The effect of *Froriepia subpinnata* on the performance, carcass characteristics, blood parameters, immune system, microbial population, intestinal morphology, and breast meat fatty acid content of broiler chickens

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Abstract

The present experiment was conducted to investigate the effects of *F. subpinnata*powder (FSP) on performance, carcass characteristics, blood parameters, immune system, microbial population, intestinal morphology, and percentage of fatty acids in the breast meat of broiler chickens. To conduct the experiment, 300 day-old male chickens from the ROSS 308 strain were used in the form of a completely random design with 4 treatments and 5 replicates, with each replicate containing 15 chickens. The experimental treatments were, respectively: 1) control with base diet (without additives), 2) base diet + 1% FSP, 3) base diet + 2% FSP, and 4) base diet + 3% FSP. The results showed that the feed intake and weight gain increased in the treatments containing 2% and 3% FSP when compared to the control ($P<0.05$). Cholesterol and ALT levels in the treatment containing 3% FSP were lower that the control ($P<0.05$), while the concentration of glutathione peroxidase enzyme in the treatment containing 3% FSP significantly increased ($P<0.05$). Thymus weight and antibodies produced against AIV in all three levels of FSP increased significantly compared to the control ($P<0.05$). The population of lactobacilli and coliforms in the treatments containing FSP increased and decreased significantly compared to the control ($P<0.05$). The length and width of the intestinal villi of the chickens that were fed with 3% of FSP had a significant increase compared to the control ($P<0.05$). The percentage of saturated fatty acids in the breast decreased significantly with the consumption of all three levels of FSP ($P<0.001$). In general, the results showed that the use of 3% FSP in the broiler diet increased the efficiency of growth performance and enzyme activity, while strengthening the immune system, favourably altering the intestinal microbial population, and reducing the fat in breast meat.

Introduction

The poultry industry has an important role to provide protein for human societies. With an anticipated in global human populations, it is necessary to increase the growth rate in the poultry industry. An important challenges for the industry is the cost of feed and to address this feed additives are used to achieve high production at low cost.

The use of antibiotic growth stimulants to increase livestock productivity has been implemented for more than 50 years. Antibiotics are used to stimulate growth, treat disease, increase meat production the absorption of substances, and aids the neutralization of toxins that are produced by harmful intestinal bacteria. Growth stimulant products work in the digestive system and improve the performance of the animal by affecting the bacterial population of the digestive system (Mehdi et al. 2018).

The increase in the prevalence of bacterial diseases in recent decades and the increase in bacterial resistance to antibiotics make it necessary to explore methods that complement or even replace antibiotics.

One of the most important additives that have been introduced as an alternative to antibiotics is medicinal plants. Medicinal plants are plants whose leaves and fruits store substances that have a

physiological effect on the body of birds (Abdelli et al. 2020).

It possible that dietary inclusion of medicinal plants in the diet can improve digestibility and intake, medicinal plants may also improve the movements of the gallbladder, secretion of pancreatic digestive enzymes in broilers and accelerate food digestion and metabolism (El-Shall et al. 2020).

Many of the beneficial properties of medicinal plants are due to the presence of effective substances in them such as carvacrol, thymol, and myrcene, and most of the proven biological activities of these substances are antioxidant and antibacterial activities (Hieu Pham et al. 2020).

F. subpinnata plant is one of the medicinal plants of the Apiaceae family, which has been used as an appetizer and antiseptic in traditional medicine. Other properties of *F. subpinnata* leaves, include antioxidant, antibacterial, and antifungal action (Nabavi et al. 2008).

In some studies, one of the aims of using plant compounds is to change the microbial population of the intestinal contents of birds to increase the population of desirable microbial flora, including *Lactobacillus* species, or to reduce the load of harmful and pathogenic microbes (Chowdhury et al. et al. 2018). Various research has shown that because of limonene and germacrene, *F. subpinnata* can have high antimicrobial properties and can be effective in eliminating the harmful microbial flora of the intestine (Verdian-Rizi, 2008). Furthermore, this plant can favourably affect the physiological and biochemical conditions of the digestive system of broilers by increasing the ilial population of beneficial bacteria and increase the ratio of villi height to the depth of the crypt.

Extensive research has shown that medicinal plants are a rich source of antioxidants and prevent harm from oxidative stress resulting from excessive free radical production in the body (Broom and Kogut, 2018). In the research of Mirzania et al. (2019), it was shown that the extract of *F. subpinnata* plant contains significant amounts of phenol, flavonoid, and carotenoid compounds with high antioxidant properties, and as a result, *F. subpinnata* may prevent diseases and maintain health by increasing the phagocytic activity of white blood cells and stimulating the immune system and can also improve in the growth rate of birds (Mirzania et al. 2019).

Herbal medicines such as *F. subpinnata*, may significantly reduce the serum lipids in broiler chickens due to their phenolic compounds (Adaszyńska-Skwirzyńska and Szczerbińska, 2017). Herbs containing flavonoid compounds reduce lipid peroxidation and protects liver cells, and also reduces protein oxidation and increases glutathione peroxidase activity (Sonkusale et al. 2011).

Considering the beneficial effects of medicinal plants and the increasing trend to use medicinal plants, it is expected that the use of *F. subpinnata* medicinal plant due to the phenolic compounds in the diet of broiler chickens can be beneficial. This experiment was performed to investigate how dietary inclusion of *F. subpinnata* leaf powder affects the performance, carcass characteristics, blood parameters, immune system, microbial population, intestinal morphology and fatty acid percentage of broiler breast.

Materials and methods

To conduct this experiment, 300 day-old male broiler chickens from the ROSS 308 strain were used in a completely randomized design with four treatments, replicated five times and including 15 chickens per replicate. Chickens were randomly assigned to experimental treatments after weighing. Experimental treatments included 1: a base diet without additives (control), 2: a base diet containing 1% dry powder of *F. subpinnata*, 3: a base diet containing 2% dry powder of *F. subpinnata*, and 4: a base diet containing 3% dry powder of *F. subpinnata*.

To prepare the powder of *F. subpinnata* plant, the necessary amount of aerial parts (leaves) of the *F. subpinnata* plant sample was prepared and dried in the shade for 1 week. It was subsequently ground to powder using an electric mill and stored at room temperature until required. Some of the available powder was sent to the botanical laboratory of Tehran University to identify and measure nutritient concentrations (Table 1).

Table 1
The ingredients of the experimental diets

Ingredients (%)	Starter (1–10 days old)	Grower (11–24 days old)	Finisher (25–42 days old)
Corn	54.58	58.99	62.49
Soybean meal	32.46	30.30	28.45
Corn gluten meal	6.4	4.43	2.10
Soybean oil	2.00	2.00	3.00
NaCl	0.25	0.3	0.32
$CaCO_3$	1.20	1.08	0.97
Dicalcium phosphate	1.85	1.72	1.59
DL-Methionine	0.25	0.22	0.19
L-Lysine-HCl	0.35	0.31	0.28
Threonine	0.16	0.14	0.12
Mineral premix[1]	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25
Chemical analyses			
Metabolizable energy (kcal/kg)	2950	3100	3150
Crude protein (%)	22.00	21.50	19.50
Lysine (%)	1.33	1.29	1.15
Methionine (%)	0.76	0.65	0.61
Methionine + Cysteine (%)	1.10	0.99	0.98
Tryptophan (%)	0.25	0.24	0.22
Ca (%)	0.94	0.87	0.79
Available Phosphorus (%)	0.64	0.59	0.55

[1] Supplied the following per kilogram of feed: Manganese: 100 mg; Iron: 50 mg; Copper: 100 mg; Zinc: 100 mg; Selenium: 0.2 mg and Iodine: 1 mg.

[2] Supplied the following per kilogram of feed: vitamin A: 11000 IU; vitamin D_3: 23000 IU; vitamin E: 121 IU; vitamin K: 2 mg; Thiamin: 4 mg; Riboflavin: 4.6 mg; Niacin: 85 mg; Pantothenic acid: 25 mg; Pyridoxine: 4 mg; Biotin: 0.03 mg; Folic acid: 1.2 mg and vitamin B_{12}: 0.020 mg.

Ingredients (%)	Starter (1–10 days old)	Grower (11–24 days old)	Finisher (25–42 days old)
Na (%)	0.18	0.17	0.17
Cl (%)	0.24	0.22	0.20
[1] Supplied the following per kilogram of feed: Manganese: 100 mg; Iron: 50 mg; Copper: 100 mg; Zinc: 100 mg; Selenium: 0.2 mg and Iodine: 1 mg.			
[2] Supplied the following per kilogram of feed: vitamin A: 11000 IU; vitamin D_3: 23000 IU; vitamin E: 121 IU; vitamin K: 2 mg; Thiamin: 4 mg; Riboflavin: 4.6 mg; Niacin: 85 mg; Pantothenic acid: 25 mg; Pyridoxine: 4 mg; Biotin: 0.03 mg; Folic acid: 1.2 mg and vitamin B_{12}: 0.020 mg.			

Experimental diets of different periods (starter: 1–10 days old, grower: 11–24 days old, and finisher: 25–42 days old) were adjusted according to the needs of the ROSS 308 strain using UFFDA software (Table 2).

Table 2
Chemical composition of *F. subpinnata*

	%
α-Pinene	0.78
Carvacrol	0.9
Thymol	0.3
α-Terpinolene	8.12
Terpinene	0.15
Limonene	7.1
β-Myrcene	0.8
Borneol	1.4
Anisole	1.7
Isobornyl Formate	28.6
Camphor	6.7
Germacrene	0.6

At the end of each rearing period, all chickens were weighed individually. Before weighing, the birds were curfewed from feeding for 3 hours to provide the same state of the digestive system.

To determine the weight gain of each unit in each period, the difference in weight at the end and beginning of the breeding period was calculated. To calculate the amount of feed consumed by each experimental unit, the amount of feed remaining at the end of each rearing stage was subtracted from the total feed given during the period. The conversion ratio was calculated by dividing the average feed intake by the average weight gain of chickens for each period.

To investigate the characteristics of the carcass at the end of the period (42 days), 3 birds were randomly selected for each replicate and their weight was recorded, and then they were slaughtered by cutting the jugular vein. After removing the feathers, cutting off the head and feet, and removing the intestines and viscera, the weight of the whole carcass and its different parts (chest and thighs), the weight of the internal organs (liver, pancreas, heart, and gizzard), and weight of abdominal fat were measured. The percentage of each component was measured by dividing the weight of each section by the pre-slaughter live weight before slaughtering.

To measure blood parameters, at the end of the 42nd day, 3 birds were selected from each replicate and 5 cc blood samples were collected through the wing vein. Then 2.5 cc of this blood was transferred to test tubes without anticoagulant to extract the serum and another 2.5 cc to tubes containing anticoagulant to prepare plasma. After 10 minutes of centrifugation at 3000 rpm, serum and plasma were separated from the blood samples and kept at -20°C. Quantitative diagnostic kits of Pars Azmoon company and the spectrophotometer model Ce1010 of England were used to measure glucose, cholesterol, triglycerides, LDL, HDL, and VLDL. Also, the activity of liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were evaluated by Biolis 24i autoanalyzer.

The activities of plasma glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzymes were also measured by a spectrophotometer using a Randox Ransel commercial kit at wavelengths 340 and 505 nm, respectively. To measure malondialdehyde in plasma, 7.5 grams of trichloroacetic acid, 187 mg of TBA, and 6.2 ml of hydrochloric acid were combined and placed in boiling water. 3 ml of this mixture with 300 µL of plasma was placed inside the test tube and placed at 90 degrees Celsius water for 20 minutes. The tubes were removed from the the water and were quickly cooled with cold water, after which, 2 mL of isobutanol were added and the tube was exposed to centrifugation at 2500 rpm for 10 minutes and optical absorption was calculated at a wavelength of 532 nm.

The weight of lymphoid organs and titer of antibodies produced against sheep red blood cells (SRBC), Newcastle (NDV), and avian influenza (AIV) were used to check immunity. The percentages (by weight) of the spleen, thymus, and bursa of Fabricius of slaughtered birds were calculated.

To determine the antibody titer against SRBC, two birds were selected on days 28 and 36 of each replicate and 0.2 ml of 10% SRBC suspension was injected into the wing vein of each bird. Blood samples were collected 35 and 42 days later and their serum used to determine the titer of total antibody and immunoglobulin G and M against SRBC by hemagglutination assay (HA). Also, to investigate the antibody production against Newcastle virus (NDV) and influenza (AIV), 2 birds were selected on days 35 and 42 of each replicate, and blood samples were taken. After separating the serum, the

hemagglutination inhibition test was performed on the serums, and the immune function of the chickens was evaluated by producing antibodies against the Newcastle vaccine virus and influenza.

To investigate intestinal microbial populations, the contents of the ileum of each slaughtered bird were collected. Approximately 2 g of the contents of the ileum were poured into sterile microtubes. One gram of which was subsequently poured into a test tube that contained 9 mL of phosphate-buffered saline and was vortexed for 3 minutes. Further dilutions continued with a dilution factor of 10 until having 10^{-5}, 10^{-7}, and 10^{-9} dilutions were available, from which 0.1 mL was applied to the surface of an agar culture medium inside the plates. For counting lactobacilli from Man rogosa sharpe agar (MRS), Eosine methylene blue agar (EMB) was used for Escherichia coli, Mac Conkey agar for coliform, BSM agar for Bifidobacterium and Wilkins-Chalgren agar for total anaerobic bacteria. The culture media related to anaerobic bacteria, lactobacilli, and bifidobacterium were placed in an anaerobic, CO_2 (containing 5%) incubator and the rest of the culture media were placed in an aerobic incubator. The incubation temperature for all culture media was set at 37°C. Iincubation continued for 48 h for all culture mediums. The bacterial count was calculated using a colony counter device by counting the number of colonies in each pellet and multiplying it by the dilution factor, and it was expressed based on colony forming units (CFU) per gram of sample.

On day 42, to investigate the morphology of the intestine of broiler chickens, a portion of jejunum was separated from the small intestine of each slaughtered chicken. A 1 cm tissue sample was prepared from the midpoint of the jejunum and fixed in 10% formalin. Shaved samples with a thickness of five micrometers were placed on a glass slide using a rotating semi-automatic microtome and stained with hematoxylin and eosin. An optical microscope (Olympus Corporation, BX Model U TV 0.5) was used to examine the morphology of tissue samples. Histomorphometric measurements of intestinal villi were measured on three healthy villi selected from each sample. The investigated morphological indicators including the length of the villi (from the tip of the villus to the junction of the crypt (µm), the width of the villus, calculated as the average of the beginning, middle and end of the villus(µm), the depth of the Libercon or crypt glands (from the base of the villus to the submucosal layer in terms of micrometers), and the ratio of villus length to crypt depth (villus length divided by crypt depth) were measured.

To measure the percentage of saturated fatty acids in the breast muscle tissue, the breast samples of the slaughtered birds were separated and stored in zipped plastic bags at -20°Cuntil chemical analysis. In the laboratory, after melting, the samples were ground twice and completely mixed and stored at a temperature of four degrees Celsius. The method of Folch et al. (1957) was used to extract saturated fatty acids from the meat samples.

Results

The performance of broiler chickens on each diet are presented in Table 3. In the starter period, feed intake and body weight gain of chickens fed diets containing *F. subpinnata* increased when compared to

the control ($P < 0.05$). In the same period, the feed conversion ratio of chickens on the 3% of *F. subpinnata* diet decreased when compared to the control ($P < 0.05$).

Table 3
The effect of experimental treatments on performance of broiler chickens on feed intake (FI), body weight gain (BWG and feed conversion ration (FCR)

Treatments	**Starter (1–10 days)**		
	FI (g.chick.day)	**BWG (g.chick.day)**	**FCR**
Control	30.93 ± 0.08^{b}	20.64 ± 0.69^{b}	1.50 ± 0.04^{a}
1% *F. subpinnata*	31.79 ± 0.19 ^{a}	22.00 ± 0.25^{a}	1.44 ± 0.009 ^{ab}
2% *F. subpinnata*	31.99 ± 0.50 ^{a}	22.41 ± 0.41^{a}	1.42 ± 0.007^{ab}
3% *F. subpinnata*	32.30 ± 0.08 ^{a}	23.28 ± 0.31^{a}	1.38 ± 0.01^{b}
SEM	0.276	0.451	0.025
P-value	0.018	0.006	0.030
Treatments	Grower (11–24 days)		
	FI (g.chick.day)	BWG (g.chick.day)	FCR
Control	82.54 ± 0.27^{c}	45.63 ± 0.83 ^{c}	1.81 ± 0.02 ^{a}
1% *F. subpinnata*	82.94 ± 0.21^{c}	46.20 ± 0.24 ^{b}	1.79 ± 0.005 ^{a}
2% *F. subpinnata*	84.85 ± 0.57^{b}	47.34 ± 0.27 ^{ab}	1.79 ± 0.004 ^{a}
3% *F. subpinnata*	86.41 ± 0.17^{a}	48.63 ± 0.26 ^{a}	1.77 ± 0.006 ^{a}
SEM	0.346	0.475	0.015
P-value	0.001	0.002	0.449
Treatments	Finisher (25–42 days)		
	FI (g.chick.day)	BWG (g.chick.day)	FCR
Control	161.60 ± 1.74^{b}	68.13 ± 2.04^{b}	2.37 ± 0.05^{a}
1% *F. subpinnata*	163.66 ± 0.46^{b}	73.96 ± 4.03^{b}	2.23 ± 0.11^{ab}
2% *F. subpinnata*	173.92 ± 1.13^{a}	82.95 ± 1.87^{a}	2.10 ± 0.03^{b}
3% *F. subpinnata*	179.006 ± 2.83^{a}	89.45 ± 2.15^{a}	2.002 ± 0.01^{c}

FI: feed intake; BWG: body weight gain and FCR: feed conversion ratio.

The means within the same column with at least one common letter, do not have significant difference ($P < 0.05$).

SEM: standard error of the means.

Treatments	Starter (1–10 days)		
	FI (g.chick.day)	**BWG (g.chick.day)**	**FCR**
SEM	1.774	2.675	0.066
P-value	0.001	0.001	0.005
Treatments	Total period (1–42 days)		
	FI (g.chick.day)	BWG (g.chick.day)	FCR
Control	91.69 ± 0.51^{c}	50.43 ± 0.68^{c}	1.82 ± 0.01^{a}
1% *F. subpinnata*	92.80 ± 0.16^{c}	53.43 ± 1.70^{c}	1.74 ± 0.05^{b}
2% *F. subpinnata*	96.92 ± 0.41^{b}	57.80 ± 0.81^{b}	1.67 ± 0.01^{bc}
3% *F. subpinnata*	99.23 ± 0.94^{a}	61.18 ± 0.94^{a}	1.62 ± 0.01^{c}
SEM	0.580	1.110	0.030
P-value	0.001	0.001	0.002
FI: feed intake; BWG: body weight gain and FCR: feed conversion ratio.			
The means within the same column with at least one common letter, do not have significant difference ($P < 0.05$).			
SEM: standard error of the means.			

During the grower period, feed intake of diets containing more than 1% *F. subpinnata* increased when compared to the control ($P < 0.01$). The weight gain of all diets containing *F. subpinnata* powder increased were greater than the control ($P < 0.01$).

In the finished period, intake, weight gain, and feed conversion ratio in the diets containing 2 and 3% of *F. subpinnata* improved ($P < 0.01$). Across the study, the chickens that were fed with 2% and 3% *F. subpinnata* had higher feed intake and weight gain ($P < 0.05$). Furthermore, the feed conversion ratio in the broilers fed diets containing *F. subpinnata* were significantly lower when compared to the control ($P < 0.01$).

Table 4 reports the percentage of carcass and breast meat of broiler chickens fed diets containing 3% *F. subpinnata* were greater when compared to the control ($P < 0.05$). In contrast, the percentage of liver in broilers fed diets containing *F. subpinnata* decreased ($P < 0.05$).

Table 4
The effect of experimental treatments on carcass characteristics of broiler chickens

Treatments	Carcass (%)	Breast (%)	Thigh (%)	Liver (%)	Pancreas (%)	Heart (%)	Gizzard (%)	Abdominal Fat (%)
Control	60.77 ± 4.14[b]	20.32 ± 1.59[b]	17.72 ± 0.77[a]	2.73 ± 0.10[a]	0.18 ± 0.01[a]	0.55 ± 0.029[a]	1.13 ± 0.06[a]	1.06 ± 0.11[a]
1% *F. subpinnata*	62.67 ± 2.96[b]	21.83 ± 1.49[b]	17.26 ± 1.55[a]	2.40 ± 0.12[b]	0.20 ± 0.02[a]	0.55 ± 0.01[a]	1.03 ± 0.11[a]	0.96 ± 0.08[a]
2% *F. subpinnata*	62.97 ± 2.62[b]	21.89 ± 1.41[b]	18.11 ± 1.65[a]	2.30 ± 0.10[b]	0.16 ± 0.01[a]	0.54 ± 0.01[a]	1.09 ± 0.07[a]	0.84 ± 0.09[a]
3% *F. subpinnata*	77.58 ± 3.46[a]	24.39 ± 1.44[a]	18.43 ± 0.69[a]	2.28 ± 0.07[b]	0.24 ± 0.02[a]	0.55 ± 0.05[a]	1.34 ± 0.11[a]	0.77 ± 0.10[a]
SEM	3.34	1.48	1.25	0.10	0.02	0.03	0.09	0.10
P-value	0.048	0.039	0.92	0.02	0.07	0.003	0.13	0.24
The means within the same column with at least one common letter, do not have significant difference (P < 0.05).								
SEM: standard error of the means.								

Cholesterol content in treatments containing 3% *F. subpinnata* were lower when compared to the control (P < 0.05), but no differences were found in the concentrations of glucose, triglyceride, HDL, LDL, and VLDL (Table 5, P > 0.05).

Table 5
The effect of experimental treatments on blood biochemical parameters of broiler chickens

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	135.66 ± 18.18[a]	145.00 ± 10.97[a]	136.00 ± 17.32[a]	56.66 ± 4.33[a]	107.66 ± 11.83[a]	27.00 ± 3.46[a]
1% *F. subpinnata*	134.66 ± 13.56[a]	137.00 ± 10.97[a]	117.66 ± 17.61[a]	56.66 ± 2.02[a]	99.66 ± 9.52[a]	23.66 ± 3.75[a]
2% *F. subpinnata*	134.00 ± 3.46[a]	97.00 ± 15.58[ab]	101.66 ± 9.52[a]	55.00 ± 0.57[a]	96.00 ± 13.27[a]	20.66 ± 2.028[a]
3% *F. subpinnata*	127.66 ± 0.88[a]	81.00 ± 3.46[b]	97.00 ± 2.88[a]	61.00 ± 2.88[a]	92.00 ± 6.35[a]	19.00 ± 0.57[a]
SEM	11.485	11.132	13.316	2.809	10.578	2.764
P-value	0.958	0.041	0.233	0.511	0.759	0.259
HDL: high-density lipoprotein; LDL: low-density lipoprotein and VLDL: very low-density lipoprotein.						
The means within the same column with at least one common letter, do not have significant difference (P < 0.05).						
SEM: standard error of the means.						

The concentration of serum ALT (Table 6) decreased in the treatments containing 2% and 3% of *F. subpinnata* (P < 0.05) but the concentrations of AST and ALP did not differ (P > 0.05). The concentration of glutathione peroxidase enzyme in the treatment containing 3% *F. subpinnata* was higher when compared to the control (P < 0.05) but the concentration of superoxide dismutase and malondialdehyde did not differ (P > 0.05).

Table 6
The effect of experimental treatments on liver enzymes and antioxidant activity of blood broiler chickens

Treatments	AST (mg/dL)	ALT (mg/dL)	ALP (mg/dL)	GPX (mg/dL)	SOD (mg/dL)	MDA (mg/dL)
Control	350.00 ± 93.85^{a}	12.66 ± 1.45^{a}	7148.00 ± 707.83^{a}	358.14 ± 8.12^{b}	41.72 ± 0.79^{a}	0.59 ± 0.11^{a}
1% *F. subpinnata*	393.66 ± 24.53^{a}	11.66 ± 0.33^{ab}	4107.66 ± 578.79^{a}	361.56 ± 9.71^{b}	41.75 ± 0.14^{a}	0.59 ± 0.03^{a}
2% *F. subpinnata*	352.00 ± 32.33^{a}	8.60 ± 0.57^{b}	5625.00 ± 1498.22^{a}	361.90 ± 8.15^{b}	41.86 ± 0.51^{a}	0.58 ± 0.07^{a}
3% *F. subpinnata*	339.66 ± 25.11^{a}	8.00 ± 0.33^{b}	5703.00 ± 1433.56^{a}	488.59 ± 12.98^{a}	42.67 ± 0.91^{a}	0.58 ± 0.05^{a}
SEM	52.646	0.816	1133.115	9.075	0.782	0.098
P-value	0.890	0.031	0.370	0.042	0.141	0.601
AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline Phosphatase; GPX: Glutathione peroxidase; SOD: Superoxide dismutase and MDA: malondialdehyde.						
The means within the same column with at least one common letter, do not have significant difference ($P < 0.05$).						
SEM: standard error of the means.						

The relative weight of the thymus was higher in all treatments containing *F. subpinnata* (Table 7) ($P < 0.01$). The relative weight of the bursa of Fabricius and the spleen weres not different from the control ($P > 0.05$).

Table 7
The effect of experimental treatments on weight of lymphoid organs of broiler chickens

Treatments	Thymus (%)	Bursa of Fabricius (%)	Spleen (%)
Control	0.05 ± 0.005 ^{c}	0.05 ± 0.009 ^{ab}	0.06 ± 0.008 ^{a}
1% *F. subpinnata*	0.10 ± 0.009 ^{b}	0.04 ± 0.004 ^{b}	0.09 ± 0.009 ^{a}
2% *F. subpinnata*	0.12 ± 0.012^{b}	0.06 ± 0.009 ^{ab}	0.10 ± 0.012 ^{a}
3% *F. subpinnata*	0.19 ± 0.01^{a}	0.07 ± 0.006 ^{a}	0.10 ± 0.020 ^{a}
SEM	0.011	0.007	0.013
P-value	0.0001	0.048	0.116
The means within the same column with at least one common letter, do not have significant difference (P < 0.05).			
SEM: standard error of the means.			

Antibodies produced against NDV at the 23rd day of age was significantly higher in the treatments containing 2% and 3% *F. subpinnata* when compared to the control (Table 8, P < 0.01). The difference in antibodies produced against influenza at day 35 of age and NDV at day 42 did not differ (P > 0.05). The antibodies produced against influenza at day 42 of age in all the diets containing *F. subpinnata* were elevated (P < 0.05).

Table 8
The effect of experimental treatments on antibodies titer produced against Newcastle Disease Virus (NDV) and Avian Influenza Virus (AIV) of broiler chickens

Treatments	35 days		42 days	
	NDV (Log2)	AIV (Log2)	NDV (Log2)	AIV (Log2)
Control	5.00 ± 0.00^{b}	5.66 ± 0.33^{a}	6.66 ± 0.33^{a}	5.66 ± 0.33^{c}
1% *F. subpinnata*	5.00 ± 0.00^{b}	5.66 ± 0.33^{a}	7.00 ± 0.00^{a}	6.00 ± 0.00^{b}
2% *F. subpinnata*	5.66 ± 0.33^{a}	5.66 ± 0.33^{a}	7.00 ± 0.00^{a}	6.66 ± 0.33^{ab}
3% *F. subpinnata*	6.00 ± 0.00^{a}	6.00 ± 0.00^{a}	7.00 ± 0.00^{a}	7.00 ± 0.00^{a}
SEM	0.167	0.289	0.167	0.236
P-value	0.006	0.802	0.441	0.014
The means within the same column with at least one common letter, do not have significant difference (P < 0.05).				
SEM: standard error of the means.				

The effect of diet on total immunoglobulins produced against SRBC, IgG, and IgM are presented in Table 9. The total immunoglobulins produced against SRBC at 35th and 42nd days of age in broilers fed 2% or 3% *F. subpinnata* were higher ($P < 0.05$), as was the concentrationof IgG at the day 35($P < 0.01$). IgM at day 35, and IgG and IgM at day 42 were not different ($P > 0.05$).

Table 9
The effect of experimental treatments on antibodies titer produced against SRBC of broiler chickens

Treatments	35 days			42 days		
	Total	IgG	IgM	Total	IgG	IgM
Control	1.00 ± 0.00^{b}	0.00 ± 0.00^{b}	1.00 ± 0.00^{a}	3.66 ± 0.33^{b}	1.66 ± 0.33^{a}	2.00 ± 0.00^{a}
1% *F. subpinnata*	1.66 ± 0.00^{b}	0.66 ± 0.33^{b}	1.00 ± 0.33^{a}	3.66 ± 0.33^{b}	2.00 ± 0.00^{a}	1.66 ± 0.33^{a}
2% *F. subpinnata*	2.66 ± 0.33^{a}	1.66 ± 0.33^{a}	1.00 ± 0.00^{a}	4.66 ± 0.33^{a}	2.66 ± 0.33^{a}	2.00 ± 0.00^{a}
3% *F. subpinnata*	2.67 ± 0.33^{a}	1.66 ± 0.33^{a}	1.01 ± 0.00^{a}	5.00 ± 0.00^{a}	2.66 ± 0.33^{a}	2.66 ± 0.33^{a}
SEM	0.236	0.289	0.167	0.289	0.289	0.236
P-value	0.003	0.009	0.052	0.022	0.095	0.085

The means within the same column with at least one common letter, do not have significant difference ($P < 0.05$).

SEM: standard error of the means.

The population of lactobacilli increased, while coliforms decreased in the diets containing *F. subpinnata* ($P < 0.05$). The population of Escherichia coli in the treatments containing 2% and 3% *F. subpinnata* decreased when compared to the control (Table 10, $P < 0.05$). The bifidobacterium population increased in the treatment containing 3% *F. subpinnata* ($P < 0.05$). The total population of anaerobic bacteria did not differ ($P > 0.05$).

Table 10
The effect of experimental treatments on intestinal microbial population of broiler chickens

Treatments	*Lactobacillus*	*Escherichia coli*	*Coliform*	*Bifidobacterium*	Total anaerobic bacteria
Control	9.10 ± 0.003 [b]	9.58 ± 0.147 [a]	9.82 ± 0.064 [a]	9.13 ± 0.029 [b]	9.13 ± 0.003 [a]
1% *F. subpinnata*	9.36 ± 0.046 [a]	9.14 ± 0.263 [ab]	8.96 ± 0.107 [b]	9.25 ± 0.081 [b]	9.28 ± 0.133 [a]
2% *F. subpinnata*	9.43 ± 0.144 [a]	8.93 ± 0.095 [b]	8.98 ± 0.101 [b]	9.47 ± 0.185 [ab]	9.29 ± 0.352 [a]
3% *F. subpinnata*	9.52 ± 0.012 [a]	8.79 ± 0.089 [b]	9.20 ± 0.274 [b]	9.81 ± 0.075 [a]	9.78 ± 0.110 [a]
SEM	0.076	0.164	0.159	0.109	0.196
P-value	0.024	0.041	0.017	0.010	0.184
The means within the same column with at least one common letter, do not have significant difference (P < 0.05).					
SEM: standard error of the means.					

Table 11 shows that the length and width of the intestinal villi increased in diets containing 3% *F. subpinnata* (P < 0.05).

Table 11
The effect of experimental treatments on intestinal morphology of broiler chickens

Treatments	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/Crypt depth
Control	921.00 ± 139.57^{b}	149.00 ± 6.35^{b}	242.66 ± 5.20^{a}	4.03 ± 0.62^{a}
1% *F. subpinnata*	928.00 ± 42.72^{b}	178.00 ± 13.85^{b}	229.00 ± 14.43^{a}	4.07 ± 0.07^{a}
2% *F. subpinnata*	995.00 ± 56.00^{b}	184.00 ± 17.32^{b}	233.00 ± 10.97^{a}	4.39 ± 0.00^{a}
3% *F. subpinnata*	1173.00 ± 54.84^{a}	258.00 ± 27.71^{a}	233.00 ± 4.04^{a}	5.02 ± 0.14^{a}
SEM	82.841	18.030	9.645	0.324
P-value	0.023	0.015	0.781	0.120
The means within the same column with at least one common letter, do not have significant difference (P < 0.05).				
SEM: standard error of the means.				

The percentage of saturated fatty acids C14:0, C16:0, C18:0, C20:0, and C21:0 were reduced in the breast meat of all chickens fed diets containing *F. subpinnata* (Table 12, P < 0.001). The percentage of C17:0 also decreased when compared to the control, but only in the diets containing 2% and 3% *F. subpinnata* (P < 0.001).

Table 12
The effect of experimental treatments on percentage of fatty acid on breast meat of broiler chickens

Treatments	C14:0 (%)	C16:0 (%)	C17:0 (%)	C18:0 (%)	C20:0 (%)	C21:0 (%)
Control	0.81 ± 0.006 [a]	27.35 ± 0.006 [a]	0.24 ± 0.006 [a]	6.41 ± 0.006 [a]	0.37 ± 0.006 [a]	0.25 ± 0.006 [a]
1% *F. subpinnata*	0.61 ± 0.006 [b]	25.24 ± 0.006 [b]	0.23 ± 0.006 [a]	6.15 ± 0.006 [b]	0.34 ± 0.006 [b]	0.15 ± 0.006 [b]
2% *F. subpinnata*	0.60 ± 0.006 [b]	25.05 ± 0.006 [c]	0.16 ± 0.006 [b]	6.07 ± 0.006 [c]	0.30 ± 0.006 [c]	0.16 ± 0.006 [b]
3% *F. subpinnata*	0.49 ± 0.006 [c]	22.38 ± 0.006 [d]	0.13 ± 0.006 [c]	5.44 ± 0.006 [d]	0.22 ± 0.006 [d]	0.16 ± 0.006 [b]
SEM	0.006	0.006	0.006	0.006	0.006	0.006
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
C14:0: Myristic acid; C16:0: Palmitic acid; C17:0: Margaric acid; C18:0: Stearic acid; C20:0: Arachidic acid and C21:0: Heneicosanoic acid.						
The means within the same column with at least one common letter, do not have significant difference ($P < 0.05$).						
SEM: standard error of the means.						

Discussion

As seen in the results, the addition of *F. subpinnata* powder at levels of 2% and 3% increased the feed intake and weight gain in the finisher period as well as the entire breeding period, when compared to the control. While the conversion ratio of the entire period was also reduced in all treatments containing *F. subpinnata*. Different results in the performance of broilers fed different medicinal herbs have been reported. Some studies report that the use of thymus medicinal plant offering 60 mg/kg in the diet increased weight and improve feed conversion ratio (Denli et al. 2004), similar to the results of the present experiment. Also, carvacrol as one of the main components of the thymus plant when fed at 200 mg/kg in the diet favourably improved feed conversion ratio of broiler chickens (Lee et al. 2003).

The three main compounds of thymus essential oil are carvacrol, thymol, and terpinene (Laila et al. 2017), which, due to their antioxidant, antimicrobial, and antiseptic properties, can provide better digestion and absorption of nutrients and increase the body weight and feeding efficiency in poultry by reducing the harmful microbial population of the digestive system and improving the health and immunity of chickens. The presence of harmful bacteria causes chronic intestinal inflammation and nutrient absorption disorders. The effective compounds of medicinal plants can play a significant role in weight gain by eliminating these bacteria.

Also, previous studies have shown that some compounds of medicinal plants such as carvacrol can affect the quality of feed intake by stimulating the digestive system and regulating appetite control centers (Mansoub, 2011).

Due to the presence of similar compounds of *F. subpinnata* and thymus, the reason for the improvement in the growth performance of broiler chickens in the present experiment could be the antioxidant and antimicrobial properties of *F. subpinnata* powder. The phenolic substances of medicinal plants appear to stimulate digestive secretions such as salivary amylase, bile acids, and pancreatic enzymes (amylase, lipase, and protease) and the increase in the activity of these enzymes leads to the increase of nutrient availability (Badiri and Saber, 2016), and may lead to improved feed conversion ratio.

The group receiving 3% *F. subpinnata* powder had the highest significant percentage of carcass and breast. Consistent with the present experiment, Hoseinyan Bilandi et al. (2018) reported in their experiments that the active compounds of herbal medicines can increase the growth of broiler chicken carcasses. The disadvantages of the existence of harmful microbes in the digestive system are the increase the deamination activity of dietary proteins and amino acids, as well as the increase in the speed of their decomposition due to the secretion of the substances such as urease enzyme produced by microbes. Since medicinal plants reduce the microbial population in the ileum, they can reduce the rate of protein and digested amino acids, and as a result, more of them are absorbed and stored in the body, which ultimately reduces the accumulation of fat in the body and improves the carcass percentage.

As seen in the results, the percentage of liver was lower in all chickens fed *F. subpinnata*, which is consistent with the results of Tiihonen et al. (2010). Liver is one of the organs in body that performs detoxification and can filter a significant part of the toxins produced by harmful microbes. Since the use of medicinal plants in the diet of broilers reduces the population of harmful microbes, it reduces the detoxification activity of the liver leading to a decerase in its weight (Tiihonen et al. 2010). It may be taken that the decrease in the percentage of liver observed in thepresent study can be taken as one of the positive effects of using *F. subpinnata* in the diet.

The effect of plant-based flavonoids to lower blood cholesterol has been shown in numerous experiments. In an experiment, eucalyptus leaves a level of 0.5% in the broiler diet, reduced cholesterol levels (Hassan et al. 2011). In another study, it was found that feeding dill essential oil to rats reduced plasma cholesterol levels (Hajhashemi and Abbasi, 2008). The presence of terpenoid compounds in herbal medicines can reduce serum cholesterol by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) activity (Hanafy et al. 2016). It has also been reported that herbal medicines may reduce the acidity of the intestine and thus reduce the solubility of unconjugated bile acids, and their excretion, and ultimately reduce serum cholesterol levels (Hanafy et al. 2016). Additionally, medicinal plants in the diet of poultry, may also cause the production of bile-decomposing enzymes through the activity of lactobacilli, and deconjugating them can reduce blood cholesterol. Further, lactobacilli located in the small intestine can metabolize cholesterol and reduce its absorption (Ebadollahi et al. 2017).

In the present experiment, the ALT was significantly decreased in those diets containing more than 1% *F. subpinnata* powder in the diet. In an experiment conducted on broiler chickens, the effect of 800 mg/kg on the reduction of alanine aminotransferase enzyme was significant, which is consistent with the results of the present experiment. Also, the beneficial effects of herbal medicines on the function of liver enzymes have been stated by Ajayi and Odutuga (2004). These researchers reported that herbal medicines strengthen the function of liver and kidney organs. Liver cells contain high concentrations of ALT and AST enzymes, where ALT is present in the cytoplasm and AST in the mitochondria of liver cells (Elwan et al. 2019). Free radicals and toxic substances cause the accumulation of fat in the liver by disrupting the metabolism of fats, resulting in liver necrosis. During liver necrosis, the membrane of liver cells are destroyed, and the concentration of liver enzymes increases into the blood (Moawad, 2007). Although intracellular mechanisms prevent the destruction of liver cells, some factors such as stress and poisoning cause the inefficiency of these mechanisms (Lieber, 1997). Active compounds such as flavonoids and phenols found in medicinal plants can play a role in the protection of liver cells due to their antioxidant nature (Al-Sagheer et al. 2018). The phenolic compounds of *F. subpinnata* may prevent superoxide anion production, and thus prevents the oxidation of the liver membrane and the release of liver enzymes into the blood (Alagawany et al. et al. 2021). Furthermore, the the concentration of glutathione peroxidase enzyme in the broiler chickens fed in the present study with 3% *F. subpinnata* was elevated, supporting the antioxidant nature of the diet. The use of artichoke drugs in mice reduces lipid peroxidation and protein oxidation and increases glutathione peroxidase activity (Jimenez-Escrig et al. 2003). It has also been reported that the consumption of 60 mg/kg of thymol and carvacrol (active components of thymus) in the diet of broilers improved the activity of antioxidant enzymes (Hashemipour et al. 2013). Furthermore, supplementing with herbal medicine in the diet of broiler chickens caused a significant increase in glutathione peroxidase activity and serum antioxidant capacity (Placha et al. 2013).

Plants containing phenolic and flavonoid compounds have a high capacity as strong antioxidants to clear free radicals and eliminate oxidative reactions. Carvacrol and thymol (active ingredients of *F. subpinnata*) can increase the activity level of antioxidant enzymes and give hydrogen ions to free radicals, causing their destruction, reducing peroxidation, and also reducing the level of malondialdehyde (Hosseini and Meimandipour, 2018).

The results showed that the consumption of 1–3% of *F. subpinnata* caused a significant increase in thymus weight in broiler chickens. The thymus is one of the main organs of the poultry immune system where the maturation of lymphocyte cells takes place. It has been well clarified that as the relative weight of the thymus increased, there is an association with higher antibody production and an increase in the CD4/CD8 ratio of T lymphocytes (Abdulameer et al. 2017). Abdelnour et al. (2018) observed in the results of their experiments that the use of Herbal medicine (pepper) has increased the activities of T lymphocytes. Torki and Najafi (2010) announced that the combination of herbal medicines as antioxidants in the diet of broilers improves the maturation of T-CD4 lymphocytes, especially T helpers in the thymus.

It has been reported in other research that the phenolic compounds of plants can affect the weight of lymphoid organs because these compounds prevent the production of prostaglandins in the body due to their antioxidant and anti-inflammatory properties and thus strengthen immunity (Reda et al. et al. 2020).

In the investigation of experimental treatments on the humoral immune system, the titer of antibodies produced against NDV on the 35th day of age in birds fed with 2% and 3% of *F. subpinnata* was significantly higher than the other birds. Also, all levels of *F. subpinnata* in the diet of broiler chickens had a significant increase in the antibodies produced against influenza on the 42nd day of age. Total antibody titers against SRBC on the 35th and 42nd days of age, as well as IgG level on the 35nd day of age, were significantly higher in broiler chickens that received 2% and 3% *F. subpinnata* in their diet, compared to other birds. The positive effect of adding medicinal plants to the diet of broilers has been shown. For example, feeding broilers with Memordica improves the antibody titer against NDV after 14 days of immunization (Xiao et al. 2009) and alfalfa extract (polysavon) in the diet of broiler chickens increases the AIV antibody titers against AIV at the 5th week of age (Dong et al. 2007).

Christake et al. (2004) and Placha et al. (2014) showed that medicinal plants can enhance humoral immunity in broilers by producing IgG and IgM antibodies. Other studies show that herbal medicines increase the cyctoxic activity of natural killer cells (NK cells) (Luettig et al. 1989). Furthremore, herbal medicines and compounds derived from them have direct effects on macrophages, causing them to express pro-inflammatory mediators and antimicrobial agents (Ismail et al. 2021). It is likely that the use of *F. subpinnata* plant together with the NDV and AIV vaccine has caused inflammatory and specific responses against the NDV and AIV may have increased the effectiveness of the vaccine. In the results of previous tests, it has been stated that carvacrol and thymols as antioxidant compounds strengthen the immune system (Rajput et al. 2017). By donating extra electrons during energy production cycles, antioxidants can control and inhibit free radical production and prevent weakening of the immune system (Hong et al. 2012). Also, the phenolic compounds found in medicinal plants, in addition to their antioxidant properties, stimulate the immune system. The reason is that lactic acid-producing bacteria, including lactobacilli, can stimulate the bird's immune system by increasing B lymphocytes production and increase antibody production (Xin Jian et al. 2018).

Flavonoids in medicinal plants can also act as stimulants of the immune system and increase the level of interferon gamma (IFN-γ), interleukin 4 and increase the secretion of interleukin 10, and as a result, the activity of lymphocytes is stimulated and due to the reciprocal antagonistic mechanisms directs immune responses from cellular to humoral (Elbaz et al. 2021).

In the results of this experiment, *F. subpinnata* treatments showed a high lactobacillus population at all levels in the ileum samples, and this difference was quite significant compared to the control. The population of coliforms in the control was higher than diets containing *F. subpinnata*. Furthermore, Escherichia coli was decreased compared to the control by using dietary *F. subpinnata* levels greater than one percent. According to the obtained results, it seems that lactobacilli had a negative effect on the bacilli. This was also observed by Teymouri Zadeh et al. (2009) who reported that by adding 0.3% of

medicinal plants (thymus and ziziphora), the population of coliforms decreased and the population of lactobacilli increased.

In relation to the antimicrobial activity of medicinal plant compounds, it has been found that these compounds cause disturbances in the cytoplasmic membrane, stopping the penetration of stimulating protons, smoothing the flow of electrons and active transport, as well as coagulation of bacterial contents (Gangeh and Salarmoini, 2015).

Carvacrol is one of the phenolic compounds of *F. subpinnata* medicinal plant. Due to the active hydroxyl group in its chemical structure, carvacrol has antimicrobial effects against a wide range of bacteria, including mycoplasma, staphylococcus, E. coli, and campylobacter. Hydroxyls are also effective in deactivating bacterial enzymes (Ferreira et al. 2018).

In previous studies, the positive effect of carvacrol on lactic acid bacteria has been reported and it has been found that lactobacilli are effective in reducing the total population of bacilli in the digestive system of broiler chickens. Carvacrol disrupts cell function and kills harmful bacteria by changing the permeability of H+/K + channels and changing the ion gradient (Taheri et al. 2010). Therefore, the antimicrobial properties of *F. subpinnata* can be attributed to the presence of its phenolic compounds, which have been able to exert their antibacterial properties.

In the present experiment, the length and width of the intestinal villi of broiler chickens fed with 3% *F. subpinnata* were higher compared to the control group. This may be due to the altered microbial population of the small intestine of broiler chickens which has been shown to change the morphological characteristics of the intestines (Poorghasemi et al. 2017). Such that competition between the microbial population in the gastrointestinal tract and it's host for the absorption of nutrients on one hand, and the formation of growth-reducing metabolites produced by microbial activity, on the other hand, may have a negative effect on intestinal mucosa (Oladokun et al. 2021).

Studies have shown that there are factors that stimulate digestion and absorption in plant species that, in addition to stimulating digestion, also have antimicrobial activity against bacteria in the intestines, and this antimicrobial activity can reduce the harmful bacterial population in the intestines of birds (Su et al. 2021).

Considering the antimicrobial effects of carvacrol, thymol, and limonene present in *F. subpinnata* and the significant increase of beneficial intestinal bacteria (Lactobacillus and Bifidobacterium) as well as the significant decrease of harmful bacteria (Escherichia coli) in the present experiment, it is perceived that *F. Subpinnata*, by reducing the pathogenic bacterial population in the intestine has reduced the production of toxic compounds such as ammonia and prevented the destruction and damage of the mucous cells of the intestinal wall, thus increasing the length of the intestinal villi (Bakkali et al. 2008). In fact, intestinal inflammation is reduced with the reduction of harmful microorganisms, which can prevent the destruction of villi and protein leakage into the intestinal lumen and reduce the amount of regeneration (Kelly et al. 2017).

Broiler chickens fed diets containing *F. subpinnata* had lower proportions of saturated fatty acids in the breast meat, which also the reduced of blood cholesterol in these chickens. It is concluded that the *F. subpinnata* plant has supported the synthesis and storage of fatty acids in the meat. The effectiveness of the ratio of fatty acids of breast meat with different levels of phytogenic compounds in the diet of broiler chickens aligns with the findings of Scheuermann et al. (2009). Consistent with the present experiment, Al-Kassei (2009) stated that the addition of thymus medicinal plant to the diet of broiler chickens can reduce the amount of fat in the meat in addition to reducing the serum cholesterol level. This research suggested a positive relationship existed between serum cholesterol and meat fat and attributed the reason for the reduction of fat reserves to the phenolic compounds of medicinal plants (carvacrol and thymol), which have a negative effect on lipogenesis. They concluded that herbal medicines increase the oxidation of fat tissue and decrease the amount of fat storage in the body via enhanced gluconeogenesis.

Separately, it was reported the effect of herbal medicine (Satureja) on the change in the level of steroid hormones and the increase in blood testosterone in broilers. Considering the effect of these hormones on the increase of fat catabolism, it is possible that *F. subpinnata* has stimulated the reduction of fatty acid reserves in meat (Salma et al. 2007). Therefore, it was shown in the present study that the compounds of *F. subpinnata* support the absorption and excretion of lipids, and can indirectly affect the amount of fatty acids in the breast.

Conclusion

The presnt study shows that the use of different levels of *F. subpinnata* in the diet of broiler chickens significantly improved feed conversion ratio over the entire study. Weight gain and feed intake of broiler chickens improved throughout the full study period at diets containing more than 1%. Carcass percentage as well as the length and width of the intestinal villi increased compared to the control, but only at the level of 3% *F. subpinnata*. Cholesterol concentration decreased and GPX enzyme activity increasd at the level of 3% *F. subpinnata*. In the examination of the immune system, thymus weight and the antibodies produced against AIV increased at all three levels of *F. subpinnata*, but the total immunoglobulins produced against SRBC increased significantly only at levels higher than 1%. The population of lactobacilli and coliforms improved significantly in all three levels of *F. subpinnata*, but the population of Escherichia coli at levels higher than one and Bifidobacterium only at the level of 3% improved significantly compared to the control. A high percentage of saturated fatty acids in the breast was significantly reduced with the consumption of all three levels of *F. subpinnata*. In general, although a significant improvement was seen for some of the investigated parameters in the treatments fed with 1% and 2% *F. subpinnata* powder, the use of 3% of *F. subpinnata* powder in the diet showed better results for all the investigated parameters.

Declarations

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Availability of data

The authors declare that all data in the article are true and reliable and are available on demand.

Code availability: (Not applicable)

Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Babak Rostampour, Mohammad Chamani, Alireza Seidavi, Abolfazl Zarei, and Naser Karimi. The frst draft of the manuscript was written by Babak Rostampour, Mohammad Chamani, Alireza Seidavi, Abolfazl Zarei, and Naser Karimi. All authors read and approved the fnal manuscript.

Code availability (Not applicable)

Ethics approval: The experimental protocol was ratified by the Animal Ethic Committee of the Islamic Azad University, Science and Research, Tehran, Iran, and the experiment was performed with respect to the International Guidelines for research involving animals (Directive 2010/63/EU).

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