

Exploring Nutritional Evaluation of Cauliflower (Brassica Oleracea Var Botrytis) Waste on the Thyroid Hormone, Antioxidant Status, Immunity and Expression of Interleukin 6 and HSP 70 Genes in Broilers

Mahesh Theerthesh

ICAR-Indian Veterinary Research Institute: Indian Veterinary Research Institute

Avishek Biswas

Central Avian Research Institute

Gautham Kolluri

Central Avian Research Institute

Subodh Kumar Saha

subodhksaha@yahoo.com

ICAR-Indian Veterinary Research Institute: Indian Veterinary Research Institute

Ashvini Pundalik Bansod

Indian Veterinary Research Institute

Rajeshwar Khandare

Indian Veterinary Research Institute

Research Article

Keywords: Antioxidant, Cauliflower leaf meal, Immunity, Heat stress, Polyphenol, Interleukin 6, Heat shock protein 70

Posted Date: August 22nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7145387/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Waste and Biomass Valorization on February 10th, 2026. See the published version at <https://doi.org/10.1007/s12649-026-03499-x>.

Abstract

The present study was investigated effect of CLM on thyroid hormone profile, immunity, expression of IL6 & HSP70 gene and antioxidant status of broiler. 240 day-old chicks were distributed into 5 groups having 8 birds in each replicate. The diet was formulated as per BIS (2007) standards for T1, while T2, T3, T4, T5 and T6 were given diets substituted with CLM @ 50, 75, 100, 125, and 150 g/kg, respectively for 42 days. The weight of the spleen, thymus and humoral immune response was significantly ($P < 0.05$) higher in T6 group. On 21st day, serum IL6 levels was significantly ($P < 0.05$) lower in T4, T5, and T6. On 42nd day, it was significantly ($P < 0.05$) lower in T6. Relative expression of the HSP70 gene in liver tissue was significantly ($P < 0.05$) down regulated in T6. The serum SOD and catalase levels were significantly ($P < 0.05$) higher in T4, T5, and T6. Serum FRAP and DPPH values were significantly ($P < 0.05$) higher in T5 and T6. The thyroid hormone profile, weight of bursa, cell-mediated immune response and serum GPx levels were remained comparable. Hence, it is concluded that CLM inclusion in a diet up to 15% improves immunity and antioxidant and alleviates heat stress in chickens.

Introduction

Leaf meal is a protein-rich feed ingredient made from the leaves of plants that can be used in broiler production as a protein source to supplement traditional feed ingredients, such as corn and soybean meal. The use of leaf meal in broiler production can have several benefits considering the shorter production cycle of broilers. It is a highly digestible protein source, which can help improve the growth rate and feed efficiency of broilers, resulting in profitable broiler production [68]. The leaf meal can be a cost-effective alternative to traditional protein sources, as it is often available at a lower price.

The presence of health-promoting substances such as vitamins, carotenoids, phenols, flavonoids, and minerals in leaf meals is responsible for their good health qualities [6, 54]. Along with protein, most leaf meals have high ash content, making them a rich source of minerals for poultry [68]. Cauliflower leaf meal (CLM) is rich also rich in essential amino acids and would offer enough of these nutrients for poultry and animal diets [43]. Brassicas are known for their antioxidant properties, attributed to the presence of polyphenols and flavonoids [19]. Efforts have been stepped up to find an alternative to antibiotics for in-feed use. It has been found that using leaf meals improves the health and physiological conditions of broilers on farms. As a result, leaf meal could be used as an alternative to in-feed antibiotics in broiler chickens [68]. Among the active substances, phenols have been shown to aid in the development of immunological organs and boost the immune system of chicks [34]. Phenols may also function as antimicrobials and antioxidants, protecting broilers' immune systems from the harmful effects of reactive oxygen species [34].

One of the most pleiotropic interleukins generated in areas of damage or infection is interleukin 6. High levels of IL-6 have been associated with an increased risk of disease and mortality in broiler chickens [72]. The HSP70 is the most conserved and well-studied member of the HSP family, and its expression is altered by a wide range of substances or stressors. The primary inducer of HSP-related genes is the high

ambient temperature [77]. HSP70 gene expression has been extensively researched and utilised as a marker for heat stress in chickens. With this above brief background, the study was planned to investigate the effect of the inclusion of CLM on the thyroid hormone profile, immunity, heat stress, and antioxidant status of broiler chickens.

Materials and Methods

Ethics statement

The experimental procedures carried out in the present study were approved by the Institutional Animal Ethics Committee (IAEC). The guidelines of the 'committee for the purpose of control and supervision of experiments on animals (CPCSEA) 2012' established under the 'Prevention of Cruelty to Animals Act 1960' of the Indian Penal Code were followed.

Preparation of Cauliflower leaf meal

The cauliflower leaves waste was collected from the nearby vegetable market, in Delapeer, Bareilly, India. These wastes were chopped using a chaff cutter. The chopped waste is then sun-dried on a tarpaulin followed by milling using an attrition mill and stored in an airtight container.

Proximate analysis and determination of the chemical composition of cauliflower leaf meal

The proximate analysis was carried out as per the method suggested by AOAC, (1990). The Crude Protein (CP) was estimated by the Kjeldahl method by taking a pooled sample, ether extract using soxhlet ether extraction apparatus, and crude fibre was estimated by acid (2.04N H₂SO₄) digestion followed by alkali (2.5 N NaOH) digestion, expressed as a difference in oven-dry weight and weight after ashing. The total ash (TA) was determined by ashing the samples in a muffle furnace at 550°C after decarbonisation. Total phosphorus (TP) was estimated by the calorimetric method using phospho molybdo-vanadate reagent [9] and Calcium as per the methods of [71]. Glucosinolates and the total phenolic content were estimated by the method suggested by Abaza *et al.* [1] and Makkar *et al.* [47], respectively. Gross energy (kcal/kg) was estimated by using Gallenkamp adiabatic bomb calorimeter. The metabolisable energy was calculated by multiplying the gross energy value by a factor of 0.82 [50].

Experimental birds

The experiment was conducted for a total duration of 42 days. 240 straight run day old CARI BRO VISHAL chicks were allotted to six groups in a completely randomized design, forty in each group. Each group had five replicates and each replicate had eight birds. These birds were fed increasing level of cauliflower leaf meal (CLM) at 5, 7.5, 10, 12.5 and 15% from the day old.

Feeds and feeding

The ingredients and nutrient composition of the basal experimental diet are given in the Table 1. The BIS (2007) feeding standards were followed to fulfill the nutrient requirements of broilers. As per BIS standard, the birds were fed three types of diet during the 42-day trial *i.e.*, pre-starter (0–7 days), starter (8–21 days), and finisher (22–42 days). The T1 group of birds was fed a control diet and T2, T3, T4, T5 & T6 groups were fed CLM included diet by partial replacement of maize and soya bean meal in the experimental diet at the level of 50, 75, 100, 125, and 150 g/kg, respectively. The diet of all the groups were made iso-caloric and iso-nitrogenous.

Table 1
Ingredient and chemical composition of the control experimental diets

Ingredients (g/kg)	Broiler pre-starter (0–7 days)	Broiler starter (8–21 days)	Broiler finisher (22–42 days)
Maize	534.1	579.9	656.4
SBM	410	364.1	279
Oil	18	28	36
Limestone	9	12.5	11
DCP	17	4.2	6
Methionine	1.7	0.3	0.6
Lysine HCL	0	1	1
Salt	5	5	5
Mineral mix*	1	1.5	1.5
Vitamin premix**	2.2	1.5	1.5
Coccidiostat	1	1	1
Toxin binder	1	1	1
Analysed (% DM)			
CP	22.8	22.2	20.3
CF	2.61	3.8	3.7
EE	3.56	3.7	3.9
AIA	0.28	0.78	0.58
Calculated			
ME (kcal/kg)	3017.88	3106.4	3198.8
E:P ratio	131.2	140.8	160.2
AP (g/kg)	4.5	4.5	4.5
Lys (g/kg)	13	12	10.8
SBM soya bean meal; DCP digestible crude protein; CP crude protein; CF crude fibre; EE ether extract; AIA acid insoluble ash; AP available phosphorus; Lys lysine; Met methionine			
*Mineral mix supplied mg/kg diet: Mg 300, Mn 55, I 0.4, Fe 56, Zn 30, Cu 4.2.			
**Vitamin premix supplied per kg diet: Vit. A 8250 IU, Vit. D ₃ 2400 IU, Vit. E 15 mg, Vit. K 1 mg, Vit. B ₁ 6 mg, Vit. B ₁₂ 8 mg, Niacin 12 mg, Pyridoxin 1.6 mg, Choline chloride 500 mg.			

Ingredients (g/kg)	Broiler pre-starter (0–7 days)	Broiler starter (8–21 days)	Broiler finisher (22–42 days)
Met (g/kg)	5	5	4.5
SBM soya bean meal; DCP digestible crude protein; CP crude protein; CF crude fibre; EE ether extract; AIA acid insoluble ash; AP available phosphorus; Lys lysine; Met methionine			
*Mineral mix supplied mg/kg diet: Mg 300, Mn 55, I 0.4, Fe 56, Zn 30, Cu 4.2.			
**Vitamin premix supplied per kg diet: Vit. A 8250 IU, Vit. D ₃ 2400 IU, Vit. E 15 mg, Vit. K 1 mg, Vit. B ₁ 6 mg, Vit. B ₁₂ 8 mg, Niacin 12 mg, Pyridoxin 1.6 mg, Choline chloride 500 mg.			

Housing and management

The chicks of different experimental groups were reared in cages with a wire mesh bottom. The electrically heated battery brooders were used to brood the chicks. 23 hrs lighting program was practiced. The experimental birds were fed in the morning every day. *Ad-libitum* feed and water were made available throughout the day. All the birds were reared under uniform and standard management practices.

Thyroid hormone profile

Thyroid hormone profile namely, T3, T4 and TSH was estimated by Quanti Microlisa® (J. Mitra and Co. Pvt. Ltd) based on the principle of competitive ELISA. Microwells are coated with respective anti-antibodies. Sample is added to microwell followed by addition of enzyme conjugate. Incubation is followed by a washing step to remove unbound compounds. The colour reaction is started by addition of substrate and stopped after a definite time. The colour intensity is proportional to the concentration of hormone in the sample.

Weight of immune organs

At the end of forty two days of experimental period, 10 birds from each dietary group (2 birds per replicate) were randomly selected and sacrificed after 17 hrs of fasting. Spleen, bursa, and thymus were collected and weighed. The weight of immune organs was expressed as % live weight (Pre-slaughter live weight) as shown below.

$$Spleen (\%) = \frac{Weight\ of\ spleen\ (g)}{Pre\ slaughter\ live\ weight\ (g)} \times 100$$

$$Bursa (\%) = \frac{Weight\ of\ bursa\ (g)}{Pre\ slaughter\ live\ weight\ (g)} \times 100$$

$$Thymus (\%) = \frac{Weight\ of\ thymus\ (g)}{Pre\ slaughter\ live\ weight\ (g)} \times 100$$

Cell mediated immunity

Using a method recommended by Cheng and Lamont (1988), the CMI response to the Phytohemagglutinin-P (PHA-P) mitogen was evaluated on day 27 of the experiment. PHA-P (1.0 mg/ml of PBS, 100microgram /bird) was administered intradermally to right foot web of ten birds in each treatment using a tuberculin syringe. Foot web of selected birds was cleaned using the cotton before injection. The left foot web of same bird was given 0.1 ml of freshly prepared autoclaved PBS serving as a control. The mean skin thickness of the right and left foot webs of the injected birds of each treatment was measured using a micrometre at 0 hours and 24 hours after PHA-P mitogen injection. The difference in thickness of the control foot web at 0 and 24 hours was subtracted from the difference in thickness of the mitogen-injected foot web to calculate the foot web index (FWI).

$$FWI (mm) = (R_2 - R_1) - (L_2 - L_1)$$

Where,

R_1 = Thickness (millimetre) before injection of PHA-P injection

R_2 = Thickness (millimetre) after 24 hours of PHA-P injection

L_1 = Thickness (millimetre) before injection of PBS solution

L_2 = Thickness (millimetre) after 24 hours of PBS injection

Humoral immune response

As a measure of antibody titre against 1% SRBC suspension, humoral immunity was assessed. 10 birds in each treatment which were not involved in cell mediated immunity test were injected 1% SRBC via intravenous route on 29th day of the experiment. After five days post-inoculation, about 2.0 ml of blood was collected in serum vacutainers from immunized birds. The separated serum was used for microtiter haemagglutination (HA) test following a method reported by Siegel and Gross [64].

Estimation of serum IL6 and HSP70

To evaluate the serum IL6 and HSP70, blood was collected on 21st day and 42nd day from 10 birds/treatment in serum vacutainers. The separated serum was transferred to 2ml Eppendorf tubes and stored at -20°C until analysed. Amount of IL6 in serum was quantified using Fine Test® Chicken Interleukin-6 ELISA kit (Catalogue no ECH0046). Likewise, serum HSP70 using Fine Test® Chicken HSP70 ELISA kit (Catalogue no ECH0072).

Gene expression

Gene expression study of IL6 gene in spleen and HSP 70 gene in liver was done at 21st and 42nd day of experiment, respectively. Four birds per treatment were randomly chosen and sacrificed to collect 100

mg of spleen and liver samples (following standard protocol) from each bird. Collected samples were immediately transferred to nuclease-free, 2.0 ml Eppendorf tubes that contained 1.0 ml of RNeasy Protect[®] reagent. The tissues were homogenised using an automated homogenizer (Polytron) on a high setting for 30 to 60 seconds. The RNA was isolated from the homogenised samples using the TRIzol[™] reagent. Isolated RNA samples were checked for purity in a Nano drop UV spectrophotometer (Nano Drop 1000[®], Thermo-Scientific, Singapore). The concentration of each RNA sample was calculated after obtaining the O.D. at 260/280. Revert Aid[™] first-strand cDNA synthesis kit (K 1622, Thermo Fisher Scientific, U.S.) was used to create the first-strand cDNA from the appropriate amount of RNA using random hexamer primers. Already reported primers (Table 2) were used to quantify IL6 and HSP70 genes. mRNA expression folds of target genes were quantified relative to GAPDH and 28S RNA as housekeeping genes respectively. The 2^{-ΔΔCt} method was used to analyse the real-time PCR data. Relative expression of genes IL-6 and HSP-70 in spleen and liver tissues respectively were quantified by a real-time PCR detection system (QIAquant 96[®] thermocycler) using 2X SYBR green master mix (RR420A, Takara TB Green[™] Premix Ex Taq[™]). All molecular reactions were performed by using a nuclease-free 8 tube-strips containing optically clear flat caps (Axygen Scientific[®], Inc. USA).

Table 2
Primers (forward and reverse) used for real-time assay

S. No	Gene	Sequence (5'-3')	Annealing Temperature (°C)	Length (bp)	Accession no
1	IL6	F-AAATCCCTCCTCGCCAATCT R-CCCTCACGGTCTTCTCCATAAA	61	106	NM204628.1
2	HSP70	F-ACGACCAGGGGAACCGCACCCAC R-GGCTTGCCACCCTCGTTCACCAC	56	74	HM587997
3	GAPDH	F-CCGTCCTCTCTGGCAAAGTCC R-AGCCCCAGCCTTCTCCATG	58	266	NM204305
4	28S RNA	F-CAGGTGCAGATCTTGGTGGTAGTA R- GCTCCCGCTGGCTTCTCC	58	273	JN639848
IL6 Interleukin 6; HSP70 Heat shock protein 70; GAPDH Glyceraldehyde 3-phosphate dehydrogenase; RNA Ribonucleic acid; F Forward; R Reverse					

Temperature humidity index (THI)

THI was calculated using the formula suggested by Habeeb *et al.* [29]Z. $THI = db^{\circ} F - [(0.55 - 0.55 \times RH)(db^{\circ} F - 58)]$. Where, $db^{\circ} F$ = dry bulb temperature (in Fahrenheit) and RH = relative humidity percentage

(RH%)/100. THI is classified as: <82 = absence of heat stress, 82 to < 84 = moderate heat stress, 84 to < 86 = severe heat stress, and 86 and more = very severe heat stress.

Antioxidant status

Serum superoxide dismutase was quantified using Amplite™ colorimetric superoxide dismutase assay kit by AAT Bioquest® (Catalogue no 11305). Serum catalase enzyme using SIGMA-ALDRICH® catalase assay kit (Catalogue no CAT100). Serum glutathione peroxidase enzyme using Cayman® glutathione peroxidase assay kit (Item no. 703102). Total antioxidant capacity of serum is evaluated by ferric reducing ability of plasma (FRAP) as suggested by Benzie *et al.*, 1999 and 2,2-diphenyl-1-picrylhydrazyl (DPPH) by Brand *et al.*, [14].

Statistical Analysis

The experimental data were analysed using the SPSS computer package (IBMSPSS software version 26.0, SPSS Inc., Chicago, USA) adopting standard statistical procedures of Snedecor and Cochran, [66]. The parameters were analysed by one-way ANOVA using the general linear model and Tukey post-hoc analysis. Significance was declared at $P < 0.05$ unless otherwise stated.

Results

Chemical composition of cauliflower leaf meal

The chemical composition of cauliflower leaf meal (CLM) was given in the Table 3. The dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), nitrogen-free extract (NFE), total ash (TA), and acid insoluble ash (AIA) content of the fresh cauliflower waste were 13.6, 22.3, 6.93, 9.47, 49.76, 11.54 and 1.37 percent, respectively. Similarly, the calcium (Ca), phosphorus (P), total phenolics (TP), and glucosinolates were 3.60, 0.6, 7.68, and 0.042 percent respectively. The gross energy (GE) and metabolisable energy (ME) content of CLM was 2560 kcal/kg and 2099 kcal/kg respectively.

Table 3
Chemical composition (%) of the cauliflower leaf meal

Particulars	%
CP	22.3
EE	6.93
CF	9.47
NFE	49.76
TA	11.54
AIA	1.37
Ca	0.61
P	0.28
TPC	7.68
Glucosinolates	0.042
Gross Energy (kcal/kg)	2560
Metabolisable energy (kcal/kg)	2099
CP crude protein; EE ether extract; CF crude fibre; NFE nitrogen free extract; TA total ash; AIA acid insoluble ash; Ca calcium; P phosphorus; TPC total phenolic content	

Thyroid hormone profile

The thyroid hormone profiles (T3, T4, and TSH) of experimental group of birds fed CLM in the present investigation were comparable with the control group of birds on the 21st and 42nd day of the experiment (Table 4).

Table 4
Effect of feeding cauliflower leaf meal on the thyroid hormone profile

Treatment	21st day			42nd day		
	T3 (ng/ml)	T4 (µg/ml)	TSH (µIU/ml)	T3 (ng/ml)	T4 (µg/ml)	TSH (µIU/ml)
T1	1.12	19.61	0.86	1.99	21.79	1.25
T2	1.21	17.70	0.91	2.08	19.55	1.52
T3	1.33	17.75	0.87	2.16	18.80	1.28
T4	1.82	17.77	0.94	1.78	19.07	1.19
T5	1.25	18.08	0.98	2.05	18.88	1.20
T6	1.45	17.85	0.91	2.22	19.04	1.18
SEM	0.452	0.283	0.025	0.259	0.452	0.058
P-value	0.851	0.242	0.779	0.745	0.289	0.618
T1 Control diet; T2 (50 mg/kg CLM); T3 (75 mg/kg CLM); T4 (100 mg/kg CLM); T5 (125 mg/kg CLM); T6 (150 mg/kg CLM); CLM Cauliflower; T3 Triiodothyronine; T4 Thyroxine; TSH Thyroid stimulating hormone; SEM standard error mean.						

Weight of immune organs

The weight of different immune organs in the current investigation was presented in the Table 5. The weight of the spleen was significantly ($P < 0.05$) higher in T6 group as compared to the control group. The weight of the thymus was significantly ($P < 0.05$) higher in the T6 group of birds as compared to the control group. The weight of bursa of experimental group of birds fed CLM was comparable with the control group of birds.

Table 5
Effect of feeding cauliflower leaf meal on the weight of immune organs

Live weight (%)			
Treatment	Spleen	Bursa	Thymus
T1	0.245 ^a	0.146	0.282 ^a
T2	0.250 ^a	0.140	0.300 ^a
T3	0.248 ^a	0.148	0.313 ^a
T4	0.258 ^a	0.144	0.343 ^{ab}
T5	0.271 ^a	0.158	0.354 ^{ab}
T6	0.320 ^b	0.151	0.440 ^b
SEM	0.041	0.010	0.017
P-value	0.038	0.517	0.029
T1 Control diet; T2 (50 mg/kg CLM); T3 (75 mg/kg CLM); T4 (100 mg/kg CLM); T5 (125 mg/kg CLM); T6 (150 mg/kg CLM); CLM Cauliflower leaf meal; SEM standard error mean			
^{abc} Mean with superscript in a column differ significantly (P < 0.05)			

Cell-mediated and Humoral immune response

The cell-mediated immune (CMI) response of the experimental group of birds fed CLM in the current investigation was comparable with the control group (Table 6). The humoral immune response of experimental group of birds against sheep RBCs (SRBCs) was significantly (P < 0.05) higher in T6 group when compared with the control group of birds (Table 6).

Table 6

Effect of feeding cauliflower leaf meal on cell-mediated immunity, humoral immunity, serum IL6 levels, and relative expression of IL6 gene

Treatment	FWI (mm)	HA titre (log2)	IL6 ELISA (pg/ml)		IL6 Fold change
			21st day	42nd day	
T1	0.283	18.98 ^a	171.71 ^b	287.81 ^b	1 ^a
T2	0.410	19.29 ^a	144.57 ^{ab}	284.00 ^b	0.96 ^a
T3	0.355	19.06 ^a	120.57 ^{ab}	278.29 ^{ab}	1.10 ^a
T4	0.321	25.33 ^{ab}	76.00 ^a	276.86 ^{ab}	1.18 ^{ab}
T5	0.372	26.57 ^{ab}	68.76 ^a	259.72 ^{ab}	1.27 ^{ab}
T6	0.390	31.22 ^b	70.42 ^a	193.52 ^a	1.77 ^b
SEM	0.015	1.055	9.340	10.08	0.076
P-value	0.182	0.003	< 0.001	0.049	0.012
T1 Control diet; T2 (50 mg/kg CLM); T3 (75 mg/kg CLM); T4 (100 mg/kg CLM); T5 (125 mg/kg CLM); T6 (150 mg/kg CLM); CLM Cauliflower leaf meal; FWI Foot web index; HA Haemagglutination; IL6 Interleukin 6; SEM standard error mean					
^{abc} Mean with superscript in a column differ significantly (P < 0.05).					

Serum IL-6 levels

The serum interleukin-6 (IL6) levels on 21st & 42nd day of the experiment were presented in the Table 6. In the current investigation, the serum IL-6 level (pg/ml) on the 21st day was significantly (P < 0.05) lower in T4, T5 and T6 group of birds. On the 42nd day, the serum IL 6 was significantly (P < 0.05) lower in T6 group and the serum IL6 levels in the other experimental group of birds fed CLM were comparable with the control group.

IL-6 gene expression study

The results of relative expression of IL6 gene were presented in the Table 6. The relative expression of IL6 gene was significantly (P < 0.05) higher in T6 group of birds as compared to the control group.

Temperature humidity index

The weekly temperature humidity index (THI) during the experimental were 80.69, 82.01, 83.02, 85.20, 85.61, and 89.32 during the 1st, 2nd, 3rd, 4th, 5th, and 6th week of the experiment, respectively. The weekly temperature humidity index (THI) was significantly (P < 0.05) higher during 4th, 5th, and 6th week of the experiment.

HSP 70 gene expression study

The results of relative expression of HSP70 gene was presented in the Table 7. The relative expression of HSP70 gene was significantly ($P < 0.05$) lower in T6 group of birds as compared to the control group.

Table 7

Effect of feeding cauliflower leaf meal on relative expression HSP70 gene and serum HSP70 levels

Treatment	HSP70 Fold change	Serum HSP 70 (ng/ml)	
		21st day	42nd day
T1	1 ^b	1.187	1.227 ^b
T2	0.93 ^{ab}	1.1617	1.193 ^{ab}
T3	0.79 ^{ab}	1.1718	1.172 ^{ab}
T4	0.81 ^{ab}	1.163	1.155 ^{ab}
T5	0.68 ^{ab}	1.1383	1.093 ^a
T6	0.52 ^a	1.137	1.077 ^a
SEM	0.048	0.009	0.014
P value	0.025	0.557	0.005
T1 Control diet; T2 (50 mg/kg CLM); T3 (75 mg/kg CLM); T4 (100 mg/kg CLM); T5 (125 mg/kg CLM); T6 (150 mg/kg CLM); CLM Cauliflower leaf meal; HSP70 Heat shock protein 70; SEM Standard error mean			
^{abc} Mean with superscript in a column differ significantly ($P < 0.05$).			

Serum HSP 70 levels

On the 21st day, the serum HSP70 levels of the experimental group of birds fed CLM were comparable with the control group however, on the 42nd day of the experiment, the mean serum levels of HSP 70 were significantly ($P < 0.05$) lower in T5 and T6 groups than T2, T3, T4, and the control group (Table 7).

Antioxidant status

The results pertaining to the concentration of serum antioxidant enzymes and total antioxidant capacity of serum was presented in the Table 8. The serum SOD levels were significantly ($P < 0.05$) higher in the T6 group of birds. Catalase level was significantly ($P < 0.05$) higher in the T4, T5 and T6 group of birds. The serum GPx levels in the experimental group of birds were comparable with the control group. The serum FRAP values were significantly ($P < 0.05$) higher in T5 and T6 group as compared to the control group of birds (Table 8). The serum DPPH values were significantly ($P < 0.05$) higher in the T6 group of

birds and the DDPH values of the other experimental group of birds fed CLM were comparable with the control group (Table 8).

Table 8
Effect of feeding cauliflower leaf meal on the antioxidant status

Treatment	SOD (U/ml)	Catalase ($\mu\text{mol}/\text{min}/\text{ml}$)	GPx ($\text{nmol}/\text{min}/\text{ml}$)	FRAP (mM/ml)	DPPH (% Antioxidant activity)
T1	66.16 ^a	11.24 ^a	6.64	2.822 ^a	14.82 ^a
T2	67.43 ^{ab}	11.51 ^{ab}	5.09	3.157 ^{ab}	15.03 ^a
T3	69.05 ^{ab}	11.67 ^b	5.10	3.177 ^{ab}	16.07 ^a
T4	68.64 ^{ab}	11.72 ^c	4.97	3.147 ^{ab}	15.38 ^a
T5	70.34 ^{bc}	12.29 ^c	4.25	3.388 ^b	17.19 ^{ab}
T6	73.88 ^c	12.54 ^c	5.82	3.444 ^b	18.66 ^b
SEM	0.625	0.113	0.662	0.045	0.637
P-value	< 0.001	0.003	0.169	< 0.001	0.042
T1 Control diet; T2 (50 mg/kg CLM); T3 (75 mg/kg CLM); T4 (100 mg/kg CLM); T5 (125 mg/kg CLM); T6 (150 mg/kg CLM); CLM Cauliflower leaf meal; SOD Super oxide dismutase; GPx Glutathione peroxidase; FRAP Ferric reducing ability of plasma; DPPH 2,2-diphenylpicrylhydrazyl; SEM Standard error mean					
^{abc} Mean with superscript in a column differ significantly (P < 0.05).					

Discussion

Chemical composition of cauliflower leaf meal

The dry matter (DM) content of cauliflower leaves in the present experiment was similar to the DM content reported by Wadhwa and Bakshi [73] and (2016) however, it was found to be higher than values reported by Sharma and Prasad [62] and Raza *et al.* [58]. The crude protein (CP) content of CLM in the current investigation, was very close to the value reported by Mahgoub *et al.* [45] and Gupta and Wagle [28]. However, the CP content was lower than the value reported by Wani *et al.* [75] and higher than the value reported by Mogra *et al.* [48] and Bakshi *et al.* [11]. Similarly, the ether extract (EE) content of CLM was higher than the EE content reported earlier [28, 48 and 11]. The crude fibre (CF) content of CLM (9.47%) was comparable to the values reported by Gupta and Wagle [28] and lower than the value reported by Mogra *et al.* [48]. The nitrogen-free extract (NFE) of CLM was comparable to the value reported by Wani *et al.* [75] and Ahmed *et al.* [5]. The total ash (TA) content of CLM was consistent with the findings of Ahmed *et al.* [5] & Gupta and Wagle [28] and lower than the value reported by Mogra *et al.*

[48]. The acid insoluble ash (AIA) of the CLM was higher than the reports of Gupta and Wagle [28] and it may be due to different ways of collection and drying. The calcium (Ca) content of CLM was comparable to the value reported by Revathi *et al.* [59] and higher than the value reported by Ahmed *et al.* [5]. The phosphorus (P) content of CLM was also comparable to the value reported by Revathi *et al.* [59] and higher than the value reported by Ahmed *et al.* (2013). The total phenolic content (TPC) of CLM in the present experiment was higher than the values reported by Abul-Fadl [3] and Wu *et al.* [76]. However, the TPC values in the current study were lower than those reported by Collado-Alvarado *et al.* [17]. The glucosinolates content of CLM in the present investigation was comparable to the value reported by Gupta and Wagle [28] & Drabińska *et al.* [20] and slightly lower than the levels reported by Abul-Fadl [3] & Chandra [15].

Thyroid hormone profile

The thyroid hormone profile of birds in the current investigation were consistent with the findings of Rabie *et al.* [56] who reported that the feeding canola meal (*Brassica napus*) upto 15% in broilers did not affect the plasma thyroid hormone profile. Further, Abdel-Moneim *et al.* [2] reported that the serum T3 concentration was unaffected in the Japanese quail fed a diet containing full-fat canola seeds at 150g/kg. Kermanshahi and Pour [36] reported no significant difference in thyroid hormone profile of broilers fed rapeseed meal upto 20% level. Similarly, Pena [53] reported that there was no significant difference in serum T3, and T4 hormone levels of laying hens fed Broccoli Stems and Leaves Meal upto 15% level.

Weight of immune organs

The higher weight of immune organs in the 15% CLM fed group of birds in the current experiment corroborated with that of Ao and Kim [7] who reported higher relative weight of the spleen in broilers fed grape seed extract rich in polyphenols. The similar findings were also reported by Omar *et al.* [52] who reported a higher weight of thymus in broilers fed onion extract rich in polyphenols. The improved humoral immune response against sheep red blood cells (SRBC) seen in this experiment may be correlated with the increased weight of immune organs. Similar co-relation between greater spleen, thymus, and other immune organ weight and higher antibody titre against SRBCs was also reported by Kabir *et al.* [33]. Increased weight of immune organs may be attributed due to the proliferation of lymphocytes. When challenged with an antigen, lymphocytes respond by creating lymphokines, proliferating their antigen-specific lymphocyte clones, and enhancing immune responses [65]. Yang *et al.* [78] reported an increased splenic lymphocyte proliferation response and spleen weight in broilers supplemented with quercetin (phenolic compound).

Cell-mediated and Humoral immune response

The cell mediated immune response of experimental group of birds recorded in the current investigation was consistent with the findings of Kumar *et al.* [39] who reported that there was no significant difference in CMI response of broilers fed pomegranate peel extract rich in polyphenols during the

summer. Similar findings were reported by Rao *et al.* [57] where he reported that there was no significant difference in cell mediated immune response to PHA-P in broilers fed *Moringa oleifera* leaf meal and pomegranate peel meal at 1000mg/kg & 500mg/kg of basal diet respectively. In contrast, to the present findings, Gopi *et al.* [27] reported that pigeons fed polyphenols isolated from pomegranate peels had better cell mediated immune response against intradermal inoculation of PHA-P. Dumaup and Ampode [23] also reported significantly ($P < 0.05$) higher CMI in broiler chickens fed diets containing water hyacinth meal.

The humoral immune response of experimental group of birds recorded in the current investigation were consistent with the findings of Pourhossein *et al.* [55], who reported the enhanced immune response against SRBCs in birds fed sweet orange peel extract rich in polyphenols. Emadi and Kermanshahi [25] reported that the broiler chicks fed turmeric rhizome powder rich in polyphenols upto 0.75% level had a higher antibody titre against SRBCs. In further support of these findings, Gholamrezaie *et al.* [26] reported that broiler chickens supplemented with *Artemisia annua* extract and leaf powder (contains flavonoids and quercetin) at 1.5% level increased total antibody titre against SRBC in the experimental groups when compared with the control group. Increased antibody titre against SRBC in the current study is suggestive of increase in humoral immunity of experimental birds when compared with the control group.

Serum IL-6 levels

IL6 is a pleiotropic cytokine, and its concentration is used as an indicator to study the inflammatory status of animals [60]. In the present investigation, the results were in line with the reports of Sun *et al.* [70], who reported that the supplementation of a polyphenol-rich *Yucca schidigera* extract at 300mg/kg of basal diet in broiler chickens, resulted in lower serum IL-6 concentrations when compared with the control group. Adeyemi *et al.* [4] also reported lower serum IL6 levels in broiler chickens fed diets supplemented with *Anacardium occidentale* leaf powder upto 4g/kg. In further support of these findings, Liu *et al.* [42] reported that the grape seed extract rich in polyphenols reduced inflammation in mice fed a high-fat diet by significantly ($P < 0.05$) lowering serum pro-inflammatory cytokine (IL6) concentrations. Similarly, Kilany *et al.* [38] reported that there was a significant decrease in serum IL6 levels of broilers fed a diet containing 5% *Moringa oleifera* leaf meal and 0.5% sweet basil.

The findings of the present investigation were consistent with the results of Nootash *et al.* [49], who reported that the Interleukin-6 gene expression in the spleen of rainbow trout was upregulated on feeding green tea extract (*Camellia sinensis*) rich in polyphenol at a rate of 500 mg/kg. Further, Shekarabi *et al.* [63] reported that the transcription levels of interleukin-6 genes were up-regulated in the fish fed dandelion flower extract (DFE) at 3 and 4 g/kg of basal diet. In further support of this study, Awad *et al.* [10] reported that the IL-6 gene was up-regulated in the spleen of broiler chickens fed rutin a bioactive chemical found in apple and tea leaves at a rate of 1g/kg diet. Similarly, Khalid *et al.* [37] reported significantly increased IL6 gene expression in rabbits fed *Moringa oleifera* leaf powder at 200 mg/kg body weight.

Temperature humidity index

During the last 3 weeks of the experimental trial the THI was in severe to very severe heat stress. The effects of heat stress on laying hens can be compared between various study conditions by utilising THI, which takes into account both ambient temperature and humidity [35]. El-Tarabany, [24] reported that, Japanese quail exposed to heat stress showed decreased immunological and production characteristics as well as negative welfare consequences.

HSP 70 gene expression study

The heat shock transcription factor activity or concentration can be increased at high temperatures to cause the synthesis of HSP70 mRNA [18]. The HSP70 is the most conserved and well-studied members of the HSP family, and their expression is altered by a wide range of substances or stressors. It is reported that the primary inducer of HSP-related gene expression is high ambient temperature [77]. The results of the present investigation were consistent with the findings of Hajati *et al.* [29], who reported that the broilers supplemented with grape seed extract rich in polyphenols and vitamin C during chronic heat stress conditions resulted in a down-regulation of HSP-70 gene expression in the liver and heart when compared with the control group. Further, Sugito *et al.* [69] reported the downregulation of HSP-70 gene in the kidney of broilers fed with salix extract under heat stress conditions. Wan *et al.* [74] reported that the mRNA expression levels of HSP70 was decreased in breast muscle of broilers supplemented with *Artemisia annua* L. when compared with the control group. Similarly, Song *et al.* [67] reported significantly ($P < 0.05$) decreased HSP70 mRNA expression in the intestine of broilers supplemented with *Artemisia annua* at 1g/kg of diet.

Serum HSP 70 levels

When analysing the broiler's response to heat stress, it is crucial to take oxidative stress into account [40]. Bird's maintaining heat homeostasis under stressful conditions results in higher reactive oxygen species (ROS) concentrations. It is reported that the body begins producing heat shock proteins to defend itself from ROS' damaging effects on the cells [22]. The results of the present findings were consistent with the findings of Hassan and Asim [30], who reported that the broilers supplemented with vitamin C and acetylsalicylic acid had lower serum HSP70 levels than controls when raised in heat stress conditions (40°C for 4 hours per day). Hu *et al.* [32] reported that the serum HSP70 levels were significantly ($P < 0.05$) reduced in broilers fed the diet supplemented with phloretin (a type of natural phenol that is found in apple tree leaves) at 200 mg/kg. In further support of these findings, Zhang *et al.* [79] and Liu *et al.* [41] reported the significant ($P < 0.05$) decrease in HSP70 levels in broilers fed a diet supplemented with the plant antioxidants like curcumin and resveratrol. The reduced serum HSP70 levels in treatment groups may also be attributed to the fact that the polyphenols present in the CLM will scavenge the ROS which is also one of the factors responsible for HSP production as suggested by Mahmoud *et al.* [46].

Antioxidant status

Broilers require higher antioxidant capacity since low antioxidant capacity has been linked to cellular damage, immune system degeneration, and metabolic abnormalities [68]. The results of present investigation were similar with the Drazbo *et al.* [21] who reported that there was a significant ($P < 0.05$) increase in serum SOD levels of turkey fed rape seed meal at a 15% level. Wan *et al.* [74] reported that the broilers fed *Artemisia annua L.* leaves rich in polyphenols showed greater blood levels of SOD and catalase. Further, Hu *et al.* [31] reported that the serum catalase and glutathione peroxidase concentration were significantly ($P < 0.05$) increased in broilers fed the diet supplemented with phloretin (natural phenol in apple tree leaves) at 200 mg/kg diet. These results corroborated with the findings of Drazbo *et al.* [21], who reported significantly ($P < 0.05$) higher FRAP in the turkey fed a rape seed meal at a 15% level. Similar results were reported by Ognik *et al.* [51] in turkey hens fed a diet supplemented with the quercetin (phenolic compound). Llorach *et al.* [44], reported that the testing of a cauliflower by-product extract using FRAP method revealed a significant antioxidant potential. He also reported that there is a linear correlation between FRAP readings and the overall phenolic content of cauliflower leaves. These results are consistent with the findings of Wan *et al.* [74] who reported the broilers fed *Artemisia annua L.* leaves rich in polyphenols showed significantly ($P < 0.05$) higher serum DPPH levels when compared with the control. Llorach *et al.* [44] reported that an extract of cauliflower by-products effectively scavenges DPPH free radicals. Similarly, Seong *et al.* [61] reported the significantly higher DPPH free radical scavenging capacity of Chinese cabbage extract.

Conclusions

Therefore, it is concluded that cauliflower leaf meal (CLM) inclusion in the diet upto 15% improves immunity, which is evident by increased immune organ weight, higher haemagglutination titre against sheep RBCs, and upregulation of IL6 gene in the spleen. CLM feeding also increases the antioxidant status and alleviates heat stress in broiler chickens.

Declarations

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The work has been done under the institute project entitled “Studies on the utilization of fruit and vegetable waste as an alternative feed” and funded by Indian Veterinary Research Institute, Bareilly, India.

Author Contributions

TM is involved in conducting experiment, analysis, and writing the manuscript. SS and AB planned and executed of both experiments, edited manuscript, coordinated studies. GK and AB are involved in conducting experimental trial and analysis part. RM edited manuscript.

Data Availability

Corresponding author should be contacted to get data available on request.

References

1. A.O.A.C. Methods of analysis. Association of Official Analytical Chemists. (15th Edn.) Washington, USA : 20–44. (1990)
2. A.O.A.C. Official Methods of analysis. AOAC international. 18th edn., Virginia, USA (2000)
3. Abaza, R.H., Blake, J.T.: Fisher. Oxalate determination: Analytical problems encountered with certain plant species. *J. Association Official Anal. Chem.* **51**, 5 963–967 (1968)
4. Abdel-Moneim, Abdel-Moneim, E., Sabic, E.M., Abu-Taleb, A.M., Ibrahim, N.S.: Growth performance, hemato-biochemical indices, thyroid activity, antioxidant status, and immune response of growing Japanese quail fed diet with full-fat canola seeds. *Trop. Anim. Health Prod.* **52**(4), 1853–1862 (2020). <https://doi.org/10.1007/s11250-020-02200-1>
5. Abul-Fadl, M.M.: Nutritional and chemical evaluation of white cauliflower by-products flour and the effect of its addition on beef sausage quality. *J. appl. sci. res.* **8**(2), 693–704 (2012)
6. Adeyemi, K., Sola-Ojo, F., Ishola, J., Ahmed, M., Lawal, M.: Influence of Anacardium occidentale leaf supplementation in broiler chicken diet on performance, caecal microbiota, blood chemistry, immune status, carcass, and meat quality. *Br. Poult. Sci.* **62**(4), 552–561 (2021). <https://doi.org/10.1080/00071668.2021.1894321>
7. Ahmed, F.A., Rehab, F.M., Ali: Bioactive compounds and antioxidant activity of fresh and processed white cauliflower. *Biomed. Res. Int.* (2013). <https://doi.org/10.1155/2013/367819>
8. Aires, A., Fernandes, C., Carvalho, R., Bennett, R.N., Saavedra, M.J., Eduardo, A.S.R.: Seasonal effects on bioactive compounds and antioxidant capacity of six economically important Brassica vegetables. *Molecules.* **16**(8), 6816–6832 (2011). <https://doi.org/10.3390/molecules16086816>
9. Ao, X., Kim, I.H.: Effects of grape seed extract on performance, immunity, antioxidant capacity, and meat quality in Pekin ducks. *Poult. Sci.* **99**(4), 2078–2086 (2020)
10. Awad, A., Zaglool, A.W., Samah, R.: Khalil. Immunohaematological status and mRNA expression of the genes encoding interleukin-6, nuclear-factor kappa B, and tumor-necrosis factor- α in the spleen of broilers supplemented with dietary rutin. *Anim. Prod. Sci.* **59**(8), 1454–1461 (2018). <https://doi.org/10.1071/an18102>

11. Bakshi, M.P.S., Wadhwa, M., Harinder, P.S., Makkar: Waste to worth: vegetable wastes as animal feed. *CABI Reviews* 1–26, (2016). <https://doi.org/10.1079/pavsnr201611012>
12. Benzie, I.F.F., Strain, J.J.: Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In *Methods in enzymology*, ((15–27), Academic press (1999). [https://doi.org/10.1016/s0076-6879\(99\)99005-5](https://doi.org/10.1016/s0076-6879(99)99005-5)
13. BIS Product manual for poultry feeds – specifications, IS 1374: 2007, New Delhi: (2007)
14. Brand-Williams, W., Cuvelier, M.-E.: W. T. Berset. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **28**(1), 25–30 (1995). [https://doi.org/10.1016/s0023-6438\(95\)80008-5](https://doi.org/10.1016/s0023-6438(95)80008-5)
15. Chandra, A.K.: Goitrogen in food: cyanogenic and flavonoids containing plant foods in the development of goiter. In *Bioactive foods in promoting health*, 691–716. Academic Press (2010). <https://doi.org/10.1016/b978-0-12-374628-3.00042-6>
16. Cheng, S., Lamont, S.J.: Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poult. Sci.* **67**(7), 989–995 (1988). <https://doi.org/10.3382/ps.0670989>
17. Collado-González, J., Piñero, M.C., Ginés, Otalora, J., López-Marín, Francisco, M.: Del Amor. Enhancement of Bioactive Constituents in Fresh Cauliflower By-Products in Challenging Climate Conditions. *Antioxidants* 11(5), 958 (2022). <https://doi.org/10.3390/antiox11050958>
18. Craig, E.A., Carol, A., Gross: Is hsp70 the cellular thermometer? *Trends Biochem. Sci.* **16**, 135–140 (1991). [https://doi.org/10.1016/0968-0004\(91\)90055-z](https://doi.org/10.1016/0968-0004(91)90055-z)
19. Deng, G.-F., Lin, X., Xu, X.-R., Gao, L.-L., Xie, J.-F., Li, H.-B.: Antioxidant capacities and total phenolic contents of 56 vegetables. *J. Funct. Foods.* **5**(1), 260–266 (2013). <https://doi.org/10.1016/j.jff.2012.10.015>
20. Drabińska, N., Jež, M., Nogueira, M.: Variation in the Accumulation of Phytochemicals and Their Bioactive Properties among the Aerial Parts of Cauliflower. *Antioxidants.* **10**(10), 1597 (2021). <https://doi.org/10.3390/antiox10101597>
21. Dražbo, A., Ognik, K., Zaworska, A., Ferenc, K., Jankowski, J.: The effect of raw and fermented rapeseed cake on the metabolic parameters, immune status, and intestinal morphology of turkeys. *Poult. sci.* **97**(11), 3910–3920 (2018). <https://doi.org/10.3382/ps/pey250>
22. Droge, W.: Free radicals in the physiological control of cell function. *Physiol. Rev.* **82**(1), 47–95 (2002). <https://doi.org/10.1152/physrev.00018.2001>
23. Dumaup, H.J.J., Keiven Mark, Ampode: Inclusion of water hyacinth meal in broiler chicken diets: Potential on the production performance and cell-mediated immunity. *Int. J. Biosci.* **17**(6), 469–479 (2020)
24. El-Tarabany, M., Salah: Impact of temperature-humidity index on egg-laying characteristics and related stress and immunity parameters of Japanese quails. *Int. J. Biometeorol.* **60**(7), 957–964 (2016). <https://doi.org/10.1007/s00484-015-1088-5>

25. Emadi, M., Kermanshahi, H.: Effect of turmeric rhizome powder on immunity responses of broiler chickens. *J. Anim. Ve t Adv.* **2** (2007). <https://doi.org/10.3923/ijps.2007.345.348>
26. Gholamrezaie, S.L., Mohammadi, M., Jalali, S.J., Abolghasemi, S.A.: Roostaei. Extract and leaf powder effect of *Artemisia annua* on performance, cellular and humoral immunity in broilers. *Iran. J. Vet. Res.* **14**(1), 15–20 (2013)
27. Gopi, M., Dutta, N., Elaiyaraja, G., Velusamy, M., Kolluri, G., Tyagi, J.S., Jaydip, J.R.: Govindasamy Prabakar, and Ramasamy Dhinesh Kumar. Polyphenols improved the heat stress induced immune suppression in broilers. *Indian J. Poult. Sci.* **54**(3), 245–249 (2019). <https://doi.org/10.5958/0974-8180.2019.00044.8>
28. Gupta, K., Wagle, D.S.: Nutritional and antinutritional factors of green leafy vegetables. *J. Agric. Food Chem.* **36**(3), 472–474 (1988). <https://doi.org/10.1021/jf00081a016>
29. Habeeb, A., Alnaimy, A.E., Gad, Mostafa Abas Atta: Temperature-humidity indices as indicators to heat stress of climatic conditions with relation to production and reproduction of farm animals. *Int. j. biotechnol. recent. adv.* **1**(1), 35–50 (2018). <https://doi.org/10.18689/ijbr-1000107>
30. Hajati, H., Hassanabadi, A., Golian, A., Nassiri-Moghaddam, H., Mohammad Reza, Nassiri: The effect of grape seed extract and vitamin C feed supplementation on some blood parameters and HSP70 gene expression of broiler chickens suffering from chronic heat stress. *Ital. J. Anim. Sci.* **14**(3), 3273 (2015). <https://doi.org/10.4081/ijas.2015.3273>
31. Hassan, A.A., Rana, A., Asim: Effect of vitamin C and acetylsalicylic acid supplementation on some hematological value, heat shock protein 70 concentration and growth hormone level in broiler exposed to heat stress. *Iraqi J. Vet. Sci.* **34**(2), 357–363 (2020). <https://doi.org/10.33899/ijvs.2019.125950.1195>
32. Hu, H., Bai, X., Xu, K., Zhang, C., Chen, L.: Effect of phloretin on growth performance, serum biochemical parameters and antioxidant profile in heat-stressed broilers. *Poult. Sci.* **100**(8), 101217 (2021). <https://doi.org/10.1016/j.psj.2021.101217>
33. Hu, R., He, Y., Arowolo, M.A., Wu, S., He, J.: Polyphenols as potential attenuators of heat stress in poultry production. *Antioxidants.* **8**(3), 67 (2019). <https://doi.org/10.3390/antiox8030067>
34. Kabir, S.M., Lutful, M.M., Rahman, M.B., Rahman, M.M., Rahman, Ahmed, S.U.: The dynamics of probiotics on growth performance and immune response in broilers. *Int. J. Poult. Sci.* **3**(5), 361–364 (2004). <https://doi.org/10.3923/ijps.2004.361.364>
35. Kamboh, A.A., Arain, M.A., Mughal, M.J., Zaman, A., Arain, Z.M., Soomro, A.H.: Flavonoids: Health promoting phytochemicals for animal production-a review. *J. Anim. Health Prod.* **3** (1), 6–13 (2015). <https://doi.org/10.14737/journal.jahp/2015/3.1.6.13>
36. Kang, S., Kim, D.-H., Lee, S., Lee, T., Lee, K.-W., Moon, H.-H.C.B.: Tugay Ayasan, and Yang-Ho Choi. An acute, rather than progressive, increase in temperature-humidity index has severe effects on mortality in laying hens. *Front. Vet. Sci.* **568093**, 7 (2020). <https://doi.org/10.3389/fvets.2020.568093>

37. Kermanshahi, H., Abbasi, A.R., Pour: Replacement value of soybean meal with rapeseed meal supplemented with or without a dietary NSP-degrading enzyme on performance, carcass traits and thyroid hormones of broiler chickens. *Int. J. Poult. Sci.* **5**(10), 932–937 (2006).
<https://doi.org/10.3923/ijps.2006.932.937>
38. Khalid, A., Rauf, T.B., Yasoob, Z., Zhang, D., Yu, J., Feng, X., Zhu, Suqin Hang: Supplementation of *Moringa oleifera* leaf powder orally improved productive performance by enhancing the intestinal health in rabbits under chronic heat stress. *J. Therm. Biol.* **93**, 102680 (2020)
39. Kilany, O.E., Youssef, F., Mabrouk, M., Fares, I.M.: Clinicopathological studies on the effect of some antibacterial medicinal plants in broilers. *J. Clin. Pathol.* **1003** (2018).
<https://doi.org/10.1016/j.jtherbio.2020.102680>
40. Kumar, K., Prashanth, V., Ravinder Reddy, Gnana Prakash, M.: Effect of supplementing pomegranate (*Punica granatum*) peel extract on serum biochemical parameters and immune response in broilers during summer. *Pharma innov.* **7**(1), 591–601 (2018). <https://doi.org/10.5455/ijlr.20180228050827>
41. Lin, H., Decuyper, E., Buyse, J.: Acute heat stress induces oxidative stress in broiler chickens. *Comp. biochem. physiol., Mol. Part. A: Mol. Integr. Physiol.* **144**(1), 11–17 (2006).
<https://doi.org/10.1016/j.cbpa.2006.01.032>
42. Liu, L., Fu, C., Xie, M.Y.H., Li, S., Yu, Q., He, S., He, J.: Resveratrol modulates intestinal morphology and HSP70/90, NF- κ B and EGF expression in the jejunal mucosa of black-boned chickens on exposure to circular heat stress. *Food Funct.* **7**(3), 1329–1338 (2016). <https://doi.org/10.1039/c5fo01338k>
43. Liu, W., Zhao, S., Wang, J., Shi, J., Sun, Y., Wang, W., Ning, G., Jie Hong, and, Liu, R.: Grape seed proanthocyanidin extract ameliorates inflammation and adiposity by modulating gut microbiota in high-fat diet mice. *Mol. Nutr. Food Res.* **61**(9), 1601082 (2017).
<https://doi.org/10.1002/mnfr.201601082>
44. Livingston, A., Lyle, R.E., Knowles, J., Page, D.D., Kuzmicky: Kohler. Processing of cauliflower leaf waste for poultry and animal feed. *J. Agric. Food Chem.* **20**(2), 277–281 (1972).
<https://doi.org/10.1021/jf60180a039>
45. Llorach, R., Espín, J.C., Francisco, A., Tomás-Barberán, Ferreres, F.: Valorization of cauliflower (*Brassica oleracea* L. var. *botrytis*) by-products as a source of antioxidant phenolics. *Agric. Food Chem.* **51**(8), 2181–2187 (2003). <https://doi.org/10.1021/jf021056a>
46. Mahgoub, O., Kadim, I.T., Eltahir, Y., Al-Lawatia, S., Al-Ismaili, A.M.: Nutritional value of vegetable wastes as livestock feed. *Sultan Qaboos Univ. j. sci.* **2**, 78–84 (2018).
<https://doi.org/10.24200/squjs.vol23iss2pp78-84>
47. Mahmoud, K.Z., Edens, F.W., Eisen, E.J., Havenstein, G.B.: Ascorbic acid decreases heat shock protein 70 and plasma corticosterone response in broilers (*Gallus gallus domesticus*) subjected to cyclic heat stress. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **137**(1), 35–42 (2004).
<https://doi.org/10.1016/j.cbpc.2003.09.013>
48. Makkar, Harinder, P.S., Blümmel, M., Borowy, N.K., Becker, K.: Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric.* **61**(2),

- 161–165 (1993). <https://doi.org/10.1002/jsfa.2740610205>
49. Mogra, R., Banga, J., Preeti, R.: Nutrient composition of cauliflower (*Brassica oleracea* var. *Botrytis*) leaf powder and its acceptability in fast food snacks. *Food Sci. Res. J.* **3**(2), 167–171 (2012)
 50. Nootash, S., Sheikhzadeh, N., Baradaran, B., Oushani, A.K., Moghadam, M.R.M., Nofouzi, K., Monfaredan, A., Aghebati, L.: Fatemeh Zare, and Sadigheh Shabanzadeh. Green tea (*Camellia sinensis*) administration induces expression of immune relevant genes and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). *Fish. Shellfish Immunol.* **35**(6), 1916–1923 (2013). <https://doi.org/10.1016/j.fsi.2013.09.030>
 51. NRC-National Research Council. Nutrient Requirements of Poultry. 9th rev. ed., National Academy Press, Washington, DC 57–63: (1994)
 52. Ognik, K., Cholewinska, E., Czech, A.: The effect of adding hesperidin, diosmin, quercetin and resveratrol extracts to feed for turkey hens on selected immunological and biochemical blood indices. *Ann. Anim. Sci.* **16**(4), 1101 (2016). <https://doi.org/10.1515/aoas-2016-0035>
 53. Omar, A.E., Hanan, S., Al-Khalaifah, W.A.M., Mohamed, Heba, S.A., Gharib, A., Osman, N.A., Al-Gabri, Shima, A.: Amer. Effects of phenolic-rich onion (*Allium cepa* L.) extract on the growth performance, behavior, intestinal histology, amino acid digestibility, antioxidant activity, and the immune status of broiler chickens. *Front. vet. sci.* **7**, 582612 (2020). <https://doi.org/10.3389/fvets.2020.582612>
 54. Peña, G.: Broccoli Stems and Leaves Meal as a Feed Ingredient for Laying Hens. PhD diss., University of California, Davis (2020)
 55. Podsędek, A.: Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT - Food Sci. Technol.* **40**(1), 1–11 (2007). <https://doi.org/10.1016/j.lwt.2005.07.023>
 56. Pourhossein, Z., Qotbi, A.A.A., Seidavi, A., Laudadio, V., Centoducati, G., Tufarelli, V.: Effect of different levels of dietary sweet orange (*Citrus sinensis*) peel extract on humoral immune system responses in broiler chickens. *J. Anim. Sci.* **86**(1), 105–110 (2015). <https://doi.org/10.1111/asj.12250>
 57. Rabie, M.H., Hayam, M.A., Abo El-Maaty, M.R., El-Gogary: Sh Abdo. Nutritional and physiological effects of different levels of Canola Meal in broiler chick diets. *Asian J. Anim. Vet. Adv.* **4**, 161–172 (2015). <https://doi.org/10.3923/ajava.2015.161.172>
 58. Rao, S.V., Rama, M.V.L.N., Raju, B., Prakash, U., Rajkumar, Reddy, E.P.K.: Effect of supplementing moringa (*Moringa oleifera*) leaf meal and pomegranate (*Punica granatum*) peel meal on performance, carcass attributes, immune and antioxidant responses in broiler chickens. *Anim. Prod. Sci.* **59**(2), 288–294 (2018). <https://doi.org/10.1071/an17390>
 59. Raza, A., Hussain, J., Hussain, F., Zahra, F., Mehmood, S., Mahmud, A., Amjad, Z.B., et al.: Vegetable Waste Inclusion in Broiler Diets and its Effect on Growth Performance, Blood Metabolites, Immunity, Meat Mineral Content and Lipid Oxidation Status. *Braz J. Poult. Sci.* **21**(1) (2019). <https://doi.org/10.1590/1806-9061-2018-0723>
 60. Revathi, D., Sashidevi, G., Hemalatha, G., Kanchana, S.: Formulation of cookies from cauliflower leaves. In III International Symposium on Underutilized Plant Species 1241, 659–662 (2015).

<https://doi.org/10.17660/actahortic.2019.1241.96>

61. Scheller, J., Chalaris, A.: Dirk Schmidt-Arras, and Stefan Rose-John. The pro-and anti-inflammatory properties of the cytokine interleukin-6. *Biochim. Biophys. Acta Mol. Cell. Res.* **1813**(5), 878–888 (2011). <https://doi.org/10.1016/j.bbamcr.2011.01.034>
62. Seong, G.-U., Hwang, I.-W., Shin-Kyo, Chung: Antioxidant capacities and polyphenolics of Chinese cabbage (*Brassica rapa L. ssp. Pekinensis*) leaves. *Food chem.* **199**, 612–618 (2016). <https://doi.org/10.1016/j.foodchem.2015.12.066>
63. Sharma, R., Prasad, R.: Nutritional evaluation of dehydrated stems powder of cauliflower incorporated in Mathri and Sev. *J. Nutr. Food Sci.* **8**(1), 1000651 (2018). <https://doi.org/10.4172/2155-9600.1000651>
64. Shekarabi, S.P., Hosseini, Z.S., Mostafavi: Mehdi Shamsaie Mehrgan, and Houman Rajabi Islami. Dietary supplementation with dandelion (*Taraxacum officinale*) flower extract provides immunostimulation and resistance against *Streptococcus iniae* infection in rainbow trout (*Oncorhynchus mykiss*). *Fish. Shellfish Immunol.* **118**, 180–187 (2021). <https://doi.org/10.1016/j.fsi.2021.09.004>
65. Siegel, P.B., Gross, W.B.: Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poult. Sci.* **59**(1), 1–5 (1980). <https://doi.org/10.3382/ps.0590001>
66. Singh, V., Kumar, P., Dwivedi, B.R., Chaudhary, Singh, R.: Gymnemic acid stimulates in vitro splenic lymphocyte proliferation. *Phytother Res.* **30**(2), 341–344 (2016). <https://doi.org/10.1002/ptr.5514>
67. Snedecor, G.W., Cochran, W.G.: Statistical methods, 9th edn. Iowa State University, Ames, Iowa, USA (2004)
68. Song, Z., Cheng, K., Zhang, L., Wang, T.: Dietary supplementation of enzymatically treated *Artemisia annua* could alleviate the intestinal inflammatory response in heat-stressed broilers. *J. Therm. Biol.* **69**, 184–190 (2017). <https://doi.org/10.1016/j.jtherbio.2017.07.015>
69. Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H.I., Sartono, T.A.: Recent advances in the incorporation of leaf meals in broiler diets. *Livest. Res. Rural Dev.* **31**(7) (2019). <https://doi.org/10.1007/s11250-019-02147-y>
70. Sugito, S., Etriwati, M., Akmal, E., Rahmi, M., Delima: Zainal Abidin Muchlisin, and Denny Irmawati Hasan. Immunohistochemical Expression of AQP2 and HSP70 in Broiler Kidney Tissue Treated with *Salix tetrasperma* Roxb. Extract under Heat Exposure. *Sci. World J.* (2021). <https://doi.org/10.1155/2021/8711286>
71. Sun, D.: Bin-lin Shi, Man-man Tong, and Su-mei Yan. Improved performance and immunological responses as a result of dietary *Yucca schidigera* extract supplementation in broilers. *Ital. J. Anim. Sci.* **17**(2), 511–517 (2018). <https://doi.org/10.1080/1828051x.2017.1358593>
72. Talapatra, S.K., Ray, S.C., Sen, K.C.: The analysis of mineral constituents in biological materials. 1. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in food-stuffs. *Indian J. Vet. Sci.* **10**, 243–258 (1940)

73. Verdrengh, M., Tarkowski, A.: Role of neutrophils in experimental septicemia and septic arthritis induced by *Staphylococcus aureus*. *Infect. Immun.* **65**(7), 2517–2521 (1997).
<https://doi.org/10.1128/iai.65.7.2517-2521.1997>
74. Wadhwa, M., Kaushal, S., Bakshi, M.P.S.: Nutritive evaluation of vegetable wastes as complete feed for goat bucks. *Small Rumin Res.* **64**(3), 279–284 (2006).
<https://doi.org/10.1016/j.smallrumres.2005.05.017>
75. Wan, X.L., Niu, Y., Zheng, X.C., Huang, Q., Su, W.P., Zhang, J.F., Zhang, L.L., Wang, T.: Antioxidant capacities of *Artemisia annua* L. leaves and enzymatically treated *Artemisia annua* L. in vitro and in broilers. *Anim. Feed Sci. Technol.* **221**, 27–34 (2016).
<https://doi.org/10.1016/j.anifeedsci.2016.08.017>
76. Wani, T.A., Sood, M., Raj Kumari Kaul: Nutritional and sensory properties of roasted wheat noodles supplemented with cauliflower leaf powder. *Ann. Food Sci. Technol.* **12**(2), 102–107 (2011).
<https://doi.org/10.5897/ajb12.2346>
77. Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B., Gebhardt, S.E.: Prior. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* **52**(12), 4026–4037 (2004). <https://doi.org/10.1021/jf049696w>
78. Xie, J., Tang, L., Lu, L., Zhang, L., Xi, L., Liu, H.-C., Odle, J., Luo, X.: Differential expression of heat shock transcription factors and heat shock proteins after acute and chronic heat stress in laying chickens (*Gallus gallus*). *PloS one.* **9**(7), e102204 (2014).
<https://doi.org/10.1371/journal.pone.0102204>
79. Yang, J.X., Maria, T.C., Zhou, B., Xiao, F.L., Wang, M., Mao, Y.J., Li, Y.: Quercetin improves immune function in Arbor Acre broilers through activation of NF-κB signaling pathway. *Poult. sci.* **99**(2), 906–913 (2020). <https://doi.org/10.1016/j.psj.2019.12.021>
80. Zhang, G.F., Yang, Z.B., Wang, Y., Yang, W.R., Jiang, S.Z.: Gai. Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poult. sci.* **88**(10), 2159–2166 (2009).
<https://doi.org/10.3382/ps.2009-00165>