Effect of dietary supplementation of seaweed (*Ulva lactuca*) and *Azolla* on growth performance, haematological and serum biochemical parameters of *Aseel* chicken

Vijayalingam Thavasi Alagan*, Rajesh Nakulan Vatsala, Ilavarasan Sagadevan, Vairamuthu Subbiah and Venkataramanan Ragothaman

**Abstract**

**Background:** An effort was made to assess the effectiveness of dietary supplementation of *Ulva lactuca* (*U. lactuca*), a seaweed and *Azolla* individually and in combination on different physiological parameters of *Aseel* chicken. A total of 40 *Aseel* chicks of 8 weeks old were allocated into four groups, C₁ (control), T₁ (*Azolla* alone), T₂ (seaweed alone) and T₃ (seaweed plus *Azolla*). *Aseel* chicks in C₁ were fed with formulated grower feed alone, birds in T₁ had control diet with 5% *Azolla*, birds in T₂ had control diet with 3% *U. lactuca* and birds in T₃ had control diet admixed with 5% of *Azolla* and 3% of *U. lactuca*. Parameters were recorded for a continuous period of 2 months in 15 days interval.

**Results:** The growth performance was found to be significant (*P* ≤ 0.05) during 30 days of feed trials and highly significant (*P* ≤ 0.01) during 45 and 60 days of treatment. Birds in T₃ had a higher body weight gain, shank length and feed conversion efficiency followed with T₁ and T₂ compared to C₁. PCV, RBC, WBC and heterophil counts were not influenced (*P* ≥ 0.05) by dietary treatments. Uric acid, creatinine, AST, glucose, triglycerides and magnesium levels revealed a high significant (*P* ≤ 0.01) variation, and cholesterol level showed significant (*P* ≤ 0.05) changes during 30th and 60th day of feed trials between the control and treatment groups. Total protein, globulin, phosphorus and electrolytes like Na, K and Cl levels were not significant (*P* ≥ 0.05) during 30th day and were highly significant (*P* ≤ 0.01) during the 60th day of the trial.

**Conclusions:** It could be noted that inclusion of *U. lactuca* and *Azolla* as feed supplement in grower chicken had a better body weight gain when given in combination (*U. lactuca* and *Azolla*) rather than supplemented with the sole entity. Based on the haematological and serum biochemical analysis, the supplementation of *U. lactuca* and *Azolla* at the levels included in this trial did not pose any threat to the physiological well-being of *Aseel* chicken.

**Keywords:** *Aseel* chicken, *Azolla*, Growth parameter, Haematology, Serum biochemistry, *Ulva lactuca*
1 Background

Seaweed has a long history of feeding as a supplement in livestock. Seaweeds in animal rations were a controversial topic, since situation and fodder scarcity favours the use of various macroalgae as an edible food source for human and animals [1, 2]. Algae, both micro and macro, have been used since the beginning of 45 BC where the Greeks collected seaweed from seashore, washed and gave it to their livestock and thus prolonged their lives [3]. The brown and red seaweeds were often fed to sheep, horses and cattle for 6 to 8 weeks or even 18 weeks when fodder scarcity exists in winter in temperate countries [4]. Large numbers of reports were published on the effect of seaweeds on growth performance, immune status and blood profile parameters of different varieties of fishes [5–7]. Effects of dietary supplementation of seaweeds in sheep [8, 9] and rabbits [10, 11] were also reported. Seaweeds had been used in poultry to improve the immune status and decrease microbial load in digestive tract and enhance the quality of poultry meat and eggs at the inclusion level of 1-5% [12–16]. Although effects on dietary supplement of Ulva sp. in poultry were reported, only a limited report contained the mention on the blood profile and body weight gain. The effect of this seaweed as a sole supplementation and in combination with Azolla was very meagre.

Seaweeds gather more attention due to higher content of essential amino acids, minerals, vitamins and trace metals [10, 17]. Makkar et al. [2] stated that the Ulva sp. contained 18.6% crude protein, 6.9% crude fibre, 26.2% neutral detergent fibre, 8.7% acid detergent fibre, 3.5% lignin, 1.2% ether extract, 23% ash and 14.7 MJ/kg gross energy on dry matter basis. Zahid Phool et al. [18] reported that supplements of seaweed enhance the nutritive quality and growth of small animals and birds in terms of body weight gain, fats and protein contents. Okab [19] stated seaweed can be used as an alternative source of feed for animals. Seaweed was also a richest source of antioxidants [20, 21], which enhances the immune status. The digestibility studies on seaweeds, particularly Ulva lactuca, as a feed supplement to animals were scarce [12, 22]. Seaweed usage appeared to be economically, ecologically, sociologically and etiologically viable [23, 24]. More studies are needed to evaluate the effects of feeding a diet supplemented with various combinations of seaweeds.

Azolla, a free-floating water fern that fixes atmospheric nitrogen in association with nitrogen-fixing blue-green alga Anabaena azollae was very much used as a sustainable source of feed substitute for livestock especially dairy cattle, poultry, piggery and fish [25]. Azolla was a very rich source of protein (25-35%) on dry matter basis and were rich in essential amino acids, minerals, vitamins and carotenoids including the β carotene [26]. Alalade and Iyayi [27] performed chemical analytical studies on the nutritional composition of Azolla meal and reported a presence of 21.4% crude protein, 12.7% crude fibre, 2.7% ether extract, 16.2% ash and 47.0% carbohydrate on DM basis with a gross energy value of 2039 kcal/kg. There were various reports on the usage of Azolla as feed supplement in livestock and poultry and its effect on body weight gain performance, immune condition, haematological and biochemical parameters [28–32]. This present paper deals the effect of feeding Ulva lactuca and Azolla as individual supplement and in combination on different performance parameters of Aseel chicken.

Aseel chicken is one of the fifteen recognised native breeds of chicken in India [33] and is known for its fighting ability, hardiness, lean meat and better flavour of meat and eggs. Aseel is a dual-purpose breed. This breed can achieve 1.0 kg live body weight during 12th week of age with FCR at 3.5 and liveability of 95%. The breed evolved had specific features like long face and slender neck, uniformly thick but not fleshy. The eyes are compact, well set and present a bold look. Wattles and ear lobes are bright red and the beak is hard. The body length is elongated measuring 58.5 cm from head to tail and 60.25 cm head to toe. The legs are strong, straight and set well apart. Aseel lays dark brown-shelled eggs with thick shell measuring 0.33 mm. Reduced broodiness with resultant more egg number (160) and more chicks (112) per dam are characteristic features of this birds. Aseel chicken can withstand even very adverse climatic situation and hence, it is slowly getting popularised in this district as a form of backyard poultry.

Modern intensive poultry production has achieved phenomenal gains in the efficient and economical production of high quality and safe chicken meat, eggs and poultry by-products. At the same time as making gains in production and efficiency, the industry has to maximise the health and well-being of the birds and minimise the cost of production and the impact of the industry on the environment. Supplementing the diet with naturally available matter could achieve this success. More work is required to identify the positive effects of such naturally available matters and to understand the levels of their inclusion as feed supplements as a sole or in combination to see the synergistic action of those supplements in the body weight gain, feed conversion ratio and on the haematological and biochemical parameters.

2 Methods

2.1 Ulva lactuca

About 20 kg of seaweed Ulva lactuca was collected from Mandapam and Therkutharavai coastal area of Ramanathapuram District. The collected Ulva lactuca was washed 5 times in freshwater to remove salts and shade.
dried. The dried seaweed was finely powdered in a special pulveriser.

2.2 Azolla
About 20 kg of Azolla was received from a goat farmer of Bodinaickenur, Theni District. The collected Azolla was washed 5 times in freshwater to remove the odour and shade dried. The dried Azolla was finely powdered in a special pulveriser.

2.3 Feed
The grower mash mixed with 5.0% Azolla was used in T1 group, the mash mixed with 3.0% of green seaweed was used in T2 group and the mash mixed both with Azolla and green seaweed at the level of 3.0% and 5.0% respectively was used in T3 group. The plain grower mash without the addition of any supplements was used in control (C1) group and the details are given in Table 1.

2.4 Chicks
A total of 40 numbers of 8 weeks old grower Aseel chicken were used for this study. Each bird weighed about 270 to 320 g. They were randomly distributed into three dietary treatment groups and one control group. Each bird was taken as an experimental unit and the number of replicates per group was 10.

2.5 Experimental site
The study was conducted in a poultry farm at Sathirakudi Village, Ramanathapuram District during the month of April and May 2019.

2.6 Proximate analysis
Proximate analysis of formulated and mixed ration, U. lactuca and Azolla, was done at AFAQAL, Namakkal, a constituent unit of TANUVAS, Chennai, to estimate the dry matter, moisture, crude protein, crude fibre, ether extract, total ash, acid insoluble ash, calcium, phosphorus and gross energy content as per standards of Association of Official Agricultural Chemists (AOAC) [34].

2.7 Experimental feeding
The birds of the control group (C1) were fed with the formulated grower poultry feed as a basal diet. Birds in group T1 were fed with 5% Azolla, T2 with 3% U. lactuca and T3 with combination of 5% Azolla and 3% U. lactuca on DM basis mixed with the basal diet. For identification of the birds, wing bands were used in all the (C1, T1, T2 and T3) groups. The date of start of the feed trial is considered as day 0. The weight of individual birds in each group were recorded in an interval of 15 days (0th day, 15th day, 30th day, 45th day and 60th day) to assess the growth performance, shank length, average daily body weight gain, feed intake and feed conversion ratio. Blood samples were collected from wing vein of 3 birds from each group on 30th day and on 60th day of the trial for haematological and serum biochemical studies. After the successful completion of the study, the birds in the study were sold by the farmer.

2.8 Haematology and serum biochemical analyses
Haematological parameters like haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count and differential counts (heterophils, lymphocyte, monocyte and eosinophil) were determined by using standard methods [35]. Serum biochemical parameters like uric acid, creatinine, total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), glucose, triglycerides, calcium, phosphorus, magnesium, cholesterol, sodium, potassium and chloride were estimated as per the procedure given in the commercial kits procured from Agappe Diagnostics Ltd., using CECIL CE 2021 UV spectrophotometer. Globulin concentration was obtained by deducting the albumin content from total protein.

2.9 Statistical analysis
The data were statistically analysed using one-way ANOVA (analysis of variance) [36] to study the effect of treatment on various parameters. Software used was SPSS ver. 25.0. This was a field study and the number of birds available was a limitation. Since the genetic group was Aseel, which was hardy and adapted to the local area, we did not consider more number of birds per replicate. The individual bird was the experimental unit.

Table 1 Inclusion level of dietary ingredients

| Experimental diets | C1 | T1 | T2 | T3 |
|--------------------|----|----|----|----|
| Ingredients (%)    |    |    |    |    |
| Maize              | 30 | 30 | 30 | 30 |
| Pearl millet       | 13 | 13 | 13 | 13 |
| Rice               | 8  | 8  | 8  | 8  |
| Soyabean oil cake  | 10 | 8  | 10 | 7  |
| Groundnut oil cake | 16 | 16 | 16 | 16 |
| Fish meal          | 10 | 7  | 7  | 5  |
| Rice bran          | 10 | 10 | 10 | 10 |
| Limestone          | 1  | 1  | 1  | 1  |
| Mineral mixture    | 2  | 2  | 2  | 2  |
| Seaweeda           | 0  | 0  | 3  | 3  |
| Azolla             | 0  | 5  | 0  | 5  |
| Total              | 100| 100| 100| 100|

C1: control diet alone, T1: control diet with 5% Azolla, T2 control diet with 5% seaweed, T3: control diet with 5% Azolla and 3% seaweed

*Green algae—U. lactuca
3 Results

3.1 Proximate analysis of feed samples

The inclusion level of 5% Azolla (T1), 3% Ulva lactuca (T2) and combination of 5% Azolla and 3% Ulva lactuca (T3) as feed supplement in poultry feed ration (C1) is given in Table 1, and the chemical composition, viz. dry matter (%), moisture (%), crude protein (%), crude fibre (%), ether extract (%), total ash (%), calcium (%), phosphorus (%), and metabolizable energy (Kcal/kg) of experimental diets (C1, T1, T2 and T3), Azolla and Ulva lactuca, is given in Table 2.

3.2 Growth performance and feed conversion efficiency

During the study period, no mortality was recorded both in the control group (C1) and treatment groups (T1, T2 and T3). The average body weight gain, shank length and feed efficiency during the 2-month trial period were highest in T3 group and least in T2 (Table 3). Birds in T3 required only 3.39 kg of feed to achieve a body weight gain of 1 kg, and it was 5.08 kg in T2 group. Birds in T1 group took 4.00 kg of feed to achieve a body weight gain of 1 kg. However, in case of the control group, it was 4.76 kg of feed for 1 kg of body weight gain. There was no significant difference (P ≥ 0.05) in growth performance during 0 to 15 days of the trial. Significant difference (P ≤ 0.05) in growth performance could be recorded during 30th day, and it was highly significant (P ≤ 0.01) during 45th and 60th day of the trial (Table 4).

3.3 Haematology and serum biochemistry

The effect of experimental diets on individual haematological and serum biochemical parameters during 30th and 60th day of feed trials in all the treatment groups. No significant changes (P ≥ 0.05) could be observed in albumin, ALT and serum calcium level during 30th and 60th day of feed trials. However, the levels of total protein, globulin and serum phosphorus and electrolytes like Na, K and Cl did not show any significant changes (P ≥ 0.05) during 30th day and were highly significant (P ≤ 0.01) during the 60th day of the trial.

4 Discussion

4.1 Growth performance

There was no significant variation in body weight gain in all the treatment groups till 15th day of the trial. The feed intake in the treatment groups was not affected which indicated that the feed was palatable and the level of inclusion of the testing materials did not affect the wholesomeness of the feed as stated by Abudabos et al. [14]. Abudabos et al. [14] followed a 3.0% dietary inclusion level of green seaweed in broiler chicken and reported a better growth performance, carcass characteristics and serum constituents. Similarly, Mamata et al. [37] had followed an inclusion level of 5-10% Azolla as dietary replacement in broiler chicken and reported a positive impact on improvement in terms of body weight and net returns per bird. These earlier reports formed the base for the assessment of inclusion levels of green seaweed (3.0%) and Azolla (5.0%) in the present study. Ventura et al. and Carrillo et al. [12, 38] reported a reduction in feed intake and body weight gain in broilers fed with the diet admixedtured with Macrocystis

Table 2 Chemical composition of experimental diets, Azolla and green algae (Ulva lactuca)

| Chemical composition            | C1   | T1  | T2  | T3  | Azolla | *Green algae |
|---------------------------------|------|-----|-----|-----|--------|--------------|
| Dry matter (%)                  | 88.80| 88.55| 88.86| 89.05| 91.10  | 82.56        |
| Moisture (%)                    | 11.20| 11.45| 11.14| 10.95| 8.90   | 17.44        |
| Crude protein (%)               | 18.58| 19.63| 18.52| 19.89| 17.45  | 6.55         |
| Crude fibre (%)                 | 9.76 | 10.35| 9.67 | 10.12| 14.24  | 4.93         |
| Ether extract (%)               | 2.24 | 2.71 | 2.26 | 2.79 | 3.24   | 0.97         |
| Total ash (%)                   | 7.43 | 8.36 | 8.77 | 8.98 | 20.37  | 20.83        |
| Calcium (%)                     | 1.10 | 1.13 | 1.08 | 1.18 | 1.50   | 0.90         |
| Phosphorus (%)                  | 0.58 | 0.62 | 0.60 | 0.68 | 0.91   | 0.37         |
| Metabolizable energy (Kcal/kg)  | 2517 | 2491| 2481| 2483| 2839   | 2157         |

C1, control diet alone; T1, control diet with 5% Azolla; T2, control diet with 3% seaweed; T3 control diet with 5% Azolla and 3% seaweed

*Green algae—Ulva lactuca

respectively. Haematological analysis showed a high significant (P ≤ 0.01) variation in PCV, RBC and WBC count during 30th and 60th day of feed trials in all the treatment groups.

Serum biochemistry study revealed a high significant (P ≤ 0.01) variation in uric acid, creatinine, AST, glucose, triglycerides and magnesium and a significant (P ≤ 0.05) variation in the level of serum cholesterol during 30th and 60th day of feed trials between the control and treatment groups. No significant changes (P ≥ 0.05) could be observed in albumin, ALT and serum calcium level during 30th and 60th day of feed trials. However, the levels of total protein, globulin and serum phosphorus and electrolytes like Na, K and Cl did not show any significant changes (P ≥ 0.05) during 30th day and were highly significant (P ≤ 0.01) during the 60th day of the trial.
pyrifera at 15% level and Ulva rigida at 10-15% level respectively. The highest body weight gain, increased shank length and feed efficiency in T3 group, showed the synergistic action of combined feeding of Azolla (5%) and U. lactuca (3%). The non-significant difference in the first 15 days was perhaps due to the period of adaptation for the birds to the newly introduced diet. The increase in weight gain in birds fed with Azolla and U. lactuca combination might be due to the increased availability and utilisation of protein and other micronutrient. Moreover, the abundantly available antioxidants and antimicrobial constituents in U. Lactuca [39] might have made the birds to utilise the proteins from both Azolla and U. lactuca efficiently and availability of higher fibre content in U. lactuca might have enhanced the digestibility of the protein-rich diet. The T2 group showed comparatively low body weight gain, which was in accordance with the report of Madibana et al. [6] who reported reduced growth in fish fed with diet contained U. lactuca at a higher level. He also opined that the reduced growth in group fed the diet mixed with U. lactuca only might be due to higher fibre and lower protein content. In chickens, quantity and quality of protein would determine the success of body weight gain. Birds in T1 group showed a better body weight gain compared to C1 group. This might be due to the better availability of protein and other micro nutrients from Azolla in the diet.

4.2 Haematology and serum biochemistry
Blood parameters were an ideal tool for the assessment of health, nutritional and physiological status of animals during feed trials [40]. Blood parameters of birds in this trial were within normal range except PCV, RBC and WBC which showed a highly significant difference (P ≤

### Table 3 Effect of seaweed (Ulva lactuca) and Azolla feed supplement on growth, shank length, feed intake, average daily gain and feed conversion (mean ± SE)

| Parameters                          | Experimental diets |
|-------------------------------------|--------------------|
|                                     | C1 | T1 | T2 | T3 |
| Period (days)                       | 60 | 60 | 60 | 60 |
| Initial weight (g)                  | 273 ± 21.294<sup>a</sup> | 319.4 ± 18.025<sup>a</sup> | 305.6 ± 21.220<sup>a</sup> | 292.4 ± 15.863<sup>a</sup> |
| Final weight (g)                    | 841.7 ± 20.579<sup>a</sup> | 1020.8 ± 54.861<sup>b</sup> | 840.2 ± 18.274<sup>a</sup> | 1108.8 ± 58.686<sup>a</sup> |
| Gain weight (g)                     | 568.7 ± 8.723<sup>b</sup> | 701.4 ± 48.045<sup>a</sup> | 534.6 ± 13.733<sup>b</sup> | 816.4 ± 44.509<sup>a</sup> |
| Initial shank length (g)            | 64.2 ± 0.929<sup>ab</sup> | 61.5 ± 1.035<sup>bc</sup> | 66.2 ± 1.073<sup>a</sup> | 57.1 ± 1.140<sup>c</sup> |
| Final shank length (g)              | 111.9 ± 1.629<sup>ab</sup> | 114.8 ± 2.426<sup>a</sup> | 106.9 ± 1.84<sup>b</sup> | 117.2 ± 1.381<sup>a</sup> |
| Gain in shank length (g)            | 47.7 ± 1.868<sup>c</sup> | 53.3 ± 2.508<sup>b</sup> | 40.7 ± 2.418<sup>d</sup> | 57.1 ± 1.545<sup>d</sup> |
| Average daily gain (g)              | 9.48 ± 0.146<sup>b</sup> | 11.69 ± 0.801<sup>a</sup> | 8.91 ± 0.229<sup>b</sup> | 13.61 ± 0.742<sup>d</sup> |
| Feed intake (g)                     | 2707.01 ± 41.521<sup>b</sup> | 2805.60 ± 192.182<sup>a</sup> | 2715.77 ± 69.765<sup>d</sup> | 2767.60 ± 150.886<sup>a</sup> |
| Feed conversion                     | 4.76 ± 0.005<sup>b</sup> | 4.00 ± 0.068<sup>b</sup> | 5.08 ± 0.270<sup>d</sup> | 3.39 ± 0.008<sup>d</sup> |

C<sub>1</sub>, control diet alone, T<sub>1</sub> control diet with 5% Azolla, T<sub>2</sub> control diet with 3% seaweed; T<sub>3</sub> control diet with 5% Azolla and 3% seaweed. Means within same row bearing different superscripts are significantly different at P ≤ 0.05

### Table 4 Effect of seaweed (Ulva lactuca) and Azolla feed supplement on weight gain of Aseel chicken

| Weight gain (in days) | Experimental diets |
|-----------------------|--------------------|
|                       | C<sub>1</sub> | T<sub>1</sub> | T<sub>2</sub> | T<sub>3</sub> |
| 0<sup>NS</sup>         | 273 ± 21.294<sup>a</sup> | 319.4 ± 18.025<sup>a</sup> | 305.6 ± 21.220<sup>a</sup> | 292.4 ± 15.863<sup>a</sup> |
| 15<sup>NS</sup>        | 380.20 ± 20.612<sup>a</sup> | 411.40 ± 22.173<sup>a</sup> | 429.40 ± 29.457<sup>a</sup> | 427.20 ± 16.919<sup>a</sup> |
| 30<sup>*</sup>         | 510 ± 30.333<sup>a</sup> | 610 ± 35.636<sup>ab</sup> | 549.70 ± 30.675<sup>ab</sup> | 640.30 ± 24.699<sup>b</sup> |
| 45<sup>**</sup>        | 649 ± 25.784<sup>a</sup> | 802 ± 41.800<sup>d</sup> | 656.80 ± 23.983<sup>d</sup> | 856 ± 32.183<sup>b</sup> |
| 60<sup>**</sup>        | 841.70 ± 20.579<sup>a</sup> | 1020.80 ± 54.861<sup>b</sup> | 840.20 ± 18.274<sup>d</sup> | 1108.80 ± 58.686<sup>b</sup> |

C<sub>1</sub>, control diet alone, T<sub>1</sub> control diet with 5% Azolla, T<sub>2</sub> control diet with 3% seaweed, T<sub>3</sub> control diet with 5% Azolla and 3% seaweed; NS = P ≥ 0.05

*P ≤ 0.05
**P ≤ 0.01
<sup>ab</sup>Means within same row bearing different superscripts are significantly different at P ≤ 0.05
between the control and treatment groups during 30th and 60th day of evaluation. There was a highly significant change in Hb level at 30th day and not on 60th day of the trial.

The serum biochemical analysis showed an increase in uric acid content in all the treatment groups (T1, T2 and T3) when compared to the control (C1) during 30th and 60th day of trial period. Madibana et al. and Knoph and Olsen [6, 41] reported similar changes in ammonia toxicity conditions of fish and in fishes fed with U. lactuca. The increase in uric acid might be due to the presence of a good amount of protein in the diet of treatment groups. The creatinine level was also marginally higher in all the treatment groups compared to the control diet as reported by earlier workers [6] in fish fed with Ulva. Similarly, Madibana et al. [6] reported decreased cholesterol level in dusky hob fish, fed with various graded levels of Ulva. Yu et al. [42] stated feeding 500 mg/kg polysaccharides from Ulva pertusa, significantly lowered cholesterol levels in mice. The significant reduction in the levels of cholesterol and triglycerides in this present study showed that the meat produced by the supplementation of U. lactuca and Azolla would result in the production of lean meat which could be consumable for patients of cardiac disorders and hypertensive conditions. The levels of albumin, ALT and calcium were within the normal range in all the treatment groups during 30th and 60th day of trial. The total protein, globulin, phosphorus and electrolytes like Na, K and Cl did not show any significant change on 30th day and showed highly significant variation on 60th day of the trial. The results revealed that the birds showed marked changes only after the 30th day of the trial. In general, the dietary supplementation of Azolla and U. lactuca individually and in combination in Aseel grower chicken did not elicit much physiological alterations and the supplementation in combination found to be more effective in terms of weight gain.

### Table 5  Effect of seaweed (Ulva lactuca) and Azolla feed supplement on haematological parameters of Aseel chicken

| Haematological parameters (in days) | Experimental diets |
|------------------------------------|-------------------|
|                                    | C1    | T1       | T2       | T3       |
| 30                                 |       |          |          |          |
| **Hb (g/dL)**                      | 9.2 ± 0.153a   | 10.5 ± 0.153b | 9.5 ± 0.120a | 9.6 ± 0.240a |
| **PCV (%)**                        | 26.4 ± 0.200a  | 28.4 ± 0.115b | 25.6 ± 0.240a | 26.4 ± 0.230a |
| **RBC (m/μL)**                     | 2.5 ± 0.120a   | 3.3 ± 0.074b  | 2.8 ± 0.042a  | 2.9 ± 0.038a  |
| **WBC (cmm)**                      | 16466.7 ± 176.383c | 16033.3 ± 88.192b | 13466.7 ± 202.759a | 14300 ± 114.470b |
| *H (%)                             | 31 ± 0.577a    | 38 ± 0.577c   | 36 ± 0.577bc | 34 ± 0.577b  |
| *L (%)                             | 61 ± 0.577a    | 59 ± 0.577a   | 59 ± 0.577a  | 61 ± 0.577a  |
| NSM (%)                            | 3.7 ± 0.333a   | 2.7 ± 0.333a  | 2.3 ± 0.333a  | 2.7 ± 0.333a |
| NSE (%)                            | 3.7 ± 0.333a   | 2.3 ± 0.333a  | 2.7 ± 0.333a  | 2.3 ± 0.333a |
| 60                                 |       |          |          |          |
| **Hb (g/dL)**                      | 9.5 ± 0.203a   | 9.6 ± 0.145a  | 9.5 ± 0.185a  | 9.5 ± 0.173a  |
| **PCV (%)**                        | 28.0 ± 0.088d  | 26.4 ± 0.145a | 26.9 ± 0.577c | 27.5 ± 0.577c |
| **RBC (m/μL)**                     | 2.6 ± 0.015b   | 2.7 ± 0.042b  | 2.8 ± 0.018a  | 2.8 ± 0.022bc |
| **WBC (cmm)**                      | 18466.7 ± 176.383d | 17900 ± 57.735c | 15366.7 ± 88.192a | 16500 ± 57.735b |
| *H (%)                             | 34 ± 1.155b    | 35 ± 1.155b   | 34 ± 0.577ab  | 30 ± 0.882a   |
| *L (%)                             | 61.3 ± 0.662a  | 62.7 ± 0.882a | 61.7 ± 1.202a | 64.7 ± 0.882a |
| *M (%)                             | 3.7 ± 0.333ab  | 2.7 ± 0.333a  | 3.7 ± 0.333ab | 4.3 ± 0.333b  |
| NSE (%)                            | 2.3 ± 0.333a   | 2.7 ± 0.333a  | 2.3 ± 0.333a  | 2.7 ± 0.333a  |

H heterophil, L lymphocyte, M monocyte, E eosinophil

C1 control diet alone, T1 control diet with 5% Azolla, T2 control diet with 3% seaweed, T3 control diet with 5% Azolla and 3% seaweed; NS = P ≥ 0.05

*P ≤ 0.05

**P ≤ 0.01

Means within same row bearing different superscripts are significantly different at P ≤ 0.05

Chicken fed with Ulva lactuca at 3% level. Similarly, Madibana et al. [6] reported decreased cholesterol level in dusky hob fish, fed with various graded levels of Ulva. Yu et al. [42] stated feeding 500 mg/kg polysaccharides from Ulva pertusa, significantly lowered cholesterol levels in mice. The significant reduction in the levels of cholesterol and triglycerides in this present study showed that the meat produced by the supplementation of Ulva lactuca and Azolla would result in the production of lean meat which could be consumable for patients of cardiac disorders and hypertensive conditions. The levels of albumin, ALT and calcium were within the normal range in all the treatment groups during 30th and 60th day of trial. The total protein, globulin, phosphorus and electrolytes like Na, K and Cl did not show any significant changes on 30th day and showed highly significant variation on 60th day of the trial. The results revealed that the birds showed marked changes only after the 30th day of the trial. In general, the dietary supplementation of Azolla and Ulva lactuca individually and in combination in Aseel grower chicken did not elicit much physiological alterations and the supplementation in combination found to be more effective in terms of weight gain.
5 Conclusions

It could be noted that inclusion of *U. lactuca* and *Azolla* as feed supplement in grower chicken had a better body weight gain when given in combination (*U. lactuca* and *Azolla*) rather than supplemented with the sole entity. Based on the haematological and serum biochemical analysis, the supplementation of *U. lactuca* and *Azolla* at the levels included in this trial did not pose any threat to the physiological well-being of Aseel chicken. In this present study, the Aseel grower bird could achieve better gain in body weight and no symptoms of digestive disturbances observed during the trial period. It was interesting to note that supplementation of *U. lactuca* alone in the diet could not achieve significant gain in body weight. Therefore, it was concluded that combination of *Azolla* and *U. lactuca* supplementation in Aseel could achieve a better result rather than as a separate supplementation with either *Azolla* or *U. lactuca*.

### Table 6 Effect of seaweed (*Ulva lactuca*) and *Azolla* feed supplement on serum biochemical parameters of Aseel chicken

| Serum biochemical parameters (in days) | Experimental diets |
|---------------------------------------|---------------------|
|                                       | C₁  | T₁  | T₂  | T₃  |
| 30 **Uric acid (mg/dL)**               | 13.0 ± 0.404ₐ       | 15.4 ± 0.783ₐ | 24.9 ± 0.777ₜ | 22.6 ± 0.869ₚ       |
| **Creatinine (mg/dL)**                 | 0.18 ± 0.032ₐ       | 0.065 ± 0.053ₜ | 0.33 ± 0.025ₚ | 0.046 ± 0.012ₚ       |
| NSTotal protein (g/dL)                 | 4.3 ± 0.406ₚ         | 4.0 ± 0.498ₚ | 3.2 ± 0.338ₚ | 4.0 ± 0.473ₚ         |
| **Albumin (g/dL)**                     | 2.4 ± 0.120ₜ         | 2.2 ± 0.176ₜ | 2.0 ± 0.088ₜ | 2.4 ± 0.233ₜ         |
| **Globulin (g/dL)**                    | 1.8 ± 0.441ₜ         | 1.9 ± 0.338ₜ | 1.1 ± 0.267ₜ | 1.6 ± 0.240ₜ         |
| **ALT (IU/L)**                         | 31.0 ± 3.512ₐ       | 44.3 ± 6.119ₚ | 48.3 ± 2.603ₚ | 36.3 ± 1.764ₚ         |
| **AST (IU/L)**                         | 437.0 ± 14.22ₐ      | 303.7 ± 14.81ₜ | 242.0 ± 19.46ₜ | 215.3 ± 14.25₂       |
| **Glucose (mg/dL)**                    | 39.7 ± 0.384ₚ        | 62.3 ± 13.09ₜ | 71.0 ± 3.78ₜ | 90.3 ± 6.17ₜ         |
| **Triglycerides (mg/dL)**              | 218.0 ± 27.15ₚ       | 110.3 ± 8.09ₚ | 204.3 ± 6.17ₜ | 145.7 ± 3.33ₜ         |
| **Calcium (mg/dL)**                    | 11.0 ± 0.91ₐ        | 11.6 ± 0.55₁ₚ | 12.4 ± 0.04₁ₚ | 11.8 ± 0.32₂         |
| **Phosphorus (mg/dL)**                 | 5.9 ± 0.439ₚ        | 6.1 ± 0.332ₜ | 6.5 ± 0.20ₚ | 6.4 ± 0.26₉         |
| **Magnesium (mg/dL)**                  | 3.7 ± 0.049ₚ        | 4.8 ± 0.32₀ₜ | 4.5 ± 0.20₀ₚ | 4.8 ± 0.06₇         |
| **Cholesterol (mg/dL)**                | 134.1 ± 1.96ₐ       | 120.5 ± 4.22₅ | 116.1 ± 4.21ₙ | 118.4 ± 2.48ₙ         |
| **Sodium (mmol/L)**                    | 144.9 ± 2.18ₗ       | 150.2 ± 4.24ₗ | 155.1 ± 4.71ₙ | 155.3 ± 6.9₀ₙ         |
| **Potassium (mmol/L)**                 | 5.1 ± 0.44₁ₖ       | 5.6 ± 0.23₂ₚ | 6.0 ± 0.48ₚ | 5.9 ± 0.46₆         |
| **Chloride (mmol/L)**                  | 112.4 ± 2.5₆ₙ       | 119.3 ± 4.4₂₉ | 121.5 ± 4.8₂ₙ | 124.₃ ± 6.1₅ₙ         |
| 60 **Uric acid (mg/dL)**               | 10.7 ± 0.31₁ₖ       | 14.6 ± 0.49₁ₙ | 18.2 ± 0.32₁ₚ | 17.7 ± 0.32₉         |
| **Creatinine (mg/dL)**                 | 0.42 ± 0.018ₐ       | 0.55 ± 0.02₝ₙ | 0.47 ± 0.01₈ₚ | 0.45 ± 0.01₈ₙ        |
| **Total protein (g/dL)**               | 3.8 ± 0.11₅ₚ        | 4.1 ± 0.08₅ₚ | 3.5 ± 0.08₅ₚ | 4.₇ ± 0.1₄₅         |
| **Albumin (g/dL)**                     | 2.1 ± 0.08₅ₚ        | 2.2 ± 0.1₂₀ₚ | 1.₉ ± 0.₆₆₀ₚ | 2.₄ ± 0.1₂₀ₙ         |
| **Globulin (g/dL)**                    | 1.7 ± 0.3₃ₑₚ        | 1.₉ ± 0.1₁₅ₙ | 1.₆ ± 0.1₁₅ₙ | 2.₃ ± 0.0₅₉         |
| **ALT (IU/L)**                         | 30.0 ± 1.₁₅ₖₚ       | 30.7 ± 0.₆₆₇ₙ | 34.7 ± 1.₇₆₄ₙ | 31.₀ ± 1.₁₅₅ₙ         |
| **AST (IU/L)**                         | 250.0 ± 3.₇₈ₖₚ      | 2₈₃.₃ ± 1.₃₃₉ₚ | 2₇₃.₇ ± 2.₇₂₈ₚ | 1₉₂.₇ ± 3.₇₁₂ₙ         |
| **Glucose (mg/dL)**                    | 45.7 ± 0.₃₃₅ₚ       | 4₈.₀ ± 0.₅₇₇ₚ | 7₈.₀ ± 0.₅₇₇ₚ | 8₁₀.₇ ± 5.₇₇₉ₚ         |
| **Triglycerides (mg/dL)**              | 11₄.7 ± 1.₄₅₂ₚₚ     | 1₀₃.₇ ± 3.₉₃₀ₘₚ | 1₁₄.₀ ± 1.₁₅₅₂ₘₚ | 1₀₁.₃ ± 1.₇₆₄ₚ         |
| **Calcium (mg/dL)**                    | 1₂₆. ± 0.₂₆₂ₚₚ      | 1₁₈. ± 0.₂₁₇ₚ | 1₂₈. ± 0.₁₇₈ₚ | 1₂₄. ± 0.₅₈₇ₙ         |
| **Phosphorus (mg/dL)**                 | 6.₁ ± 0.₁₆₃ₚ        | 6.₃ ± 0.₂₁₄ₚ | 6.₉ ± 0.₅₅₈ₚ | 6.₉ ± 0.₀₂₂ₙ         |
| **Magnesium (mg/dL)**                  | 3.₈ ± 0.₀₇₆ₚ       | 3.₉ ± 0.₄₀₄ₚ | 4.₇ ± 0.₁₁₈ₙ | 4.₈ ± 0.₀₆₁ₙ         |
| **Cholesterol (mg/dL)**                | 1₂₈.₀ ± 1.₁₅₅ₘₚ      | 1₁₈.₇ ± 2.₃₃₉ₚ | 1₀₉.₀ ± 2.₆₄₆ₘ | 1₁₀.₇ ± 5.₆₉₆ₘ         |
| **Sodium (mmol/L)**                    | 1₄₁.₉ ± 0.₃₁₈ₙ      | 1₅₇.₂ ± 1.₂₀₁ₜ | 1₆₂.₈ ± 0.₇₈₄ₙ | 1₆₆.₈ ± 1.₁₀₂ₙ         |
| **Potassium (mmol/L)**                 | 1₄.₉ ± 0.₀₅₂ₚ       | ₅.₂ ± 0.₀₃₅ₙ | ₅.₁ ± 0.₀₇₆ₙ | ₅.₁ ± 0.₀₆₄ₙ         |
| **Chloride (mmol/L)**                  | 1₀₇.₉ ± ₀.₃₄₈ₙ      | ₁₂₅.₁ ± ₂.₆₅₃ₙ | ₁₂₇.₆ ± ₁.₄₀₅ₙ | ₁₃₄.₉ ± ₀.₈₁₁ₙ         |

*ₚ* < 0.05
*ₚ < 0.01
*ₚ < 0.001

Means within same row bearing different superscripts are significantly different at *P* ≤ 0.05.
Abbreviations

U. lactuca: Ulva lactuca; AFAQAL: Animal Feed Analytical and Quality Assurance Laboratory; TANUVAS: Tamilnadu Veterinary and Animal Sciences University; ANOVA: Analysis of variance; SEM: Standard error of the mean; PDIIFF: Power dffraction; DM: Dry matter; NS: Not significant; PCV: Packed cell volume; RBC: Red blood cell; WBC: White blood cell; H: Heterophils; L: Lymphocytes; M: Monocytes; E: Eosinophils; Hb: Haemoglobin; Na: Sodium; K: Potassium; Cl: Chloride; ALT: Alanine transaminase; AST: Aspartate transaminase

Acknowledgements

Authors would like to express their gratitude to the Vice-Chancellor, Registrar and the Director of Extension Education, Tamil Nadu Veterinary and Animal Sciences University for providing this wonderful opportunity to bring out this On-Farm Trials (OFT) work as a research data sources for the benefits and welfare of scientific community and farmers.

Authors’ contributions

TAV designed, outlined, drafted, and revised the manuscript of this study. NVR conducted the feed trials and blood collection in poultry. SI carried out the collection of Azolla and Ulva lactuca and formulation of the feed. SV conducted the haematology and serum biochemistry analysis. RV worked out on the statistical interpretations of the study. All authors have approved the manuscript for submission.

Funding

The research project was completed under On-Farm Trials (OFT) scheme funded by Tamilnadu Veterinary and Animal Sciences University sanctioned with a total amount of Rs.10,000/- (INR).

Availability of data and materials

All the data generated during the current study are included in this research article and the datasets used during the study are available from the authors upon reasonable request and permission from the corresponding author. All the data generated during the current study are included in this research article and the datasets used during the study are available from the authors upon reasonable request and permission from the corresponding author.

Ethics approval and consent to participate

We would like to state that a written consent and approval has been obtained from the Directorate of Extension Education, Tamilnadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai—51, to use the farm animals for conducting this On-Farm Trials study. Since no birds were killed/euthanized during the study, ethical approval is not applicable for this study. After the conduction of feed trials, the farmer sold the chicken to the consumer at an appropriate market value.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

1Veterinary University Training and Research Centre, Ramanathapuram, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
2Regional Research and Educational Centre, Pudukottai, Tamilnadu Veterinary and Animal Sciences University, Chennai, India. “Centralized Clinical Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.”
3Livestock Farm Complex, Madhavaram Milk Colony, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
4Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
5Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
6Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
7Centralized Clinical Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
8Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
9Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
10Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
11Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
12Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
13Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
14Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
15Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
16Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
17Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
18Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
19Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
20Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
21Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
22Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
23Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
24Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
25Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
26Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
27Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
28Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
29Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
30Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
31Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
32Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
33Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
34Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
35Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
36Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
37Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
38Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
39Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
40Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
41Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
42Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
43Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
44Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
45Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
46Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
47Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
48Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
49Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
50Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
51Laboratory, Madras Veterinary College, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
23. Halama D (1990) Single cell protein. In: Boda K (ed) Nonconventional feedstuff in the nutrition of farm animals. Elsevier, New York, p 34
24. Phang SM (1992) Role of algae in livestock-fish integrated farming system. In: Mukherjee TK, Moi PS, Panandam JM, Yang YS (eds) Proceedings of the FAO/IPT workshop on integrated livestock-fish production system, 16-20 Dec., 1991, University of Malaya, Kuala Lampur, p 49
25. Lakshmanan A, Kumar K, Latha P (2017) Azolla – a low cost and effective feed supplement to poultry birds. Int J Curr Microbiol App Sci 6:3622–3627
26. Ivan DT, Thuget TQ (1989) Use of Azolla in rice production in Vietnam. In: Nitrogen and rice. International Rice Research Institute, Philippines, p 395
27. Alalade OA, Iyayi EA (2006) Chemical composition and the feeding value of Azolla (Azolla pinnata) meal for egg-type chicks. Int J Poult Sci 5:137–141
28. Basak B, Pramanik MAH, Rahman MS, Tarafdar SU, Roy RC (2002) Azolla (Azolla pinnata) as a feed ingredient in broiler ration. Int J Poult Sci 1:29–34
29. Kathirvelan C, Banupriya S, Purushothaman MR (2015) Azolla – an alternate and sustainable feed for livestock. Int J Sci Environ Tech 4:1153–1157
30. Bhattacharyya A, Shukla PK, Roy D, Shukla M (2016) Effect of Azolla supplementation on growth, immunocompetence and carcass characteristics of broilers. J Anim Res 6:941–945
31. Kumar M, Dhusta RK, Jain D, Sharma T, Nehra R, Gupta L (2018) Effect of supplementation of Azolla on the hematology, immunity and gastrointestinal profile of broilers. Int J Liv Res 8:184–191
32. Shukla M, Bhattacharyya A, Shukla PK, Roy D, Yadav B, Siroh R (2018) Effect of Azolla feeding on the growth, feed conversion ratio, blood biochemical attributes and immune competence traits of growing Turkeys. Vet World 11: 459–463. https://doi.org/10.14202/vetworld.2018
33. NBAGR, 2013. National Bureau of Animal Genetic Resources, Karnal, Haryana, 30th November 2013. http://www.nbagr.res.in/regchi.html.
34. AOAC (2000) Official methods of analysis, 17th edn. Association of Official Analytical Chemists, Washington D.C.
35. Jain NC (2000) Schalm’s veterinary haematology. Joh Wiley Publications, Philadelph. pp 35–36
36. SAS (2004) SAS user’s guide. Statistics. SAS Institution, Cary
37. Marnata J, Abdul A, Anadamoy M, Shiv Mohan M, Satyanarayana B, Bhogeshwar C, Bhagchand C (2018) Effect of (Azolla pinnata) feed on the growth of broiler chicken. J Entomol Zool Stud 6:391–393. https://doi.org/10.22271/jento
38. Carrillo DS, Casas VMW, Castro GMW, Perez GL, Garcia VR (1990) The use of Macrocystis pyrifera seaweed in broiler diets. Invest Agraran Prod Sanidad Anim 5:137–142 ISSN No: 0213-5035
39. Vijayalingam TA, Rajesh NV, Kalpana Devi R (2017) Bioactive chemical constituent analysis, in vitro antioxidant and antimicrobial activity of whole plant methanol extracts of Ulva lactuca Linn. Br J Pharm Res 15:3–14. https://doi.org/10.1007/s00580-011-1259-7
40. Satheeshkumar P, Ananthan G, Senthil Kumar D, Jagadeesan L (2011) Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. Comp Clin Path 5:1–5. https://doi.org/10.1007/s00580-011-1259-7
41. Knoph MB, Olsen YA (1994) Subacute toxicity of ammonia to Atlantic salmon (Salmo salar L.) in seawater effects on water and salt balance, plasma cortisol and plasma ammonia levels. Aquat Toxicol 30:295–310. https://doi.org/10.1016/0166-445x(94)00046-8
42. Yu P, Li N, Liu X, Zhou G, Zhang Q, Li P (2003) Anti-hyperlipidemic effects of different molecular weight sulfated polysaccharides from Ulva pertusa (Chlorophyta). Pharmacol Res 48:543–549. https://doi.org/10.1016/s1043-6618(03)00215-9