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Research Article

Effects of Inclusion of Fermented Carrageenan By-products in the Basal Diet of Broiler Chickens on Growth Performance, Blood Profiles and Meat Composition

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Abstract

Background: Carrageenan by-products may be a potential source of nutrients that may improve the health and growth performance of broiler chickens. **Objective:** This study aimed to evaluate the effects of fermented carrageenan by-products (FCB) on the growth performance, blood profiles and meat composition of broilers. **Methodology:** Two hundred and fifty one day old chicks (DOC) were distributed in a completely randomized design consisting of 5 treatments and 5 replicates with 10 birds per replicate. The basal diet mainly consisted of corn, soybean, rice bran and fish meal and the treatments were T1 (basal diet only as a control), T2 (97.5% basal diet+2.5% FCB), T3 (95.0% basal diet+5% FCB), T4 (92.5% basal diet+7.5% FCB) and T5 (90.0% basal diet+10% FCB). Experimental birds were kept under standard management conditions for 5 weeks. **Results:** The inclusion of FCB in the basal diet significantly affected feed intake, Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) and it significantly reduced the concentration of blood cholesterol, triglycerides and Low Density Lipoproteins (LDL) while significantly elevating High Density Lipoproteins (HDL). However, blood haemoglobin values were statistically similar among all treatments. Furthermore, FCB significantly reduced the fat and cholesterol content of the breast meat but did not significantly affect the water, protein and ash contents. **Conclusion:** Up to 7.5% FCB can be used in broiler chicken diets to improve growth performance and the quality of the breast meat.

Key words: Breast meat, cholesterol, carrageenan by-products, conversion, fibers, haemoglobin, lipoprotein

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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

Eucheuma cottonii (*Kappaphycus alvarezii*) is a cultivated red seaweed that grows well in coastal waters and can generally be harvested every 45 days. Furthermore, it is a commercial source of carrageenan that is used as a gelling agent and stabilizer by the food and pharmaceutical industries^{1,2} and it also contains high amounts of dietary fibers, minerals, vitamins, antioxidants, polyphenols, phytochemicals, proteins and polyunsaturated fatty acids and may have medicinal uses^{3,4}. In fact, some of these bioactive components have been used as a functional feed to improve the health of livestock⁴.

The production process that converts *Eucheuma cottonii* to carrageenan produces large amounts of by-products. Manuhara *et al.*⁵ recently reported a 34.4% carrageenan extraction efficiency from seaweed using a 2.5% KCl solution, indicating that 65.5% of the seaweed material remained as a by-product. However, this by-product can potentially be used as a source of nutrients for other productive processes.

Previous studies have indicated that the inclusion of seaweed in diets had no anti-nutritive effects on broiler chickens⁶ but improved their feed intake, live weight gain and feed efficiency^{7,8}. Some seaweeds could serve as sources of protein and energy, while others may have bioactive compounds that could be used as prebiotics to improve livestock productivity and health⁴. However, the inclusion of these materials in livestock diets could reduce the bioavailability of dietary components⁹ and seaweed also tends to accumulate heavy metals⁴.

If we assume that seaweed and carrageenan by-products have similar nutrient compositions and characteristics, carrageenan by-products may be a potential source of nutrients to promote the health and growth of animals. In addition, the nutritional value of carrageenan by-products may be improved through additional treatments, such as fermentation¹⁰, but more research is needed to more fully realize the potential of the inclusion of fermented carrageenan by-products (FCB) in animal feeds. Therefore, this study aimed to investigate the addition of FCB to the basal diet of broiler chickens affects growth performance, blood profiles and the chemical composition of the meat.

MATERIALS AND METHODS

Broiler chickens were housed in pens whose floors were covered with wood shavings. Each cage was 120×100×100 cm and was equipped with a feeding and drinking trough as well as lamps for heating and lighting. A

Table 1: Basal diet composition

Feed ingredients	Percentage	
	Starter (0-3 weeks)	Finisher (3-5 weeks)
Corn	56.00	56.50
Soybean meal	25.00	26.00
Fish meal	11.40	6.00
Rice bran	5.00	9.36
Palm oil	1.00	0.00
Limestone	1.00	0.90
Salt	0.02	0.07
Premix ¹	0.30	0.20
Lysine	0.08	0.18
DL-methionine	0.20	0.09
Total	100.00	100.00
Calculated composition		
Metabolizable energy (kcal kg ⁻¹)	3207.00	3201.00
Crude protein	23.00	20.00
Crude fiber	3.13	3.70
Calcium	1.13	0.75
Phosphate	0.29	0.35
Lysine	1.02	1.07
Methionine	0.51	0.38

¹The diet supplied the following amounts per kilogram: Calcium 32.5%, phosphorous 10%, iron 6 g, manganese 4 g, iodine 0.075 g, copper 0.3 g, zinc 3.75 g, vitamin B₁₂ 0.5 mg, vitamin D₃ 50,000 IU, vitamin A 1,200,000 IU, vitamin E 800 IU, vitamin K 200 mg, vitamin B₁ 200 mg, vitamin B₂ 500 mg, vitamin B₆ 50 mg, vitamin C 2500 mg, Ca-D-pantothenate 600 mg, niacin 4,000 mg, methionine 3,000 mg, lysine 3,000 mg, santonin 1,000 mg, zinc bacitracin 2,100 mg

total of 250 one day old chicks (DOC) were allocated to 5 treatments in a Completely Randomized Design (CRD) with 5 replicates (cages) per treatment and 10 birds per cage for a total of 25 cages. Experimental diets and drinking water were provided freely throughout the study period and the birds were kept under standard management conditions for 5 weeks.

Carrageenan by-products were fermented as outlined in Hasanuddin and Rusdi¹⁰ and the FCB were dried and ground into a powder. In this form, these FCB were used as a substitute for part of the basal diet, which consisted of corn, rice bran, soy bean, fish meal, coconut oil, premix, methionine and lysine (Table 1). Experimental diets were the basal diet mixed with FCB in varying proportions, including a control consisting of the basal diet only (T1), 97.5% basal diet+2.5% FCB (T2), 95.0% basal diet+5% FCB (T3), 92.5% basal diet+7.5% FCB (T4) and 90.0% basal diet+10% FCB (T5).

The following parameters were measured: Feed intake, Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) as well as blood profiles, including blood cholesterol, Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), triglycerides and haemoglobin levels. The chemical composition of the breast meat was also analyzed, including the concentrations of water, protein, fat and ash as well as cholesterol levels.

Body weight and feed intake were recorded weekly and used to calculate the BWG and Feed Conversion Ratio (FCR). At the end of the experimental period, one bird from each cage was randomly selected for blood sample collection; approximately 3 mL of blood was collected into a labelled sterilized tube using a needle and syringe and was immediately centrifuged at 1,500 ×g for 15 min. Blood samples were also taken using 3 mL tubes containing ethylenediaminetetraacetic acid (EDTA) to measure haemoglobin and enzymatic colorimetric methods were applied to analyze blood parameters. Cholesterol and HDL were determined using the cholesterol oxidase-p-aminophenazone (CHOD-PAP) enzyme and triglyceride levels were determined using the glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) enzyme. Furthermore, LDL was calculated using an equation [i.e., cholesterol-(HDL-triglyceride/5)] described by Baraas and Jufri¹¹. Two birds were randomly selected from each cage and slaughtered to determine meat composition. The breast meat was separated from each carcass and weighed and the meat samples were transferred to the laboratory for further analysis. The water, crude ash, crude protein and fat contents of the breast meat were determined using an AOAC¹² method and the cholesterol content of the breast meat was determined using the Liebermann-Buchard method¹³.

Statistical analysis: Data were analysed by one-way analysis of variance¹⁴ and Duncan's multiple range test¹⁵ was applied to compare the treatment means at a significance level of 5%.

RESULTS AND DISCUSSION

Growth performance: Our previous *in vitro* study found the chemical composition of FCB to be 16.78% protein, 0.25% lipid, 17.53% fibre and 20.60% ash¹⁰, but whether their inclusion affects the overall nutrient composition of the diet and, in turn, the growth performance of broiler chickens has not been confirmed. In this study, the inclusion of FCB in a basal diet significantly increased ($p < 0.05$) feed intake, Body Weight Gain (BWG) and the Feed Conversion Ratio (FCR) (Table 2), which is consistent with the results of previous studies showing that the addition of fermented plant products

in livestock diets had a significant positive effect on growth performance¹⁶⁻¹⁸. Here, feed intake increased linearly with increasing amounts of FCB in the diet, but not all treatments were statistically different from the control group. For example, feed intake is likely mediated by the improved flavour of the diet conferred by FCB, as previously reported for fermented seaweed^{17,18}, even though taste perception in poultry is known to be less developed than in mammals¹⁸.

Interestingly, FCB at 10% of the basal diet reduced BWG and FCR relative to the control group even though the birds consumed more feed (Table 2). The negative effects of dietary fibre on broiler growth performance are associated with a decrease in nutrient availability and digestion as well as the concentration of Metabolizable Energy (ME) in the diet¹⁹. This increased fibre could act to dilute nutrients while elevating the digesta passage rate, which reduces nutrient digestibility²⁰ and decreases the availability of nutrients for absorption in the digestive tract. Our result is in agreement with that of Tabook *et al.*¹⁹, who found that experimental birds manifested an adaptation process as feed intake increased to compensate for the reduction in nutrient concentrations that would negatively affect growth performance. In contrast, Jimenez-Moreno *et al.*²¹ revealed that fibre inclusion improved BWG and FCR by promoting growth performance and nutrient digestibility in young birds and Mourao *et al.*²² found that fibre from dehydrated pasture material increased FCR and reduced growth but did not influence feed intake. The present study suggested that as much as 7.5% FCB can be used to supplement a broiler diet without negatively affecting growth performance.

Blood profiles: The inclusion of FCB in broiler chicken diets was associated with significant decreases in the blood concentration values of cholesterol, low-density lipoprotein (LDL) and triglycerides (Table 3), while high-density lipoprotein (HDL) values significantly increased as more FCB was included in the diet ($p < 0.05$, Table 3). These findings are in accordance with those of Chen and Anderson²³, Astawan *et al.*²⁴ and Van Bennekum *et al.*²⁵, who found that the inclusion of fibre in diets reduced plasma cholesterol and triglyceride concentrations and increased the level of HDL in

Table 2: Mean values of feed intake, body weight gain and feed conversion ratio

Parameters	Treatments				
	T1	T2	T3	T4	T5
Feed intake (kg bird ⁻¹)	2.14±0.14 ^a	2.51±0.24 ^b	2.25±0.18 ^a	2.39±0.19 ^{ab}	2.31±0.13 ^{ab}
Body weight gain (kg bird ⁻¹)	1.32±0.16 ^{ab}	1.33±0.15 ^{ab}	1.38±0.11 ^a	1.36±0.13 ^{ab}	1.17±0.10 ^b
Feed conversion ratio	1.64±0.15 ^a	1.90±0.19 ^b	1.63±0.08 ^a	1.77±0.15 ^{ab}	1.92±0.19 ^b

Mean ± SD within the rows followed by different superscript letters are significantly different at $p < 0.05$

Table 3: Mean concentration values of blood cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), triglyceride and haemoglobin

Parameters	Treatments				
	T1	T2	T3	T4	T5
Cholesterol (mg dL ⁻¹)	147.0±1.00 ^a	134.0±1.00 ^b	131.0±2.65 ^{bc}	131.0±1.00 ^{bc}	128±2.65 ^d
HDL (mg dL ⁻¹)	20.6±0.85 ^a	22.3±0.76 ^b	24.7±0.63 ^c	27.7±0.69 ^d	28.0±0.50 ^d
LDL (mg dL ⁻¹)	94.3±2.42 ^a	92.9±1.72 ^{ab}	92.4±0.90 ^{ab}	90.7±0.72 ^{ab}	89.9±0.91 ^b
Triglyceride (mg dL ⁻¹)	153.0±2.02 ^a	150.0±1.07 ^{ab}	149.0±0.98 ^{ab}	148.0±1.58 ^{bc}	144±1.75 ^c
Haemoglobin (mg dL ⁻¹)	10.5±0.57	10.7±1.19	10.2±0.89	10.1±0.63	11.1±1.22

Mean±SD within the rows followed by different superscript letters are significantly different at p<0.05

mice and rats. However, our results differed from those of other authors, who found that the addition of fermented plant products¹⁶ or fermented seaweed¹⁷ to broiler chicken diets had no effect on plasma concentrations of cholesterol, triglyceride, HDL, LDL or haemoglobin. The decreased blood cholesterol seen in the present study was presumably due to the presence of more fibre from FCB, which may have inhibited hepatic cholesterol synthesis from fermentation metabolites by intestinal microflora. Indeed, Stupanuk²⁶ stated that fibre fermentation in the colon produces volatile fatty acids, including propionic acid, which is immediately absorbed through the hepatic portal vein and is transported to the liver where it inhibits the activity of HMG-CoA-reductase and, in turn, the rate of cholesterol biosynthesis. Dietary fibre also binds bile acids that are synthesized from cholesterol in the digestive tract, so less cholesterol reaches the blood vessels²⁴. Additionally, Van Bennekum *et al.*²⁵ reported that dietary fibre lowered cholesterol in mice through satiation, leading to lower overall energy intake and moderate or low bile acid-binding capabilities. Less energy intake resulted from the reduction of feed intake by 15-20% relative to the control group²⁵.

Increasing dietary fibre reduces blood cholesterol levels through various mechanisms: (i) Fibre binds bile acids in the small intestine, thus increasing their faecal excretion, (ii) Fibre reduces the absorption of fat and cholesterol, (iii) Fibre reduces the rate of carbohydrate absorption, which reduces serum insulin and, in turn, decreases the stimulation of cholesterol and lipoprotein synthesis and (iv) Fibre hampers cholesterol synthesis due to the presence of short-chain fatty acids generated by fermentation in the colon²⁷. Indeed, there is a negative relationship between HDL, LDL and cholesterol; low HDL concentrations promote LDL uptake and cholesterol release into tissues. Meanwhile, higher concentrations of HDL reduce LDL uptake and cholesterol internalization²³.

Incorporation of FCB in the diets of broiler chickens reduced the levels of LDL in the blood samples (p<0.05). The LDL is often called "Bad" cholesterol because it is largely composed of cholesterol and phospholipids²⁸, but in many animals, including poultry, very-low-density lipoprotein (VLDL)

and LDL play a role in cholesterol transport. Before entering the cells to form lipoprotein, cholesterol circulates in the blood. In the blood vessels, VLDL is converted to LDL and triglycerides and the protein component is eliminated²⁹. Therefore, high levels of LDL in the blood indicate the presence of excess cholesterol that can penetrate the walls of blood vessels. The conditions that influence LDL can also impact triglyceride and protein levels.

Here, we found haemoglobin values that were consistent with those reported by Talebi *et al.*³⁰ and Lokaewmanee *et al.*¹⁶. Moreover, we found that inclusion of FCB in the diet did not significantly affect blood haemoglobin concentrations (p>0.05), which could be because the nutrient content of the diet retained its capacity to promote the formation of blood haemoglobin. Tehrani *et al.*³¹ reported similar findings; broilers provided with different levels of the brine shrimp *Artemia urmiana* in their diet had levels of haemoglobin comparable to that of the control group. Lokaewmanee *et al.*¹⁶ found no differences in the haemoglobin parameters of birds given fermented plant products. The above results suggested that haematological values in poultry can be influenced by factors not related to diet, including age, sex, breed, climate, nutritional status and other physiological factors^{30,32}.

Meat composition: Diet composition is one of the most important external factors that can affect the chemical composition of broiler meat³³ and diets rich in undigested structural carbohydrates can yield low-fat meat. Similarly, diets that have a higher protein-energy ratio will tend to produce low-fat or lean meat. Therefore, we examined the effects of including FCB in the diet of broiler chickens on the chemical composition of the breast meat (Table 4). The results showed that FCB inclusion reduced the cholesterol and fat contents of breast meat (p<0.05), which is consistent with the results of Ponte *et al.*³⁴, who found that inclusion of alfalfa as a fibre source reduced cholesterol levels in the breast meat of chickens. Ponte *et al.*³⁴ also reported that lower cholesterol levels could result from restricted intake of high-energy feed. Alternatively, the observed decrease in cholesterol could be

Table 4: Mean values of chemical composition of breast meat

Parameters	Treatments				
	T1	T2	T3	T4	T5
Water (%)	73.0±0.96	73.70±1.04	73.20±0.98	73.30±1.47	73.70±1.40
Protein (%)	21.7±0.74	20.30±2.30	21.60±1.43	20.80±1.40	20.90±0.87
Fat (%)	3.71±0.09 ^a	3.13±0.51 ^{ab}	2.37±0.33 ^b	2.62±0.75 ^{ab}	1.92±0.33 ^b
Ash (mg g ⁻¹)	1.06±0.11	1.02±0.09	1.13±0.25	1.12±0.14	1.09±0.19
Cholesterol (mg g ⁻¹)	1.18±0.07 ^a	1.16±0.12 ^a	0.76±0.04 ^b	0.74±0.05 ^{bc}	0.55±0.09 ^c

Mean±SD within the rows different superscript letters are significantly different at p<0.05

related to fibre-related reductions in the activity of digestive tract enzymes, such as bile salt hydrolase, that in turn decreased cholesterol levels³⁵. Increased amounts of fibre in the diet of broiler chickens fed FCB might not be digested in the digestive tract, so more lipids might be bound and excreted in the form of bile salts and neutral steroid hormones. Indeed, in this study, less cholesterol and lipids were available for metabolic processes at the tissue level, which reduced the fat content of the breast meat (Table 4). A similar trend was reported by Ponte *et al.*³⁴ for birds that consumed alfalfa.

The results of the current study also indicated that the inclusion of FCB in the diet of broiler chickens did not affect the water, protein or ash contents of the breast meat (p>0.05). This result could be due to the similar protein levels of the experimental diets. This evidence is supported by the fact that increasing the protein content of the diet significantly improve the protein content of the breast meat while not affecting the water content³⁶. Moreover, Marcu and Opris³⁷ found that the protein content of the breast meat increased by 0.48% when the dietary protein content was raised by 2%.

Overall, our results indicate that FCB can be used as an alternative feed ingredient for broiler chickens and its inclusion improved the quality of the breast meat while reducing both its cholesterol and fat contents. In fact, FCB supplementation could be a novel approach to produce breast meat that has lower cholesterol and fat contents, which are qualities that consumers value.

CONCLUSION

This study demonstrated that fermented carrageenan by-products are a promising alternative feed ingredient that can be added to the diet of broiler chickens at concentrations up to 7.5% without significant negative effects on growth performance. Moreover, FCB improved broiler breast meat quality by reducing the fat and cholesterol contents of both the blood and the meat, but the protein, ash and water contents were not altered.

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