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The digestibility value of local chicken rations fed with feed containing fermented catfish waste

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Abstract

The study aims to determine the effect of fermented patin fish waste (FPFW) by microbes *Lactobacillus paracasei, Bacillus subtilis*, and *Saccharomyces cerevisiae* (LBS) in rations on the digestibility of dry matter and crude protein, as well as obtain the level of use that produces the best digestibility of dry matter and protein in local chickens. The study used SNC(SNC) aged 14 weeks, as many as 24 tails, kept for 14 days. The experimental design was randomized, with six treatments and four repeats. Ration treatment is R0 (Lower control ration, protein 15% and EM 2750 kcal/kg, without the use of FPFW); R1 (R0 contains 5% FPFW); R2 (R0 contains 10% FPFW); R3 (R0 contains 15% FPFW); R4 (R0 contains 20% FPFW); and RS (Upper control ration, protein 18% and EM 2750 kcal/kg, without use of FPFW). The results showed that the use of FPFW by LBS microbes had a significant effect ($P<0.05$) on the digestibility of dry matter and ration protein. Using 10% FPFW in the ration formula provides the best digestibility value of dry matter and crude protein of the ration on SNC.

Keywords: Patin fish waste; Fermentation; Protein digestibility; Dry matter digestibility; Super free-range chicken

1. Introduction

The catfish fillet industry can produce a yield of around 33%; the remaining 67% is waste in the form of tail, skin, bones, stomach contents, and belly [1]. If not utilized properly, the waste does not rule out the possibility of causing odor and environmental pollution due to decay. This waste is deficient but can add significant value if utilized optimally. One of the catfish waste treatments that can be done is by making fish meal. Catfish waste can be used as animal feed. Patin waste still contains protein and fat that is high enough so that it has the potential to be used as a feed source of amino acids and essential fatty acids. Catfish fillet waste contains about 12.51% protein [2]. The total amino acid content of catfish tail hydrolysate is 32.68%, and in the bones is 25.65% [3]. The dominant essential amino acids on the tail are leucine 2.72% and lysine 2.30% [4]. The dominant essential amino acid in the catfish bone is lysine 4.8% [3]. The fat content of catfish is 11.20% on the tail, 13.10% on the bones and tail, 6.63% on the trimming residual meat, 36.21% on the belly flap, and 7.90% on the skin. The dominant fatty acids contained in catfish are palmitic acid and oleic acid. The percentage of unsaturated fatty acids on the tail is 53.24%, and on the belly flap is 54.38% [5].

Supplementation of amino and essential fatty acids in poultry feed can improve ration quality, performance, health, and livestock product quality. Lysine and methionine supplementation in native chicken rations significantly affects breast, thigh, and back weight [6]. Supplementation of essential amino acids such as lysine, methionine, and threonine can improve egg production, weight, and mass of single-comb laying hens [7]. Supplementation of essential amino acids can increase the protein content in Arabian chicken eggs [8]. Supplementation of feed ingredients rich in omega-3 can improve the physical quality of broiler chicken meat and increase the intensity of color and omega-3 content in eggs [9].

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Fish waste silage is feed from fish industry waste, processed using chemical hydrolysis or fermentation. The fermentation method is more advantageous than chemical hydrolysis because it can produce amino acids without removing the content of specific amino acids [10]. The fermentation process can improve the nutrient profile of fish waste [11]. *Lactobacillus paracasei, Bacillus subtilis*, and *Saccharomyces cerevisiae* have proteolytic and lipolytic activities, so fermentation using these microbes can improve the profile of amino acids and fatty acids [12].

L. paracasei is a probiotic that has proteolytic and lipolytic activity and produces a number of enzymes including dipeptidase, prolidase, aminopeptidase, and esterase [12]. The use of *L. paracasei* showed an increase in total essential amino acids of 10.25% in fermentation of soybean flour for 72 hours. This fermentation also increased the linolenic acid content which was 14.98% of the control [13]. *B. subtilis* is a probiotic commonly used in animal feed processing because it is safe, highly effective, and relatively cheap. *B. subtilis* can produce several enzymes, including proteases, lipases, and carboxypeptidases [14]. Using *B. subtilis* in fermentation can increase amino and fatty acid content. Soybean okara fermented by *B. subtilis* produces an increase in several essential amino acids, including aspartic acid, phenylalanine, glycine, leucine, lysine, proline, serine, threonine, tyrosine, and valine. It shows increased linoleic and oleic acid content [15]. *S. cerevisiae* is a yeast commonly used to ferment animal feed. One of the benefits of *S. cerevisiae* is that it can increase the digestibility of feed [16]. *S. cerevisiae can* produce several enzymes, namely amylase, protease, and other enzymes that can help digest food substances in the digestive organs of livestock [17].

Super native chickens (SNC) result from a cross between native chickens and laying hens. SNC can reach weights of up to 1.5 kg/tail with a maintenance duration of around 2.5 months [18]. The advantages of SNC are that they can be kept in large numbers with uniform weight, have faster growth compared to ordinary native chickens, have low mortality rates, and can adapt to the environment [7]. However, this super native chicken is still raised on a small business scale, its productivity is still low, its slow growth, and it still has incubating properties [19]. Efforts can be made to maximize the use value of feed, which can be given essential nutrient feed substituted in the ration. Digestibility is one of the benchmarks in determining feed quality. Feeding fermented products can increase the digestibility value. Fish waste silage substituted as much as 6–12% in rations can increase the digestibility of dry matter and protein in broiler chickens [20]. Fermented freshwater pomfret waste *(Colossoma macropomum*) given as much as 5% in the ration can increase the digestibility of dry matter in laying hens but does not affect protein digestibility [21].

2. Materials and methods

2.1. Fermentation with LBS microbes (*L.paracasei, B.subtilis, and S.cerevisiae*)

The LBS microbial inoculum is made by fermenting catfish waste with pure cultures to multiply LBS microbes so that each microbe becomes accustomed to the catfish waste substrate. Further fermentation is carried out.

2.2. Fermented catfish waste (FPFW)

Fermented Patin Fish Waste (FPFW) is obtained after fermentation of catfish waste with LBS microbes for five days at a dose of 10%. After it is harvested, the fermented product is dried and ground into FPFW.

2.3. Ransum

The ration used is prepared based on the nutritional needs of SNC finisher period, namely crude protein 18-19%; crude fat 4-7%; crude fiber 3-5%; calcium 1-1.2%; phosphorus 0.35%; lysine 0.6%; methionine 0.8%; and EM 2750 kcal/kg [22]. The treatment ration consisted of two kinds of controls, for control below R0 with a crude protein content of 15% and control over RS ration with a crude protein content of 18%, and the energy content of both control rations was made the same at 2750 kcal/kg. The nutrient content and metabolizable energy of the feed ingredients used are shown in Table 1, the formulation of the treatment ration is shown in Table 2, and the nutrient content and metabolizable energy of the treatment ration in Table 3.

Table 1 Metabolizable energy content and nutrients of research feed ingredients

Feed Ingredients	ME	CP	EE	CF	Ca	P	Lys	Meth
	(Kcal/kg)	…………………….. (%)……………………..						
FPFW*	2239	37.27	10.51	1.15	5.56	8.60	1.95	0.50
Yellow corn	3350	8.60	3.80	2.20	0.02	0.08	0.26	0.18
Soybean meal	2230	44.00	0.80	7.00	0.29	0.27	2.69	0.62
Fine bran	1630	10.80	5.81	10.80	0.11	0.19	0.64	0.24
Meat bone meal	2375	38.84	10.93	2.46	9.80	4.50	2.08	0.54
Bone meal	-	-	-	-	24.00	12.00	-	-
Stone flour	-	-	-	-	40.00	-	-	-
PrMEix	-	-	-	-	30.87	1.11	-	-

Source: Analysis of the Ruminant Animal Nutrition and Fodder ChMEistry Laboratory, Faculty of Animal Husbandry, Padjadjaran University (2023). FPFW, fermented catfish waste.

Table 2 Trial Ration Formulation

Feed Ingredients	R0	R1	R2	R3	R4	RS
	……………………………%………………………………………					
FPFW*	0.00	5.00	10.00	15.00	20.00	0.00
Yellow corn	57.00	58.00	58.00	58.00	58.00	53.00
Soybean meal	8.00	6.00	5.00	3.50	1.50	14.00
Fine bran	23.00	21.00	20.50	19.50	18.00	18.00
Meat bone meal	10.00	8.00	4.50	2.00	0.00	13.00
Bone meal	0.75	0.75	1.00	1.50	2.00	0.75
Stone flour	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
Total	100	100	100	100.	100	100

FPFW, fermented catfish waste; R0, bottom control ration without FPFW; R1, ration contains 5% FPFW; R2, rations contain 10% FPFW; R3, ration contains 15% FPFW; R4, ration contains 20% FPFW; RS, upper control ration without FPFW.

Table 3 Metabolizable energy and nutrients content of experimental rations

Ration	metabolizable energy and nutrient content								
	CP	EE	CF	Ca	P	Lys	Meth	Sys	ME
	……………………………%……………………………………								k.cal/kg
R0	15.07	5.19	4.20	1.09	0.53	1.10	0.49	0.30	2750
R1	15.09	5.27	3.92	1.25	0.90	1.04	0.47	0.29	2763
R2	15.00	5.39	3.83	1.37	1.18	0.94	0.44	0.29	2748
R3	15.06	5.50	3.67	1.59	1.45	0.88	0.42	0.29	2742
R4	15.09	5.59	3.43	1.95	1.82	0.81	0.41	0.29	2746
RS	18.07	5.61	3.96	1.26	0.62	1.37	0.54	0.33	2751

R0, bottom control ration without FPFW; R1, ration contains 5% FPFW; R2, rations contain 10% FPFW; R3, ration has 15% FPFW; R4, ration contains 20% FPFW; RS, upper control ration without FPFW.

2.4. Super native chichen (SNC)

The SNC used was 14 weeks old, totaling 24 tails. The battery cage is 30 cm x 30 cm x 35 cm, which can hold as many as 24 units.

2.5. Testing of treatment rations on super free-range chickens

Chickens are kept in individual cages and given treatment rations two times a day, namely 50 grams in the morning and 50 grams in the afternoon. Chickens were given treatment rations for 14 days, then cut and taken part of the large intestine to take samples of dry matter digestibility test and crude protein.

2.6. Sampling and testing

Samples are taken in the large intestine; then stool samples are removed. The fecal samples were weighed and then analyzed for dry matter content and crude protein, while the indicators (ration lignin and feces) were analyzed by the [23]. This test is carried out to determine the digestibility of dry matter and protein.

2.7. Observed modifiers

The modifiers measured in this study were the digestibility of dry matter and protein ration. The digestibility measurement refers to the method of [24], cited by [25] and modified by [26]).

2.7.1. Dry matter digestibility (DMD) measurement method

The treatment ration and fecal samples are analyzed for dry matter content and its indicator (lignin), then the digestibility of dry matter can be measured using the following formula:

$$\mathrm{DMD} = 100\% - \left[100\left\{\frac{\%\ \text{lignin ransom}}{\%\ \text{lignin feces}} \times \frac{\%\ \text{DM feces}}{\%\ \text{DM ransom}}\right\}\right]$$

2.7.2. Crude protein digestibility (CPD) measurement method

Treatment rations and fecal samples are analyzed for crude protein content, and its indicator (lignin), then protein digestibility can be measured using the following formula:

$$\mathrm{CPD} = 100\% - \left[100\left\{\frac{\%\ \text{lignin ransom}}{\%\ \text{lignin feces}} \times \frac{\%\ \text{CP feces}}{\%\ \text{CP ransom}}\right\}\right]$$

2.8. Trial plan and statistical analysis

The study was conducted using the experimental method with a randomized design complete with six treatments. Each test was repeated four times, resulting in 24 experimental units. The treatment given is in the form of the level of use of FPFW in the ration. The difference between treatments was tested using the Duncan multiple distance test.

3. Results and discussion

3.1. Effect of treatment on dry matter digestibility

The results of research on the effect of treatment on dry matter digestibility are presented in Table 4.

The results of the variety analysis showed that the treatment had a natural effect ($P<0.05$) on the digestibility of dry matter. Furthermore, a Duncan multiple distance test was carried out to determine the differences between treatments. Based on Table 4, it can be seen that the lowest dry matter digestibility is R0 (upper control ration without the use of FPFW; CP 15%), which is 43.00%, and the highest is R2 (ration contains 10% FPFW; CP 15%) which is 63.76%. This suggests that using FPFW can improve the digestibility of dry matter. This increase in dry matter digestibility is caused by LBS (*L. paracasei, B. subtilis, S. cerevisiae*) able to remodel complex compounds such as carbohydrates, proteins, and fats into more uncomplicated so that the digestive organs of chickens more easily digest them. According to [27]), the process of chemical changes from complex compounds to more superficial ones due to enzymes produced by microbes reflects improving the quality of feed nutrients, which causes increased nutrient digestibility.

The increase in dry matter digestibility in this study is also inseparable from the role of LBS, which can produce several enzymes during fermentation to help the digestive process in the digestive organs of chickens. This is supported by the opinion of [28] that lactic acid-producing bacteria can produce enzymes that help the digestive process. According to [12], *L. paracasei* can produce several enzymes, including dipeptidase, prolidase, aminopeptidase, and esterase. According to [14] state that *B. subtilis can* produce protease enzymes, lipases, and carboxypeptidases. According to [17] state that *S. cerevisiae* can produce amylase and protease enzymes.

Table 4 Digestibility of dry matter of each treatment

Treatment	Treatment					
	R0	R1	R2	R3	R4	RS
	....%..					
1	44.44	58.47	66.21	54.98	46.94	50.00
2	41.72	58.03	65.60	61.30	48.10	49.70
3	41.44	52.50	58.95	62.18	51.59	45.08
4	44.38	57.11	64.30	58.31	47.82	46.31
Average	43.00^{a}	56.53^{c}	**63.76^{d}**	59.19^{c}	48.61^{b}	47.77^{b}

R0, bottom control ration without FPFW; R1, ration contains 5% FPFW; R2, rations contain 10% FPFW; R3, ration has 15% FPFW; R4, ration contains 20% FPFW; RS, upper control ration without FPFW.

The increase in digestibility of dry matter in this study is also thought to be due to an increase in enzyme activity in the digestive organs of chickens. The research results by [20] showed that rations containing fermented fish waste increased amylase and protease activity in broiler chicken jejunum. According to [29], the increase in digestive enzyme activity is caused because organic acids in fermentation products can increase the production of secretin and free protons and lower digesta pH, thereby stimulating the secretion of pancreatic and small intestine enzymes. This is in line with the opinion of [30] that a mixture of probiotics and prebiotics in feed can help the digestive tract by lowering intestinal pH so that it can stimulate the secretion of pancreatic enzymes.

The increase in the digestibility value of dry matter in this study is thought to be due to a rise in the structure of the small intestine's villi so that the absorption surface is wider. This is supported by [31] opinion that lactic acid bacteria in feed can increase nutrient absorption by increasing intestinal epithelial structure and expanding the absorption surface area. Research by [20] showed that fish waste silage of as much as 3-12% in the ration increased the villi height ratio and broiler chickens' crypt depth. According to [31], maximum digestion and absorption of food substances occurs when the high villi-to-crypt depth ratio increases. In line with [32], nutrient absorption is more efficient if the abspsy field is more comprehensive. The zigzag structure of the villi can also cause the feed rate to be slower so that absorption will be maximized. According to [31], the increased structure of intestinal villi can be caused by fermented feed with low pH and high lactic acid bacteria, which can improve intestinal health by balancing intestinal microflora.

The highest digestibility value of dry matter in this study was in rations containing 10% FPFW (R2), while rations containing FPFW >10% (R3 and R4) decreased digestibility value. This is in line with the opinion of [33] that the rate of use of fish waste silage in rations for local chickens ranges from 8-10%. This decrease in digestibility value is due to catfish waste containing histamine. The research results by [34] showed that the histamine content of freshwater fish was 4,291 mg/kg. According to [35], histamine stimulates excessive acid production by the proventricle, and this acidic condition can cause erosion in the next digestive organ, the gizzard. Gizzard erosion can infect the intestines due to increased Clostridium perfringens. According to [36], the amount of C. perfringens in the intestine increases significantly with the severity of injury to the gizzard mucosa. According to [37], C. perfringens is a typical microorganism that lives in the intestines of poultry, but if the concentration is too high, it can cause necrotic enteritis. According to [38] reported that animal protein from fish can contain histamine, which can damage the intestinal mucosa. In line with [39], necrotic enteritis is more common in chickens fed animal feed such as fish, cows, and so on compared to chickens with plant protein. According to [40] state that digestive tract disorders can interfere with nutrient digestibility.

The results showed that the highest dry matter digestibility value was in the R2 treatment. This means that rations with a CP content of 15% but containing 10% FPFW can compete with rations (without using FPFW) with a CP content of 18% when viewed from the digestibility of dry matter. In other words, a ration with a CP content of 18% can be reduced to 15% if the ration contains 10% FPFW. The use of FPFW can be a consideration in the efficiency of ration use.

3.2. Effect of treatment on protein digestibility

The results of research on the effect of treatment on protein digestibility are presented in Table 5.

Table 5 Digestibility of crude protein of each treatment

Treatment	Treatment					
	R0	**R1**	**R2**	**R3**	**R4**	**RS**
	…………………………………………%…………………………………………					
1	49.05	57.74	74,32	57.10	64.48	49.10
2	45.27	59.70	73.91	63.15	64.05	49.34
3	43.36	54.77	70.11	64.01	64.46	52.50
4	48.32	56.83	73.28	60.30	62.11	49.87
Average	46.50^{a}	57.29^{c}	**72.90^{e}**	61.14^{d}	63.78^{d}	50.20^{b}

R0, bottom control ration without FPFW; R1, ration contains 5% FPFW; R2, rations contain 10% FPFW; R3, ration has 15% FPFW; R4, ration contains 20% FPFW; RS, upper control ration without FPFW.

The results of the variety analysis showed that the treatment had a natural effect (P<0.05) on protein digestibility. Furthermore, a Duncan multiple distance test was carried out to determine the differences between treatments. Based on Table 5, it can be seen that the lowest protein digestibility is R0 (lower control ration without the use of FPFW; CP 15%) which is 46.50%, and the highest is R2 (ration contains 10% FPFW; CP 15%) which is 72.90%. This suggests that the use of FPFW can improve protein digestibility. This increase in protein digestibility is caused by LBS microbes' ability to make the protein contained in catfish waste simpler. According to [41], the degradation of proteins into peptides and amino acids during fermentation increases the protein available and is easily digested by livestock. In line with research by [20], the fermentation of fish waste by *B. subtilis* can increase nutrient content, including amino acids. According to [42], the digestibility value can be influenced by the nature of the feed and its suitability to be hydrolyzed by chicken digestive enzymes.

The increase in protein digestibility in this study can be caused by LBS microbes that can produce protease enzymes that help digest proteins in the digestive organs of chickens. In line with the opinion of [28], lactic acid-producing bacteria can produce enzymes that help the digestive process. According to [43] state that *L. paracasei* can produce aminopeptidase and oligopeptidase enzymes. According to [44], *Bacillus* sp. can produce serine protease, cysteine protease, and metalloprotease. According to [45], *S. cerevisiae* can produce aspartate protease enzymes.

The increase in protein digestibility in this study is also thought to be due to the rise in protease enzyme activity in the digestive organs of chickens. In line with the research results by [20], rations containing fermented fish waste can increase protease enzyme activity in broiler chicken jejunum and increase the digestibility of crude protein. The results of research by [46] show that rations containing fermented feed can increase the activity of protease enzymes in the intestines and pancreas of broilers.

The increase in digestibility of crude protein in this study is also thought to be due to the rise in the structure of the villi so that the absorption field is more comprehensive and able to increase the absorption of amino acids. In line with the research results by [20], fish waste silage as much as 3-12% in the ration can increase the ratio of villi height and crypt depth of broiler chickens. According to [47], the ratio of villi height to crypt depth is an essential indicator of digestion and nutrient absorption capacity in the intestine, so an increase in the ratio of villi height to crypt depth indicates an increase in digestion and nutrient absorption in the intestine. According to [31], the increased structure of intestinal villi can be caused by fermented feed with low pH and high lactic acid bacteria, which can improve intestinal health by balancing intestinal microflora.

Like dry matter digestibility, the highest digestibility value of crude protein in this study was also in treating rations containing 10% FPFW (R2). In comparison, rations containing FPFW >10% (R3 and R4) decreased digestibility values. This decrease in digestibility value is due to catfish waste containing histamine. The research results by [34] showed that the histamine content of freshwater fish was 4,291 mg/kg. According to [35]), histamine is capable of causing erosion in gizzards. Gizzard erosion can infect the intestines due to increased Clostridium perfringens. According to [36], the severity of injury to the gizzard mucosa increases the amount of C. perfringens in the intestine. According to

[37], too high concentrations of C. perfringens can cause necrotic enteritis. According to [39], necrotic enteritis is more common in chickens fed animal feed such as fish, cows, and so on than in chickens given plant protein. According to [40] state that digestive tract disorders can interfere with nutrient digestibility.

Like dry matter digestibility, the highest protein digestibility value is in the R2 treatment. This means that rations with a CP content of 15% but containing 10% FPFW can exceed rations (without using FPFW) with a CP content of 18% when viewed from the digestibility of crude protein. In other words, a ration with a CP content of 18% can be reduced to 15% if the ration contains 10% FPFW. The use of FPFW can be a consideration in the efficiency of ration use.

4. Conclusion

Fermented patin fish waste by microbes *Lactobacillus paracasei, Bacillus subtilis, Saccharomyces cerevisiae* can improve the digestibility of dry matter and protein ration in super native chickens. The rate of use of FPFW by LBS microbes is 10%, resulting in the highest digestibility of dry matter and protein rations in super native chickens.

Compliance with ethical standards

Conflict of interest statement

No conflict of interest to be disclosed.

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