THE EFFECT OF FERMENTED SOYBEAN MEAL ON THE GROWTH OF SYRIAN HAMSTERS (*Mesocricetus auratus)*

By

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DECLARATION

I hereby declare that the work embodied in this thesis is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the thesis of this final year project entitled "The Effect Of Fermented Soybean Meal On The Growth Of Syrian Hamsters (*Mesocricetus auratus)"* by Adibah Najihah binti Ab Raman, matric number F14A0005 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science), Faculty of Agro Based Industry, University Malaysia Kelantan.

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_____

Supervisor

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
SBM	Soybean Meal
FSBM	Fermented Soybean Meal
SSF	Solid State Fermentation
ANFs	Anti Nutritional factor
VRI	Veterinary Research Institute
HCL	Hydrochloric acid
LAB	Lactic Acid Bacteria

LIST OF SYMBOL

ºC	Degree Celcius
%	Percent
M	Mol
g	gram

The Effect of Fermented Soybean Meal on Growth of Syrian Hamsters,

Mesocricetus Auratus

ABSTRACT

The present study is to determine the effect of fermented soybean meal towards growth of Syrian Hamster, *Mesocricetus auratus*. Fermented soybean meal had been introduced and famous in aquaculture feeding and swine feed in replacing fish meal. Use of fermented feed is to increase the animal digestibility. This study was carried out in eight weeks period. It is focusing on three different treatments which the hamsters were fed with fermented soybean meal, non fermented soybean meal and commercial feed were set up. Nine hamster were used in total with no replication. The parameter taken in this study is the animal growth and its survival rate. One Way Anova ($P<0.05$) used to determine the result and significance of this experiment. The results show that the fermented soybean meal had the lowest growth of hamster compared to other two treatments. It shows no significance in this study. The result of this study is different from previous study which shows that feed with fermented is better. Weight gain of hamster in fermented soybean meal treatment is 6.05g, while in SBM and Control are 18.98g and 18.75g respectively. Least preference of hamster towards fermented soybean meal is due to its strong odour which molasses was used to make the feed. Thus indicates behaviour character of the hamster in feed preference. The survival rate is 100% for control, while 66.67% in both FSBM and SBM. Nutritional analysis also done in this study which shows that percentage value of SBM in crude protein, ash and crude fat is higher that FSBM which the value are 35.8%, 5.31% and 1.63% respectively. Later research could be done on fermented feed with different formulated.

Keywords : *Mesocricetus auratus*, fermented, soybean meal, animal feed

Kesan Perapan Makanan Kacang Soya terhadap Pertumbuhan Hamster Syria,

Mesocricetus Auratus

ABSTRAK

Kajian ini dijalankan untuk menentukan kesan perapan serbuk kacang soya terhadap pertumbuhan berat Syrian Hamster, mesocricetus auratus. Perapan serbuk kacang soya telah diperkenalkan dan digunakan dalam pembuatan makanan akuakultur dan makanan babi dengan menggantikan serbuk ikan. Penggunaan perapan serbuk kacang soya adalah untuk meningkatkan penghadaman haiwan. Kajian ini dijalankan dalam tempoh lapan minggu. Fokus kepada tiga rawatan berbeza iaitu hamster yang diberi makanan perapan serbuk kacang soya, serbuk kacang soya dan makanan komersial telah disediakan. Sembilan ekor hamster digunakan secara keseluruhan tanpa ulangan. Parameter dalam kajian ini adalah pertumbuhan haiwan dan tahap jangka hayat. Anova ($P<0.05$) digunakan bagi menentukan keputusan dan signifikasi eksperimen ini. Keputusan menunjukkan perapan serbuk kacang soya mempunyai pertumbuhan berat paling sedikit dibandingkan dengan dua rawatan lain. Ia tidak menunjukkan signifikasi iaitu pertumbuhan berat ketiga rawatan. Keputusan berat yang ditunjukkan dalan kajian ini adalah berbeza dari kajian yang terdahulu yang menunjukkan makanan perapan lebih baik. Pertambahan berat hamster dalam rawatan FSBM ialah 6.05g sementara dalam SBM dan control adalah 18.98g dan 18.75g masing- masing.Pemilihan hamster terhadap perpan serbuk kacang soya adalah kerana bau yang kuat dimana 'molasses' digunakan dalam pembuatan. Ini menunjukkan tingkah laku hamster dalam pemilihan makanan. Kadar jangka hayat ialah 100% pada control, sementara 66.67% pada FSBM dan SBM.Analisa nutrisi juga dilakukan dalam kajian ini dimana peratus yang ditunjukkan dalam SBM untuk protein, abu, dan lemak adalah lebih tinggi berbanding FSBM dengan nilai 35.8%, 5.31% dan 1.63% masing-masing. Kajian seterusnya boleh dijalankan dengan perapan makanan dengan berbeza formulasi.

Kata Kunci: mesocricetus auratus, perapan, kacang soya, makanan haiwan

CHAPTER 1

INTRODUCTION

1.0 Research background

One of the biggest and widely used protein ingredients is soybean. Soybean is an annual crop which is easy to grow and can produce high amount of protein compared to other crop. In addition, using soybean as one of the protein source in human and animal feeding is common because the crop are able to supply most nutrient. According to United States Department of Agriculture in 2007, US, Brazil, Argentina, China and India are countries which are producing high soybean which estimate around 206.4 million tons. Soy bean also recognized as an important in production on biodiesel.

Soy bean meal is mainly used and important feed ingredient to produce animal feeds in most of the countries. Production of soybean meal increasing due to increase in demands as soybean is readily available at a reasonable price. According to El-shemy (2011), dependence on soy bean meal as ingredient in non ruminant feeding rise due to recent ban in European Union to terrestrial feed Ingredient for instance fish meal, bone meal in poultry and pig feeding.

Substance of life, reproduction, growth, and health of an animal dependent on the feed they consume. Most commercial pellet especially for non ruminant is to provide nutrient to the animal. Thus, to make sure the ingredient used in making feed important so that the nutrient contained can be fully utilised to the animal. Bioavailability is referring to the amount of nutrient available in ingredient for the productive purpose of the animal (El-shemy, 2011).

Process of utilizing microorganism to convert solid or liquid substrate into other and various product is called fermentation. Commonly fermented products that available are beer, wine glutamic acid and soy sauce. Types of fermentation can be divided into either solid state fermentation or submerged fermentation. In solid state fermentation, the condition is free water as the microorganism grew in a moist solid. Examples of solid state fermentation manufacturing are processing of soy sauce and soy paste. Besides that, usage of dissolve substrate such as sugar solution or suspended in a large amount of water is a condition of submerge fermentation. Examples of submerge fermentation are pickling vegetables and producing yogurt.

There are 24 species of hamsters that can be found in Europe and Asia. Some of the species are Siberian hamster (*Cricetulus griseus*), black-bellied or European hamster (*C. cricetus*), and Armenian hamster *(C. migratorius*). Syrian Golden Hamster or Syrian Hamster (*Mesocricetus auratus*) is a common hamster that being used in study and research. It is from order of *Rodentia* and suborder *Myomorpha*. The hamster origin is from northwest region of Syria. The advantage of Syrian hamster as model for research is because of its ability and susceptibility to disease and infection. Besides hamster, animals that being used as model for disease infection is guinea pig and rats (Suckow et al., 2017).

1.1 Problem statement

Soybean meal has already been widely use as one of the main protein source in making feed. Compared to fish meal, the soybean meal is highly available but the anti nutritional factor in soybean meal prevent the content readily absorb is low if given in high quantity and can give effect to the animal. Thus, by using fermented soybean meal, the anti nutritional factor can be reduce thus the nutritional value can be improve for better animal growth.

1.2 Hypothesis

H_o = Fermented soybean meal feed increase the growth of Syrian hamster, *M. auratus* compared to non-fermented feed.

H_1 = Fermented soybean meal feed not increase the growth of Syrian hamster, *M. auratus* compared to non-fermented feed.

1.3 Objective

The present study objectives are :

1. To study the effect of fermented soybean meal and determine the survival rate on the growth of Golden Syrian Hamster (*Mesocricetus auratus*).

1.4 Scope of study

The scope of the study is growth of animals with feeding fermented feed and its survival rate.

1.5 Limitation of study

The limitation of this study is cost to conducting the experiment on the Syrian hamster which is costly. Thus, replication cannot be done. Besides that, nutritional requirement reference regarding hamster is limited.

1.6 Significant of study

The purpose of this study is to test and compare on the fermented soy bean meal and non fermented soybean meal on hamster and its effect to the growth of the animal. Throughout this study, the preference of the hamster on the feed can be determine. Other than that, this study will explain on the fermentation process and the fermentation effect on feed. Previous study mostly had apply fermentation in feed of aquaculture or swine, but on pets food is still less.

CHAPTER 2

LITERATURE REVIEW

2.1 Soybean meal

Feed mills production is expanding, yet the requirement for fish meal is increasing. Method of replacing fish meal with other protein source of plants had been in research such as cottonseed meal or soybean meal (Hassaan et al., 2015). Soybean meal is gained by crushing and grinding the flakes after the oil from soybeans has removed by solvent extraction process. They are rich in amino acids, which the amino acids will match other ingredient to have a balanced diet.

Fish meal is a major protein source in feeding diet. Other plant protein that has a relatively high content of protein, and availability in supply is soybean meal. According to Lee et al. (2016), In recent years, fish meal cost increased which the supply become insufficient in supporting demands. Alternative sources of protein in feed should be finds to lowering the costs. Several studies to find alternative source of protein from plant origin had attempt. By reason that they are reasonable price, high quality and availability consistency, soybean received most attention.

Soybean is another vegetable source that has high content of protein and widely used in feed industry. Major protein contain in soybean are glycinin and betaconglycinin. Soybean meal had been used to replace fish meal but the usage is

limitless due to its anti nutritional factor and insufficient level of methionine and lysine in fish feed. Extruded, full fat, solvent extracted, soy protein concentrate and fermented soy are types of different soy bean meal product that being used for animal feeding. In aqua feed, some problems may occur if using plant based protein in for instance less digestibility, certain amino acids is limited, and presence of anti nutritional factors, ANFs. (Jones et al., 2010) According to Yamamoto (2010), changes on intestine of fish occurred when given feed with soybean meal with high anti nutritional factor (Hassaan et al., 2015).

Figure 2.1 Soybean Meal obtained from Animal Lab, Umk Jeli

Guzmán et al. (2016) stated that, high digestible amino acid and high energy content available in soybean meal makes SBM is an ingredient choice in make up diets for pigs . Extraction of nutrient from ingredient is not fully absorb to the gastro intestinal tract which some of its will be lost through faeces. However, the nutrient recovery in soy bean meal is high that will makes it as one of the main ingredient in animal feeding. Soybean meal maintain as the most protein resource use in animal feeding. Thus, the study to maximise the usages of this ingredient for instance

creating methods to improve its utilisation is aggressively conducted by researchers around the world (El-shemy, 2011).

Table 2.1 shows the basic nutrient in soybean product. According to Banaszkiewicz (2011) the content of crude fibre in soybean is lower compared to other vegetable protein, comparison of crude protein and crude fat in soybean seeds and soybean meal, soybean meal contain higher crude protein which is about 40-49% compared with soybean seed that is about 40%. Table 2.2 shows the percentage specification of composition of soybean meal.

Table 2.1 Basic nutrient in soybean product (El-shemy, 2011)

Nutrient	**Soybean seeds % of DM**	**Soybean meal**	
		44% CP, % of DM	**49% CP, % of DM**
Crude protein	37.08	43.8 – 49.9	52.8 – 56.3
Crude ash	4.86	5.6 – 7.2	5.2 – 9.1
Crude fat	18.38	0.55 – 3.0	1.0 – 3.3
Crude fibre	5.12	4.3 – 7.2	3.1 – 4.1
NDF	12.98	12.3 – 18.9	7.4 – 12.2
ADF	7.22	8.9 – 11.9	5.2 – 6.7
N-free-extractive	24.00	34.3	33.2
Starch	4.66	5.51	5.46

Table 2.2 Composition of Soybean Meal (Banaszkiewicz, 2011)

Specification %	SBM
Dry matter	92
Ash	6.5
Crude Protein	44
Crude Fat	1.9
Fibre	6.7
NDF	14.5
ADF	9.5

2.2 Anti nutritional effect of soybean meal

However, there are some anti nutritional factor and antigenic protein in soybean meal which makes it application in feed is limited. Anti nutritional factor that present in raw soybean are lectins, trypsin inhibitors, allergens and non digestible oligosaccharide. Anti nutritional factor such as phytic acid, allergens, protease inhibitors, saponins, antivitamins and phytostrogens are used by plants for their defence against disease causing by foreign organism which, these anti nutritional factor also available in soybean meal. Particularly, presence of trypsin inhibitor and some oligosaccharides will lowering the efficiency of nutrient utilization when fed to animals(Cho et al., 2007). Presence of anti nutritional factor will affect the absorption

of nutrient that will decrease the feed efficiency and lower the growth rate (Lee et al., 2016).

Presence of trypsin inhibitor in animal will decrease the performance of the animal and also increase the metabolic nitrogen excretion. Other than that, presence of lectins in soybean meal will increase the mortality rate in animals also lowering its growth. On the other hands, extruded soybean meal, soy protein isolate and soy protein concentrate have low concentration of the anti nutritional factors. Thus these product mostly be used in animal and human feeding (Seo & Cho, 2016).

According to Wang et al. (2016), a few methods for instance fermentation, mechanical process, mating and soaking can be applied to eliminate ANFs and excite the bioavailability of micronutrient in the plant based diets. Other than that, process such as heat treatment, extraction, purification into protein concentrate and isolates could be used to reduce the ANFs in soybean but it is costly. One of the methods that are lower in cost but can increase the protein concentration and decrease the ANFs level is by fermentation (Lee et al., 2016).

The anti nutritional activity will give effect for examples reduce protein digestibility, toxicity, and development of gastrointestinal tract in young animal. Other than that, the efficiency of utilisation and metabolism of protein will decrease as well as stimulate pancreas organ to increase gastric secretion due to presence of protein inhibitors.

2.3 Effect of Fermented Soybean meal

According to Chisti (1999), fermentation process could be influenced by several factors that are pH, nature, temperature, composition of medium, dissolve oxygen and carbon dioxide. The rate of fermentation, the nutritional quality and generation of toxicity can be affected by those factors. Cho et al. (2007) stated that, trypsin inhibitor was decreased after a fermentation of soybean meal.

Solid state fermentation (SSF) was defined as a fermentation process of a non soluble material that will acts as a sources of nutrients and physical support in the condition of no free flowing liquid. Production of metabolites for example antibiotics and enzymes of solid sate fermentation were higher compare to submerged fermentation. Moselhy et al. (2015) stated that, as organic acids, which is lactic acid is formed by spontaneous solid state fermentation. Methods of microbial fermentation and heat treatment had been used to reduce anti nutritional factor and eliminate it (Hassaan et al., 2015). Fermentation of soybean meal was able to enhance the nutritional value and digestibility (Kim et al., 1999).

According to Demecková et al. (2002), liquid fermented feed given to lactating sows rise the number of lactic acid bacteria in piglet while lowered the number of coliforms. Study by Giraffa (2004) stated that, fermentation which involves molds yeast, and bacillus as inoculums which is controlled fermentation may achieved more predicted result.

According to this Wang et al. (2017), fermentation in ruminant feed has become the solution for shortage in feedstuff especially during winter seasons. One of the by product used widely to rise the feeding quality also rate of fermentation is molasses (Lima et al., 2010). Previous study by Yunus et al. (2000), shows that, concentration of Amino Acid of silage could be decrease the pH by molasses addictive which inhibit the growth of microorganism.

Lactobacillus used for previous research was showed the effective in preventing diarrhea in E-coli challenged for piglets (Hu et al., 2008). Besides that, Tonheim et al. (2007) informed that, digestibility in water soluble protein is higher compare to water insoluble protein which occurs in the fermentation process that would increase the digestibility of crude protein.

According to Ding et al. (2015), the inactivated or removal of ANFs in SBM can be done by fermentation . A previous study reported that SBM that is fermented with *B.subtilis* may improve morphology and digestive enzyme in weaned pigs. Fermentation in soybean meal is to increase nutrient digestibility also helps in prevent diarrhea in piglets by degradation of protein and carbohydrate into low molecular weight and water soluble compound. Reducing diarrhea in weaning pigs increase feed efficiency and susceptibility of the animals to Escherichia coli infection (Yuan et al., 2016).

Figure 2.3 Fermented Soybean Meal

Besides that, by using microorganism such as *Lactobacillus spp., Bacillus spp., and Aspergillus spp*. the quality of SBM through fermentation is improved. Based on previous study, fermented soybean meal using lactobacillus reported displayed higher dry matter and protein digestibility than commercial soybean meal in white shrimp (Lin & Mui, 2017).

Seo & Cho (2016) recorded that, fermentation of soybean meal with *Bacillus subtilis* was introduced to Asian market recently. Fermented soybean meal believed can reduce the concentration of anti nutritional factor increase digestibility and bioactivity by hydrolyze intact protein into smaller peptides. Several studies reported that immune reactivity and allergic reaction cause by soy product can be reduced and weight gain in animals can be decrease by decreasing trypsin inhibitors. However, the information about changes that protein undergo during fermentation is limited.

Previous research by Refstie et al. (2005) suggested that, fermentation is an alternative to improve the nutritional value in fish feed. Other than that, fermentation

of lactobacillus sp. achieves to reduce the levels of non starch carbohydrates in wheat and barley whole meal. Lactic acid fermentation also showed that it can reduce phytic acid in cereal, sesame seed whole meals. Table 5.1 shows the chemical composition and anti nutritional factors available in commercial soybean meal and yeast fermented soybean meal. Table 2.3 shows the chemical composition and anti nutritional factor of soybean and yeast fermented soybean meal.

Table 2.3 Chemical compositions and anti nutritional factors of commercial soybean meal and yeast fermented soybean meal. (Hassaan et al., 2015)

Items	**Commercial soybean meal**	**Yeast Fermented soybean meal**
Crude protein (g/kg)	440	500
Crude lipid (g/kg)	43	48
Ash (g/kg)	61	71
Crude fibre (g/kg)	63	32
Nitrogen free extract (g/kg)	393	349
Phytic acid(g/100g)	0.56	0.04
Trypsin inhibitor (IU/mg protein)	2080	1902

2.4 ***Mesocricetus auratus***

Golden Syrian Hamster had been introduced to laboratory since 1930's. Election of Golden Syrian hamster was due to its physiology aspects which their energy balance regulations more similar to human than other rodent. According to

previous study, stated that, cardiovascular disease and diabetes is more vulnerable to human and hamster (Ross et al., 2017). Other than that, also recommended that hamster is a perfect species that could be used for stress study. Albers (2012) stated that, both male and female hamster will show their communitive behaviour and rich social behaviour, also capability to discriminate complex social. Losing in a fought is evidence which the hamster become stressful.

Based on Veterinary Research Institute (VRI), there are six species of laboratory animals that are rabbit (New Zealand breed), white mice (Swiss Albino), hamster (Golden Syrian), guinea pig (Hartlay Strain), mice and Sprague Dawley Rat. Commonly, a white mice has higher demand as laboratory animals because of their short gestation time, bred well and have a large size of litter.

Hamster is a member of family Muridae and Cricetinae is its subfamily. Across Europe and Asia, there contain 24 species in five genera. Average body weight of a Syrian hamster is 120g. Current golden hamster were believed to be descended from original 13 hamster that were captured in Syria which the female and its young were found in Syria in 1930 before taken and bred to England and United States. The golden hamster that mostly being found today is in wild type which is having a reddish golden brown hair coat. Other coat colour such as dark brown, cream, white and long haired were resulted from genetic mutation. Hamsters have body temperature range 37-38 ºC and lifespan maximum up to 36 month. Other than that, the average body weight for adult male is 87-130g and 95-130g for female. Besides, they preferred environmental temperature range 21-24ºC. Body of Syrian hamster have length about 15-20cm long with short legs, thick fur, large ears and sharp claws (Heatley & Harris, 2009).

The characteristics of Syrian hamster makes it's commonly used as a model especially in biomedicine, or biochemical aspects is they have check pouch which relate to studies of microbes. Based on VRI, feeding rate given to hamster is 5g/2 times per day. Bedding use is sawdust, it is dried and disinfected also changes 2 times per week. After bred, hamster have a survivability rate at 87% (Chandrawathani, & Ramlan, 2012).

Compartment in hamster stomach consists of fore stomach and glandular stomach. In the fore stomach contains a large number of bacteria and protozoa. Hamster has a fine developed caecum which will help in food utilization (Sakaguchi & Matsumoto, 1985). Previous study by Kiku- chi-Hayakawa et al. (1998) stated that, levels of blood cholesterol in rats are decrease given fermented soybean milk, also antherosclerosis in hamster is prevented given LAB fermented milk. Using LAB products begins with yogurt then it diverse into pickles and beverages. Fermented product using LAB showing press forward of food industry (Wang et al, 2010).

Table 2.4 Taxonomy of Golden Syrian Hamster

Kingdom :	Animalia
Phylum :	Chordata
Class :	Mammalia
Order:	Rodentia
Family:	Cricetidae
Genus:	Mesocricetus
Species:	*Mesocricetus auratus*

CHAPTER 3

MATERIALS AND METHODS

3.1 Location

This study involved preparation of fermented soybean meal, preparation of feed, proximate analysis and feeding trial. These methods were conducted in Animal Laboratory, Faculty Agro Based Industry, Universiti Malaysia Kelantan.

Soybean meal was obtained from Animal Laboratory, Faculty of Agro Based Industry, Universiti Malaysia Kelantan. Meanwhile, animas were bought from pet shop located in Kota Bharu, Kelantan.

3.2 Materials

3.2.1 Raw Materials and Equipment

List of raw materials that were used in feeding trial were soybean meal, molasses, distilled water, starter culture (*Lactobacillus plantarum* spp.), oil, tapioca flour, and commercial pallet.

List of equipment that was used for feeding trial were aquarium, drinker, measuring cylinder, closed container, basin.

Besides that, the list of machineries that were use in the experiment were weighing machine, Kjedhal machine, moisture analyzer, furnace and oven. For approximate analysis, soxhlet, rotary evaporator also been used.

3.3 Methods

3.3.1 Preparation of the fermentation

3.3.1.1 Culture of *Lactobacillus spp.*

Oxoid MRS agar was used as nutrient media. The bacteria species that was used *Lactobacillus plantarum spp*. Nutrient agar and nutrient broth was prepared to culture the *Lactobacillus spp.* bacteria. Strains of *Lactobacillus spp*. obtained from laboratory, Faculty of Agro Based Industry, Universiti Malaysia Kelantan. The culture was streaked on the nutrient agar and left in incubator for a week to grow. Single colony of the bacteria was then transferred to nutrient broth and let for three days in incubator.

3.3.1.2 Preparation of fermented soybean meal

Two kilogram of soybean meal was fermented by adding of molasses, bacteria and water. The mixture was stirred and mixed thoroughly so all the mixture blend together. Then, it was kept in dark containers covered with plastic on top before seal and kept in dark place for three months fermentation. Hygienic procedure needed in conducting the fermented soybean meal as it is easily become moulded.

3.3.2 Pelletizing the pellet

3.3.2.1 Preparation of feed

Soybean meal and fermented soybean meal feed was prepared by mixing of the meal with tapioca starch as binder, water and oil. The mixture was then pallet and heated in an oven at 50ºC (Wang et al., 2016).

3.3.3 Proximate Analysis method

3.3.3.1 Moisture

Five gram of powder of soybean meal and powder of fermented soybean meal were analyzed using SHS MX-50 moisture analyzer. The temperature reached 160º. Once the analyzer done, the value will be appear on the screen of the machine.

3.3.3.2 Ash

One gram of soybean meal powder and 1g fermented soybean meal powder was weighed and put in a crucible. The crucible was put in the furnace at 600ºC for 3 hours. After that, the crucible was put in dessicator to prevent moist air from getting inside. The equation used to calculate percentage of is:

$$\%Ash = \frac{W3 - W1}{W2} \times 100$$

Where; W1= Weight of empty container

W2 = Weight of sample

W3= Weight of Sample after dry

3.3.3.3 Crude Protein

Kjehdahl method was used to determine crude protein. 0.1M HCL was prepared. For digestion process, Sample of FSBM and SBM was put in a digestion tube. Replication of two was made for each sample. In each tube contain 10ml distilled water, 12 ml sulphuric acid, and Kjehdal tablet.

For distillation process, each conical flask was added with 30ml boric acid, 3ml bromocressol green and 2.1 ml methyl red. Distilled water and sodium hydroxide NaOH was poured into the digestion tube that contained the sample. (Bradley, 2010) The conical flask and the tube was run in the Kjeltec 8200 Manufacturer Foss North America Kjehdahl machine.

Then, titration process takes place which the conical flasks from distillation process were titrated with 0.1M HCL. The amount of HCL used was recorded. The equation used to calculate percentage of crude protein is:

$$\%\text{Nitrogen} = (\text{mL sample} - \text{mL blank}) \times 0.1 \times 14.007$$

$$\%\text{Crude Protein} = \%\text{Nitrogen} \times 6.25$$

3.3.4 Crude Fat

Thirty gram of SBM powder and 30g of FSBM powder was put in timble which were put in Soxchlet machine. Solution used as solvent was petroleum ether. The soxhlet machine was run for 5 hours. Then, the solvent in the flask was filter using brand Buchi Rotary Evaporator till the oil collected (Bradley, 2010). The equation used to calculated the percentage of crude fat is :

$$\%\text{Crude Fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

3.3.4 Feeding trial

The research was conducted in animal laboratory. Suitable temperature for hamsters is between 21ºC to 26ºC and humidity between 30% to 70%. The *M.auratus* housing was away from direct sunlight, or fireplace.

Nine hamsters, male and female, were used in this research. The initial weight of each hamster was taken before carry out the experiment. The *Mesocrietus auratus* were put in three different aquarium representing three different treatment.

The hamsters were placed in an aquarium as cages. The hamster were feed with Control, FSBM and SBM (Yuan et al., 2016). Drinking water was available all the time. The hamsters were fed 5g per day for each hamster as according to VRI.

Weight of the hamsters was taken and record once a week and the average for each treatment was calculated.

3.3.5 Growth indices

The initial body weight and final body weight each of individual hamsters in each treatment were recorded. The growth rate and of the hamster were calculated using formula and equation. The survival rate of the hamsters also recorded. (Hassaan et al., 2015a)

Formula

Weight gain : (final body weight – initial body weight)

Source :(Wang et al., 2016)

Survival rate : (final hamster number/initial hamster number) x 100

Source : (Lin & Mui, 2017)

3.3.6 Statistical analysis

The growth rate and nutrition of the fermented soybean meal (FSBM), and non fermented soybean meal (SBM) was analysed using SPSS software version 22, one way ANOVA to determine the result.

CHAPTER 4

RESULT

4.1 Feeding trial analysis

Figure 4.1 show the mean weight of hamster (g) in each treatment for every week. By comparing the treatments, FSBM shows the least weight gain after eight weeks of study. There is slightly drop in weight of the hamster in FSBM in the first four weeks, but it is increasing steadily through week 8. Graph of SBM rise in the first three weeks, but there is slightly fell through week 4. However, the weight is increasing gradually from week 5 to week 8.

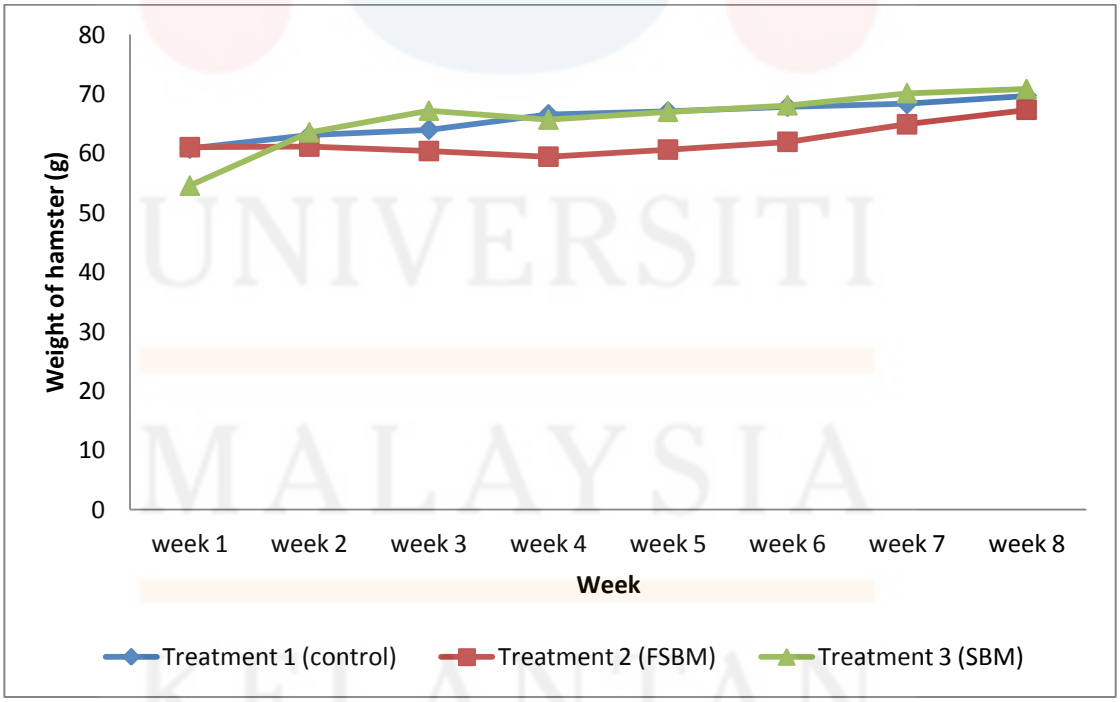

Figure 4.1 Mean weight of hamster in each treatment against week

Table 4.1 Initial and final weight of hamster in different experimental diet

Parameter	Treatment 1	Treatment 2	Treatment3
Initial Weight (g)	56.69±3.79[a]	61.25±3.95[a]	51.80±0.1[a]
Final Weight (g)	75.44±1.87[a]	67.30±3.10[a]	70.79±4.11[a]
Weight gain (g)	18.75±5.65[a]	6.05±0.85[a]	18.98±4.01[a]

Values are means ± SE. Values in the same row with different superscripts are significantly different ($p<0.05$)
*Treatment 1 : Control; Treatment 2 : Fermented soybean meal; Treatment 3 : Non fermented soybean meal

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Table 4.1 shows the initial weight, final weight, weight gain of the hamster in eight weeks period. Weight gain in FSBM is the lowest with 6.05±0.85 compared to weight gain is SBM and Control which are 18.98±4.01 and 18.75±5.65 respectively. The significance value of weight gain is 0.171 which is more than the value of P(<0.05). Thus, the weight gain value is not significance.

4.2 Survival rate analysis

Graph 4.2, shows the mean survival rate of the hamsters for Control, FSBM and SBM. Control has a good survival rate which it is 100% with no mortality. For FSBM and SBM, they shows same survival rate which are 66.67%.

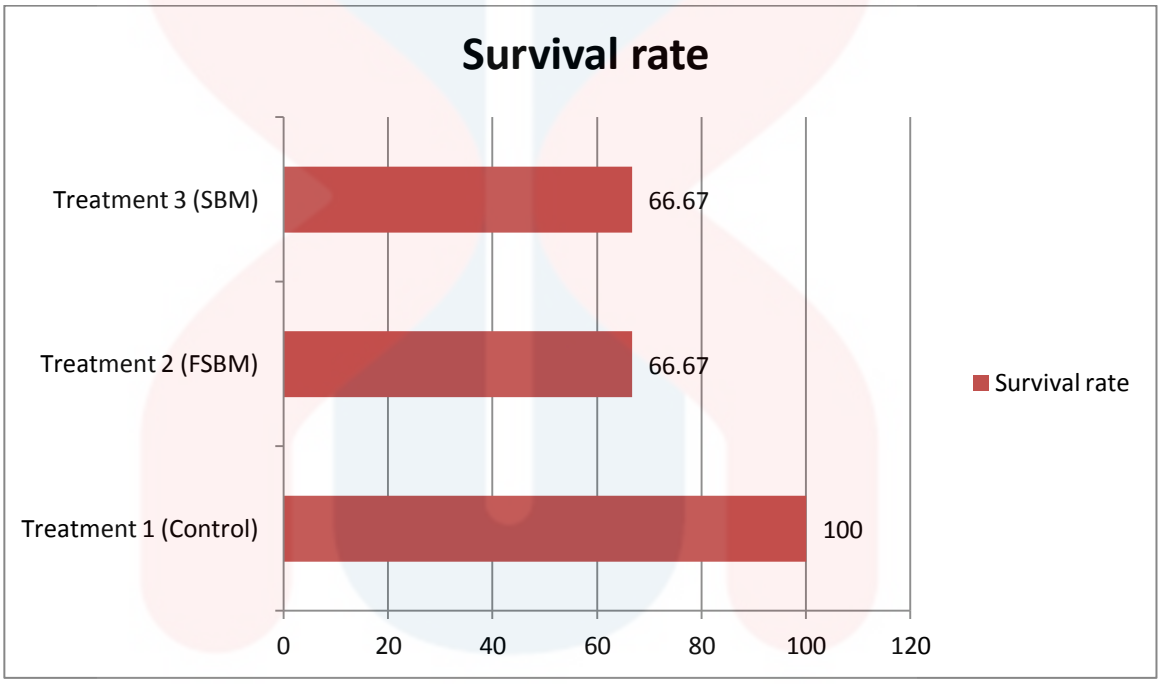

Figure 4.2 Survival rate of hamster in different treatment

4.3 Nutritional Analysis

Based on table 4.3, comparing between the two different feed, method use to analyse the nutrition was referring to AOAC (1995). Ash, crude protein, and crude fat had a higher value of percentage for SBM meal with 8.41%, 35.98% and 1.63% respectively. Meanwhile, FSBM had a higher moisture value of percentage which is 8.20%.

Table 4.3 Nutritional analysis of different feed

Element	Fermented soybean meal, FSBM (%)	Non fermented soybean meal, SBM (%)
Moisture	8.20	7.83
Ash	5.31	8.41
Crude Protein	6.42	35.98
Crude Fat	0.63	1.63

CHAPTER 5

DISCUSSION

5.1 Feeding trial analysis

From current study, hamsters were used as a model to check on the effect of fermentation soybean meal (FSBM) to its weight. Weight of the hamster was taken every week and the result of the mean weight is plot as in Figure 4.1. For each treatment, the average of the weight was calculated and analysed.

The result of weight for each treatment shows that treatment with fermented soybean meal (FSBM) has the least weight gained compared to non fermented soybean meal (SBM) and control treatment.

Based on previous study, FSBM should result the most weight gain compared to SBM. Yuan et al. (2016) stated, the feed conversion rate and average daily gain of newly weaned piglet in diet of replacement 10% FSBM with SBM better than control group. Average daily gain and FCR of pig fed FSBM greater than control group. (Kim et al., 2005; Joneset et al., 2010; Yuan et al., 2016)

Referring to Figure 4.1, weight of hamster for FSBM is decreasing for the first 4 weeks. In week 4, the reading of the weight is at the lowest. This may due to the suitability and acceptance of the hamster to the feed. This shows that hamsters in

FSBM needs 4 weeks to accept feed made up from fermented soybean meal. Other than that, housing condition also can affect their emotional and lead them to stress which will affect their growth (Ross et al., 2017) Then, the graph is increasing in week 5, to week 8. However, increasing of the weight is still not reach as the weight of SBM and control.

Other than that, weight from SBM rose from week 1 to week 3. This shows that hamster in this treatment were able to accept feed that made up of non fermented soybean meal. Then, moving to week 4, the graph fell a bit due to changes of environment which cannot be controlled. Stress in animal also lead to decreasing the feed intake of the animal. Ross et al., (2017) stated that, stress due to social defeat will effects the animals such as changes in food intake also changes in sleep. The animal will also avoid contacting in social. The author also stated that comparing social isolation and social housing, social housing is more stressful. Mainly the female hamster undergo social separation will impact the body mass, declining food intake and rising the anxiety-like behaviour. (Shannonhouse et al., 2014) Having difference sexes in a housing of hamster is important to examine the housing effects for both sexes and condition of its body weight.

However, the weight went up gradually from week 5 to week 8 showing that the feed was palatable to the hamsters. Comparing weight of hamster given FSBM and SBM to weight of Control which given commercial feed, hamsters in control shows they have weight that almost close to weight in SBM. This indicates that hamster in FSBM did not affect fully weight gain of the hamster in two months period.

As for this experiment, the result obtained was different compared to previous study. Previous studies showed that the animal given with fermented feed should increase the animal growth. A previous study stated that *Lactobacillus plantarum* fermented Soybean meal could swap with fish meal in feeding juvenile turbot with no influencing the juvenile turbot growth (Wang et al., 2016). However, test of fermented product on hamster was not in record. Most of research conducted on aquaculture feed and pig.

After eight weeks of feeding, by referring to table 4.1, result obtained is no treatment shows significance difference ($p<0.05$) in their body weight. The result shows no significance difference which the value is exceeding value of P(<0.05).

From Table 4.1, shows the average number of initial and final weight of the *mesocricetus auratus* after consuming feed of fermented soybean meal (FSBM), soybean meal (SBM), and commercial pallet for 8 weeks. Hamster fed with FSBM shows the least increase in weight with a difference of 6.05±0.85g only. Comparing the treatment of SBM and commercial pallet, the difference is almost close. The difference number of initial and final weight of SBM is 18.98±4.01g while the difference weight of hamster consumed commercial pallet is 18.75±5.65g.

Other than that, the possible reasons for the result to be different from previous study are the time interval of the fermentation process. FSBM was fermented for 3 months which, it should increase more the palatibility, but it happened to be that the hamsters did not preferred on fermented food. Besides that, the odour which come from molasses do effect the preference of the hamster. Hamster

behaviour in feeding is specific. Natural fermentation of soybean silage lead to unpleasant odour and high butyric acid content, which possible due to low level of water soluble carbohydrate content. (Budakli et al., 2016).

5.2 Survival rate analysis

The treatment was placed at Animal Lab, Faculty of Agro Based Industry, UMK Jeli throughout the research. Weather during the experiment was not consistent. In the middle of the experiment, raining seasons happened which makes the temperature of surrounding drops and disturbed the animal housing environment. By that, the animals become stressed and avoid eating.

From figure 4.2, showing that control treatment has a 100% survival rate means no mortality. But, for FSBM and SBM treatment, the survival rate is only 66.67%.

The mortality happened in SBM treatment during the experiment believed to be due to “wet tail” disease infected to one of the hamster. It can cause high mortality rate and animal become diarrhea. The reasons for this wet tail are anorexia, irritability and finally death occurred. Escherichia coli could be the reason its occurred. Piglet which given FSBM diet significantly increase *Lactobacillus* counts, which decrease *E.coli* counts in fecal thus lowering the diarrhea rate and FCR. Phatogenic microbial proliferation were inhibit where the beneficial microbes present (Yin et al., 2012; Yuan et al., 2016).

Other than that, changes of surrounding temperature and cannibalism among the animals also affected. The mortality that occurred in FSBM treatment is due to cannibalism. Delville et al. (2003), justify an animal that have a territorial and solitary attitude is male hamster. After 25 days, hamsters isolated themselves in the cages, and are ready to show aggression to mainly the same sexual category. Aggressive attitude is depending on the environmental conditions. Fresh fruits and vegetables are examples of crucial feed for hamster. The animal given pellets comes with a possibility of not balance nutrient ingestion. Reducing in implantation rate and rising in cannibalism could occur by exclusion of fruits as hamster diet.

4.3 Nutritional Analysis

From Table 4.3, Moisture value of FSBM is 8.20% higher compared to SBM which is 7.83%. This shows that FSBM contain higher number of moisture. The moisture could come from the fermentation process which was used molasses and water. High number of moisture percentage indicates that possibility for FSBM to spoil is higher than SBM.

Percentage of ash in FSBM is 5.31% lower than SBM which is 8.41%. Analysing ash is to determine the mineral content available in the sample. From the result, this shows that the SBM contains higher mineral than FSBM.

The value of crude protein showing in table 4.3 indicates that crude protein in SBM has a big difference with FSBM. CP in SBM is 35.8% while in FSBM is 6.42% only. A sufficient level of protein to keep supplies is minimum 16% for adult hamster.

For appropriate growth and pregnancy, 24% protein is essential. Thus, the percentage of crude protein in SBM was exceeding the recommended. However, the amount of crude protein left in FSBM is less. Some of the nutrient could loss during fermentation process. Butyric acid was produced in between the fermentation process by undesirable microorganism which break down amino acid resulting nutrition loss (Ni et al., 2017). Other than that, percentage value of crude fat in SBM 1.63% while percentage value of crude fat in FSBM is 0.63%.

Food wastage could be decrease by increasing the hardness of the diet pellets. This paper stated that when mash feed were given to rats, the food intake in lower than pellet (Ford, 1977). According to Newkirk (2010), the author stated that, the pet food industry rely producing products which is high quality because the owner and the animals are sensitive to rancid fat, off colour and smell. Soybean product have high digestibility which it is also being used as source of protein. Most soybean products contain oligosaccharides, carbohydrates and some animal has the possibility to develop soybean allergies

CHAPTER 6

CONCLUSION

The aim of this study is to observe the effect of fermented soybean meal, FSBM to the growth of the hamster. Based on the result, it shows that FSBM did not affect fully the growth of the hamsters compared to on fermented soybean meal SBM. Comparison between FSBM and SBM growth, weight gain in FSBM is 6.05±0.85 while SBM is 18.98±4.01. Growth of the hamsters given FSBM did not show a wide increase of weight. It can be concluded that hamster in FSBM treatment did not preferred fermentation product from this study. It could be due to presence of odour in fermented feed. H_0 hypothesis in this experiment is rejected.

Based on previous study on aquaculture and swine, the result expected should be the hamsters with FSBM feed have highest weight comparing to SBM and control. But, the results turn out vice versa. There are some effect of fermentation that cannot be adapted to the hamster which be one of factor that it did not increase its weight. Besides that, stress and environment condition which occurred during study resulting the graph of weight for each treatment fluctuated. It is also can be concluded that hamster need some time to be adapt with the feed given due to graph of FSBM which the weight drops at the 4 weeks earlier.

For the approximate analysis activity, SBM shows higher percentage in ash, crude protein and crude fat. The value of SBM is almost as previous study by referring to table 2.1.2. Meanwhile, FSBM shows higher percentage in moisture

compared to SBM. Effect of higher percentage in moisture will makes the feed easily become spoil. It may be due to the solution of molasses and water available in the meal.

RECOMMENDATION

This study shows as not expected result as previous study. However, study regarding fermentation product to hamster is still in research. Most of previous studies are focusing to aquaculture feed and pig feeding. Thus, as recommendation, more study and research about fermentation product on hamster should do by researcher using other method. Present study could have some lack or deficiency while conducting. Later research about this study could be improved by improving the formulated feed by replacing element in formula and treatment of fermentation with using other formula and different time interval of fermentation. This study will help in improving nutrient in rodent feed especially small animal and pets and improving pet industry.

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APPENDIX A

A.1 ONE WAY ANOVA

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Initialweight	Between Groups	89.337	2	44.668	2.238	.254
	Within Groups	59.877	3	19.959		
	Total	149.214	5			
Finalweight	Between Groups	66.631	2	33.315	1.665	.326
	Within Groups	60.043	3	20.014		
	Total	126.674	5			
Weightgain	Between Groups	219.106	2	109.553	3.370	.171
	Within Groups	97.530	3	32.510		
	Total	316.637	5			

APPENDIX B

List of feed sample