

**EVALUATION OF DIFFERENT TREATMENTS  
FOR IMPROVING EFFICIENCY OF  
UTILIZATION OF AGRICULTURAL  
BY-PRODUCTS IN RUMINANT  
FEEDING**

By

**MARWA ATEF SAYED MADKOUR**

B.Sc. Agric. Sc., (Animal production), Cairo University, 1999

M.Sc. Agric. Sc., (Animal nutrition), Ain Shams University, 2013

**A Thesis Submitted in Partial Fulfillment  
Of  
The Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY**

**in**

**Agricultural Sciences  
(Animal Nutrition)**

**Department of Animal Production  
Faculty of Agriculture  
Ain shams University**

**2018**

**Approval Sheet**

**EVALUATION OF DIFFERENT TREATMENTS  
FOR IMPROVING EFFICIENCY OF  
UTILIZATION OF AGRICULTURAL  
BY-PRODUCTS IN RUMINANT  
FEEDING**

By

**MARWA ATEF SAYED MADKOUR**

B.Sc. Agric. Sc., (Animal production), Cairo University, 1999  
M.Sc. Agric. Sc., (Animal nutrition), Ain Shams University, 2013

**This thesis for Ph.D. degree has been approved by:**

**Dr. Mounir Mahmoud Ebrahim El-Adawy** .....  
Prof. Emeritus of Animal Nutrition, Faculty of Agriculture, Alexandria  
University

**Dr. Hussein Saad Hussein Soliman** .....  
Prof. Emeritus of Animal Nutrition, Faculty of Agriculture, Ain Shams  
University

**Dr. Nasr Elsayed Yahya El-Bordeny** .. .....  
Prof. of Animal Nutrition, Faculty of Agriculture, Ain Shams University

**Dr. Hamdy Mohamed Mohamed Khattab** .....  
Prof. Emeritus of Animal Nutrition, Faculty of Agriculture, Ain Shams  
University

**Date of Examination: 4/10/2018**

**EVALUATION OF DIFFERENT TREATMENTS  
FOR IMPROVING EFFICIENCY OF  
UTILIZATION OF AGRICULTURAL  
BY-PRODUCTS IN RUMINANT  
FEEDING**

By

**MARWA ATEF SAYED MADKOUR**

B.Sc. Agric. Sc., (Animal production), Cairo University, 1999  
M.Sc. Agric. Sc., (Animal nutrition), Ain Shams University, 2013

**Under The Supervision of:**

**Dr. Hamdy Mohamed Mohamed Khattab**

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

**Dr. Nasr Elsayed Yahya El-Bordeny**

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

**Dr. Badr Elbastawisy Mattar.**

Chief Researcher of Animal Nutrition, Department of Animal  
nutrition, Animal Production Research Institute, Agriculture  
Research Center.

# CONTENTS

	Page
<b>1 INTRODUCTION.....</b>	1
<b>2. REVIEW OF LITERATURE.....</b>	2
2-1- Chemical composition and characterization of crop residuals...	2
2-2- Bio-upgrading of low quality roughages.....	2
2-2-1- Biological treatments.....	4
2-2-1-1-Microorganisms used in biological treatments.....	5
2-2-1-2- Mode of action of biological treatments.....	5
2-2-1-3- Effect of biological treatments on chemical composition of crop residues.....	7
2.3- Effect of crop residuals bio upgrading on <i>in vitro</i> fermentation parameters.....	8
2.4- Effect of biological treatments on animal performance.....	11
2-4-1-Effect on feed intake.....	11
2-4-2- Effect on nutrients digestibility and nutritive values.....	12
2-4-3- Effect on rumen liquor parameters.....	12
2-4-4- Effect of biological treatments on some blood parameters....	14
2-4-5- Effect on growth performance.....	15
2-5- Microbial feed additives (Direct fed microbials, DFM).....	16
2-5-1 Definition of probiotic or direct-fed microbials (DFM).....	16
2-5-2-Types of Direct-Fed Microbial.....	16
2-5-3 General mode of action for DFM.....	17
2-5-4 Effect on milk yield and its composition in lactating animals..	17
2-5-5- Effect on beef production.....	20
2-5-6- Spore forming bacteria as DFM sources.....	20

<b>3- MATERIALS AND METHODS.....</b>	<b>25</b>
3.1. Biological treatments and microbial media extracts (DFM)...	25
3.1.1. Biological treatments.....	25
3.1.2. Microbial media extracts as a direct fed microbes (DFM)....	26
3.2. <i>In Vitro</i> evaluation.....	27
3.2.1. <i>In Vitro</i> batch culture technique.....	27
3.2.1.1. Incubation and sampling procedures.....	28
3.2.1.2 Determination of basic rumen fermentation parameters.....	28
3.2.1.2.1. Determination of pH value and ammonia concentration....	28
3.2.1.2.2. Total gas production.....	29
3.2.1.2.3. Determination of microbial population.....	29
3.2.1.2.4. In vitro calculation.....	30
3.2.1.2.5. Relative gas production parameters calculation.....	30
3.3. Rustic system.....	31
3.3.1 Rumen inoculum and artificial saliva.....	32
3.3.2 RUSTIC fermenter set-up.....	32
3.3.3. Sampling.....	33
3.3.4 Analytical methods.....	34
3.4. <i>In vivo</i> trial.....	34
3.4.1. Preparing of the direct fed microbial (DFM) products.....	34
3.4.2. The experimental animals.....	35
3.4.3. The experimental rations.....	35
3.4.4. Digestibility trials .....	37
3.4.5. Rumen activity.....	37
3.4.6. Blood samples.....	37
3.4.7. Feed conversion calculation.....	38
3.4.8. Chemical analysis.....	38
3.4.8.1 Feed and feces sample .....	38
3.4.8.2. Rumen fermentation parameters.....	39

3.4.8.3. Blood plasma parameters.....	39
3.4.8.4. Statistical analysis.....	39
<b>4- RESULTS AND DISCCUTIONS.....</b>	<b>41</b>
4.1. Biological treatments of rice straw.....	41
4.1.1. Effect of different biological treatments on chemical composition of rice straw.....	41
4.1.2. Effect of different biological treatments on recovery rate of solid substrate and degradation rate of cell wall contents.....	44
4.2. <i>In vitro</i> evaluation trials.....	46
4.2.1. <i>In vitro</i> evaluation of ration containing biological treated rice straw using batch culture technique.....	47
4.2.1.1. Effect on experimental rations chemical composition.....	47
4.2.1.2. Effect on <i>in vitro</i> DM and OM Degradability.....	47
4.2.1.3. Effect on <i>in vitro</i> gas production .....	51
4.2.1.4. Effect on <i>in vitro</i> methane production.....	54
4.2.1.5 Effect on <i>in vitro</i> calculated microbial biomass production (MBP), efficiency of microbial biomass production (EMBP) Short Chain Fatty Acid (SCFA) and metabolisable energy (ME) .....	56
4.2.1.1.6. Effect on <i>in vitro</i> fermentation parameters and rumen flora count.....	59
4.2.2. Evaluation of the best two biological treatments using rumen stimulation technique (RUSITEC).....	62
4.2.2.1. Effect on nutrients degradability .....	62
4.2.2.2. Effect on gas and methane production .....	65
4.2.2.3. Effect on fermentation parameters.....	65
4.2.3. <i>In vitro</i> evaluation of ration containing untreated rice straw and supplemented with different direct feed microbes (DFM) using batch culture technique.....	68
4.2.3.1 Effect on <i>in vitro</i> DM and OM degradability .....	68

## IV

4.2.3.2. Effect on gas production.....	70
4.2.3.3 Effect on methane production.....	73
4.2.3.4. Effect on calculated microbial biomass production (MBP), efficiency of microbial biomass production (EMBP) Short Chain Fatty Acid (SCFA) and metabolisable energy (ME).....	75
4.2.3.5. Effect on fermentation parameters and total rumen bacteria count.....	77
4.2.4. Evaluation of the best two DFM supplementation using rumen stimulation technique (RUSITEC).....	79
4.2.4.1. Effect on nutrients degradability .....	79
4.2.4.2. Effect on gas and methane production.....	81
4.2.4.3. Effect on fermentation parameters and flora counts .....	83
4.3. <i>In vivo</i> trial .....	85
4.3.1. Effect of inclusion DFM's in lamb's ration on nutrient digestibility.....	85
4.3.2. Effect of inclusion DFM's in lamb's ration on feed intake.....	88
4.3.3. Effect of inclusion DFM's in lamb's ration on rumen fermentation parameters .....	89
4.3.3.1. Total volatile fatty acids concentration.....	90
4.3.3.2. Ammonia nitrogen concentration.....	90
4.3.3.3. pH value.....	92
4.3.3.4. Cellulose activity.....	93
4.3.4. Effect of inclusion DFM's in lamb's ration on Blood metabolic parameters.....	93
4.3.4.1. Blood plasma total protein concentration.....	93
4.3.4.2. Blood plasma albumin concentration.....	95
4.3.4.3. Blood plasma globulin concentration.....	95
4.3.4.4. Blood plasma albumin: globulin (A: G) ratio.....	96
4.3.4.5. Blood plasma urea concentration.....	96
4.3.4.6. Blood plasma tri-glyceride concentration.....	96
4.3.4.7. Blood plasma creatinine concentration.....	97
4.3.4.8. Blood plasma ALT, AST and alkaline phosphatase activity.....	97

4.3.5. Effect of inclusion DFM in lambs ration on growth performance.....	98
4.3.6. Economic efficiency.....	100
4.4. Conclusion .....	100
<b>5- SUMMARY AND CONCLUSION.....</b>	<b>102</b>
<b>6. REFERENCES.....</b>	<b>118</b>
<b>ARABIC SUMMARY.....</b>	



## LIST OF TABLES

No.	Title	Pages
1.	Chemical composition of some agricultural by-products.....	3
2.	Effect of biological treatments on chemical composition of crop residues.....	9
3.	Microorganisms used in DFM products.....	18
4.	The effects of various strains of DFM on adult ruminant performance.....	21
5.	Effects of DFM containing bacilli on ruminant performance....	24
6.	Cellulases Enzymes activity of different fungal and bacterial direct fed microbes.....	27
7.	Chemical composition of the experimental rations Ingredients (%).....	36
8.	Effect of different biological treatments on chemical composition of rice straw on DM basis.....	42
9.	Effect of different biological treatments on DM and OM recovery rates and CF, NDF and ADF degradation of rice straw after three-week incubation period.....	45
10.	Effect of fungal treatment versus bacterial treatments on DM and OM recovery rates and CF, NDF and ADF degradation rates of rice straw after three-week incubation period.....	46
11.	Effect of inclusion different biological treated rice straw in tested rations on its calculated chemical composition on DM basis.....	48
12.	Effect of different biological treated rice straw on <i>in vitro</i> DM and OM degradability after 24 hours.....	49
13.	Effect of fungal and bacterial treated rice straw on <i>in vitro</i> DM and OM degradability after 24 hours .....	50
14.	Effect of inclusion different biological treated rice straw in tested rations on total gas production after 24 hrs. <i>in vitro</i>	

## VII

	fermentation.....	52
15.	Effect of fungal and bacterial biological treated rice straw on total gas production after 24 hrs <i>in vitro</i> fermentation .....	53
16.	Effect of different biological treated rice straw on methane (CH <sub>4</sub> ) production after 24 hrs <i>in vitro</i> fermentation.....	55
17.	Effect of inclusion fungal and bacterial biological treated rice straw in tested rations on methane (CH <sub>4</sub> ) production after 24 hrs <i>in vitro</i> fermentation.....	56
18.	Effect of inclusion biological treated rice straw in experimental ration on calculated microbial biomass production (MBP), efficiency of microbial biomass production (EMBP) Short Chain Fatty Acid (SCFA) and metabolisable energy (ME).....	58
19.	Effect of inclusion fungal and bacterial treated rice straw in experimental ration on calculated microbial biomass production (MBP), efficiency of microbial biomass production (EMBP) Short Chain Fatty Acid (SCFA) and metabolisable energy (ME).....	59
20.	Effect of different biological treated rice straw on <i>in vitro</i> fermentation parameters and total rumen bacteria count after 24 hrs.....	60
21.	Effect of inclusion fungal and bacterial treated rice straw in experimental ration on <i>in vitro</i> fermentation parameters and total rumen bacteria count after 24 hrs .....	61
22.	Effect of inclusion <i>P. ostreatus</i> and <i>P. polymexa</i> treated rice straw and untreated rice straw in experimental ration on nutrients degradability (%) after 48 hrs. of fermentation in rumen stimulation technique.....	63
23.	Effect of inclusion <i>P. ostreatus</i> and <i>P. polymexa</i> treated rice straw in experimental ration on gas production and methane emission after 48 hrs. of fermentation in rumen stimulation technique.....	66
24.	Effect of inclusion <i>P. ostreatus</i> and <i>P. polymexa</i> treated rice	

## VIII

straw in experimental ration on fermentation parameters and rumen flora count after 48 hrs. of fermentation in rumen stimulation technique.....	67
25. Effect of DFM supplementation on DM and OM degradability after 24 hrs. <i>in vitro</i> fermentation.....	69
26. Effect of fungal and bacterial DFM supplementation on DM and OM degradability of (%) after 24 hrs. <i>in vitro</i> fermentation.	70
27. Effect of different DFM supplementation on total gas production after 24 hrs. <i>in vitro</i> fermentation .....	71
28. Effect of fungal and bacterial DFM supplementation on total gas production after 24 hrs. <i>in vitro</i> fermentation .....	73
29. Effect of different DFM supplementation on methane (CH <sub>4</sub> ) produced after 24 hrs. <i>in vitro</i> fermentation.....	74
30. Effect of fungal and bacterial DFM supplementation on methane (CH <sub>4</sub> ) produced after 24 hrs. <i>In vitro</i> fermentation...	75
31. Effect of different DFM supplementation on calculated MBP (mg/g DM), EMBP (mg/mg dDM), SCFA (mmol/g DM) and ME (Mcal/kg DM).....	76
32. Effect of fungal and bacterial DFM supplementation on calculated MBP, EMBP, SCFA and (ME).....	77
33. Effect of different DFM supplementation on fermentation parameters and total rumen bacteria count after 24 hrs. <i>in vitro</i> fermentation.....	78
34. Effect of fungal and bacterial DFM supplementation on fermentation parameters and total rumen bacteria count after 24 hrs. <i>in vitro</i> fermentation.....	79
35. Effect of ration supplementation with <i>Ph. Chrysosporium</i> , <i>B. subtilis</i> and mixed DFM on nutrients degradability (%) after 48 hrs. of fermentation in rumen stimulation technique.....	80
36. Effect of ration supplementation with <i>Ph. Chrysosporium</i> , <i>B. subtilis</i> and mixed DFM on gas and methan production after 48 hrs. of fermentation in rumen stimulation technique.....	82

37.	Effect of ration supplementation with <i>Ph. Chrysosprium</i> , <i>B. subtilus</i> and mixed DFM on fermentation parameters and rumen flora count after 48 hrs. of fermentation in rumen stimulation technique .....	84
38.	Effect of lamb's ration supplementation with different DFM on nutrient digestibility coefficients .....	86
39	Effect of lamb's ration supplementation with different DFM on feed intake .....	89
40.	Effect of lamb's ration supplementation with different DFM on rumen fermentation parameters .....	91
41.	Effect of lamb's ration supplementation with different DFM on some blood plasma parameters .....	94
42.	Effect of lamb's ration supplementation with different DFM on growth performance .....	99
43.	Effect of lamb's ration supplementation with different DFM on economic efficiency .....	101

## LIST OF FIGURES

<b>Fig.</b>		<b>Page</b>
1	Factors affecting roughage material quality .....	4
2	Fungal growth on solid substrate.....	7
3	Schematic presentation of the mode of cellulose action on crystalline cellulose fibrils.....	7
4	Effect of biological treatments on roughage material quality	10

## ABSTRACT

**Marwa Atef Sayed Madkour: Evaluation of Different Treatments for Improving Efficiency of Utilization of Agricultural By-Products in Ruminant Feeding. Unpublished Ph.D. Thesis, Department of Animal production, Faculty of Agriculture, Ain Shams University, 2018.**

The objective of this study was to select the best method to improve agriculture by-product utilization, as well as *in vivo* studies to the elected treatments. This study was carried out in 5 steps; in the first one, nine different microorganisms were used in bio-upgrading the chemical composition and nutritive values of rice straw. In the second *in vitro* evaluation for effect of inclusion of the bio-upgraded rice straw in ruminant rations using two techniques (batch culture and RUSITEC technique). In the third step the nine microorganisms media were used to produce direct fed microbes (DFM). In the fourth step *in vitro* evaluation for effect of supplementing ruminant rations with different DFM using two techniques (batch culture and RUSITEC). The fifth step was to evaluate *in vivo* trials for the best DFM supplementation. Biodegradation of rice straw with different fungal and bacterial microorganisms decreased organic matter (OM), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose content compared to untreated rice straw. However, crude proteins (CP), ash, acid detergent lignin (ADL), hemicellulose and non-fiber carbohydrate (NFC) contents were increased for biological treated rice straw compared to untreated. All rations containing biologically treated rice straw had no significant higher dry matter degradability (DMD) than that of the control ration. A significant increase was observed in OMD and the amount of total gas accumulated per g DM, OM, NDF and ADF for the rations containing biological treated rice straw compared to that containing untreated rice straw. The best fungal biological treatment was *P. ostreatus*, as well as the best bacterial biological treatment was *P. polymexa*. No significant differences in DMD % were observed among the experimental rations. However, a significant increase in OMD% was recorded for the

rations supplemented with different DFM compared to control except *T. viride* and *B. Circulance* treatments which were not significantly higher than control. *In vitro* total gas production per g DM, OM, NDF and ADF (ml/g) of rations supplemented with direct fed microbes after 24 hours in vitro fermentation were significantly increased with DFM supplementation except for *T. viride* and *B. circulance* which were not significantly different from the control. The best fungal DFM supplementation response was recorded for *Ph. chrysosporium*, As well as the best bacterial DFM response was noticed for *B. Subtilus*. Utilization *P. chrysosporium*, *B. Subtilus* DFM supplementation recorded higher dDM and dOM % as well as total accumulated gas production. In the *in vivo* experiment, thirty-two Barkey lambs (3 months old,  $22.31 \pm 1.57$  kg) were randomly assigned into four groups, 8 lambs of each. The first group (control) was given control rations without DFM supplementation, while treated groups (Fungal, Bacterial and Mixed DFM) were fed the control ration plus 2.5 g fungal, bacterial or mixed (1:1) DFM; respectively. Results of chemical composition showed that all biological treated rice straw recorded lower ( $P \leq 0.05$ ) OM, CF, NDF, ADF and cellulose content than untreated rice straw (RSc). The groups fed ration supplemented with DFM (fungal, bacterial and mixed) recorded higher DM, TDN and digestible CP intake than control group. Supplementation of lambs ration with DFM (fungal, bacterial and mixed) did not showed significant ( $P > 0.05$ ) effects on rumen liquor TVFA's and ammonia concentration at 0, 3 and 6 hrs post feeding. While, DFM supplemented rations showed significantly higher rumen liquor pH at 0, 3 and 6 hrs after feeding compared to the control group. Numerically increase was recorded in cellulose activity as unit per ml rumen liquor for groups fed ration supplemented with bacterial and mixed DFM. Direct fed microbial supplementation significantly ( $P \leq 0.05$ ) 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. Bacterial or mixed DFM supplementation showed numerically increased ( $P > 0.05$ ) in plasma total protein concentration compared to lambs

fed non-supplemented ration. Albumin, globulin, urea, triglycerides, creatinine ALT and AST, alkaline phosphates activity were not significantly ( $P>0.05$ ) affected by DFM supplementation. Total gain and ADG were significantly increased ( $P\leq 0.05$ ) for groups received rations supplemented with DFM compared to control group. Also DFM supplementation significantly ( $P\leq 0.05$ ) improved feed conversion as DM, TDN, CP and DCP compared to the control group.

It may be concluded that supplementing lambs ration with fungal, bacterial or mixed DFM improved feed intake, digestibility, average daily gain and feed conversion ratio without any adverse effects on animal health and performance.

**Key words:** lambs, DFM, feed intake, digestibility, rumen contents activity, growth performance.