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The Feeding Effects of Uronic Acid Extraction from Sargassum Crassifolium on Unsaturated Fatty Acids and the Immunity of Lohman Chicken Eggs

V. G. Kereh*), L. M. Untu*), Ch. Pontoh*), T.F.D. Lumy*), N.J. Kumajas*), M. Rotinsulu*), G.J.V. Assa*)

*Faculty of Animal Science,Sam Ratulangi University, Jln Kampus Bahu, Manado, 95115

veybekereh@unsrat.ac.id

Abstract

The current recommendation is to avoid the use of antibiotics, as this can result in the development of resistance among harmful microorganisms and the presence of residues in the product. This study sought to determine whether uronic acid, which was extracted from Sargassum crassifolium and added to drinking water, had an impact on the immune system of Lohman chicken eggs. Two groups of twenty-two laying hens were provided with different types of commercial feed, one containing antibiotics and the other antibiotic-free. Subsequently, the hens were randomly allocated to receive the brown seaweed content in the drinking water of each of the five treatments (A1 = 0.0% S. crassifolium (control); A2 = 2.5% S. crassifolium; A3 = 5.0% S. crassifolium; A4 = 7.5% S. crassifolium; and A5 = 10.0% S. crassifolium). The study employed a completely randomized design with three replications, two variables, five treatments, and six laying hens per replication. The outcomes revealed that while Salmonella sp. infection persisted, antibody titers and levels of unsaturated fatty acids exhibited variation between treatments. It can be concluded that S. crassifolium uronic acid has the potential to elevate Lohman chicken eggs' amounts of unsaturated fatty acids and boost their immunity.

Keywords: *Sargassum crassifolium*, uronic acid, Lohman chicken, immunity, fatty acid

1. Introduction

Sargassum crassifolium, or brown algae, are classified within the Phaeophyceae family. This seaweed exhibits several shapes, a predominate hue that is either brown or yellow and remains that way after the drying process, and a whole structure with real stems, leaves, also root systems (Yenusi et al., 2014). (Merdekawati & Susanto, 2009). A number of studies have demonstrated the antiviral and antibacterial properties of S. crassifolium, it contains important components including uronic acid, sucrose, and sulfate (Mandal et al., 2007). Furthermore, this seaweed is rich in polysaccharides and carotenoids. S. crassifolium contains polysaccharides that have been proven to possess anti-inflammatory, antiproliferative, anticancer, anticoagulant, antithrombotic, and antioxidant effects, in addition to facilitating digestion and reducing blood fat and cholesterol levels (Zhao et al., 2005). According to several previous studies, a variety of other antioxidants are also present in this seaweed, such as polysaccharides, carotenoids, minerals, proteins, amino acids, and dietary fiber (Burtin, 2007).

Seaweed has the potential to be converted into animal feed, as its uses have yet to be fully realized. An organic source of non-starch polysaccharides with high crude fiber is seaweed. (March et al., 2013; Anggadiredja et al., 1996). Its bioactive content enables laying hens to absorb nutrients more effectively, alter the bacteria in their cecum, and impact their digestive system.

Those who consume eggs as a food product must consider the quality of the eggs in question. Identifying the various unsaturated fatty acids that the body requires to combat ailments represents a

significant research priority. Typically, consumers demonstrate a preference for eggs of superior quality. Another crucial element is the provision of feed that is rich in nutrients, particularly those that can enhance the eggs' quality (Kereh et al., 2019). However, microorganisms that may prove harmful to cattle include viruses and bacteria that may be present in food, the air, or the feed itself. Bacteria, particularly those belonging to the Salmonella species group, have the potential to contaminate chickens and subsequently spread to customers throughout the incubation, development, and post-harvest phases (Gantois, 2009). These microorganisms not only pose a threat to animal welfare but also represent a potential hazard to the safety of meat or eggs intended for human consumption. Various strategies have been employed to address this challenge, including the use of pharmaceuticals, adherence to good hygiene practices, and immunization.

While this endeavor offers certain advantages, it also presents a number of drawbacks. For instance, a number of bacterial strains have demonstrated antibiotic resistance (Devegowda et al., 1997). The typical objective of antibiotics is to impede the proliferation of infections (Farhad and Farida, 2011; Teteh et al., 2016). But recentlyantibiotic usage in feeding has been restricted as a result of the possibility of harmful microorganisms to develop heightened resistance (Abdulhasan, 2018; Santoso et al., 2018). To create safer, healthier, and more competitive meat and egg products (Mattjik and Sumertajaya, 2002; Rusli et al., 2015), it is necessary to develop alternatives made of natural ingredients that can replace antibiotics in feed formulation (Abaza, 2007; Winsisch et al., 2008; Abbas, 2013; Aqil, 2016; Mahfuz et al., 2017; Voemesse et al., 2018). S. crassifolium, which has a high concentration of uronic acid, which might be used in place of antibiotics. This research aims to investigate the effects of uronic acid-derived from S. crassifolium on the immune system of Lohman chicken eggs as a substitute for antibiotics. Although there does not seem a lot of data available on the usage of this plant as an extra for feeding, particularly as a potential feed additive, the investigation is warranted.

2. Materials and Methods

Research Material

In this investigation, brown seaweed (S. crassifolium) was utilized in conjunction with 120 Lohman strain hens that were 22 weeks old. Both commercial feed and feed lacking antibiotics were employed. The drinking water was treated with a brown seaweed extract (S. crassifolium) at percentages of 5.0%, 0%, 10%, 2.5%, and 7.5%. Table 1 presents the nutritional composition of the feed. The chickens were housed in 35 by 36 by 42 centimeter cages with battery-operated 16L/8D lighting system lights. The hens were provided with water and feed for a period of one week prior to the commencement of the trial in order to facilitate their adaptation to the experimental conditions. The course of treatment lasted for a period of 3 months.

Table 1. Nutrient content of feed

	Nutrient composition
Dry mater (%)	93.02
Ash (%)	10.77
Crude protein (%)	18.12
Ether extract (%)	5.63
Crude fibre (%)	6.16
BETN (%)	52.34
Gross energy (ccal/kg)	37.34
Calcium (%)	5.85
Phosphor (%)	0.71

Preparation of Seaweed extract

A combination of 90% ethanol and 100 grams of dried seaweed was prepared in a 5:1 ratio. The mixture was then swirled for three hours and subsequently allowed to rest at room temperature for a full day. Subsequently, the mixture was heated to 50 degrees Celsius, which facilitated the melting of the seaweed.

Feeding Trial

A total of 22 Lohman hens were randomized into two experimental groups at the age of eighteen weeks. The initial cohort was provided with commercial feed that included antibiotics, whereas the subsequent cohort was administered feed that was free of antibiotics. The five treatments, comprising 0%, 2.5%, 5.0%, 7.5%, and 10% brown seaweed in drinking water, were randomly allocated to each group. Water and food were available to the animals at all times, at 7 a.m. and 7 p.m. respectively.

Variables observed

This investigation examined several elements, such as the quantity of unsaturated fats in eggs, and the coagglutination tests' capacity to measure laying hens' resistance to Salmonella sp. and the ability to produce antibody titers through serological testing.

Trial Design and Data Analysis

A 5x2 factorial arrangement was employed to conduct a fully randomized design experiment with three replications. Six genuine laying chickens were used in each reproduction. First, the quantity of brown seaweed in the drinking water had to be determined. The control group, designated A1, was provided with no brown algae. The remaining groups, A2 through A5, were provided with the following levels of S. crassifolium: 2.5%, 5%, 7.5%, and 10%, respectively. The second component was the inclusion of feed contains antibiotics. B1 represented feed treated with antibiotics, while the feed without antibiotics represented by B2.

The data were evaluated utilized SPSS® 21.0 statistical software, which included orthogonal polynomial test, analysis of variance, and Duncan's multiple range test.

3. Results and Discussion

3.1. Results

The immune systems of Lohmann hens were affected by the use of uronic acid produced by brown algae in chicken's water rather of antibiotics, as shown by serology and coagglutination tests. Coagglutination tests were conducted on hens fed with S. crassifolium uronic acid with and without antibiotics in their diet. The results demonstrated that the chickens exhibited resistance to Salmonella sp. The outcomes showed that every hen administered uronic acid derived from brown algae was resistant to Salmonella sp.

Antibody titers in chicken blood serum are quantified through serological testing. The statistical analysis revealed that the feed treated without antibiotics and containing 10% S. crassifolium (B2A5) at week 34 of the trial produced most elevated antibody titer in contrast to alternative methods. Conversely, at the outset of the trial (week 3), there was no notable distinction in uronic acid levels between the treatments (P>0.05).

Table 2. Effect of uronic acid level on chicken antibody titer.

Factor	3th week			34th week		
	B1	B2	average	B1	B2	Average
A1	2.33	0.33	1.33	2.33[a]	2.33[a]	2.33
A2	2.67	2.00	2.33	3.67[b]	2.33[a]	3.00
A3	3.00	1.67	2.33	2.67[a]	3.00[a]	2.84
A4	2.67	2.67	2.67	3.33[a]	3.00[a]	3.17
A5	3.00	3.33	3.17	3.00[a]	4.67[b]	3.84
Average	2.73	2.00		3.00	3.07	

A2 contains 2.5% uronic acid, A3 contains 5% uronic acid, A4 contains 7.5% uronic acid, and A5 contains 10% uronic acid. A1 serves as the control with 0% uronic acid. Meanwhile, B1 refers to feed

with antibiotics, and B2 refers to feed without antibiotics. Statistically significant differences (P<0.05) are shown by various superscripts in the same row.

Table 3 demonstrates the impact of providing brown seaweed extract to laying hens in their water on the levels of palmitic acid present in their eggs. The 10% concentration of Sargassum crassifolium generated considerably more unsaturated fatty acids than the 0% and 2.5% concentrations ($P < 0.01$). Besides, there was no noticeable change between the 7.5% and 10% concentrations ($P > 0.05$).

Table 3. Effect of uronic acid level on egg fatty acid.

Factor	palmitic		oleic		linoleic	
	B1	B2	B1	B2	B1	B2
A1	19.20	19.28	33.41	33.35	7.67	7.40
A2	20.37	20.78	36.68	36.18	10.27	10.81
A3	20.66	22.18	37.80	39.71	10.28	11.14
A4	23.27	22.68	38.07	38.69	10.50	9.62
A5	21.52	22.04	38.98	38.38	10.03	10.43
Average	21.00	21.39	36.99	37.26	9.75	9.88

A2 has 2.5% uronic acid, A3 has 5% uronic acid, A4 has 7.5% uronic acid, and A5 has 10% uronic acid. A1 is the control, having 0% uronic acid. Antibiotic-containing feed B1 and antibiotic-free feed B2 are available in the meanwhile. Statistically significant differences (P<0.05) are shown by various superscripts in the same row.

3.2 Discussion

Without the use of antibiotics, feed intake per head per day (g/head/day) is often higher than that of animals taking antibiotics when drinking water is mixed with uronic acid that has been separated from S. crassifolium. This indicates that S. crassifolium uronic acid has the potential to enhance feed intake and accelerate the digestive process by increasing water intake. According to Zhao et al. (2005), S. crassifolium has the ability to accelerate the digestive process of feed. The study's findings indicate that uronic acid that has been separated from S. crassifolium, which is used to make alginate, is a key element in the Lohman hens' increased meal consumption. It has been demonstrated that the soluble fiber alginate has several advantageous impacts on the digestive tract. These include the reduction of blood glucose levels, the elimination of harmful microbial colonies, the absorption of toxins in the large intestine, alterations to the intestinal microbiota, and a reduction in toxicity in the intestinal lumen (Brownlee et al., 2005). This means there is a rise in feed intake and a quicker pace of digestive tract emptying.

Due to the nature of the fatty acid transfer mechanism, eggs typically have a low amount of unsaturated fatty acids. Egg yolk linoleic content will rise and oleic content will decrease when a supply of polyunsaturated fatty acids (PUFA) is provided. A comparable outcome is observed when S. crassifolium's isolated uronic acid is combined with water for consumption.

The results demonstrated that Salmonella sp. protection was shown by all hens given S. crassifolium uronic acid. This outcome is likely attributable to the immunomodulatory effects of polysaccharides present in brown seaweed. These immunomodulators stimulate non-specific cellular and humoral defensive processes, as well as reinforcing the immune system's general defenses. Due to of their inability to be broken down by saliva and hydrolyzed in the mouth, small intestine, and stomach, these non-starch polysaccharides remain intact when they enter the gut and operate as immunostimulants (Ale et al., 2011).

The investigation concluded that higher levels of uronic acid in laying hens often correlated with higher antibody titer values (see Table 1). This indicates that S. crassifolium's uronic acid has the potential to enhance laying hens' antibody levels. Uronic acid that produced by S. crassifolium contributes to the development of antibodies that can stop the spread of viruses. As stated by Han and Marasco (2011), the immune response mediated by antibodies contributes significantly to the body's defense against viruses. Antibodies function by attaching themselves to viral proteins, which stops the virus from replicating and prevents the process from continuing.

4. Conclusion

As an alternative to antibiotics, From S. crassifolium, uronic acid is extracted and that was diluted and mixed with water, thereby increasing the concentration of unsaturated fatty acids and enhancing immune function in Lohman chicken eggs.

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