Effect of Addition of *Pediococcus pentosaceus* Strain N6 Isolate and Cassava Peel on Total Microbes and Gastrointestinal Organ Weight of Super Native Chicken

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Abstract. The use of commercial antibiotics causes residues in chicken meat. The study aims to determine the best concentration between Pediococcus pentosaceus Strain N6 isolate and cassava peel flour on the weight of the digestive tract organs of super native chickens The study consisted of 2 stages. Stage I. Determination of the best synbiotic concentration. using a completely randomized design (CRD) 4 treatments and 5 replicates. Treatments consisted of R0 = 1 ml isolate without the addition of synbiotics, R1 = 1 ml + 3% isolate (0.3 g cassava peel flour), R2 = 1 ml + 6% isolate, and R3 = 1 ml + 9% isolate. The parameter observed was a total microbial colony (TPC). Phase II: addition of synbiotics to super native chicken feed. using RAL 4 treatments and 5 replicates. Treatment P0 = basal ration without synbiotic, P1 = basal ration + synbiotic 150 ml/kg ration, P2 = basal ration + synbiotic 300 ml/kg ration, and P3 = basal ration + synbiotic 450 ml/kg ration. Parameters measured: TPC, weight of digestive tract organs: duodenum, jejunum, and ileum, test results, R3 produced TPC: 64 X 109 CFU/ml, decreased the weight of duodenum, jejunum and ileum (0.73%, 1.79% and 0.32%).

1 Introduction

The demand for native chicken continues to increase along with public awareness of healthy foods that are free from antibiotics. One of the poultry that has the potential to be developed is the super native chicken. Super native chicken is a cross between roosters and laying hens so that it grows faster than ordinary native chickens, has a low mortality rate, easily adapts to the environment, and easily adapts to livestock growth (growth promoter). The use of synthetic antibiotics can pose a health risk to consumers due to the presence of antibiotic residues in poultry meat. The use of antibiotic residues also results in bacterial resistance, so bacteria can contaminate pens, feed, and drinking water causing livestock to become infected

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with bacteria and difficult to cure. Very serious bacterial resistance can also occur in humans in the form of treatment failure for gastrointestinal infections caused by Campylobacter and Salmonella bacteria [1].

One alternative to antibiotics that can be developed is to use a combination of synbiotics between prebiotics and probiotics to increase chicken productivity. One of the synbiotics that can be used is *Pediococcus pentosaceus* Strain N6 isolate as a probiotic and cassava peel as a prebiotic. This isolate was isolated from the hot springs of Rimbo Panti, West Sumatra. This isolate has high antimicrobial activity against pathogenic bacteria (Listeria monocytogenes, Salmonella Typhimurium, and *E.Coli O157:H7),* can grow at an optimum pH of 3-7, and grows at an optimum temperature of 50 ^{0}C [2].

Cassava peel is an agricultural waste that continues to increase in number along with the community's need for cassava as a food ingredient. Cassava peels contain oligosaccharides that can be used as a medium for microbial growth so that it functions as a prebiotic. Synbiotics as antibiotics function to improve digestive conditions and increase nutrient absorption. The quality of synbiotics is determined by the concentration of microbes used as probiotics and the amount of fiber or prebiotics used as probiotic feed. The best synbiotics are those that produce more microbial growth.[3], stated that this isolate produces bacteriocin Pediosin N6 whose production is optimal if grown on a medium containing a carbon source in the form of glucose. So adding cassava peel containing oligosaccharides will increase the growth of isolates.

The combination of the two additives can work together to improve digestive conditions and result in more optimal nutrient absorption so that it can affect the growth and productivity of super-native chickens. Optimal feed absorption will affect the weight of the digestive tract organs. The weight of the digestive tract organs will decrease if the absorption of feed is getting better.[4], stated that the relative weight of the digestive tract is influenced by the quality of ration protein content, digestibility, feed form, and chicken age.

The purpose of this study was to determine the best concentration between *Pediococcus pentosaceus* Strain N6 isolate and cassava peel flour as a synbiotic added to feed on the weight of the digestive tract organs of super-native chickens.

2 Materials and Methods

2.1 Research Materials

2.1.1 Preparation of the isolate starter Pediococcus pentosaceus strain N6

Pediococcus pentosaceus Strain N6 isolates were grown on MRSA and then incubated for 24 hours at 37 0C. After that, 1 ose isolate was taken and grown in 9 ml of MRS broth medium then incubated at 45-50 0C for 24 hours. Liquid culture of *Pediococcus pentosaceus* Strain N6 isolate is ready to be used as a probiotic [3]

2.1.2 Cassava peel processing

Modified method [5], cassava peels are obtained from opak factory waste and tape making. The outer cassava skin is separated from the inner cassava skin, then the inner part (epidermis) is taken. After that, it was boiled for 1 hour, then the cassava skin was soaked for 3x24 hours in water added with salt (NaCl) with a concentration of 5%. After that, the cassava skin is dried in the sun. After that, it is ground into flour and sieved with a sieve. Cassava peel flour was then autoclaved at 121^{o}C for 15 minutes—fermentation of goat milk with a

single starter isolates of *Pediococcus pentosaceus* strain N6. Fresh goat milk has to be filtered, then pasteurized or heated to a temperature of 70-80^{o}C for 5 to 10 minutes wait until it cooled. After the temperature of goat's milk decreased to around 40-45^{0}C, a yogurt starter was added according to the treatment dose and then incubated according to the treatment. After, the resulting yogurt is tested for microbiological quality and fatty acids of goat milk yogurt.

2.2 Research Methods

2.2.1 Stage 1. Determination of the best concentration between Pediococcus pentosaceus Strain N6 isolates and cassava peel flour (feed additive)

Tests using Completely Randomised Design (CRD) 4 treatments 5 replicates. The treatment consisted of R0 = 1 ml isolate without the addition of cassava peel flour, R1 = isolate 1 ml + 3% (0.3 gr cassava peel flour), R2 = isolate 1 ml + 6% (0.6 gr cassava peel flour), R3 = isolate 1 ml + 9% (0.9 gr cassava peel flour).

2.2.2 Stage 2. Use of synbiotics as supplementary feed in super native chickens.

The method used 100 super chickens aged 1 day (DOC). The study lasted for 10 weeks, using a completely randomized design (CRD) with 4 treatments and 5 replicates, each plot filled with 5 chickens. Treatments consisted of: T0= No feed additive (Control) or basal ration, T1= Basal ration + feed additive 150 ml/kg ration, T2= Basal ration + feed additive 300 ml /kg ration and T3 = Basal ration + feed additive 450 ml /kg ration

2.3 Research variable

2.3.1 Best Total Plate Count (TPC) combination of cassava peel and isolates.

Each treatment sample was taken as much as 1 gram, then diluted in physiological saline solution as much as 9 ml as dilution 10-1 and so on until dilution 10-6. In this dilution, 0.1 ml was taken and grown in MRS Agar medium and incubated for 24 hours at 36 0C. The number of colonies of microbes grown was calculated by the formula: CFU/ml = number of colonies x 1/factor dilution x 1/many samples [6].

2.3.2 Organ weights of the digestive tract of super native chickens.

In the modified method of [7], the percentage weight of the digestive tract organs of chickens consisting of duodenum, jejenum, and ileum was obtained by weighing the weight using analytical scales of each sample experimental unit divided by the live weight of the chicken multiplied by 100%.

2.4 Data analysis

The data were evaluated using Analysis of Variance (ANOVA), if there was a significant difference between treatments, further tested with Duncan's test. Data processing using SPSS 20 software, at 5% level ($p<0.05$).

3 Results and Discussion

3.1 Total Plate Count (TPC)

The average results of Total Plate Count (TPC) in testing the concentration between Cassava Peel Flour and *Pediococcus pentosaceus* Strain N6 isolates as synbiotics can be seen in Table 1. below:

Table 1. Average Total Plate Count (TPC) concentration between cassava peel flour and *Pediococcus pentosaceus* strain N6 isolate

Treatments	Replications					Total	Average ($x10^8$ CFU/ml)
	1	2	3	4	5		
R0 (Control)	1.3	2.4	1.8	2.5	1.9	9.9	1.98^a
R1 (3%)	2	3.2	3	2.2	2.6	13	2.6^a
R2 (6%)	3.1	2.8	3.5	3.1	8.3	20.8	4.16^a
R3 (9%)	11.2	6	5.4	6.7	8.9	38.2	7.64^b

Note: Numbers with superscripts in the same row and column show no significant difference (P>0.05). Meanwhile, numbers with different superscripts show a very significant effect (P<0.05).

The results of the Analysis of Variance (ANOVA) tested with SPSS), showed a significant effect ($P < 0.05$) between the addition of cassava peel flour and isolate. The results of Duncan's further test (Table 1), showed that the R0 treatment was not significantly different ($P>0.05$) with R1 and R2, but R3 showed a significantly different effect ($P < 0.5$) with other treatments. The average results of Total Microbial Colony (TPC) from a dilution of 10^7 in each treatment consisted of: T0 was 1.98 X 10^8 CFU/ml, T1 was 2.6 X 10^8 CFU/ml, T2 was 4.16 X 10^8 CFU/ml and T3 was 7, 64 X 10^9 CFU/ml.

The R3 treatment had a significant effect on increasing Total Microbial Colony (TPC) compared to the control, T1 and R2 treatments because the cassava peel flour added was more and more, namely 9 gr where this cassava peel flour in the fermentation process served as a source of nutrients for the growth of *Pediococcus pentosaceus* Strain N6 isolates besides that in the poultry digestive process it is hoped that this prebiotic will help the food digestion process. This is according to [8], prebiotics are a source of energy for microbes to improve microbial balance in the digestive tract.

The results of this study are higher than the research of [9], who tested the addition of cassava peel flour as much as 6 grams and lactic acid bacteria by producing the highest total microbial colonies, namely 6.5×10^9 CFU / ml, this is because in this study using more cassava peel flour, namely 9 gram.

3.2 Duodenum Weight

The average weight percentage of the duodenum digestive tract of native chickens during the research can be seen in Table 2. The highest average duodenal weight during the study was found in the T0 treatment (basal ration without synbiotics) which was 0.91% and the lowest average was found in P3 (basal ration + synbiotics 450 ml/kg ration) which was 0.73%. Increasing the dose of synbiotics in the ratio increases the population of isolates that have high antimicrobial activity to inhibit the growth of pathogenic bacteria in the digestive tract and increase the work of the duodenum organ in absorbing feed. The results of the study are by the research of [10], which obtained duodenum weight ranging from 0.77-1.03%.

Table 2. Average duodenal weight (%)

Treatments	Replications					Total	Average
	1	2	3	4	5		
R0 (Control)	0.82	0.97	1.16	0.8	0.81	4.56	$0.912^{a} \pm 95{,}49$
R1 (3%)	0.81	0.74	0.92	0.97	0.78	4.22	$0.844^{a} \pm 91{,}86$
R2 (6%)	0.75	0.78	0.74	0.78	0.81	3.86	$0.772^{ab} \pm 87{,}86$
R3 (9%)	0.78	0.7	0.75	0.77	0.66	3.66	$0.732^{b} \pm 85{,}55$

Note: Numbers with superscripts in the same row and column show no significant difference (P>0.05). Meanwhile, numbers with different superscripts show a very significant effect (P<0.05).

The results of the Analysis of Variance (ANOVA) tested with SPSS showed a significantly different effect (P <.0.05) between the synbiotic treatment of *Pediococcus pentosaceus* Strain N6 isolate and cassava peel on the duodenum weight of super native chickens. The results of Duncan's further test (Table 2), showed that the treatment of T3 = basal ration + feed additive (450 ml /kg ration) was significantly different (P <.0.05) from the treatment of T0 (Control) and T1 = basal ration + feed additive (150 ml /kg ration). Treatment (T3) was not significantly different (P>.0.05) with T2 = basal ration + feed additive (300 ml /kg ration).

The treatment of T3 and T2 showed a significant effect on reducing duodenal weight because Lactic Acid Bacteria (LAB) functions to suppress the growth of pathogenic bacteria to increase the absorption of food substances according to [11], state that LAB functions to regulate microbial composition by suppressing pathogenic microbes in the digestive tract, reducing duodenal weight to increase digestibility and absorption of nutrients. Furthermore, feeding with a high crude fiber content tends to have a larger organ because it is influenced by the quality of the feed so it has an impact on duodenal digestibility. Furthermore, according to [12], feeding with high crude fiber tends to have larger organs, due to the increased amount of feed and has an impact on digestibility and absorption, thus affecting the physiological condition of the digestive organs.

3.3 Jejunum Weight

The average weight percentage of the yeyenum digestive tract of native chickens during the research can be seen in Table 3. below:

Table 3. Average yeyenum weight (%)

Treatments	Replications					Total	Average
	1	2	3	4	5		
R0 (Control)	2.95	2.27	2.36	2.92	1.67	12.17	$2.434^{a} \pm 1.56$
R1 (3%)	2.08	2.27	1.81	2.08	1.98	10.22	$2.044^{a} \pm 1.42$
R2 (6%)	2.19	2.15	2.08	1.69	1.73	9.84	$1.968^{ab} \pm 1.40$
R3 (9%)	2.03	1.73	1.78	1.79	1.64	8.97	$1.794^{b} \pm 1.33$

Note: Numbers with superscripts in the same row and column show no significant difference (P>0.05). Meanwhile, numbers with different superscripts show a very significant effect (P<0.05).

The highest average weight of jejunum was found in treatment T0 (basal ration without synbiotics) which was 2.43% and the lowest average weight of jejunum was found in T3 (basal ration + 450 ml/kg ration synbiotics) which was 1.79%. The use of synbiotics can increase the number of Lactic Acid Bacteria (LAB) in the jejunum and increase the ability to inhibit the growth of pathogenic bacteria in the digestive tract so as to increase the absorption

of feed on the jejunum wall. According to [10], acidic conditions cause the growth of *Escherichia coli* to be inhibited so that the host is protected from pathogenic bacteria.

The results of the Analysis of Variance (ANOVA) tested with SPSS showed a significantly different effect (P <.0.05) between the synbiotic treatment of *Pediococcus pentosaceus* Strain N6 isolate and cassava peel on the yeyenum weight of super native chickens. The results of Duncan's further test (Table 2), showed that the treatment of T3 = basal ration + feed additive (450 ml /kg ration) was significantly different (P <.0.05) from the treatment of T0 (Control) and T1 = basal ration + feed additive (150 ml /kg ration). Treatment (T3) was not significantly different (P>.0.05) with T2 = basal ration + feed additive (300 ml /kg ration).

The treatment of T3 and T2 showed a significant effect on reducing the weight of the jejenum because the increase in the dose of synbiotics caused an increase in the population of LAB isolates which would increase the absorption of nutrients and reduce the weight of the jejenum. This is to the opinion of [11], that LAB serves to regulate microbial composition by suppressing pathogenic microbes in the digestive tract to increase the digestibility and absorption of nutrients.

3.4 Ileum Weight

The average weight percentage of the duodenum digestive tract of native chickens during the research can be seen in Table 4. Below:

Table 4. Average ileum weight (%)

Treatments	Replications					Total	Average
	1	2	3	4	5		
R0 (Control)	0.59	0.37	0.83	0.83	0.33	2.95	$0.59^{b} \pm 776.81$
R1 (3%)	0.28	0.35	0.6	0.47	0.46	2.16	$0.43^{ab} \pm 765.72$
R2 (6%)	0.53	0.38	0.45	0.37	0.39	2.12	$0.42^{b} \pm 761.11$
R3 (9%)	0.41	0.22	0.39	0.3	0.29	1.61	$0.32^{a} \pm 756.74$

Note: Numbers with superscripts in the same row and column show no significant difference (P>0.05). Meanwhile, numbers with different superscripts show a very significant effect (P<0.05).

The highest average ileal weight during the study was found in the T0 treatment (basal ration without synbiotics) which was 0.59% and the lowest average ileal weight was found in T3 (basal ration + 450 ml/kg ration synbiotics) which was 0.32%. This is different from the results of research by [13], which stated that the relative weight of the ileum ranged from 0.63 - 0.89%. The decrease in the weight of the ileum is because synbiotics play a role in the dilation of intestinal villi in the ileum. This is to the opinion of [14], that the addition of natural synbiotics to the ratio of male Sentul chickens can increase the number of lactic acid bacteria in the duodenum, jejunum, and ileum, reduce the number of escherichia colli in the ileum and elevate and dilate the intestinal villi in the ileum.

The results of the Analysis of Variance (ANOVA) tested with SPSS showed a significantly different effect (P <.0.05) between the synbiotic treatment of *Pediococcus pentosaceus* Strain N6 isolate and cassava peel on the ileum weight of super native chickens. The results of Duncan's further test (Table 4), showed that the treatment of T3 = basal ration + feed additive (450 ml /kg ration) was significantly different (P <.0.05) from the treatment of T0 (Control). Treatment (T3) was not significantly different (P>.0.05) with T1 = basal

ration + feed additive (150 ml /kg ration) and T2 = basal ration + feed additive (300 ml /kg ration).

The T3 treatment showed a significant effect on reducing ileal weight because the addition of synbiotics caused an increase in the population of LAB isolates in the ileum to help the absorption of food substances. This increased absorption activity causes a decrease in ileal weight. According to [15], the ileum is a functioning part of the process of nutrient absorption because the absorption of the largest nutrients occurs in the ileum, the ileum has a role in absorbing nutrients such as amino acids, vitamins, and monosaccharides. Furthermore, [16] states that the main role of the ileum is as a place of absorption of water and minerals although some absorption of advanced nutrients still occurs here.

4 Conclusion

The research resulted in the best concentration between *Pediococcus pentosaceus* Strain N6 isolate and cassava peel flour in the R3 treatment with a total microbial colony of 7.64 x 10^9 CFU/ml. The addition of synbiotics to super native chicken feed has a real effect on reducing the weight of the duodenum, yayenum and ileum with a value of 0.73%, 1.79% and 0.32% respectively.

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References

1. Casburn-Jones, A. C., & Farthing, M. J. G. (2004). Management of infectious diarrhoea. Gut, 53(2) :296–305.
2. Yah N, Marlida Y, Arnim and Yuherman. (2014). Antimicrobial activity of lactic acid bacteria thermophilic isolated from hot spring rimbo panti of West Sumatera for food preservatives. Pak. J. Nutri. 13(8): 465-72.
3. Ketaren N Y B., Y. Marlida, Arnim and Yuherman. (2018), Optimization of Pediocin N6 Production by Isolate *Pediococcus Pentosaceus* Strain N6 With Modification of Carbon and Nitrogen Sources. Pakistan Journal of Biotechnology. 15 (4): 901-908
4. Naufa, A. M, H. I. Wahyuni & I. Mangisah. (2013). Bobot relatif organ pencernaan ayam kedu petelur diberi ransum dengan berbagai level protein. Jurnal Veteriner. 8 (4): 1-13.
5. Wahyuni, S., Agustina, A. S., Natsir, H., & Raya, I. (2019). The prebiotic production by using cassava peels with the addition of both K+ and Mg2+ metal ions as an activator and their potential to enhance broiler quality. Journal of Physics: Conference Series, 1341(3).
6. Harley, J. P and Prescott, L. M. 1993, Laboratory Exercises In Microbiology Second Edition, Eastern Kentucky University Melborne. Australia.
7. Kieronczyk, B., Sassek, M., Oszmalek, E. ., Kolodziejski, P., Rawski, M., Swiatkiewicz, S., & Józefiak, D. (2017). The physiological response of broiler chickens to the dietary supplementation of the bacteriocin nisin and ionophore coccidiostats. Poultry Science, 96(11) :4026–4037.
8. Haryati, T. 2011. Probiotik dan Prebiotik sebagai pakan imbuhan nonruminansia. Wartazoa 21(3) : 125-132.
9. Purwinarto, Suprijatna, E., & Kismiati, S. (2020). Pengaruh Penambahan Kulit Singkong dan Bakteri Asam Laktat sebagai Aditif Pakan terhadap Profil Saluran Pencernaan Ayam Pedaging. Jurnal Peternakan Indonesia (Indonesian Journal of Animal Science),

22(1):101.
10. Badrussalam, A., Isroli, & T. Yudiarti. 2020. Pengaruh Penggunaan Aditif Kunyi terhadap Bobot Relatif Organ Pencernaan Ayam Kampung Super. Jurnal Sain Peternakan Indonesia, 15(3): 273-279.
11. Sarwono, S.R., T. Yudiarti & E. Suprijatna. (2013). Pengaruh Pemberian Probiotik Terhadap Trigliserida Darah, Lemak Abdominal, Bobot dan Panjang Saluran Pencernaan Ayam Kampung. Animal Agriculture Journal, Vol. 1. No.2: 157-167.
12. Iskandar, S. 2004. Pertumbuhan dan perkembangan alat pencernaan ayam anak Silangan Pelung xKampung terhadap kandungan protein ransum. JITV. 9 (4): 217-225.
13. Pertiwi, D.D.R., R. Murwani & T. Yudiarti. 2017. Bobot relatif saluran pencernaan ayam broiler yang diberi air rebusan kunyit dalam air minum. JPI 19(2): 60 – 64.
14. Hartono, E.F., N. Iriyanti, & S. Suhermiyati. (2016). Efek penggunaan sinbiotik terhadap kondisi miklofora dan histologi usus ayam sentul jantan. Jurnal Agripet 16(2) : 97-105.
15. Siagian Y. A. 2016. Gambaran Histologis Dan Tinggi Vili Usus Halus Bagian Ileum Ayam Ras Pedaging Yang Di Beri Tepung Daun Kelor (Moringa Oleifera) Dalam Ransum. Universitas Hasanuddin. Makasar
16. Shivus, B. (2014). Function of the digestive system. J. Appl. Poult. Res. 23: 306 – 314.