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Effects of Phytochemical Antioxidants and Amino Acid Supplement (X-VIRUS) on the Growth Performance, Carcass Traits, Immune Response, Blood Biochemical Parameters and Antioxidant Capacity of Broilers

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ABSTRACT:
Background: The poultry industry continues to grow due to the availability, affordability, and nutritional value of eggs and meat. Factors influencing this growth include genetics, feed quality, disease prevention, and the use of antibiotics. However, the search for alternatives to antibiotics has gained momentum due to concerns about antibiotic resistance and consumer demand. This study aimed to evaluate the effectiveness of adding various doses of the commercial compound X-VIRUS solution (containing antioxidants and essential amino acids) to drinking water, with the goal of determining the optimal dose for improving broiler productivity.
Methods: A total of 360 unsexed Ross 308 broilers were assigned to four treatments. Each treatment group was divided into six pens, with 15 chicks per pen. Treatments included: T1) control group (did not receive any additives), T2) 0.5 ml of X-VIRUS per liter of water, T3) 1 ml of X-VIRUS per liter of water, and T4) 1.5 ml of X-VIRUS per liter of water. Performance, carcass traits, biochemical blood variables and antioxidant capacity of broilers were assessed.
Results: Administering X-VIRUS solution at a dose of 1.5 ml/l in broilers' drinking water increased body weight and weight gain compared to the lower doses. Additionally, higher X-VIRUS doses improved feed intake and feed conversion ratios. X-VIRUS solution increased significantly the relative weight of carcass, breast, and thigh in the fifth week. Higher X-VIRUS doses (1 and 1.5ml/l) led to higher antibody titers compared to lower doses. X-VIRUS solution reduced triglyceride, cholesterol, LDL, glucose, ALT and MDA levels and increased HDL, total protein levels and GSH-Px activity, while albumin and globulin levels remained unchanged.
Conclusions: administering a dose of 1.5ml/l of X-virus solution showed the best results in the performance, carcass characteristics, blood parameters and antioxidant capacity of broilers.

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KEYWORDS: Antioxidant; broiler; performance; X-VIRUS; biochemical

1. INTRODUCTION

The poultry industry has experienced significant growth over the past three decades. The widespread availability, affordability, and high nutritional value of eggs and meat, which supply essential nutrients such as minerals, vitamins, and amino acids, have led to an increase in their consumption (Dhama et al., 2014). Key factors driving this growth include optimizing the genetic potential of birds, ensuring access to quality feed rich in essential amino acids, maintaining ideal environmental conditions, and using plant-based antioxidants to prevent disease outbreaks. Although the use of antibiotics as growth promoters and for medicinal purposes has increased, the demand for alternatives to antibiotics has also gained significant momentum. The goal is to identify substitutes that can sustain productivity and product quality while addressing concerns about antibiotic resistance, antibiotic residues in poultry products, and the increasing consumer demand for antibiotic-free options. Some alternatives in poultry production include the use of vitamins and minerals, herbal medicines, phytobiotics rich in antioxidants, and antimicrobial peptides (Yadav et al., 2016). Phytobiotics are plant-derived compounds with diverse biological activities in plants, animals, and humans. They have antimicrobial effects, modify gut microflora population, and demonstrate antiviral, antiprotozoal, and antifungal functions. The use of plant-based antioxidants can enhance intestinal health by reducing the presence of pathogens and preventing subclinical infections commonly observed in poultry. The ability of plant antioxidants to conserve nutrients becomes more evident when birds are exposed to unsanitary situations and are provided with a diet that is deficient or unbalanced in vitamins and amino acids. This unequivocally highlights the crucial role of plant antioxidants in alleviating the detrimental effects of such circumstances on avian health and maximizing nutrient utilization (Andersson and Macgowan, 2003; Luangtongkum et al., 2006; Martinez et al., 2006; Billah et al., 2015).

Feed and its manufacturing process constitute a substantial proportion of the overall expenses in broilers production. Typically, in minimum-cost feed formulations, the nutrient availability of ingredients is considered; however, the impact of heat processing on the nutrient availability is often overlooked (Gehring et al., 2011). Previous studies by Loar et al. (2014) and Boney and Moritz (2017) have shown that the use of temperatures above 85°C significantly reduces the digestibility of essential amino acids, including methionine, valine, isoleucine, leucine, and lysine, in birds. It is speculated that thermal processes like pelleting have the potential to solubilize dietary fiber and modify the structure of starches and proteins in the feed (Østergard et al., 1989). Conformational changes in the diet ingredients can either enhance enzyme access or reduce nutrient availability by forming new compounds, such as those produced by the Maillard reaction (Thomas et al., 1997). Martinez-Amezcua et al. (2007) reported that excessively high temperatures during pellet production could compromise nutrient accessibility, particularly reducing the availability of amino acids (AA). Nutritionists anticipate that feed pelleting methods could result in the loss of AA. Therefore, it may be necessary to increase the amount of AA in bird diets before the pelleting process (Lynch et al., 2023) or through over-formulation in water. According to these findings, the aim of this study was to investigate the effects of phytochemical antioxidants and amino acid supplements on growth performance, carcass characteristics, immune response, and blood biochemical parameters in broilers.

2. MATERIALS AND METHODS

2.1 Ethical approval

The present experiment received approval from the Animal Ethics Committee of Department of Animal Production, College of Agriculture / Al-Qasim Green University. These procedures were conducted with the utmost consideration for animal rights and welfare, ensuring minimal stress to the livestock. All procedures were performed in strict accordance with the applicable guidelines and regulations pertaining to animal research.

2.2 Birds, diets, and housing

A total of 360 one-day-old unsexed Ross 308 broilers, with an average body weight (BW) of 41.12 ± 0.97 g, were acquired from a local hatchery and allocated to four dietary treatments. Each treatment had six replicates, with 15 birds per replicate, based on a completely randomized design (CRD). The treatments included: T1= a basal diet without X-VIRUS solution (control), T2= 0.5 ml of X-VIRUS per liter of water, T3= 1 ml of X-VIRUS per liter of water, and T4= 1.5 ml of X-VIRUS per liter of water. The chicks were fed a three-stage diet consisting of starter mash, grower and finisher pellets. All birds were given a starter (0-7 d), grower (8-14 d) and (15-35 d) finisher diets. All diets were formulated based on the National Research Council (NRC, 1994) guidelines. The components and chemical composition of the starter and finisher feeds are outlined in Table 1. Feed and water were provided ad libitum throughout the trial period. The total weight of the broilers in each pen was recorded at one day of age, and subsequently at the completion of the starter phase (0-7 d), the grower phase (7-14 d) and (14-35 d) the finisher phase. The temperature and minimal airflow requirements were diligently monitored and documented twice daily throughout the study to ensure proper maintenance (Aviagen, 2018). During the first five days of the breeding phase, each pen was equipped with a tray feeder. Subsequently, a cylindrical feeder and drinker were provided in each pen. The X-VIRUS compound, produced by the Chinese company (Henanchuavqriu Biological Technology), contains a high concentration of five different types of medicinal herbs: honeysuckle, scrophulariaceae, radix scutellariae, radix rehmanniae, and multiple amino acids.

Table 1. Composition and calculated analysis of basal diets

Ingredient (%)	Starter (0-7 d)	Grower (8-14 d)	Finisher (15-35)
Corn	39.42	43.00	47.45
Soya meal (44%)	44.36	40.11	34.55
Wheat	10	10	10
Concentrate[1]	2.5	2.5	2.5
Calcium carbonate	1.26	1.07	1.25
Sunflower oil	2.29	3.15	4.08
Salt (NaCl)	0.17	0.17	0.17
Sum	100	100	100
Composition analysis			
ME (kcal/Kg)	3000	3.100	3.200

Crude protein (%)	23	21.5	19.5
Ca (%)	0.96	0.87	0.79
Available P (%)	0.48	0.43	0.39
Na (%)	0.16	0.16	0.16
Lysine (%)	1.44	1.29	1.15
Methionine (%)	0.77	0.70	0.64
Threonine (%)	0.97	0.88	0.78
Met + Cys (%)	1.08	0.99	0.90
DCAB[2] (mEq kg^{-1})	236.23	220.74	200.20
Crude fiber (%)	5.05	4.84	4.55
Fat (%)	3.10	3.08	3.04
Linoleic acid (%)	1.02	1.09	1.17

[1]Provime concentrate produced by Provime / Dutch: Jordanian origin Each kg contains: 17% crude protein, 1.5% crude fat, 1% fiber, 14% calcium, 6.7% available phosphorus, 4.8% sodium, 5.8% chloride, 13.7% total phosphorus, 9.3% non-lysine, 7.8% methionine + cysteine, 0.4% tryptophan, 2100kcal/kg energy representative, 480000 IU vitamin A, 220000 IU vitamin D3, 3000 mg Vitamin E, 138 mg Vitamin K3, 138 mg Vitamin B1, 280 mg Vitamin B2, 600 mg Vitamin B5, 48 mg Vitamin B6, 1000 mg Vitamin B9, 1800 mg Niacin, 6900 mg Biotin, 2400 mg Iron, 3500 mg Zinc, 3200 mg Manganese, 480 mg Copper, 10 mg Selenium, 48 mg Iodine, 250 mg Antioxidant. (2) According to each of the energy represented,crude protein, crude fiber,fat, lysine, and methionine + cysteine calcium, and bio phosphorus. Feed is designing according guid ross 308 company according to UFFDA 2019 programs. Note. ME: metabolizable energy. [2]DCAB = Na + K – Cl.

2.3 Estimation of total phenolic content

The measurement of total phenolic compounds (Table 2) was conducted using the Folin–Ciocalteu colorimetric technique, as described by Ui-Haq et al. (2012). The results are expressed in terms of gallic acid equivalents, represented as milligrams of gallic acid per gram of extract.

Table 2. Chemical analysis of X-VIRUS solution dietary supplement

No	Phenol compound	Concentrate (ppm)
1	Apigenin	85.9
2	Caffeic acid	145.9
3	Chlorogenic acid	66.9
4	Ellagic acid	85.4
5	Gallic acid	174.8
6	Kaempferol	96.9
7	Qurcetine	88.9
8	Rutin	74.9

Table 2. Chemical analysis of X-VIRUS solution dietary supplement- continue

No	Amino acid	Concentrate (µg/gm)
1	Histidine	36.98
2	Alanine	44.58
3	Arginine	50.14
4	Asparagine	55.98
5	Aspartic Acid	49.05
6	Cysteine	63.59
7	Glutamic Acid	70.58
8	Glutamine	65.25
9	Glycine	55.08
10	Isoleucine	60.14
11	Leucine	65.28

12	Lysine	74.11
13	Methionine	80.25
14	Phenylalanine	65.98
15	Proline	33.25
16	Serine	60.25
17	Threonine	61.44
18	Tyrosine	55.29

2.4 Amino acid examination

Amino acid analysis was conducted in the laboratory using a Korean-made amino acid analyzer. The method was based on the approach described by Scriver CR in 2001. The carrier phase consisted of a mixture of methanol, acetonitrile, and 5% formic acid in the ratios of 20:60:20, with a flow rate of 1 ml/min. A ZORBAX Eclipse-AAA segregation column (150 x 4.6 mm, particle size 3.5 μm) was utilized for the separation of amino acids. A fluorescence detector identified the amino acids at different wavelengths (Ex = 445 nm, Em = 465 nm). The analysis was performed using the Clarity 2015 program. High-performance liquid chromatography (HPLC) analysis was conducted on a SYKAM HPLC system (Germany) equipped with a C18-ODS column (250 × 4.6 mm, 5 µm).

2.5 Growth performance

The average body weight gain was calculated by subtracting the initial group weight from the final group weight for each period and then dividing that difference by the number of birds present at the end of the phase. To calculate feed intake (FI), the residual feed was subtracted from the total feed provided to each pen throughout the study. Daily mortality rates were recorded and adjusted. Feed conversion ratio (FCR) was corrected for mortality and represented as grams of feed consumed by all chickens in each pen divided by grams of body weight gain. This methodology was based on the approach described by Imari et al. (2020).

2.6 Carcass traits and blood sampling

At 35 days of age, a male chick with a body weight (BW) approximately equivalent to the average live BW of the pen was selected. The chosen chick was weighed and subsequently sacrificed. After feather removal, the carcass and its various components, including the bursa of Fabricius, gizzard, spleen, liver, pancreas, abdominal fat, breast, thighs, wings, back, and neck, were individually weighed and documented. The findings were expressed as percentages of the live body weight of the chicks. Blood samples were centrifuged at 3000 rpm for 15 minutes, and the resulting plasma was stored at -20°C until the assessment of various metabolic parameters using commercially available colorimetric kits (Biodiagnostic Company, Giza, Egypt). The measured parameters included cholesterol, HDL, LDL, and triglycerides (TG), alanine transaminase (ALT), aspartate transaminase (AST), glucose, total protein, albumin and globulin. Additionally, commercially available kits and a spectrophotometer (Shimadzu, Japan) were used to determine the levels of plasma malondialdehyde (MDA), catalase (CAT), and glutathione peroxidase (GSH-Px).

2.6 Statistical analysis

The data were analyzed using the SAS Institute software with a one-way analysis of variance (ANOVA). Mean differences were compared using Duncan's multiple range test, and statistical significance was set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Growth performance

The outcomes of BW, BWG, FI and FCR are shown as the mean ± standard error of the mean over the five-week experimental phase in Table 3. The results demonstrated that broilers fed with the X-VIRUS solution at a dosage of 1.5 ml/l in their drinking water showed a significantly greater increase in BW and BWG compared to the control group and those treated with 0.5 ml/l and 1 ml/l during the trial period ($P \leq 0.05$). In addition, the inclusion of 1 ml/l and 1.5 ml/l doses of the X-VIRUS solution resulted in higher feed intake compared to the birds treated with 0 ml/l and 0.5 ml/l doses of the supplement ($P \leq 0.05$). The FCR of broilers decreased with increasing levels of X-VIRUS solution during the 1-5 week period ($P \leq 0.05$).

The inclusion of commercially available phytonutrient blends of carvacrol, capsicum oleoresin, cinnamaldehyde, and essential oils in poultry diets has been observed to give variable results. An analysis of broiler chickens- thirteen clinical trials was applied to evaluate the impact of using these standardized phyto- compounds. They further stated that under different standards adopted for broilers, supplementation of these compounds improved weight gain, feed conversion ratio and percent mortality. This may also explain why even after so much research, the mechanism through which these phytochemicals work is not accurately known but there is speculation on it being an important role like that of an antioxidant or an antimicrobial. The dietary inclusion of phytochemicals would help to stabilize intestinal microbiota and inhibit the production of microbial metabolites which harm the chickens and also inhibit the growth of harmful pathogenic bacteria in the chickens' bodies which again would lead to better performance by chickens (Kim et al., 2015). agreed with this study. BW, BWG, and FI of broilers (1-35 d) increased with different phenolic-rich onion extract levels (1-3 g/kg diet) without showing any adverse effects on birds as was reported similarly by Hilliar et al. (2020). On the other hand, Comi et al. (2014) found no significant difference in the body weight, average daily feed intake, and average daily gain of birds when fed with olive polyphenol extract. Amino acids as supplements increased the synthesis of cecal butyric acid as well as total short-chain fatty acid production by Hilliar et al. (2020) and Chrystal et al. (2020). This supplementation not only supported the growth performance and feed conversion efficiency but also enhanced immunity. The application of higher temperatures during feed processing can induce considerable degradation of cysteine, recognized as the most thermolabile amino acid. This degradation subsequently affects lysine, leading to the depletion of arginine, threonine, and serine from the feed (Papadopoulos, 1989). The X-VIRUS compound, consisting of medicinal plants, contains essential amino acids required by poultry to address the significant challenge faced by poultry farming in Iraq, particularly the prevalence of viral diseases that affect fast-growing commercial breeds. These birds need continuous antioxidants and essential amino acids for their well-being. Furthermore, the pelleting process in feed manufacturing can lead to a loss of amino acid specificity due to the effects of heat and pressure. To mitigate this issue, it may be necessary to supplement these amino acids in the drinking water using X-VIRUS.

Table 3. Effects of dietary supplementation of phytochemical antioxidants and amino acid (X-VIRUS) on growth performance in broiler chickens (Means ± standard error)

Treatments (ml/l)	BW(g)	FI(g)	BWG(g)	FCR
0	1634.33 ± 39.64 d	2773.33± 11.20 c	1600.33± 40.20 d	1.73± 0.03 a
0.5	1779.33± 48.46 c	2918.33 ± 20.62 b	1744.67± 48.70 c	1.67± 0.05 ab
1	1906.67± 15.89 b	2986.67 ± 13.61 a	1872.33±15.23 b	1.59± 0.01 bc
1.5	2058.33± 30.59 a	3030.67± 2.02 a	2023.33± 30.38 a	1.49± 0.02 c
Significance level	**	**	**	**

*** Averages with different letters within one column indicate differences at a significant level.**

3.2 Carcass and internal organs

Table 4 presents that the effects of adding various doses of the X-VIRUS solution on the mean carcass weight of broilers in the fifth week of the experiment. The inclusion of 1.5 ml/l of the X-VIRUS solution significantly increased the weights of the carcass, breast, and thighs compared to the control group ($P \leq 0.05$).

Table 4. Effects of dietary supplementation of phytochemical antioxidants and amino acid (X-VIRUS) on characteristics of carcass (g) of broilers ROSS 308 (math. Mean ± standard error)

Treatments	Chick weight	Chest Weight	Thighs weight	Liver weight	Heart weight	Gizzard weight
0	1265.00 ± 24.66 b	751.66± 76.50b	573.33±36.55c	40.66±2.1	9.00±1.5	40.00± 1.73
0.5	1325.00±28.86 b	836.66±32.82ab	671.66±46.93bc	38.33±0.88	11.66±0.33	44.33±5.20
1	1466.67 ± 65.08b	861.66±26.82ab	631.66±15.89ab	41.33±3.33	11.66±1.66	39.66±2.60
1.5	1626.67±74.23a	976.66±39.29a	795.00±10.40 a	37.66±1.33	10.33±0.88	39.66±0.33
Significance level	**	**	**	NS	NS	NS

*** Averages with different letters within one column indicate differences at a significant level.**

In comparison to the findings of the current study, Aji et al. (2011) indicated that adding onion and garlic to the diet did not have a significant effect on the carcass yield of broiler chickens. Similarly, Bampidis et al. (2005) reported that incorporating oregano did not substantially impact body weight, carcass weight, or the relative weights of the heart and liver in broiler chickens. However, Sahu et al. (2017) found that treatment with *C. tora* positively affected dressing percentage and sensory evaluation compared to non-treated birds. Consistent with the current results, Omar et al. (2020) documented that supplementation with phenolic-rich onion extract did not significantly impact the percentages of gizzard, liver, and heart on day 35. According to Rahimi et al. (2011), the increased dressing percentage in broilers can be attributed to the stimulatory impacts of coriander, which contains certain phytochemical components that enhance pancreatic secretions. This stimulation promotes the secretion of digestive enzymes, improving the availability of supplementary nutrients, including amino acids, that are digested and absorbed in the gastrointestinal tract.

Supplementing with threonine and lysine has been shown to improve carcass traits and growth efficiency in broilers, presumably because threonine and lysine act as limiting amino acids for breast muscle production (Estalkhzir et al., 2013; Belloir et al., 2019). However, providing broilers with high AA diets can be costly, given the minimal enhancements in yield and the increased feed expenses, especially when combined with high feed component prices. Sufficient concentrations of AA, including lysine, are crucial for optimizing protein accretion and increasing meat yield (Kidd et al., 2004). Furthermore, it has been reported that increasing amino acid levels and utilizing pelleted diets positively influence breast meat yield, indicating that both pelleted diets and a higher AA density of 96% have beneficial effects (Rubio et al., 2020).

3.3 Immune response

The effect of diets supplemented with phytochemical antioxidants and the amino acid supplement (X-VIRUS) on the titers of antibodies for Newcastle Disease Virus (NVD), Avian Influenza Virus (AIV), and Infectious Bronchitis Virus (IBV) at 7 days post-vaccination was highly significant ($P \leq 0.05$) (Table 5). The groups fed a diet containing 1 and 1.5 ml/l of the X-VIRUS solution showed significantly higher antibody titers against NVD, AIV, and IBV compared to the other groups (T1 and T2) at different periods post-vaccination ($P \leq 0.05$). Medicinal herbs rich in phenols, flavonoids, tannins, essential oils, apigenin, caffeic acid, chlorogenic acid, ellagic acid, gallic acid, kaempferol, quercetin, and rutin possess antioxidant properties (Acamovic and Brooker, 2005; Hashemipour et al., 2013). Due to their antibacterial, antiviral, and antioxidant characteristics, these compounds are anticipated to enhance the immune response in broilers (Botsoglou et al., 2002). Separately, Yalçın et al. (2020) and their co-workers measured specific antibody titer (SRBC– sheep red blood cells) in the serum of laying hens and recorded that thyme-supplemented diets developed high rates in these parameters. This effect was deemed to be the consequence of the favorable impact of thyme on maintaining physiological balance immunocompetent cells. The supplementation of 120 and 240 mg/kg essential oil, respectively, increased serum antibody titers against NDV significantly over controls that were receiving the basic diet; as reported by Du et al. (2016). Liu et al. (2021) found that capsaicin-rich chili extract inclusion at 80 mg/kg in broiler diets improved antioxidant status, boosted immunological functions, and positively affected carcass traits. It acts as precursors for protein synthesis relative to having sulfur-containing amino acids such as methionine, and cysteine (Brosnan et al., 2006). Besides, the immune system function among many other physiological functions, these metabolites act as precursors for protein synthesis by methionine and cysteine. Basophil from human blood smear, Wright's stain The immune functions of the two types of immunity, cellular and humoral, involvement of methionine have been supported by various experiments (Métayer et al., 2008). It was found that broiler chickens receiving methionine and choline have increased levels of antibodies in the blood (Lopes et al., 2019). For instance, cysteine has been associated with lymphocyte and macrophage functions. Lymphocyte and macrophage functions in broilers have been found to respond well along with inflammatory responses to feed added cysteine and anti-Eimeria IgA levels in broilers fed the new diet were reported higher as compared to the traditionally fed broilers (Ren et al., 2020). Arginine's requirement supplementation above NRC recommendations improves growth performance and immune competence against avian infectious diseases, which has been corroborated in numerous studies (Jahanian et al., 2018). Valine has an intermediary role between myofibrilar muscle proteins, and weight reduction in lymphoid organs in chicken. Supplementing the diets of birds with high levels of valine has been shown to enhance antibody production when exposed to viruses (Baracos et al., 2006).

Table 5. Effects of dietary supplementation of phytochemical antioxidants and amino acid (X-VIRUS) on antibodies titer (Ab) against viral diseases ND, AI, IB ($\log_2$) at periods post vaccination (math. Mean ± standard error)

Treatments ml/l	Immunity Level AI HI titer	Immunity Level ND HI titer	Immunity Level IB HI titer
0	3.00 ± 0 c	6.00 ± 0.57 c	6.33 ± 1.20 b
0.5	3.33 ± 0.33 c	7.66 ± 0.33 b	6.66 ± 0.33 b
1	5.33 ± 0.66 b	8.33 ± 0.33 ab	8.33 ± 0.33 ab
1.5	7.66 ± 0.33 a	9.00 ± 0 a	9.66 ± 0.33 a
Significance level	**	**	**

*** Averages with different letters within one column indicate differences at a significant level.**

3.4 Blood biochemical parameters

3.4.1 Serum lipids

Table 6 presents the results of the X-VIRUS solution's effect on the serum lipid fractions of broilers at 35 days of age. The administration of 0.5, 1, and 1.5 ml/l of the X-VIRUS solution significantly reduced the levels of cholesterol, triglycerides, and LDL, while increasing HDL levels in broiler serum ($P \leq 0.05$). The bioactivity of flavones has been associated with various anti-obesity effects. For instance, luteolin, a flavone, acts as a strong inhibitor of triglyceride lipase in preadipocytes and enhances insulin sensitivity by activating the peroxisome proliferator-activated receptor γ (PPARγ) (Zheng et al., 2010). Moreover, flavones have the ability to reduce inflammation linked to obesity by inhibiting the nuclear factor kappa-B (NF-κB)-mediated pathway (González-Castejón and Rodriguez-Casado, 2011). According to Mirzaei-Aghsaghali (2012), onion extract stimulates the synthesis of bile acids and enhances the activity of pancreatic enzymes, primarily lipase and amylase, resulting in improved fat digestion. In agreement with our study, Gazwi et al. (2022) found that adding 500 to 1000 mg/kg of Coriandrum sativum grains and Cichorium intybus roots to the diet significantly reduced plasma concentrations of total cholesterol, triglycerides, and LDL. Additionally, Cao et al. (2012) indicated that extracts of C. sativum and C. intybus contain flavonoids capable of enhancing lipid metabolism in broiler chickens. Several researchers have demonstrated that the addition of fructans from chicory in the diet of broiler chickens brings a significant decrease in serum levels of total cholesterol, LDL, and triglycerides (Faramarzzadeh et al., 2017; Yusrizal and Chen, 2003; Mirza Aghazadeh et al., 2015; Khoobani et al., 2019). Polyphenols have been recognized for their ability to bind with cholesterol, thereby facilitating cholesterol fecal elimination (Roy and Schneeman, 1981). Furthermore, they also raise the cholesterol 7-alpha hydroxylase activity associated with the enzyme that has some connection with bile synthesis and cholesterol homeostasis (Chen et al., 2012). The reduction in serum cholesterol, LDL, and triglycerides with increase HDL levels observed in this case study suggests anti-atherosclerotic effect of X-VIRUS solution.

Table 6. Effects of dietary supplementation of phytochemical antioxidants and amino acid (X-VIRUS) on serum lipid fractions of broiler chickens (Means ± standard error)

Treatments ml/l	Cholesterol (mg/100ml)	Triglyceride (mg/100ml)	Concentration of high-density lipoproteins HDL (mg/100ml)	Concentration of low-density lipoproteins LDL (mg/100ml)
0	205.62±3.34 a	216.64±11.06 a	99.25 ± 2.28 b	63.03 ± 6.81 a
0.5	187.19 ± 1.54 b	186.21 ± 2.35 b	134.38±2.13 a	20.63± 8.07 b
1	190.85 ±1.51 b	156.56 ± 6.00 c	135.73 ±1.19 a	20.14± 2.83 b
1.5	185.60 ±5.19 b	152.91± 5.79c	136.05±3.15 a	17.55± 3.90 b
Significance level	**	**	**	**

*** Averages with different letters within one column indicate differences at a significant level.**

El-Katcha et al. (2018) reported that supplementing the diet with excess methionine (30%) led to the lowest concentrations of serum triglycerides. Additionally, the elevated serum cholesterol levels observed with 30% lysine supplementation may be attributed to lysine's role as a precursor of L-carnitine. This process could enhance the supply of carnitine for metabolic functions, particularly in fatty acid oxidation, thereby reducing the accumulation of long-chain fatty acids typically stored in adipose tissues. Furthermore, lysine may stimulate insulin release from the pancreas, potentially affecting lipid metabolism. Fouad et al. (2013) demonstrated that plasma total cholesterol levels were significantly lower in broilers receiving L-arginine treatments (0.25%, 0.50%, or 1.00% L-Arg) compared to the control. In addition, HDL-C levels were not affected by the treatments, while LDL-C levels were significantly lower with 1.00% L-Arg in compared to the control. Our findings also indicate that the amino acids content in the X-VIRUS solution lowers blood total cholesterol, triglyceride, and LDL levels but as for now, we have little information on how these amino acids really work on the serum lipids more especially to what extent L-arginine supplementation can reduce cholesterol. Chicks on a broiler diet and supplemented with L-arginine (0.25% or 1.00%) showed depressions in triglycerides but raised NEFAs hence indicating a possible role of L-arginine in converting the triglycerides into glycerol and free fatty acids (Fouad et al., 2013).

3.4.2 Total protein, albumin, globulin, and glucose levels

Table-7 Effect of Phytochemical Antioxidants and Amino Acids (X-VIRUS) on Serum Total Protein, Albumin, Glubulin, and Glucose Levels in Broilers at 35 Days of Age The effect of the X-VIRUS solution on serum total protein, albumin, globulin, and glucose in broilers at 35 days of age is presented in Table 7. It can be observed that supplementation with the X-VIRUS solution significantly enhanced total protein levels on an average basis ($P \leq 0.05$) in birds over the control group. This enhancement in total protein mainly resulted from increased globulin levels indicating a better immune status of the broiler birds as influenced by the supplementation of X-VIRUS solution. The findings of Mathivanan and Edwin (2012) and Hassan et al. (2016) which were in congruence with the present study also showed through their results that the Andrographis paniculata and moringa leaf supplementation increased levels of total proteins. The rise is generally associated with improved protein metabolism and synthesis but does not always translate into better growth performance. However, the X-VIRUS solution significantly decreased serum glucose levels ($P \leq 0.05$). No significant effect, however, was observed on albumin and globulin concentrations by the X-VIRUS treatment ($P > 0.05$).

Contrary to blood serum carried parameters such as total protein, albumin, globulin and glucose. the results of Ghazalah et al. (2007) and Mansoub (2011) who carried black pepper supplementation did not significantly impact serum parameters in agreement with the results of the present study, stated that chicory incorporation into broiler diets lowered serum glucose levels. Khoobani et al. (2019) also commented that there were lower serum glucose levels when chicory was incorporated into broiler diets. Gazwi et al. (2022) found reduced serum glucose levels in broilers fed Coriandrum sativum and Cichorium intybus extracts at six weeks of age, while this hypoglycemic effect could be as a result of some diterpenoids (Jain et al., 2000) and flavonoids (Gupta et al., 1983) which are reported to demonstrate glucose regulatory activities.

Table 7. Effects of dietary supplementation of phytochemical antioxidants and amino acid (X-VIRUS) on serum Total protein, Albumin, Globulin and glucose of broiler chickens (Means ± standard error)

Treatments ml/l	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)
0	3.57 ± 0.24 b	2.01 ± 0.08	1.563 ±0.18	304.95 ±2.04 a
0.5	4.47 ± 0.07 a	2.32 ± 0.05	2.15 ± 0.02	265.44 ±6.42 b
1	4.37± 0.07 a	2.35 ± 0.29	2.01 ± 0.36	251.59± 9.68 b
1.5	4.63 ± 0.1 a	2.56 ± 0.11	2.07 ± 0.05	225.03±7.2 c
Significance level	**	NS	NS	**

*** Averages with different letters within one column indicate differences at a significant level.**

Blood parameters can provide critical insight into various metabolites and physiological functions in poultry. For example, Soltan (2009) and Xue et al. (2018) found that L-glutamine supplementation could improve growth performance and serum biochemical indices, especially in animals affected by necrotic enteritis. However, contrasting with the current study, Daramola (2019) reported that supplementing broiler diets with medicinal plants, such as bitter leaf meal and moringa leaf meal, did not affect total protein levels. Abou-Kassem et al. (2022) reported that the highest concentrations of total protein were observed in the group fed a diet containing 0.90% Met + Cyst, compared to the control. An increase in TP and Alb was observed with increasing dietary sulfur amino acids, particularly DL-methionine, according to Ghavi et al. (2020). Therefore, the anabolic action of methionine would be related to an increased availability of substrates at the sites where protein synthesis takes place (Majdeddin et al., 2019). The same amino acid has been reported to enhance muscle protein synthesis, and lower catabolism in rapidly growing broilers (Zeitz et al., 2019). The increase in total protein levels in broilers is therefore a further possible effect of X-VIRUS solution whereby amino acids are made more available and this favors anabolism over catabolism..

3.4.3 Antioxidant capacity

in blood serum were significantly high (P<0.01) at 35 days of age from the groups fed with Mequindox alone Table 8. Effect of dietary supplementation with phytochemical antioxidants and amino acids (X-VIRUS) on AST, ALT, CAT, GSH-Px activities and MDA concentration in plasma of broilers at 35 days of age. Serum ALT activities, and MDA concentration in blood serum were significantly high (P<0.01) at 35 days of age from the groups fed Mequindox alone, were significantly affected by the inclusion of X-VIRUS in the diet compared to the control group (P≤0.05). The concentrations of ALT and MDA in the serum of broilers decreased, while GSH-Px increased with 1 and 1.5 ml/l of X-VIRUS solution (P≤0.05).

Table 8. Effects of dietary supplementation of phytochemical antioxidants and amino acid (X-VIRUS) on antioxidant enzymes activity of broiler chickens (Means ± standard error)

Treatments ml/l	ALT (U/l)	AST (U/l)	CAT (µg/ml)	GSH-Px (U/gHb)	MDA (mmo/ml)
0	12.09±0.29 a	21.51± 0.64	146.28±4.15	859.39±28.24 d	5.27± 0.22 a
0.5	11.84 ±0.26 a	20.16± 0.38	149.49±1.90	1020.73± 67.6 c	4.66 ±0.34 ab
1	10.84± 0.25 b	21.01± 0.33	150.05±2.29	1233.65±42.45 b	4.15± 0.04 b
1.5	10.60± 0.35 b	20.87± 0.38	152.36±0.60	1382.75±4.87 a	4.09±0.02 b
Significance level	**	NS	NS	**	**

*** Averages with different letters within one column indicate differences at a significant level.**
ALT =alanine transaminase, AST =aspartate aminotransferase, CAT= catalase and GSH-Px= Glutathione peroxidase, MDA= Malondialdehyde

The reduction in serum ALT levels following X-VIRUS supplementation suggests that the solution may have hepatoprotective effects, as it reduces liver enzyme discharge, unlike the control group. This emphasizes the importance of utilizing natural and organic antioxidants. In this study, X-VIRUS supplementation increased GSH-Px activity and decreased MDA levels, indicating improved oxidative stress management in the broilers. High GSH levels indicate that broilers vaccinated with X-VIRUS have a better ability to scavenge reactive oxygen species than the control, which is reflected by lower lipid peroxidation (MDA concentration). The increase in antioxidant status in X-VIRUS-treated broilers is due to the presence of polyphenolic compounds within the X-VIRUS solution. The higher dose of polyphenolic-rich X-VIRUS solution had a more potent effect in improving the redox status of broilers on all tested oxidative stress markers, excluding AST and CAT. This emphasizes how young chickens are highly vulnerable to oxidative stress and, thus, warrant antioxidant supplementation against the associated pathological conditions. Polyphenols and their metabolites, upon release of conjugates within cells, would provide powerful antioxidant activities (DE LA Torre et al., 2008). Other polyphenols may be of small quantity, though all act synergistically to enhance their bioactivity greatly (Pignatelli et al., 2006). The X-VIRUS remedy is rich in flavonoids such as apigenin, caffeic acid, chlorogenic acid, gallic acid, kaempferol, quercetin, and rutin. These said compounds provide the body with potent antioxidant defense as reported by this study. Corresponding to the present findings, Sahu et al. (2017) reported that chickens treated with Cassia tora extract had significantly reduced levels of MDA and increased GSH-Px activity in relation to lowered oxidative stress and lipid peroxidation. In another experiment by Papadopoulou et al. (2017), poultry was supplied with polyphenols extracted from olive mill wastewater in drinking water, which caused a remarkable improvement in the antioxidant capability.

Afkhami et al. (2020) also underlined the antioxidant functions of sulfur-containing amino acids like methionine and cysteine because they are direct precursors for the synthesis of glutathione in the body, while Ghavi et al. (2020) supported this finding. Their findings indicated that higher dietary sulfur amino acid (DSAA) levels were associated with decreased blood serum AST levels, hence indicating a more favorable condition of liver oxidative damage lowered ..

5. CONCLUSION

Finally, inclusion of different doses of the X-VIRUS supplement in broilers' drinking water was beneficial. It enhanced growth performance, immune response to NDV, AIV, and IBV, and had favorable effects on the serum lipid profile through decreased levels of serum glucose cholesterol, triglycerides, and LDL levels, while increasing HDL levels and total protein. In a word, X-VIRUS supplementation has the potential to lower glucose, a notable antioxidant index like ALT and MDA, while upping GSH-Px enzyme activity in the blood. These findings suggest that X-VIRUS could serve as a valuable dietary supplement for promoting the health and performance of broilers.

Competing interests

The authors declared no conflicts of interest or competing interests to disclose.

REFERENCES

1. Abou-Kassem, D. E., El-Abasy, M. M., Al-Harbi, M. S., Abol-Ela, S., Salem, H. M., El-Tahan, A. M., El-Saadony, M. T., Abd El-Hack, M. E., & Ashour, E. A. (2022). Influences of total sulfur amino acids and photoperiod on growth, carcass traits, blood parameters, meat quality and cecal microbial load of broilers. *Saudi journal of Biological Sciences*, 29(3),1683-1693. https://doi.org/10.1016/j.sjbs.2021.10.063
2. Acamovic, T., & Brooker, J. D. (2005). Biochemistry of plant secondary metabolites and their effects in animals. *Proceedings of The Nutrition Society*, 64(3), 403-412. https://doi.org/10.1079/PNS2005449
3. Afkhami, M., Kermanshahi, H., & Majidzadeh Heravi, R. (2020). Evaluation of whey protein sources on performance, liver antioxidants and immune responses of broiler chickens challenged with ethanol. *Journal of Animal Physiology and Animal Nutrition* 104(3), 898-908. https://doi.org/10.1111/jpn.13327
4. Aji, S. B., Ignatius, K., Asha'Adatu, Y., Nuhu, J. B., Abdulkarim, A., Aliyu, U., Gambo, M. B., Ibrahim, M. A., & Abubakar, H. (2011). Effects of feeding onion (Allium cepa) and garlic (Allium sativum) on. *Research Journal of Poultry Science,* 4, 22-27.
5. Amezcua, C. M., Parsons, C. M., Singh, V., Srinivasan, R., & Murthy, G. S. (2007). Nutritional characteristics of corn distillers dried grains with solubles as affected by the amounts of grains versus solubles and different processing techniques. *Poultry Science*, 86(12), 2624-2630. https://doi.org/10.3382/ps.2007-00137
6. Amezcua, C. M., Parsons, C. M., Singh, V., Srinivasan, R., & Murthy, G. S. (2007). Nutritional characteristics of corn distillers dried grains with solubles as affected by the amounts of grains versus solubles and different processing techniques. *Poultry science*, 86(12), 2624-2630. https://doi.org/10.3382/ps.2007-00137
7. Andersson, M. I., & MacGowan, A. P. (2003). Development of the quinolones. *Journal of Antimicrobial Chemotherapy*, 51(1), 1-11. https://doi.org/10.1093/jac/dkg212
8. Attia, Y. A., Hassan, R. A., Shehatta, M. H., & Abd-El-Hady, S. B. (2005). Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to diets containing 2. Different levels of methionine. *International Journal of Poultry Science*, 4(11), 856-865. https://doi.org/10.3923/ijps.2005.856.865
9. Aviagen. (2018). Ross Broiler Management Handbook-2018. In Aviagen Ross Management Guide, 1–147. Huntsville, AL: Aviagen Group. https://aviagen.com/assets/Tech_Center/Ross_Broiler/Ross- BroilerHandbook2018-EN.pdf.

10. Ayssiwede, S. B., Chrysostome, C., Ossebi, W., Dieng, A., Hornick, J. L., & Missohou, A. (2010). Digestibility and metabolic utilisation and nutritional value of Cassia tora (Linn.) leaves meal incorporated in the diets of indigenous Senegal chickens. *Revue de Médecine Vétérinaire,* 549-558. https://doi/full/10.5555/20113044568
11. Baker, D. H. (2009). Advances in protein-amino acid nutrition of poultry. *Amino Acids,* 37, 29-41. https://doi.org/10.1007/s00726-008-0198-3
12. Bampidis, V. A., Christodoulou, V., Florou-Paneri, P., Christaki, E., Chatzopoulou, P. S., Tsiligianni, T., & Spais, A. B. (2005). Effect of dietary dried oregano leaves on growth performance, carcase characteristics and serum cholesterol of female early maturing turkeys. *British Poultry Science,* 46(5), 595-601. https://doi.org/10.1080/00071660500256057
13. Baracos, V. E., & Mackenzie, M. L. (2006). Investigations of Branched-Chain Amino Acids and Their Metabolites inAnimal Models of Cancer. *The Journal of Nutrition,* 136(1), 237S-242S. https://doi.org/10.1093/jn/136.1.237S
14. Bartell, S. M., & Batal, A. B. (2007). The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract, and humoral immune response of broilers. *Poultry Science,* 86(9), 1940-1947. https://doi.org/10.1093/ps/86.9.1940
15. Belloir, P., Lessire, M., Lambert, W., Corrent, E., Berri, C., & Tesseraud, S. (2019). Changes in body composition and meat quality in response to dietary amino acid provision in finishing broilers. *Animal,* 13(5), 1094-1102. https://doi.org/10.1017/S1751731118002306
16. Billah, M. M., Rana, S. M., Hossain, M. S., Ahamed, S. K., Banik, S., & Hasan, M. (2015). Ciprofloxacin residue and their impact on biomolecules in eggs of laying hens following oral administration. *International Journal of Food Contamination,* 2,1-7. https://doi.org/10.1186/s40550-015-0019-x
17. Boney, J. W., & Moritz, J. S. (2017). The effects of Spirulina algae inclusion and conditioning temperature on feed manufacture, pellet quality, and true amino acid digestibility. *Animal Feed Science and Technology,* 224, 20-29. https://doi.org/10.1016/j.anifeedsci.2016.11.008
18. Botsoglou, N. A., Florou-Paneri, P., Christaki, E., Fletouris, D. J., & Spais, A. B. (2002). Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *British Poultry Science,* 43(2), 223-230. https://doi.org/10.1080/00071660120121436
19. Bravo, D., & Ionesco, C. (2008). Meta analysis of the effect of a mixture of carvacrol, cinnamaldehyde and capsicum oleoresin in broilers. In Book of Abstracts for the 10th World Conference on Animal Production. 150-150. Wageningen Academic. https://doi.org/10.3920/9789086865789_300
20. Brosnan, J., & Brosnan, M. (2006). 5th Amino Acid Assessment Workshop—The Sulfur-Containing Amino Acids: An Overview. *The Journal of Nutrition*, 136, 16365-16405. https://doi.org/10.1093/jn/136.6.1636S
21. Cao, F. L., Zhang, X. H., Yu, W. W., Zhao, L. G., & Wang, T. (2012). Effect of feeding fermented Ginkgo biloba leaves on growth performance, meat quality, and lipid metabolism in broilers. *Poultry Science*, 91(5), 1210-1221. https://doi.org/10.3382/ps.2011-01886
22. Chen, Q., Wang, E., Ma, L., & Zhai, P. (2012). Dietary resveratrol increases the expression of hepatic 7α-hydroxylase and ameliorates hypercholesterolemia in high-fat fed C57BL/6J mice. *Lipids in Health and Disease,* 11, 1-8. https://doi.org/10.1186/1476-511X-11-56
23. Chrystal, P. V., Moss, A. F., Khoddami, A., Naranjo, V. D., Selle, P. H., & Liu, S. Y. (2020). Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. *Poultry Science,* 99(3), 1421-1431. https://doi.org/10.1016/j.psj.2019.10.060
24. Comi, M. (2014). The role of some additives in enhancing feed efficiency and health in monogastric animals. M. S. thesis, Università degli Studi di Milano.
25. Daramola, O. T. (2019). Medicinal plants leaf meal supplementation in broiler chicken diet: effects on performance characteristics, serum metabolite and antioxidant status. *Animal Research International,* 16(2), 3334-3342.
26. De la Torre, R. (2008). Bioavailability of olive oil phenolic compounds in humans. *Inflammopharmacology*, 16(5), 245-247. https://doi.org/10.1007/s10787-008-8029-4
27. Du, E., Wang, W., Gan, L., Li, Z., Guo, S., & Guo, Y. (2016). Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with Clostridium perfringens. *Journal of Animal Science and Biotechnology*, 7, 1-10. https://doi.org/10.1186/s40104-016-0079-7
28. El-Katcha, M. I., Soltan, M. A., El-Shall, N. A., & El-Desoky, A.M. (2018). Effect of high dietary level of some amino acids and coccidial infection on growth performance and health status of broiler chicken. *Alexandria Journal of Veterinary Sciences*, 58(1). https://doi.org/110.5455/ajvs.281585

29. Estalkhzir, F. M., Khojasteh, S., & Jafari, M. (2013). The effect of different levels of threonine on performance and carcass characteristics of broiler chickens. *Journal of Novel Applied Sciences*, 2(9), 382-386.
30. Faramarzzadeh, M., Behroozlak, M., Samadian, F., & Vahedi, V. (2017). Effects of chicory powder and butyric acid combination on performance, carcass traits and some blood parameters in broiler chickens. *The Iranian Journal of Applied Animal Science,* 7(1), 139-145.
31. Fouad, A. M., El-Senousey, H. K., Yang, X. J., & Yao, J. H. (2013). Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. *Animal*, 7(8), 1239-1245. https://doi.org/10.1017/S1751731113000347
32. Gazwi, H. S., Mahmoud, M. E., & Toson, E. M. (2022). Analysis of the phytochemicals of Coriandrum sativum and Cichorium intybus aqueous extracts and their biological effects on broiler chickens. *Scientific Reports,* 12(1), 6399. https://doi.org/10.1038/s41598-022-10329-2
33. Gehring, C. K., Lilly, K. G. S., Shires, L. K., Beaman, K. R., Loop, S. A., & Moritz, J. S. (2011). Increasing mixer-added fat reduces the electrical energy required for pelleting and improves exogenous enzyme efficacy for broilers. *The Journal of Applied Poultry Research,* 20(1), 75-89. https://doi.org/10.3382/japr.2009-00082
34. Ghavi, S., Zarghi, H., & Golian, A. (2020). Effect of dietary digestible sulphur amino acids level on growth performance, blood metabolites and liver functional enzymes of broilers 1–11 days of age. *The Italian Journal of Animal Science,* 19(1), 1439-1449. https://doi.org/10.1080/1828051X.2020.1847606
35. Ghazalah, A. A., El-Hakim, A. S. A., & Refaie, A. M. (2007). Response of broiler chicks to some dietary growth promoters throughout different growth periods. *Egyptian Poultry Science Journal,* 53-77. https://doi.org/doi/full/10.5555/20083151188
36. González-Castejón, M., & Rodriguez-Casado, A. (2011). Dietary Phytochemicals and Their Potential Effects on Obesity: a Review. *Pharmacological research*, 64 (5), 438–455. https://doi.org/10.1016/j.phrs.2011.07.004
37. Gupta, K. K., Taneja, S. C., Dhar, K. L., & Atal, C. K. (1983). Flavonoids of Andrographis paniculata. *Phytochemical,* 22(1), 314-315. https://doi.org/10.1016/S0031-9422(00)80122-3
38. Hashemipour, H., Kermanshahi, H., Golian, A., & Veldkamp, T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry Science*, 92(8), 2059-2069. https://doi.org/10.3382/ps.2012-02685
39. Hassan, H. M. A., El-Moniary, M. M., Hamouda, Y., El-Daly, E. F., Youssef, A. W., & Abd El-Azeem, N. A. (2016). Effect of different levels of Moringa oleifera leaves meal on productive performance, carcass characteristics and some blood parameters of broiler chicks reared under heat stress conditions. *Asian Journal of Animal and Veterinary Advances,* 11(1), 60-66. https://doi.org/10.3923/ajava.2016.60.66
40. Hilliar, M., Keerqin, C., Girish, C. K., Barekatain, R., Wu, S. B., & Swick, R. A, (2020). Reducing protein and supplementing crystalline amino acids, to alter dietary amino acid profiles in birds challenged for subclinical necrotic enteritis. *Poultry Science,* 99(4), 2048-2060. https://doi.org/10.1016/j.psj.2019.11.042
41. Imari, Z. K., Hassanabadi, A., & Nassiri Moghaddam, H. (2020). Response of broiler chickens to calcium and phosphorus restriction: Effects on growth performance, carcase traits, tibia characteristics and total tract retention of nutrients. *Italian journal of animal science,* 19(1), 929-939. https://doi.org/10.1080/1828051X.2020.1808101
42. Jahanian, R., & Khalifeh-Gholi, M. (2018). Marginal deficiencies of dietary arginine and methionine could suppress growth performance and immunological responses in broiler chickens. *Journal of Animal Physiology and Animal Nutrition,* 102(1), e11-e20. https://doi.org/10.1111/jpn.12695
43. Jain, D. C., Gupta, M. M., Saxena, S., & Kumar, S. (2000). LC analysis of hepatoprotective diterpenoids from Andrographis paniculata. *Journal of Pharmaceutical and Biomedical Analysis*, 22(4), 705-709. https://doi.org/10.1016/S0731-7085(99)00297-6
44. Khoobani, M., Hasheminezhad, S. H., Javandel, F., Nosrati, M., Seidavi, A., Kadim, I. T., Laudadio, V., & Tufarelli, V. (2019). Effects of dietary chicory (Chicorium intybus L.) and probiotic blend as natural feed additives on performance traits, blood biochemistry, and gut microbiota of broiler chickens. *Antibiotics,* 9(1), 5. https://doi.org/10.3390/antibiotics9010005
45. Kidd, M. T., McDaniel, C. D., Branton, S. L., Miller, E. R., Boren, B. B., & Fancher, B. I. (2004). Increasing amino acid density improves live performance and carcass yields of commercial broilers. *Journal of Applied Poultry Research,* 13(4), 593-604. https://doi.org/10.1093/japr/13.4.593
46. Kim, J. E., Lillehoj, H. S., Hong, Y. H., Kim, G. B., Lee, S. H., Lillehoj, E. P., & Bravo, D. M. (2015). Dietary Capsicum and Curcuma longa oleoresins increase intestinal microbiome and necrotic enteritis in three commercial broiler breeds. *Research journal of Veterinary Sciences,* 102, 150-158. https://doi.org/10.1016/j.rvsc.2015.07.022
47. Kuldeep Dhama, K. D., Ruchi Tiwari, R. T., Khan, R. U., Sandip Chakraborty, S. C., Marappan Gopi, M. G., Kumaragurubaran Karthik, K. K., Mani Saminathan, M. S., Desingu, P. A., & Sunkara, L. T. (2014). Growth promoters and

novel feed additives improving poultry production and health, bioactive principles and beneficial applications: the trends and advances-a review. *International Journal of Pharmaceutics*, 10, 129-159. https://doi/full/10.5555/20143296417

48. Liu, S. J., Wang, J., He, T. F., Liu, H. S., & Piao, X. S. (2021). Effects of natural capsicum extract on growth performance, nutrient utilization, antioxidant status, immune function, and meat quality in broilers. *Poultry Science,* 100(9), 101301. https://doi.org/10.1016/j.psj.2021.101301
49. Loar II, R. E., Wamsley, K. G. S., Evans, A., Moritz, J. S., & Corzo, A. (2014). Effects of varying conditioning temperature and mixer-added fat on feed manufacturing efficiency, 28-to 42-day broiler performance, early skeletal effect, and true amino acid digestibility. *Journal of Applied Poultry Research,* 23(3), 444-455. https://doi.org/10.3382/japr.2013-00930
50. Lopes, M. G., Dominguez, J. H. E., Peter, C. M., Santos, E., Rodrigues, P. A., Rodrigues, P. R. C., Picoli, T., Corrêa, M. N., Schmitt, E., de Lima, M., & Fischer, G. (2019). Resposta imune humoral em novilhas de corte suplementadas com sal mineral com ou sem adição de metionina protegida da degradação ruminal. Semina: *Revista de Ciências Agrárias,* 40, 3057-3068. https://doi.org/10.5433/1679-0359.2019v40n6Supl2p3057
51. Luangtongkum, T., Morishita, T. Y., Ison, A. J., Huang, S., McDermott, P. F., & Zhang, Q. (2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of Campylobacter spp. in poultry. *Applied and Environmental Microbiology,* 72(5), 3600-3607. https://doi.org/10.1128/AEM.72.5.3600-3607.2006
52. Lynch, E., Bowen, K., Ayres, V., Boltz, T., Wamsley, K. G. S., Boney, J. W., & Moritz, J. S. (2023). Hygienic pelleting can decrease Hubbard× Ross 708 apparent ileal amino acid digestibility, broiler performance, and increase digestible amino acid requirement. *Journal of Applied Poultry Research,* 32(3),100355. https://doi.org/10.1016/j.japr.2023.100355
53. Majdeddin, M., Golian, A., Kermanshahi, H., Michiels, J., & De Smet, S. (2019). Effects of methionine and guanidinoacetic acid supplementation on performance and energy metabolites in breast muscle of male broiler chickens fed corn-soybean diets. *British Poultry Science,* 60(5), 554-563. https://doi.org/10.1080/00071668.2019.1631447
54. Mansoub, N. H. (2011). Comparison of using different level of black pepper with probiotic on performance and serum composition of broiler chickens. *Journal of Basic and Applied Sciences,* 1, 2425–2428.
55. Martinez, M., McDermott, P., & Walker, R. (2006). Pharmacology of the fluoroquinolones: a perspective for the use in domestic animals. *The veterinary journal,* 172(1), 10-28. https://doi.org/10.1016/j.tvjl.2005.07.010
56. Mathivanan, R., & Edwin, S. C. (2012). Hematological and serum biochemical parameters of broilers fed with Andrographis paniculata as an alternative to antibiotic growth promoter. *Journal of Medicinal Plants Research,* 6(44), pp.5647-5650. https://doi.org/10.5897/JMPR12.430
57. Métayer, S., Seiliez, I., Collin, A., Duchêne, S., Mercier, Y., Geraert, P. A., & Tesseraud, S. (2008). Mechanisms through which sulfur amino acids control protein metabolism and oxidative status. *The Journal of Nutritional Biochemistry,* 19(4), 207-215. https://doi.org/10.1016/j.jnutbio.2007.05.006
58. Mirza Aghazadeh, A., & Nabiyar, E. (2015). The effect of chicory root powder on growth performance and some blood parameters of broilers fed wheat-based diets. *Journal of Applied Animal Research,* 43(4), 384-389. https://doi.org/10.1080/09712119.2014.978778
59. Mirzaei-Aghsaghali A. (2012). Importance of medical herbs in animal feeding: A review. *Annals of Biological Research,* 3(2), 918-923.
60. National Research Council, and Subcommittee on Poultry Nutrition. Nutrient requirements of poultry: 1994. National Academies Press. Nottingham, UK.
61. Omar, A. E., Al-Khalaifah, H. S., Mohamed, W. A., Gharib, H. S., Osman, A., Al-Gabri, N. A., & Amer, S. A. (2020). 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. *Frontiers in Veterinary Science*, 7, 582612. https://doi.org/10.3389/fvets.2020.582612
62. Østergård, K., Björck, I., & Vainionpää, J. (1989). Effects of extrusion cooking on starch and dietary fibre in barley. *Food Chemistry,* 34(3), 215-227. https://doi.org/10.1016/0308-8146(89)90142-8
63. Papadopoulos, M.C. (1989). Effect of processing on high-protein feedstuffs: a review. *Biological wastes,* 29(2), 123-138. https://doi.org/10.1016/0269-7483(89)90092-X
64. Papadopoulou, A., Petrotos, K., Stagos, D., Gerasopoulos, K., Maimaris, A., Makris, H., Kafantaris, I., Makri, S., Kerasioti, E., Halabalaki, M., & Brieudes, V. (2017). Enhancement of antioxidant mechanisms and reduction of oxidative stress in chickens after the administration of drinking water enriched with polyphenolic powder from olive mill waste waters. Oxidative medicine and cellular longevity. https://doi.org/10.1155/2017/8273160
65. Pignatelli, P., Ghiselli, A., Buchetti, B., Carnevale, R., Natella, F., Germano, G., Fimognari, F., Di Santo, S., Lenti, L., & Violi, F. (2006). Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. *Atherosclerosis,* 188(1), 77-83. https://doi.org/1016/j.atherosclerosis.2005.10.025

66. Rahimi, S., Teymori Zadeh, Z., Torshizi, K., Omidbaigi, R., & Rokni, H. (2011). Effect of the three herbal extracts on growth performance, immune system, blood factors and intestinal selected bacterial population in broiler chickens. *Journal of Agricultural Science and Technology*, 13(4), 527-539. https://doi.org/10.1001.1.16807073.2011.13.4.6.5
67. Ren, Z., Bütz, D. E., Whelan, R., Naranjo, V., Arendt, M. K., Ramuta, M. D., Yang, X., Crenshaw, T. D., & Cook, M. E. (2020). Effects of dietary methionine plus cysteine levels on growth performance and intestinal antibody production in broilers during Eimeria challenge. *Poultry Science*, 99(1), 374-384. https://doi.org/10.3382/ps/pez503
68. Roy, D. M. & Schneeman, B. O. (1981). Effect of soy protein, casein and trypsin inhibitor on cholesterol, bile acids and pancreatic enzymes in mice. *Journal of Nutrition*, 111(5), 878-885. https://doi.org/10.1093/jn/111.5.878
69. Rubio, A. A., Hess, J. B., Berry, W. D., Dozier III, W. A., & Pacheco, W. J. (2020). Effects of feed form and amino acid density on productive and processing performance of broilers. *Journal of Applied Poultry Research*, 29(1), 95-105. https://doi.org/10.3382/japr/pfz032
70. Sahu, J., Koley, K. M., & Sahu, B. (2017). Attribution of antibacterial and antioxidant activity of Cassia tora extract toward its growth promoting effect in broiler birds. *Veterinary world*, 10(2), 221. https://doi.org/10.14202/vetworld.2017.221-226
71. Scriver, C. R. (2001). Garrod's foresight; our hindsight. *Journal of inherited metabolic disease*, 24, 93–116. https://doi.org/10.1023/A:1010351630856
72. Soltan, M. A. (2009). Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and some blood parameters of broiler chickens. *International Journal of Poultry Science*, 8(1), 60-68. https://doi.org/10.3923/ijps.2009.60.68
73. Thomas, M., Van Zuilichem, D. J., & Van der Poel, A. F. B. (1997). Physical quality of pelleted animal feed. 2. Contribution of processes and its conditions. *Animal Feed Science and Technology*, 64(2-4), 173-192. https://doi.org/10.1016/S0377-8401(96)01058-9
74. Ul-Haq, I., Ullah, N., Bibi, G., Kanwal, S., Ahmad, M. S., & Mirza, B. (2012). Antioxidant and cytotoxic activities and phytochemical analysis of Euphorbia wallichii root extract and its fractions. *Iranian Journal of Pharmaceutical Research*, 11(1), 241. https://doi.org/10. 22037/ IJPR. 2011. 1030
75. Xue, G. D., Barekatain, R., Wu, S. B., Choct, M., & Swick, R. A. (2018). Dietary L-glutamine supplementation improves growth performance, gut morphology, and serum biochemical indices of broiler chickens during necrotic enteritis challenge. *Poultry Science*, 97(4), 1334-1341. https://doi.org/10.3382/ps/pex444
76. Yadav, A. S., Gautham Kolluri, G. K., Marappan Gopi, M. G., Kumaragurubaran Karthik, K. K., Malik, Y. S., & Kuldeep Dhama, K. D. (2016). Exploring alternatives to antibiotics as health promoting agents in poultry-a review. https://doi.org/10.18006/2016.4(3S).368.383
77. Yalçin, S., Handan, E. S. E. R., Onbaşilar, İ., &Yalçin, S. (2020). Effects of dried thyme (Thymus vulgaris L.) leaves on performance, some egg quality traits and immunity in laying hens. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 67(3), 303-311.
78. Yusrizal, Y., & Chen, T. C. (2003). Effect of adding chicory fructans in feed on fecal and intestinal microflora and excreta volatile ammonia. *International Journal of Poultry Science*, 2, 188-194. https://doi.org/10.5555/20033119231
79. Zeitz, J. O., Mohrmann, S., Käding, S. C., Devlikamov, M., Niewalda, I., Whelan, R., Helmbrecht, A., & Eder, K. (2019). Effects of methionine on muscle protein synthesis and degradation pathways in broilers. *Journal of Animal Physiology and Animal Nutrition*, 103(1), 191-203. https://doi.org/10.1111/jpn.13026
80. Zheng, C. D., Duan, Y. Q., Gao, J. M. & Ruan, Z.G. (2010). Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs. *Journal of the Chinese Medical Association*, 73(6), 319-324. https://doi.org/10.1016/S1726-4901(10)70068-X