Effect of fermented shrimp shell supplementation of low protein diet on the performance of Indonesian native chicken

Abun Abun, Tuti Widjastuti and Kiki Haetami

Departement of Animal Nutrition and Feed Technology, Padjadjaran University, Sumedang-West Java, Indonesia; Departement of Animal Production, Padjadjaran University, Sumedang-West Java, Indonesia; Departement of Fisheries, Padjadjaran University, Sumedang-West Java, Indonesia

ABSTRACT
A high-quality diet during the growth period is necessary to support poultry performance. Shrimp shell fermentation with Bacillus licheniformis, Lactobacillus sp., and Saccharomyces cerevisiae (SSFBLS) can improve the quality of low protein diet in Indonesian native chickens. This study was conducted to evaluate the effect of SSFBLS supplementation in a low protein diet on the growth performance of Indonesian native chickens. Three hundred native chickens were assigned to six treatments with five replications using a completely randomized design. The treatments consisted of a low protein diet formula (15%) with SSFBLS levels 0, 5, 10, 15, and 20% and RS as a high protein recommended diet (18% protein). The evaluation results showed that the use of SSFBLS supplementation of 10% in low-protein diets, in (P < 0.05) improved body weight gain, feed conversion, and feed efficiency than at 0-5%, and was similar to the high-protein diet. SSFBLS 20% increased the protein content of meat and reduced cholesterol levels of meat and ammonia excreta. Revenue on feed costs was highest at 10% SSFBLS. The added value of SSFBLS, which contains nutrients and chitosan, can be used as an ingredient in feed formulas to support the growth performance of Indonesian native chickens.

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Highlights
- Native chicken is a livestock commodity that can be adapted to various types of feed. Still, its superiority as a source of low-cholesterol food protein needs to be supported by high-quality diets.
- Chitin protein material from microbiologically engineered shrimp waste can be transformed into a high-quality feed source used in low-protein diet formulas.
- Fermented shrimp waste products (SSFBLS) can replace the use of fish meal, improve chicken growth performance reduce meat cholesterol, and increase income. They can be used up to 10% in feed formulas.

Introduction
The Indonesian native chicken type of Sentul is one of the local chicken family that has been cultivated among Indonesian indigenous native chickens (Gallus domesticus). Still, the level of their productivity needs to be increased (Widjastuti et al., 2020). Sentul chickens are primarily fighting cocks; however, they are kept by farmers as dual-purpose chickens and currently as meat producers. The plumage colours of Sentul chickens are dominated by grey with significant variation, and male chickens can reach a bodyweight of around 500 g with a carcass percentage of 66.5% within eight weeks (Asmara et al., 2017).

To support the productivity of Sentul chickens, high-quality feed is necessary Indonesian native chicken has high adaptability to the environment, giving it great potential to be developed. However, farmers must pay attention to the time required to reach the harvesting ages of Indonesian native chickens to meet the market’s demands by considering the efficiency of the nutritional ratios used for producing a high body weight gain (Widjastuti et al., 2020). However, to date, there is little information about the rations needed for Indonesian Sentul chickens, which require balanced nutritional intake to maintain performance.

Development of the environment-friendly livestock sub-sector would help optimize the use of natural resources, especially the industrial, agricultural, and fishery wastes that are processed into feed. Indonesia is the third-largest shrimp-producing country in the world. Crustacean shell ensembles production in situ are alternatives to the use of low-cost, environment-friendly technologies (Wahyuntari et al., 2011). It was reported that approximately 6–8 million tons of crustacean waste is produced worldwide every year, and only a small portion is used as chitosan products (Mao et al., 2017). Each year, about 80-90% of the total amount of shrimp meat is exported in the form of frozen (headless) shrimp and shrimp skin (peeled) (Judawisastra et al., 2012). The shells and exoskeletons of shrimp are industrial waste from shrimp freezing plants and can reach 45-48% of the shrimp’s intact weight, featuring enough protein and phosphorous content (Mao et al., 2017; Kumari and Rupak, 2020).
However, shrimp waste is used for various industrial purposes related to production and characterization, leading to deproteinization from the chitin material. In recent years, there has been much success in making waste useful for biomass protein. In utilizing shrimp shells’ nutrients, treatment is required via gradual liquid-state fermentation through microbes. It is better to characterize some bacterial species capable of degrading specific substrates to improve their nutrient quality (Alshelmani et al., 2013; Alshelmani et al., 2015). Bacillus licheniformis is a bacterium capable of producing relatively high amounts of protease and chitinase, allowing it to free some nitrogen or proteins from chitin bonds (Haddar et al., 2011; Alshelmani et al., 2014).

Chitinous structures are mainly of ectodermal origin in multicellular animals and form the characteristic exoskeleton of most invertebrates. Chitin constitutes more than half of the total organic matter in chitinous structures (Tharanathan et al., 2003). Chitosan is a compound derived from the deacetylation of chitin which is sourced from shrimp shell waste (Cira et al., 2000). According to Mao et al. (2017), chitin is the most abundant natural biopolymer derived from crustaceans’ exoskeletons, including shrimp shells. Furthermore, as the shrimp by-products have been identified as an animal protein source of great potential, more researchers are focused on how to extract proteins from shrimp-shell efficiently. In recent years, there has been much success in making waste useful for biomass protein. New challenges to agricultural research and development include shifting focus to less favorable environments through fermentation and providing inputs to processes that find sustainable solutions.

The main objective of this research was based on using an available shrimp-shell product-low protein diet in Indonesian native chicken performance. The outcome of this research is expected to reduce fish meal use, ammonia excreta, and meat cholesterol in terms of increasing the economic efficiency of Indonesian Sentul chicken farms.

Shrimp waste can be used as an alternative source of animal protein to replace a fish meal because it is processed first through solid substrate fermentation (SSFBLs). Bioprocess utilization of waste can improve nutritional value, so the purpose of using SSFBLS is to increase the efficiency of rations and IOPC. Health status is influenced by cholesterol and blood hematologic content; with the level of SSFBLS, we will determine what level of SSFBLS is safe for health and improve performance.

Materials and method

A step of the experiment includes (1) Preparation of shrimp shell fermentation with Bacillus licheniformis, Lactobacillus sp., and Saccharomyces cerevisiae (SSFBLS) in liquid state fermentation and (2) Main experiment (feeding trial) of shrimp shell-low protein diet.

Fermentation of shrimp shell

Shrimp shells were collected from cephalothorax and washed and dried under the sun until the moisture content was around 10%. B. licheniformis, Lactobacillus sp., and S. cerevisiae isolates were stored and maintained on nutrients for 24 h at 37 °C. The inoculum starter included glucose, yeast extract, tryptone, NaCl, NaOH, CaCO3, pH buffer solution (pH 4, pH 7, and pH 9), and bovine serum albumin. A standard mineral solution (SMS) was added gradually during the fermented process in a shaker water bath (Julabo Shaking Water Bath SW-23, Yatherm Sci. Germany) at rotary 120 rpm, using B. licheniformis (dose 2% v/w, incubated for two days at 45 °C), further Lactobacillus sp. (2% v/w, two days, 35–37 °C) and S. cerevisiae (3% v/w, two days, 30 °C). Culture conditions through the process gradually fermented were as follows:

- Fermented by B. licheniformis includes: preparing the starter inoculum of the bacteria and then cultivating the starter in a 125 ml Erlenmeyer flask containing 50 ml of sterile broth solution set to pH 7, regulated using HCl 1N. The broth solution was supplemented with B. licheniformis and then incubated at 45 °C with adding standard mineral solution. 0.5% (b/v) yeast extract, 0.5% (b/v) KH2PO4, 0.1% (b/v) CaCl2, 0.5% (b/v) NaCl, and 0.05% (b/v) MgSO4.
- Fermentation by Lactobacillus sp.: prepared the starter inoculum Lactobacillus sp. and then cultivated the starter in an Erlenmeyer flask. Lactobacillus sp. was inserted into the broth solution, which was then incubated at 35-37°C with adding a mineral solution, consisting of 0.5% (b/v) yeast extract, 0.5% NH4NO3, 0.05% KCl, 0.05% MgSO4, 0.01% FeSO4, and 0.001% SePO4.
- Fermented by S. cerevisiae: prepared the starter inoculum S. cerevisiae with a broth solution and then incubated the starter in an incubator for 2 days at 30 °C, with adding a SMS consist of 0.5% (b/v) yeast extract, 0.5% NH4NO3, 0.05% KCl, 0.05% MgSO4, 0.01% FeSO4, 0.001% SePO4, and 0.5% CaCO3.

Feeding trial

Heaters remained on to keep the room temperature constant. The chicks were allocated randomly into 30 units with ten birds per cage (100 × 100 × 100 cm3) made of an iron frame and a wireram. Each group of pens was equipped with a feeding and drinking area made from plastic. Collected data included the initial body weight, consumed feed, and the weight gained every week. The feeding trial was carried out with 300 birds for ten weeks. The blood sampling was carried out when the chickens were ten weeks of age to examine the total erythrocytes, leukocytes, hematocrits, and hemoglobin. The meat quality was determined according to the sample of a carcass and then the cholesterol and protein content was determined. Ammonia was excreted once every two weeks. The study was male and had an initial weight variation coefficient of 7.57% (10 birds per pen). The feed ingredients for the rations included yellow corn, soybean meal, rice bran, fish meal, CaCO3, and SSFBLS, which are shown in Table 1.

The feeding trial used an experimental method with a completely randomized design (CRD), consisting of five SSFBLS levels in the low diet formula/negative control (R0 = CP 15%); R1 = ration containing 5% SSFBLS/CP 15%; R2 = 10% SSFBLS/CP 15%; R3 = 15% SSFBLS/CP 15%; R4 = 20% SSFBLS/CP 15%;
and R₅ = standard ration as positive control CP 18%), and each treatment was repeated five times. The standard diet (R₅) was compiled based on the recommendations of the Indonesian National Standard (2013).

**Growth performance, blood metabolites, and carcass yields**

Every week during the experiment, feed intake (FI) and body weight gain (BWG) of the native chickens were determined on a replicate basis after 4-h fasting. Using the FI and BWG, feed efficiency (FE), and feed conversion ratio (FCR) (FCR = g feed intake/g body weight gain) were calculated. Blood samples were collected from two Sentul native chickens per group through wing veins on d 70 of the experiment. These samples were collected into clean plastic tubes containing anticoagulant EDTA. They were kept for two hours at room temperature and centrifuged at 2000g for 5 min at 4°C, and levels of erythrocytes, leucocytes, hematocrits, and hemoglobin were determined.

Day-old chickens, as many as two native chickens from each replicate with a bodyweight close to the treatment average, were randomly chosen and weighted individually after 4-h fasting before feeding trials. Rearing period for each treatment along 70 days. Chickens were sacrificed by cervical dislocation and eviscerated carcass (Alshelmani et al. 2016), and levels of erythrocytes, leucocytes, hematocrits, and hemoglobin were determined.

Excreta was removed every two weeks and collected. Ammonia (NH₃) was measured fortnightly following APHA 1992 and chemical kits.

The carcass yield and income over feed of cost (IOFC) were calculated as follows:

\[
\text{Carcass yield} = \left( \frac{\text{empty carcass weight} + \text{edible offal}}{\text{live pre-slaughtering weight}} \right) \times 100.
\]

\[
\text{IOFC} = \left[ \frac{\text{average weight of bird, kg} \times \text{price/kg live weight}}{\text{price of day-old chick} + \text{total feed consumed} \times \text{price of feed}} \right] \times 100.
\]

Ammonia value is obtained by taking excreta samples after feeding and then testing in the laboratory.

**Statistical analysis**

Feed intake of the native chickens and their average body weight gain were recorded at weekly intervals, compiled, and compared upon the completion of the experiment. A completely randomized design (CRD) was used for the six treatments, and each treatment was replicated five times. The obtained data were tabulated and compiled in the excel worksheet. And reported feeding treatment data were statistically examined via a one-way analysis of variance (ANOVA). Furthermore, treatment differences were tested using a Duncan Multiple Range Test. Statistical significance was accepted at \( P < 0.05 \).

### Table 1. Diets formula

| Raw material | R₀ | R₁ | R₂ | R₃ | R₄ | R₅ |
|--------------|----|----|----|----|----|----|
| SSFBLS*      | 0.00 | 5.00 | 10.00 | 15.00 | 20.00 | 0.00 |
| Fish meal    | 8.00 | 6.50 | 3.75 | 1.25 | 0.00 | 9.25 |
| Yellow corn  | 58.00 | 58.00 | 58.00 | 58.00 | 60.00 | 56.00 |
| Rice bran    | 28.00 | 26.75 | 24.75 | 23.00 | 18.00 | 21.50 |
| Soybean meal | 4.75 | 2.50 | 2.25 | 1.50 | 0.00 | 12.00 |
| Bone meal    | 0.75 | 0.75 | 0.75 | 0.75 | 1.00 | 0.75 |
| CaCO₃        | 0.50 | 0.50 | 0.50 | 0.50 | 1.00 | 0.50 |
| Total        | 100 | 100 | 100 | 100 | 100 | 100 |

| Crude protein (%)** | 15.08 | 15.03 | 15.05 | 15.03 | 15.18 | 18.04 |
| Extract ether (%)** | 6.66 | 6.70 | 6.54 | 6.43 | 6.09 | 6.92 |
| Crude fibre (%)**   | 4.89 | 4.97 | 5.08 | 5.19 | 4.92 | 4.51 |
| Calcium (%)**       | 1.05 | 1.27 | 1.39 | 1.54 | 2.03 | 1.16 |
| Phosphor (%)**      | 0.58 | 0.65 | 0.68 | 0.72 | 0.84 | 0.63 |
| Lysine (%)***       | 0.97 | 0.95 | 0.90 | 0.86 | 0.86 | 1.21 |
| Methionine (%)***   | 0.35 | 0.38 | 0.40 | 0.42 | 0.45 | 0.40 |
| Meth. + cyst. (%)*** | 0.67 | 0.69 | 0.70 | 0.71 | 0.73 | 0.75 |
| ME (kcal/kg)**      | 2775 | 2770 | 2781 | 2792 | 2838 | 2781 |
| Chitosan (%)***     | 0 | 0.160 | 0.479 | 0.638 | 0.638 | 0 |

*SSFBLS, Shrimp shells gradually fermented using B. licheniformis, Lactobacillus sp., and S. cerevisiae
**Laboratory of Feed Chemistry and Nutrition of Ruminant Padjadjaran University (2019); ***SIG Laboratory (2019)
Results

Description of SSFBLS in liquid state fermentation

The preparation of processing conditions of the shrimp shell was done with microbes, in the previous study about the dissolved nutrient released from the chitin bonds, and the optimum was obtained at a solid–liquid ratio of 1:9 with a containing of entire protein content (wet material) as much as 35.62%, 13.91% chitin, 5.75% calcium, 1.34% phosphorous, and 7.79% fat (Abun et al., 2021).

Performance of native chickens fed a diet with SSFBLS

The average feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), and feed efficiency (FE) during the ten-week feeding trial are presented in Table 3.

Collected data included the initial body weight, the feed intake, and the weight gained every week. Data on the measurement of these factors relative to the performance of the Indonesian Sentul native chicken growth phase (10 weeks) An increase in dry matter (DM) and its components did not influence (P > 0.05) feed intake of a diet containing different levels of SSFBLS (Table 3). The mean of BWG and feed efficiency were significant (P < 0.05) and increased following feed intakes of Sentul chickens.

The average value of the Indonesian native chicken feed efficiency during the study ranged from 36.91–39.44%. Based on this information, the value of the feed efficiency of the chickens was in a normal range. The use of SSFBLS supplementation in a low protein diet at level 10% resulted in BWG, FCR, and FE significantly (P < 0.05) better than 0, 5%, and 20%, but not significant (P > 0.05) with the level SSFBLS 15% and the standard feed.

Meat and blood parameters

The weight of the carcass and its quality, as well as the cholesterol and protein content of the chicken’s meat-growth phase, are shown in Table 4.

The absolute weight of the carcass was significantly different (P < 0.05) among the six dietary treatments, but the percentage of the carcass was similar (P > 0.05). Significantly higher carcass weight was obtained for R3 (CP 15%) and R4 (CP 18%) (P < 0.05) better than R2, R3, and R4 but not significant (P > 0.05) with R2 and R3 (Table 4). The meat protein in the carcass of Sentul chickens with treatment with 20% SSFBLS was significantly (P < 0.05) higher than with no use of SSFBLS in rations (R0) treatments (R0, R1, and R5). The average IOFC for R2 and R4 was higher (P < 0.05) than for R2 and R4. At the same time, R3 was not significantly (P > 0.05) from R4 and R5 (P > 0.05). In terms of the IOFC, chickens with a feed of the SSFBLS 10% was the best of the other at a value of 27,755 IDR/birds/starter period.

No significant differences (P > 0.05) were shown in the mean haematologic parameters of the blood (Table 5). The number of erythrocytes and leukocytes was standard in the chickens, ranging from 2.0–3.2 × 10^6/mm³ and 16–40 × 10^3 items/mm³ respectively (Weiss and Wardrop, 2010).

Ammonia excreta was significantly different (P < 0.05) among treatments, i.e. decreased along with increasing use of SSFBLS (Table 5). The highest (P < 0.05) ammonia excreta (11.21 ml M) were obtained in the excreta of the chicken diet with a higher proportion of protein (18%) without SSFBLS; otherwise, the lowest (P < 0.05) was in the ammonia excreta of chickens (4.02 ml M) fed a diet containing SSFBLS 20%, and both of them significantly different with the other treatments.

Discussion

Ration descriptive

Table 1 and Table 2 informed the composition of nutrient content at each treatment and the nutrient of raw feed shrimp shells compared with SSFBLS. In Table 2, information about metabolizable energy (kcal/kg) improved significantly (P < 0.05) from 2,599–2,904 (11.74%) and an increase in energy efficiency (28.8%) in this study chicken before bigger. The other essential nutrients also increased significantly (P < 0.05) after fermentation, with % of change including lysin (44.08%), methionine (15.87%), calcium (22.92%), phosphorous (116.03%), organic acid (45.61%) and chitosan (68.78%); this result indicates the breakdown of chitin bonds, which makes the material’s nutrients more available for use by living organisms. The nutritional content and metabolizable energy necessarily reflect the quality of the nutrients used as feed for meat conversion in livestock (Liermann et al., 2019).

Table 2 showed that fermentation using microbes in liquid media released the whole fermentation product and then dried and yielded dry material SSFBLS with nutrient content, i.e. 47.45% crude protein. Compared to other reports, including the production of high protease activity, bacterial strains were selected for producing chitosan from fishery by-products via bioprocessing. Recovered chitin from biological treatment

| Table 2. The average nutrient content and energy of shrimp shells and SSFBLS |
|-------------------------------|---------------------------------|-----------------|
| Ingredients                  | Shrimp shells                    | SSFBLS*         |
| Crude Protein (%)            | 43.41 a                          | 47.45 b         |
| Crude Lipid (%)              | 13.54 a                          | 7.03 b          |
| Crude Fibber (%)             | 18.25 a                          | 7.79 b          |
| Calcium (%)                  | 5.54 a                           | 6.81 b          |
| Phosphorous (%)              | 1.31 a                           | 2.83 b          |
| Lysine (%)                   | 2.11 a                           | 3.04 b          |
| Methionine (%)               | 1.26 a                           | 1.46 b          |
| Cysteine (%)                 | 0.51                             | 0.54            |
| Organic Acid (%)             | 1.14 a                           | 1.66 b          |
| Chitosan (%)                 | 1.89 a                           | 3.19 b          |
| Gross Energy (kcal/kg)       | 3,892 a                          | 3,379 b         |
| Metabolizable Energy (kcal/kg)| 2,599 a                          | 2,904 b         |
| Energy Efficiency (%)        | 66.78 a                          | 86.01 b         |

Approximate analyzed % dry basis

*Means with different superscripts within the same row is significantly different (P < 0.05)

**SEM: Standard error of the mean
recorded a wide range of residual protein, for instance, 7.17%–22%, with the yield mainly depending on shrimp body parts in which shrimp shell revealed the highest recovery rate (22.12%, non-cook giant tiger shrimp; 22.66%, non-cook tiger shrimp; 23.23%, cooked tiger shrimp) and generally, the yield of chitin extraction from shrimp could be found at a range from 5% to 20% (Doan et al., 2019). These wet materials were part of the whole choice SSFBLS then dried and yielded the final content of crude protein, a slight increase compared to the initial matter, i.e. from 43.41% to 47.45% (9.31%), and the degree of change in calcium, phosphorous respectively 22.92% and 116.03% (Table 2). According to Doan et al. (2019), before fermentation, the ratio of protein/ash/chitin in shrimp waste (head and shell) was 44.77/25.10/30.13 (%/%/%.); during fermentation, the ratio of protein was decreased, while ash (calcium and phosphorous) and chitin were increased. Pre-digestion using three types of microbes gradually breaks down the protein in the shrimp shells via \( B. licheniformis \), pre-digestion of minerals via \( Lactobacillus \) sp., and then fermentation using microbial activity via \( S. cerevisiae \). It was further explained by Abun et al. (2021) that in a stepwise bioprocess using three types of microbes, pre-digestion and decomposition occur through microbial enzymes the chitin bonds are released.

**Table 3.** Effect of SSFBLS in rations on feed intake, weight gain, feed conversion ratio, and feed efficiency of the native chicken growth phase over ten weeks

| Treatment | Initial weight | Final weight | Feed intake | Body weight gain | Feed conversion (index) | Feed efficiency (%) |
|-----------|---------------|--------------|-------------|-----------------|-------------------------|---------------------|
| R0        | 26.92         | 1,095.00     | 2,892.69    | 1,068.08        | 2.71                    | 36.91               |
| R1        | 28.22         | 1,095.55     | 2,845.04    | 1,076.33        | 2.67                    | 37.51               |
| R2        | 28.18         | 1,158.18     | 2,872.59    | 1,130.00        | 2.54                    | 39.33               |
| R3        | 28.12         | 1,137.51     | 2,849.42    | 1,109.30        | 2.57                    | 38.93               |
| R4        | 28.14         | 1,101.69     | 2,817.76    | 1,073.55        | 2.63                    | 38.09               |
| R5        | 28.10         | 1,177.36     | 2,913.61    | 1,149.26        | 2.54                    | 39.44               |
| SEM**     | 0.38          | 20.93        | 27.221      | 20.76           | 0.035                   | 0.507               |

**Table 4.** The effect of SSFBLS in rations on carcass weight and carcass percentage, as well as the cholesterol and protein content of the native chicken growth phase

| Treatment | Carcass Weight (g) | Carcass percentage (%) | Meat Cholesterol (mg/g) | Meat Protein (%) | IOFC** | IDR/bird |
|-----------|--------------------|------------------------|-------------------------|------------------|--------|----------|
| R0        | 777.38             | 71.01                  | 9.46                    | 22.50            | 25.007  |
| R1        | 784.47             | 71.58                  | 9.39                    | 23.03            | 25.508  |
| R2        | 825.20             | 71.26                  | 8.90                    | 23.23            | 27.755  |
| R3        | 828.36             | 72.83                  | 8.83                    | 23.27            | 27.348  |
| R4        | 792.60             | 71.94                  | 8.78                    | 23.30            | 26.175  |
| R5        | 844.45             | 71.74                  | 9.36                    | 23.06            | 26.299  |
| SEM**     | 16.433             | 0.597                  | 0.192                   | 0.197            | 611.380  |

**Means with different superscripts within the same column are significantly different \((P < 0.05)\)**

**SEM: Standard error of the mean**

**IOFC: Income over feed of cost**

Chitosan in this ration with a level of SSFBLS 5-20% was about the range 0.160-0.638%(Table 1). According to Tharanathan et al. (2003), the use of crustacean shell ensembles and lactic acid production in situ are alternatives to low-cost, environmentally friendly technologies. Bacillus sp. has been used for shrimp by-product fermentation and chitosan production. The meat processing industry contains an appreciable amount of protein that can be recovered with the use of chitosan; this protein, after drying and sterilization, makes a great source of feed additives for farm animals (Rout, 2001). According to Skorik et al. (2017), some essential properties of biomedicinal use of Chitosan, such as antioxidant activity and haemostatics effect, were evaluated, one of them being that chitosan can initiate hemostasis.

The level of 10% SSFBLS in a low protein diet over ten weeks results in gain performance (1,130 g/head/day). The performance of Sentul chickens in the main research (feeding trial) was obtained the highest \((P < 0.05)\) BWG with 10% SSFBLS in a low protein diet (CP 15%), equivalent to standard feed with a control/CP 18% (R3), respectively 1,130 and 1,149 g/head/day, and the lowest weight gain \((P < 0.05)\) was obtained in the 0-5% SSFBLS treatment amounting to 1,067-1,068 g/head/day. The Duncan test results showed that using 10-15% SSFBLS product in the diets resulted in the same BWG and feed conversion as the diet with high protein content (18%). From Table 1, it can be calculated that Ca:P = R0 1.81:1, R3 1.84:1, and treatment ration 5% 1.95:1, R3 (10% SSFBLS) 2.04:1, R3 2.14:1, and R4 2.42:1, which indicates that the ration with SSFLS 10-15%, has a balance of Ca and P at R2 according to the required standard, which is 2:1 for growing chickens (Brito et al., 2020; Liermann et al., 2019). When viewed from the lysine and methionine balance, (lys: methionine) = R0 2.07:1, R3 3.03:1, and treatment ration 5% 2.50:1, R2 2.25:1, R3 (15% SSFBLS) 2.05:1, and R3 1.91:1, R3 has the best balance 2:1. This indicates that using 10-15% SSFBLS products can support the optimal growth phase of Sentul chickens (Abun et al., 2021; Trela et al., 2020).

Level use of up to 15% SSFBLS products in the growth phase of the native chicken did not hurt BWG. Although the diet had a lower protein content than the positive control (R3), the weight gain when using up to 15% of the SSFBLS product improved growth performance. Fibre components that are difficult to digest (chitin bonds in shrimp waste) can be overcome by the presence of the proteolytic enzymes produced by \( B. licheniformis \), followed by mineralization via \( Lactobacillus \) sp.
and fermentation by *S. cerevisiae*. When viewed based on the composition of the raw materials and the nutrient content of the ration (Table 2), the use of SSFBLS products can reduce (primarily) the use of fish meals without changing the nutrient content of Sentul chicken rations. Overall, the range of nutrients other than protein (fat, crude fibre, calcium, and phosphorus) fulfills the needs for the growth phase of Sentul chickens. SSFBLS products can reduce fish meal protein sources that are still imported.

Based on the results of measuring the BWG every week, there was an increase in growth, which shows that the use of SSFBLS consumed can support the performance of Sentul chickens. This led to Table 1, which showed the iso-energy content at all treatments, such that there was no difference in the consumed. The information on the not significant feed intake among treatments in Table 3 indicated that the use of SSFBLS products did not cause a decrease in ration consumption compared to the basal and standard feed (R0 and RS). This shows that the level of SSFBLS up to 20% in the diet does not yield odors, tastes, colours, or textures disliked by Sentul chickens, leading to a decrease in ration consumption. According to Lierman et al. (2019) and Lyu et al. (2019), the physical structures of the feed ingredients also determine the amount of feed intake and so to nutrient digestibility (Alshelmani et al. 2021). The chemical structure provides an overview of the physical form of chitin from tiger shrimp waste. Native poultry prefers a fibrous physical condition; it was further emphasized that including flour as a feed ingredient makes it difficult for chickens to swallow food and causes livestock to drink more.

Bioprocessing has a positive effect on reducing crude fibre (Zamani et al., 2016) from 18.25% to 7.79%, which the chitinous in SSFBLS few of them remove become chitosan. The use of chitin as a carbon source in bioprocessing using microorganisms will result in the production of two extracellular end products simultaneously: microbial enzymes, including chitinolytic enzymes as the main product, and chitin-protein hydrolysis products as by-products that can increase the use-value of the shell. Zaki et al. (2015) showed that chitosan could improve intestinal epithelial health and produce longer intestinal villi length in sea bass (*Dicentrarchus labrax*).

The health status of chickens can be determined by the number of erythrocytes and leucocytes and the amount of hematocrit and hemoglobin in chicken blood (Movahhedkhah et al., 2019). Weiss and Wardrop (2010) range from 2.0 - 3.2 × 10⁶ grains/mm³ for erythrocytes and between 16 - 40 × 10³ grains/mm³ for leucocytes. The normal hematocrit and hemoglobin values in chickens were in the range of 24-43% and 7.0 - 13.0 g/dL, respectively (Widjastuti et al., 2020). The study results on the hematological value of chicken blood remained normal; therefore, the chickens did not experience disturbances in their physiological blood systems. A good haematological condition following livestock health standards causes oxygen and food substances in the body smooth. The metabolic processes in the body improve and can increase the productivity of Sentul chicken.

Table 5 showed that the use of SSFBLS had a positive effect on decreasing ammonia disposal. Ammonia is a final waste from organisms, including chickens. Ammonia discharge is toxic and causes environmental pollution if the amount exceeds the tolerance limit. The ammonia content of the ration was significantly influenced by the level of SSFBLS administration. This shows a role for SSFBLS processing technology inefficient nitrogen utilization that exchange of chitin material became chitosan. During the enzymatic process, lactic acid-producing bacteria demineralize crustacean shells instead of acidic treatment. The obtained lactic acid reacts with calcium carbonate yielding calcium lactate, which can be precipitated and removed. *Bacillus* sp. are bacteria that also produce chitin deacetylase and can be used to generate chitosan (Tharanathan et al., 2003). Shi-bin and Hong (2012) stated that for land animals, chitosan could be used as a feed additive because it has low side effects on growth, increases growth, improves immune function, inhibits intestinal pathogenic microbes, and lowers cholesterol.

The cholesterol content of Sentul chicken meat, based on the results of this study, ranges from 8.78–9.46 µg/mg, and the content of protein ranges from 22.50-23.30%. Chicken cholesterol ranges from 100 to 120 mg (Yadav and Jha, 2019), and the cholesterol content in the blood of a small animal is considered safe if it does not exceed 225 mg/dL (Sugano et al., 1994). The cholesterol content and the results of this study indicate that the use of SSFBLS products fermented via *B. licheniformis*, followed by demineralization with *Lactobacillus* sp, and lastly, fermentation with the microbial intervention of *S. cerevisiae* did not reduce the percentage of the carcass. Similarly, Senz et al. (2019) showed that using *Lactobacillus acidophilus* as an inoculant in ration fermentation significantly improved carcass growth and quality.

Protein carcass result using 10, 15, and 20% SSFBLS was significantly (*P*< 0.05) higher than 0% SSFBLS (basal diet).

**Table 5. Hematological value of blood and nitrogen exhaust of native chicken feces**

| Treatment* | Erythrocytes (x 10⁶/mm³) | Leukocyte (x 10⁶/mm³) | Haematocrit (%) | Hemoglobin (g/dL) | NH₃ (µg/mg) |
|------------|--------------------------|-----------------------|----------------|------------------|-------------|
| R₀         | 2.36                     | 36.09                 | 32.60          | 9.46             | 9.43        |
| R₁         | 2.39                     | 34.95                 | 33.40          | 9.80             | 7.53        |
| R₂         | 2.40                     | 34.65                 | 33.80          | 10.20            | 7.00        |
| R₃         | 2.46                     | 34.74                 | 34.20          | 10.44            | 4.80        |
| R₄         | 2.38                     | 34.44                 | 33.60          | 10.06            | 4.02        |
| RS         | 2.42                     | 36.39                 | 34.40          | 10.74            | 11.21       |
| SEM*       | 0.027                    | 0.743                 | 0.460          | 0.390            | 1.133       |

*abc*, *def* means with different superscripts within the same column are significantly different (*P* < 0.05)

*R₀ = CP 15%; R₁ = ration containing 5% SSFBLS/CP 15%; R₂ = 10% SSFBLS/CP 15%; R₃ = 15% SSFBLS/CP 15%; R₄ = 20% SSFBLS/CP 15%; RS = standard ration/CP 18%.

**SEM**: Standard error of the mean.
treatment, meaning that the protein quality in SSFBLS can supply the single-cell protein requirements of birds. Probiotics play an essential role in stabilizing the intestinal ecosystems of animals by enhancing the growth of beneficial bacteria and competing with pathogenic bacteria in the intestine (Azzam et al., 2019). According to the research by Widjastuti et al. (2021) and Saleh et al. (2020), the nutrients derived from feed ingredients will be used by living meat protein. The wastewater released from food processing plants, typically seafood, dairy, or meat processing industries contain an appreciable amount of protein which can be recovered with the use of chitosan; this protein, after drying and sterilization, makes a great source of feed additives for farm animals (Haetami et al., 2020; Rout, 2001). Nutritional content and digestibility necessarily reflect the quality of the nutrients used as feed for meat conversion in livestock (Liermann et al., 2019).

The results of this study indicated that SSFBLS as a protein ration supplement produced a carcass presentation that was not significantly different \((P > 0.05)\) from that of rations featuring more fish meal content. The preparation of feed consisting of a mixture of several kinds of feed ingredients could be beneficial by obviating the shortcomings of each feed ingredient (Cullere et al., 2019; Ross et al., 2019). Alternate SSFBLS feed ingredients were obtained from gradual fermentation using three types of microbes, protein decomposed by \(B. \text{licheniformis}\), the pre-digestion of minerals by \(Lactobacillus\) sp., and, at the end of the process, fermentation carried out via the microbial intervention of \(S. \text{cerevisiae}\), which can be used as a substitute for protein fish meal in the ration arrangement.

The results showed that increasing the application of fermented shrimp shells can improve the average IOFC in the diets of Sentul chickens. In terms of the IOFC, chickens with fed of the SSFBLS 10% was the best of the other at a value of 27,755 IDR/birds/starter period. IOFC is used to determine the profit of a business compared to the feed cost, i.e. the difference in Total Revenue and Total Feed Cost (Purba et al., 2018; Hassan et al., 2019). A diet with high protein content entails an increased risk of increasing the cost and pollution of ammonia waste. The use of SSFBLS can reduce ammonia emissions and also reduce environmental pollution. Therefore, raising chickens using SSFBLS is a safer alternative applied in residential areas.

**Conclusion**

The level of 10% SSFBLS in a low protein diet over ten weeks results in the gained performance and feed efficiency of Indonesian Sentul chickens. Levels up to 20% can use as a feed ingredient to obtain a meat protein and to get low ammonia excreta, without reducing the quality of the feed and health status. SSFBLS 10-20% can use as raw material feed in the formulation as a source of protein and chitosan. This SSFBLS product with nutrients and chitosan content is suggested to reduce fish meal on diet formula and ammonia excreta.

**Conflict of interest**

The authors declare no conflicts of interest.

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**Data availability statement**

No data availability

**ORCID**

Abun Abun <http://orcid.org/0000-0003-1017-4365>

Tuti Widjastuti <http://orcid.org/0000-0003-1437-5164>

Kiki Haetami <http://orcid.org/0000-0001-6055-4601>

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