

THE FEEDING EFFECTS OF URONIC ACID EXTRACTION FROM *Sargassum crassifolium* ON UNSATURATED FATTY ACIDS AND THE IMMUNITY OF LOHMAN CHICKEN EGGS

Veybe KEREH, Ivonne UNTU, Cherly PONTOH, Tilly LUMY,
Nontje Juliana KUMAJAS, Meity IMBAR

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

Corresponding author email: veybekereh@unsrat.ac.id

Abstract

Antibiotics are currently not allowed to be used because they can make pathogenic bacteria resistant and leave residues in products. The purpose of this study was to determine whether Lohman chicken eggs' immunity was affected by drinking water containing uronic acid extracted from *Sargassum crassifolium* (*S. crassifolium*). Sixty laying hens were divided into two groups: 1) chickens fed commercial feed with antibiotics, and 2) chickens fed feed without antibiotics. The chickens were randomly assigned to one of five treatments that included brown seaweed in the drinking water, A1 = 0.0% (control); A2 = 2.5%; A3 = 5.0%; A4 = 7.5%; A5 = 10.0%. Five treatments, two factors, and three replications were used in the completely randomized study design. Six laying hen heads were included in each replication. Titer antibody and unsaturated fatty acid were different between treatments, but *Salmonella* sp. infection was the same. It came to the conclusion that the lohman chicken eggs' immunity and unsaturated fatty acid levels were both enhanced by the uronic acid extracted from *S. crassifolium*.

Key words: fatty acid, immunity, Lohman chicken, *Sargassum crassifolium*, uronic acid.

INTRODUCTION

Sargassum crassifolium (*S. crassifolium*) is a member of the Phaeophyceae (brown algae) family. It has true roots, stems, and leaves (Yenusi et al., 2014), a variety of forms, and a predominant brown or blonde color that does not change with drying (Merdekawati & Susanto, 2009). It has been demonstrated that brown seaweed (*S. crassifolium*), which has major components such as sugar, sulfate, and uronic acid, acts as an antiviral and antibacterial agent (Mandal et al., 2007). Carotenoids and polysaccharides are found in *S. crassifolium*. Polysaccharides aid in digestion, reduce blood lipid and cholesterol levels, and possess anti-thrombotic, anticancer, antioxidant, antiproliferative, anti-inflammatory, anticoagulant, and antiproliferative properties (Zhao et al., 2005). Polysaccharides, dietary fiber, minerals, proteins, amino acids, vitamins, polyphenols, and carotenoids (Burtin, 2007) are among the antioxidants found in seaweed, according to numerous studies.

Because it hasn't been used to its full potential, seaweed can be processed into ingredients for

animal feed. According to March et al. (2013) and Anggadiredja et al. (1996), seaweed is a natural source of non-starch polysaccharides that contains a lot of crude fibers. Its bioactive factors affect the digestive process, changing the microflora in the caecum and allowing laying hens to use nutrients effectively.

If you want to eat eggs, you should look at their quality. The search for various forms of unsaturated fatty acids, which the body needs to prevent a variety of diseases, began. Customers tend to prefer eggs of higher quality. Also, very important for getting good egg parts is feeding, especially feed with a lot of nutrients in it (Kereh et al., 2019). However, livestock can be harmed by microbes found in food, water, or the air, such as viruses or bacteria. Bacteria, such as *Salmonella* species group, frequently transmit contamination of chickens to consumers through the hatching, growth, and post-harvest stages (Gantois, 2009). These bacteria will have an impact not only on the health of livestock but also on the safety of meat or egg products that humans will consume. This has been overcome through vaccination, sanitation, and antibiotic use, among other methods.

This effort is useful, but it has some limitations, like some bacteria strains that are resistant to antibiotics (Devegowda et al., 1997).

Antibiotics are used to stop pathogens from growing (Farhad & Farida, 2011; Tete et al., 2016). However, the use of antibiotics in feed has been restricted due to their tendency to increase pathogen bacteria's resistance (Abdulhasan, 2018; Santoso et al., 2018). As a result, antibiotic alternatives made from natural ingredients are required in feed formulas (Abaza, 2007; Winsisch et al., 2008; Abbas, 2013; Aqil, 2016; Mahfuz et al., 2017; Voemesse et al., 2018) in order to produce meat and egg products that are safe, healthy, and competitive (Mattjik & Sumertajaya, 2002; Rusli et al., 2015).

Utilizing *S. crassifolium* containing uronic acid may become an alternative to antibiotics. *S. crassifolium* has not yet been reported as a potential seaweed additive for feed ingredients, particularly feed additives. Therefore, the purpose of this study was to investigate how Lohman chicken eggs' immunity was affected by uronic acid extracted from *S. crassifolium* as a substitute for antibiotics.

MATERIALS AND METHODS

Research Material

This study used 120 Lohman strains aged 22 weeks and brown seaweed (*S. crassifolium*). Antibiotic-containing feed and antibiotic-free feed were the commercial feeds used in the study. Brown seaweed (*S. crassifolium*) extract was added to drinking water at concentrations of 0, 2.5, 5.0, 7.5, and 10%.

Table 1 displays the feed's nutritional content. A 35 x 36 x 42 cm individual battery cage with a feeding area, drinking water, and lights (16L/8D lighting system) was used.

Before beginning treatment, the chickens were introduced to the provisional feed and water for a week. For three months, the chicken needed to be looked after.

Preparation of Seaweed extract

100 grams of dried seaweed were mixed with ethanol (90 percent) (5:1), stirred for three hours, left to stand for 24 hours at room temperature, and then concentrated at 50 degrees Celsius to produce seaweed extract.

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 (kcal/kg)	37.34
Calcium (%)	5.85
Phosphor (%)	0.71

Feeding Trial

Twenty-two 18-week-old Lohman strains were divided into two groups: 1) chickens fed antibiotic-laden commercial feed and 2) chickens fed antibiotic-free feed. One of the five treatments containing brown seaweed was randomly assigned to the chickens: 0, 2.5, 5.0, 7.5, or 10%) in the water used for drinking. In the morning (at 7 a.m.) and afternoon (at 17 p.m.), ad libitum feed and water were given to the animals.

Variables observed

The variables observed in this study included the following: egg unsaturated fatty acid, the immunity of laying hens to *Salmonella* sp. Detected by coagglutination test and antibody titer detected by serological tests

Trial Design and Data Analysis

An experiment with a completely random design and three replications was carried out in a 5x2 factorial arrangement. Six laying hens lived in each replication. The first factor was the amount of brown seaweed (*S. crassifolium*) present in the water used for drinking (A1 = 0% *S. crassifolium* in the control); 2.5% *S. crassifolium* in A2; A3 contains 5% *S. crassifolium*; 7.5% *S. crassifolium* in A4; A5 is *S. crassifolium* 10.0%. The presence or absence of antibiotics in the feed was the second factor (B1 indicates feed with additional antibiotics, B2 indicates feed without additional antibiotics). The data were analyzed using analysis of variance followed by Duncan's multiple range test and the orthogonal polynomial test using the SPSS® 21.0 statistical software program.

RESULTS AND DISCUSSIONS

Coagglutination and serological tests revealed that Lohmann chickens' immunity was affected

by the administration of uronic acid extracted from *S. crassifolium* in drinking water as an alternative to antibiotics. The chicken's resistance to *Salmonella* sp. detected by a test of coagglutination. All of the research chickens treated with the uronic acid level of *S. crassifolium* with or without antibiotics in their feed had positive reactions to *Salmonella* sp., according to the analysis of chicken blood coagglutination. All chickens treated with *S. crassifolium* uronic acid levels exhibited immunity against *Salmonella* sp., as demonstrated by these results. Serological tests revealed the contained antibody titer of the poultry blood serum. According to the findings of the statistical analysis performed on the antibody titer of Lohman chickens, the treatment of feed without antibiotics with a level of 10% *S. crassifolium* (B2A5) yielded the highest antibody titer in comparison to other treatments at the end of the study (34th week), whereas the treatment of uronic acid levels had non-significant differences ($P>0.05$) at the beginning of the study (3rd week) (Table 2).

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

Factor	3 th week			34 th week		
	B1	B2	Ave rage	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
Ave rage	2.73	2.00		3.00	3.07	

A1 = 0% Uronic acid (control), A2 = 2.5% Uronic acid, A3 = 5% Uronic acid, A4 = 7.5% Uronic acid, A5 = 10% Uronic acid, B1 = Feed with antibiotic; B2 = Feed without antibiotic. A different superscript in the same row shows a significant difference ($P<0.05$).

The effect of the treatment of brown seaweed extract (*Sargassum crassifolium*) in the drinking water of laying hens on egg palmitic acid levels can be seen in Table 3. *Sargassum crassifolium* 10% were significant ($P<0.01$) have higher unsaturated fatty acid than level of *Sargassum crassifolium* 0% and 2.5%, but have non significantly ($P>0.05$) than level of *Sargassum crassifolium* 7.5%.

With a level of uronic acid extracted from *S. crassifolium* present in drinking water, feed intake (g/head/d) tended to be higher without antibiotics than with antibiotics. This shows that

S. crassifolium - derived uronic acid can increase feed intake and facilitate feed digestion in drinking water.

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

A1 = 0% Uronic acid (control), A2 = 2.5% Uronic acid, A3 = 5% Uronic acid, A4 = 7.5% Uronic acid, A5 = 10% Uronic acid, B1 = Feed with antibiotic; B2 = Feed without antibiotic. A different superscript in the same row shows a significant difference ($P<0.05$).

Zhao et al. (2005) mentioned that *S. crassifolium* might make it easier to digest food. According to the findings of this study, the alginate that is derived from the uronic acid that is extracted from *S. crassifolium* probably played a significant role in increasing the feed intake of Lohman chickens. According to Brownlee et al. (2005), alginate is a soluble fiber that reduces blood glucose levels, reduces intestinal lumen toxicity, eliminates harmful microbial colonies, absorbs toxins in the colon, and alters intestinal microflora. Because of these conditions, more feed is taken in and the rate at which the digestive tract is emptying faster.

Eggs have a low percentage of unsaturated fatty acids due to the transfer of those fatty acids. The linoleic content of egg yolks will rise when fed a source of polyunsaturated fatty acids (PUFA), whereas the oleic content will decrease. The same effect can be seen when uronic acid extracted from *S. crassifolium* is added to drinking water.

Salmonella sp. immunity is present in all chickens treated with *S. crassifolium* uronic acid levels. This is possible due to the immunomodulatory properties of brown seaweed polysaccharides. Both specifically and non-specifically, the immune system's defenses are strengthened by immunomodulators, which also induce non-specific cellular and humoral defense mechanisms. These non-starch polysaccharides arrive in the intestines intact and serve as immunostimulants (Ale et al., 2011) because they are resistant to saliva's digestion and hydrolysis in the mouth, stomach, and small intestine.

At the conclusion of this study, the antibody titer value generally increased when the uronic acid

level of *S. crassifolium* in laying hens was higher (Table 1). This demonstrates that layer hens' antibody can be increased with the uronic acid from *S. crassifolium*. Antibodies may be produced by the *S. crassifolium* uronic acid, which may prevent viral replication. According to Han & Marasco (2011), the body's defense against viruses relies heavily on antibody-mediated immune responses. By binding to viral proteins, antibodies prevent viral replication, thereby preventing the replication process.

CONCLUSIONS

Unsaturated fatty acid levels and immunity in Lohman chicken eggs have been raised by administering uronic acid extracted from *S. crassifolium* in water as an alternative to antibiotics.

REFERENCES

- Abaza, I.M (2007). Effects of using fenugreek, camomile and radish as feed additives on productive performance and digestibility coefficients of laying hens. *Poult. Sci.*, 27, 199-218.
- Abbas, T.E. (2013). The use of *Moringa oleifera* in poultry diets. *Turk. J. Vet. Anim. Sci.*, 37, 492-496.
- Abdulhasan, S.D. (2018). Effect of Digestrom® and Poultry Star® on the body performance and immunity status of broiler chickens. *Int. J. Poult. Sci.*, 17, 385-391.
- Ale, M.T., Mikkelsen, J.D., Meyer, A.S. (2011). Important determinants for fucoidan bioactivity: A critical review of structure-function relation and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. *Mar Drugs*, 9, 2106-2130.
- Anggadiredja, H., Sidiq, A.S, Pratomo, S., Rudyansyah, A. (1996). Screening of marine algae from Warambadi Seachore Sumba Island of Indonesia for antibacterial activity. *Photomedicine*, 3, 1-37
- Aqil, A.A. (2016). Effect of adding Dietary Fenugreek (*Trigonella foenum graecum* L.) powder on productive performance and egg quality of laying hens. *Int. J. Poult. Sci.*, 15(7), 259-263.
- Brownlee, I.A., Allen, A., Pearson, J.P., Dettmar, P.W., Havler M.E., & Atherton, M.R. (2005). Alginate as a source of dietary fiber. *Critical review i Food Science and Nutrition*, 45, 497-510.
- Burtin, P. (2003). Nutritional value of seaweeds. *Electron. J. Environ. Agric. Food Chem.*, 2, 498-503.
- Devegowda, G., Aravind, B.I.R., & Morton, M.G. (1997). Immunosuppression in poultry caused by aflatoxin and its alleviation by *Saccharomyces cerevisiae* (Yea Sacc, 1026) and Mannan oligosaccharides. *Proc. Alltech 11th Annual Asia Pacific Lecture Tour*, 121-132.
- Farhad, A., & Farida, R.A. (2011). Factor affecting quality and quantity of egg production in laying hens; A review. *World Appl. Sci. J.*, 12, 372-384.
- Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey T.J., & Immerseel, F.V. (2009). *Mechanisms of egg contamination by Salmonella enteritidis*. Federation of European Microbiological Societies, Belgium, Blackwell publishing.
- Han, T., & Marasco, W.A. (2011). Structural basis of influenza virus neutralization. *Ann NY Acad Sci.*, 1217, 178-90.
- Mahfuz, S.U., Nahar, M.J., Mo, C., Ganfu, Z., Zhongjun L., & Hui, S. (2017). Inclusion of probiotic on chicken performance and immunity: A review. *Int. J. Poult. Sci.*, 16, 328-335.
- Mandal, P., Mateu, C.G., Chattopadhyay, K., Pujol, C.A., Damonte E.B., & Ray, B. (2007). Structural features and antiviral activity of sulphated fucans from the brown seaweed *Cystoseira indica*. *Antiviral Chemistry & Chemotherapy*, 18, 153-162.
- March, W., Hamid, N., Liu, T., Lu J., & White, W.L. (2013). Fucoidan from New Zealand *Undaria Pinnatifida*: monthly variations and determination of antioxidant activities. *Carbohydr Polym.*, 95, 606-614.
- Mattjik, A.A., & Sumertajaya, M. (2002). *Perancangan Percobaan dengan Aplikasi SAS dan Minitab*. Ed ke-2. Bogor, ID: IPB Press Publishing House.
- Merdekawati, W., & Susanto, A.B. (2009). Kandungan dan komposisi pigmen rumput laut serta potensinya untuk kesehatan. *Squalen.*, 4(2), 41-47.
- Rusli, R.K., Wiryawan, K.G., Toharat, T., Jakaria, & Mutia, R. (2015). Supplementation of mangosteen pericarp meal and vitamin e on egg quality and blood profile of laying hens. *Media Peternakan.*, 38(3), 198-203.
- Santoso, U., Fenita Y., & Kusuyisya (2018). The effect of fermented *Sauropus androgynus* plus bay leaf inclusion on the hematologic and lipid profiles of female broiler chicken. *International Journal of Poultry Science*, 17(9), 1-8.
- Teteh, A., Gbeassor, M., Decuypere E., & Tona, K. (2016). Effects of *Moringa oleifera* leaf on laying rate, egg quality and blood parameters. *Int. J. Poult. Sci.*, 15(7), 277-282.
- Voemesse, K., Teteh, A., Nideou, D., N'nanlé, O., Gbeassor, M., Decuypere E., & Tona, K. (2018). Effect of *Moringa oleifera* leaf meal on growth performance and blood parameters of egg type chicken during juvenile growth. *Int. J. Poult. Sci.*, 17, 154-159.
- Winsisch, W., Shadle, K., Plitzner C., & Kroismayr, A. (2008). Use of pyrogenic product as feed additives for swine and poultry. *J. Anim. Sci.*, 86, 140-148.
- Yenusi, T.N.B., Sabdono A., & Widowati, I. (2014). Studi komposisi dan potensi antioksidan dari pigmen rumput laut *Turbinaria conoides* yang berasal dari perairan pantai Hamadi Jayapura Papua. *Seminar Nasional Kimia dan Pendidikan Kimia VI : "Pemantapan Riset Kimia dan Asesmen dalam pembelajaran Berbasis pendekatan Saintifik"* Surakarta, 316-325.
- Zhao, X., Xue, C.H., Cai, Y.P., Wang D.F., & Fang, Y. (2005). The study of antioxidant activities of fucoidan from *Laminaria japonica*. *High Technology Letters*, 11, 91-94