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Research Journal of Pharmaceutical, Biological and Chemical Sciences  
Addition Of Weissella Paramesenteroides As Probiotic In Liquid Soap From Abdominal Fat Cattle. Sri Melia<sup>1</sup>, Afriani Sandra<sup>1</sup>, Arif Trisman<sup>2</sup>, Hendri Purwanto<sup>2</sup>, and Endang Purwati<sup>1\*</sup>. <sup>1</sup>Laboratory of Technology of Animal Product Processing, Faculty of Animal Science, Andalas University, Limau Manis Campus, Padang, Indonesia <sup>2</sup>Post Graduate Student, Department of Biotechnology, Andalas University, Limau Manis Campus, Padang, Indonesia **ABSTRACT** This research was aimed to determine the effect of Weissella paramesentroides on the physical properties (pH and foam power) and microbiology (inhibition of Escherichia coli O157, the number of aerobic bacteria colonies and the number of lactic acid bacteria) in the probiotic liquid soap from abdominal fat cattle (tallow). The bacteria was Weissella paramesentroides 80 ml (1 x 10<sup>8</sup> CFU /ml). Randomize block design consisted of 5 treatments with 4 groups as replication. The variation of the added Weissella paramesentroides were [A \(0\)](#), [B \(2 × 10<sup>8</sup> CFU /ml\)](#), [C \(4 × 10<sup>8</sup> CFU /ml\)](#), [D \(6 × 10<sup>8</sup> CFU /ml\)](#), and [E \(8 × 10<sup>8</sup> CFU /ml\)](#). The results showed that the addition of Weissella paramesentroides probiotic in liquid soap significantly lowered the pH, enhanced the foam, inhibited the growth of Escherichia coli O157 bacteria, lowered the number of the aerobic bacteria colonies and increased the number of the lactic acid bacteria colony. The optimum addition of the Weissella paramesentroides was at pH 9.75, which resulted in a foam power of 5.225 cm, a strong inhibition of bacteria (19.25 mm), the number of



aerobic bacteria colonies of  $4.25 \times 10^3$  CFU/ml, and the number of lactic acid bacteria colonies  $1.51 \times 10^{10}$  CFU/ml. Keywords: Weissella paramesenteroides, liquid soap, tallow, inhibition bacteria

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**INTRODUCTION** Cattle is one of the mainstay commodity in the farming world, as one of the cattle product is meat that consumed by many people. Cattle also produced which is called by-products as a complementary ingredient in making up the body composition of beef cattle. By-products produced include skin, blood, abdominal fat, bone, gastrointestinal tract and horns. Increasing population and economic income communities, as well as public awareness of the benefits of farm commodities to health then scale farm businesses will increase, with increased consumption of meat, by-product from cattle that are generated also increases. By-products of cattle slaughter if treated effectively, will increase the economic value of the by-product. Abdominal fat cattle is one of the by-products that can be made into soap by saponification reaction. Abdominal fat is extracted in the form of oil called tallow, which is a solid at room temperature and liquid at a temperature of  $64^{\circ}\text{C}$ . Other types of animal fat, which can be obtained by extraction (The warming) that pork fat (lard) dan suet [1]. The main function of soap as a disinfectant soap or kill pathogens, To fulfill these functions needs the addition of Probiotic called Weissella paramesenteroides, This is lactic acid bacteria (LAB), which able to convert carbohydrates (glucose) into lactic acid and bacteriocins that can inhibit pathogenic bacteria such as Escherichia coli O157. Use of Weissella paramesenteroides with dose of  $2 \times 10^8$  CFU/g can reduce the activity of Escherichia coli O157 as Enteropathogenic bacterium Escherichia coli (EPEC), a pathogen bacteria causing diarrhea [2]. Weissella species are a member of LAB. There are 14 species officially recognize. All Weissella species are nonspore formers, Gram-positive, producing lactic acid and carbon dioxide and catalase negative [3]. Weissella paramesenteroides was isolated from tempoyak (fermented Durian) made in Philipines has potential as a probiotic product [4]. Probiotics are characterized as live microorganisms which when managed in satisfactory sums present a medical advantage on the host [5]. Probiotic microscopic organisms have been appeared to have a hostile movement against nourishment borne ailment operators, for example, S. aureus, Salmonella spp., E. coli, L. monocytogenes and Cl. Perfringens. [6]. Liquid soap can be used as sanitary materials and hygiene at slaughterhouses. Slaughterhouses are processing place of livestock products are safe and healthy for consumption by people from germs, especially diseases caused by pathogenic bacteria. Cattle, goats, sheep, and chickens can carry pathogens Escherichia coli O157, if not treated with proper sanitation, can cause the disease for humans. Based on the fact described, we will reported about making liquid soap that qualify for national standard of Indonesia product from abdominal fat cattle to increased the economic value of byproduct, so that will create innovation and new technologies in the field of animal husbandry and to aimed the effect of adding probiotic Weissella paramesenteroides on physical properties and microbiology in this product.

**MATERIALS AND METHODS** Research design and Variable Measured The study was conducted by an experimental method, using a randomized block design (RAK) with 5 treatments and 4 replications, where groups as replication. Treatment of additions Weissella paramesenteroides with dose  $1.01 \times 10^{10}$  CFU/ml, ml A = 0, B = 2 ml, C = 4 ml, D = 6 ml and E = 8 ml.



Statistical models of this design according to Steel and Torrie (1995). The treatments were significant ( $P < 0.05$ ) or highly significant ( $P < 0.01$ ) carried out a further test with Duncan's Multiple Range Test (DMRT). In this study, the measured variable is the pH, foam, total aerobic bacteria colonies, colonies total lactic acid bacteria and the inhibition of *Escherichia coli* O157. Research Procedures Probiotic liquid soap must qualify to the national standard of Indonesia for a liquid product, that called SNI 06-4085-1996, which used method [7], with some modification.

**RESULTS AND DISCUSSION pH**

The average pH of the probiotic liquid soap [can be seen in Table 1](#). [Table 1: The Average of pH of Probiotic Liquid Soap Treatments](#)

Treatment	Average of pH
A	12.49a
B	11.67b
C	11.12c
D	10.46d
E	9.75e

Description: different superscripts in the same column showed a highly significant ( $P < 0.01$ ). Table 1 showed that the average pH of the probiotic liquid soap range between 9.75-12.49, the pH of the highest in treatment A is 12.49 and the lowest in treatment E are 9.75. [Results of analysis of variance showed that the](#) treatment effect is highly significant ( $P < 0.01$ ) in the pH. The treatment A significantly different with B, C, D and E. This shows that the addition of probiotic *Weissella paramesenteroides* significance on the pH of the liquid soap probiotic Peof tallow. The mechanism of decrease in pH in the liquid soap probiotics by *Weissella paramesenteroides*, namely liquid soap is the result of the saponification reaction with KOH abdominal fat cattle which produce soaps and glycerol. In accordance with the opinion of Barrels (2009) that the soap is saponification reaction (saponification) between a fatty acid with a base that will produce soaps and glycerol. Glycerol is formed from side reactions of liquid soap making can be fermented by *Weissella paramesenteroides*, where *Weissella paramesenteroides* including lactic acid bacteria group. The result of the fermentation of glycerol by LAB will produce organic acids, one of which is lactic acid. This is supported by research Purwati, Arief, and Rakhmadi (2011), which managed to isolate bacteria *Weissella paramesenteroides* which is lactic acid bacteria in the manufacture of curd. The ability to ferment or use a type of sugar is a way to identify LAB, where one type of sugar that can be processed by LAB is glycerol [9]. Results fermentation of glycerol as a by-product of the manufacture of soap this will then be processed by *Weissella paramesenteroides*, thus producing lactic acid to lower the pH of liquid soap. [10] found *Weissella* strain which isolated from human feces and potential as probiotics. It survived at pH 3.0 and grew well between 15 – 45°C. Based on SNI 06-4085-1996 regulated quality requirements of liquid soap, wherein the pH is allowed in liquid soap ranged between 8-11. The results of the probiotic liquid soap making tallow with the addition of *Weissella paramesenteroides*, showed that the liquid soap qualifies to SNI are treatment D and treatment E with pH 10.46 and 9.79. Thus, the soap that has the best pH of the result of the addition of probiotic *Weissella paramesenteroides* is treatment E = 8 ml (8.08 x 10<sup>10</sup> CFU/ml) with pH 9.79.

**Foam Power**

The average of foam power of probiotic liquid soap [can be seen in Table 2](#). [Table 2: The Average of Foam Power of Probiotic Liquid Soap Treatments](#)

Treatment	Foam Power of Probiotic Liquid Soap (cm)
A	4.225a
B	4.350a
C	4.775b
D	5.075c
E	5.225c

Description: different superscripts in the same column showed a significant ( $P < 0.05$ ). The average of foam power of liquid soap with the addition of probiotic *Weissella paramesenteroides* range between 4,225-5,225 cm (Table 2). Foam power highest obtained at treatment E



is 5,225 cm, while its foam power is largest than treatment A with 4,225 cm. Results of analysis of variance showed significant treatment effect ( $P < 0.05$ ) against the foaming power of probiotic liquid soap. This means that the provision of *Weissella paramesenteroides* effects on foam power of liquid soap. The increased capacity of liquid soap foam due to the increased addition of probiotic *Weissella paramesenteroides* caused by increased the stability of the foam that formed if the pH soap near 7 it will form a more stable foam. This caused of a soap bubble will be formed maximal if the pH is not too acidic or too alkaline. pH is too acidic for example in soil containing hardness ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ) will reduce the foam formed, so if the pH is too alkaline, the  $\text{OH}^-$  ion will be difficult to unravel because there is no  $\text{H}^+$  ion for equilibrium reactions of acids and bases in a solution, so that the soap does not work optimally and foam that formed less than the soap at normal pH (7) that would be perfect equilibrium between  $\text{H}^+$  and  $\text{OH}^-$  ions. In accordance with the opinion of Day and [11], that the pH value of an element is the ratio between the concentration of hydrogen ions  $[\text{H}^+]$  ion concentration of hydroxyl  $[\text{OH}^-]$ . If the concentration of  $\text{H}^+$  is greater than  $\text{OH}^-$ , the material is called acid : the pH value is less than 7. If the  $\text{OH}^-$  concentration is greater than  $\text{H}^+$ , the material is called alkaline, with pH value greater than 7. If the concentration of  $\text{H}^+$  equals  $\text{OH}^-$  the material is referred to as a neutral material. Added by [12] for the purpose of laundering the use of soaps with hard water will cause a decrease in the foam because hard water conditions are acidic, so use more soap and effectiveness of soap to be reduced. Treatment A generated foam power that lowest compared than treatment B, C, D and E. This is due to the treatment of A (0 ml) no replenishment *Weissella paramesenteroides*, resulting in lower power foam. In accordance with the results of testing the pH of the liquid soap probiotics in the treatment of A also produces the highest pH, so the higher pH makes foam power formed will be low. When compared with all treatments, the best foam power is treatment E, this is due to produce higher foam power because the pH of probiotic liquid soap in the treatment E is lowest. The best results are obtained at treatment E with the addition *Weissella paramesenteroides* of 8 ml ( $8.08 \times 10^{10}$  CFU/ml). When compared with pH, the best result was also similar in the treatment E with a pH of 9.75. If the pH of the soap is almost close to neutral, it will form a foam that is more stable if compared with pH in treatment D, C, B and A are further away from the normal pH, so the foam power on treatment E more than the other interventions namely *Weissella paramesenteroides* with the addition of 8 ml ( $8.08 \times 10^{10}$  CFU/ml). The number of Aerobic Bacteria Colonies The average total colonies of aerobic bacteria of probiotics liquid soap [can be seen in Table 3](#). Table 3: [The number of Aerobic Bacteria colonies of Probiotic Liquid Soap Treatments Total Colonies of Aerobic Bacteria \( \$1 \times 10^3\$  CFU/ml\)](#) A 27.75a B 21.75b C 15.50c D 9.50d E 4.25e Description: different superscripts in the same column showed a highly significant ( $P < 0.01$ ). Table 3 showed that the average the number of aerobic bacteria colonies of probiotic liquid soap range between  $4.25 \times 10^3$  CFU/ml -  $27.75 \times 10^3$  CFU/ml, where the number colony of aerobic bacteria was highest in treatment A is  $27.75 \times 10^3$  CFU/ml and the lowest in treatment E is  $4.25 \times 10^3$  CFU/ml. [Results of analysis of variance showed that the treatment effect is highly significant \( \$P < 0.01\$ \) in total colonies of aerobic bacteria of probiotic liquid soap.](#) This shows that the addition of probiotic *Weissella paramesenteroides* very effect on total colonies of aerobic



bacteria of probiotic liquid soap (tallow). The decline in total colonies of aerobic bacteria in probiotic liquid soap along with the increase of addition of *Weissella paramesenteroides*. Its probiotic contained antimicrobial activity (bacteriocins). Aerobic bacteria consisting mostly of bacteria pathogens and pollutants, so with reduced total colonies of aerobic bacteria, This shows good results to the quality standard of liquid soap. This is in accordance with the opinion by Surono (2004) that lactic acid bacteria also lowers the pH of the environment and excrete compounds that serve to inhibit pathogenic microorganisms such as  $H_2O_2$  compounds, diacetyl,  $CO_2$ , acetaldehyde, d-isomer, amino acids, and bacteriocins. Bacteriocins produced *Weissella paramesenteroides* which is bacteriostatic (inhibit) that growth of aerobic bacteria, but in large quantities will be bakteriocidal that kill the growth of aerobic bacteria. This is supported by the opinion Harijani [13], one of the results of LAB metabolites are bacteriocins, able to produce like antibiotic. Bacteriocins have the nature bakteriocidal and bacteriostatic characteristics and have activity on bacteria that are ecologically similar and closely related to each other in a single family. In addition, bacteriocins produced pentosan which is stable on gram positive and able to maintain the quality of lactic acid bacteria based microbiology. Bacteriocins are primary metabolites have antagonistic activity on bacteria that caused decay and bacteria pathogens. SNI 06-4085-1996 regulated the quality requirements of liquid soap, where a maximum of the total aerobic bacteria colonies or total plate count that allowed the liquid soap is  $1 \times 10^5$  CFU/ml. The results of the study of liquid soap with the addition of *Weissella paramesenteroides*, showed that all treatments resulted in the average of total colonies of aerobic bacteria in probiotic liquid soap that qualifies to SNI 06-4085-1996, wherein the best liquid soap with the number colonies of aerobic bacteria are the lowest, namely in treatment E 8 ml ( $8.08 \times 10^{10}$  CFU/ml) by the number of aerobic bacteria colonies  $4.25 \times 10^3$  CFU/ml. The number of Lactic Acid Bacteria Colonies Table 4: The number of LAB Colonies of Probiotic Liquid Soap Total Colonies of LAB Treatments ( $1 \times 10^8$  CFU/ml) A 0.00a B 15.75b C 26.25c D 71.00d E 151.00e Description: different superscripts in the same column showed a highly significant ( $P < 0.01$ ). The average the number colonies of [lactic acid bacteria in probiotic liquid soap can be seen in Table 4](#). Table 4 showed that the average the number colonies of lactic acid bacteria in probiotic liquid soap range from  $0-151.00 \times 10^8$  CFU/ml, which the number colonies of lactic acid bacteria is highest on treatment E is  $1.51 \times 10^{10}$  CFU/ml and the lowest in treatment A  $0 \times 10^8$  CFU/ml. Results of analysis of variance (indicating that the treatment effect is highly significant ( $P < 0.01$ ) in on number colony of lactic acid bacteria of probiotic liquid soap. It's showed that the addition of *Weissella paramesenteroides* very effected on total colonies of lactic acid bacteria of tallow. Increased the number colonies of LAB along with the addition of bacteria *Weissella paramesenteroides*, because these bacteria include the type of lactic acid bacteria (LAB). This is supported by the opinion Rostini [14], which said that the bacteria that belong to the LAB is *Aerococcus*, *Alloccoccus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Streptococcus*, *Weissella*, *Tetragenococcus*, and *Vagococcus*. Added by [15], where the results of the research concluded that LAB which dominates in Sumbawa horse milk, the bacteria have form like *Weissella paramesenteroides* which is short rod cells. The increase in number



colonies of LAB along with the addition of bacteria *Weissella paramesenteroides* also to bacteriocins produced by *Weissella paramesenteroides* that serve as a compound inhibiting the growth of pathogenic bacteria. Extra *Weissella paramesenteroides* probiotic in the manufacture of liquid soap will suppress the growth of pathogenic bacteria, so as to decrease the number of pathogenic bacteria along with the addition of *Weissella paramesenteroides*, then the amount of lactic acid bacteria will increase. This is supported by the opinion of [16], that LAB is the kind of heterofermentatif, decomposition of glucose is done through the pentose phosphate and fermentation of these works is the enzyme fosfoketolase and can produce lactic acid 40-50%, ethanol, bacteriocins, acetic acid and CO<sub>2</sub>. This is why *Weissella paramesenteroides* can grow in the liquid soap so that more its additions, the liquid soap will also increase the growth of LAB, thus the high addition of *Weissella paramesenteroides* in the manufacture of liquid soap also increases the total colonies of LAB. The results of the average number colonies of LAB lowest in treatment A (0.00). The absence of colony growth LAB liquid soap in the treatment of A (0.00) caused by in this treatment no added bacteria *Weissella paramesenteroides*. The results of this study showed total LAB in treatment B ( $15.75 \times 10^8$  CFU/ml) to the treatment E ( $151.00 \times 10^8$  CFU/ml) that qualify doses for probiotic products in the manufacture of liquid soap can provide moisture to the skin and can serve to suppress pathogenic bacteria the cause of the disease. The best treatment is treatment E as many as 8 ml ( $8.08 \times 10^{10}$  CFU/ml) with total colonies of LAB high of  $151.00 \times 10^8$  CFU/ml or  $1.51 \times 10^{10}$  CFU/ml, so that the total colonies of LAB that satisfies criteria stated FAO/WHO (1997) as a probiotic is currently on number 106-1010 CFU / ml. Inhibitory Power of Probiotic Liquid Soap For *Escherichia coli* O157 The average inhibitory power of probiotic liquid soap for *Escherichia coli* O157 can be seen in Table 5. Table 5: Inhibitory Power of Probiotic Liquid Soap for *E.coli* O157 (mm) Treatments Inhibitor zones of Probiotic Liquid Soap A 3.00a B 11.50b C 14.38c D 17.75d E 19.25d Description: different superscripts in the same column showed a significant ( $P < 0.05$ ). In Table 5 showed that the average of the inhibition power for *Escherichia coli* O157. The highest inhibition obtained at treatment E is 19.25 mm, while the lowest is treatment A 3.00 mm. Results of analysis of variance showed the addition of *Weissella paramesenteroides* provides significant effect ( $P < 0.05$ ) inhibition on *Escherichia coli* O157. The ability of liquid soap to inhibit the growth of bacteria *Escherichia coli* O157 as pathogenic bacteria along with the addition of *Weissella paramesenteroides*, caused by the activity of anti- bacteria / bacteriocins produced by *Weissella paramesenteroides*. This is in accordance with the opinion of [17], that the lactic acid bacteria of which includes a group heterofermentative is *Leuconostoc*, *Weissella*, and some *Lactobacilli*. [18] reported that *Weissella paramesenteroides* isolated from South Indian fermented foods, koozh. The inhibition may primarily be due to the production of inhibitory compounds such as bacteriocins, hydrogen peroxide or organic acids as well as competitive adhesion to the epithelium [18]. The heterofermentative LAB, glucose decomposition do pentose phosphate pathway. In this fermentation enzymes that work is fosfoketolase and can produce 40-50% lactic acid, ethanol, bacteriocins, acetic acid and CO<sub>2</sub>. [16]. *Weissella paramesenteroides* DX was isolated from traditional greek sausage has been appeared to create a 4450-Da



class IIa bacteriocin, weissellin A, made out of 43 amino acids. Weissellin A showed against all gram-positive microorganisms tried, but was not dynamic against Salmonella enteric Enteritidis [20]. Figure 1: Inhibition Power of Probiotic Liquid Soap for Escherichia coli O157 The Growth of Escherichia coli O157, with the strength of  $39 \times 10^5$  CFU/ml hampered due to inherent bactericidal (killing bacteria) pathogens by Weissella paramesenteroides. Bacteriocidal nature is derived from the bacteriocins produced by Weissella paramesenteroides. Bacteriocins of lactic acid bacteria according to [21] is defined as an active protein or protein complex that showed bactericidal action. Martin et al. (2009) were studied in the isolation of lactobacilli from sow milk and identified as Weissella paramesenteroides was showed a clear inhibition antimicrobial activity against E .coli CECT4076, E .coli RJM1 and E .coli RJM2, Enterococcus faecium P21, Enterococcus faecalis TAB28, Listeria monocytogenes Ohio. LAB produces H<sub>2</sub>O<sub>2</sub> under aerobic growth conditions. LAB also excrete H<sub>2</sub>O<sub>2</sub> as personal protective equipment which is capable of bacteriostatic or bactericidal. H<sub>2</sub>O<sub>2</sub> can be used as an antimicrobial agent against bacteria, fungi, and even viruses. In accordance with the opinion of [16], that LAB excreting compounds capable of inhibiting pathogenic microorganisms such as H<sub>2</sub>O<sub>2</sub>, diacetyl, CO<sub>2</sub>, acetaldehyde, d-isomer, amino acids and bacteriocins.

**CONCLUSIONS** Addition probiotic Weissella paramesenteroides affected the physical properties and microbiological of liquid soap on abdominal fat cattle and qualify to National Standard of Indonesia requirements for liquid soap product which is SNI 06-4085-1996. The addition of probiotic Weissella paramesenteroides best at 8 ml ( $8.08 \times 10^{10}$  CFU/ml) resulted in pH 9.75, foam power 5,225 cm, total colonies of aerobic bacteria  $4.25 \times 10^3$  CFU/ml, total colonies of lactic acid bacteria  $1.51 \times 10^{10}$  CFU/ml and having inhibition power for Escherichia coli O157 19.25 mm.

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