Effects of Probiotic, Prebiotic, and Synbiotic Mixed Culture Based on Wheat Pollard on Productivity of Kampung's Chicken

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ABSTRAK

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Penelitian bertujuan menguji efektivitas ransum berbasis *wheat pollard* terolah untuk meningkatkan produktivitas ayam kampung sampai umur 8 minggu. Penelitian menggunakan rancangan acak lengkap pola searah dengan lima perlakuan dan empat ulangan. Perlakuan terdiri dari ransum berbasis *wheat pollard* (WP), ransum berbasis *wheat pollard* plus probiotik *mixed culture* (WPPro), ransum berbasis *wheat pollard* prebiotik *mixed culture* (WPPre), ransum berbasis *wheat pollard* sinbiotik *mixed culture* 60% (WPS60). Parameter yang diamati adalah konsumsi ransum, bobot badan akhir, konversi pakan (FCR), pertambahan bobot badan, retensi nitrogen, *income over feed chick cost* (IOFCC) dan profil vili usus. Hasil penelitian memperlihatkan bahwa bobot badan akhir, pertambahan bobot badan, retensi nitrogen, IOFCC dan profil vili usus halus (duodenum, jejenum, ileum) nyata (P<0,05) dipengaruhi oleh perlakuan. Penambahan sinbiotik *mixed culture* yang dibuat dari *wheat pollard* sebanyak 40% (WPS 40) dalam ransum mampu meningkatkan produktivitas ayam kampung sampai umur 8 minggu.

Kata Kunci: Wheat Pollard, Ayam Kampung, Sinbiotik Mixed Culture

ABSTRACT

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This research was aimed to assess the effectiveness of processed wheat pollard -based rations to increase the productivity of Kampung chickens raised until 8 weeks old. The research was carried out in a completely randomized design with 5 treatments and 4 replications. The treatments consisted of wheat pollard based ration (WP), wheat pollard based ration plus probiotic mixed culture (WPPro), wheat Pollard as prebiotic mixed (WPPre), wheat pollard as synbiotic mixed culture ration 40% (WPS40), wheat pollard as synbiotic mixed culture ration 60% (WPS60). The parameters observed were feed consumption, final body weight, feed conversion ratio (FCR), weight gain, nitrogen retention, income over feed and chick cost (IOFCC) and profiles of intestinal villus. Results showed a significant effect of the treatments on the final body weight, weight gain, nitrogen retention, IOFCC and profiles of small intestinal villus (duodenum, jejenum, ileum). It was concluded that the inclusion of 40% wheat pollard synbiotic mixed culture (WPS 40) in the ration was able to increase the productivity of kampung chickens reared until 8 weeks old.

Key Words: Wheat Pollard, Kampung Chicken, Synbiotic Mixed Culture

INTRODUCTION

Kampung chicken is one of the native chickens of Indonesian. Various methods are used to improve the productivity of kampung chickens, one of which is the utilization of quality feed. The feed contained 20% protein and 2800 kcal/kg of metabolic energy produced the highest body weight and ration efficiency in kampung chicken (Permadi et al. 2020). Resnawati & Bintang (2014) stated that the ingesta in the crop of 6 weeks old kampung chicken could be used as a

reference to calculate the needs of fiber, fat, calcium, and phosphorus. In the feed formulation that consisted of local feed ingredients, the addition of functional feed to improve the performance of the chickens may be needed. The functional feed is a feed that contains active ingredients other than nutrients. One of them is a mixed culture of synbiotic feed. Synbiotic mixed culture is created from probiotic and prebiotic. The use of synbiotics is more efficient than a single use of probiotics or prebiotics separately (Gourbeyre et al. 2011).

Utama & Setiani (2014) stated that synbiotic is a fermented product derived from the enhancement in ability of probiotic bacteria due to the availability of specific substrates (prebiotics) for fermentation. The addition of synbiotic in rations was very effective in promoting growth, endurance, and beneficial microflora composition in poultry (Alloui et al. 2013; Mookiah et al. 2014). Hartono et al. (2016) stated that the addition of synbiotics influenced the condition of intestinal microflora, it increased the number of lactic acid bacteria, decreased the number of Escherichia colli, increased the height and width of villi. The addition of synbiotics could improve intestinal performance so that the absorption of nutrients is more optimal (Solis de los Santos et al. 2005). The addition of synbiotics to chickens rations could affect the histology of the duodenum caused by the enhancement of beneficial bacterial populations and stimulates vascularization and the development of villi. Synbiotics increased the thickness of the intestine, increased the nutrients absorbed, and reduced the metabolic needs of the digestive system (Iannitti & Palmieri 2010).

Intestinal histomorphology indicates the health status and productivity of livestock. Increase villi length, villi width, crypt depth, and crypt width in the intestine are indicated by livestock productivity and health status (Hidayat et al. 2016). The provision of multi-strain probiotics can improve immunity and control pathogenic bacteria in native chickens. Shortchain fatty acid (SCFA) produced by probiotics play a role in the multiplication of intestinal epithelial cells (Sugiharto et al. 2018). SCFA in particular, butyrate is a component of cell membrane phospholipids produced by Bifidobacteria and Lactobacilli through anaerobic fermentation processes in the intestine (den Besten et al. 2013). Intestinal villi profile strongly influenced the feed flow rate so it will affect the absorption of feed nutrients, epithelial, and enterocyte cell production (Perić et al. 2010; Abdel-Raheem et al. 2012; Gómez et al. 2012).

The innovation of this research was processing waste into additives that functioning as probiotics, prebiotics, and synbiotic mixed culture with fermented cabbage as the source of mixed culture probiotic and wheat pollard as a source of mixed prebiotics. Besides this research provides information on methods or ways of making additives that are easy and inexpensive through the application of fermentation technology, so it can be applied by farmers easily. The mixed cultures in this study were a combination of probiotic microorganisms from fermented cabbage (Lactobacillus brevis, Lactobacillus plantarum, Rhizopus oryzae and Saccharomyces cerevisiae) as well as a combination of food fiber such as arabinosa, mannosa, raffinosa, and starch resistant which are contained in wheat pollard processed as prebiotic. This study was done to assess the effectiveness of wheat pollard-based rations to improve the productivity of native chicken until the age of 8 weeks.

MATERIALS AND METHODS

The research material consisted of 200 day old chicks (DOC) of kampung chicken, where 160 were allocated for wheat pollard study, and the rest of 40 chicks were used for nitrogen retention study. The average initial weight of kampung chicken was 38.0 g. The DOCs were obtained from the Maron chicken Satker (Government Unit under the Regency of Livestock Services) of Temanggung Regency. The feed was formulated consisted of ground corn, wheat pollard, prebiotic mixed, mixed culture synbiotic, soybean meal, mixed culture probiotics from fermented cabbage, mineral-vitamin mix, L-lysin HCl and DL-methionine.

The chemicals used were HCl 0.2 N, formalin, neutral buffer 10% formalin (BNF), and disinfectant. The equipment used is fermenters, litter cages for raising native chickens equipped with feed and drinking containers, battery cages, brooders for chicken warmers, sprayers, controlled dryers, autoclaves, disc mills, digital scales with an accuracy of 0.1 g and microscopes.

Methods

The research was carried out in a completely randomized design with 5 treatments and 4 replications. Each unit of replication consisted of 8 DOC. The treatments were applied for 8 weeks with the combination as: WP (Wheat pollard based ration), WPPro (Wheat pollard based ration plus probiotic mixed culture), WPPre (Wheat Pollard as prebiotic mixed), WPS40 (Wheat pollard as synbiotic mixed culture ration 40%), WPS60 (Wheat pollard as synbiotic mixed culture ration 60%)

Research procedure

The research was started by producing additives (probiotic mixed culture, prebiotic mixed, and synbiotic mixed culture). All dietary treatments were adjusted to meet the nutrient requirements of the chickens.

Production of mixed prebiotics made from wheat pollard

It started by filtering wheat pollard with a 20 mash filter to separate wheat pollard from other materials. After that added water up to 45% water content. After mixing evenly, put wheat pollard into the autoclave and heated to 121°C for 15 minutes. After that, dried the

wheat pollard, in an oven at 50° C for \pm 48 hours, then mashed the wheat pollard using a disk mill until it was in the form of flour and ready to be used as prebiotic wheat pollard (Utama et al. 2019). The formula for adding 45% water-based on Utama et al. (2017) was as follows:

$$\frac{((material\ water\ content\ (\%)x\ material\ weight\ (g))+A)}{material\ weight\ (g)+A\ (added\ water)}\chi\ 100$$

Making fermented cabbage probiotics

Fermented cabbage probiotics (mixed culture probiotics) begun with cutting the cabbage as thin as possible, then blended and added 8% salt and 6.7% molasses then fermented for 6 days in facultative anaerobes condition (Utama et al. 2018a; 2018b). After that, the results of fermentations were dismantled and ready to be used as probiotics containing *Lactobacillus brevis*, *Lactobacillus plantarum*, *Rhizopus oryzae* and *Saccharomyces cerevise*.

Making mixed culture synbiotics

It started with making probiotics from fermented cabbage juice (Utama et al. 2018a; 2018b). and made prebiotics from wheat pollard (Utama et al. 2019). Prebiotic wheat pollard had 45% water content so it needed to be added with water up to 70% water. The formula for adding 70% water according to Utama et al. (2017) is as follows:

$$\frac{((\textit{material water content (\%)} x \; \textit{material weight (g)}) + \textit{A})}{\textit{material weight (g)} + \textit{A} \; (\textit{added water})} \chi \; 100$$

The addition of fermented cabbage by 40% was calculated based on the amount of water added to meet the water content of 70%. Fermentation was carried out for 4 days under facultative anaerobic conditions and stored at room temperature. The fermented wheat pollard (synbiotic mixed culture) was then harvested and dried in a controlled dryer at 40° C for \pm 72 hours, after which the fermented wheat pollard (synbiotic mixed culture) was mashed and ready to be used as a feed mixture.

The process of making feed

The feed used contains 20-21% protein with metabolizable energy of 2900-3100 kcal/kg and arranged according to the formulation (Table 1). All feed ingredients were mixed evenly and weighed according to treatment. The feed was given starting from DOC until the age of 8 weeks. The form of feed was uniform in the form of a mash with a size of 20

mash. The composition and chemical composition of the treatment ration is presented in Table 1.

In vivo Test

The DOCs on arrival were weighed to find out the initial body weight then distributed into the experimental cages and gave an isotonic solution with a ratio of 500 ml isotonic: 2 liters of clean water to restore body energy lost during transportation. Each experimental unit contained 8-9 DOCs placed in a cage with 1 x 1 m² size. Feed was given according to the needs of the chicken and the residual of the feed were weighed every day. The chickens were weighed every week. Drinking water was given ad libitum. The mixed culture probiotics were added during the study (every 4 days) by 50 ml of fermented cabbage probiotics diluted in 500 ml of clean water (10⁷cfu/ml). Mixed culture fermented cabbage probiotics contained Lactobacillus brevis, Lactobacillus plantarum, Rhizopus oryzae and Saccharomyces cerevise (Utama et al., 2018a; 2018b). The provision of mixed culture fermented cabbage probiotics, with a population of 10⁷ cfu / ml probiotic bacteria. No vaccination programs nor the use of antibiotics, drugs, and other additives were applied during the study. The experiment was carried out for 8 weeks.

Parameters Measured

The parameters observed in this experiment were feed consumption, final body weight, body weight gain, feed conversion ratio (FCR), nitrogen retention, income over feed and chick cost (IOFCC), and intestinal villi profile.

Consumption of rations

The amount of ration consumed was obtained from the calculation of the amount of ration consumed every week.

Final body weight

The final body weight was obtained by subtracting the final 8 week weight by initial body weight.

Average Daily Weight Gains

Measurement of body weight gain (ADG) in g/bird/day was calculated following formula:

Table 1. Composition of treatment ration

	Composition of Treatment Ration					
Feed Ingredients	WP	WPPro	WPPre	WPS40	WPS60	
			%			
Corn	33.00	33.00	33.00	33.00	13.00	
Wheat pollard	40.00	40.00	0.00	0.00	0.00	
Wheat pollard Prebiotic	0.00	0.00	40.00	0.00	0.00	
Wheat pollard Synbiotic	0.00	0.00	0.00	40.00	60.00	
Soybean meal	25.00	25.00	25.00	25.00	25.00	
VitMin-Mix	0.20	0.20	0.20	0.20	0.20	
NaCl	0.25	0.25	0.25	0.25	0.25	
L-Lysin HCL	0.10	0.10	0.10	0.10	0.10	
DL-Metionin	0.10	0.10	0.10	0.10	0.10	
CaCO ₃	1.35	1.35	1.35	1.35	1.35	
Total	100.00	100.00	100.00	100.00	100.00	
Crude Protein (%)	20.911	20.911	20.721	20.621	20.741	
Metabolic Energy (kcal/kg)	3097^{2}	3195^{2}	3036 ²	3126^{2}	3148^{2}	
Crude Fat (%)	2.25^{1}	2.25^{1}	2.56^{1}	2.34^{1}	2.49^{1}	
Crude Fiber (%)	4.231	4.23^{1}	4.41^{1}	4.10^{1}	4.68^{1}	
Ca (%)	0.84^{2}	0.84^{2}	0.94^{2}	0.91^{2}	0.93^{2}	
P(%)	0.50^{2}	0.50^{2}	0.58^{2}	0.53^{2}	0.58^{2}	
L-Lysin HCl ³	0.80	0.80	0.80	0.80	0.80	
DL-Metionin ³	0.40	0.40	0.40	0.40	0.40	
Resistant Starch ⁴ (%)	37.25	37.25	37.84	38.18	30.36	
Strach ⁵ (%)	51.63	51.63	51.17	51.46	37.46	
Amylose ⁵ (%)	14.11	14.11	13.45	13.59	8.31	
Amylopectin ⁵ (%)	37.52	37.52	37.72	37.87	29.15	
Total Lactic Acid Bacteria (cfu)	-	$2x10^{7}$	-	$12x10^{7}$	12x10 ⁷	
Total yeast (cfu)	-	$12x10^{8}$	-	$24x10^{7}$	$24x10^{7}$	

^{1:} Analysis Results from the Feed and Nutrition Science Laboratory of the Faculty of Animal Husbandry and Agriculture Diponegoro University

²: Analysis Result from the Integrated Research and Testing Laboratory of Gajah Mada University

^{3:} Analysis Result based on Calculations using a table composition of feed ingredients
4: Analysis Results from PAU Gajah Mada University

^{5:} Analysis Result from Laboratory of Food Technology and Agricultural Products Gajah Mada University
WP: Wheat pollard based ration, WPPro: Wheat pollard based ration plus probiotic mixed culture, WPPre: Wheat Pollard as prebiotic mixed, WPS40: Wheat pollard as synbiotic mixed culture ration 40%, WPS60: Wheat pollard as synbiotic mixed culture ration 60%

Feed conversion ratio (FCR)

The measurement of feed conversion ratio (FCR) was calculated based on the ratio between the amount of ration consumed and the weight gain measured during the study.

Nitrogen retention

Measurements of nitrogen retention values were performed on 8-week-old chickens. The measurement of nitrogen retention in kampung chickens was carried out by the total collection method. Each group of chickens fed with treatment ration was kept in a battery cage and filled with two chicks per cage and repeated 4 times for each treatment (2 birds per repetition). Six (6) chickens were placed in one cage to get endogenous excreta. All treated chickens were fasted for the first 24 hours to remove the remainder of the feed in the digestive tract. The chickens were given 100g of feed and the excreta were collected for 48 hours. Chickens for collection of endogenous excreta were also fasted for the first 24 hours and provided with drinking water adlibitum. The endogenous chicken excreta collected for 44 hours. The excreta were sprayed once every hour with 1N HCl to capture and reduce nitrogen evaporation. The excreta were then dried, ground, and analyzed for nitrogen content. The formula for calculating nitrogen retention (RN) is as follows (Sibbald, 1980):

$$\frac{(FdxNf) - ((E \times Ne) - (En \times Nen))}{(Fd \times Nf)} \times 100$$

Where, RN: Nitrogen Retention (%); Fd: Feed Consumed (g); Nf: Nitrogen Feed (%); Ne: Nitrogen Excreta (%); E: Total Excreta (g); En: Total Ekskreta Endogenous (g); Nen: Nitrogen Endogenous (%).

Income over feed and chick cost (IOFCC)

Income over feed and chick cost is the difference between the average income (in rupiah) obtained from the price of one chicken and the average expenditure of one chicken which includes the price of feed and the price of day old chicken (DOC) during the study.

Profiles of kampung chickens intestinal

The profile of intestinal kampung chickens measured in this experiment were villi length, width, into crypt and crypt width. The morphological profile of chicken's intestines was obtained through all intestinal samples i.e., the duodenum, jejunum, and ileum. About 1 cm² for the histological evaluation the intestinal samples were fixed in BNF solution for 24 hours. After

fixation, it was then dehydrated with 70, 80, 90, 95% alcohol solution and absolute alcohol I, II, III. Then clarified with xylol I, II, III solution, then the samples were filtered with paraffin I, II, III, and blocked with paraffin. The blocks were cut with a thickness of 4 - 5 μ m using a microtome and stained with Hematoxylin-Eosin staining. Measurement of the length and width of villi and measurement of the width and depth of the intestinal crypt were carried out by observing them under a 400x magnification binocular microscope.

Data analysis

The data of ration consumption, final body weight, body weight gain, FCR, income over feed and chick cost (IOFCC), nitrogen retention and intestinal profile were analyzed using a complete randomized design in a unidirectional pattern and if there was a significant effect it was then continued with Duncan's Multiple Range Test (Steel & Torrie 1989). The mathematical model used is as follows:

$$Yij = \mu + \tau_i + \varepsilon_{ij}$$

Where, $Y_{ij} =$ value observed in the i treatment and j replication; $\mu =$ the influence of the average value of the general treatment; $\tau_i =$ level influence *wheat pollard* treatment I; $\epsilon_{ij} =$ influence the level errors of wheat pollard treatment i and replication j

RESULTS AND DISCUSSION

Results showed that there were significant effects (P < 0.05) of the treatment on body weight gain, final body weight, and nitrogen retention as presented in Table 2.

Feed consumption

Probiotic, prebiotic, and synbiotic mixed culture administration did not affect feed consumption. Based on the composition of the ration (Table 1) the fiber content in the treatment feed were fairly the same, as well as the content of amylose, starch, and amylopectin. Utama et al. (2019) stated that the content of hemicellulose, lignin, starch, amylose, and amylopectin in wheat pollard was still considered normal for poultry feed. Consumption of the rations with similar pollard levels (40%) also resulted in the same nitrogen retention.

Average daily weight gain

The effect of probiotics, prebiotics, and symbiotic mixed culture based on wheat pollard had a significant

Tabel 2. Effect of probiotic, prebiotic and symbiotic mixed culture based on wheat pollard on the performances of kampung chickens up to 8 weeks of age

Treatment		Parameter				
	Feed Consumption (g/bird/day)	ADG(g/bird/day)	Final Weight (g/bird)	FCR	Nitrogen Retention (%)	
WP ¹⁾	83.75 <u>+</u> 12.62	12.32 ^{c2)} ±0.63	727.46° <u>+</u> 35.59	4.05 <u>+</u> 0.75	80.73° <u>+</u> 6.52	
WPPro	90.37 <u>+</u> 12.69	13.35 ^b <u>+</u> 0.61	785.72 ^b <u>+</u> 34.13	4.03 <u>+</u> 0.56	82.98 ^a +3.48	
WPPre	93.20 <u>+</u> 12.33	12.92 ^{bc} ±0.13	766.56 ^{bc} ±7.41	4.28 <u>+</u> 0.54	81.25 ^a ±1.74	
WPS40	83.95 <u>+</u> 18.57	14.69° <u>+</u> 0.41	860.86 ^a ±23.21	3.43 <u>+</u> 0.83	83.12 ^a ±1.09	
WPS60	88.44 <u>+</u> 28.12	10.94 ^d +0.38	650.62 ^d ±21.07	4.79 <u>+</u> 1.64	75.08 ^b ±2.05	
Average	87.94 <u>+</u> 16.87	12.84 <u>+</u> 1.38	757.24 <u>+</u> 24.28	4.12 <u>+</u> 0.86	80.63 <u>+</u> 2.98	

WP: Wheat pollard based ration; WPPro: Wheat pollard based ration plus probiotic mixed culture; WPPre: Wheat Pollard as prebiotic mixed; WPS40: Wheat pollard as synbiotic mixed culture ration 40%; WPS60: Wheat pollard as synbiotic mixed culture ration 60%; a.b.c.d Different superscript in the same row means significantly different (P<0.05)

effect on body weight gain (P <0.05). The highest body weight gain was found in the group of chicks fed WPS40 treatment i.e., 14.69 ± 0.41 g / bird/day while the lowest body weight gain was found in the WPS60 treatment i.e., 10.94 ± 0.38 g / bird/day. The increase in body weight gain is greatly influenced by the quality of the ration given. The quality of the ration can be seen from nitrogen retention (Table 2). Abdel-Raheem et al. (2012) and Mookiah et al. (2014) stated that the administration of synbiotic ration was very effective in increasing the growth of broiler chickens. The combination of *Bacillus subtilis* and *mannan oligosacharida* (MOS) in broilers caused an increase in the ratio of length to the depth of the crypt in the duodenum and ileum (Sen et al. 2011; Bai et al. 2013).

The WPS40 feed was the best feed from other treatments in facilitating high daily weight gain. The WPS40 was also more efficient compared to other treatments. Feeding a higher dose of synbiotics did not necessarily improve livestock productivity. This can be shown in the treatment of WPS40 and WPS60.

Feed Conversion Ratio (FCR)

Probiotic, prebiotic, and synbiotic mixed culture administration did not affect FCR. The use of mixed culture synbiotic rations improved (P <0.05) the body weight gain even though the FCR value was the same. Microorganisms captured in mixed culture synbiotics were known to help the digestive process by producing several enzymes such as protease, beta-mannanase, and several enzymes that are useful in helping the digestion of feed (Gourbeyre et al. 2011). Sari et al. (2017) reported that the administration of 0 to 5% mixed culture synbiotic in laying hens had no significant effect

on egg chemical content and the feed conversion ratio (FCR).

Nitrogen retention

Probiotic, prebiotic, and synbiotic mixed culture administration in the feed, influenced the nitrogen retention parameters significantly (P<0.05). The highest nitrogen retention value (83.12 \pm 1.09%) was in group of chicks fed WPS40 treatment while the lowest (75.08) \pm 2.05%) was showed in WPS60 treatment. The highest nitrogen retention value in the WPS40 treatment may be due to a better-balanced nutrients component and under the nutrient requirements of the birds as well as the presence of a mixed culture synbiotic that improved the performance of the digestive organs. Utama et al. (2017) stated that food fibers such as arabinosa, mannosa, raffinosa, and resistant starch could modulate microorganisms in the intestine, producing SCFA which had the potential to stimulate the growth of villi. Resistant starch was very effective in producing SCFA especially butyrate in the large intestine and could reduce (Utama et al. 2019). Abdel-Raheem et al. (2012) and Mookiah et al. (2014) also reported that the application of probiotics and prebiotics in broiler diets significantly increased the use of dietary nitrogen.

Income Over Feed and Chick Cost (IOFCC)

Income over feed and chick cost (IOFCC) is an economic variable that illustrates the magnitude of the benefits derived from each treatment. The effect of the use of mixed culture synbiotic rations on income over feed and chick cost in kampung chicken during 8 weeks trial is presented in Table 3.

Tabel 3. Effect of probiotic, prebiotic and synbiotic mixed culture based on wheat pollard based on income over feed and chick cost (IOFCC) of kampung chicken up to 8 weeks of age

Treatments	Revenue from Chicken Selling (IDR)	Expenditures during rearing (Feed+DOC) (IDR)	IOFCC (IDR)
WP ¹⁾	25,461	19,691	5,770 ^{b2)} ±481
WPPro	27,500	21,366	6,135 ^b ±306
WPPre	26,655	21,831	4,824 ^b <u>+</u> 251
WPS40	30,130	21,191	8,939 ^a <u>+</u> 482
WPS60	22,772	21,976	795° <u>+</u> 881
Average	26,504	21,211	5,293 <u>+</u> 480

WP: Wheat pollard based ration; WPPro: Wheat pollard based ration plus probiotic mixed culture; WPPre: Wheat Pollard as prebiotic mixed; WPS40: Wheat pollard as synbiotic mixed culture ration 40%; WPS60: Wheat pollard as synbiotic mixed culture ration 60%; a.b.c.d Different superscript in the same row means significantly different (P<0.05)

Feeding probiotic, prebiotic, and synbiotic mixed culture to kampung chickens significantly influenced (P<0.05) the Income over feed and chick cost (IOFCC). The highest IOFC was in group of chicken fed WPS40 treatment, i.e., of 8939±482 (IDR/bird) while the lowest was in WPS60 treatment i.e., 795±881 (IDR/bird). Factors affecting IOFCC included the price of rations, consumption of rations, final body weight, and the selling price of chickens per kg of live weight. The production price of wheat pollard-based on synbiotic mixture culture ration was IDR5000/kg while the selling price of live chickens at the end of the treatment period was IDR 35000/bird. Feed containing WPS40 was more efficiently utilized compared to the commercial diet as the price of the commercial ration was expensive (IDR8500/kg).

Histomorphology of the intestine of Kampung chicken age 8 week

The use of wheat pollard mixed culture-based synbiotic ration affected length, width, crypt width, and duodenal crystalline depth, jejunum, and ileum (P<0.05). The effect of giving probiotic, prebiotic, and synbiotic mixed culture based on wheat pollard on the intestinal profile of kampung chicken at 8 weeks old is presented in Table 4.

Villi are an absorptive place and secretion of digestive enzymes so it is assumed that the size of the villi will affect the level of feed digestibility. The length of villi in the duodenum was the longest in the group of chicken fed WPS40 treatment (1959 \pm 47µm) while the shortest in the WPS60 treatment (1491 \pm 76µm). Whilst the length of villi in the jejunum was the longest in the group of chicken fed WPPre treatment (1521 \pm 71µm) and the shortest in the WP treatment (1182 \pm 67µm). The length of villi in the ileum was the longest in the WPPre treatment (1984 \pm 58µm) while the shortest was in the WP treatment (1595 \pm 45µm). In general villi length of the group of chicken fed synbiotic culture

mixture treatment was 40% better than control, probiotic, prebiotic, and commercial feed treatments chickens groups. Hidayat et al. (2016) stated that intestinal histomorphology reflected the health status of livestock. The increase in villi length, villi width, crypt depth, and crypt width in the intestine was an indication of the growth and health status of livestock.

This condition reflected the healthy digestive tract that comes from probiotic metabolism. *Lactobacillus* creates acidic conditions and produces antimicrobials (free fatty acids, low pH, and bacteriocin), competition for attachment locations in the intestinal epithelium and stimulation of the immune system to protect livestock from pathogenic bacteria by lowering the pH of the intestinal part hindsight thereby disrupting the growth of these bacteria (Tellez et al. 2006; Hill et al. 2014).

The intestinal profile was presented in Table 4 and Figure 4. The nitrogen retention of WPS40 treatment was $83.12 \pm 1.09\%$ with the depth and width of the crypts better than other treatments. The width of intestinal in birds fed the synbiotic treatment was longer than other treatments. This cannot be separated from the two components of a synbiotic composition, namely mixed culture probiotics and prebiotic mixed culture.

Utama & Setiani (2014) stated that synbiotic was a fermentation product that came from an increase in the ability of probiotic bacteria caused by the availability of specific prebiotics to be fermented. Starch resistance plays a role in stimulating the speed of crypts cell production. The depth and width of the crypts treatment of WPS40 were better compared to other treatments. Utama et al. (2017) and Utama et al. (2019) stated that food fibers such as arabinosa, mannosa, raffinosa and resistant starch could modulate microorganisms in the intestine, produced short chain fatty acids (SCFA) which had the potential to stimulate the growth of villi and reduced ammonia.

The width of the villi in the duodenum and jejunum showed no significant difference between the groups of treated chicken, whilst the ileum was significantly

Tabel 4. Influence of probiotic, prebiotic and synbiotic mixed culture based on wheat pollard on the profile of small intestinal of Kampung chickens at 8-weeks-old

D	Treatments						
Parameters	WP ¹⁾	WPPro	WPPre	WPS40	WPS60		
	μm						
Villi length							
Duodenum	1,738 ^b <u>+</u> 65	1,781 ^{ab} <u>+</u> 53	$1,760$ ab ± 75	1,959 a <u>+</u> 47	1,491° <u>+</u> 76		
Jejunum	1,182° <u>+</u> 67	1,308 ^{bc} <u>+</u> 58	1,521 ^a <u>+</u> 71	1,454 ^{ab} <u>+</u> 67	1,266 ^{bc} ±62		
Ileum	1,595 ^b <u>+</u> 45	1,758 ^b <u>+</u> 67	1,984° <u>+</u> 58	1,802 ^{ab} +56	1,667 ^b <u>+</u> 58		
Top width of villi							
Duodenum	257 <u>+</u> 26	245 <u>+</u> 24	275 <u>+</u> 33	252 <u>+</u> 29	221 <u>+</u> 15		
Jejunum	226 <u>+</u> 24	216 <u>+</u> 29	240 <u>+</u> 25	231 <u>+</u> 32	195 <u>+</u> 28		
Ileum	125° <u>+</u> 34	143 ^{bc} <u>+</u> 36	161 ^{ab} ±27	180° <u>+</u> 18	136° <u>+</u> 18		
Bottom width of villi							
Duodenum	$297^{ab} + 10$	$262^{bc} \pm 22$	324 ^a <u>+</u> 23	313° <u>+</u> 31	242° <u>+</u> 28		
Jejunum	262 <u>+</u> 27	313 <u>+</u> 13	266 <u>+</u> 19	293 <u>+</u> 25	252 <u>+</u> 16		
Ileum	233 ^b ±17	238 ^b ±25	330° <u>+</u> 31	349 ^a <u>+</u> 36	317ª <u>+</u> 25		
Crypts depth							
Duodenum	370 <u>+</u> 33	417 <u>+</u> 18	449 <u>+</u> 37	440 <u>+</u> 52	383 <u>+</u> 36		
Jejenum	442 ^b ±18	330° <u>+</u> 20	497° <u>+</u> 21	$456^{ab} \pm 26$	330° <u>+</u> 47		
Ileum	301 <u>+</u> 14	311 <u>+</u> 13	319 <u>+</u> 41	339 <u>+</u> 18	323 <u>+</u> 16		
Crypts width							
Duodenum	45° <u>+</u> 7	61 ^b <u>+</u> 6	66 ^b ±5	81ª <u>+</u> 3	62 ^b ±5		
Jejenum	43 ^b <u>+</u> 4	53 ^b <u>+</u> 5	55 ^b <u>+</u> 7	80° <u>+</u> 2	70° <u>+</u> 6		
Ileum	44 <u>+</u> 5	50 <u>+</u> 7	53 <u>+</u> 8	54 <u>+</u> 6	50 <u>+</u> 6		

WP: Wheat pollard based ration; WPPro: Wheat pollard based ration plus probiotic mixed culture; WPPre: Wheat Pollard as prebiotic mixed; WPS40: Wheat pollard as synbiotic mixed culture ration 40%; WPS60: Wheat pollard as synbiotic mixed culture ration 60% a.b.c.d Different superscript in the same row means significantly different (P<0.05)

different (P<0.05). The chicks fed WPS40 treatment was significantly different the width of villi from the treatment of WPPro, WP, and WPS60. The bottom width of the villi showed a marked difference in the duodenum and ileum while the jejunum was not significantly different. At the bottom width of villi of the kampung fed WPPre and WPS40 treatments had the same effects. The depth of crypts on jejunum showed a significant difference (P<0.05) between treatment groups while on the duodenum and ileum was no difference. The highest crypt depth showed at WPPre and WPS40 treatments while the lowest was showed at Probiotics triggered the WPPro and WPS60. production of SCFA which played a role in the process of intestinal epithelial cell proliferation (Hung et al. 2012).

The width of the crypts in the duodenum and jejunum of all treatment groups showed a significant difference (P<0.05) whereas in the ileum was not significantly different. The width of the crypts in the duodenum and jejunum of the chicken group fed WPS40 treatment was significantly different from the groups either fed WPPro, or WP, or WPPre, and or WPS60. Pelicano et al. (2005) stated that the villous profile greatly influenced the feed rate so that it affected the absorption of feed nutrients. New epithelial and enterocyte cells were produced by crypts which then migrate to the villi (Perić et al. 2010; Abdel-Raheem et al. 2012; Gómez et al. 2012). The surface of the intestinal tract was coated by viscoelastic mucous gel which acts as a natural defense system and also helps in the absorption of nutrients. In detail, the image of intestinal villi can be seen in figures 1 to 6.

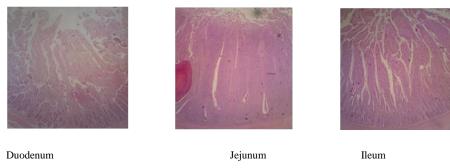


Figure 1. Profile of small intestinal villi of chickens fed wheat pollard (WP) at 8-week-old at 400x magnification

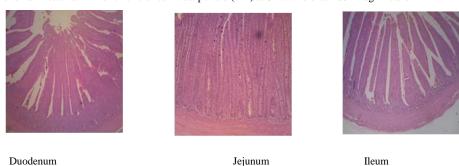


Figure 2. Profile of small intestinal villi of chickens fed Wheat pollard plus probiotic mixed culture-based ration (WPPro) at 8-weeks-old at 400x magnification

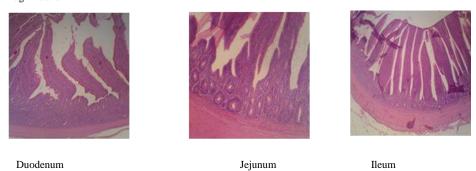


Figure 3. Profile of small intestinal villi of chickens fed Wheat Pollard probiotic mixed culture-based ration (WPPre) at 8-weeks-old at 400x magnification

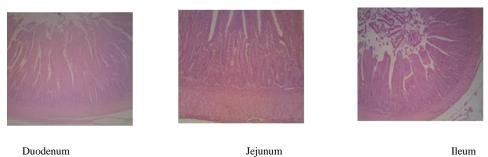
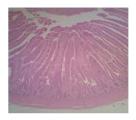
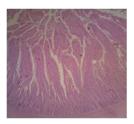


Figure 4. Profile of small intestine villi of chickens fed wheat pollard synbiotic feed mixed culture 40% (WPS40) at 8 weeks-old at 400x magnification







Duodenum Jejunum Ileum

Figure 5. Profile of small intestine villi of chickens fed wheat pollard feed 60% mixed culture synbiotic (WPS60) at 8 weeks old at 400x magnification

CONCLUSION

It is concluded that the addition of 40% fermented wheat pollard (WPS40) in the ration was able to increase the productivity of kampung chickens until 8 weeks of age as indicated by an increase in body weight, nitrogen retention, intestinal villi profile and IOFCC.

REFERENCES

- Abdel-Raheem S, AbdAllah S, Hassanein K. 2012. The effects of prebiotic, probiotic and synbiotic supplementation on intestinal microbial ecology and histomorphology of broiler chickens. Int J Agro Vet Med Sci. 6: 277–289.
- Alloui MN, Szczurek W, Świątkiewicz S. 2013. The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. Ann Anim Sci. 13:17–32.
- Bai SP, Wu AM, Ding XM, Lei Y, Bai J, Zhang KY, Chio JS. 2013. Effects of probiotic-supplemented diets on growth performance and intestinal immune characteristics of broiler chickens. Poult Sci. 92:663–670.
- den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud D-J, Bakker BM. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 54:2325–2340.
- Gómez S, Angeles ML, Mojica MC, Jalukar S. 2012. Combination of an enzymatically hydrolyzed yeast and yeast culture with a direct-fed microbial in the feeds of broiler chickens. Asian-Australasian J Anim Sci. 25:665–673.
- Gourbeyre P, Denery S, Bodinier M. 2011. Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. J Leukoc Biol. 89:685–695.
- Hartono EF, Iriyanti N, Suhermiyati S. 2016. Efek Penggunaan sinbiotik terhadap kondisi miklofora dan histologi usus ayam Sentul jantan. J Agripet. 16:97–105.
- Hidayat SCM, Harimurti S, Yusiati LM. 2016. Pengaruh suplementasi probiotik bakteri asam laktat terhadap histomorfologi usus dan performan puyuh jantan. Bul Peternak. 40:101–106.

- Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, et al. 2014. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 11:506–514.
- Hung AT, Lin S-Y, Yang T-Y, Chou C-K, Liu H-C, Lu J-J, Wang B, Chen S-Y, Lien T-F. 2012. Effects of Bacillus coagulans ATCC 7050 on growth performance, intestinal morphology, and microflora composition in broiler chickens. Anim Prod Sci. 52:874–879.
- Iannitti T, Palmieri B. 2010. Therapeutical use of probiotic formulations in clinical practice. Clin Nutr. 29:701–725.
- Mookiah S, Sieo CC, Ramasamy K, Abdullah N, Ho YW. 2014. Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J Sci Food Agric. 94:341–348.
- Pelicano ERL, Souza PA, Souza HBA, Figueiredo DF, Boiago MM, Carvalho SR, Bordon VF. 2005. Intestinal mucosa development in broiler chickens fed natural growth promoters. Rev Bras Ciência Avícola. 7: 221-220
- Perić L, Milošević N, Žikić D, Bjedov S, Cvetković D, Markov S, Mohnl M, Steiner T. 2010. Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. Arch Anim Breed. 53:350–359.
- Permadi ANN, Kurnianto E, Sutiyono S. 2020. Karakteristik morfometrik ayam kampung jantan dan betina di Desa Tirtomulyo Kecamatan Plantungan, Kabupaten Kendal, Jawa Tengah. J Peternak Indones. 22:11-20.
- Resnawati H, Bintang I. 2014. Kebutuhan pakan ayam kampung periode pertumbuhan. In: Prosiding Lokakarya Nas Inov Teknol Pengemb Ayam Lokal. Bogor (Indones): Pusat Penelitian dan Pengembangan Peternakan; p. 138–141.
- Sari EMA, Suprijatna E, Sarengat W. 2017. Pengaruh sinbiotik untuk aditif pakan ayam petelur terhadap kandungan kimiawi telur. J Peternak Indones. 19:16–22.
- Sen S, Ingale SL, Kim JS, Kim KH, Kim YW, Khong C, Lohakare JD, Kim EK, Kim HS, Kwon IK, Chae BJ. 2011. Effect of supplementation of *Bacillus subtilis* LS 1-2 grown on citrus-juice waste and corn-soybean meal

- substrate on growth performance, nutrient retention, caecal microbiology and small intestinal morphology of broilers. Asian-Australasian J Anim Sci. 24:1120–1127.
- Sibbald IR. 1980. Metabolizable energy in poultry nutrition. Bio Sci. 30:736-741
- Solis de los Santos F, Farnell MB, Tellez G, Balog JM, Anthony NB, Torres-Rodriguez A, Higgins S, Hargis BM, Donoghue AM. 2005. Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. Poult Sci. 84:1092–1100.
- Steel RG, Torrie JH. 1989. Prinsip dan Prosedur Statistik Suatu Pendekatan Biometrik. 4th ed. Translator: Sumantri B. Jakarta (Indones): Gramedia Pustaka Utama.
- Sugiharto S, Yudiarti T, Isroli I, Widiastuti E, Wahyuni HI. 2018. Hematological parameters and selected intestinal microbiota populations in the Indonesian indigenous crossbred chickens fed basal diet supplemented with multi-strain probiotic preparation in combination with vitamins and minerals. Vet World. 11:874–882

- Tellez G, Higgins SE, Donoghue AM, Hargis BM. 2006. Digestive physiology and the role of microorganisms. J Appl Poult Res. 15:136–144.
- Utama CS, Setiani BE. 2014. Production of Probiotic Supplement Based on Agriculture Industrial Waste. Pakistan J Nutr. 13:386–389.
- Utama CS, Sulistiyanto B, Kismiyati S. 2017. The effects of water addition and steaming duration on starch composition of wheat pollard. Reaktor. 17:221-225.
- Utama CS, Zuprizal, Hanim C, Wihandoyo. 2018a. Probiotic Testing of *Lactobacillus brevis* and *Lactobacillus plantarum* from Fermented Cabbage Waste Juice. Pakistan J Nutr. 17:323–328.
- Utama CS, Zuprizal, Hanim C, Wihandoyo. 2018b. Isolasi dan identifikasi Bakteri asam laktat selulolitik yang berasal dari jus kubis terfermentasi. J Apl Teknol Pangan. 7:1–6.
- Utama CS, Zuprizal, Hanim C, Wihandoyo. 2019. Pengaruh lama autoclave terhadap kualitas kimia *wheat pollard* yang berpotensi sebagai prebiotik. J Apl Teknol Pangan. 8:113-123.