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Effect of shrimp waste fermentation feed supplement use on the balance of protein efficiency and color of native chicken egg yolk

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Abstract

Background: Supplement feed with an optimal composition will increase livestock productivity through increased feed digestibility and feed consumption that will provide a balance between amino acid supply and energy to grow, and produce. One of the fishery wastes that can be used as feed supplements is shrimp waste. The purpose of the study was to determine and obtain the level of use of feed supplements of fermented shrimp waste in the ration which resulted in a balance value of protein efficiency and the best egg yolk colour in the native chickens.

Materials and Methods: The experiment used 20 native chickens aged 40 weeks in 20-unit cages. The study used the Complete Randomized Design method with 5 kinds of treatment, namely R0 (ration without the use of feed supplement fermentation of shrimp waste), R1 (ration with the use of feed supplement 0.5% fermentation of shrimp waste), R2 (ration with the use of feed supplement 1.0% fermentation of shrimp waste), R3 (ration with the use of feed supplement 1.5% fermentation of shrimp waste), and R4 (ration with the use of feed supplement 2.0% fermentation of shrimp waste), each treatment is repeated four times. The changes observed were ration consumption, protein consumption, egg weight, protein efficiency balance, and yolk colour.

Results: Shrimp waste fermentation products as feed supplements have a significant influence ($P<0.05$) on feed consumption, protein consumption, egg weight, the balance of protein efficiency, and the colour of the yolk. The use of shrimp waste fermentation products gives optimal results at the rate of 1.5% (R3) in the ration against feed consumption and protein consumption, the weight of eggs optimal results at the level of 2.0 % (R4), and the balance of protein efficiency and against the colour of the yolk optimal results at the level of 1.0% (R2).

Conclusions: The use of shrimp waste fermentation products of 1.0 levels in the ration results in an optimal balance of protein efficiency and yolk colour. The pattern of the relationship between treatments to the balance of protein efficiency is obtained in linear form with the equation $y = 0.1494x + 3.7593$ (R2 =93.71%). and in the colour of the yolk in linear form with the equation $y = 0.85x + 7.4883$ (R2=96.33%). Shrimp waste fermentation products can be used at a rate of 1.0-2.0% in the feed formula of the local poultry layer phase and livestock other than local poultry.

Keywords: Feed supplement; Fermentation; Shrimp waste; Free-range chicken; Protein efficiency balance; Egg yolk colour

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1. Introduction

Feed is one of the important factors in supporting the productivity of native chickens. Productivity will be achieved efficiently if the feed provided meets the needs of chickens according to age and maintenance management [1,2]. One way to produce the productivity of native chickens is by giving feed supplements. Feed supplements are useful for supplementing food substances needed by the livestock body so that there is a balanced composition to produce optimally. Supplement feed with an optimal composition will increase livestock productivity through increased feed digestibility and feed consumption that will provide a balance between amino acid supply and energy to grow, and produce [3–5]. One of the fishery wastes that can be used as Feed supplements is shrimp waste.

Shrimp waste is the rest of the body consisting of the head, skin and tail. The main composition of shrimp waste is protein, chitin, fats and minerals [6]. The skin part contains more chitin and less protein, while the head part contains less chitin but more protein [7–9]. The weakness of shrimp waste is the presence of an inhibitory factor for antinutrient substances in the form of chitin. The average chitin content in the dry shell of arthropods is 20-25% [10–12], so it is necessary to carry out processing first, one of which is biologically processing using microbes. Chitin is protein binding to shrimp waste physically. Chitin limits digestive enzymes to proteins, causing low digestibility when consumed by livestock [13,14]. Shrimp waste undergoing fermentation processing techniques will reduce part of the chitin content with the help of the enzyme chitinase [13,15].

The fermentation process can change feed ingredients that are difficult to digest for easier, provide aromas and flavours that are preferred by livestock, and can increase their nutrient levels [16–18]. Fermentation of shrimp waste using microbes including the bacteria *Bacillus licheniformis*, *Lactobacillus* sp. and yeast *Saccharomyces cerevisiae*. *B. licheniformis* produces relatively high amounts of chitinase enzymes and protease enzymes with deproteination properties where the enzyme degrades the glycosidic β (1,4) bonds in chitin and will free some nitrogen or protein in the form of N-Acetyl-D-glucosamine monomers as well as acetylamino so that the protein is detached from the chitin bond [5,19–22]. *Lactobacillus* sp. functions to break down glucose, sucrose, maltose, and lactose into lactic acid so that mineral deposits occur [4,23]. *S. cerevisiae* is a yeast that produces the enzymes amylase, lipase, protease, and other enzymes that can help the digestive process of food substances in the digestive organs [14,24].

Shrimp waste fermented with the bacteria *B. licheniformis, Lactobacillus* sp., and yeast in the form of *S. cerevisiae* can convert complex organic matter such as proteins, carbohydrates and fats into molecules - simpler molecules so that they are easily digested, turn the taste and aroma of the dislike into favour, and in some certain ways add durability [18,25]. The fermentation process in shrimp waste with microorganisms *B. licheniformis, Lactobacillus* sp., and *S. cerevisiae* yeast can improve the quality of shrimp waste proteins containing chitin by increasing the completeness and balance of essential amino acids and having optimal digestibility [3].

Improving the nutritional quality of fermented shrimp waste causes the quality of nutrients to increase as well. Shrimp waste has a crude protein content of 25-40%, calcium carbonate of 45-50% and chitin of 15-20% [26]. The nutrient content of fermented shrimp waste is crude protein 39.29%, crude fat 7.03% and crude fibre 6.81% [14]. The nutrient content of shrimp waste is 42.65% crude protein, 8.07% crude fat, and 18.18% crude fibre while after processing through fermentation the nutrient content of fermented shrimp waste becomes 44.73% crude protein, 8.71% crude fat, and 16.11% crude fibre [27].

The protein efficiency balance describes the quality of protein in the ration because it can show how much protein is used in the feed to produce eggs. Protein efficiency balance is a method of testing protein quality that can be done directly [28]. The balance value of protein efficiency is also obtained from the ratio of egg period divided by protein consumption [29]. The protein efficiency balance is used to test the effectiveness of ration proteins, which means that if the protein efficiency balance value noticeably decreases then the effectiveness of protein used in the ration is also low [28].

Amino acids contained in the feed are useful for use directly to produce or become other metabolic precursors. One of the amino acids contained in shrimp waste is lysine. Lysine is used directly for production and maintenance [28]. The lysine content in shrimp waste is quite high, namely 4.58% and a source of sulphurous amino acids (S) with a methionine content of 1.26% [22]. The lysine content in shrimp can be an essential amino acid supplementation that can be used to increase egg weight. Egg weight is influenced by proteins, fats and essential amino acids contained in the ration [30].

In addition to the completeness of essential amino acids, shrimp waste also contains a yellow pigment in the form of astaxanthin. Egg yolk or commonly called yolk has a variety of colours ranging from yellow to orange. The colour difference is influenced by beta carotene or the content of vitamin A in the feed. The colour of the yolk in eggs is influenced by the content of xanthophylls and carotenoids contained in the chicken ration [31]. The accumulation of natural pigment astaxanthin is found in many types of shrimp so that if the feed contains more pigment substances it can give a reddish-orange colour [32]. The increase in the yellow colour of the yolk is due to the presence of carotenoid pigments that the shrimp head contains [33]. The use of shrimp waste flour can also improve the colour score of the yolk. This is because shrimp waste flour contains astaxanthin dye which affects pigmentation in the colour of the yolk.

Various studies have been conducted related to the use of feed supplements to balance protein efficiency and egg yolk colour in poultry. Fermented shrimp waste using the bacteria *B. licheniformis, Lactobacillus* sp., and yeast in the form of *S. cerevisiae* can improve the quality of shrimp waste protein containing chitin by increasing the completeness and balance of essential amino acids and having an optimal digestibility [14]. According to [34], the supplementation of various levels of chitosan in duck rations up to 1.5% still has a significant influence on egg production, feed consumption and conversion of duck rations quite well. According to] The addition of garlic flour supplements or shrimp waste of 1.5% each can increase the colour score of the yolk batter. Based on the description, it can be hypothesized that the use of feed supplements fermentation of shrimp waste at a level of 1.5% can result in the balance of protein efficiency and the highest colour of chicken egg yolks.

2. Material and methods

The materials used are Shrimp waste, Isolates of *B. licheniformis, Lactobacillus* sp. and *S. cerevisiae*, aquadest, glucose, yeast extract, tryptone, NaCl, NaOH, $CaCO_3$, pH buffer 4, buffer pH 7, buffer pH 9, and bovine serum albumin. The tools used are stainless jars (reactors), water baths, shaker baths, cup cups, bunsen burners, petri dishes, porcelain cups, centrifuge, funnels, pH-meters, spectrophotometers, test tubes, furnaces, shearing machines, and 40-week-old layer-phase native chickens. The cage used in the form of a cage system with a size of 22 cm× 40 cm × 40 cm is made of bamboo. Each cage is equipped with a feed bin and a place for drinking water.

The treatment rations used in the study were rations without the use of feed supplements fermentation of shrimp waste (R0), rations with the use of feed supplements of 0.5% fermentation of shrimp waste (R1), ration with the use of feed supplement 1.0% fermentation of shrimp waste (R2), ration with the use of feed supplement 1.5% fermentation of shrimp waste (R3), ration with the use of feed supplement 2.0% fermentation of shrimp waste (R4). The ration is made based on the crude protein content of 15% and metabolic energy of 2,750 kcal/kg.

2.1 The procedure for making shrimp waste fermentation products is

- Deproteination. Fermenting Autho-Shaker-Bath (ASB). Shrimp waste is put into a jar of stainless, then inoculated with the inoculum *B. licheniformis* at a dose of 2.0 % (v / w). It is then put into the ASB engine for 2 days at a temperature of 45ºC with a revolution of 120 rpm.
- Demineralization. Deproteination products, then added *Lactobacillus* sp inoculum as much as 2.0 % (v / b), then incubated for 2 days at a temperature of 35ºC, using an ASB engine with a rotation of 120 rpm.
- Fermentation with *S. cerevisiae*. The demineralized product was then fermented using *S. cerevisiae* as much as 3.0 % (v / b), then incubated for 2 days at a temperature of 30ºC, using an ASB engine with a revolution of 120 rpm.
- Binding. The bioprocess product was further supplemented with the mineral selenium (Se) as much as 0.15 ppm (73 ppm in the form of selenite), and the addition of Na-Alginate 5%. Further grinding with a particle size of 100 mash.

The feed ingredients that make up the ration consist of corn, fine bran, soybean meal, fish meal, shrimp waste fermentation feed supplement (SWFFS), bone meal, $CaCO_3$ and Grit. The content of food substances and metabolic energy of the feed ingredients that make up the ration can be seen in Tabel 1. The arrangement of experimental rations used in the study is presented in Table 2. Based on the arrangement of the ration, the nutrient content and metabolic energy of the experimental ration were obtained as presented in Table 3.

Table 1 Metabolizable energy content and nutrient ingredients of experimental feed ingredients

feed ingredients	metabolizable energy	crude protein	crude Fat	crude fibre	calcium	Phos-phorous	lysine	Meth-ionine
	(Kcal/kg)	……………………………. ……… .. (%)………………………………...						
yellow corn	3,370	8.60	3.90	2.00	0.02	0.10	0.24	0.18
rice bran	1,630	12.00	13.00	12.00	0.12	0.21	0.71	0.27
soybean meal	2,240	44.00	0.90	6.00	0.32	0.29	2.90	0.65
Fish meal	2,970	58.00	9.00	1.00	7.70	3.90	6,50	1.80
SWFFS*	2,614	39.29	7.03	7.79	6.81	2.83	3.04	1.46
bone meal	-	-	-	-	29.00	14.00	-	-
$CaCO_3$	-	-	-	-	40.00	-	-	-
Grit	-	-	-	-	30.87	1.11	-	-

Source: Results of the analysis of the Laboratory of Ruminant Animal Nutrition and Animal Feed Chemistry, Faculty of Animal Husbandry, Padjadjaran University (2021); *SWFFS, shrimp waste fermentation feed supplement

Table 2 Experimental ration formulation

no	feed ingredients	R0	R1	R2	R3	R4
		………….…………. (%)…………………..				……
1	yellow corn	63.00	62.68	62.37	62.06	61.74
2	soybean meal	7.50	7.46	7.43	7.39	7.35
3	rice bran	14.25	14.18	14.10	14.04	13.97
4	SWFFS*	0.00	0.50	1.00	1.50	2.00
5	Fish meal	8.00	7.96	7.92	7.88	7.84
6	bone meal	2.50	2.49	2.48	2.46	2.45
7	Grit	2.50	2.49	2.48	2.46	2.45
8	$CaCO_3$	2.25	2.24	2.22	2.21	2.20

*SWFFS, shrimp waste fermentation feed supplement

Table 3 Metabolizable energy content and experimental ration nutrients

nutrient content	R0	R1	R2	R3	R4
metabolizable energy (kcal/kg)	2,761	2,760	2,760	2,759	2,758
Crude protein (%)	15.07	15.18	15.31	15.43	15.55
Crude fat (%)	5.10	5.11	5.12	5.13	5.14
Crude fibre (%)	3.50	3.52	3.54	3.57	3.59
Calcium (%)	3.07	3.09	3.10	3.12	3.14
Phosphorous (%)	0.80	0.82	0.83	0.83	0.85
Lysine (%)	0.99	1.00	1.01	1.02	1.03
Methionine (%)	0.35	0.35	0.36	0.36	0.37

2.2 Observed changes include

2.2.1 Feed consumption

Feed consumption (g) is the amount of feed given minus the residual feed

Feed consumption (g) = Initial feed (g) – Residual Feed (g)

2.2.2 Protein consumption

Protein consumption (g) is the amount of protein consumed by chickens and is calculated by the formula as follows:

Protein consumption (g) = Feed consumption (g) x Ration crude protein content (%).

2.2.3 Egg weight

Egg weights are weighed using digital scales.

2.2.4 Protein efficiency balance (PEB) is the ratio between egg weight and protein consumption.

$$\text{PEB} = \frac{Egg\ weight}{protein\ consumption}$$

2.2.5 Egg yolk score

The yolk score was measured using the *Egg Yolk Colour Chart* on a scale of 1- 15.

2.3 Experimental design and statistical analysis

The study was conducted using an experimental method and used a Complete Randomized Design with 5 kinds of ration treatment and repeated 4 times. The data obtained were analysed with Anava using fingerprints and differences between treatments tested using the Tukey test and to determine the relationship pattern of each treatment using Polynomials Orthogonal.

3. Results and discussion

3.1 Effect of Treatment on Feed Consumption

Table 4 Feed Consumption, Protein Consumption, Egg Weight, Protein Efficiency Balance, and Yolk Colour

Parameters	Treatment				
	R0	R1	R2	R3	R4
Feed consumption (g/b/day)	73.23[a]	74.41[a]	74.42[a]	77.45[ab]	81.25[b]
Protein consumption (g/b/day)	11.04[a]	11.30[a]	11.39[a]	11.95[ab]	12.63[b]
Egg weight (g/grain)	41.75[a]	43.27[a]	44.05[a]	47.36[b]	51.68[c]
Protein Efficiency Balance	3.79[a]	3.83[a]	3.87[a]	3.96[ab]	4.09[b]
Egg Yolk Colour	7.50[a]	8.00[ab]	8.25[ab]	8.58[ab]	9.33[b]

[a, b, c] means different superscripts within the same column significantly (P < 0.05)

Based on the results of statistical analysis of the five treatment rations, namely rations without the use of shrimp waste fermentation products, the ration contains 0.5%, 1.0%, 1.5% and 2.0%. Shrimp waste fermentation products as feed supplements have a significant influence (P<0.05) on feed consumption. The results of the Orthogonal Polynomial test showed that the equation in the linear form produced a noticeable difference with the equivalent of the linear receipt reg result obtained at Y= 72.335 + 3.8179x, and the coefficient of determination was 0.8622 (R2 = 86.22%). The use of shrimp waste fermentation products gives optimal results at the rate of 1.5% (R3) in the ration against feed consumption. This is to improve the quality of nutrients in fermented shrimp waste, namely by decreasing the chitin content in fermented shrimp waste. Decrease in chitin content from the fermentation process of shrimp waste with the help of the bacteria *B. licheniformis*, which frees part of the protein in the form of the monomer N-Acetyl-D-glucosamine and acetylamino from chitin [35,36], as well as the fermentation process by *S. cerevisiae* which helps the process of digestion of food substances in the digestive organs [37]. This causes an increase in palatable in the ration so that there is a difference in the amount of feed consumption that uses shrimp waste fermentation products. As per the opinion of [38] that shrimp waste is fermented with the bacteria *B. licheniformis, Lactobacillus* sp., and yeast in the form of *S. cerevisiae* improving its quality and palatability by providing aromas and flavours that are preferred by livestock. By the opinion of [3] that the amount of ration consumed is also influenced by the palatability of the ration.

3.2 Effect of Treatment on Protein Consumption

Results statistical analysis of the five treatment rations, namely rations without the use of shrimp waste fermentation products, the ration contains 0.5%, 1.0%, 1.5% and 2.0%. Shrimp waste fermentation products as feed supplements have a significant influence ($P<0.05$) on protein consumption. The results of the Orthogonal Polynomial test showed that the equation in the linear form produced a noticeable difference with the equivalent of the linear receipt reg result obtained at $Y= 0.9123 +0.7707x$, and the coefficient of determination was 0.9123 ($R2 = 91.23\%$). The use of shrimp waste fermentation products gives optimal results at the rate of 1.5% (R3) in the ration against protein consumption. According to [37], that protein consumption is influenced by the amount of ration consumption. Feed whose energy is higher the less consumed, vice versa, if the feed energy is low, it will be consumed more and more to meet its needs. Some of the factors that affect protein consumption include the level of energy and protein in the ration. This statement is supported by the opinion of [39], that protein consumption is influenced by metabolic energy content and ration protein. Protein consumption depends on the level of protein ration and the number of rations consumed [29]. This study found that increased feed consumption also affected protein consumption in the Sentul chicken layer phase.

3.3 Effect of Treatment on Egg Weight

The results of the statistical analysis of the five treatment rations, namely rations without the use of shrimp waste fermentation products, the rations contain 0.5%, 1.0%, 1.5% and 2.0%. Shrimp waste fermentation products as feed supplements have a significant influence ($P<0.05$) on egg weight. The results of the Orthogonal Polynomial test showed that the equation in the quadratic form produced a noticeable difference with the equation of the result of quadratic regression obtained at $Y = 41.992 + 0.1505x + 2.3209x\ 2$, and the coefficient of determination was 0.9898 ($R2 = 98.98\%$). The use of shrimp waste fermentation products gives optimal results at the level of 2.0 % (R4) in the ration against the weight of eggs. According to [40], the protein and fat content in the feed will affect the weight of eggs, while according to [41], the protein and amino acid content in the feed also affects the weight of eggs. The types of amino acids that greatly affect the weight of eggs are lysine and methionine. The content of lysine and methionine in the feed in a row range from 0.99%-1.031% and 0.345%-0.367%. This is by the opinion of [4], that an increase in lysine in feed can increase the egg weight of native chickens by 2 g / grain and is higher than that of heavy eggs from the control treatment. A good balance of amino acids and obtaining optimal egg weight in the treatment ration with the use of shrimp waste fermentation products as feed supplements also illustrate the improvement in quality protein ration by fermentation technique on shrimp waste so that it affects the weight of eggs in Sentul chicken. Other factors affect egg weight, namely genetics, feed, age, type of livestock, seasonal changes when livestock lay eggs and body weight of livestock [37,39].

3.4 Effect of Treatment on Protein Efficiency Balance

Based on the results of statistical analysis of the five treatment rations, namely rations without the use of shrimp waste fermentation products, the ration contains 0.5%, 1.0%, 1.5% and 2.0%. Shrimp waste fermentation products as feed supplements have a significant influence ($P<0.05$) on the balance of protein efficiency. The results of the Orthogonal Polynomial test showed that the equation in the linear form produced a noticeable difference with the equivalent of the linear receipt reg result obtained at $Y = 3.7593 + 0.1494x$, and the coefficient of determination was 0.9371 ($R2 = 93.71\%$). The use of shrimp waste fermentation products gives optimal results at the level of 1.0% (R2) in the ration against the balance of protein efficiency. This proves that the fermentation process in shrimp waste with the bacteria *B. licheniformis, Lactobacillus* sp., and yeast *S. cerevisiae* can improve the quality of ration proteins by increasing the completeness and balance of essential amino acids contained in them and having optimal digestibility. The balance of the amino acids methionine and lysine in the treatment ration with the rate of use of shrimp waste fermentation products as feed supplements between 0.5%-2% is still within the limits of the normal balance of the amino acids methionine and lysine. In line with [28] opinion, the balance of the amino acids methionine and lysine between 0.38:1 and 0.42:1 in the chicken ration is still within normal limits, so the balance value of the efficiency of the resulting protein is optimal. The greater the balance value of protein efficiency, the better because livestock can utilize protein effectively. This is to the opinion of [42], that the higher the value of the protein efficiency ratio, the more efficiently livestock utilizes the protein consumed.

3.5 Effect of Treatment on Egg Yolk Colour

Based on the results of statistical analysis of the five treatment rations, namely rations without the use of shrimp waste fermentation products, the ration contains 0.5%, 1.0%, 1.5% and 2.0%. Shrimp waste fermentation products as feed supplements have a significant influence ($P<0.05$) on the colour of the yolk. The results of the Orthogonal Polynomial test showed that the equation in the linear form produced a noticeable difference with the equivalent of the linear receipt reg result obtained at $Y = 7.4883 + 0.85x$, and the determination coefficient 0.9633 ($R2=96.33\%$). The use of shrimp waste fermentation products gives optimal results at the level of 1.0% (R2) in the ration against the colour of the yolk. According to that the colour score of the egg yolk is in the range of a score of 7-12. The egg yolk colour index score is increasing with the increase in the level of the feeding of shrimp waste fermentation products in the ration. This indicates that the stalking pigment contained in the shrimp head plays a very important role in increasing the colour index of the yolk so that with the increase in the level of giving fermentation products of shrimp waste, the colour index of the yolk also increases. This is because shrimp contains a yellow pigment in the form of astaxanthin. The accumulation of natural pigment astaxanthin is found in many types of shrimp so that if the feed contains more pigment substances it can give a reddish-orange colour [3]. The results of the study reported by [43], astaxanthin is the largest pigment composition in crustaceans (crabs, lobsters, and shrimp). Factors that can influence the increase in the colour of the yolk are the content of chitosan, carotenoids and pigment substances in the ration. This is to the opinion [32], that the colour of the yolk is influenced by substances contained in the ration such as chitosan, xanthosis, carotenoids and chlorophyll.

4. Conclusion

Fermentation products of shrimp waste from bioprocess processing by the bacteria *B. licheniformis, Lactobacillus* sp., and *S. cerevisiae* yeast can be used as a feed supplement in native chicken ration formulations. The use of shrimp waste fermentation products of 1.0 levels in the ration results in an optimal balance of protein efficiency and yolk colour. The pattern of the relationship between treatments to the balance of protein efficiency is obtained in linear form with the equation $y = 0.1494x + 3.7593$ ($R2 = 93.71\%$). and in the colour of the yolk in linear form with the equation $y = 0.85x + 7.4883$ ($R2=96.33\%$).

Suggestion

Shrimp waste fermentation products can be used at a rate of 1.0-2.0% in the feed formula of the local poultry layer phase and livestock other than local poultry.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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