PROBIOTIC MICROBE ACTIVITY FOR APPLICATION AS ANTI- FUNGAL AND FEED INTAKE RATE OF RUMINANTS

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ABSTRACT

In industry *Lactobacillus* and yeast (*Saccharomyces cerevisiae*) have been added to animal feed to increase ruminant feed intake. In the current study, we aimed to investigate the symbiotic effect between *Lactobacillus rhamnosus* (LAB) and yeast on antifungal activity in relation to increase feed intake of mix-bred *Jamnapari* goats. First, maximum biomass yield of innoculum LAB and *S.cerevisiae* was determined by using different ratio of initial substrate (molasses) concentration and percent of innoculum. A mixed culture of 4.94% LAB and 4.60% *S. cerevisiae* with 6.72 g/l molasses resulted in highest biomass yield of cell 3.18 ± 0.25 g/l. The formulation of mix culture was found to produce the highest anti-fungal activity 37.08% ± 2.53 mycelium growth of *Aspergillus flavus* as compared with single culture of LAB 63.07% ± 0.81 and *S. cerevisiae* 64.24%. The formulation was used for silage production through solid-state fermentation. Then the effect of silage on ruminant feed intake was studied by comparing ruminant feed of silage S3 (100% silage) and S2 which was 25% silage added with 75% mix feed (50% tapioca leaves + 50% napier grass) with commercial feed (non-silage content) as control parameter such as S5 90% mix feed (50% tapioca leaves + 50% napier grass) added with 10% soy waste, S1 100% mix feed (50% tapioca leaves + 50% napier grass) and S4 99% mix feed (50% tapioca leaves + 50% napier grass) added with 1% urea which applied through four male mix-bred *Jamnapari* goat. Both feed with silage content were able to increase feed intake activity (100% consumption) as compared with commercial feeds. The result of this study showed that mixed culture LAB and *S. cerevisiae* could provide advantage to the animal feed industry in term of improving the process of anti-fungal with stimulating appetite in ruminants without any chemical supplement.

ABSTRAK

Didalam industri makanan ternakan, *Lactobacillus* dan yis (*saccharomyces cerevisiae*) telah di tambah ke makanan ternakan bertujuan untuk meningkatkan kadar pengambilan makanan ruminan. Kajian kesan simbiotik antara *Lactobacillus rhamnosus* (LAB) dan yis dilakukan terhadap aktiviti antikulat di samping meningkatkan kadar pengambilan makanan kambing kacukan *Jamnapari*. Hasil akhir kepekatan biojisim maksimum inokulasi LAB dan *S.cerevisiae* telah ditentukan dengan menggunakan nisbah berbeza kepekatan awal substrat (molases) dan peratus inokulasi. Inkulasi kultur campuran 4.94% LAB dan 4.60% *S. cerevisiae* dengan 6.72 g/l substrat (molases) menunjukkan hasil akhir kepekatan biojisim sel yang tertinggi sebanyak 3.18 ± 0.25 g/l berbanding dengan kultur tunggal. Aplikasi kultur campuran terhadap aktiviti anti-kulat didapati menghasilkan kadar perencatan tertinggi pertumbuhan miselium kulat *Aspergillus flavus* iaitu sebanyak 37.08% ± 2.53 berbanding dengan aplikasi kultur tunggal LAB sebanyak 63.07% ± 0.81 dan *S.cerevisiae* sebanyak 64.24%. Formulasi kultur campuran diaplikasikan untuk pengeluaran silaj melalui proses fermentasi keadaan pepejal. Kesan silaj terhadap kadar pengambilan makanan ruminan telah dikaji dengan membuat perbandingan di antara makanan ruminan silaj S3 (100% silaj) dan S2 iaitu 25% silaj di tambah dengan 75% campuran makanan (50% daun ubi + 50% rumput napier) dengan makanan komersial (tanpa silaj) sebagai parameter kawalan iaitu S5 90% campuran makanan (50% daun ubi + 50% rumput napier) ditambah dengan 10 % sisa soya, S1 100% campuran makanan (50% daun ubi + 50% rumput napier) dan S4 iaitu 99% campuran makanan (50% daun ubi rumput + 50% rumput napier) ditambah dengan 1% urea yang diaplikasikan terhadap empat ekor kambing kacukan *Jamnapari*. Keputusan menunjukkan bahawa kedua-dua suapan makanan yang mengandungi silaj dapat meningkatkan aktiviti pengambilan makanan (100% pengambilan makanan) berbanding dengan suapan komersial. Hasil kajian ini menunjukkan bahawa kultur campuran LAB dan *S.cerevisiae* dapat memberi kelebihan terhadap industri makanan haiwan dalam aspek penambahbaikan proses anti-kulat serta berupaya menjadi peransang selera makan haiwan ruminant.

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LIST OF ABBREVIATIONS

CO_2	Carbon dioxide
C.T	Centrifuge tube
H_3BO_3	Boric Acid
H_2SO_4	Sulfuric Acid
HCl	Hydrochloric Acid
LAB	*Lactobacillus Rhamnosus* NRRL B-442
MRS	Mann Ranggosa Sharp
MCB	Master Cell Bank
Mix	Mix culture
Mix (O)	Mix culture after optimization
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NH_3	Ammonia gas
OD_{600}	Optical density at wavelength 600 nm
Opt	Optimization
O_2	Oxygen

LIST OF SYMBOLS

[B]	Biomass concentration
°C	Temperature unit, Degree Celsius
dN / dt	Differential number of cell N at certain time, t
dX / dt	Differential concentration X, at certain time, t
g	Weight unit (gram)
l	Volume unit (liter)
g/l	Concentration unit (gram per liter)
ml	Volume unit (milliliter)
N	Number of cell
N_0	Number of cell at, t=0
[S]	Substrate concentration
t	Time (h)
t_0	Starter time t=0
t_d	Doubling time
μ	Specific growth rate
X	Cell concentration
X_0	Cell concentration at, t=0
$\int$	Integration
%	Percentage
% v/w	Percent volume per total weight
% w/v	Percent weight per total volume

LIST OF EQUATION

CHAPTER 1

INTRODUCTION

1.1 Research Background

Use of microorganism in animal feed industries is mainly to solve feed poisoning lead to disease of livestock. This study presents the usage of mix culture of *Lactobacillus rhamnosus NRRL B-442* (LAB) and *Sacchromyces cerevisiae* (Sc) with regard to increase the yield productivity of culturing process in complex carbon sources and to apply probiotic technology towards animal feed production. LAB plays a role for its broad spectrum effectiveness on anti-fungal activity. It is potentially inhibits 73% of *Aspergillus s.p.* group at 37 °C (Munoz *et al*., 2010). This study also represents an increasing of the effectiveness of Sc towards antifungal activity which works as a growth promoter to increase the yield of productivity. Using Sc as an additional inoculation in animal feed is safe and potentially degrading aflatoxin producer, *Aspergillus flavus* as reported in (Kusumaningtyas *et al*., 2006). Sc genome which encodes a flo has been commonly studied as a single-cell organism, living freely in suspension protein cell walls which reveal the presence of up to 20 protein bands with molecular masses in the range between 60-220 kDa.

This research proposes the kinetic parameter on specific growth rate and doubling time through the mix culture (LAB + Sc) to obtain efficiency and stability of the optimal growth for both microorganisms in complex nutrient and maximize the nutrient utilization from cane molasses.

Novel formulation is proposed to apply an aerobic fermentation through silage fermentation process in the production of animal feed by using mix culture as inoculants during the initial process. Then it will follow with anaerobic fermentation which reducing the fermentation period to fourteen days to ensure the nutrient and energy level (MJ/kg) achieve the optimal value required by ruminant. This formulation is based on aerobic growth during the initial process could increase the number of LAB and Sc. High level of oxygen is necessary for respiration due to the growth of mix culture activity, thus releasing of carbon dioxide (CO_2) and Adenine Triphosphate (ATP). Continuous low oxygen level in anaerobic condition may catalyze the mix culture to secrete metabolites which can utilize the substrate to optimize the nutrient and energy level. The purpose of the study is also to optimize the crude fiber percentage while increasing the silage acidic level which benefits the feed intake activity on ruminant.

1.2 Problem Statement

Mycotoxin presence of inhibition is one of the problems in the feed industry around the world. Previous publication estimated that up to 25 % of the worlds crops are contaminated with mycotoxin (Chuckwuka *et al*., 2010). Mycotoxin can inhibit the entire activity, including the purchase of raw materials such grain buyers, import and export materials, feed and food processing, users of the commodities such as livestock, poultry and dairy production, consumers and national government (Chuckwuka *et al*., 2010). Mycotoxin can affect considerable economic particulars, not only the feedstuffs, but also for intermediaries. Despite the economic importance, mycotoxin would cause problems to

the presence of economic development efforts on a larger scale. Aflatoxin one types of the mycotoxin can cause illness or death when it contaminates feed or food (CAST 2003; Kusumaningtyas *et al*., 2006). Alfatoxicosis secreted from aflatoxin is related to anorexia, which can cause reduction of weight. Other among reasons arising from alfatoxicosis are poor feed utilization, hemorrhage and susceptibility to environmental and microbial stress (Navid and Aidin, 2011).

Chemical supplementary is a source of protein fed to cattle as supplementary formulation in commercial application. For example, urea is a source of non-protein nitrogen (NPN) which is fed to cattle. Farmers use urea in order to save cost. However, excessive use of urea may result in the presence of excess ammonia poisoning in the circulatory system. Urea is converted to ammonia after entering the rumen. This ammonia can be used by bacteria for digestive process to produce energy which can generate proteins, but may enter the bloodstream. Excess ammonia will enter the circulatory system which can cause toxicity to cattle. The capacity of urea which enters to the bloodstream goes through the assimilation process in the liver before being removed. Excessive urea consumption in cattle can cause ammonia toxicity with death resulting in less than 30 minutes (Sewell, 1993).

Difficulty of nutrient utilization through molasses medium of single culture LAB and Sc will reduce the yield productivity of cell because lack of complex carbon sources utilization such as molasses. Unfortunately, it does also will reduce the number of binding agent through inhibition mycelium growth. This study proposes the use of mix culture LAB + Sc through complex carbon sources such as molasses to increase the nutrient utilization of molasses compare with single culture through growth kinetic study to increase the biomass yield. The application of the culture through anti-fungal susceptibility technique of *Aspergillus flavus* wills also investigate to monitor the improvement of binding agent for mycelium inhibition growth for mix culture (LAB+Sc) compared with single culture. The processing of animal feed, proposed to use mix culture for silage inoculants to improve the energy intake compared with the others feed through

percent feeding intake rate per hour of male goat jamnapari to reduce the wastage of non-intake feed.

1.3 Objectives of Study

i. To determine efficacy growth rate with maximum yield of microbial activity by comparing single culture and mix culture strain of *Lactobacillus rhamnosus NRRL B-442* and commercial *Saccharomyces cerevisiae* into complex sugar molasses.

ii. To improve the inhibition of indigenous Aflatoxin-producing *Aspergillus flavus* growth using mix culture (*Lactobacillus rhamnosus* NRRL B-442 + *Saccharomyces cerevisiae*).

iii. To improve feed intake activity of *Jamnapari* by using mix culture after conducting solid-state fermentation on hay (silage)

1.3.1 Scopes of Study

i. Screen and attenuation the range parameter of the growth rate of microbes using integration method with different ratios of molasses: medium namely mixed cultures (*Saccharomyces cerevisiae and Lactobacillus rhamnosus NRRL B-442*) using Potato Dextrose Broth and Man Rogosa Shrape broth, respectively as starter culture.

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