

# **DECREASE NITROGEN EXCRETION BY RUMINANTS TO MITIGATE GREENHOUSE**

**Submitted By**

**Abo Bakr Tawfik Muhran Soliman**

B.Sc. of Agricultural Sciences (Public Division), Faculty of Agriculture, Asuit University,  
1996

M. Sc. in Environmental Sciences, Institute of Environmental Studies & Research,  
Ain Shams University, 2010

A thesis submitted in Partial Fulfillment  
Of  
The Requirement for the Doctor of Philosophy Degree  
In  
Environmental Sciences

Department of Environmental Agricultural Sciences  
Institute of Environmental Studies and Research  
Ain Shams University

**2018**

## APPROVAL SHEET

# **DECREASE NITROGEN EXCRETION BY RUMINANTS TO MITIGATE GREENHOUSE**

**Submitted By**

**Abo Bakr Tawfik Muhran Soliman**

B.Sc. of Agricultural Sciences (Public Division), Faculty of Agriculture, Asuit University,

1996

M. Sc. in Environmental Sciences, Institute of Environmental Studies & Research,

Ain Shams University, 2010

A thesis submitted in Partial Fulfillment

Of

The Requirement for the Doctor of Philosophy Degree

In

Environmental Sciences

Department of Environmental Agricultural Sciences

This thesis Towards a Doctor of Philosophy Degree in

Environmental Science Has been Approved by:

Name

Signature

**1-Prof. Dr. Fouad Metawie El Shouny**

Prof. of Biochemistry

Faculty of Agriculture

El-Menofia University

**2-Prof. Dr. Salwa Mahmoud Hamdy**

Prof. of Animal Nutrition

Faculty of Agriculture

Ain Shams University

**3-Prof. Dr. Hamdy Mohamed Ahmed El Sayed**

Prof. of Animal Nutrition

Faculty of Agriculture

Ain Shams University

**4-Prof. Dr. Nasr El Sayed Yehia El-Bordeny**

Prof. of Animal Nutrition

Faculty of Agriculture

Ain Shams University

**2018**

# **DECREASE NITROGEN EXCRETION BY RUMINANTS TO MITIGATE GREENHOUSE**

**Submitted By**

**Abo Bakr Tawfik Muhran Soliman**

B.Sc. of Agricultural Sciences (Public Division), Faculty of Agriculture, Asuit University,  
1996

M. Sc. in Environmental Sciences, Institute of Environmental Studies & Research,  
Ain Shams University, 2010

A thesis submitted in Partial Fulfillment  
Of  
The Requirement for the Doctor of Philosophy Degree  
In  
Environmental Sciences  
Department of Environmental Agricultural Sciences  
Under The Supervision of:  
**1-Prof. Dr. Hamdy Mohamed Ahmed El Sayed**  
Prof. of Animal Nutrition  
Faculty of Agriculture  
Ain Shams University

**2- Prof. Dr. Nasr El Sayed Yehia El-Bordeny**  
Prof. of Animal Nutrition  
Faculty of Agriculture  
Ain Shams University

**3-Dr. Hemmat Abd El Fattah Ebrahim Saeed**  
Associate Prof. of Biochemistry  
Faculty of Agriculture  
Ain Shams University

## ACKNOWLEDGEMENT

Praise and thanks be to **ALLAH**, the most merciful for directing me to the right way and provides me all I have.

I would like to express my deepest and sincere and appreciate gratitude to the former supervisor **Prof. Dr. Hamdy Mohamed Ahmed El-Sayed**, Professor of Animal Nutrition, Animal Production Dept., Ain Shams University, for his supervision, guidance, valuable help, continuous support and encouragement during preparing this work and study up to the last minutes of his life.

I would like to express my deepest and sincere and appreciate gratitude to **Prof. Dr. Nasr Elsayed Yehia El-Bordeny**, Professor of Animal Nutrition, Animal Production Dept., Ain Shams University, for his supervision, guidance, valuable help and preparation of this manuscript.

I am indebted and sincere thanks to **Dr. Hemmat Saied**, Assistant Prof. Biochemistry, Faculty of Agriculture, Ain Shams University, for his supervision, and continuous encouragement during the study and preparation of this manuscript.

Special thanks to my colleagues in the Agricultural Science Department, Institute of Environmental Studies and Research, Ain Shams University, for their co-operation and friendly atmosphere.

Special thanks are due to the staff of Animal Production Department, Faculty of Agriculture, Ain Shams University, for their cooperation and friendly atmosphere.

I would like to express my gratitude and my deep thanks to my family for their great support and enhancing me throughout my life.

Finally, I wish to express my sincere gratitude to every one cooperate me during this work.

## ABSTRACT

**Abo Bakr Tawfik Muhran : Decrease nitrogen excretion by ruminants to mitigate greenhouse. Unpublished Ph.D. Thesis, Agricultural Science, Institute of Environmental Studies and Research, Ain Shams University, 2017.**

Exogenous fibrolytic enzyme (EFE) have been shown to improve nitrogen digestibility and feed efficiency in feedlot animals. So, this study aimed to evaluate effect of using EFE on nitrogen excretion as well as productive performance of growing lambs. Sixteen Barkey lambs (3 months old,  $22.31 \pm 1.57$  kg) were randomly assigned into two groups, 8 lambs for each according to live body weight. The first group (control) was fed control rations without EFE supplementation, while treated group were fed the control ration plus 2.5 g exogenous fibrolytic enzyme. The groups fed ration supplemented with EFE recorded higher DM, TDN and digestible CP intake. Supplementation of lambs ration with EFE showed no significantly effect on rumen liquor TVFA's and ammonia concentration at 0, 3 and 6 hrs post feeding. While Supplementation of lambs ration with EFE significantly increased rumen liquor pH at 0, 3 and 6 hrs after feeding compared to the control group. Exogenous fibrolytic enzyme significantly improved nutrients digestibility as dry matter, organic matter, crude protein, crude fiber, nitrogen free extract, neutral detergent fiber (NDF) and acid detergent fiber (ADF) as well as feeding values as TDN and digestible crude protein. Exogenous fibrolytic enzyme supplementation to lambs ration showed numerically increased ( $P > 0.05$ ) in plasma total protein concentration compared to lambs fed ration not supplemented. While albumin, globulin, triglycerides and creatinine concentration and Alanine Transaminase (ALT), Aspartate Transaminase (AST), alkaline phosphates activity were not significantly ( $P > 0.05$ ) affected by EFE supplementation. Total gain and average daily gain significantly increased ( $P \leq 0.05$ ) for group received rations supplemented with

EFE compared to control group. Also supplementation lambs rations with EFE significantly ( $P \leq 0.05$ ) improved feed conversion as DM, TDN, CP and DCP compared to the control group. It could be concluded that supplementing lambs ration with EFE resulted in increased feed intake and digestibility, consequently increased average daily gain and feed conversion without any adverse effect on animal health and performance. As well as exogenous fibrolitic enzyme to animal ration resulted in decrease nitrogen excretion in animal feces which maybe had a positive effect on the environments

**Key words:** lambs, Exogenous fibrolytic enzyme, feed intake, digestibility, growth performance.

# CONTENTS

	Page
<b>1. INTRODUCTION</b>	<b>1</b>
<b>2. REVIWE OF LITERATURE</b>	<b>4</b>
2-1- Environmental pollution caused by animal production	4
2-1-1 Nitrogen losses	6
2.1.2 Phosphorus Losses	11
2-1-3 Potassium losses	13
<b>2.2. Using exogenous fibrolytic enzyme in ruminant feeding</b>	<b>14</b>
2-2-1 Types, sources and extraction of enzymes	14
2-2-2 Application of enzymes	18
2-2-3 Production responses in ruminants	20
2-2-3-1 Dairy cattle:	20
2-2-3-2 Beef cattle:	22
2-2-3-3 Sheep:	23
2-2-3-4 Goat:	26
2-2-3-5 Buffalo:	27
<b>3. MATERIALS AND METHODS</b>	<b>28</b>
3-1 Exogenous fibrolytic enzyme	28
3-2 Animals, diets, feeding and experimental design	28
3-3 Digestibility trials	29
3-4 Rumen activity	30
Feed conversion:	30
Chemical analysis:	30
Blood parameters:	31
Statistical analysis:	31
<b>4. RESULTS AND DISCUSSION</b>	<b>33</b>
4-1 Effect of the experimental treatment on Feed intake	33
4-2 Effect of experimental treatments on rumen fermentation parameters: -	35

4-2-1 Total volatile fatty acids concentration: -	35
4-2-2 Ammonia nitrogen concentration:-	36
4-2-3 pH value:	41
4-3 Effect of experimental treatments on nutrients digestibility and nutritive values:	42
4-4 Effect of experimental treatments on blood plasma parameters:	46
4-5 Effect of treatments on growth performance: -	47
<b>5. SUMMARY AND CONCLUSION</b>	<b>50</b>
5-1 Exogenous fibrolytic enzyme	50
5-2The results are summarized as follows	50
5-2-1 Effect of the experimental treatment on Feed intake	50
5-3 Effect of experimental treatments on rumen fermentation parameters:	50
5-3-1 Total volatile fatty acids concentration: -	51
5-3-2 Ammonia nitrogen concentration: -	51
5-3-3 pH value: -	51
5-4 Effect of experimental treatments on nutrients digestibility and nutritive values:	51
5-5 Effect of experimental treatments on blood plasma parameters: -	51
5-6 Effect of treatments on growth performance: -	52
<b>Conclusion:</b>	<b>52</b>
<b>6. REFERENCES</b>	<b>53</b>
<b>ARABIC SUMMEARY</b>	



## LIST OF TABLES

No.	Title	Pages
1.	Cellulase and xylanase producing microorganisms and optimum conditions for the production ( <b>Motta <i>et al.</i>, 2013; Sadhu and Maiti, 2013</b> ) (modified).....	16
2.	Chemical composition of the experimental rations Ingredients (%). ....	29
3.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on feed intake.....	33
4.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on rumen fermentation kinetics.....	37
5.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on nutrient digestibility coefficients.....	43
6.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on some blood plasma parameters.....	47
7.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on growth performance .....	48

## LIST OF FIGURES

No.		Pages
1.	Effect of the experimental treatment on Feed intake	34
2.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on feed intake	34
3.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on feed intake	35
4.	Effect of the experimental treatment on TVFA's concentration	38
5.	Effect of the experimental treatment on pH value	38
6.	Effect of the experimental treatment on ammonia concentration	39
7.	Effect of treatments on Cellulose activity	39
8.	Effect of treatments on Specific activity of cellulose	40
9.	Effect of the experimental treatment on Digestibility coefficients	44
10.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on nutrient digestibility coefficients	44
11.	Effect of lamb's ration supplementation with exogenous fibrolytic enzyme on nutrient digestibility coefficients	45
12.	Effect of treatments on growth performance	48
13.	Effect of treatments on Dry matter and TDN conversion	49
14.	Effect of treatments on Crude protein and Digestible protein	49

## **LIST OF ABBREVIATION**

<b>ADF</b>	Acid Detergent Fiber
<b>ADL</b>	Acid Detergent lignin
<b>CF</b>	Crude Fiber
<b>CFM</b>	Concentrate Feed Mixture
<b>CP</b>	Crude Protein
<b>DCP</b>	Digestible crude Protein
<b>dDM</b>	degraded Dry Matter
<b>DE</b>	Digestible Energy
<b>DM</b>	Dry Matter
<b>DMI</b>	Dry Matter Intake
<b>ECE</b>	Exogenous cellulitic enzyme
<b>EE</b>	Ether Extract
<b>EFE</b>	Exogenous fibrolytic enzyme
<b>GE</b>	Gross Energy
<b>ME</b>	Metabolizable Energy
<b>NDF</b>	Natural Detergent Fiber
<b>NFC</b>	Non fiber carbohydrate
<b>NH<sub>3</sub>-N</b>	Ammonia concentration
<b>OM</b>	Organic Matter
<b>TDN</b>	Total Digestible Nutrient
<b>TDNI</b>	Total Digestible Nutrient Intake
<b>TVFA's</b>	Total Volatile Fatty acids

## 1. INTRODUCTION

Nitrogen is one of the elements of amino acids (AAs) that form proteins allowances by all animals; animals consume nitrogen in all its forms (protein, AAs and non-protein nitrogen) and then excrete various nitrogen forms. But these forms of nitrogen, if directly discharged into water surface in runoff or deposited in the water from aerial emissions, may be cause water pollution. Furthermore, nitrogen volatilization in the form of ammonia (NH<sub>3</sub>) from the animal manures is one of the important environmental concern. Which the volatilized nitrogen returns to the water or land dry precipitation, via rainfall, or direct absorption.

Although the ammonia nitrogen may be beneficial as a fertilizer for agricultural fields (plant fertilizer), it may be not benefit in another ecosystem. Most of livestock are produced in open feedlots, which the losses of nitrogen can be represent about 70% of the N excreted by those animals. The excess of Nitrogen over required protein amounts is excreted primarily as urea in the urine, and urease activity in feces resulted in volatilize much of the excreted nitrogen (**Hutson *et al.* 1998; Rotz *et al.* 1999**).

The Council for Agricultural Science and Technology (CAST) published a report entitled Integrated Animal Waste Management. One of the most important recommendations in this report was to “change animal diets to decrease nutrient outputs” The nutrients were nitrogen and phosphorus (**CAST 1996, 1**).

Improvements of feed utilization and animal productivity are the aims of most of nutrition studies. One of the important of the environment issues is decrease nitrogen pollution through improveing nitrogen utilization in ruminant. These aims could be achieved by producing exogenous fibrolytic enzyme products to use it as feed additives. Many of the feed additives have been used to improve animal performance and feed utilization efficiency.

Recent research has demonstrated that supplementation diets of dairy animal and feedlot with fiber degrading enzymes can improve feed utilization

and animal performance by enhancing fiber degradation in vitro (**Hristove *et al.*, 1996; Gado *et al.*, 2007; El-Adawy *et al.*, 2008; Rodrigues *et al.*, 2008**), in situ ( **Feng *et al.*, 1996; Lewis *et al.*, 1996; Tricarico *et al.*, 2005; Krueger *et al.*, 2008**) and in vivo (**Yang *et al.*, 1999; Gado *et al.*, 2007; Salem *et al.*, 2007; Gado and Salem, 2008**). Feeding enzymes is often accompanied by increased feed intake, which may partly be due to increased palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis. However post-ingestive enzyme effects, such as increased digestion rate and /or extent of digestion (**Beauchemin and Rode, 1996; Feng *et al.*, 1996; Gado and salem, 2008; Krueger *et al.*, 2008**) may increase hydrolytic activity in the rumen to reduce gut fill and enhance feed intake (**Adesogan, 2005**).

Positive effects of adding exogenous enzymes to ruminant diets have been reported for lactating dairy cows and growing cattle. Dairy cows fed forage treated with a fibrolytic enzyme additive ate more feed and produced 5–25% more milk (**Lewis *et al.*, 1995; Tricarico *et al.*, 2005; Stella *et al.*, 2007**), improved the energy balance of transition dairy cows (**DeFrain *et al.*, 2005**) and increased milk production in small ruminants (**Titi and Lubbadah, 2004; Stella *et al.*, 2007**). In feedlot cattle, fibrolytic enzymes have improved live weight (LW) gain by as much as 35% and feed conversion ratio by up to 10% (**Beauchemin *et al.*, 1995**).

Work on the exploitation of the largest proportion of nitrogen in the feed in addition to after economic, it less than the amount of nitrogen volatile and added to the air of air and prevent the transformation of the nitrogen oxides that contribute to the destruction of the ozone layer while reducing acid rain pollution and caused by adverse changes in neighborhoods of the aquatic environment.

So, the objective of this study was to evaluate effect of using exogenous cellulitic enzyme on efficiency of protein utilization and productive performance of growing lambs.

## **2. REVIEW OF LITERATURE**

### **2-1- Environmental Pollution Caused by Animal Production**

As indicated earlier, environmental pollution by animal production systems is caused by two imbalances. The intensity of the nutrient flux through the animal part of the soil-plant-animal system often exceeds the capacity of the other components of the system to efficiently utilize the elements C, N, P, and K. Besides, the ratio between C, N, P, and K required in animal production differs from the ratio that can be efficiently handled by the soil and plant components of the system. Apart from deposition on the soil, the imbalances also cause the escape of C, N, P, and K to two other components essential for the system, the atmosphere and subsoil and surface water.

The physical appearance of the four elements differs. This not only influences their availability as a nutrient for plants and animals but is also important for the ease with which they may become dispersed in the environment. Availability in the different compartments of the soil-plant-animal system is influenced by many different factors and interactions. Nutrient availability in the soil is beyond the scope of this paper, and discussion here is restricted to some aspects of dispersion in the environment. The elements C and N appear in solid, solute, and gaseous forms, whereas only solid and solute forms of P and K are known to occur in nature. Dispersion of solid forms are least difficult to control, solute forms cause more problems, and controlling the dispersion of gaseous forms is almost impossible.

A continuous exchange of C from solid through solute to gaseous forms and vice versa takes place through photosynthetic, respiratory, and fermentative processes occurring in the soil simultaneously. Comparable, but more complicated, conversions take place with N through processes known as N-fixation, ammonification, nitrification, and denitrification. The ammonification of urea, excreted onto the soil as urine, causes ammonia to escape into the air followed by deposition and nitrification to  $\text{NO}_3^-$ , which

easily leaks into subsoil water reservoirs. Alternatively, the denitrification of nitrate may cause the escape of nitrous oxides (NO<sub>x</sub>) . Ammonia is believed to contribute to the acid deposition, and nitrous oxides are known for their harmful effects on the ozone layer.

A special situation occurs in ruminants when C is converted into methane (CH<sub>4</sub>) . Methane is also considered harmful to the ozone layer as well as contributing to the greenhouse effect (**Tamminga, 1992a**). Methane escapes to the air in relatively small quantities, because of its much higher intensity of infrared energy absorption than CO<sub>2</sub>, and CH<sub>4</sub>'s contribution to the greenhouse effect per gram is around 30 times higher than that of CO<sub>2</sub> (**Johnson *et al.*, 1991**). Less desired effects of CO<sub>2</sub> from animal production are restricted to those resulting from the use of fossil energy.

Phosphorus is an important nutrient in primary production. This element often starts its entry in the soil-plant-animal system as an ingredient of the feed, particularly when a high concentrate to milk ratio is applied. It is more serious for concentrate feeds if they are based on by-product ingredients. Such ingredients have a relatively high P content, of which a relatively small proportion is absorbed and retained from it by ruminants, and a major part is excreted in feces. When this is subsequently used to fertilize the soil, less P is leaving the system in animal products than enters the system with imported (concentrate) feed ingredients. In the long run this may make P accumulate in the soil until the soil becomes saturated, after which P will filter into groundwater or remain in surface water.

The element K is highly soluble in water. Compared with those of animals, K requirements of plants are relatively high and again concentrate ingredients may contain high amounts, much higher than the animal can efficiently use. Accumulation in the soil does occur only to a limited extent and excess K leaves the system in ground and surface water. Although maximum values of 12 mg K/L are used for drinking water, harmful effects are not well documented. Many foods contain much higher levels; for instance, milk contains 1,500 mg K/L (**Van Boheemen *et al.*, 1991**).