Prebiotics\textsuperscript{BLS} from encapsulated of extract of shrimp waste bioconversion on feed supplement quality and its implication of metabolizable energy and digestibility at Indonesian local chicken

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ABSTRACT
Bioconversion product of shrimp waste with BLS microbes (\textit{Bacillus licheniformis}, \textit{Lactobacillus} spp. and \textit{Saccharomyces cerevisiae}) holds digestive proteases with a great potential to be used as prebiotics for native chicken. In the present work, the ratio of liquid extract bioconversion product and binders formulated both 4:1 (T\textsubscript{1}), 5:1 (T\textsubscript{2}), and 6:1 (T\textsubscript{3}) to entrap prebiotics enzymes from BLS microbes have been characterized. The trials were needed to verify contained in prebiotics from encapsulated liquid extract on quality of feed supplement. The overall results indicated that binders formulated T\textsubscript{2} (5:1) capsules of prebiotics\textsuperscript{BLS} extract were better vehicles to deliver shrimp enzymes in native chickens. The implication of metabolizable energy and digestibility at Indonesian local chicken showed that T\textsubscript{2} binders formulated improve protein digestibility (80\%) and metabolizable energy (3033 kcal/kg) of local chicken. Feeding trial prebiotic\textsuperscript{BLS} used at four levels (0, 0.5, 1, 1.5, or 2\%) at basal feed (protein 28.22\%), with one treatment of standard feed (protein 32.18\%). Supplementing diets with prebiotic\textsuperscript{BLS}, especially at 1.5\%, has a variety of growth-promoting effects. The results demonstrate that the observed benefits of prebiotic\textsuperscript{BLS} on low protein diets can be achieved.

1. Introduction
Indonesian native chicken meat is a food commodity whose preference is increasing farmers must pay attention to the production period by paying attention to the efficiency of the ration used to produce high weight gain. Weight gain is influenced by the availability of tissue-forming amino acids, so that protein consumption is directly related to the growth process. Protein supplements in the form of prebiotic enzyme sources can improve the quality of the ration protein and can improve the effects of the given feed. It is necessary to find alternative protein sources as an effective protein supplement from waste materials, one of which is shrimp waste. Indonesia is a shrimp exporter in the form of frozen shrimp without skin and head (headless) or peeled so that 60–70\% of the shrimp weight becomes waste. The specialty of shrimp waste is that it has good nutritional content, especially protein (42.65\%) (Gernat 2001), however, the constraint on the use of shrimp waste protein is chitin, which is covalently bound to glucosides, making it difficult to digest by poultry digestive enzymes (Abun and Haetami 2016). Utilization of shrimp waste in the form of chitosan produces liquid waste which, if not handled properly, will have a negative impact on the waters, which can increase the biological oxygen demand (BOD) and chemical oxygen demand (COD). Microbiological processing uses microbes in Shrimp processing waste holds digestive proteases with a great potential to be used as feed supplement for Indonesian of native chickens.

Microbiological processing through fermentation techniques has often been carried out by previous researchers (Alshelmani et al. 2014, 2021; Alshelmani et al. 2016, 2017). \textit{Bacillus licheniformis} bacteria produce chitinase and protease enzymes with deproteinization properties that can free some nitrogen or protein from chitin bonds (Austin et al. 1981).
Lactobacillus spp. serves to break down glucose, sucrose, maltose, and lactose, and the mineralization process (Cira et al. 2000). Saccharomyces cerevisiae is a yeast that produces amylase, lipase, protease, and other enzymes that can aid in the digestion of nutrients in the digestive organs (Doan et al. 2019; Huezo et al. 2019).

This research was designed with a breakthrough stepwise fermentation technique using three types of microbes B. licheniformis, Lactobacillus spp., and S. cerevisiae, and mineral supplements during the bioconversion process to produce a feed supplement product called Prebiotics\textsuperscript{BLS}. In the prebiotics\textsuperscript{BLS} manufacturing process, an effective encapsulation technology from extracted bioconversion of shrimp waste is needed as a practical alternative to further processing, and the results are favoured by livestock, the price is low, and the nutritional value increases (especially protein). Prebiotics\textsuperscript{BLS} encapsulated from extracts of bioconversion product of shrimp waste have a great potential to be used as a feed supplement in poultry nutrition. The bio-conversion extract product (prebiotics\textsuperscript{BLS}) functions as an emulsifier in the digestive tract of chickens, thereby increasing absorption and nutrient efficiency. Thus, it is effective in converting feed protein to be converted into meat (growth promoter).

We descriptive evaluate where encapsulation methods as a strategy to improve intestinal delivery of exogenous enzymes in native chicken to enhance their digestion process. Alginic acid also called olin is a polysaccharide distributed widely in the cell walls of brown algae that is hydrophilic and forms a viscous gum when hydrated. With metals such as sodium and calcium, its salts are known as alginate. Calcium alginate is a water-insoluble, gelatinous, cream-colored substance that can be created through the addition of aqueous calcium chloride to aqueous sodium alginate. Calcium alginate is also used for entrapment of enzymes and forming artificial seeds in plant tissue culture.

2. Materials and methods

The research consisted of (1) Product preparation (covering bioconversion and extraction), (2) Product testing (quality product as a physical and biological test), and (3) Feeding trial the level of BLS prebiotic as feed supplement on the growth phase local chicken.

2.1. Bioconversion of shrimp waste in stages using BLS microbes

Materials used in this experiment include shrimp waste, B. licheniformis, Lactobacillus spp. isolate, and S. cerevisiae, selenium, glucose, yeast extract, tryptone, NaCl, NaOH, CaCO\textsubscript{3}, pH 4 buffer, pH 7 buffer, pH 9 buffer, and Bovine Serum Albumin. The tools used are stainless-les tubes (reactors), water-bath, shaker-bath, autoclave, trophi, ‘Bunsen’ burners, petri dishes, porcelain dishes, centrifuges, funnels, PH-meter, and spectrophotometer.

The bioconversion stages include:

- The process of deproteination of shrimp waste by a B. licheniformis inoculum with a dose of 2\% (bv/bw) in a stain-les jar container and put into the ASB (auto-shaker-bath) machine for 2 days at 45°C with a rotation of 120 rpm (Abun and Haetami 2016).
- Demineralization of deproteinated products by the inoculum of Lactobacillus spp. 2\% (bv/bw), then incubated for 2 days at 35°C, using an ASB machine with a rotation of 120 rpm (Abun and Haetami 2016).
- The bioconversion of demineralized products by S. cerevisiae as much as 3\% (bv/bb), which is incubated for 2 days at 30°C, using an ASB machine with a rotation of 120 rpm (Abun and Haetami 2016).

2.2. Encapsulating of product of liquid extract of bioconversion: making prebiotics\textsuperscript{BLS} feed supplement

- The encapsulation of the bioconversion liquid product was carried out through adding of binder.
- Bio-conversion products \textsuperscript{BLS} were supplemented with selenium (Se) of 0.15 ppm. The liquid extract of fermented shrimp waste is then added with a compactor.
- Binder formulation as additional energy sources and binder with 5\% Na-alginate, 5\% zeolite, and 90\% corn starch.
- The formulation of the ratio of the extract from the bioconversion results and the compacting agent, as a treatment is as follows:
  (a) 1 referred to as T\textsubscript{1} (treatment-1)
  (b) 1 referred to as T\textsubscript{2} (treatment-2)
  (c) 1 referred to as T\textsubscript{3} (treatment-3)
- The milling feed supplement with a particle size of 60 μm and dried in a drying oven at 50°C until constant weight (90\% dry matter) and then used as a feed supplement for local chickens.
- Released the product called Prebiotics\textsuperscript{BLS} (Figure 1)

2.3. Quantitative analysis of prebiotics\textsuperscript{BLS} products by UV-Vis spectroscopy and FTIR

The prebiotic BLS products were observed for their wavelength absorption using UV-vis spectroscopy. The complex compound that is expected to be detected is astaxanthin, its absorption is measured at a wavelength of 300 nm–600 nm to get the spectrum pattern and maximum wavelength. The analysis was continued qualitatively using FTIR. Astaxanthin and astaxanthin complexes were measured using FTIR with the ATR method. Measurements were made at wave numbers 350 cm\textsuperscript{-1}– 4000 cm\textsuperscript{-1}.

2.4. Analysis of quality and digestibility of prebiotics\textsuperscript{BLS} proteins

The prebiotic quality of BLS from the encapsulated with binder treatment was tested descriptively including the measurement of the content of active substances and amino acids. Furthermore, the protein digestibility test was carried out through measurement with the indicator method. The measurement of prebiotics\textsuperscript{BLS} protein digestibility value based on shrimp waste extract was carried out on local chickens (as test
animals), with 3 prebiotics BLS treatments and each of which was repeated 7 times. To determine the difference in effect between treatments, analysis was carried out using Duncan’s multiple range tests. The study used 120 tail of Sentul day-old (DOC) type local chickens without straight runes obtained from the Development Centre for Poultry Breeding, Jatiwangi, Majalengka-West Java, Indonesia. DOC had an average coefficient of variation of initial weight of 8%. The cage used was a cage-shaped cage of 24 units with a length of 0.7 m, a width of 0.5 m, and a height of 0.7 m. Each cage unit consists of 5 chicks and was equipped with a feed place in the form of a round feeder and around water drinking water place made of plastic, and a 15-watt incandescent lamp. Chicken maintenance was carried out from day one to 8 weeks, giving rations and drinking water is done in ad-libitum.

Furthermore, the prebiotics BLS quality test was carried out biologically on local chickens by determining the metabolic energy value and digestibility (protein and dry matter). Chemical analysis is carried out in the laboratory by measuring:

- Dry matter, gross energy, nutrient content (protein), and lignin of prebiotics BLS
- Dry matter, gross energy, nutrient content (protein), and lignin content of excreta

### 2.5. Feeding trial of supplementing prebiotic BLS on performance of local chickens

The best prebiotics from the results of physical, chemical, and digestibility tests are then used as feed supplements in the ration formula. Prebiotic BLS added in the treatment rations were as follows:

- $R_0 =$ basal ration, without prebiotic BLS (18% protein and 2800 kcal/kg ME);
- $R_1 = R_0 + 0.5\%$ prebiotic BLS
- $R_2 = R_0 + 1.0\%$ prebiotic BLS
- $R_3 = R_0 + 1.5\%$ prebiotic BLS
- $R_4 = R_0 + 2.0\%$ prebiotic BLS
- $R_S =$ Standard ration, without prebiotic BLS (22% protein and 2800 kcal/kg ME);

Parameters observed include gain, feed conversion ratio, and the relative growth rate (RGR) which as calculated as described by Hassan et al. (2019):

$$RGR = \frac{(\text{final weight} - \text{initial weight})}{0.5 \times \text{(final weight} - \text{initial weight})} \times 100$$

The feed ingredients for the basal and standard ration consist of yellow corn, fine bran, soybean meal, fish meal, CaCO₃, coconut oil (Table 1).

### 2.6. Data analysis

Data of the study for digestibility, metabolic energy value, and performance variables were subjected to analysis of variance (ANOVA) as a completely randomized design. To determine the difference in effect between treatments, analysis was carried out using Duncan’s multiple range tests.

### 3. Results and discussion

#### 3.1. The content prebiotic BLS active substances and encapsulated amino acids

The results of LC-MS analysis showed that the active substance content of fermented shrimp waste (LNC) is astaxanthin, was 26.75% (Indonesian Police Forensic Laboratory, 2019). LC-MS analysis is a chemical analysis technique that combines the physical separation capabilities of liquid

| Feed ingredient (%) | Basal ration ($R_0$) | Standard ration ($R_S$) |
|---------------------|-----------------------|-------------------------|
| Cornmeal             | 60.00                 | 54.00                   |
| Soybean meal         | 23.50                 | 25.00                   |
| Fish meal            | 14.00                 | 11.00                   |
| Rice bran            | 0.00                  | 0.00                    |
| Coconut oil          | 0.00                  | 0.00                    |
| CaCO₃                | 1.00                  | 1.00                    |
| Total                | 100.00                | 100.00                  |
| Metabolizable energy (kcal/kg) | 2802 | 2826 |
| Crude protein (%)    | 18.33                 | 22.18                   |
| Crude lipid (%)      | 4.14                  | 5.29                    |
| Crude fibre (%)      | 4.55                  | 3.56                    |
| Lysine (%)           | 0.96                  | 0.39                    |
| Methionine (%)       | 0.32                  | 0.58                    |
| Methionine + cystine (%) | 0.63 | 0.91 |
| Phenylalanine (%)    | 0.87                  | 1.00                    |
| Threonine (%)        | 0.69                  | 0.86                    |
| Ca (%)               | 0.55                  | 0.69                    |
| Se (mg)              | 0.11                  | 0.26                    |
chromatography (HPLC) with the mass analysis capabilities of mass spectrometry, which functions to separate several compounds or mixtures of compounds based on their polarity. Other substances detected from the LC-MS analysis of prebiotic liquid products are solvents and impurities, namely: L methyl ester 31.28%, nickel protoporphyrin disodium 26.36%, 4,9-epoxy-1H-benzelen 14.85%, and propaneamine 0.76%. Based on the data, the astaxanthin content in prebiotic liquid waste resulted from encapsulation with different binder formulations, obtained the astaxanthin and amino acid content of the product (Table 2).

Table 2 showed that T2 treatment has the advantage of total amino acids, and several types of essential amino acids are higher in content than T1. Bio-process shrimp waste by *B. licheniformis, Lactobacillus spp.* and *S. cerevisiae* can improve the quality of protein ratio by increasing the completeness and balance of essential amino acids contained (Reddy et al. 1996; Rao et al. 1998) so Prebiotics can be used as a feed supplement in Indonesian local poultry feed formulas. Thus, the higher ratio of liquid extract, the total amino acid increases, and at the level of the ratio of 6:1, this is the optimal ratio.

The balance of amino acids, especially methionine and lysine in the treatment ratio with the addition of feed supplement at the level of 1.5-2.0%, is in the ideal balance methionine: lysine was ((0.49–0.52): 1) (Abun et al. 2018). In line with Soares et al. (2020), that the balance of the methionine and lysine amino acids in the ration formula is between 0.48 and 0.52: T1 and T3 were near. Bio-process shrimp waste by *B. licheniformis, Lactobacillus spp.* and *S. cerevisiae* can improve the quality of protein ratio by increasing the completeness and balance of essential amino acids contained (Reddy et al. 1996; Rao et al. 1998). Therefore, Prebiotic can be used as a feed supplement in Indonesian local poultry feed formulas.

Table 2. The contents of astaxanthin and amino acids of shrimp waste bioconversion products encapsulated with various binder formulations.

| Ingredient         | Astaxanthin (%) | Treatments        |
|--------------------|-----------------|-------------------|
|                    |                 | T1  | T2  | T3  |
|                    |                 | 375 ppm | 428 ppm | 535 ppm |
| Amino acids (%)    |                 |       |       |      |
|                    |                 |       |       |      |
| L-Serine           | 1.25            | 1.36  | 1.37  |
| L-Glutamic acid    | 4.51            | 5.35  | 5.35  |
| L-Phenylalanine*   | 0.90            | 1.10  | 1.10  |
| L-Isoleucine*      | 0.94            | 0.88  | 0.87  |
| L-Valine*          | 1.37            | 1.34  | 1.34  |
| L-Alanine          | 2.17            | 2.08  | 2.09  |
| L-Lysine*          | 0.94            | 1.48  | 1.44  |
| Glycine            | 1.09            | 1.19  | 1.17  |
| L-Lysine*          | 0.99            | 0.99  | 0.85  |
| L-Aspartic Acid    | 1.64            | 2.12  | 2.10  |
| L-Leucine*         | 2.71            | 2.58  | 2.58  |
| L-Tyrosine**       | 0.45            | 0.78  | 0.78  |
| L-Proline          | 1.79            | 1.92  | 1.90  |
| L-Threonine*       | 0.97            | 1.15  | 1.13  |
| L-Histidine*       | 0.59            | 0.60  | 0.59  |
| L-Cystine**        | 0.08            | 0.09  | 0.09  |
| L-Methionine*      | 0.14            | 0.28  | 0.28  |
| Total              | 22.38           | 25.29 | 25.04 |

Note: *Essential amino acids, **Semi essential amino acids.

3.2. Active astaxanthin substance result of LC-MS and FT-IR analysis

Astaxanthin is a carotenoid pigment, with a molecular structure like that of β-carotene (Figure 2).

Astaxanthin is a natural carotenoid and has antioxidant power that is much higher than other well-known antioxidants such as vitamins E and C (Salem et al. 2020). In binding to oxygen, astaxanthin is 550 times stronger than vitamin E and 40 times stronger than β-carotene. Astaxanthin content in prebiotics liquid nutrient concentrate (LNC) was higher with the reduced presentation of alginate use. The prebiotic containing astaxanthin can function as an oxygen scavenger so that it does not support oxidation reactions, better than the vitamin C mechanism. Astaxanthin antioxidants bind to metals which can catalyze the oxidation reaction of citric acid and amino acids. To inhibit lipid peroxidation, astaxanthin is even stronger than vitamin E (Chintong et al. 2019; Hu et al. 2019). The graph of the LC-MS reading results regarding the active substance astaxanthin in the prebiotic product is shown in Figure 3. The findings showed the potential of the active substance possessed by the prebiotics product because of encapsulation with various ratios of shrimp waste and binder extract formulations.

Figure 3 showed the results of LC-MS astaxanthin profiles in three different formulations of LNC and Na-alginate ratios. Absorbance profile vs. time on astaxanthin extraction assisted by ultrasonic wave 21 KH at 475 nm. Based on the reading results on the graph of the potential of the active substance, it is known that the astaxanthin content as the peak area in the prebiotic liquid waste extract, which first appeared was in the T2 treatment, namely on wavelength of 450 nm. The results of the encapsulation of LNC products were carried out by LC-MS analysis (Figure 3(a–c)) showed multiple frequencies and times. Astaxanthin has the characteristic of absorbing in areas with a maximum wavelength of 476 nm.

Shrimp waste liquid extract product material has the potential to be used as a feed supplement for poultry, but an encapsulation process is needed to bind the active ingredients and nutrients. Binder or encapsulation treatment according to Kumari and Kishor (2020, Sulistyani 2017), aims to protect the core from environmental influences, regulate the release of the core material and maintain the stability of the core material.

The encapsulation process is influenced by the ratio of the core material (shrimp waste concentrate) to the coating material (alginate). The astaxanthin contained in the encapsulated bioconversion liquid product (prebiotics) with the original material (shrimp shells and bioconversion liquid waste) needs to be studied for molecular changes, to determine its effectiveness by using FTIR. Astaxanthin is an organic compound consisting of two-terminal ring systems connected by a polyene group (Figure 2). The molecular structure which
Figure 3. The results of LC-MS readings on the Prebiotics with shrimp waste extract formulations and binder ratio; (a) 4:1; (b) 5:1; (c) 6:1.
contains a lot of carbon atoms makes astaxanthin very non-polar (log $K_w = 13.27$). The conjugated double bonds in the astaxanthin molecule provide the ability to absorb visible light at a maximum wavelength of about 476 nm in ethanol solvents and are red (Fox et al. 1994; Kusmayadi et al. 2018, 2019).

The conversion from the results of the analysis of astaxanthin into LNC products was 26.75%, the resulting content of astaxanthin from the encapsulated product quantitatively and further tested using FT-IR (Fourier Transform Infrared) to see the sharpness of the functional groups. The FT-IR 8300/8700 spectrophotometer is a tool that can be used to identify compounds, especially organic compounds, both qualitatively and quantitatively. The analysis is carried out by looking at the shape of the spectrum, namely by looking at the specific peaks that indicate the type of functional group possessed by the compound.

The results of the interpretation of the absorption or peak of FTIR analysis of chitin isolated from tiger shrimp shell waste (*Penaeus monodon*) showed that the liquid extract of shrimp waste processing (LNC) was unstable during storage and transportation. The binder treatment was intended to encapsulate the active ingredients contained therein. The results of the analysis through the reading of the FTIR method on the material from the shrimp shell, the bioconversion liquid product (LNC), and the encapsulated prebiotics were shown in Figure 4a–c. Whereas Table 3 showed the product wave number and the bond intensity of the astaxanthin functional group.

Table 3 showed the infrared light absorption which shows the characteristics of the astaxanthin functional groups, namely C–H, C=C, C=O, and O–H which appear in Figures 1–3. The astaxanthin functional group also appears in the synthesis results where each result of the synthesis shows the C–H, C=C, C=O, and O–H functional groups represent there is astaxanthin in the encapsulated product as a ligand for complex compounds. Astaxanthin is a xanthophyll carotenoid derivative compound that is very potent and has antioxidant activity. The astaxanthin functional group in all complexes and the appearance of the bond intensity of the astaxanthin functional group.

**3.3. Measurement of metabolic energy value and digestibility of prebiotics products**

The experimental results pointed that prebiotics (based on shrimp waste) can be used as a feed supplement in the local chicken feed formula. The results of the analysis of variance showed that the treatment had a significant effect ($P < 0.05$) on the value of metabolic energy, dry matter digestibility, and protein digestibility (Table 4).

Measurement of the value of metabolic energy, dry matter digestibility, and protein digestibility of the prebiotics resulting from the encapsulated of liquid products with various binder formulations was carried out on local chickens (as test animals), with 3 treatment ratios of extract and binder, each of which was repeated 7 times. Metabolic energy measured is nitrogen corrected pseudo metabolic energy (AMEn). Prebiotics ($T_2$) (ratio of extract and solids 84%: 16%), produced the highest metabolic energy value of 3,033.49 kcal/kg.

Table 4 showed that the prebiotics ($T_3$) and $P_2$ (ratio of extract and solids 5:1), produced the highest protein digestibility value of 80.00%. The shrimp waste extract was treated with various comparisons of the extract and the compaction agent and then tested on local chickens as test animals. Protein digestibility is devoted to local chickens because the technology for developing and improving feed in these chickens is being pursued, to obtain high growth performance.

Rations that have a good balance of amino acids show optimal growth performance. The results of the protein digestibility test of the prebiotics ($T_3$) extract encapsulation treatment in the ration had a high digestibility value (ranging from 71.94 to 80%). This illustrates an increase in the quality of protein rations with the addition of prebiotics. Deproteination by *B. licheniformis* which produces chitinase enzymes and protease enzymes to degrade β (1,4) glycosidic bonds in chitin and release several proteins in the form of N-acetyl-D-glucosamine and acetylamino monomers, whereby increasing protein digestibility (Tharanathan and Kittur 2003; Shintani 2019). *Lactobacillus* spp. bacteria that function in the demineralization process, and break down glucose, sucrose, maltose, and lactose into lactic acid (Liu et al. 2020). Fermentation with the help of *S. cerevisiae* yeast which produces amylase, lipase, protease, and other enzymes can help digest nutrients in the digestive organs (Wagstaff 1989).

**3.4. Effect of dietary prebiotic on performance traits**

The present study concludes that prebiotic ($T_3$) supplementation is capable of improving ($P < 0.05$) body weight gain (BWG), the protein efficiency ratio (PER), and feed conversion ratio (FCR) of local chickens (Table 5).

Local chickens fed a diet supplemented with the highest level of prebiotic ($T_3$) (1.5–2.0%) exhibited a greater body weight, body weight gain, protein efficiency ratio, and a lower feed conversion ratio (FCR) ($P < 0.05$) compared to the other groups (Table 5).
Figure 4. Graph of readings using FTIR, (4a) Shrimp shells, (4b) Liquify extract bioconversion, (4c) Prebiotics
Moreover, Lim et al. (2019) reported that numerous bacteria and different protease isozymes were identified to contribute to the relief of crude protein deficiency (Kidd and Tillman 2016). Prebiotic BLS supplementation likely favours rations that have a good balance of amino acids, which are extremely important in maintaining health and preventing disease.

### 4. Conclusions

1. Shrimp waste can be used as a culture medium for the growth of BLS microbes (B. licheniformis, Lactobacillus spp., and S. cerevisiae) in producing secondary metabolites (enzymes, vitamins, minerals). The astaxanthin (antioxidant) content in the fermented shrimp waste extract was 26.75%.

2. Extract of fermented shrimp waste supplemented by the mineral selenium can be used as a basic material for making BLS prebiotics by adding compaction materials (5% Na-alginate, 5% zeolite, and 90% corn starch) with a ratio of 84%: 16%, and then it can be used as a feed supplement in local poultry (chicken) feed formulas.

3. Nutritional content and biological value of shrimp waste-based prebiotics BLS are as follows:
   (a) Astaxanthin of 428 ppm
   (b) Total dissolved protein (amino acids) is 25.15%
(c) Metabolic energy of 3.033 kcal/kg
(d) Digestibility of dry matter is 74.39% and protein digestibility is 80.00%.

(4) Local chickens fed a diet supplemented with the highest level of prebiotic BLS (1.5–2.0%) exhibited a greater body weight, body weight gain, protein efficiency ratio, and a lower feed conversion ratio.

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No potential conflict of interest was reported by the author(s).

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