EFFECT OF MICROCAPSULES TO PROTECT SUPPLEMENTS FROM RUMEN BIOHYDROGENATION

By

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B.Sc. Agric. Sc. (Animal Production), Cairo University, 2012

A Thesis Submitted in Partial Fulfillment

Of

The Requirements for the Degree of

MASTER OF SCIENCE
In
Agricultural Sciences
(Animal Nutrition)

Department of Animal Production

Faculty of Agriculture

Ain Shams University

2019

Approval Sheet

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ABSTRACT

Mahmoud Abdelkader Mahmoud Mohamed. Effect of Microcapsules to Protect Supplements From Rumen Biohydrogenation. Unpublished Master Thesis, Department of Animal Production, Faculty of Agriculture, Ain Shams University, 2019.

Forty-eight lactating Holstein Friesian cows were utilized to assess impact of supplementing probiotic ZAD (mixture of live bacterial cells and their enzymes) compared with T5X (antitoxins product) on some productive, reproductive and antitoxins activity parameters. We have divided the animals into three experimental categories (16 each). The first category, control treatment, was fed basal diet without any supplements. The second category, ZAD treatment, was fed basal diet supplemented with ZAD probiotic (1.1 L/ton). The third category, T5X treatment, was fed basal diet supplemented with T5X antitoxins product (1.1 Kg/ton). Milk yield was recorded. Milk samples, blood samples and feed samples were collected and analyzed. Time-interval between calving and fertilizing artificial insemination and pregnancy rate were recorded. The gained outcomes demonstrated that milk yield (2.2kg/d) was significantly raised by ZAD treatment than other treatments. Milk components yield were significantly ($P<0.05$) raised by ZAD probiotic and T5X treatments than control. Blood serum total protein, albumin, globulin, glucose, urea and total lipids were not significantly ($P>0.05$) influenced by treatments. AFB1 in concentrate mixture and AFM1 in milk were significantly ($P<0.05$) diminished by ZAD and T5X than control. Numbers of days needed to fertilized artificial insemination were diminished by treatments than control. Pregnancy rate was significantly ($P<0.05$) raised by ZAD probiotic than T5X and control treatments, respectively. The overall results of this study illustrated that probiotic ZAD, potentially, has antitoxins activity leading to enhancing the productive and reproductive performance of lactating cows.

Keywords: Probiotic, Antitoxins Activity, Milk Yield, Reproductive Performance, Blood.

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 Prof. Dr. Hany Mahmoud 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 Prof. Dr. Hamdy Mousa Metwally, 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 Dr. Ramadan Mohammed AbdelGawad, researcher of Dairy Science, Dairy Science Department, National Research Centre, for his continuous help during the experimental work of the research.

Thanks and deep gratitude is due to Dr. Mohammed Waeer and Dr. Mohammed Hussen Elgammal for their supporting and helping me in the veterinary information and treated the sick animals.

I wish to express my sincere appreciation and deepest gratitude to my father Prof. Dr. Abd El-Kader M. Kholif, Professor of Dairy Animal Production, Dairy Science Department, National Research Centre, for continuous all support, valuable helps, guidance and patient throughout my life.

Many thanks are also due to my family, mother, brothers, sisters and uncles. I can't forget my wife and my son.

Grateful acknowledgment should be also extend to the staff members Dairy Science Department, National Research Centre for all facilities they offered to make this work possible.

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LIST OF ABBREVIATIONS

Abbreviation	Means
ADF	Acid detergent fiber
AFB1	Aflatoxin B1
AFM1	Aflatoxin M1
AFs	Aflatoxins
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CF	Crude fiber
CP	Crude protein
DCAD	Dietary cation anion difference
DIM	Days in milk
DM	Dry matter
EE	Either extract
GIT	Gastro intestinal tract
LAB	Lactic acid producing bacteria
LB	*Lactobacillus spp.*
LBY	*Lactobacillus spp.* Plus yeast
LUB	Lactic acid utilizing bacteria
ME	Metabolizable energy
NEg	Net energy for growth
NEL	Net energy for lactation
NDF	Neutral detergent fiber
NFE	Nitrogen free extract
OTA	Ochratoxin A
SMS	A proprietary adsorbent containing sodium montmorillonite with live yeast, yeast culture, mannan oligosaccharide, vitamin E
ZEN	Zearalenone

INTRODUCTION

Mycotoxins are defined as secondary metabolites produced by some of fungi species. Mycotoxins are produced mainly by five species of fungi (*Fusarium* sp, *Penicillium* sp. *Aspergillus* sp. *Claviceps* sp. *Alternaria* sp.) **(Xiong *et al.* 2015; Zouagui *et al*., 2017).** One of the most common types of mycotoxins exists in livestock feeds is aflatoxins. Aflatoxins are produced mainly by members of *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* by the polyketide pathway **(Battacone *et al*., 2012, Xiong *et al*., 2018).** Natural forms of aflatoxin, including forms B1, B2, G1, and G2, are often found in feeds. Monohydroxylated Aflatoxin M1 (AFM1), is derivate of aflatoxin B1 (AFB1), occurs in milk from dairy cows fed an AFB1 contaminated diet and may be subsequently transferred into other dairy products **(Battacone *et al*., 2005; Firmin *et al*., 2011 and Xiong *et al*., 2015).** The aflatoxins AFB1 and AFM1 were classified as hepatotoxic and carcinogenic substances, and they are considered as group 1 human carcinogens by the International Agency for Research on Cancer (IARC) of the World Health Organization **(IARC, 2002).** Many studies were conducted using different methods to lessen to decrease the transfer of aflatoxins from feed to milk. These methods are including physical, chemical, and biological methods. Organic and inorganic adsorbents have been used to decrease absorbance of AFB1 and transforming as AFM1 to milk (**Khattab *et al.* 2009; Xiong *et al*., 2015; Zouagui *et al*., 2017).** Also, the antitoxic activities for microorganisms have been documented. Several studies reported that certain strains of bacteria and yeasts have parietal structures capable of binding to mycotoxins **(Zouagui *et al*., 2017).**

Probiotics are defined as “live microorganisms that have a positive effect by improving the balance and activity of the digestive tract microflora and thereby host health and productivity **(Chiquette, 2009; Xu *et al*., 2017).**

INTRODUCTION

The probiotic product, ZAD, is a biotechnical product made from anaerobic bacteria which convert the polysaccharide into monosaccharide by specific enzymes. **(Gado *et al*. 2011)** reported that ZAD improved nutrients digestibility, live body weight gain and feed conversion of wheat straw in sheep and increased milk production for lactating animals **(Gado *et al*., 2009; Salem *et al*., 2011; Khattab *et al*., 2011).**

As mentioned earlier, using bacterial probiotics creates an acidic environment which it detrimental to pathogens. In addition, the production of bacteriocins by some probiotic strains helps maintain intestinal health and working as adsorbents for mycotoxins **(Firmin *et al*., 2011; Zouagui *et al*., 2017, Xiong et al., 2018)**. In this context, the main objective of this study was evaluating the antitoxic activity for ZAD probiotic product comparing to the commercial one (T5X). Productive and reproductive performances of lactating cows were evaluated.

REVIEW OF LITERATURE

Dairy farming sustainable is one of the most important goal to achieve dairy food security, should be food police maker and specialist promoting and supporting dairy farming sustainability to meet current economic, environmental and social needs **(Daniele et al., 2012).** Probiotics are being explored as substitutes of antibiotic feed additives that improve gut health, promote animal performance, and thereby, dairy farmers profits **(Hillal et al., 2011)**. Conversely, mycotoxins are known to cause intoxications in animals and adversely affect their growth and reproduction causing serious economic losses (**Fink, 1999; CAST, 2003 and Bryden, 2012).** Recently, detoxification of mycotoxins using probiotics is considered one of the most promise strategies in dairy farms.

1. Probiotics

1.1. Definition and history of probiotics:

The probiotic word comes from the Greek words “pro” (in favour) and “biotic” (life). Probiotics can be defined as some kinds of live microorganisms that may beneficially affect the host digestive process by improving the balance of the intestinal microflora” **(Fuller, 1989).** However, more recently definition explained that probiotics (or direct fed microbial) are mono or mixed cultures of live microorganisms which, when applied to animal or human, beneficially affect the host by improving the properties of the indigenous microflora **(Sanders, 2008; Ezema, 2013, Dlamini et al., 2017).** This latest definition is more specific in terms of the host and types of microorganisms and not restricted to the intestinal microbial community.

The interest of probiotics was started too early. one hundred years ago, some studies reported that *lactobacilli* that survive in the intestinal tract was desirable for health. Also, some reports attributed the longevity of Bulgarians to their consumption of lactobacilli from fermented milk product (**Chiquette, 2009)**. After the First World War and discovering of antibiotics, the popularity of probiotics decreased, however they were still used to reestablish the intestinal microflora following aggressive antibiotic treatments (**Chiquette, 2009)**. The interest in using probiotics for humans and animals and for the understanding of their mode of action have been renewed. The renewed interest in probiotics has emerged from a general public and scientific concern about the widespread use of antibiotics and the possibility for transfer of antibiotic resistance to human pathogenic bacteria. For this reason, the European Union banned the use of antibiotics for non-therapeutic purposes in January 2006. Therefore, the imperative to find safe alternatives to the use of antibiotics is raised (**Chiquette, 2009, Dlamini et al., 2017)**.

1.2. Types of Probiotics:

1.2.1. Bacterial Probiotics:

The major and frequently studied bacterial microorganisms used as probiotics in ruminant production include those derived from *Lactobacillus, Streptococcus, Entrococcus, Bacillus, Clostrididium, Bifidobacterium* species, *Propionibacterium, E.coli* Nissle 1917**(Kruis *et al.*, 2004)**, *Megasphaera elsdenii* and *Prevotella bryantii* **(Seo et al., 2010),** *Ruminococcus spp.* **(Gado et al., 2011)**. The bacterial probiotic strains can be classified as lactic acid producing (LAB) and lactic acid utilizing bacteria (LUB). Lactic acid production and utilization in the rumen is related to feed efficiency and animal health **(Seo et al., 2010).**

1.2.2. Fungal Probiotics:

Yeasts and fungal probiotics such as *Saccharomyces* and *Asperillus* respectively have given better results in adult ruminants **(Fuller, 1999; Seo et al., 2010)**. The combinations of probiotics strains could increase the beneficial health effects compared with individual strains, because of their synergetic adhesion effects **(Collado et al., 2007).**

1.3. Characteristics of a Good Probiotic:

According to the Food and Agriculture Organization of the United Nations **(FAO, 2002)** and the World Health Organization **(WHO, 2002)**, probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host. This definition of probiotics is also adopted by the International Scientific Association for Probiotics and Prebiotics and is used in most scientific publications. A number of criteria are used to select probiotic strains. According to **(Fuller, 1989)** an effective probiotic should be a strain that is capable of exerting a beneficial effect on the host animal, increased growth or resistance to disease, non-pathogenic and non-toxic, should be present as viable cells, and capable of surviving and metabolizing in the gut environment (resistant to low pH and organic acids). Moreover, probiotics should compete along with a highly-diverse and competitive environment presented by the gut microflora **(Bezkorovainy, 2001);** adhere to the intestinal epithelial cell lining **(Guarner and Schaafsma, 1998);** produce antimicrobial substances towards pathogens; remain viable during storage and use; have good sensory properties; be isolated from the same species as it is intended to use (**Collins and Gibson, 1999)**. However, the results obtained in field trials have been variable.

1.4. Probiotics Mechanism of Action :

Some proposed mechanisms of probiotics when fed to the host animals were:

(i) Production of a wide variety of antimicrobial substances **(Vandenbergh, 1993)** and inhibitory metabolites such as organic acids, bacteriocins, diacetyl, antibiotics and H2O2 **(Rolfe, 2002);**

(ii) Competition with the pathogen for adhesion sites or nutritional sources **(Guillot, 2003);** the presence of some bacteria in the intestinal tract is dependent on their ability to adhere to the gut epithelium, such that they become immobilized on the gut wall and resist being flushed out by peristalsis, as well as occupying a