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## Research Article

# Bioprocessing of Shrimp Waste and its Effect on the Production and Quality of Eggs from Domestic Laying Hens

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## Abstract

**Background and Objective:** The processing of waste materials from the frozen shrimp industry in the form of shrimp skins and heads has the potential to be used as an alternate feed additive in poultry ration formulas. The limiting factor for the use of shrimp waste is the presence of chitin, which binds strongly to proteins, fats and minerals through  $\beta$  (1-4) covalent bonds so that it is difficult to digest by chicken digestive enzymes. Waste-product bio-processing can be carried out through deprotonation stages with *Bacillus licheniformis* as well as demineralization with *Lactobacillus* sp. and *Saccharomyces cerevisiae* to convert materials to concentrated feed nutrient products (hereafter termed Nutrient Concentrates<sup>BL5</sup>). This biological test of Nutrient Concentrates<sup>BL5</sup> in ration formula was conducted to determine the effectiveness of the product in the production and quality of chicken eggs. **Materials and Methods:** The study was carried out using domestic laying hens on a test farm. The experimental design was completely randomized; it consisted of 6 ration treatments and each treatment was replicated 4 times. The variances of the obtained data were analysed and differences between treatments were tested with Duncan's multiple range test. **Results:** The results of the study were obtained: (a) Nutrient Concentrates<sup>BL5</sup> could be stored up to 8 weeks at room temperature (pH: 7.36, ammonia: 9.60%, water content: 9.67% and energy conversion: 77.36%), (b) The laying performance of domestic hens in poultry production using Nutrient Concentrates<sup>BL5</sup> as a feed additive at a level of 2% in the ration formula was equivalent to that of the hens fed the standard ration (egg weight 44.71 g grain<sup>-1</sup>, hen-day production 51.79% and ration efficiency 35.49%) and (c) The quality of chicken eggs from hens fed Nutrient Concentrates<sup>BL5</sup> was better than that of hens fed the standard ration (shape index: 79.34, albumin index: 0.20, Haugh-unit: 94.11, yolk index: 0.37, yolk colour: 9.00 and egg cholesterol levels: 96.45 mg dL<sup>-1</sup>). **Conclusion:** Nutrient Concentrates<sup>BL5</sup>, made from shrimp waste bioprocessed by *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae*, can be used as feed additives in the feed formula of domestic laying hens. To support the production and quality of chicken eggs, Nutrient Concentrates<sup>BL5</sup> can be used up to levels of 2% in the feed formula.

**Key words:** Egg production, egg quality, feed additives, laying hens, shrimp waste

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The waste products from the cold storage processing industry in the form of shrimp skins and heads are materials that have the potential to be used as alternative feed ingredients for chickens. According to Abun *et al.*,<sup>1</sup> incorporation of these materials is based on the nutritional content, namely, crude protein 43.41%, crude fibre 18.25%, crude fat 7.27%, calcium 5.54%, phosphorus 1.31%, lysine 3.11%, methionine 1.26% and cysteine 0.51%, as well as gross energy 3892 kcal kg<sup>-1</sup>. The limiting factor for the use of shrimp waste products as chicken feed ingredients is the presence of chitin; shrimp waste contains 15-20% chitin. Chitin binds strongly to proteins, fats and minerals in  $\beta$  (1-4) covalent bonds so that it is difficult for poultry enzymes to digest diets containing chitin<sup>2-4</sup>. Poultry do not have enzymes that can breakdown glycoside bonds [ $\beta$ -glycosidic (1-4)]; thus, the waste products must be processed first. One approach to converting organic matter into new products that are useful and that have better nutritional value is to use microbes to bioprocess materials.

Waste-product bioprocessing can be carried out in two stages: deproteinization with *Bacillus licheniformis* microbes and demineralization with *Lactobacillus* sp. and *Saccharomyces cerevisiae*. *Bacillus licheniformis* is a bacterium capable of producing relatively high amounts of protease and chitinase<sup>1,5</sup>. *Lactobacillus* sp. is capable of microbially decomposing glucose, sucrose, maltose and lactose into lactic acid so that mineral deposits occur<sup>3</sup>. *Saccharomyces cerevisiae* is a yeast that can produce amylase, lipase, protease and other enzymes that can help digest food substances in the digestive organs of chickens<sup>6</sup>.

The composition of animal rations, in addition to seeds and livestock maintenance procedures, is a determining factor of animal growth. The optimal performance of domestic laying hens can only be realized if hens are given a quality ration that meets nutritional requirements by delivering sufficient and balanced amounts. Fulfilling the food requirements of the ration can be accomplished by adding bioprocessed products as feed additives. This needs to be considered because feed additives can improve the quality of domestic laying hen rations and benefit product value. Therefore, efforts are needed to find alternative feed additives that are cheap, easily available, of good quality and that are non-food. One feed additive is shrimp waste, which is processed with fermentation technology<sup>7,8</sup>. Indonesia is listed as the third largest shrimp producing country in the world, producing approximately 0.08 million tons annually from 380,000 ha of shrimp ponds. Approximately 80-90% of the shrimp are exported in the form of headless, skinless (peeled) and frozen shrimp. Shrimp skins, heads and tails are generated as industrial waste by shrimp

freezing plants and this waste can reach 30-40% of the whole weight<sup>5,9</sup>. The use of processed product shrimp waste is a source of alternative feed ingredients that can be utilized in the preparation of domestic laying hen rations. Shrimp skin contains carotenoids (colour pigments) in the form of astaxanthin, which is a provitamin A ( $\beta$ -carotene) used in the formation of the chicken egg yolk<sup>2,10</sup>. The protein and mineral contents are quite high, illustrating the potential of using shrimp waste as an alternative feed source of protein, vitamins and minerals for domestic laying hens. However, the obstacle in using shrimp waste is the strong binding of nutrients by chitin; thus, it is difficult for chicken digestive enzymes to digest nutrients.

Domestic laying hens have long been reared by the people of Indonesia as local chickens, vegetable chickens, or domestic laying hens; in Latin, these poultry are known as *Gallus domesticus*. Domestic laying hens account for 20-40% of eggs and 25% of the meat consumed domestically<sup>11</sup>. Domestic laying hens are more likely to be reared on community farms given that domestic laying hens do not require large amounts of capital, are easy to maintain and have a high degree of adaptability. Furthermore, the meat and eggs of domestic laying hens are preferred by the community. In general, domestic poultry is traditionally maintained extensively (its production is low and the mortality rate is quite high), which causes the population to fluctuate over time. The potential and prospects of domestic poultry are very good but until now, information and research on the development of domestic poultry is still relatively limited. The low level of productivity of domestic poultry is influenced by genetic and environmental factors. Inadequate genetic factors coupled with maintenance and feeding methods that are still traditional are the causes of low production of domestic poultry, both in their growth and egg production. Egg production only reaches 30-60 eggs per year, with an average egg weight of 37.5 g per egg<sup>11</sup>.

The use of bioprocessed products (Nutrient Concentrates<sup>BL5</sup>) in the ration formula is expected to improve the quality of domestic laying hens' rations. A good ration can increase egg production and quality as well as the economic value of hens so that domestic laying hens can be intensively maintained by the community. This study aimed to determine the effectiveness of the waste-product in the production and quality of chicken eggs.

## MATERIALS AND METHODS

### Processing of nutrient concentrates<sup>BL5</sup>

**Deproteination:** A starter *Bacillus licheniformis* inoculum was cultivated in 50 mL of broth and incubated for two days at a

temperature of 50°C and a dose of 2% inoculum (v/w)<sup>2</sup> and fermentation liquid substrates with standard solution were placed in an auto-shaker bath for one, two and three days at 45°C and 120 rpm<sup>12</sup>.

**Deminerlization:** A starter *Lactobacillus* sp. inoculum was cultivated in a mixed standard solution (0.5% (w/v) yeast extract, 0.5% NH<sub>4</sub>NO<sub>3</sub>, 0.05% sodium-chloride, 0.05% MgSO<sub>4</sub>, 0.01% FeSO<sub>4</sub> and 0.001% CuSO<sub>4</sub>) and fermented in an auto-shaker bath. *Lactobacillus* sp. inoculum was added to the deproteination products according to treatment (one, two and three days at a temperature of 45°C at 120 rpm)<sup>12,13</sup>.

**Fermentation with *Saccharomyces cerevisiae*.** A *Saccharomyces cerevisiae* isolate was maintained in an incubator for three days and an inoculum was prepared with a standard solution (0.5% NH<sub>4</sub>NO<sub>3</sub>, 0.05% KCl, 0.05% MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.01% FeSO<sub>4</sub>·7H<sub>2</sub>O and 0.001% CuSO<sub>4</sub>·5H<sub>2</sub>O) and fermented in an auto-shaker bath. After obtaining its deminerlization product, a *Saccharomyces cerevisiae* inoculum at 3% (v/w)<sup>2</sup> was added and then incubated for one, two and three days at 35°C<sup>2</sup>.

**Mineralization:** The bioprocessed product was supplemented with 0.15 ppm of mineral selenium (Se) (73 ppm in Selene) and 5% Na-Alginate. Then, the product was ground to a particle size that could be filtered through a 60 mesh sieve.

**Storage period of nutrient concentrates<sup>BL5</sup>:** Nutrient Concentrates<sup>BL5</sup> was generated using shrimp waste and then stored at room temperature for 2, 4, 6 and 8 weeks. After storage, the pH level, NH<sub>3</sub> content and moisture content were measured. The selected product (which had a stable storage period) was then analysed for nutrient content and energy and used as feed additive in the ration formula of domestic laying hens.

**Feeding trial:** The study used domestic laying hens ("Sentul chicken") from the Jatiwangi Poultry Livestock Research Part, Majalengka-Indonesia. The chickens were divided randomly into 24 cage units; each cage contained 1 hen and the birds were numbered in each cage. Chickens used in the study had an initial weight variation coefficient of 4.07%. Each of the 24 cages was 50 cm long, 50 cm wide and 80 cm high. Each

group of cages was equipped with a place to feed and drink made of plastic. The feed ingredients of the ration consisted of yellow corn, fine bran, soybean meal, coconut cake, fish meal, grit (mussel flour), CaCO<sub>3</sub>, coconut oil<sup>14,15</sup> and Nutrient Concentrates<sup>BL5</sup>. In the experiment ration, the nutrient ratios were prepared based on SNI recommendations<sup>16</sup>. The protein and energy content of the control ration (R<sub>0</sub>) were 15% and 2750 kcal kg<sup>-1</sup>, respectively and those of the standard ration (R<sub>5</sub>) were 18% and 2750 kcal kg<sup>-1</sup>, respectively. Treatment rations were as follows:

- R<sub>0</sub> = Control ration (15% protein and EM 2750 kcal kg<sup>-1</sup>)
- R<sub>1</sub> = Control ration+0.5% nutrient concentrates<sup>BL5</sup>
- R<sub>2</sub> = Control ration+1.0% nutrient concentrates<sup>BL5</sup>
- R<sub>3</sub> = Control ration+1.5% nutrient concentrates<sup>BL5</sup>
- R<sub>4</sub> = Control ration+2.0% nutrient concentrates<sup>BL5</sup>
- R<sub>5</sub> = Standard ration (protein 18% and ME 2750 kcal kg<sup>-1</sup>)

**Trial procedure:** Domestic laying hens, starting from chickens laying eggs (5% hen day) were maintained with standard procedures. Each chicken was placed in one of the 24 cage units. The data collection and recording phase started with weighing each chicken to determine the initial body weight. Measurements of the feed consumption, the number of laid eggs and the egg weight were carried out every day. Measurement of the egg quality (egg yolks and cholesterol content) was carried out in the 4th and 8th weeks.

**Observed variables:** The following variables were observed: (1) Egg weight (g grains<sup>-1</sup>), (2) Number of eggs (grains), (3) Daily egg production (%), (4) Feed efficiency (%), (5) Shape index of eggs, (6) Albumin index, (7) Haugh Unit, (8) Yolk index, (9) Yolk colour and (10) Egg cholesterol content (mg dL<sup>-1</sup>).

**Experimental design and statistical analysis:** The experiments were performed in the laboratory using a complete randomized design consisting of six treatment rations (R<sub>0</sub>, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub>) that were replicated four times. The data were analysed using the Statistical Analysis System (SAS, version 9.1, SAS Institute Inc., Cary, NC, USA). Variables were analysed by one-way analysis of variance (ANOVA) using the statistical programmed SPSS version 19 (SPSS Inc., Chicago, IL). Significantly different means were determined by Duncan's multiple comparison test at the level of p<0.05.

## RESULTS

### Effect of storage time on pH value, NH<sub>3</sub> content and moisture content of nutrient concentrates<sup>BLS</sup>:

The average pH value, ammonia content (NH<sub>3</sub>) and moisture content over 10 weeks of storage are shown in Table 1 and the nutrient and energy values of the Nutrient Concentrates<sup>BLS</sup> are shown in the Table 2.

Table 1 shows that the pH level and moisture content of Nutrient Concentrates<sup>BLS</sup> decreased with the length of storage time, while the NH<sub>3</sub> (ammonia) content increased. Storage of Nutrient Concentrates<sup>BLS</sup> up to 8 weeks is safe for use as feed ingredients.

The bioprocessed shrimp waste product (Nutrient Concentrates<sup>BLS</sup>) can be held up to 2 months (8 weeks) at room temperature storage conditions. The following results were obtained: the pH approached neutral (7.36), the NH<sub>3</sub> (ammonia) content was less than 10% (9.60%) and the water content was stable (below 10%). Nutrient Concentrates<sup>BLS</sup> was of good quality, given that the energy conversion (gross energy to metabolizable energy) was 77.36% and the value of the protein digestibility was 72.91% (Table 2).

### The Influence of the Incorporation of Nutrient Concentrates<sup>BLS</sup> in the Ration on the Performance of Egg-Laying Domestic Hens

The average egg weight, number of eggs, daily production (hen-day production), feed efficiency and income over feed cost of the domestic laying hens used in the experiment are shown in Table 3.

The average weight of the eggs of the domestic laying hens from the experiment ranged from 40.56-44.71 g egg<sup>-1</sup>. The results of the analysis showed that the ration treatment did not have a significant impact ( $p>0.05$ ) on the egg weight of domestic laying hens. The use of Nutrient Concentrates<sup>BLS</sup> up to the level of 2% in the rations of the domestic laying hens did not affect ( $p>0.05$ ) the egg weight.

The average number of eggs laid by the hens used in the experiment ranged from 2.63-3.50 points. The results of the analysis showed that the ration treatment did not significantly impact ( $p>0.05$ ) the number of eggs laid by hens. The use of Nutrient Concentrates<sup>BLS</sup> up to a level of 2% in the ration of the domestic laying hens did not affect the number of eggs.

The average hen-day production of eggs from the experiment ranged from 37.50-51.79%. The results of the

Table 1: The average pH value and ammonium (NH<sub>3</sub>) and moisture contents of the nutrient concentrates<sup>BLS</sup> product over ten weeks of storage

Parameters	Initial	2nd week	4th week	6th week	8th week	10th week
pH	8.26	7.96	7.72	7.48	7.36	7.30
NH <sub>3</sub>	3.49	4.42	6.44	8.84	9.60	11.64
Moisture	16.63	14.88	11.65	10.80	9.67	9.52

Table 2: Nutrient contents of products of bioprocessed shrimp waste (nutrient concentrates<sup>BLS</sup>)

Chemical and biological value of bioprocessed products (nutrient concentrates <sup>BLS</sup> )	Contents
Crude protein (%)	39.29
Extract ether (%)	7.03
Crude fibre (%)	7.79
Calcium (%)	6.81
Phosphor (%)	2.83
Lysin (%)	3.04
Methionine (%)	1.46
Cystine (%)	0.54
Free organic acid (%)	1.66
Gross energy (kcal kg <sup>-1</sup> )	3,379.00
Metabolized energy (kcal kg <sup>-1</sup> )	2,614.00
Dry matter digestibility (%)	72.91

Table 3: The average egg weight, hen-day production, feed efficiency and income over feed cost of domestic laying hens of the experiment

Parameters	Feed treatments					
	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
Egg weight (g grain <sup>-1</sup> )	40.560 <sup>A</sup>	41.72 <sup>A</sup>	42.500 <sup>A</sup>	42.970 <sup>A</sup>	44.710 <sup>A</sup>	42.99 <sup>A</sup>
Number of eggs (eggs weeks <sup>-1</sup> )	2.630 <sup>C</sup>	2.88 <sup>BC</sup>	2.880 <sup>BC</sup>	3.250 <sup>AB</sup>	3.630 <sup>A</sup>	3.50 <sup>A</sup>
Hen-day production (%)	37.500 <sup>C</sup>	41.07 <sup>BC</sup>	41.070 <sup>BC</sup>	46.430 <sup>AB</sup>	51.790 <sup>A</sup>	50.00 <sup>A</sup>
Feed efficiency (%)	23.450 <sup>D</sup>	25.88 <sup>CD</sup>	26.900 <sup>CD</sup>	30.650 <sup>B</sup>	35.490 <sup>A</sup>	33.20 <sup>AB</sup>
IOFC (IDR hens <sup>-1</sup> weeks <sup>-1</sup> )	3.562 <sup>C</sup>	4.087 <sup>C</sup>	4.172 <sup>BC</sup>	5.112 <sup>AB</sup>	6.001 <sup>A</sup>	5.73 <sup>A</sup>

Table 4: Average shape index values, albumin index, Haugh unit, yolk index, yolk colour index, eggshell thickness and cholesterol levels of eggs from domestic laying hens fed with nutrient concentrates<sup>BLS</sup>

Parameters	Feed treatments					
	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
Shape index	76.48 <sup>A</sup>	76.24 <sup>A</sup>	76.70 <sup>A</sup>	78.64 <sup>A</sup>	79.34 <sup>A</sup>	78.71 <sup>A</sup>
Albumin index	0.14 <sup>A</sup>	0.15 <sup>A</sup>	0.17 <sup>A</sup>	0.18 <sup>A</sup>	0.20 <sup>A</sup>	0.18 <sup>A</sup>
Haugh unit index	86.03 <sup>A</sup>	88.92 <sup>A</sup>	91.72 <sup>A</sup>	92.12 <sup>A</sup>	94.11 <sup>A</sup>	93.29 <sup>A</sup>
Yolk index	0.28 <sup>C</sup>	0.31 <sup>BC</sup>	0.32 <sup>AB</sup>	0.34 <sup>AB</sup>	0.37 <sup>A</sup>	0.35 <sup>AB</sup>
Yolk colour index	7.44 <sup>D</sup>	7.72 <sup>CD</sup>	8.02 <sup>BCD</sup>	8.56 <sup>ABC</sup>	9.00 <sup>A</sup>	8.74 <sup>AB</sup>
Eggshell thickness (%)	10.68 <sup>C</sup>	11.06 <sup>BC</sup>	11.32 <sup>BC</sup>	11.68 <sup>BC</sup>	12.79 <sup>A</sup>	12.05 <sup>AB</sup>
Egg cholesterol (mg dL <sup>-1</sup> )	184.21 <sup>A</sup>	153.45 <sup>C</sup>	148.24 <sup>C</sup>	117.03 <sup>C</sup>	96.45 <sup>D</sup>	184.21 <sup>B</sup>

analysis showed that the ration treatment did significantly affect ( $p < 0.05$ ) the hen-day production of eggs by domestic laying hens. The use of Nutrient Concentrates<sup>BLS</sup> up to a level of 2% in the rations of domestic laying hens did not influence the hen-day production of eggs.

The average efficiency of the use of domestic laying hens from the experiment ranged from 23.45-35.49%. The results of the analysis showed that the ration treatment did show a significant difference ( $p < 0.05$ ) in the efficiency of the use of domestic laying hen feed. The use of Nutrient Concentrates<sup>BLS</sup> up to a level of 2% in the feed of domestic laying hens did not affect the efficiency of ration use.

**Influence of nutrient concentrates<sup>BLS</sup> in the ration on the quality of eggs of domestic laying hens:** The average shape index value, albumin index, Haugh unit, yolk index, yolk colour and cholesterol level of the egg from domestic laying hens fed rations containing Nutrient Concentrates<sup>BLS</sup> in the experiment are presented in Table 4.

The average shape index value of eggs from the experiment ranged from 76.2-79.34. The results of the analysis showed that the ration treatment had no significant effect ( $p > 0.05$ ) on the shape index value of the egg from domestic laying hens. The use of Nutrient Concentrates<sup>BLS</sup> in the rations (crude protein 15%) at levels ranging from 0.5-2% did not significantly ( $p > 0.05$ ) affect the basal ration (R<sub>0</sub>/15% protein) and the standard ration (R<sub>5</sub>/17.5% protein) of the shape index value of eggs. The same egg shape index values indicated that the use of Nutrient Concentrates<sup>BLS</sup> did not influence egg air space.

The average albumin index value of eggs from the domestic laying hens used in the experiment ranged from 0.14-0.18. The results of the analysis showed that the ration treatment had no significant effect ( $p > 0.05$ ) on the albumin index values of the eggs from domestic laying hens. The use of Nutrient Concentrates<sup>BLS</sup> did not significantly affect the

standard ration (R<sub>5</sub>/18% protein) to the albumin index value of domestic laying hen eggs. The albumin index value of good chicken eggs is clear and concentrated.

The average value of the Haugh-unit (HU) of eggs from domestic laying hens from the experiment ranged from 86.03-94.11. The results of the analysis showed that the ration treatment had no significant effect ( $p > 0.05$ ) on the value of the Haugh unit of the eggs. The Haugh unit (HU) is a unit used to determine the freshness of the egg contents, especially the egg white.

The average yolk index value of the eggs from the experiment ranged from 0.28-0.37. The results of the analysis showed that the ration treatment had a significant effect ( $p < 0.05$ ) on the yolk index value of the eggs from the domestic laying hens. The use of Nutrient Concentrates<sup>BLS</sup> in rations up to a level of 2% (R<sub>4</sub>/15% protein) resulted in relatively good egg quality.

The average colour value of the egg yolks from the experiment ranged from 7.44-9.00. The results of the analysis showed that the ration treatment had a significant effect ( $p < 0.05$ ) on the colour value of the eggs yolk. The use of Nutrient Concentrates<sup>BLS</sup> in rations at a level of 2% (R<sub>4</sub>/15% protein) resulted in an egg yolk colour index that was significantly ( $p < 0.05$ ) higher than that of the eggs resulting from hens fed on basal rations (R<sub>0</sub>/15% protein). To determine the quality of egg yolks, a troche yolk colour fan can be used. Based on measurements with these tools, the colour of the yolk was within the normal range (yolk index 7-12).

The average cholesterol content of the eggs from the domestic laying hens used in the experiment ranged from 96.45-184.21 mg dL<sup>-1</sup>. The results of the analysis showed that the ration treatment had a significant effect ( $p < 0.05$ ) on the cholesterol content of the eggs. The use of Nutrient Concentrates<sup>BLS</sup> in rations at a level of 2% (R<sub>4</sub>/15% protein) resulted in a cholesterol content that was significantly ( $p < 0.05$ ) lower than that of eggs resulting from hens fed on basal rations (R<sub>0</sub>/15% protein) and standard rations (R<sub>5</sub>/18% protein).

## DISCUSSION

### Effect of Storage time on pH value, NH<sub>3</sub> content and moisture content of nutrient concentrates<sup>BLS</sup> stored for 10 weeks

**Storage:** Figure 1 shows that the bioprocessed shrimp waste products (Nutrient Concentrate<sup>BLS</sup>) can be held up to 2 months (8 weeks) in storage conditions at room temperature. The following results were obtained: the pH was close to neutral (7.36), the NH<sub>3</sub> (ammonia) content was less than 10% (9.60%) and the water content was stable (below 10%). A storage time of up to 8 weeks was still safe.

**Egg production:** The use of Nutrient Concentrates<sup>BLS</sup> up to the level of 2% in rations did not have a negative impact on the egg production performance of domestic laying hens and the results were equivalent to those attained with standard rations (18% protein). Body weight of laying hen during the study was relatively homogenous, with the coefficient of variation in body weight ranging from 3.80-4.07%, with ration consumption of each hen at 75 g hen<sup>-1</sup> day<sup>-1</sup>.

**Egg quality:** High cholesterol content in the blood predisposes individuals to atherosclerosis, a condition where cholesterol and lipids enter the inner blood vessel walls, resulting in the accumulation (deposition) of ester cholesterol and lipids in the connective tissue of the arterial walls<sup>17</sup>. Development of atherosclerosis is closely related to the consumption of foods that are high in cholesterol and saturated fat<sup>11,17</sup>. Certain cardiovascular diseases, such as atherosclerosis or hardening of the arteries, are partly due to high cholesterol levels in the blood<sup>11,18</sup>. The use of a bioprocessed product supplemented with Se (Nutrient Concentrates<sup>BLS</sup>) as an antioxidant component (glycation-peroxidase) in chicken rations can reduce fat accumulation and suppress the activity of the 3-hydroxy-3-methylglutaryl Co-A reductase enzyme, which functions to synthesize cholesterol in the liver. Shrimp skin contains carotenoids (colour pigments) in the form of astaxanthin, which is a provitamin A.

Changes in egg cholesterol content are governed by the food consumed by hens. The consumption of feed that results in high fat accumulation is associated with high cholesterol deposits in meat and eggs<sup>11,17</sup>. The excess energy content that is not utilized by the body is stored in the form of fat. The cholesterol contained in chicken eggs is thought to cause ill-health effects in consumers suffering from certain diseases.

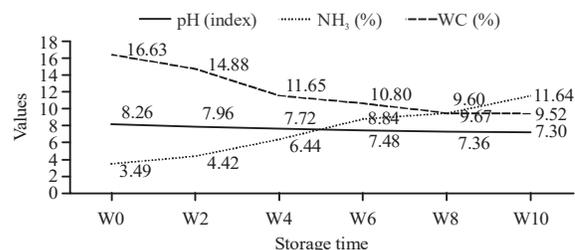


Fig. 1: The effect of storage time on pH value, NH<sub>3</sub> content and water content of bioprocessed shrimp waste products (Nutrient Concentrates<sup>BLS</sup>) over 10 weeks of storage

However, cholesterol is still needed by the human body and these needs range from 1,000-1,500 mg per day. The results of cholesterol metabolism are used as follows: (1) The formation of cell membrane structures and plasma lipoproteins, (2) The plasma membrane permeability and the activity of enzymes related to membranes and (3) the generation of precursors in the synthesis of a number of steroid hormones needed for the production of vitamin D<sub>3</sub> and bile salts used to emulsify fatty foods digested in the small intestine<sup>11,18</sup>.

The physical and chemical quality of the eggs from the domestic laying hens in general was better due to consumption of rations containing Nutrient Concentrates<sup>BLS</sup> compared to those eggs produced by hens that consumed rations without Nutrient Concentrates<sup>BLS</sup>. Nutrient Concentrates<sup>BLS</sup> can be used up to a level of 2% in the feed formula.

Figure 2 shows the different patterns of egg quality among the different levels of feed supplements with Nutrient Concentrates<sup>BLS</sup>. The level of Nutrient Concentrates<sup>BLS</sup> that produced the eggs of the best quality was 2% (R<sub>4</sub>).

Nutrition is a contributing factor to the quality and colour of eggs, both internally and externally<sup>19-21</sup>. Feeding probiotics to layers could improve eggshell colour, particularly in brown-shelled eggs<sup>3</sup>. Supplements containing *Bacillus subtilis* were administered to 63-week-old Lohman Brown commercial hybrids, resulting in increased pigmentation for up to 2 weeks after the first delivery<sup>22</sup>. The weight of each egg, as well as the weight of the shell, the strength of the shell and any abnormalities in the egg structure, yolk colour and properties of the albumen, are indicators of the uniformity of the shell physical properties and the internal standards of the eggs. The cuticle on the shell serves as the first line of defence against pathogens and acts as a waterproofing agent<sup>18</sup>. Removal of the cuticle increased penetration of eggs by

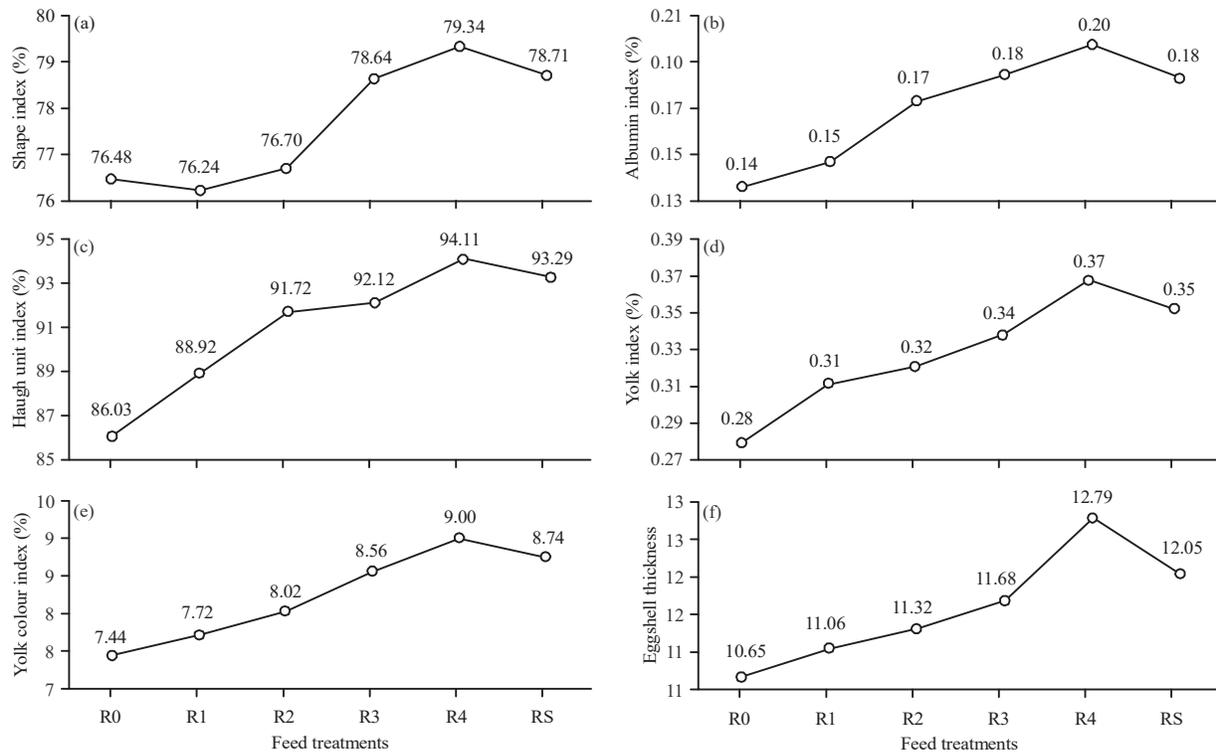


Fig. 2(a-f): Quality charts (a) Shape index, (b) Albumin index, (c) Haugh unit, (d) Yolk colour index, (e) Eggshell thickness and (f) Egg cholesterol of the eggs from domestic laying hens fed rations containing Nutrient Concentrates at the level of 0-2% compared to standard rations

20-60%. Eggs with poor-quality cuticles were found to have 40% more contamination than eggs with more intact cuticles (26%).

### CONCLUSION

Bioprocessed shrimp waste products by *Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae* can be used as feed supplements in laying domestic poultry and hereinafter are referred to as Nutrient Concentrates<sup>BLS</sup>. Nutrient Concentrates<sup>BLS</sup> can be stored up to 2 months at room temperature conditions (the pH approaches a neutral value (7.36), the NH<sub>3</sub> (ammonia) content is less than 10% (9.60%) and the water content is stable below 10%). The optimal production of domestic laying hen eggs for 60 days of maintenance, namely, the treatment of rations containing 2% Nutrient Concentrates<sup>BLS</sup> with an average egg weight of 44.71 g egg<sup>-1</sup>; number of eggs 3.63 points and hen-day production 51.79%; with feed efficiency of 35.49%. The optimal egg quality of domestic laying hens was the treatment of rations containing 2% of Nutrient Concentrates<sup>BLS</sup> with a shape index value of 79.34, albumin index 0.20, Haugh unit 94.11, yolk index 0.37, yolk colour 9.00 and egg cholesterol level of 96.45 (mg dL<sup>-1</sup>).

### SIGNIFICANCE STATEMENT

This study determined a standard operational procedure for manufacturing nutrient concentrations that can be beneficial for improving egg quality (lower cholesterol) for the benefit of human health. This study will help researchers to uncover the potential utility of a combination of three types of microbes (*Bacillus licheniformis*, *Lactobacillus* sp. and *Saccharomyces cerevisiae*) in the processing of waste materials.

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