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Effects of Amphora Algae on Productive Performance and Immune Response of Broiler Chickens

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ABSTRACT

Microalgae, especially *Amphora coffeaeformis* (*A. coffeaeformis*), are introduced to poultry diets, mainly as a rich source of polyunsaturated fatty acids (PUFAs), α-linolenic acid, eicosapentaenoic (EPA) and docosahexaenoic (DHA). This study aimed to investigate the effect of dietary supplementation of *A. coffeaeformis* on broiler chickens' productive performance, physiological status, and immune response. A total of 180 (Ross 508) broiler chickens aged one day were wing banded and randomly divided into three treatments and a control group according to the form of *A. coffeaeformis*, with 45 chickens each. Each treatment had three replicates (15 chickens for each replicate). Chickens from the three treatments were fed a diet supplemented with *A. coffeaeformis* algae at levels of 0.15, 0.45, and 0.75% of the diet from the first week to the fifth weeks of age. The obtained results indicated a significant difference in live body weight (LBW), body weight gain (BWG), and growth rate (GR) at the different experimental periods due to the effects of *A. coffeaeformis* treatments compared to the control group. Chickens fed basal diet and diet with *A. coffeaeformis* at levels of 0.45%, and 0.75% significantly increased LBW, BWG, and GR% at all intervals (1-3), (3-5), and (1-5) weeks of age compared to *A. coffeaeformis* algae at levels of 0.15%. Chickens fed a diet supplemented with *A. coffeaeformis* 0.45% and AC 0.75% recorded higher plasma total protein insignificantly, albumin significantly, at five weeks of age compared to the other *A. coffeaeformis* treatments and control group. Moreover, the lower levels of plasma triglycerides, total cholesterol, LDL, and significantly higher levels of plasma HDL were found at a basal diet supplemented by *A. coffeaeformis* 0.15% and the control group. Also, AC 0.15% and *A. coffeaeformis* 0.45% recorded insignificantly lower plasma levels of Glutathione and Superproxedase (58.55 and 71.43 mg/l, respectively) when compared with other *A. coffeaeformis* treatments and control group. Dietary supplementation of chickens' feed with *A. coffeaeformis* microalgae can promote the proliferation of beneficial bacteria (microbiota).

Keywords: *Amphora coffeaeformis*, Antioxidative status, Broiler chickens, Blood parameter, Immune response, Microalgae

INTRODUCTION

According to estimates, in 2025, the consumption of animal products will rise as an increasing population in the World, therefore, the consumption of proteins (FAO 2020). When the continued meat consumption is increased, the consumption of animal proteins will increase, which leads to overuse and subsequent limitation of traditional sources used for livestock and aquatic animal feed, such as corn, rice, soybeans, and fish meal which causes overexploitation (Cardinaletti et al., 2018; Valente et al., 2021). Due to the perfect nutritional structure, microalgae are hopeful of overcoming overuse of the consumption of animal products (Wild et al., 2019). Microalgae are a diverse category of photosynthetic organisms that live in freshwater and marine habitats and can be unicellular, multicellular, or eukaryotic (Bhuvana et al., 2019). Additionally, it gains a greater yield than conventional crops and does not involve using pesticides or causing land disputes with agricultural activities (Koyande et al., 2019; Wild et al., 2019). Substances necessary for feed during the growth of animals, including polysaccharides, polyunsaturated fatty acids (PUFAs), protein, essential amino acids, minerals, vitamins, lipids, phenolic, and antioxidant pigments are formed by Microalgae (Bhuvana et al., 2019; Santhakumaran et al., 2020). After being incorporated into animal feed, the microalgae biomass produces chemicals that are essentially required for the protein and energy for animal growth (de Tonnac et al., 2018, Kibria and Kim, 2019). Due to microalgae, biomass increases animal feed digestibility and immune response, improving meat's nutritional, technological, and sensory qualities (de Tonnac et al., 2018, Kibria and Kim, 2019).

Algae can manufacture powerful and advantageous natural substances (such as polyphenols, sterols, polyunsaturated fatty acids, proteins, sulfated polysaccharides, alkaloids, agonic acid, and carotenoids). For this reason, international pharmaceutical companies have recently been interested in using algae (Ayoub et al., 2019). *Amphora*

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coffeaeformis (*A. coffeaeformis*) is most frequently seen in brackish and alkaline freshwater environments (Bhosle et al., 1993). Microalgae are the source of Long-chain polyunsaturated fatty acids (LC-PUFAs); therefore, it is used as food in humans, animals, and aquaculture (Lee et al., 2019). As well as algae antioxidant capacity, especially AC revealed rich concentrations of PUFAs, particularly DHA, linoleic acid, and EPA (El-Bahr et al., 2020). So *A. coffeaeformis* dietary appeared to be more beneficial than *Chlorella vulgaris* and *Spirulina platensis* in antioxidant status, performance, and nutritional value (El-Bahr et al., 2020). This study aimed to investigate the effect of *A. coffeaeformis* supplement on broiler chickens' productive performance and physiological status and the impact on the immune response.

MATERIAL AND METHODS

Ethical approval

All samples were chosen per standard protocol without any animal stress or injury. Moreover, the study was done according to Cairo University Institutional Animal Care and Use Committee (CU- IACUC) Veterinary Medical and Agricultural Sciences Sector in Egypt, under the approval code # CU/II/F/19/21#.

Study design

A total of 180 unsexed chicks aged one-day (Ross 508) broiler chickens were purchased from a local hatcher, a Gold Breeder Company. They were weighed individually (42 ± 1 g), given wing bands, and randomly assigned to three treatments and a control group according to the level of *A. coffeaeformis*, with 45 chickens each. Each treatment had three replicates (15 chickens in each replicate). Chickens from the three treatments were fed a diet with levels of *A. coffeaeformis* algae 0.15, 0.45, and 0. 75 %, respectively. The environment and hygienic conditions under which the chickens were kept in cages (1 m in length, 0.6 m in width, and 0.4 m in height) were similar. According to the vaccination program, the vaccinations were given to chickens against Newcastle, Gumboro diseases, and avian influenza. At 6, 10, and 14 days of age, we used the Hitchner B1 strain, H5N1, and Gumboro vaccines. Then, at 20 days of age, the chickens were revaccinated the samples against Newcastle virus disease (CEVA company, France). In the first three days of age, chickens were brood at 35°C inside Batteries with electric heaters and then decreased temperature of 2°C weekly till the end of the fourth week. The lighting schedule was 24 hours of light at three days, then reduced to 22 hours, and a 2-hour dark was applied from 6 to 35 days of age (the end of the experiment). Feed (as mash) and water were offered *ad libitum*. We fed starter and grower diets formulated as shown in Table 1.

Table 1. Composition and chemical analyses of starter and grower diets of broiler chickens (Ross 508)

Ingredients (%)	Starter (1-14 days)	Grower (15-35 days)
Yellow corn	56	59.89
Soybean meal (46% protein)	32	28.42
Corn gluten	6.05	4.95
Soya oil	1.5	2.53
Mono-calcium phosphate	1.55	1.38
Limestone	1.75	1.7
Premix (Vitamin+Mineral)*	0.2	0.2
D.L. Methionine	0.22	0.22
L. Lysine Hcl	0.25	0.25
Salt	0.40	0.40
Chemical analyses (%)		
Choline Chloride	0.06	0.06
Crude protein	23	21
Metabolizable energy (kcal/kg)	3000	3100
Calcium	1.0	0.94
Available phosphorus	0.49	0.44
Lysine	1.4	1.3
Methionine	0.67	0.61
Methionine + Cystine	1.04	0.95
Sodium	0.18	1.8
Total of diet	100	100

*Every 2 grams of premix mixture contained: Vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopherol acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron (FeSO4 H2O), 40 mg; copper (CuSO4 5H2O), 7 mg; iodine [Ca(IO3)2], 0.7 mg; selenium (Na2SeO3), 0.3 mg

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Data collection

Body weight, weight gain, and growth rate

Chickens were weighed individually at the first, third, and fifth weeks of age. Weight gain and growth rate were calculated separately with the formula reported by Broody **(**1949**).**

Feed intake and Feed conversion ratio

The experimental diets were provided regularly and measured daily. The feed intake was counted by subtracting the weighted given feed and remainder portion, further divided by the number of chickens for every experimental treatment, then expressed in grams per chicken at the period from (0-3), (3-5) and (0-5) weeks of age.

Mortality rate

The mortality rate percentage was calculated by subtracting the number of live chickens at the end of the experiment from the initial number.

European production efficiency factor

After the calculation of the Feed conversion ratio (FCR) and viability percentage, the European production efficiency factor (EPEF) was used to assess the growing process of broiler chickens, as found by Van (2003) and Marcu et al. (2013). European production efficiency factor was calculated according to Marcu et al. (2013) by Formula 1.

$$\text{EPEF} = \frac{\text{Viability (\%)} \times \text{BW(kg)}}{\text{Age (day)} \times \text{FCR (kg feed} \div \text{kg gain)}} \times 100 \qquad \text{Formula 1}$$

Blood parameters

After five weeks of age chickens, four blood samples were obtained randomly from each treatment from the wing vein for chemical analyses. Ten ml of blood samples were collected without anticoagulant into a clean centrifuge tube, and then heparinized blood samples were centrifuged at 2500 rpm for 15 minutes. Plasma samples were stored in the deep freezer at approximately -20°C until the time of chemical analyses. A commercial kit (Bio Diagnostic Company, Egypt) was used for the chemical analyses utilizing a colorimetric approach to determine the plasma protein fractions (albumin, total protein) and kidney function test (uric acid). The lipid profile includes triglycerides, total cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The liver function had Aspartate aminotransferase (AST) and alanine aminotransferase, glucose, thyroid hormones (T3 and T4), measurements of antioxidant capacities of plasma (total antioxidants, glutathione peroxidase (GPx), and super peroxidase).

Thyroid hormones

The concentrations of T3 and T4 were calculated by Radioimmunoassay in plasma, as mentioned in previous studies by Huybrechts et al.(1989) and Darras et al. (1992). Intra assay of the variation of T3 and T4 at coefficients was 4.5 and 5.4%, respectively.

Antioxidant capacities of plasma

The samples were measured with GPx kits (Randox, Crumlin, UK). Total antioxidant status in plasma (Miller et al., 1993) and the activity of glutathione peroxidase in the blood (GPx, EC 1.11.1.9) was measured based on the technique by Wang et al. (2011).

Immunity response

The measurement of anti-Newcastle diseases vaccine antibody titers was done during weeks third and fifth to measure the humoral immunity by using a method described by Swayne (1998) that six serum samples that were subjected to hemagglutination inhibition.

Bacteriological examination

Ten samples of 5 grams of broiler intestine 2 cm long were isolated and cut-opened within complete sterilization conditions. After that, they were weighed and transmitted into Falcon tubes 50 ml which were sterile after adding 30 ml of saline solution (NaCl: 0.85%). At maximum speed, the samples were mixed for one minute by vortexing, and then tenfold serial dilutions from each sample by the same saline solution were prepared. Finally, the dilutions were used to detect and list various groups of bacteria. One ml from each of the previous dilutions was added into two individual aseptic Petri dishes, then added sterile plate agar that was melted and cooled. After mixing, counting total bacterial per gm, which incubated the inoculated plates at 30°C for 48 hours, and counting each plate that contained 30-300 colonies.

In two individual aseptic Petri-dishes that added one ml from each of the previous dilutions, sterile Eosin methylene blue agar (EMB) mediums were added that were melted and cooled. After mixing, the inoculated plates were incubated at 37°C for 48 hours. Based on the differential counts of green, colorless, and pink colonies, the *Escherichia*

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coli (*E. coli*), *Proteus* species., and *Enterobacter* species were counted per gram (Downes and Ito, 2001). The Xylose-lysine-Deoxycholate medium used to detect *Salmonella* as red colonies with the black center was recorded as positive for *Salmonella*.

Statistical analysis

One-way analysis of variance was used to determine the effect of different levels of *A. coffeaeformis*: 0.15%, 0.45%, and 0.75% on the performance of growth, some blood analysis, and evaluation of humoral immunity. Data were statistically analyzed by the general linear model procedure of the SAS software (SAS 2004). The comparison of mean values was made by Duncan's multiple range test (Duncan 1955), and significant differences appeared to be ($p < 0.05$).

RESULTS AND DISCUSSION

Productive traits

Body weight, body weight gains, and growth rate as influenced by levels of *A. coffeaeformis* on broiler performance are shown in Table 2. Both *A. coffeaeformis* 0.45% and *A. coffeaeformis* 0.75% supplementation significantly improved broiler performance throughout the experiment period than *A. coffeaeformis* 0.15% and the control group. At weeks 3 and 5, *A. coffeaeformis* 0.45% and 0.75% had significant ($p < 0.05$) body weights (824.17 and 1955.61 g, respectively). Body weight gain and growth rate in all groups were similar at (1-5) weeks, and all treatments of *A. coffeaeformis* recorded significantly ($p < 0.05$) low values during the same periods obtained in Table 2.

This result may be due to *A. coffeaeformis* significantly improved appetite, which gave rise to higher feed intake and progressed growth (Ayoub et al., 2019). The absorption of minerals and vitamins was improved (Gružauskas et al., 2004, Mariey et al., 2012) as Kaoud (2012) reported dietary *Spirulina platensis* ($p < 0.05$) raised Life body weight. These results agreed with those discovered by Zhao et al. (2004), who indicated that chickens fed with *Amphora* showed a higher average daily gain. The growth rate improved due to the efficiency of *A. coffeaeformis* in getting better immune status and serum composition (Abudabos et al., 2013) and reducing microbial load in the gastrointestinal tract (Costa et al., 2022). The results obtained agree with those found by Kang et al. (2013), Khan et al. (2021), and Long et al. (2018), who reported that in broiler chickens, the growth parameters were improved by adding microalgae in feed.

Table 2. The effect of different levels of *Amorpha coffeaeformis* on body weight, body weight gain, and growth rate of broiler chickens (Ross 508) at first, third, and fifth weeks of age

Treatment	Body weight (g)			Body weight gain (g)			Growth rate (%)		
	1 Weeks	3 Weeks	5 Weeks	1-3 Weeks	3-5 Weeks	1-5 Weeks	1-3 Weeks	3-5 Weeks	1-5 Weeks
Control	184.2 ± 1.74^{a}	798.0 ± 15.17^{a}	1888.53 ± 22.70^{b}	613.59 ± 4.80^{c}	1087.15 ± 10.74^{b}	1700.74 ± 12.77^{b}	124.95 ± 0.44^{b}	81.02 ± 0.50^{b}	164.36 ± 0.24^{c}
AC 0.15%	172.90 ± 1.74^{b}	805.6 ± 13.84^{a}	1886.63 ± 20.93^{b}	632.69 ± 4.80^{b}	1080.41 ± 10.74^{b}	1713.10 ± 12.77^{b}	129.32 ± 0.44^{a}	80.28 ± 0.50^{b}	166.40 ± 0.24^{b}
AC 0.45%	176.45 ± 1.74^{b}	824.1 ± 13.84^{a}	1929.05 ± 20.42^{ab}	647.71 ± 4.80^{a}	1104.88 ± 10.74^{b}	1752.60 ± 12.77^{a}	129.46 ± 0.44^{a}	80.26 ± 0.5^{b}	166.48 ± 0.24^{b}
AC 0.75%	173.52 ± 1.74^{b}	749.5 ± 14.01^{b}	1955.61 ± 20.67^{a}	575.38 ± 4.80^{d}	1206.21 ± 10.74^{a}	1781.58 ± 12.77^{a}	124.72 ± 0.44^{b}	89.23 ± 0.50^{a}	167.39 ± 0.24^{a}
p-value	0.0001	0.0019	0.0613	0.0001	0.0001	0.0008	<.0001	<.0001	<.0001

^{abcd} Means different superscript letters in each column express significant differences ($p < 0.05$). AC: *Amorpha coffeaeformis*

Table 3 shows the effect of *A. coffeaeformis* supplementation levels (%) on feed intake, FCR, Mortality rate, and European broiler efficiency index. Broiler chickens fed the diet supplemented with *A. coffeaeformis* 0.75% and *A. coffeaeformis* 0.15 % were found to be consumed a lower average feed intake throughout the experiment period than 0.45% and the control group. Broiler chickens fed the diet supplemented with *A. coffeaeformis* at a level of 0.15% and *A. coffeaeformis* at 0.45% improved FCR insignificantly ($p > 0.05$) throughout the experiment than 0.75% and the control group. Broiler chickens fed the diet supplemented with *A. coffeaeformis* 0.45% recorded a significantly lower mortality rate ($p < 0.05$) during the experimental period than other treatments. Broiler chickens fed the diet supplemented with *A. coffeaeformis* 0.75% and *A. coffeaeformis* 0.45% showed significantly ($p < 0.05$) higher average EPEF% during the whole experimental period than other groups. The *A. coffeaeformis* 0.15% and the control group recorded the significantly highest average of MR% and EPEF% ($p < 0.05$), respectively, compared to the other *A. coffeaeformis* treatments.

The results obtained agree with those reported by Kharde et al. (2012), which indicated that adding microalgae *Spirulina platensis* to broiler chicken diets significantly boosted FCR compared to the control diet. This enhancement

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could be attributed to a healthy microbial community in the gastrointestinal system, which improves the absorption of dietary vitamins and minerals and plays a critical part in the health of broilers (Belay et al., 1996; Kharde et al., 2012). The results obtained may be attributed to *A. coffeaeformis* has various elements, including vitamins and minerals that may help to promote growth, improvement in the FCR (Belay et al., 1996), and getting better in the digestibility of nutrients which were in agreement with Zhao et al. (2004). It is possible that Amphora's bioactive chemicals, which include antibacterial, antiviral, anti-inflammatory, and antioxidant activities, are responsible for the favorable results that have been concluded by (Rajput and Mishra 2012, Salahuddin et al., 2017). The increased EPEF and decreased mortality rate of chickens fed on supplemented diets could be strengthened the usefulness of dietary additives (microalgae) on nutrients and feed efficiency as well as being antibacterial and pathogens (Alwaleed et al., 2021). The results agree with those reported by Abdel-Moneim et al. (2022), which indicated that in correlation with the amounts and mixtures of the dietary supplements of microalgae, the EPEF increased significantly.

Table 3. The effect of different levels of *Amorpha coffeaeformis* on feed intake, feed conversion, ratio, mortality rate and European broiler efficiency index of broiler chickens (Ross 508) at first, third, and fifth weeks of age

Treatment	Feed intake (g)			Feed conversion ratio (g)			Mortality rate (%)	EBI (%)
	1-3 Weeks	3-5 Weeks	1-5 Weeks	1-3 Weeks	3-5 Weeks	1-5 Weeks	1-5 Weeks	1-5 Weeks
Control	830.39± 17.58	1740.39± 36.28^{b}	2591.78± 83.02	1.35 ± 0.03	1.60 ± 0.02^{b}	1.53 ± 0.05	19.05 ± 1.84a	295.15 ± 11.70^{b}
AC 0.15%	849.38± 17.58	1628.21± 36.28^{c}	2539.12± 83.02	1.34 ± 0.03	1.5 0± 0.02^{c}	1.48 ± 0.05	4.76 ± 1.84b	346.31 ± 11.70^{b}
AC 0.45%	870.71± 17.58	1858.33± 36.28^{a}	2750.99± 83.02	1.34 ± 0.03	1.68 ± 0.02^{a}	1.57 ± 0.05	0.00±0.00c	351.37 ± 11.70^{a}
AC 0.75%	826.43± 17.58	1837.90± 36.28^{ab}	2686.58± 83.02	1.44 ± 0.03	1.52 ± 0.02^{c}	1.51 ± 0.05	2.38 ± 1.84b	363.63 ± 11.70^{a}
p-value	0.2917	0.0008	0.3006	0.1500	0.0002	0.7354	<.0001	0.0027

^{abc} Means different superscript letters in each column express significant differences ($p < 0.05$). AC: *Amorpha coffeaeformis*

Blood parameters

The impact of different levels of *A. coffeaeformis* supplement on blood plasma constituents at three weeks is shown in tables 4 and 5. The *A. coffeaeformis* 0.15% caused increased Total protein ($p > 0.05$), Albumin ($p > 0.05$), AST ($p < 0.005$), ALT ($p > 0.05$), T3, T4 total antioxidant insignificantly ($p > 0.05$), respectively compared to the other *A. coffeaeformis* treatments and control group. Also decreased insignificantly GPX ($p > 0.05$) and Superoxidase (SPX, $p > 0.05$), same as *A. coffeaeformis* 0.45% when compared with other *A. coffeaeformis* treatments and control group. However, *A. coffeaeformis* 0.45% decreased total plasma cholesterol insignificantly ($p > 0.05$) and LDL ($p < 0.05$). They also significantly increased plasma triglycerides ($p < 0.05$) and insignificantly HDL ($p > 0.05$) compared to the other *A. coffeaeformis* treatments and control group. Furthermore, *A. coffeaeformis* 0.75% insignificantly increased blood glucose ($p > 0.05$). However, the control group was insignificantly ($p > 0.05$) lower in plasma uric acid levels at three weeks than those in the *A. coffeaeformis* treatments, and also increased AST ($p < 0.05$) showed in the control group.

The effect of different levels of *A. coffeaeformis* supplement on blood plasma constituents at five weeks are shown in tables 6 and 7. Plasma albumin levels were affected significantly ($p > 0.05$) by experimental treatments only at five weeks of age at all levels of *A. coffeaeformis* treatment. The *A. coffeaeformis* 0.45% significantly increased TP ($p < 0.05$) compared to the other *A. coffeaeformis* treatments and control group. The *A. coffeaeformis* of levels 0.45% and 0.75% were significant decreases of GPX ($p < 0.05$) and SPX and insignificant increases of antioxidants ($p < 0.05$). However, *A. coffeaeformis* 0.75% increased blood glucose ($p > 0.05$), T3, and T4 ($p > 0.05$) and also increased AST ($p < 0.05$) compared to the other *A. coffeaeformis* treatments and control group. Although *A. coffeaeformis* 0.15% decreased plasma Triglycerides ($p < 0.05$), Total cholesterol ($p < 0.05$) and LDL ($p < 0.05$) also increased ALT ($p > 0.05$) compared to the other *A. coffeaeformis* treatments and control group. Furthermore, the control group was significantly ($p < 0.05$) lower in uric acid levels at five weeks. Those in the *A. coffeaeformis* treatments also significantly increased HDL in the control group ($p < 0.05$).

These results agree with Long et al. (2018), who discovered that the addition of microalgae (MA) to broiler chicken's diet led to higher levels of plasma albumin/globulin ratio, lower levels of plasma total cholesterol and LDL compared to the control group, and higher levels of plasma glucose. Brown and Cline (1974) reported that the microalgae reduced plasma uric acid, exciting microalgae-assisted chickens for more efficient nitrogen utilization. These results did not agree with those reported by Sugiharto et al. (2018), who noticed that the serum biochemical parameters such as AST and AST were not significantly different ($p > 0.05$) across the microalgae treatments.

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Table 4. The influence of different levels of *Amorpha coffeaeformis* on plasma total protein, albumin, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, aspartate aminotransferase and alanine aminotransferase in broiler chickens (Ross 508) aged three weeks

Treatment	T.P (g/dl)	Al (g/dl)	TCH (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AST (mg/dl)	ALT (mg/dl)
Control	7.42 ± 0.80	3.97 ± 0.24	281.346 ± 16.4^{a}	63.42 ± 13.70^{b}	209.70 ± 18.3^{a}	58.97 ± 5.53	37.97 ± 2.00^{a}	8.45 ± 0.46
AC 0.15%	8.23 ± 0.80	3.68 ± 0.24	256.88 ± 16.4^{ab}	117.5 ± 13.70^{a}	158.99 ± 18.3^{ab}	74.37 ± 5.53	31.34 ± 2.00^{b}	8.57 ± 0.46
AC 0.45%	8.17 ± 0.80	3.71 ± 0.24	223.24 ± 16.4^{b}	125.0 ± 13.70^{a}	123.30 ± 18.3^{b}	74.94 ± 5.53	30.49 ± 2.00^{b}	7.26 ± 0.46
AC 0.75%	7.33 ± 0.80	3.74 ± 0.24	245.87 ± 16.4^{ab}	98.15 ± 13.70^{ab}	160.03 ± 18.3^{ab}	66.21 ± 5.53	26.92 ± 2.00^{b}	7.32 ± 0.46
p-value	0.7837	0.8292	0.1262	0.0214	0.0272	0.1667	0.0075	0.0975

^{ab} Means different superscript letters in each column express significant differences ($p \leq 0.05$). AC: *Amorpha coffeaeformis*, TP: Total protein, AL: Albumin, TCH: Total cholesterol, TG: Triglycerides, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase.

Table 5. The effect of different levels of *Amorpha coffeaeformis* on plasma triiodothyronine, thyroxine, glutathione, superoxidase, total antioxidant, glucose and uric acid in broiler chickens (Ross 508) aged three weeks

Treatment	T3 (mg/dl)	T4 (mg/dl)	GPx (mg/dl)	SPX (mg/dl)	TAX (mg/dl)	Glucose (mg/dl)	UA (mg/dl)
Control	5.67 ± 0.37	2.52 ± 0.03	91.08 ± 11.45	103.97 ± 8.80^{a}	0.620 ± 0.07	95.10 ± 6.24	3.95 ± 0.44
AC 0.15%	6.00 ± 0.37	2.63 ± 0.03	58.55 ± 11.45	76.98 ± 8.80^{ab}	0.637 ± 0.07	85.29 ± 6.24	4.07 ± 0.44
AC 0.45%	6.47 ± 0.37	2.62 ± 0.03	58.55 ± 11.45	71.43 ± 8.80^{b}	0.603 ± 0.07	89.71 ± 6.24	4.77 ± 0.44
AC 0.75%	5.93 ± 0.37	2.62 ± 0.03	78.06 ± 11.45	84.43 ± 8.80^{ab}	0.557 ± 0.07	103.68 ± 6.24	4.75 ± 0.44
p-value	0.5101	0.0648	0.1564	0.0800	0.898	0.2192	0.4153

^{ab} Means different superscript letters in each column express significant differences ($p \leq 0.05$). AC: *Amorpha coffeaeformis*, T3: Triiodothyronine, GPx: Glutathione, SPX: Superoxidase, TAX: Total antioxidant, T4: Thyroxine, UA: Uric acid, MSE: Mean standard error

Table 6. The effect of different levels of *Amorpha coffeaeformis* on plasma total protein, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, aspartate aminotransferase, alanine aminotransferase, and Albumin in broiler chickens (Ross 508) at five weeks of age

Treatment	TP (g/dl)	Al (g/dl)	TCH (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AST (mg/dl)	ALT (mg/dl)
Control	4.33 ± 0.37	3.87 ± 0.16^{b}	304.59 ± 4.50^{a}	130.55 ± 11.76^{ab}	233.64 ± 5.73^{a}	56.66 ± 2.31^{a}	31.34 ± 1.99^{a}	10.58 ± 0.64
AC 0.15%	4.58 ± 0.37	4.5 ± 0.16^{a}	242.20 ± 4.50^{b}	64.35 ± 11.76^{c}	181.63 ± 5.73^{b}	47.70 ± 2.31^{b}	21.15 ± 1.99^{b}	11.89 ± 0.64
AC 0.45%	5.50 ± 0.37	3.94 ± 0.16^{b}	300.92 ± 4.50^{a}	108.80 ± 11.76^{b}	223.64 ± 5.73^{a}	55.52 ± 2.31^{a}	33.04 ± 1.99^{a}	11.76 ± 0.64
AC 0.75%	5.29 ± 0.37	3.53 ± 0.16^{b}	296.64 ± 4.50^{a}	151.85 ± 11.76^{b}	224.02 ± 5.73^{a}	51.49 ± 2.31^{ab}	35.08 ± 1.99^{a}	10.83 ± 0.64
p-value	0.1142	0.0018	<.0001	0.0003	<.0001	0.0492	0.0004	0.3912

^{ab} Means different superscript letters in each column express significant differences ($p \leq 0.05$). AC: *Amorpha coffeaeformis*, TP: Total protein, AL: Albumin, TCH: Total cholesterol, TG: Triglyceridesö LDL: low-density lipoprotein, HDL: high-density lipoprotein, AST: aspartate aminotransferase and ALT: alanine aminotransferase.

Table 7. The effect of different levels of *Amorpha coffeaeformis* on plasma triiodothyronine, thyroxine, glutathione, superoxidase, total antioxidant, glucose and uric acid in broiler chickens aged five weeks

Treatment	T3 (mg/dl)	T4 (mg/dl)	GPx (mg/dl)	SPX (mg/dl)	TAX (mg/dl)	Glucose (mg/dl)	UA (mg/dl)
Control	7.07 ± 0.27	2.59 ± 0.03	84.57 ± 5.75^{a}	76.99 ± 3.69	0.597 ± 0.04	94.12 ± 5.59^{b}	2.8 ± 0.26^{b}
AC 0.15%	7.00 ± 0.27	2.59 ± 0.03	58.55 ± 5.75^{b}	70.24 ± 3.69	0.473 ± 0.04	100.73 ± 5.59^{ab}	4.91 ± 0.26^{a}
AC 0.45%	6.07 ± 0.27	2.58 ± 0.03	50.43 ± 5.75^{b}	77.86 ± 3.69	0.490 ± 0.04	101.96 ± 5.59^{ab}	4.57 ± 0.26^{a}
AC 0.75%	6.70 ± 0.27	2.60 ± 0.03	58.55 ± 5.75^{b}	68.25 ± 3.69	0.553 ± 0.04	116.42 ± 5.59^{a}	5.04 ± 0.26^{a}
p-value	0.0675	0.9545	0.0026	0.2009	0.1974	0.0646	<.0001

^{ab} Means different superscript letters in each column express significant differences ($p \leq 0.05$). AC: *Amorpha coffeaeformis*, T3: Triiodothyronine, GPx: Glutathione, SPX: Superoxidase, TAX: Total antioxidant, T4: Thyroxine, UA: Uric acid.

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Response of humoral immune

The effect of different dietary levels of *A. coffeaeformis* supplementation (%) on the antibody titers against NDV is shown in Table 8. At 23 days of age, *A. coffeaeformis* 0.75% gained the highest titer (7.67) significantly compared with other *A. coffeaeformis* treatments and the control group. The results obtained may be referred to *A. coffeaeformis*, which was abundant in several pigments and polyphenolic chemicals, including Catechin, Gallic acid, and P-coumaric acid, which led to this immune system activation (El-Sayed et al., 2018). In addition, Jaswir et al. (2011) demonstrated the attendance of -carotene and fucoxanthin in *A. coffeaeformis*, which were frequently utilized as food additives in addition to the many nutraceuticals uses including pro-vitamin A, antioxidant, anticancer, and anti-obesity, made the plant a powerful radical scavenger. The results obtained disagree with those reported by Sugiharto et al. (2018), who noticed that there was no significant difference between the algae treatments that are found in the serum biochemical parameters and antibody titer against NDV.

Table 8. The influence of different levels of *Amorpha coffeaeformis* on antibody titer against Newcastle disease virus in Broiler Chickens (Ross 508)

ND titer (Log2) / Treatment	Day 18	Day 23	Day 26	Day 28
Control	5.00 ± 0.65	5.00 ± 0.70^{b}	10.00 ± 0.66^{a}	9.33 ± 0.63^{a}
AC 0.15%	4.67 ± 0.65	5.00 ± 0.70^{b}	8.67 ± 0.66^{ab}	9.33 ± 0.63^{a}
AC 0.45%	3.67 ± 0.65	5.67 ± 0.70^{ab}	8.33 ± 0.66^{ab}	6.33 ± 0.63^{b}
AC 0.75%	4.00 ± 0.65	7.67 ± 0.70^{a}	6.67 ± 0.66^{b}	4.00 ± 0.63^{c}
p-value	0.4695	0.0431	0.0165	<.0001

^{abc} Means different superscript letters in each column express significant differences ($p < 0.05$).AC: *Amorpha coffeaeformis*, HI: Humoral immunity, ND: Newcastle disease.

Intestinal bacteriological counts

The effect of various dietary levels of *A. coffeaeformis*% supplementation on the intestinal bacterial count is shown in Table 9. Compared to the control group, the results found a highly significant ($p < 0.05$) effect due to *A. coffeaeformis* treatments on E. coli. Broiler chickens fed the diet supplemented with *A. coffeaeformis* 0.45% and *A. coffeaeformis* 0.75 recorded the highest beneficial bacteria, absent *E. coli*, and the lowest count of *Proteus* species. as well as *Enterobacter* species which mounted 8.82, -negative 6.62 and 6.20, respectively when other levels of *A. coffeaeformis* treatments and were compared with the control group. The results obtained agree with those reported by Mariey et al. (2012) and Jamil et al. (2015), which showed that *A. coffeaeformis* activated the hens' immune systems and made them resistant to harmful microorganisms, including *E. coli*, *Enterobacter*, and *Proteus* proliferation.

Table 9. The influence of different levels of *Amorpha coffeaeformis* on intestinal bacterial counts in Broiler Chickens (Ross 508)

Treatment	Beneficial Bacteria (CFU/ml)	*Escherichia coli* (CFU/ml)	*Proteus* species (CFU/ml)	*Enterobacter* species (CFU/ml)
Control	8.620 ± 0.161^{ab}	5.136 ± 0.158^{b}	7.156 ± 0.242	7.156 ± 0.242
AC 0.15 %	8.210 ± 0.161^{b}	6.360 ± 0.158^{a}	6.706 ± 0.242	6.706 ± 0.242
AC 0.45 %	8.826 ± 0.161^{a}	-ve	6.736 ± 0.242	6.736 ± 0.242
AC 0.75 %	8.806 ± 0.228^{a}	-ve	6.620 ± 0.242	6.620 ± 0.242
p-Value	0.0001	0.0003	0.4219	0.4219

^{ab} Means different superscript letters in each column express significant differences ($p < 0.05$). AC: *Amorpha* coffeaeformis, -ve: Negative

CONCLUSION

From the productive and physiological point of view, it could be recommended that *A. coffeaeformis* microalgae at levels of 0.45% and 0.75% of diet did not have harmful effects on broiler chicken's health. In addition, these levels indicated the best product performance and immunological status, biochemical parameters, as well as suppressed *E. coli*, *Enterobacter*, and *Proteus* proliferation, at the same time increased beneficial bacteria (microbiota) proliferation in the intestine. Further investigations should be carried out on supplementing different levels of A. coffeaeformis to improve our knowledge of these microorganism's properties and evaluate their other effects on the broiler chicken's health and quality products.

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Authors' contribution

Dr. Ahmed El-Kaiaty designed this study, and Yasmina Mokhtar carried out the experiment. Hany Ramadan Contributed to the design of the study and performing the experiments. The supervisor in writing this article is Ahmed El-Kaiaty. Hamada Okasha analyzed the data under the guidance of Ahmed El-Kaiaty. All authors checked and confirmed the final analyzed data and the final draft of the manuscript.

Competing interests

All research authors agree to publish this research and do not have any conflict of interest.

Ethical considerations

This research was truthful and did not plagiarize or pattern any other papers or ideas. Any fabrication or falsification did not find in this research. This article or any scientific results did not submit to any journals except World`s veterinary Journal.

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