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RESEARCH

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

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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, C_1 (control), T_1 (*Azolla* alone), T_2 (seaweed alone) and T_3 (seaweed plus *Azolla*). *Aseel* chicks in C_1 were fed with formulated grower feed alone, birds in T_1 had control diet with 5% *Azolla*, birds in T_2 had control diet with 3% *U. lactuca* and birds in T_3 had control diet admixtured 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 ($P \leq 0.05$) during 30 days of feed trials and highly significant ($P \leq 0.01$) during 45 and 60 days of treatment. Birds in T_3 had a higher body weight gain, shank length and feed conversion efficiency followed with T_1 and T_2 compared to C_1. PCV, RBC, WBC and heterophil counts were not influenced ($P \geq 0.05$) by dietary treatments. Uric acid, creatinine, AST, glucose, triglycerides and magnesium levels revealed a high significant ($P \leq 0.01$) variation, and cholesterol level showed significant ($P \leq 0.05$) 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 ($P \geq 0.05$) during 30th day and were highly significant ($P \leq 0.01$) 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*

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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 seaweeds 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 *U. 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 *U. 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 *U. lactuca* was collected from Mandapam and Therkutharavai coastal area of Ramanathapuram District. The collected *U. 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 T_1 group, the mash mixed with 3.0% of green seaweed was used in T_2 group and the mash mixed both with *Azolla* and green seaweed at the level of 3.0% and 5.0% respectively was used in T_3 group. The plain grower mash without the addition of any supplements was used in control (C_1) 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.

Table 1 Inclusion level of dietary ingredients

Experimental diets				
Ingredients (%)	**C_1**	**T_1**	**T_2**	**T_3**
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
Seaweed[a]	0	0	3	3
Azolla	0	5	0	5
Total	**100**	**100**	**100**	**100**

C_1 control diet alone, *T_1* control diet with 5% *Azolla*, *T_2* control diet with 3% seaweed, *T_3* control diet with 5% *Azolla* and 3% seaweed
[a]Green algae—*Ulva lactuca*

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 (C_1) were fed with the formulated grower poultry feed as a basal diet. Birds in group T_1 were fed with 5% *Azolla*, T_2 with 3% *U. lactuca* and T_3 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 (C_1, T_1, T_2 and T_3) 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, platelets 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.

The means for measurements showing significant differences in the ANOVA were tested using the PDIFF option. Means ± standard error of the mean (SEM) are presented in the tables and differences were considered statistically significant at $P \leq 0.05$.

3 Results

3.1 Proximate analysis of feed samples

The inclusion level of 5% *Azolla* (T_1), 3% *U. lactuca* (T_2) and combination of 5% *Azolla* and 3% *U. lactuca* (T_3) as feed supplement in poultry feed ration (C_1) 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 (C_1, T_1, T_2 and T_3), *Azolla* and *U. 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 (C_1) and treatment groups (T_1, T_2 and T_3). The average body weight gain, shank length and feed efficiency during the 2-month trial period were highest in T_3 group and least in T_2 (Table 3). Birds in T_3 required only 3.39 kg of feed to achieve a body weight gain of 1 kg, and it was 5.08 kg in T_2 group. Birds in T_1 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 ($P \geq 0.05$) in growth performance during 0 to 15 days of the trial. Significant difference ($P \leq 0.05$) in growth performance could be recorded during 30th day, and it was highly significant ($P \leq 0.01$) 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 is presented in Tables 5 and 6 respectively. Haematological analysis showed a high significant ($P \leq 0.01$) 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 ($P \leq 0.01$) variation in uric acid, creatinine, AST, glucose, triglycerides and magnesium and a significant ($P \leq 0.05$) variation in the level of serum cholesterol during 30th and 60th day of feed trials between the control and treatment groups. No significant changes ($P \geq 0.05$) 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 ($P \geq 0.05$) during 30th day and were highly significant ($P \leq 0.01$) 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 admixtured with *Macrocystis*

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

Chemical composition	C_1	T_1	T_2	T_3	*Azolla*	[a]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

C_1 control diet alone, T_1 control diet with 5% *Azolla*, T_2 control diet with 3% seaweed, T_3 control diet with 5% *Azolla* and 3% seaweed
[a]Green algae—*Ulva lactuca*

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			
	C_1	T_1	T_2	T_3
Period (days)	60	60	60	60
Initial weight (g)	273 ± 21.294[a]	319.4 ± 18.025[a]	305.6 ± 21.220[a]	292.4 ± 15.863[a]
Final weight (g)	841.7 ± 20.579[a]	1020.8 ± 54.861[b]	840.2 ± 18.274[a]	1108.8 ± 58.686[b]
Gain weight (g)	568.7 ± 8.723[b]	701.4 ± 48.045[a]	534.6 ± 13.733[b]	816.4 ± 44.509[a]
Initial shank length (g)	64.2 ± 0.929[ab]	61.5 ± 1.035[bc]	66.2 ± 1.073[a]	57.1 ± 1.140[c]
Final shank length (g)	111.9 ± 1.629[ab]	114.8 ± 2.426[a]	106.9 ± 1.84[b]	117.2 ± 1.381[a]
Gain in shank length (g)	47.7 ± 1.868[c]	53.3 ± 2.508[b]	40.7 ± 2.418[d]	57.1 ± 1.545[a]
Average daily gain (g)	9.48 ± 0.146[b]	11.69 ± 0.801[a]	8.91 ± 0.229[b]	13.61 ± 0.742[a]
Feed intake (g)	2707.01 ± 41.521[a]	2805.60 ± 192.182[a]	2715.77 ± 69.765[a]	2767.60 ± 150.885[a]
Feed conversion	4.76 ± 0.005[b]	4.00 ± 0.068[c]	5.08 ± 0.270[a]	3.39 ± 0.008[d]

C_1 control diet alone, *T_1* control diet with 5% *Azolla*, *T_2* control diet with 3% seaweed; *T_3* control diet with 5% *Azolla* and 3% seaweed
[a, b, c, d, ab, bc]Means within same row bearing different superscripts are significantly different at $P \leq 0.05$

pyrifera at 15% level and *Ulva rigida* at 10-15% level respectively. The highest body weight gain, increased shank length and feed efficiency in T_3 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 been compromised by the excessively available protein, essential amino acids and minerals in *Azolla*. The fibre content in *U. lactuca* in T_3 diet might have enhanced the digestibility of the protein-rich diet. The T_2 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 T_1 group showed a better body weight gain compared to C_1 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 ($P \leq$

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

Weight gain (in days)	Experimental diets			
	C_1	T_1	T_2	T_3
0[NS]	273 ± 21.294[a]	319.4 ± 18.025[a]	305.6 ± 21.220[a]	292.4 ± 15.863[a]
15[NS]	380.20 ± 20.612[a]	411.40 ± 22.173[a]	429.40 ± 29.457[a]	427.20 ± 16.919[a]
30*	510 ± 30.333[a]	610 ± 35.636[ab]	549.70 ± 30.675[ab]	640.30 ± 24.699[b]
45**	649 ± 25.784[a]	802 ± 41.800[b]	656.80 ± 23.983[a]	856 ± 32.183[b]
60**	841.70 ± 20.579[a]	1020.80 ± 54.861[b]	840.20 ± 18.274[a]	1108.80 ± 58.686[b]

C_1 control diet alone, *T_1* control diet with 5% *Azolla*, *T_2* control diet with 3% seaweed, *T_3* control diet with 5% *Azolla* and 3% seaweed; NS = $P \geq 0.05$
*$P \leq 0.05$
**$P \leq 0.01$
[a,b,ab]Means within same row bearing different superscripts are significantly different at $P \leq 0.05$

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

Haematological parameters (in days)		Experimental diets			
		C_1	T_1	T_2	T_3
30	**Hb (g/dL)	9.2 ± 0.153^a	10.5 ± 0.153^b	9.5 ± 0.120^a	9.6 ± 0.240^a
	**PCV (%)	26.4 ± 0.200^a	28.4 ± 0.115^b	25.6 ± 0.240^a	26.4 ± 0.230^a
	**RBC (m/μL)	2.5 ± 0.120^a	3.3 ± 0.074^b	2.8 ± 0.042^a	2.9 ± 0.038^a
	**WBC (cmm)	16466.7 ± 176.383^c	16033.3 ± 88.192^c	13466.7 ± 202.759^a	14300 ± 115.470^b
	**H (%)	31 ± 0.577^a	38 ± 0.577^c	36 ± 0.577^{bc}	34 ± 0.577^b
	*L (%)	61 ± 0.577^a	59 ± 0.577^a	59 ± 0.577^a	61 ± 0.577^a
	^{NS}M (%)	3.7 ± 0.333^a	2.7 ± 0.333^a	2.3 ± 0.333^a	2.7 ± 0.333^a
	^{NS}E (%)	3.7 ± 0.333^a	2.3 ± 0.333^a	2.7 ± 0.333^a	2.3 ± 0.333^a
60	^{NS}Hb (g/dL)	9.5 ± 0.203^a	9.6 ± 0.145^a	9.5 ± 0.186^a	9.5 ± 0.173^a
	**PCV (%)	28.0 ± 0.088^d	26.4 ± 0.145^a	26.9 ± 0.577^b	27.5 ± 0.577^c
	**RBC (m/μL)	2.6 ± 0.015^a	2.7 ± 0.042^b	2.8 ± 0.018^c	2.8 ± 0.022^{bc}
	**WBC (cmm)	18466.7 ± 176.383^d	17900 ± 57.735^c	15366.7 ± 88.192^a	16500 ± 57.735^b
	*H (%)	34 ± 1.155^{ab}	35 ± 1.155^b	34 ± 0.577^{ab}	30 ± 0.882^a
	^{NS}L (%)	61.3 ± 0.667^a	62.7 ± 0.882^a	61.7 ± 1.202^a	64.7 ± 0.882^a
	*M (%)	3.7 ± 0.333^{ab}	2.7 ± 0.333^a	3.7 ± 0.333^{ab}	4.3 ± 0.333^b
	^{NS}E (%)	2.3 ± 0.333^a	2.7 ± 0.333^a	2.3 ± 0.333^a	2.7 ± 0.333^a

H heterophil, *L* lymphocyte, *M* monocyte, *E* eosinophil

C_1 control diet alone, T_1 control diet with 5% *Azolla*. T_2 control diet with 3% seaweed, T_3 control diet with 5% *Azolla* and 3% seaweed; NS = $P \geq 0.05$

*$P \leq 0.05$

**$P \leq 0.01$

[a,b,c,d,ab,bc]Means within same row bearing different superscripts are significantly different at $P \leq 0.05$

0.01) 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 (T_1, T_2 and T_3) when compared to the control (C_1) 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, the blood glucose and magnesium were also increased with high significant values in all the treatment groups as compared to the control both on 30th and 60th days of the trial. There was a highly significant ($P \leq 0.01$) reduction in serum triglyceride and a significant ($P \leq 0.05$) reduction in serum cholesterol levels in all the treatment groups both on 30th and 60th days of trial. Abudabos et al. [14] reported a lower cholesterol level in broiler chicken fed with *U. 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 *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 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 *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 6 Effect of seaweed (*Ulva lactuca*) and *Azolla* feed supplement on serum biochemical parameters of *Aseel* chicken

Serum biochemical parameters (in days)		Experimental diets			
		C_1	T_1	T_2	T_3
30	**Uric acid (mg/dL)	13.0 ± 0.404[a]	15.4 ± 0.783[a]	24.9 ± 0.777[b]	22.6 ± 0.869[b]
	**Creatinine (mg/dL)	0.18 ± 0.032[a]	0.65 ± 0.053[c]	0.33 ± 0.025[ab]	0.46 ± 0.012[b]
	[NS]Total protein (g/dL)	4.3 ± 0.406[a]	4.0 ± 0.498[a]	3.2 ± 0.338[a]	4.0 ± 0.473[a]
	[NS]Albumin (g/dL)	2.4 ± 0.120[a]	2.2 ± 0.176[a]	2.0 ± 0.088[a]	2.4 ± 0.233[a]
	[NS]Globulin (g/dL)	1.8 ± 0.441[a]	1.9 ± 0.338[a]	1.1 ± 0.267[a]	1.6 ± 0.240[a]
	[NS]ALT (IU/L)	31.0 ± 3.512[a]	44.3 ± 6.119[a]	48.3 ± 2.603[a]	36.3 ± 1.764[a]
	**AST (IU/L)	437.0 ± 14.224[c]	303.7 ± 14.814[b]	242.0 ± 19.468[ab]	215.3 ± 14.252[a]
	**Glucose (mg/dL)	39.7 ± 0.3.844[a]	62.3 ± 13.094[ab]	71.0 ± 3.786[ab]	90.3 ± 6.173[b]
	**Triglycerides (mg/dL)	218.0 ± 27.154[c]	110.3 ± 8.090[a]	204.3 ± 6.173[bc]	145.7 ± 2.333[ab]
	[NS]Calcium (mg/dL)	11.0 ± 0.914[a]	11.6 ± 0.551[a]	12.4 ± 0.041[a]	11.8 ± 0.322[a]
	[NS]Phosphorus (mg/dL)	5.9 ± 0.439[a]	6.1 ± 0.332[a]	6.5 ± 0.204[a]	6.4 ± 0.269[a]
	**Magnesium (mg/dL)	3.7 ± 0.048[a]	4.8 ± 0.320[b]	4.5 ± 0.200[ab]	4.8 ± 0.067[b]
	*Cholesterol (mg/dL)	134.1 ± 1.964[b]	120.5 ± 4.225[ab]	116.1 ± 4.216[a]	118.4 ± 2.486[a]
	[NS]Sodium (mmol/L)	144.9 ± 2.187[a]	150.2 ± 4.245[a]	155.1 ± 4.719[a]	155.3 ± 6.904[a]
	[NS]Potassium (mmol/L)	5.1 ± 0.441[a]	5.6 ± 0.232[a]	6.0 ± 0.483[a]	5.9 ± 0.466[a]
	[NS]Chloride (mmol/L)	112.4 ± 2.569[a]	119.3 ± 4.429[a]	121.5 ± 4.825[a]	124.3 ± 6.151[a]
60	**Uric acid (mg/dL)	10.7 ± 0.311[a]	14.6 ± 0.491[b]	18.2 ± 0.321[c]	17.7 ± 0.328[c]
	**Creatinine (mg/dL)	0.42 ± 0.018[a]	0.55 ± 0.026[b]	0.47 ± 0.018[ab]	0.45 ± 0.018[a]
	**Total protein (g/dL)	3.8 ± 0.115[ab]	4.1 ± 0.088[b]	3.5 ± 0.088[a]	4.7 ± 0.145[c]
	[NS]Albumin (g/dL)	2.1 ± 0.088[a]	2.2 ± 0.120[a]	1.9 ± 0.667[a]	2.4 ± 0.120[a]
	**Globulin (g/dL)	1.7 ± 0.333[a]	1.9 ± 0.115[ab]	1.6 ± 0.153[a]	2.3 ± 0.058[b]
	[NS]ALT (IU/L)	30.0 ± 1.155[a]	30.7 ± 0.667[a]	34.7 ± 1.764[a]	31.0 ± 1.155[a]
	**AST (IU/L)	250.0 ± 3.786[b]	283.3 ± 1.333[c]	273.7 ± 2.728[c]	192.7 ± 3.712[a]
	**Glucose (mg/dL)	45.7 ± 0.333[a]	48.0 ± 0.577[a]	78.0 ± 0.577[b]	80.0 ± 0.577[b]
	**Triglycerides (mg/dL)	114.7 ± 1.452[b]	103.7 ± 3.930[a]	114.0 ± 1.155[b]	101.3 ± 1.764[a]
	[NS]Calcium (mg/dL)	12.6 ± 0.262[a]	11.8 ± 0.213[a]	12.8 ± 0.178[a]	12.4 ± 0.587[a]
	**Phosphorus (mg/dL)	6.1 ± 0.163[a]	6.3 ± 0.214[a]	6.9 ± 0.055[b]	6.9 ± 0.022[b]
	**Magnesium (mg/dL)	3.8 ± 0.076[a]	3.9 ± 0.040[a]	4.7 ± 0.118[b]	4.8 ± 0.061[b]
	*Cholesterol (mg/dL)	128.0 ± 1.155[b]	118.7 ± 2.333[ab]	109.0 ± 2.646[a]	110.7 ± 5.696[a]
	**Sodium (mmol/L)	141.9 ± 0.318[a]	157.2 ± 1.201[b]	162.8 ± 0.784[c]	166.8 ± 1.102[c]
	**Potassium (mmol/L)	4.3 ± 0.052[a]	5.2 ± 0.035[b]	5.1 ± 0.076[b]	5.1 ± 0.064[b]
	**Chloride (mmol/L)	107.9 ± 0.348[a]	125.1 ± 0.265[b]	127.6 ± 1.405[b]	134.9 ± 0.811[c]

[1]C_1 control diet alone, T_1 control diet with 5% *Azolla*, T_2 control diet with 3% seaweed, T_3 control diet with 5% *Azolla* and 3% seaweed; NS = $P \geq 0.05$
*$P \leq 0.05$
**$P \leq 0.01$
[a,b,c,ab,bc]Means within same row bearing different superscripts are significantly different at $P \leq 0.05$

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.*

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; PDIFF: Power diffraction; DM: Dry matter; NS: Not significant; PCV: Packed cell volume; RBC: Red blood cell; WBC: White blood cell; H: Heterophils; L: Lymphocyte; M: Monocyte; E: Eosinophils; Hb: Haemoglobin; Na: Sodium; K: Potassium; Cl: Chloride; ALT: Alanine transaminase; AST: Aspartate transaminase

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Authors' contributions

TAV designed, outlined, drafted, and revised the manuscript of this study. NVR conducted the feed trails 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.

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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's institution.

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.

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