World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(Research Article)

Effect of feed containing fermented catfish waste by *Lactobacillus paracasei, Bacillus subtilis, Saccharomyces cerevisiae* on microflora intestine in super native chicken

Abun Abun [1,*], Aulia Afif [2] and Denny Rusmana [1]

[1] *Department of Animal Nutrition and Feed Technology, Padjadjaran University, Sumedang-West Java, Indonesia.*
[2] *Alumni Department of Animal Nutrition and Feed Technology, Padjadjaran University, Sumedang-West Java, Indonesia.*

World Journal of Advanced Research and Reviews, 2024, 23(02), 273–280

Publication history: Received on 23 June 2024; revised on 31 July 2024; accepted on 03 August 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.23.2.2341

Abstract

This study aimed to determine the effect of fermented catfish waste (FCW) *Lactobacillus paracasei, Bacillus subtilis*, and *Saccharomyces cerevisiae* (LBS) microbes in diets on microflora intestine on nativ super chicken. The study used 24 nativ super chickens aged 14 weeks, which were reared for 14 days. The method used was a completely randomized design with six treatments (R0 = Lower control diets without the use of FCW (15% CP and 2750 kcal/kg ME); R1 = Diets containing 5% FCW (15% CP and 2750 kcal/kg ME); R2 = Diets Containing 10% FCW (15% CP and 2750 kcal/kg ME); R3 = Diets containing 15% FCW (15% CP and 2750 kcal/kg ME); R4 = Diets containing 20% FCW (15% CP and 2750 kcal/kg ME); dan RS = Upper control diets without the use of FCW (CP 18% CP and 2750 kcal/kg ME)) and four replicates. The results showed that the use of FCW by LBS microbes did not have a significant effect (P>0,05) on total intestine microbe and total *Escherichia coli* and had a considerable impact (P<0.05) on *Staphylococcus aureus*. Using as much as 5% in the diet formula gave the best *Escherichia coli* and *Staphylococcus aureus* value with the lowest value microflora intestine for nativ super chicken.

Keywords: Fermented catfish waste; Microflora intestine; Super native chicken; *Escherichia coli; Staphylococcus aureus*

1. Introduction

The catfish fillet industry produces a yield of around 33%, and the remaining 67% is waste in the form of heads, scales, bones, intestines, and bellies that still contain nutrients [1]. The analysis results of catfish waste contain 26.05% crude protein, 20.94% crude fat, Ca 1.50%, and P 7.20% (PT. Saraswanti Indo Genetech, 2022). The remaining catfish protein and fat have the potential to be substrates in producing amino acids and fatty acids by fermentation methods. Fermented products are in the form of fish silage which is processed through a fermentation process using solid state fermentation. Solid-state fermentation is a method in which microorganisms grow on a solid substrate [2]. The fermentation process can improve the nutrient profile of catfish waste [3]. Microbes that can be used in making fish silage are *Lactobacillus paracasei, Bacillus subtilis,* and *Saccharomyces cerevisiae.*

L. paracasei is a lactic acid bacterium found in the human intestines and mouth, as well as waste and silage. Lactic acid bacteria (LAB) can produce bacteriocins and lower the pH, thus inhibiting the growth of pathogenic bacteria. Bacteriocin is a protein substance produced by LAB that can inhibit the growth of pathogenic bacteria such as *E.coli, V. choler,* and *B. cereus* ([4]. *L. paracasei* can also increase the essential amino acid profile in soybean products by 10.25% [5]. *B. subtilis* is a non-pathogenic and gram-positive bacterium that is easy to breed. *B. subtilis* is a commonly used probiotic because, in addition to its high effectiveness, it can secrete several enzymes, such as proteases, amylases, lipases, and carboxypeptidases [6]. *B. subtilis* has the potential to increase the levels of amino acids such as glycine, valine, lysine,

* Corresponding author: Abun Abun

leucine, serine, threonine, proline, phenylalanine, aspartic acid, tyrosine as well as linoleic acid and oleic acid [7]. *S. cerevisiae* is a yeast commonly used in feed fermentation. *S. cerevisiae* can grow aerobically and anaerobically, depending on its ability to use different sugars depending on its environmental conditions. S*. cerevisiae* culture probiotics can produce amylase enzymes, lipases, proteases and other enzymes that can allow livestock to digest more food and be absorbed. In addition, *S. cerevisiae* can bind oxygen and create anaerobic conditions, so it is suitable for developing microflora [8]. Fermentation using *S. cerevisiae* produces soluble organic compounds that are easily absorbed, such as essential amino acids, and *S. cerevisiae* can produce high beta carotene as an antioxidant [9].

The use of fish silage distributed by 3 to 12% in the ration of broiler chickens in the finisher phase has a positive effect on the growth and balance of intestinal microflora in broiler chickens [10]. Fermented catfish waste products can be used as prebiotics. Prebiotics are a substrate microorganisms use in the host and cause health-improving effects [11]. The intestines of chickens contain pathogenic and non-pathogenic microbes, which, if the number of pathogenic microbes increases, will hurt chicken performance. Prebiotics will work as a substrate where pathogenic bacteria attach so that they do not attach directly to the surface of the intestinal villi [12], and can act as a substrate that supports the growth of beneficial microflora so that there is a change in the number of microflora ecology in the intestine [13]. One of them is the bacterium *Lactobacillus sp.,* which functions to increase productivity and control pathogenic microorganisms that work by preventing the growth of pathogenic organisms in the small intestine so that *intestinal villi* can optimally absorb protein from feed [14]. Decreased pathogenic bacteria will impact the nutrient absorption process for livestock, such as improving performance and meat quality.

Escherichia coli can be found in the intestines and is a gram-negative bacterium. *E. coli* is anaerobic facultative, rod-shaped, does not form spores, and is a natural bacteria found in the intestines [15]. *E.coli* can cause disease when it enters the host's body and adapts in the body, then attacks the immune system so that it causes disease. *E. coli* can attack intestinal mucosal cells, disrupting the nutrient absorption process. *Staphylococcus aureus* is a gram-positive bacterium that is spherical, non-sporadic, and clustered like grapes, anaerobic facultative, and can be found in water, air, humans, and animals [16]. *S. aureus* can produce enterotoxin which is an extracellular protein that can cause food poisoning in poultry products and others [17]. *S. aureus* belongs to the pathogenic microflora that develops in the intestines. The amount of pathogenic microflora, such as *S. aureus,* in the intestines can be suppressed by adding probiotics or prebiotics.

2. Material and methods

2.1. *Inokulum mikroba* LBS *(L. paracasei, B. subtilis, dan S. cerevisiae*)

The manufacture of LBS microbial inoculum is carried out by fermenting each pure culture with catfish prepared to multiply LBS microbes so that each microbe is used to live on the catfish waste substrate.

2.2. Fermented Catfish Waste (FCW)

Fermented catfish waste was obtained after fermentation of catfish waste with LBS microbes for five days at a dose of 10%. After that, it is harvested and dried, then ground into fermented catfish waste flour (FCW).

2.3. Research Feed and Ration

The feed used to prepare the ration consists of fish meal, FCW, soybean meal, corn, rice bran, $CaCO_3$, bone meal, and premix. The rations used are prepared based on the needs of super native chickens during the finisher period, namely crude protein 18-19%, crude fat 4-7%, crude fiber 3-5%, Ca 1-1.2%, P 0.35%, lysine 0.6%, methionine 0.8%, and metabolizable energy 2750 kcal/kg [18]. The study ration consisted of a lower control ration without the addition of FCW (CP 15%; ME 2,750 Kcal/kg), four rations based on the level of use of FCW (CP 15%; ME 2,750 Kcal/kg), and Upper control ration without the addition of FCW (CP 18% with ME 2,750 Kcal/kg). The composition of the research ration can be seen in Table 1, and the nutrient content of the treatment ration in Table 2.

Table 1 Research Ration

Rations	R0	R1	R2	R3	R4	RS
Fish Meal	10.00	8.00	4.50	2.00	0.00	13.00
FCW*	0.00	5.00	10.00	15.00	20.00	0.00
Soybean Meal	8.00	6.00	5.00	3.50	1.50	14.00
Yellow Corn	57.00	58.00	58.00	58.00	58.00	53.00
Fine Bran	23.00	21.00	20.50	19.50	18.00	18.00
$CaCO_3$	0.75	0.75	1.00	1.50	2.00	0.75
Bone Meal	0.75	0.75	0.50	0.00	0.00	0.75
Premix	0.50	0.50	0.50	0.50	0.50	0.50

Description: R0, Bottom control ration without the use of FCW (CP 15%); R1, Rations containing FCW 5% (CP 15%); R2, Rations contain 10% FCW (CP 15%); R3, Rations containing FCW 15% (CP 15%); R4, Rations contain 20% FCW (CP 15%); RS, Top control ration without the use of FCW (CP 18%); FCW, Fermented Catfish Waste.

Table 2 Nutrient Content of Therapeutic Rations

Rations	Nutrient (%)								
	CP	EE	CF	Ca	P	Lys.	Meth.	Sistin	ME
R0	15.07	5.19	4.20	1.09	0.53	1.10	0.49	0.30	2.750
R1	15.09	5.27	3.92	1.25	0.90	1.04	0.47	0.29	2.763
R2	15.00	5.39	3.83	1.37	1.18	0.94	0.44	0.29	2.748
R3	15.06	5.50	3.67	1.59	1.45	0.88	0.42	0.29	2.742
R4	15.09	5.59	3.43	1.95	1.82	0.81	0.41	0.29	2.746
RS	18.07	5.61	3.96	1.26	0.62	1.37	0.54	0.33	2.751

2.4. Super Nativ Chicken

A total of 24 super native chickens are kept in individual cages and fed two times a day, 50 grams in the morning and 50 grams in the afternoon. The chickens were given a treatment ration for 14 days, then cut and taken from the intestines to take a sample of the digest of the ileum for testing the intestinal microflora of super native chickens.

2.5. Sampling

Chicken intestines were taken, samples were taken in the small intestine of the ileum, and then the digested sample was removed. The digested samples were analyzed for total microbes, the number of *E. coli* bacteria, and the number of *S. aureus bacteria* at the Laboratory of Poultry and Non-Ruminant Livestock Nutrition and Animal Food Industry and the Test and Research Laboratory of the Faculty of Animal Husbandry, Padjadjaran University.

2.6. Experimental Design

The research was carried out by experimental method. The experimental design was a completely random design with six ration treatments repeated four times each. The data obtained was analyzed using the Fingerprint Test method. To find out the difference between treatments, the Duncan Multiple Distance Test was carried out.

3. Results and Discussion

3.1. Effect of Treatment on Total Intestinal Bacteria

The results of the study regarding the effect of treatment on total ileum bacteria are presented in Table 3.

The results of the variety analysis showed that the treatment did not have a natural effect (P>0.05) on the total bacteria in the ileum. Based on Table 3, it can be seen that the total bacteria in the ileum are the lowest, namely R4 treatment, which is 1.37 x 108, and the highest, which is R0, which is 2.30 x 108. This shows that the use of FCW can reduce the total bacterial ileum so that there is a change in the microflora ecosystem in the ileum. This ecosystem change is caused by FCW being a substrate for the growth of LBS microbes (*L. paracasei, B. subtilis, S. Cerevisiae*), which can act as probiotics and can also be utilized by endogenous bacteria in the intestines [19]. Fermented feed can act as a probiotic that can have health effects on the digestion and health of poultry [20]. The balance of microflora in the intestines affects the health and function of the digestive tract [21].

Table 3 Total Number of Bacteria in the Ileum of Super Nativ Chicken Per Treatment

Deuteronomy	Total Gut Bacteria					
	R0	R1	R2	R3	R4	RS
	……………………………10^8 CFU/g……………………………					
1	2.50	2.00	2.70	1.30	1.10	1.60
2	1.40	2.40	2.50	2.60	1.70	1.00
3	2.80	2.30	2.00	0.90	2.20	1.30
4	2.50	2.00	1.30	1.80	0.50	2.50
Average	2.30 ± 0.61	2.17± 0.20	2.12 ± 0.62	1.65± 0.73	1.37 ± 0.73	1.60 ± 0.64

Description: R0, Bottom control ration without the use of FCW (CP 15%); R1, Ration contains FCW 5% (CP 15%); R2, Rations contain 10% FCW (CP 15%); R3, Rations contain FCW 15% (CP 15%); R4, Rations contain 20% FCW (CP 15%); RS, Top-control rations without the use of FCW (CP 18%).

Table 3 shows that the highest total bacteria in the ileum is in the R0 treatment, which is 2.30 x 108 cfu/g. After the addition of FCW of 5 – 20%, it can reduce the total bacteria so that there is a shift of microflora in the ileum with the lowest value in rations containing 20% FCW (R4), which is 1.37 x 108 cfu/g). The study showed that adding fish waste silage in the ration with an increase in the dose of each treatment could reduce the total bacteria in the ileum of broiler chickens. The total decrease in ileum bacteria that caused the use of FCW did not have a natural effect (P>0.05) and was thought to be due to competition from non-pathogenic and pathogenic microflora in the gut. This happens because FCW can act as a substrate where intestinal microflora, both pathogenic and non-pathogenic, grows so that there is a shift in the microflora ecosystem in the ileum. The change in the ileum microflora ecosystem in this study is suspected to be due to the dominance of beneficial microflora such as LAB. *L. bacteria* can improve the morphological conditions and intestinal microflora [22]. LAB can grow and be carried away by FCW or other materials. LAB requires proteins to grow and is a proteolytic bacterium that produces proteolytic enzymes around the cell wall, cytoplasmic membrane, and inside the cell [23].

The results of this study show that the addition of FCW in the ration can decrease the intestinal microflora in the ileum. The total bacteria counted are the total of beneficial and detrimental bacteria. This means that rations with the addition of FCW can change the microflora ecosystem in the ileum of super native chickens but do not have a noticeable effect that is expected due to a shift in the number of microflora in the ileum, both pathogenic and non-pathogenic. The change is due to the addition of FCW, which can work as a probiotic or prebiotic in the ration, which has a role in maintaining the balance of intestinal microflora.

3.2. Effect of Treatment on the Number of *Escherichia coli* Bacteria

The results of the study on the effect of treatment on the number of *E. coli bacteria* are presented in Table 4.

The results of the variety analysis showed that the treatment did not have a natural effect (P>0.05) on the number of *E. coli bacteria*. Based on Table 4, it can be seen that the lowest number of *E. coli* bacteria is the R4 treatment, which is 3.82 x 102 cfu/g, and the highest is 5.1 x 102 cfu/g. This shows that adding FCW to the ration can reduce the amount of *E. coli* in the ileum intestine. This change is due to the high characteristics of FCW LAB and lactic acid will cause a decrease in intestinal pH and balance the microflora of the gastrointestinal tract so that *E. coli* is challenging to grow. *E. coli* bacteria cannot survive in environments with low pH or acidic atmospheres and cannot withstand extreme temperature changes and osmotic pressure [24].

Table 4 shows that the highest number of *E. Coli* bacteria in the ileum is in the RS treatment, which is 5.1 x 102 cfu/g, and in rations with an addition of FCW of 5-20%, showing a decrease in *E. Coli* bacteria with the lowest value in rations containing 20% FCW (R4), which is 3.82 x 102 cfu/g. These results are not much different when compared to a study by [10], which showed that adding fish waste silage in the ration with an increase in the dose of each treatment can reduce *E. Coli bacteria* in the ileum of broiler chickens. The decrease in *E. Coli bacteria* that caused the use of FCW did not have a natural effect (P>0.05) and was suspected to be due to competition from non-pathogenic and pathogenic microflora in the intestines. The decrease in *E. Coli bacteria* in the ileum in this study is allegedly due to the increased activity of beneficial microflora such as lactic acid (LAB). According to [25], LAB will lower the pH of the intestine so that it inhibits the growth of pathogenic bacteria such as coliform in the ileum. LAB produces *short-chain fatty acids* that can reduce intestinal pH and produce antimicrobials, namely bacteriocins, that can inhibit the growth of gram-negative and gram-positive bacteria [26]. This is because FCW contains LAB, which produces lactic acid, which will lower the pH of the digestive tract and also produce bacteriocins that can inhibit the growth of pathogenic bacteria such as *E. Coli.* Bacteriocins have antibacterial activity that is sensitive to gram-positive bacteria compared to gram-negative bacteria. The difference in sensitivity is due to gram-negative bacteria such as *E. Coli* having an outer membrane that acts as a protector so that it is more difficult for bacteriocins to penetrate [27].

Table 4 Number of *Escherichia coli Bacteria* in the Ileum of Super Nativ Chicken Per Treatment

Deuteronomy	Number of Bacteria *E.coli*					
	R0	R1	R2	R3	R4	RS
	....10^2 CFU/g..					
1	2.80	5.30	4.60	4.00	1.00	2.80
2	6.00	4.50	5.00	4.00	6.70	8.00
3	8.00	3.60	5.00	4.00	1.20	6.60
4	3.00	3.80	2.40	4.10	6.40	3.00
Average	4.95 ± 2.50	4.30 ± 0.77	4.25 ± 1.24	4.02 ± 0.05	3.82 ± 3.15	5.1 ± 2.60

The results of the study showed that the addition of FCW in the ration could reduce the number of *E. coli* in the ileum. Statistically, rations with R4 treatment produced the least amount of *E. Coli,* while R0 and RS treatment produced the highest amount of *E. coli*. This means that rations with the addition of FCW can lower the amount of *E. Coli* and alter the balance of the gut microflora of super native chickens but have no noticeable effect. *E. coli* has an outer cell membrane that is difficult for bacteriocins to penetrate. Hence, the shift in the beneficial microflora ecosystem to the number of *E. coli* has no significant effect. However, adding FCW can still reduce the number of *E. coli* in the ileum of super-native chickens.

3.3. Effect of Treatment on Staphylococcus aureus Bacteria Count

The results of the study's effect of treatment on the number of *Staphylococcus aureus bacteria* are presented in Table 5.

Table 5 Number of *Staphylococcus aureus Bacteria* in the Ileum of Super Nativ Chicken Per Treatment

Deuteronomy	Number of Bacteria: *S.aureus*					
	R0	R1	R2	R3	R4	RS
	....10^2 CFU/g..					
1	67.00	30.00	37.00	82.00	68.00	123.00
2	21.00	30.00	42.00	90.00	120.00	43.00
3	5.10	31.00	97.00	62.00	118.00	108.00
4	42.00	60.00	92.00	110.00	76.00	58.00
Average	33.77 ± 26.81^{a}	37.75 ± 14.84^{ab}	67.00 ± 31.88^{bc}	86.00 ± 19.86^{bc}	95.50 ± 27.34^{c}	83.00 ± 38.51^{bc}

The results of the Duncan multiple distance test showed that the number of *S. aureus bacteria* affected the addition of FCW in the ration by 10% to 20% (R2, R3, R4) and the upper control ration with a CP of 18% (RS) was significantly higher (P<0.05) compared to the lower control ration (R0). The increase in FCW of 5% (R1) was significantly lower (P<0.05) than the addition of FCW of 20% (R4), but there was no significant difference between the addition of FCW of 5%, 10%, and 15% (P>0.05). This shows that an addition of 10% to 20% FCW can increase the number of *S. aureus bacteria* and the highest number is achieved at the addition of FCW of 20%, which is 95.50 x 102. This suggests that adding FCW to the ration increases the number of *S. aureus bacteria*. This can be because *S. aureus* has resistance at pH 4-10 and optimum at pH 6-7, in addition to being able to survive at high temperatures and resistant to human proteolytic enzymes and can survive after entering the digestive tract [28]. According to [29], The growth of intestinal microbes can also be caused by feed and feeding factors and is also supported by the opinion of [30] that type, age, feed and geographical location affect intestinal microbial populations. This suggests that *S. aureus* colonies can survive and thrive on FCW substrates. According to [31], the growth of *S. aureus* in anchovy meal with higher concentrations produces more pronounced *growth of S. aureus* caused by fishmeal, which has high protein and contains essential amino acids such as isoleucine, lysine, and valine can be used by bacteria as a source of nutrients to grow, similar to FCW which is high in protein and amino acids that can support the growth of *S. aureus*.

Table 5 shows that the highest number of *S. aureus bacteria* was in the R4 treatment with the addition of 20% FCW, which was 95.50 x 102 cfu/g, and the lowest was in the R0 treatment without the addition of FCW, which was 33.77 x 102 cfu/g. This is suspected because FCW contains bacteriocins produced by*. paracasei* is weak in reducing the number of *S. aureus bacteria* in the ileum. According to [32], *L. Casei* has weak inhibitory activity against *S. aureus,* while according to [33], *L. Plantarum* has more potent inhibitory activity against *S. aureus.* This suggests that different types and strains of bacteria have different antibacterial activity. This is supported by the opinion of [34]) that the difference in antibacterial activity can be due to differences in lactic acid bacterial isolates and bacterial strains.

This means that rations with the addition of FCW can increase the amount of *S. aureus* in the ileum of super native chickens. The increase in the number of intestinal *S. aureus* can be caused by feed, namely FCW and the effect of bacteriocin produced by *L. paracasei,* which is weak on the activity of *S. aureus* and is suspected to be due to hydrolysis of protease enzymes so that bacteriocins lose their function in suppressing *S. aureus.* The addition of FCW in the ration is still practical at a dose of 5% in the R1 treatment with the same result as the ration without the addition of FCW (R0).

4. Conclusion

Fermentation of catfish waste products by LBS microbes (*Lactobacillus paracasei, Bacillus subtilis, Saccharomyces cerevisiae*) can change the intestinal microflora ecosystem of super native chickens. The rate of use of fermentation catfish waste (FCW) products by LBS microbes of as much as 5% resulted in the lowest number of *Escherichia coli* and *Staphylococcus aureus* bacteria compared to no addition of FCW to the ration of super native chickens.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest is to be disclosed

References

[1] Suryaningrum, T. D. 2008. Catfish: Export Opportunities, Post-Harvest Handling, and Diversification of Processed Products. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 3(1): 16-23.

[2] Indriani, O., Dwi., Syamsudin, Shriherfyna, A. Wardani. 2015. Invertase of Aspergillus niger With Solid State Fermentation Method And The Application in Industry: A Review. Jurnal Pangan dan Agroindustri Vol. 3 No. 4 p.1405-1411.

[3] Ghaly, A. E., V. V. Ramakrishnan, M. S. Brooks, S. M. Budge, D. Dave. 2013. Fish Processing Waste as a Potential Source of Proteins, Amino Acid and Oils: A critical Review. Journal of Microbial and Biochemical Technology, 5(4): 107-129.

[4] Ogunbanwo, S.T., A.L. Sanni, A.A. Onilude. 2003, Characterization of Bacteriocin Produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OGI. Department of Botany and microbiology University of Ibadan, Nigeria African Journal. Academic Journals, ISSN 1684- 5315. https.//www.academicjournals.org/AJBnI

[5] Li, S., Z. Jin, D. Hu, W. Yang, Y. Yan, X. Nie, J. Lin, Q. Zhang, D. Gai, Y. Ji., X. Chen. 2020. Effect of Solid-State Fermentation with *Lactobacillus casei* on The Nutritional Value, Isoflavones, Phenolic Acids and Antioxidant Activity of Whole Soybean Flour. LWT - Food Science and Technology, 125: 1-8.

[6] Yin, H., F. Jia, and J. Huang. 2019. 'The Variation Of Two Extracellular Enzymes And Soybean Meal Bitterness During Solid-State Fermentation Of *Bacillus Subtilis'*. Grain & Oil Science And Technology. Elsevier B.V., 2(2), Pp. 39–43. Doi: 10.1016/J.Gaost.2019.05.001

[7] Mok, W. K. 2019. A Metabolomic Approach To Understand The Solid - State Fermentation Of Okara Using *Bacillus Subtilis* WX - 17 For Enhanced Nutritional Profile'. AMB Express. Springer Berlin Heidelberg.

[8] Bencardino, D., G. Amagliani., G. Brandi. 2021. Carriage of *Staphylococcus aureus* among food handlers: An ongoing challenge in public health. Food Control,130,108362.

[9] Nurhayati., Berliana., Newilda. 2019. Protein efficiency of boriler chicken fed fermented waste tofu with *Saccharomyces cerevisiae*). Journal of Animal Science Vol. 22 :95-106. eISSN:2528 0805

[10] Shabbani, A., F. Boldaji, B. Dastar, T. Ghoorchi, S. Zerehdaran, A. Ashayerizadeh. 2021. Evaluation of increasing concentration of fist waste silage in diets on growth performance, gastrointestinal microbial population, and intestinal morphology of broiler chickens. Journal Animal Feed Science and Technology. 275:114874

[11] Gibson, G. R., Hutkins, Sanders, M. E., Prescott, S. L., Reimer, R. A., Salminen, S. J., Reid, G. 2017. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology &Hepatology,14(8),491–502. https://doi.org/10.1038/nrgastro.2017.75

[12] Macfarlane, G., H. Steed, S. Macfarlane. 2007. Bacterial metabolism and health-related effects of galactooligosaccharides and other prebiotics. Journal of Applied Microbiology, 104(2), 305–344. https://doi.org/10.1111/j.1365-2672.2007.03520.

[13] O'Sullivan, L., B. Murphy, P. McLoughlin, P. Duggan, P.G. Lawlor, H. Hughes, G.E Gardiner. 2010. Prebiotics from Marine Macroalgae for Human and Animal Health Applications. Marine Drugs, 8(7), 2038–2064. https://doi.org/10.3390/md8072038

[14] Cholis M. A, Suthama, Sukamto. 2018. Feeding microparticle protein diet combined with *Lactobacillus* sp. on existence of intestinal bacteria and growth of broiler chiken. Jurnal Indonesian Tropical Animal Agriculture 43: 265–271.

[15] Yang X, H. Wang. 2014. Pathogenic *E. coli*. Lacombe Research Centre. Lacombe. Canada.

[16] Hennekinne, J. A., M.L. De Buyser, S. Dragacci. 2012. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiology Reviews, 36(4), 815–836.

[17] Zhao, Y., A. Zhu, J. Tang, C. Tang, J. Chen. 2017. Identification and measurement of staphylococcal enterotoxin M from Staphylococcus aureus isolate associated with staphylococcal food poisoning. Letters in Applied Microbiology, 65(1), 27–34.

[18] Zainuddin, D. Masyitha, F. Fitriani, S. Muharrami, Wahyuni, Roslizawaty, M. Adam. 2015. Overview of Histology of the Twisted Glands of Nativ Chickens, Ducks, and Pigeons. Journal of Veterinary Medicine, 9(1): 68-70.

[19] Abdurrahman, Z. H., Y. B. Pramono, N. Suthama. 2016. Feeding effect of inulin derived from dahlia tuber combined with *Lactobacillus* sp. on meat protein mass of crossbred kampong chicken. Journal of the Indonesian Tropical Animal Agriculture, 41(1), 37–44.

[20] Ashayerizadeh, A., B. Dastar, M. S. Shargh, M. Sadeghi, S. Zerehdaran. 2017. Fermented rapeseed meal is effective in controlling Salmonella enteric serovar Typhimurium infection and improving growth performance in broiler chicks. Vet. Microbiol. 201, 93–102.

[21] Al-Fataftah, A., A. Abdelqader. 2014. Effects of dietary *Bacillus subtilis* on heat-stressed broilers performance, intestinal morphology and microflora composition. Anim. Feed Sci. Technol. 198, 279–285.

[22] Song, J. K. Y.L Xiao, Y.L. Ke, L.F. Jiao, C.H. Hu, Q.Y. Diao, B. Shi, X.T. Zou. 2014. Effevt of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Jurnal Poultry Sci. 93:581-588.

[23] Wikandari, P.R., Suparmo, Y. Marsono, E.S. Rahayu. 2012. Characterization of proteolytic lactic acid bacteria in containers. J. Natur Indonesia 14(2): 120- 125.

[24] Rahayu. E. S., E. Harmayani, T. Utami., K. Handini. 2004. *Pediococcus acidilactici* F-11 Bacteriocin Producers as Biocontrol Agents of *E. Coli* and *S. aureus* in Cold Storage Fresh Vegetables. Agritech 24 (3) : 113 – 124.

[25] Hartono, E.F., N. Iriyanti, S. Suhermiyati. 2016. Effect of Synbiotic Use on Microflora and Intestinal Histology of Male Sentul Chickens. Agripet Journal Vol. 16. No.2: 97-105. https://doi.org/10.17969/agripet.v16i2.5179

[26] Suganya, K., T. Murugan, M. Murugan. 2013. Isolation and characterization of probiotic lactic acid bacteria from milk and curd samples. Internasional Journal of Pharma and Bio Sciences, 4(1): (B) 317-324.

[27] Prudencio, C.V., M.T. Santos, M.C.D. Vanetti. 2015. Strategies for the use of bacteriocins in Gram-negative bacteria: relevance in food microbiology Journal of Food Science and Technology, 52(9): 5408-5417.

[28] Grispoldi, L., P.A. Popescu, M. Karama, V. Gullo, G. Poerio, E. Borgogni, P. Torlai, G. Chianese, A.G Fermani, P. Sechi. 2019. Study on the growth and enterotoxin production by Staphylococcus aureus in canned meat before retorting. Toxins, 11(5), 1–11.

[29] Prasetyo, H.F.D. 2019. Effect of Addition of Bidara Leaves (*Ziziphus mauritiana* Lamk) in Broiler Ration as a Substitute for Antibiotics on Intestinal Microbes. Thesis. Faculty of Animal Husbandry, University of Brawijaya. Hapless.

[30] Anadon, A., I. Ares, M.R. Martinez-Larranaga., M.A. Martinez. 2019. Prebiotics and Probiotics in Feed and Animal Health. Jurnal Veteriner Medicine. 261-285.

[31] Novitasari, T.M., Rohmi, N. Inayati. 2019. The Potential of Jengki Anchovies as an Alternative Media for the Growth of *Staphylococcus aureus* Bacteria. Journal of Medika Bio Science Analyst Vol.6 No. 1.

[32] Khikmah N. 2015. Antibacterial test of commercially fermented milk on pathogenic bacteria. Journal of Scientific Researcher 20(1): 45-52.

[33] Hariani L. 2013. Production of bacteriocin by *Lactobacillus plantarum* DJ3 and its application as a meat preservative. El-Hayah 4(1): 17-25.

[34] Sari R., P. Apridamayanti , M. Octaviani. 2018. Optimization of bacteriocin activity produced by Lactobacillus plantarum bacteria from cehun tiau drink. PharmSciRes5(1):1-6.