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Astaxanthin Content of Chicken Feed Fortified with Blue Swimming Crab *(Portunus pelagicus)* Shell and Whiteleg Shrimp *(Litopenaeus vannamei)* Shell Meals

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Abstract

The poultry industry faces high feed costs and growing consumer demand for products of better quality, including eggs with intensely coloured yolks. Astaxanthin is a potent antioxidant carotenoid that poultry cannot synthesise and must obtain from the diet. This study evaluated blue swimming crab (Portunus pelagicus) shell meal and whiteleg shrimp (Litopenaeus vannamei) shell meal, two abundant crustacean processing by-products, as natural sources of astaxanthin in chicken feed. A completely randomised design was applied in which a basal feed was fortified with each meal at 0% (control), 10%, 20%, and 30%, with three replicates per treatment. Astaxanthin was extracted with methanol and quantified by UV–Vis spectrophotometry in the raw meals and fortified feeds. The shrimp shell meal contained 4.284 mg kg^{-1} astaxanthin, slightly more than the crab shell meal (4.137 mg kg^{-1}), while the unfortified basal feed contained 3.178 mg kg^{-1}. Fortification level significantly affected astaxanthin content for both materials ($p < 0.001$). Shrimp shell fortification produced a consistent, dose-dependent increase to 5.494 ± 0.167 mg kg^{-1} at 30%, with all levels differing significantly. Crab shell fortification produced a non-linear response, peaking at 10% (3.591 ± 0.025 mg kg^{-1}) and falling below the control at 30% (2.824 ± 0.051 mg kg^{-1}), possibly because the high calcium carbonate content hindered pigment extraction. Values in the shrimp-fortified feeds exceeded those predicted from the raw-material contents, suggesting matrix effects on extraction and indicating that the spectrophotometric values represent total carotenoids expressed as astaxanthin equivalents. Whiteleg shrimp shell meal is therefore the more effective fortificant for increasing the carotenoid content of chicken feed, whereas crab shell meal should be limited to low inclusion levels.

Keywords: astaxanthin; blue swimming crab; chicken feed; crustacean by-products; fortification

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INTRODUCTION

The poultry industry, particularly egg production, is a vital source of affordable animal protein in Indonesia and other developing countries. Feed accounts for the largest share of production costs in poultry farming, commonly estimated at 70–80%, so that any innovation that lowers feed costs or adds value to feed has a direct impact on farm profitability. At the same time, consumer expectations regarding product quality have risen, and yolk colour is one of the most visible quality attributes of eggs. A deep yellow-orange yolk is widely perceived as an indicator of freshness and nutritional value, and in many markets' yolk colour strongly influences purchasing decisions. Because poultry cannot synthesise carotenoids de novo, the pigmentation of egg yolks, skin, and meat depends entirely on the carotenoids supplied in the diet (Nabi et al., 2020). Feed manufacturers therefore add natural or synthetic pigments, such as lutein and zeaxanthin

from maize and marigold or red carotenoids such as canthaxanthin and astaxanthin, to achieve the desired colour (Saini et al., 2022).

Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione, $C_{40}H_{52}O_4$) is a red-orange xanthophyll carotenoid responsible for the characteristic colour of salmon, trout, shrimp, lobster, and other crustaceans. Its molecular structure, with hydroxyl and keto groups on each terminal ionone ring, allows it to span cell membranes and quench singlet oxygen and free radicals with exceptional efficiency, and its antioxidant activity has been reported to exceed that of β-carotene and α-tocopherol (Šimat et al., 2022; Stachowiak & Szulc, 2021). Astaxanthin has no provitamin A activity, but it has been associated with anti-inflammatory, immunomodulatory, and cytoprotective effects in animals and humans (Fakhri et al., 2018). Natural astaxanthin is produced commercially from the microalga Haematococcus pluvialis and the yeast Xanthophyllomyces dendrorhous (Phaffia rhodozyma), whereas synthetic astaxanthin is derived from petrochemical precursors and dominates the aquafeed market. Both forms are expensive, which limits their use in poultry feed (Stachowiak & Szulc, 2021).

In poultry, dietary astaxanthin has been shown to improve product quality and animal health. Supplementing laying hens with natural astaxanthin from Haematococcus pluvialis increased antioxidant capacity and led to the accumulation of astaxanthin in egg yolks (Gao et al., 2020), and yolk colour darkened in a dose-dependent manner while egg quality was better maintained during storage (Heng et al., 2020). In broilers, an astaxanthin-rich bacterial cell powder supplying 30 mg kg^{-1} astaxanthin increased muscle carotenoid content and meat redness and reduced lipid peroxidation under high ambient temperature (Inoue et al., 2019), and supplementation increased muscle antioxidant activity and meat redness and reduced lipid peroxidation in birds exposed to high temperatures (Hosseindoust et al., 2020). Astaxanthin has also been reported to improve the cytoprotective capacity of the ileum in heat-stressed broilers, illustrating its protective role in the digestive tract (Kuehu et al., 2024). These findings suggest that astaxanthin can serve simultaneously as a pigment, an antioxidant, and a health-promoting feed additive, provided that an affordable source is available.

Crustacean processing by-products represent a potentially low-cost source of natural astaxanthin. Shrimp, crab, and lobster processing generates large quantities of shells, heads, and exoskeleton residues, which can account for a large proportion of the raw material weight and are frequently discarded, causing environmental pollution in coastal areas (Nirmal et al., 2020). These residues contain chitin, protein, calcium carbonate, lipids, and carotenoids, chiefly astaxanthin in free and esterified forms, and their valorisation has been advocated as a key element of a circular bioeconomy (Šimat et al., 2022). Recent reviews have shown that protein, chitin, and carotenoids can be recovered as value-added products from shrimp waste using chemical and increasingly biotechnological methods (Mao et al., 2017). Shrimp by-products contain protein, minerals, lipids rich in polyunsaturated fatty acids, and astaxanthin, which can be recovered with green solvents such as vegetable oils (Panagiotakopoulos et al., 2023). Carotenoid yields depend strongly on the extraction solvent and technique, with acetone, solvent mixtures, and assisted methods generally more effective than single polar solvents (Saini & Keum, 2018). Carotenoid extracts from shrimp shells also exhibit significant antioxidant activity, and ultrasound-assisted extraction can substantially increase astaxanthin recovery from shrimp shells (Sharayei et al., 2021).

Indonesia is one of the world's major producers of farmed whiteleg shrimp (Litopenaeus vannamei) and a leading exporter of pasteurised blue swimming crab

(Portunus pelagicus) meat. Shrimp freezing plants and crab picking facilities in South Sulawesi generate large volumes of shells that are largely underused, apart from limited use in chitin and chitosan production. Converting these by-products into feed ingredients could add economic value, reduce waste, and provide a local alternative to imported pigments. However, the two materials differ markedly in structure and composition. Shrimp shells are thin and flexible, with a relatively high proportion of protein and chitin, whereas crab shells are thick and highly calcified, with calcium carbonate constituting the major fraction (Mao et al., 2017; Šimat et al., 2022). These differences may affect both the concentration of astaxanthin in the meals and its extractability and distribution when the meals are incorporated into feed. In addition, the high mineral content of crab shell could limit its practical inclusion in poultry diets.

Despite the potential of these by-products, few studies have directly compared shrimp and crab shell meals as astaxanthin sources in poultry feed or evaluated how their inclusion level affects the measured astaxanthin content of the fortified feed. Information on the dose–response relationship is essential to determine appropriate inclusion levels before conducting feeding trials. This study therefore aimed to (1) determine the astaxanthin content of blue swimming crab shell meal and whiteleg shrimp shell meal, and (2) evaluate the effect of fortification with each meal at 0%, 10%, 20%, and 30% on the astaxanthin content of chicken feed. The results are intended to provide a scientific basis for developing functional poultry feeds based on local crustacean by-products within a circular economy framework.

METHOD

The study was conducted from August to October 2025. Shell meals and fortified feeds were prepared at the Integrated Laboratory of Institut Teknologi dan Bisnis Maritim Balik Diwa, Makassar, and astaxanthin analyses were performed at the Basic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin. Blue swimming crab (Portunus pelagicus) shells were obtained from a crab processing mini plant in Pangkajene and Islands Regency, and whiteleg shrimp (Litopenaeus vannamei) shells from a shrimp processing company in Makassar. The shells were washed under running water to remove residual flesh and impurities, dried in an oven at 60–80 °C to a moisture content below 10% determined gravimetrically, coarsely crushed, ground in a disc mill, and sieved through an 80-mesh screen, following general procedures for processing crustacean discards into meals (Mao et al., 2017; Nirmal et al., 2020). The basal feed consisted of ground maize and a commercial premix. Crab shell meal (TCK) and shrimp shell meal (TKU) were each incorporated into the basal feed at 0% (control), 10%, 20%, and 30% (w/w), yielding the crab-fortified feeds (PATCK) and shrimp-fortified feeds (PATKU), and the mixtures were homogenised in a feed mixer. A completely randomised design was used for each fortificant, with four inclusion levels and three replicates.

Astaxanthin was extracted from the raw meals and fortified feeds with methanol, adapting solvent extraction procedures described for shrimp shells (Sharayei et al., 2021; Panagiotakopoulos et al., 2023). Weighed samples were mixed with solvent on a vortex mixer, centrifuged to separate the supernatant, and the absorbance of the extract was measured with a UV–Vis spectrophotometer at the absorption maximum of astaxanthin. Concentrations were calculated from the absorbance using the specific absorption coefficient of astaxanthin (Saini & Keum, 2018) and expressed in mg kg^{-1}. Because spectrophotometry measures the combined absorbance of all carotenoids in the extract,

the results represent total carotenoids expressed as astaxanthin equivalents (Saini & Keum, 2018; Saini et al., 2022). To evaluate the contribution of each fortificant, the expected astaxanthin content of each fortified feed was calculated by mass balance as the weighted sum of the contents of the basal feed and the shell meal at the given inclusion level. Data were analysed separately for each fortificant by analysis of variance (ANOVA), with fortification level as the treatment factor and replicate included as a blocking term to account for analytical batch effects. When the treatment effect was significant ($p < 0.05$), means were compared using Duncan's multiple range test (Lee & Lee, 2018).

RESULT AND DISCUSSION

Astaxanthin content of raw materials

The astaxanthin contents of the raw materials before fortification are shown in Table 1. Shrimp shell meal had the highest content (4.285 ± 0.026 mg kg^{-1}), followed by crab shell meal (4.137 ± 0.089 mg kg^{-1}) and the unfortified basal feed (3.178 ± 0.111 mg kg^{-1}).

Table 1.
Astaxanthin content (mg kg^{-1}) of crab shell meal, shrimp shell meal, and basal feed

Replicate	Crab shell meal (TCK)	Shrimp shell meal (TKU)	Basal feed (PA)
1	4.2257	4.3142	3.2965
2	4.1372	4.2699	3.1637
3	4.0487	4.2699	3.0752
Mean ± SD	**4.137 ± 0.089**	**4.285 ± 0.026**	**3.178 ± 0.111**

Sumber: hasil penelitian, 2026

The slightly higher content of shrimp shell meal is consistent with the thinner, less calcified exoskeleton of shrimp, which contains a higher proportion of the protein–chitin matrix in which astaxanthin is deposited, whereas a larger fraction of crab shell mass consists of calcium carbonate (Šimat et al., 2022). The carotenoid content of crustaceans also varies with species, diet, moult stage, and the body components included in the waste (Šimat et al., 2022). The absolute values obtained here, approximately 4 mg kg^{-1} (equivalent to 4 μg g^{-1}), are lower than many reported for shrimp processing waste, in which acetone extraction has yielded several tens of micrograms per gram, but are of a similar order to values reported for blue crab shell (Sharayei et al., 2021). This difference is largely attributable to the extraction solvent: methanol alone is less effective than acetone or solvent mixtures for recovering astaxanthin and its esters from crustacean waste (Saini & Keum, 2018). Oven drying at 60–80 °C may also have degraded part of the pigment, because astaxanthin is sensitive to heat, light, and oxygen (Stachowiak & Szulc, 2021). The measurable content of the basal feed (3.178 mg kg^{-1}) reflects the yellow xanthophylls of maize, chiefly lutein and zeaxanthin, which absorb in the same spectral region as astaxanthin and are therefore included in spectrophotometric measurements (Saini et al., 2022). The values reported in this study should accordingly be interpreted as total carotenoids expressed as astaxanthin equivalents.

Effect of shrimp shell meal fortification

Fortification with shrimp shell meal significantly affected the astaxanthin content of the feed ($F_{3, 6} = 756.46$, $p < 0.001$), and Duncan's test separated all four levels into distinct groups (Table 2). The content increased consistently from 3.178 ± 0.111 mg kg^{-1} in the

control to 3.945 ± 0.155, 4.476 ± 0.135, and 5.494 ± 0.167 mg kg^{-1} at 10%, 20%, and 30%, respectively, an overall increase of 73%. The replicate term was also significant ($F_{2,6}$ = 19.09, p = 0.003), indicating some variation among analytical batches, but its contribution to total variation was small relative to the treatment effect.

Table 2.
Observed and expected astaxanthin content (mg kg^{-1}) of chicken feed fortified with shrimp shell meal (PATKU) and crab shell meal (PATCK)

Inclusion level	PATKU observed (mean ± SD)	PATKU expected	PATCK observed (mean ± SD)	PATCK expected
0%	3.178 ± 0.111[a]	3.178	3.178 ± 0.111[b]	3.178
10%	3.945 ± 0.155[b]	3.289	3.591 ± 0.025[d]	3.274
20%	4.476 ± 0.135[c]	3.400	3.473 ± 0.044[c]	3.370
30%	5.494 ± 0.167[d]	3.510	2.824 ± 0.051[a]	3.466

Expected values were calculated by mass balance from the contents of the basal feed and the shell meals (Table 1). Different superscript letters within a column indicate significant differences (Duncan's test, $p < 0.05$); n = 3. PATKU: $F_{3,6} = 756.46$, $p < 0.001$; PATCK: $F_{3,6} = 201.91$, $p < 0.001$.

The dose-dependent increase confirms that shrimp shell meal contributes carotenoids to the feed and that its effect is proportional to the inclusion level. Notably, the observed values exceeded those predicted by mass balance at every level, by 20%, 32%, and 57% at 10%, 20%, and 30%, respectively, and the value at 30% inclusion was even higher than that of the pure shrimp meal. This pattern indicates that the measured contents are not simply additive and that the feed matrix influenced extraction efficiency. Astaxanthin in crustacean shells is largely bound to proteins as carotenoproteins and embedded in the chitin matrix, which limits its release into polar solvents (Šimat et al., 2022). When the meal is dispersed in maize-based feed, lipids from maize may act as co-solvents that help solubilise astaxanthin and its esters, increasing the amount recovered by methanol relative to extraction of the pure meal. Interactions between shrimp-derived and maize-derived carotenoids in the extract may also contribute to the higher absorbance. These matrix effects mean that spectrophotometric values should be confirmed by chromatographic analysis, which can separate astaxanthin from maize xanthophylls and quantify free and esterified forms individually (Saini & Keum, 2018). Nevertheless, the consistent increase with inclusion level shows that shrimp shell meal is an effective source of carotenoids for feed fortification.

Effect of crab shell meal fortification

Crab shell meal also significantly affected the astaxanthin content of the feed ($F_{3,6}$ = 201.91, p < 0.001), but the response was non-linear (Table 2). The content increased from 3.178 mg kg^{-1} in the control to a maximum of 3.591 ± 0.025 mg kg^{-1} at 10%, decreased slightly to 3.473 ± 0.044 mg kg^{-1} at 20%, and fell to 2.824 ± 0.051 mg kg^{-1} at 30%, significantly below the control. Duncan's test ranked the treatments in the order 30% < 0% < 20% < 10%, with all levels differing significantly. The replicate term was significant but small ($F_{2,6}$ = 7.13, p = 0.026).

At 10% and 20%, the observed values were close to or slightly above the mass-balance predictions, indicating that crab shell meal contributed carotenoids roughly in proportion to its content. At 30%, however, the observed value was 19% below the prediction and lower than the control, even though crab shell meal itself contained more carotenoids than the basal feed. Because simple dilution cannot explain a decrease below the control, the result points to an interference effect at high inclusion levels. Crab shell is

dominated by calcium carbonate, with chitin and protein forming a dense, mineralised matrix (Mao et al., 2017; Šimat et al., 2022). At high inclusion levels, the large amount of finely ground mineral may adsorb carotenoids or physically entrap them, reducing their extraction into methanol. Carbonate minerals can also alter the local chemical environment during extraction, potentially promoting the degradation of labile carotenoids. Demineralisation with dilute acid before extraction, or the use of more effective solvents, improves astaxanthin recovery from calcified crustacean waste (Saini & Keum, 2018; Sharayei et al., 2021), which supports the interpretation that the low value at 30% reflects reduced extractability rather than an absence of pigment. Whether a similar reduction in bioavailability would occur in the digestive tract of chickens remains to be determined.

Comparison of the two fortificants and practical implications

The two by-products differed markedly in their effectiveness as fortificants. Shrimp shell meal produced a consistent and substantial increase in the carotenoid content of the feed, whereas crab shell meal produced only a modest increase at low inclusion and a decline at high inclusion. These differences can be attributed mainly to the lower mineral content and less compact structure of shrimp shells, which allow carotenoids to be released more readily (Šimat et al., 2022). From a practical standpoint, high inclusion levels of either meal raise nutritional concerns. Crab shell meal at 20–30% would supply calcium far in excess of the requirements of broilers and growing birds and could disturb the calcium–phosphorus balance, although laying hens require considerably more calcium for eggshell formation (Li et al., 2017). Shrimp shell meal contains appreciable chitin, which is poorly digested by poultry and may reduce nutrient utilisation at high inclusion levels (Mao et al., 2017).

The carotenoid concentrations achieved in this study, up to approximately 5.5 mg kg^{-1}, are also low compared with the astaxanthin levels used in poultry feeding trials. Studies reporting improvements in meat colour and antioxidant status in broilers have used supplementation levels of 20–80 mg kg^{-1} (Hosseindoust et al., 2020), and egg yolk pigmentation studies have used astaxanthin-rich yeast or algae providing comparable or higher concentrations (Gao et al., 2020; Heng et al., 2020). Shell meals incorporated without extraction are therefore unlikely to deliver astaxanthin doses sufficient to produce strong pigmentation effects on their own. Recovering a concentrated astaxanthin extract from shrimp waste, for example by improved solvent or ultrasound-assisted extraction, and adding it to feed at lower inclusion rates could provide a more effective and nutritionally balanced approach (Sharayei et al., 2021; Panagiotakopoulos et al., 2023). The valorisation of crustacean by-products in this way would also support circular economy goals by converting processing waste into a value-added feed ingredient (Nirmal et al., 2020). This study was limited to chemical analysis of the feeds; HPLC confirmation of astaxanthin, evaluation of pigment stability during storage, and feeding trials measuring yolk colour, meat quality, and bird performance are needed to establish the practical value of these fortificants.

CONCLUSION

Blue swimming crab shell meal and whiteleg shrimp shell meal both contain carotenoids that can be used to fortify chicken feed, with spectrophotometric contents of 4.137 and 4.285 mg kg^{-1} (as astaxanthin equivalents), respectively, compared with 3.178 mg kg^{-1} in the maize-based basal feed. Fortification level significantly affected the

carotenoid content of the feed for both materials. Shrimp shell meal produced a consistent, dose-dependent increase, with all levels differing significantly and the highest value at 30% inclusion (5.494 ± 0.167 mg kg^{-1}), exceeding mass-balance predictions and indicating that the feed matrix enhanced pigment extraction. Crab shell meal produced a non-linear response, with a maximum at 10% (3.591 ± 0.025 mg kg^{-1}) and a decline below the control at 30% (2.824 ± 0.051 mg kg^{-1}), most likely because its high calcium carbonate content hindered carotenoid extraction. Whiteleg shrimp shell meal is therefore the more effective fortificant, whereas crab shell meal should be limited to low inclusion levels, also in view of its high mineral load. Because the achieved concentrations are below the astaxanthin doses used in poultry feeding trials and the spectrophotometric method cannot distinguish astaxanthin from maize xanthophylls, future work should apply more efficient extraction, confirm astaxanthin by HPLC, consider using concentrated extracts, and evaluate effects on yolk colour, meat quality, and bird performance in feeding trials.

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