

## EFFECT OF SUPPLEMENTED STARTER CULTURE ON TOFU DREG SILAGE QUALITY

Denny RUSMANA, Abun, Rachmat WIRADIMADJA, Handi BURHANUDDIN

Faculty of Animal Husbandry, University of Padjadjaran, Jalan Raya Bandung-Sumedang KM. 21,  
45363, Sumedang-West Java, Indonesia, (phone) +6222 7798241, (fax) +6222 7798212

Corresponding author email:denny.rusmana@yahoo.co.id

### *Abstract*

*Research used experimental method with split-plot design. Supplemented starterculture (without starter, molasses and Lactobacillus plantarum) were whole plot and time of observed (1, 2, 3, and 4 weeks) were subplot. Every time of observed for each treatment was replicated four times. The experiment showed that bacteria population among treatments for every time observed wasn't significant ( $P>0.05$ ). Lactic acid bacteria population from first making silage until fourth weeks for all treatments showed increased ( $P<0.05$ ). First week of observed among treatments showed significant for silage pH. pH of silage that supplemented molasses and Lactobacillus plantarum were lower significant ( $P<0.05$ ) than pH of silage without starter culture. Nevertheless, furthermore time of observed among treatment wasn't significant ( $P>0.05$ ). The first week observed N-Ammonia of silage wasn't significant ( $P>0.05$ ) among treatments. Significance N-ammonia of silage among treatments was began at second week. N-Ammonia Silage that supplemented Lactobacillus plantarum was lowest ( $P<0.05$ ) significant than others.*

**Key words:** starter culture, tofu dreg silage, molasses, Lactobacillus plantarum, lactic acid bacteria, pH, N-ammonia.

### INTRODUCTION

Tofu dreg is waste product a processing of soybeans into tofu. Protein content of tofu dreg in dry matter basis is 29.17% (Lopez, 1963). That can be used as an alternative source of feed protein because it is relatively cheap and abundant enough availability. This is evidenced by the increase in tofu dreg as much as 32,832 to 36,937 thousand tons per year by 81 companies produce tofu in 1994 and in 1999 to 116 companies in Indonesia (the Central Bureau of Statistics, 1999). Tofu often found in West Java in the area around Lembang, Sumedang, Sukabumi, Pangalengan, and Bogor.

However, the weakness of tofu dreg is to have a high water content and so can not be stored longer and easily damaged, so at normal temperature can only survive about 24 hours. Preservation efforts can be done by drying in the sun, but drying it got into trouble because of the drying process takes more than 24 hours. Due to this tofu dreg to be rotten before drying. Therefore, it is necessary preservation which is easily done without harm by way of silage as

feed material processing are stored in a fresh state with anaerobic atmosphere. The goal is to maintain nutrition, color, palatability and durable (Susetyo et al., 1977).

Silage preservation method in principle is to increase the growth of lactic acid bacteria that produce lactic acid which can provide acidic conditions, which is expected to inhibit the growth of spoilage bacteria.

For the growth of lactic acid bacteria needed water soluble carbohydrate is added to the material preserved in anaerobic atmosphere. It can also be added directly lactic acid or lactic acid bacteria.

### MATERIALS AND METHODS

#### Materials

The materials used for making of silage in this study is tofu dreg. Tofu dreg obtained from the factory in Cileunyi and Bandung area.

Tofu dreg 5kg packed in plastic with anaerobic conditions by starter culture (molasses, *Lactobacillus plantarum*) or without a starter. The amount of packaging is 36 packs.

### Addition Molasses

Tofu dreg that is gave molasses as much as twelve packs. Each package is added 3% molasses or 150 grams per pack. Molasses obtained from KUD Tanjung Sari.

### Addition inoculant *Lactobacillus plantarum*

Tofu dreg by *Lactobacillus plantarum* inoculants twelve pack. *Lactobacillus plantarum* was is obtained from the Laboratory of Microbiology and Bioprocess Department of Technical Chemistry Bandung Institute of Technology.

*Lactobacillus plantarum* grown on agar (Agar, beef extract, and skim milk) and then is incubated for 48 hours in a test tube.

Take a sample of the reaction tube randomly that have was planted and incubated, for the calculated of population with Method Total Plate Count (TPC).

Subsequently tubes are another plus physiological NaCl solution, and inoculated into tofu dreg. Provision of *Lactobacillus plantarum* inoculant as much as  $5 \times 10^5$  CFU/g fresh Tofu dreg.

### **Treatment and experimental design**

The treatment will be attempted is a wide variety of starter culture. The design used was split plot design treatment with the addition of starter culture (without the starter, molasses, and *Lactobacillus plantarum*) as the main plots and observation time (1, 2, 3, and 4 weeks) as the subplot treatment.

Each treatment was repeated three times. The treatment was :

1. Tofu dreg without starter.
2. Tofu dreg + molasses (3% of the weight of Tofu dreg).
3. Tofu dreg + *Lactobacillus plantarum* (population  $5 \times 10^5$  CFU bacteria/g Tofu dreg).

### **Variables Measured**

Every week for each treatment taken as many as three packs for further analysis:

1. pH with a pH meter brands of "Hanna"
2. The content of N-ammonia was determined by the method of "Micro Diffusion Conway" (AOAC, 1980)

3. The population of lactic acid bacteria, with "Method Total Plate Count (TPC)" according Fardiaz (1992).

Observations were made up to four weeks analysis method. Data were analyzed by analysis of variance followed by Duncan's multiple range test (Gomez and Gomez, 1995).

## **RESULTS AND DISCUSSIONS**

### **The Effect of adding Starter Culture on Lactic Acid Bacteria Population**

Observations (Figure 1) shows that the population of lactic acid bacteria from the beginning of making silage until the fourth week of the overall treatment showed increased ( $P < 0.05$ ).

Results of analysis of variance influence between administration starter every week on the population of lactic acid bacteria showed no significant differences ( $P > 0.05$ ).

Nevertheless, the observation every week tofu dreg that inoculated bacteria *Lactobacillus plantarum* decreased from the first week to the second week and the next week showed an increase in population.

The decline in the population of lactic acid bacteria compared to the beginning of making silage was reported also by Yatno (1999).

The decline in the population of lactic acid in the tofu dreg out inoculated with *Lactobacillus plantarum* possibility that the competition between the lactic acid bacteria existing in the tofu dreg out with *Lactobacillus plantarum* were inoculated into tofu dreg.

Yatno research results (1999) in the tofu dreg out existing lactic acid bacterial strain *Lactobacillus sp.* Inoculation *Lactobacillus plantarum* suppress the growth of lactic acid bacteria present in tofu dreg, so that the total population of lactic acid bacteria is reduced.

McDonald (1991) states that *Lactobacillus plantarum* is very dominant in the ensilage process, highly competitive and rapidly produce more acid.

After *Lactobacillus plantarum* achieve dominance in the second week, the next week is growing rapidly.

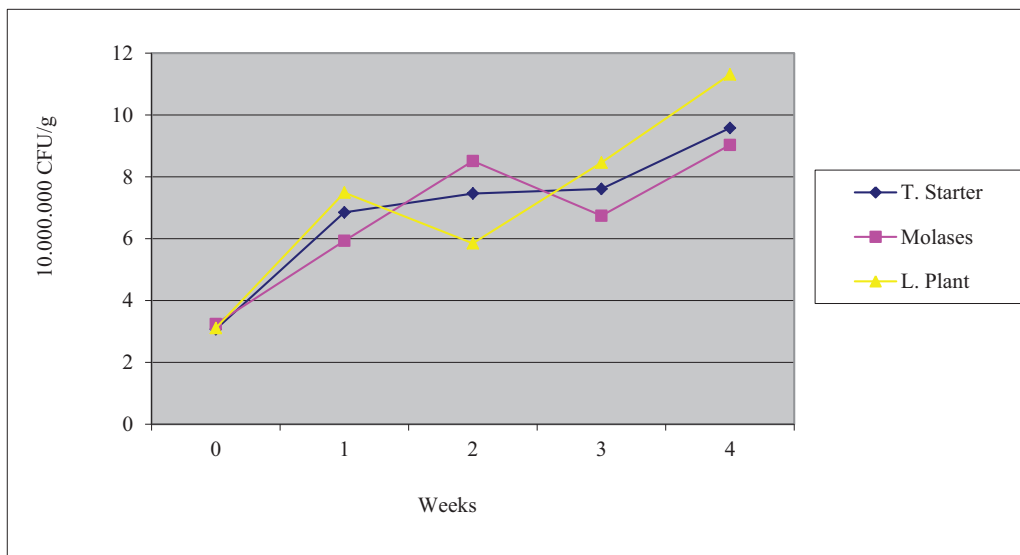


Figure 1. Population Changes Lactic Acid Bacteria ( $10^7$  CFU/ g Silage Fresh Tofu dreg) Every Weeks

Table 1. Effect of the Starter Addition on Lactic Acid Bacteria Population ( $10^7$  CFU/ g Silage Fresh Tofu Dregs) (Every Sunday)

Time	Without Starter (X $10^7$ CFU/g)	Molasses (X $10^7$ CFU/g)	<i>L. plantarum</i> (X $10^7$ CFU/g)
1 <sup>st</sup> week	6.85 a	5.93 a	7.49 a
2 <sup>nd</sup> week	7.46 a	8.51 a	7.49 a
3 <sup>rd</sup> week	7.61 a	6.74 a	8.46 a
4 <sup>th</sup> week	9.58 a	9.03 a	11.31 a

Description: The same alphabet to the row showed no significant different ( $P < 0.05$ )

### Effect of adding starter culture on pH Tofu dreg

The level of acidity (pH) of silage that has been measured by using a pH-meter indicates there has been a decrease in pH in the first week compared with the beginning of making silage. pH silage real differences among treatments occurred in the first week ( $P < 0.050$ ).

The pH of silage that was inoculated *L. plantarum* and that was added molasses was significantly lower ( $P < 0.05$ ) than without starter. But in the next few weeks between treatments showed no significant differences ( $P > 0.05$ ).

Decrease in pH than at the start of making silage, because it produces acid compounds during ensilage by lactic acid bacteria.

The acid formed during the process include lactic acid, acetic acid and butyric acid as well as several other compounds such as ethanol, carbon dioxide, methane, carbon monoxide nitrite (NO) and heat (Cullison, 1978).

In the first week seeded the pH of silage that was added molasses and that was inoculated *Lactobacillus plantarum* significantly ( $P < 0.05$ ) lower than pH of silage without starter. Molasses is a material containing a water-soluble carbohydrates.

The molasses can be used as a stimulant of microorganisms forming lactic acid.

Woolford (1998) suggested that the ensilage process requires rapid formation of lactic acid.

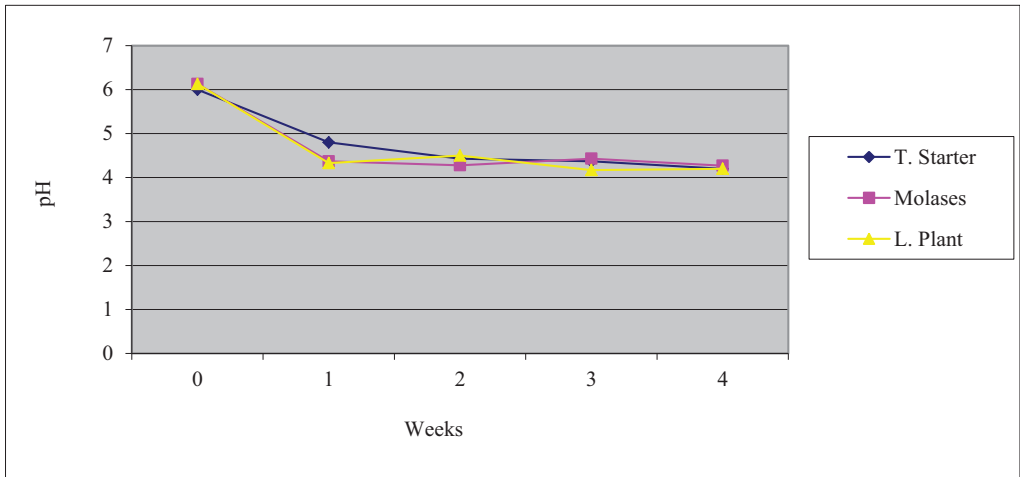


Figure 2. Changes in pH Silage Tofu Dregs Every Weeks

Table 2. Effect of the Addition Starter on Silage pH Tofu Dregs Every Week

Time	Without Starter pH	Molases pH	<i>L. plantarum</i> pH
1 <sup>st</sup> week	4.80 a	4.37 b	4.33 b
2 <sup>nd</sup> week	4.43 a	4.28 a	4.50 a
3 <sup>rd</sup> week	4.37 a	4.43 a	4.17 a
4 <sup>th</sup> week	4.20 a	4.27 a	4.20 a

Description: The not same alphabet to the rows showed significant different ( $P < 0.05$ )

Carbohydrates are readily soluble in water/WSC is a source for starting and maintaining the process of fermentation, lactic acid bacteria can multiply rapidly under conditions where available feeds rich in carbohydrates (Cullison, 1978).

To accelerate the formation of lactic acid in ensilage process can be done by direct stimulant that with the addition of lactic acid bacteria.

*Lactobacillus plantarum* is group of bacteria that have the ability to convert carbohydrates such as lactose and glucose is fermented into lactic acid in large quantities.

*Lactobacillus plantarum* is a group of bacteria homofermentatif, which will produce 2 moles of lactic acid for every mole of glucose and fructose.

According Rahayu et al. (1992) homofermentatif acid bacteria can change 95% glucose and other hexoses to lactic acid

and carbon dioxide with a small amount of volatile acids (butyric acid).

The implications of the addition molasaes or *Lactobacillus plantarum* on ensilage process is able to accelerate a decrease in pH.

#### Effect of Adding Starter Culture on N-Ammonia Silage Tofu Dreg

The content of N-Ammonia is one of the criteria considered to determine the success of the ensilage process (Wilkins, 1988). Results of analysis of variance showed that the adding of starter culture gave significant effect ( $P < 0.05$ ) on N-ammonia in silage.

Duncan's multiple range test showed that the first week N ammonia content showed nonsignificant.

The significant difference was occurred in the second week of observation, it appears that the content of the N-Ammonia lowest caused by the addition of an inoculant treatments *Lactobacillus plantarum*.

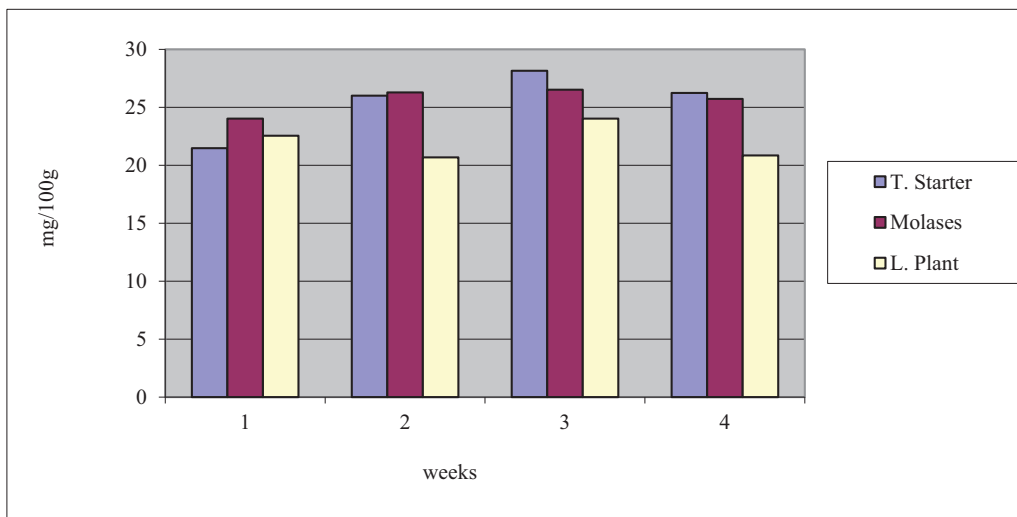


Figure 3. Production of N-Ammonia (mg/100g Silage Fresh Tofu Dregs)

Table 3. Effect of the Addition of Starter on N-Ammonia Production (mg/ 100 g Silage Fresh Tofu Dregs) every Weeks

Time	Without Starter (mg/ 100 g Silage Fresh Tofu Dregs)	Molases (mg/ 100g Silage Fresh Tofu Dregs)	<i>L. plantarum</i> (mg/ 100 g Silage Fresh Tofu Dregs)
1 <sup>st</sup> week	21.48 a	24.03 a	22.55 a
2 <sup>nd</sup> week	26.01 a	26.29 a	20.68 b
3 <sup>rd</sup> week	28.16 a	26.52 ab	24.03 b
4 <sup>th</sup> week	26.24 a	25.73 a	20.85 b

Description: The not same Alphabet to the rows showed significant different(P <0.05)

Their N-ammonia in silage is presumably because the process of deamination by bacteria other than lactic acid bacteria that are proteolytic so will outline glutamic acid and lysine into acetic acid, pyruvic acid and N compounds in the form of NH<sub>3</sub>.

According to McDonald et al (1991) that as many as 60 types of *Clostridium* and 7 species are microorganisms that are often involved in the process of fermentation of the silage.

Further explained that *Clostridia* are saccharolytic as *Clostridium butyricum* capable of fermenting organic acids and sugar and little ability to ferment proteins and amino acids, but it also is a proteolytic like *Clostridium sporogenes* which is able to ferment the amino acid glutamic acid, lysine, arginine, histidine, alanine and glycine).

Meanwhile, according Bolsen and Sapienza (1993), there are three genera, namely *Escherichia*, *Klebsiella* and *Erwinia*.

These bacteria are divided into two groups: the fermenting sugars and organic acids and the

ferment of free amino acids. This bacteria is not undesirable in the ensilage process.

These bacteria are sensitive to low pH and require wet conditions for its development. These bacteria can be suppressed as low as possible by accelerating the fermentation produced by lactic acid-producing bacteria, this is due to the optimal pH for growth of bacteria is 6-7.

The implications of N-ammonia content is that the higher N-ammonia produced from ensilage proses is mean an lot of amino acids degraded by proteolytic bacteria.

The smaller the content of N-ammonia in silage is the better, because the proteolytic process is a little occurs. From the research it appears that administration of *Lactobacillus plantarum* inoculum provide the best results because it can prevent proteolytic process, which in turn contains N-ammonia in silage is lower.

## CONCLUSIONS

### Conclusion

From the observation of silage tofu dreg for four weeks showed that the addition of *Lactobacillus plantarum* give better effect than with the addition of molasses or without a starter that is characterized by low content of N-Ammonia silage.

### Suggestion

1. To maintain the quality of the tofu dreg by way of silage suggested the addition of lactic acid bacteria *Lactobacillus plantarum*.
2. Do further research on comparing the quality of the tofu dreg with the addition of various types of lactic acid bacteria.

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