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Comparative study of *Anabaena azolla* using three different nutrient media

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Abstract
Increasing demand for protein, especially animal-based proteins and the large amounts of protein feed inputs required for production, has largely driven the research on *Anabaena azolla* (AZ) as an animal feed. Overall, AZ appears to be a prospective protein source in fish production, as well as aquaculture. The Proximate, Mineral, Phytochemical and Vitamin composition of cultured AZ was determined. The cultured samples were grouped into four as follows T_1 was the Control, and group T_2, T_3, and T_4 was treated every 24 hours with 2 mg/L urea and 40 mg Sodium bicarbonate. In the proximate analysis the T_4 group has significantly high percentage moisture content (1.73%) followed by T_3, T_2 and T_1 respectively when compared at ($p \leq 0.05$). The mineral composition shows significant increase in the Na composition of the T_4 (778.00 Mg/Kg) and T3 (775.21 Mg/Kg) (groups followed by T_2 730.30 Mg/Kg) group at ($p \leq 0.05$). The K component of T_4 (303.40 Mg/Kg) group is significantly higher than other treated groups. There is no significant difference in the composition of T_1 (159.20 Mg/Kg) and T_2 (177.40) Mg composition at ($p \leq 0.05$). The Ca content is significantly high in increasing other (T_1 (304.20 Mg/Kg) to T_4 (330.50 Mg/Kg) groups) at ($p \leq 0.05$). There is no significant difference in the Fe and P component of T_3 (17.60 Mg/Kg & 410.49 Mg/Kg) and T_4 (18.00 Mg/Kg & 427.50 Mg/Kg) as well as T_2 (15.30Mg & 390.50 Mg/Kg) and T_1 (14.00 Mg/Kg & 348.15 Mg/Kg) at ($p \leq 0.05$). The Zn and Cu composition of T_4 (5.15 Mg/Kg & 0.88 Mg/Kg) is significantly high followed by T_3, (4.77 Mg/Kg & 0.75 Mg/Kg) T_2, (4.19 Mg/Kg & 0.53 Mg/Kg) and T_1 (3.88 Mg/Kg & 0.39 Mg/Kg) at ($p \leq 0.05$) respectively. The Phyto-analysis reveals no significant difference in T_2 to T_4 groups. The T_4 (80.40 Mg of GAE/g of sample) has the highest Total phenol properties followed by T_3, 53.25 Mg of GAE/g of sample) T_2 (36.49 Mg of GAE/g of sample) and T_1 (15.45 Mg of GAE/g of sample) at ($p \leq 0.05$). T_3 (10.58 Mg of GAE/g of sample) and T_4 (12.90 Mg of GAE/g of sample) group significantly poses high Flavonoid component at ($p \leq 0.05$). The T_4 (2.06 Mg of DE/g of sample) group significantly poses high Saponin content followed by T_3 (1.89 Mg of DE/g of sample) and T_2 (1.52 Mg of DE/g of sample) at ($p \leq 0.05$). There is significantly high percentage alkaloid in T_4. (0.11% Alkaloid). The cardiac glycoside value is significantly high in T_4 (2.25 Cardiac glycoside Mg Securida side /g of sample) groups at ($p \leq 0.05$). The Vitamin compositions shows no significant difference in vitamin A component in T_1 (85.40) and T_2 (99.16) and also, T_3 (128.70) and T_4 (133.00) respectively at ($p \leq 0.05$). There is no significant difference in Vitamin C composition of T_4 (15.70) and T_3 (14.90) as well as T_2 (12.74) and T_1 (12.60) respectively at ($p \leq 0.05$). The AZ when treated with other substances existing in either organic or inorganic nature is proven to be rich in essential nutrients required for normal growth of fishes and other aquatic animals. Hence, the T_4 should be considered as the most potent sample treated with AZ. As a result of these properties, it is required that the biochemical and *in vivo* antioxidant capacity of these treatments should be further investigated.

Keywords: Component, organic, composition

Introduction
Due to the rising demand for fish food, aquaculture is the food business with the highest rate of growth in the world. Because of this rigorous cultivation, bacterial infections cause an epidemic that costs the aquaculture business billions of dollars every year globally. Many pathogenic bacteria are thought to be saprophytic in nature. These bacteria only develop into pathogenic when fish are nutritionally deficient, physically out of balance or experiencing stressors such low water quality and overstocking. A cyanobacterium, Anabaena is sometimes referred to as a blue-green alga since it produces certain colors. Antibiotic resistance, which

results from overusing drugs and raises the infection incidence by these antibiotic-resistant microbes, has made the antibacterial activity of cyanobacteria more significant in recent years.

Certain cyanobacterium strains generate compounds both within and outside of cells that have a variety of biological properties, including antibacterial and antifungal properties.

Anabaena azolla is a multicellular, filamentous, freshwater cyanobacterium that can fix nitrogen into a bioavailable form via the oxygen-labile nitrogenase complex, which is protected from molecular oxygen in specialized non-photosynthetic, non-dividing cells called heterocysts. It can be culture easily in the lab using carbon dioxide and inexpensive BG-11 medium. Anabaena has antibacterial compounds and decreases the mortality of fishes. Dietary supplementation of the microalgae enhances the growth performance, reduction of the serum enzyme activities, and improves immunity towards the A. hydrophila infection.

The growth performance of L. gonius supplemented with different algal diets is improved and the contents of lipid, protein, and carbohydrate are significantly higher than the contents of Labeo gonius fish fed with normal feed. So, in the modern aquaculture industry, the utilizing of microalgae as fish feed is expanding quickly as a suiTable alternative source. Organic waste such as chicken manure as well as Sodium bicarbonate and Urea contains high broken organic and inorganic nutrients, and high biological oxygen demand (BOD) and chemical oxygen demand (COD), total dissolved solids, total suspended solids, nitrate, phosphate and also inorganic nutrients (Habib and Kohinoor 2018). These organic and inorganic nutrients rich in carbon can help grow Anabaena in supernatant after aerobic or anaerobic digestion of chicken manure. The aim of this work is to study the production of Anabaena using DCM (Dried chicken manure) supplemented with urea and sodium bicarbonate by fed-batch addition. The proximate, Vitamins and mineral composition of the Anabaena biomass will also be investigated.

Materials and Method

The study was conducted at Prince Abubakar Audu University's Department of Fisheries and Aquaculture in Anyigba, Nigeria. Because dry chicken dung contains a high concentration of both organic and inorganic elements, particularly carbohydrates, it was chosen as the growth medium for *Anabaena azolla*. The animal farm of Prince Abubakar Audu University in Anyigba, Nigeria will provide the chicken manure.

Culture medium

The culture medium used was 2.0 kg of dry egg-laying chicken manure (DCM) collected from a closed-system house. The manure was suspended in 15 Liters of aerated tap water for 22 days before being sieved through a 30-mesh Tylor net. Industrial salt (5 mg/L) was added to prevent microbial contamination. After 24 h, 8.5 g/L of sodium bicarbonate was added before the beginning of the experiment.

Results

Percentage (%) proximate analysis

From the results in Table 4.0 below, the T_4 group has significantly high percentage moisture content followed by T_3, T_2 and T_1 respectively. The ash, Fat, Protein and carbohydrate content is significantly T_4 group than all other treated groups. Also, no significant difference in the composition T_2 and T_3 group respectively when compared at ($p \leq 0.05$).

Table 1: Percentage proximate composition of treated *Anabaena azolla*

Sample code	% Moisture content	% Ash content	% Crude fiber	% Fat content	% Protein content	% Carbohydrate
T_1	1.73±0.01^a	2.15±0.03^a	1.92±0.01^a	1.01±0.01^a	37.66±0.02^a	34.54±0.07^a
T_2	1.89±0.01^b	2.32±0.01^b	3.27±0.01^b	1.11±0.01^b	45.31±0.01^b	46.05±0.03^b
T_3	1.92±0.01^c	2.35±0.03^b	3.32±0.003^b	1.15±0.01^b	46.11±0.03^b	46.28±0.23^b
T_4	2.15±0.03^d	2.49±0.01^c	3.37±0.01^b	1.27±0.01^c	56.76±0.15^c	53.19±0.10^c
SEM	0.09	0.07	0.35	0.05	3.92	3.86

Values are expressed as Mean±SEM at (n = 3). Values with the same superscripts in the same column are not significantly different when compared at ($p \leq 0.05$).

Where: SEM: (Standard error of mean), DCM: (Dry chicken manure), B: Sodium bicarbonate, U: (Urea)

(T_1)-DCM (control): tap water was added every 24 hours

(T_2)- DCM+B: sodium bicarbonate (40 mg/L) was every 24 hour

(T_3)-DCM+U: urea (2.0 mg/L) was added every 24 hours

(T_4)-DCM+U+B: urea (2.0 mg/L) and sodium bicarbonate (40 mg/L) were added every 24 hours.

Vitamin composition

The results in Table 4.1 below shows no significant difference in vitamin A component in T_1 and T_2 and also, T_3 and T_4 respectively. T_4 has the highest composition of vitamin B_1 followed by T_3. There is no significant difference between T_2 and T_1 at ($p \leq 0.05$). There is no significant difference in Vitamin C composition of T_4 and T_3 as well as T_2 and T_1 respectively at ($p \leq 0.05$)

Table 2: Vitamins composition of the treated *Anabaena azolla*

Sample code	Vitamin A µg/RE	Vitamin B_1 Mg/100 g	Vitamin B_2 Mg/100 g	Vitamin C Mg/100 g
T_1	83.6±0.88^a	0.48±0.00^a	0.25±0.01^a	12.59±0.02^a
T_2	99.16±0.00^a	0.51±0.00^a	0.30±0.00^b	12.75±0.00^a
T_3	128.72±0.01^b	0.56±0.01^b	0.35±0.01^b	14.93±0.02^b
T_4	133.05±0.50^b	0.63±0.00^c	0.41±0.00^c	15.71±0.00^b
SEM	11.87	0.03	0.03	0.78

Values are expressed as Mean±SEM at (n = 3). Values with the same superscripts in the same column are not significantly different when compared at ($p \leq 0.05$).

Where: SEM: (Standard error of mean), DCM: (Dry chicken manure), B: Sodium bicarbonate

(T_1)-DCM (control): tap water was added every 24 hours
(T_2)- DCM+B: sodium bicarbonate (40 mg/L) was every 24 hour
(T_3)-DCM+U: urea (2.0 mg/L) was added every 24 hours
(T_4)-DCM+U+B: urea (2.0 mg/L) and sodium bicarbonate (40 mg/L) were added every 24 hours

Phytochemical composition

From Table 4.2 below, T_1 has the lowest Tannis phytochemical properties. There is no significant difference in T_2 to T_4 groups. The T_4 has the highest Total phenol properties followed by T_3, T_2 and T_3 at ($p \leq 0.05$). There is no significant difference in Flavonoid composition of T_1 and T_2. Including T_3 and T_4 which significantly poses high Flavonoid component at ($p \leq 0.05$). The T_4 group significantly poses high saponin content followed by T_3 and T_2 with T_1 which has no significant difference at ($p \leq 0.05$). The T_4 significantly poses highest Saponin content followed by the T_3 group. There is no significant difference in the Saponin content of T_2 and T_1 at ($p \leq 0.05$). The T_4 has the highest percentage alkaloid. The cardiac glycoside value is significantly high in the T_4 treated groups at ($p \leq 0.05$).

Table 3: Phytochemical composition of the treated *Anabaena azolla*

Sample code	Tannis Mg of GAE/g of sample	Total phenol Mg of GAE/g of sample	Flavonoid Mg of GAE/g of sample	Saponin Mg of DE/g of sample	% Alkaloid	Cardiac glycoside Mg Securida Side/g of sample
T_1	0.32 ± 0.00^a	15.45 ± 0.10^a	4.11 ± 0.00^a	1.52 ± 0.01^a	0.04 ± 0.00^a	0.92 ± 0.00^a
T_2	1.29 ± 0.01^b	36.50 ± 0.00^b	5.00 ± 0.06^a	1.52 ± 0.00^a	0.06 ± 0.00^a	1.16 ± 0.00^a
T_3	1.32 ± 0.00^b	53.25 ± 0.05^c	10.64 ± 0.03^b	1.90 ± 0.01^b	0.08 ± 0.00^a	1.89 ± 0.00^b
T_4	1.43 ± 0.01^b	80.41 ± 0.05^d	13.00 ± 0.07^c	2.07 ± 0.01^c	0.11 ± 0.01^b	2.25 ± 0.03^c
SEM	0.25	13.72	2.16	0.14	0.05	0.31

Values are expressed as Mean±SEM at (n = 3). Values with the same superscripts in the same column are not significantly different when compared at ($p \leq 0.05$).
Where: SEM: (Standard error of mean), GAE: (Gallie Acid Equivalent), DE: (Diospenin Equivalent), DCM: (Dry chicken manure), B: Sodium bicarbonate
T_1 = DCM (Control) - tap water was added every 24 hours
T_2 = DCM + B: 40 mg /L of Sodium bicarbonate was added every 24 hours
T_3 = DCM + U: 2 mg/L Urea was added every 24 hours
T_4 = DCM + U + B: 2 mg/L Urea and 40mg/L was added every 24 hours.

Mineral composition

Results from Table 4.3 below shows a significant increase in the Na composition of the T_4 and T_3 groups followed by T_2 group at ($p \leq 0.05$). The K component of T_4 group is significantly higher than other treated groups. There is no significant difference in the composition of T_1 and T_2 group Mg composition. The T_4 has the highest significant value followed by T_3 at ($p \leq 0.05$). The Ca content is significantly high in increasing other from T_1 to T_4 treated groups at $p \leq 0.05$. There is no significant difference in the Fe and P component of T_3 and T_4 as well as T_2 and T_1 at $p \leq 0.05$. The Zn and Cu composition of T_4 is significantly high followed by T_3, T_2, and T_1 at $p \leq 0.05$ respectively.

Discussions

The proximate analysis revealed the presence of carbohydrate, protein, fat, crude fibre, moisture, and ash content as shown in Table 4.0. The high carbohydrate and fat content in the T_4 group suggests it could promote energy boost when compared or it could also be used as a supplementary feed for fishes. Much Research has proven how the cell cycles of usual programmed cell death can be stopped by switching their metabolism to use fatty acids as fuel which suggests the potency of the stem bark extract to this regard. The high moisture content in the T_4 group reveals that it's prone to easy deterioration since food substances with a high moisture contents perishes easily (Abdel-Salam, 2013). The crude Fibre content makes feed favorable and aids in digestion and adsorption. The ash content revealed the amount of mineral contents present in a sample and it aids and promotes quick digestion and absorption of mineral content. Conversely, the high percentage of protein and moisture content suggests its quick recovery ability, reduction of muscle loss means the T_4 treated group possess tissue repair effects as stated by Kirk and Sawyer, (2013).

The Vitamin composition in Table 4.1 reveals that the T_4 and T_3 groups could be a good source of Vitamin A also known Lycopene which is a good substrate for carotenoid which is necessary for eyesight. The Vitamin B_1 and B_2 and C composition which is high in the T_4 treated groups is an indications that it could prevent Thiamine deficiency syndrome where there is severe organ damage, oxidative stress and developmental toxicity in fishes also proved that lack of thyamine (Vit.B_1) disrupt the energy metabolism through mode of actions such as reduction of ATP, Oxidative phosphorylation which causes reduced feeding in fishes.

The Phytochemical analysis revealed the presence of flavonoids, Phenol, Alkaloid, Tannin, Cardiac glycoside, and Saponin in *Anabaena* treated samples as shown in Table 4.2. The growing search for phytoplankton with high medicinal values has so far brought about better understanding of the invaluable medicinal properties of phytoplankton and their subsequent exploitation in the management of diseases. These properties are linked to the phytochemical constituent and since most phytoplankton are considered as safe products e.g Spirulina, they are reported to be safer upon consumption, accessible and affordable their usage are more recommended over synthetic products. Antioxidants are known to scavenge free radicals, prevent diseases and conditions such as oxidative stress in fishes. High saponin substances has been proven to lower the cholesterol level, have several ability to combat diseases such flu. In addition, saponins are expectorants, cough suppressant and for haemolytic activities. Relating this to our findings, it thus suggest that the amount of phenol and flavonoid estimated in *the Anabaena azolla* treated samples is an indication indications of the antioxidant and free radical scavenging potentials of the phytoplankton which indicate its antioxidant properties. Phenols play a major role as primary antioxidants or free radical terminators. Thus, suggests that the T_4 treated with

Anabaena azolla has an appreciable amount of polyphenolic compounds could serve as reliable free radical scavengers. The potentials of flavonoids present in flavonoid-rich plants that are ethno-medicinally used for treating dysfunctions has been reported to be through interaction with the cellular and molecular pathophysiologies implicated during synaptic transmission, potentiation and plasticity.

High components of Alkaloids actions are felt in the automatic nervous system, blood vessels, respiratory system, gastrointestinal tract. In addition, alkaloids are anti-pasmodic, analgesic and also have bactericidal effects which suggests the ability of the *Anabaena azolla* treated samples. The potential ability of the alkaloids has been extensively studied due to their bioactive and pharmacologic properties and is known to possess antioxidant activity by their ability to quench superoxide anions and singlet oxygen.

Tannins are well known for their anti-oxidant and anti-microbial properties as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretics properties.

The results in Table 4.3 show that the T_4 has high mineral compositions. High Na and K helps in water metabolism and acid base equilibrium as well as absorption of carbohydrate which is evident in Table 4.0 (FAO, 2016). High Mg helps in bone formation and also play critical role in metabolism of biomolecules.

Table 4: Determination of some mineral composition of the treated *Anabaena azolla*

Sample code	Na (Mg/Kg)	K (Mg/Kg)	Mg (Mg/Kg)	Ca (Mg/Kg)	Fe (Mg/Kg)	P (Mg/Kg)	Zn (Mg/Kg)	Cu (Mg/Kg)
T_1	680.40 ± 0.06^a	240.13 ± 0.07^a	159.30 ± 0.06^a	304.10 ± 0.06^a	14.10 ± 0.06^a	348.18 ± 0.02^a	3.86 ± 0.03^a	0.39 ± 0.01^a
T_2	730.30 ± 0.15^b	270.60 ± 0.09^b	177.53 ± 0.07^a	318.04 ± 0.03^b	15.37 ± 0.12^b	390.57 ± 0.07^b	4.16 ± 0.03^b	0.53 ± 0.01^b
T_3	770.20 ± 0.06^c	290.18 ± 0.06^c	189.30 ± 0.12^c	320.13 ± 0.09^b	17.60 ± 0.06^c	410.50 ± 0.00^c	4.76 ± 0.03^c	0.74 ± 0.01^c
T_4	778.20 ± 0.10^c	303.50 ± 0.09^d	207.33 ± 0.12^d	330.70 ± 0.12^c	18.13 ± 0.09^c	427.50 ± 0.17^c	5.15 ± 0.03^d	0.89 ± 0.01^d
SEM	22.39	13.76	10.10	5.47	0.95	17.09	0.29	0.11

Values are expressed as Mean±SEM at (n = 3). Values with the same superscripts in the same column are not significantly different when compared at ($p \leq 0.05$).

Where: SEM: (Standard error of mean), DCM: (Dry chicken manure), B: Sodium bicarbonate

T_1 = DCM (Control) - tap water was added every 24 hours

T_2 = DCM + B: 40 mg /L of Sodium bicarbonate was added every 24 hours.

T_3 = DCM + U: 2 mg/L Urea was added every 24 hours

T_4 = DCM + U + B: 2 mg/L Urea and 40mg/L was added every 24 hours

Conclusions

Phytoplankton such as anabaena when treated with other substances existing in either organic or inorganic nature is proven to be rich in essential nutrients required for normal growth of fishes and other aquatic animals. With the advent of scarcity and expensive nature of good feed supplements rich in protein and Carbohydrate, some of the treated groups proves to be a viable source of Vitamins and Minerals. Also, some of the treatments shows high antioxidant capacity required for scavenging free radical and prevention of oxidative stress which inflicts aquaculture.

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