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Submission date: 23-Jun-2023 03:07PM (UTC+0700)

Submission ID: 2121315560

File name: 4._IOP_Kereh_2021_788_012047.pdf (228.27K)

Word count: 2836

Character count: 14848

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To cite this article: V G Kereh *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **788** 012047

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Extraction of uronic acid from *Sargassum crassifolium* and its feeding effects on the immunity of Lohman chicken eggs

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Abstract. The use of antibiotics is currently banned because it can cause resistance to pathogenic bacteria and cause residues in products. This study aimed to see the effect of drinking water containing uronic acid extracted from *Sargassum crassifolium* (*S. crassifolium*) on the immunity of Lohman chicken eggs. One hundred and twenty laying hens were divided into 2 groups: (1) chickens given commercial feed containing antibiotics and (2) feed without additional antibiotics. The chickens were randomly assigned to one of the 5 brown seaweed supplementation treatments in drinking water A1=0.0% *S. crassifolium* (control); A2=2.5% *S. crassifolium*; A3=5.0% *S. crassifolium*; A4=7.5% *S. crassifolium*; A5=10.0% *S. crassifolium*. The study used a completely randomized factorial design of 5 treatments, 2 factors, and 3 replications. Each replication consisted of 6 heads of laying hens. There were differences between treatments on titer antibody but were no difference on infection of *Salmonella sp* the Conclusion. The uronic acid extracted from *S. crassifolium* has been able to increase the immunity of Lohman chicken eggs.

1. Introduction

Sargassum crassifolium (*S. crassifolium*) belongs to the *Phaeophyceae* (brown algae) class, which has true roots, stems, and leaves [1], varied forms, and mostly brown or blonde coloration (these colors do not change even with drying) [2]. Brown seaweed (*S. crassifolium*) contains major components including sugar, sulfate and uronic acid and has been shown to play an antiviral and antibacterial role [3]. *S. crassifolium* contains polysaccharides [2], polyphenols, and carotenoids [4]. Polysaccharides play a role in reducing blood lipid, and cholesterol levels, they aid in digestion, and they have antithrombotic, anticancer, antioxidant [5], antiproliferative (uncontrolled cell division), anti-inflammatory [6], and anticoagulants properties [7]. In particular, many researchers report the availability of various antioxidants in seaweed, such as polysaccharides, dietary fiber, minerals, proteins, amino acids, vitamins, polyphenols, and carotenoids [8].

Seaweed availability is quite abundant and has not been optimally utilized thus, it can be processed into animal feed ingredients. As stated by Anggadiredja et al. and March et al. Seaweed is a natural source of non-starch polysaccharides that contains many crude fibers and its bioactive factors affect the digestive process resulting in changes in the microflora in the caecum and the efficient use of nutrients by laying hens [9,10].

However, microbes such as viruses or bacteria in the air, food, or water have the potential to harm livestock. Bacteria, including the *Salmonella sp.* group, often contaminate chickens from the



surrounding environment to hatching, to growth and post-harvest, to the consumers' hands[11]. In addition to affecting the health of livestock, these bacteria will also affect the safety aspects of meat or egg products that will be consumed by humans. Several attempts have been made to overcome this, such as vaccination, sanitation, or antibiotic use. This effort is useful but has limitations, for example, some of the antibiotic resistance to strains of bacterial[15]. Antibiotics are used to inhibit the development of pathogens [13,14]. However, currently, the use of antibiotics in feed has been restricted because it tends to increase the resistance of pathogen bacteria [15,16], therefore antibiotic alternatives from natural ingredients are needed in feed formulas [15–20] to produce safe, healthy, and competitive meat and egg products [21,22]. The use of *S. crassifolium* with uronic acid content may become an alternative substitute for antibiotics. The use of *S. crassifolium* as a potential seaweed additive in feed ingredients, especially feed additives are still not reported. Therefore this study aimed to examine the effects of uronic acid extracted from *S. crassifolium* as an antibiotic substitute on the immunity of Lohman chicken eggs.

2. Material and method

2.1. Research Material

This study used 120 Lohman strains aged 22 weeks and brown seaweed (*S. crassifolium*). The commercial feeds used in the study were feed containing antibiotics and feed without additional antibiotics. Supplementation of brown seaweed (*S. crassifolium*) extract was 0, 2.5, 5.0, 7.5, and 10.0% given in drinking water. The nutrient content of the feed is presented in table 1. The cage used as an individual battery system (size 35x36x42 cm) equipped with a feeding area, drinking water, and lights (16L/8D lighting system). The chickens were adapted to the provisional feed for 1 month and drinking water for 1 week before treatment began. The Chicken maintenance was carried out for 3 (three) months.

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Table 1. Nutrient content of the feed.

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

2.1.1. *Preparation of Seaweed extract.* Seaweed extract was made by mixing 100 grams of dried seaweed with ethanol (90%) (5:1), stirred for 3 hours, and allowed to stand for 24 hours at room temperature and then concentrated at 50°C.

2.1.2. *Feeding Trial.* One hundred and twenty Lohman strains aged 18 weeks were divided into 2 groups: (1) chickens given commercial feed containing antibiotics and (2) chickens given feed without additional antibiotics. The chickens were randomly assigned to one of the 5 brown seaweed supplementation treatments: (0, 2.5, 5.0, 7.5, or 10.%) in the drinking water. Feed and drinking water were given *ad libitum* at 07.00 am and afternoon 17.00 pm.

2.1.3. *Variables observed.* The variables observed in this study included the following: the immunity of laying hens to *Salmonella sp.* Detected by Coagglutination test and antibody titer detected by serological tests.

2.2. *Trial design and data analysis.* An experiment was performed in a 5x2 factorial arrangement using a completely randomized design with 3 replications. Each replication consisted of six laying hens. The first factor was the level of brown seaweed (*S. crassifolium*) in drinking water (A1 = 0.0% *S. crassifolium* (control); A2 = 2.5% *S. crassifolium*; A3 = 5.0% *S. crassifolium*; A4 = 7.5% *S. crassifolium*; A5 = 10.0% *S. crassifolium*). The second factor was the presence or absence of antibiotics in feed (B1 = feed with additional antibiotics, B2 = 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.

3. Results and discussion

3.1. Results

The administration of uronic acid extracted from *S. crassifolium* in drinking water as a substitute for antibiotics to immunity of Lohmann chickens detected by Coagglutination test, and serological tests. Level of chicken immunity to *Salmonella sp.* detected by Coagglutination test. The analysis of chicken blood Coagglutination showed that all research chickens treated with the uronic acid level of *S. crassifolium* with the addition of antibiotics or without antibiotics in their feed showed positive reactions to *Salmonella sp.* These results showed that all chickens treated with uronic acid levels of *S. crassifolium* have immunity against *Salmonella sp.*

The contained antibody titer of poultry blood serum was detected by serological tests. The results of statistical analysis on the antibody titer of Lohmann chickens showed that the treatment of uronic acid levels and interaction type of feed have non-significant difference ($P > 0.05$) at the beginning of the study (3rd week) but at the end of the study (34th week 34) that have significant difference ($P < 0.05$), where the treatment of feed without antibiotics with a level of 10% *S. crassifolium* (B2A5) gave the highest antibody titer compared to other treatments (table 1).

Table 2. Effect of uronic acid level on the chicken antibody to *Salmonella sp.*

Factor	3 rd week			34 th 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	3.67 ^a	3.00 ^a	2.84
A4	2.67	2.67	2.67	3.33 ^a	3.00 ^a	3.17
A4	3.00	3.33	3.17	3.00 ^a	4.67 ^b	3.84
Average	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 ($P < 0.05$).

3.2. Discussion

Generally, feed intake (g/head/d) without antibiotics with a level of uronic acid extracted from *S. crassifolium* in drinking water tended to be higher than feed intake with antibiotics. This finding shows that uronic acid extracted from *S. crassifolium* in drinking water can increase feed intake and cause a smooth digestion process for feed. Zhao *et al.* [5] Stated that *S. crassifolium* can facilitate food digestion. This study shows that alginate from the uronic acid extracted from *S. crassifolium* probably played an important role in increasing the feed intake of Lohman chickens. Brownlee *et al.* [23] stated

that alginate is a soluble fiber that is beneficial in reducing blood glucose levels, reducing toxicity levels of the intestinal lumen, reducing microbial colonies that are not beneficial, absorbing toxins in the colon, and changing intestinal microflora. These conditions cause the feed absorption process, and the rate of digestive tract emptying to be faster and cause increased feed intake.

The use of uronic acid extracted from *S. crassifolium* in drinking water tends to show the same effect. All chickens treated with uronic acid levels of *S. crassifolium* have immunity against *Salmonella sp.* This is possible because the polysaccharides found in brown seaweed have immunomodulatory activity. The Immunomodulators have an increasing character of the body's defenses both specifically and non-specifically and non-specific induction of b10 cellular and humoral defense mechanisms. These polysaccharides are non-starch polysaccharides that are resistant to digestion and hydrolysis by saliva in the mouth, stomach, and small intestine, so that they arrive in the intestines intact and act as immunostimulators [24].

The uronic acid level of *S. crassifolium* in laying hens tended to increase the antibody titer value at the end of this research (table 1). That shows the uronic acid from *S. crassifolium* can be used to increase the antibody of layer hens. The uronic acid from *S. crassifolium* has the potential to inhibit viral replication by forming antibodies. Han and Marasco [25] stated that antibody-mediated immune responses are very important for the body's defense against viral infections. Antibody inhibits viral replication by binding viral proteins, thus inhibiting the replication function.

4. Conclusion

The administration of uronic acid extracted from *S. crassifolium* in drinking water as a substitute for antibiotics has been able to increase the immunity of Lohman chicken eggs.

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