات البيولوجية لمحتويات الكرش (الثانية والرابعة) إلى زيادة معنوية (5%) في مستوى الأحماض الدهنية الطيارة لسائل الكرش مقارنة بالمجموعة غير المعاملة (الأولى).

3.3. لوحظ ارتفاع طفيف في تركيزالامونيا- نيتروجين في سائل الكرش معنويا (5%) للمعاملات البيولوجية (الثانية والرابعة) والمجموعة الثالثة مقارنة بالمجموعة (الأولى).

4.3. جميع قياسات الكرش تأثرت معنويا بوقت أخذ العينة .

4- نتائج قياسات الدم.

تم أخذ عينات الدم من كل الحيوانات داخل المجموعات وأوضحت النتائج المتحصل عليها ما يلي:-

1.4. لوحظ ارتفاع طفيف في بروتين سيرم الدم لمجموعة المقارنة والمجموعات المعاملة (الثانية والرابعة) مقارنة بالمجموعة الأولى غير المعاملة، وكانت الفروق معنوية.

2.4. لوحظ ارتفاع طفيف في محتوى سيرم الدم من اليوريا للمجموعة المقارنة و المجموعات الثانية والرابعة (المعاملة) مقارنة بالمجموعة الثالثة (غير المعاملة) ، وكانت الفروق غير معنوية.

3.4. لم يلاحظ وجود فروق معنوية في محتوى سيرم الدم من الكرياتينين بين المعاملات المختلفة حيث سجلت المجموعة الرابعة أعلى قيمة مقارنة بباقي المعاملات سواء كانت معاملة أو غير معاملة.

4.4. لم يلاحظ أي تغير معنوي في نشاط إنزيمات الدم (GOT, GPT) نتيجة للمعاملات المختلفة و كانت جميعها في المعدل الطبيعي.

5.4. لم يلاحظ وجود فروق معنوية في محتوى سيرم الدم من الليبيدات الكلية بين مجموعة المقارنة والمجموعة الثالثة غير المعاملة. كما لم تلاحظ فروق معنوية بين المجموعتين الثانية والرابعة (المعاملة).

الخلاصة

يتضح من نتائج الدراسة أن هناك إمكانية لإستخدام قش الارز المنمى علية شعير سواء كان معامل او غير معامل بالانزيم كعلف للمجترات، إلا أن افضل النتائج تحصل عليها عند اضافة الانزيم(ZAD) وايضا عند اضافة قشر البرتقال مع الانزيم، وذلك دون وجود أي تأثيرات سلبية على الإنتاجية أو الحالة الصحية للحيوان.

زاد (ZAD)

هو مركب يتكون من مجموعة انزيمات فصلت من بكتيريا لاهوائية والتى فصلت بدورها من الكرش ، ويحتوى على خليط من الانزيمات (سليوليز، هيمى سليوليز ، بروتييز و ألفا اميليز).

إمكانية معاملة المخلفات الزراعية بإنزيمات خارجية وبكتريا لاهوائية لإنتاج اعلاف للمجترات

رسالة مقدمة من

رأفت محمود محمد جمعة

بكالوريوس علوم زراعية (إنتاج حيواني) ، جامعة عين شمس ، 2005

للحصول على

درجة الماجستير في العلوم الزراعية

(تغذية حيوان)

قسم الإنتاج الحيواني

كلية الزراعة

جامعة عين شمس

2012

صفحة الموافقة على الرسالة

إمكانية معاملة المخلفات الزراعية بإنزيمات خارجية وبكتريا لاهوائية لإنتاج اعلاف للمجترات

رسالة مقدمة من

رأفت محمود محمد جمعة

بكالوريوس علوم زراعية (إنتاج حيواني) ، جامعة عين شمس ، 2005

للحصول على

درجة الماجستير في العلوم الزراعية

(تغذية حيوان)

وقد تمت مناقشة الرسالة والموافقة عليها

اللجنة:

د. سلوى حمدي محمود .....
أستاذ تغذية الحيوان، كلية الزراعة، جامعة عين شمس

د. محمد سيد فرغلى .....
أستاذ تغذية الحيوان، كلية الزراعة، جامعة القاهرة

د. هاني محمود جادو .....
أستاذ تغذية الحيوان، كلية الزراعة، جامعة عين شمس

تاريخ المناقشة : / / 2012

جامعة عين شمس
كلية الزراعة

رسالة ماجستير

اسم الطالب : رأفت محمود محمد جمعة
عنوان الرسالة : إمكانية معاملة المخلفات الزراعية بإنزيمات خارجية وبكتريا لاهوائية لإنتاج اعلاف للمجترات
اسم الدرجة : ماجستير في العلوم الزراعية (تغذية حيوان)

لجنة الإشراف

د. هانئ محمود أحمد جادو
أستاذ تغذية الحيوان ، قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة عين شمس (المشرف الرئيسي).

د. حمدى محمد أحمد السيد
أستاذ تغذية الحيوان ، قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة عين شمس.

د. سليمان محمد سليمان عبد المولى
أستاذ تغذية الحيوان ، قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة عين شمس.

تاريخ التسجيل : 19 / 9 / 2007

الدراسات العليا

ختم الإجازة **أجيزت الرسالة بتاريخ** / /2012

موافقة مجلس الكلية **موافقة مجلس الجامعة**
/ /2012 / / 2012