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RESEARCH ARTICLE

Growth Performance and Serum Biochemical Profile of Japanese Quail as Affected by Pine Needles and Vitamin E Powder

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ABSTRACT

This study was conducted to evaluate the effects of dietary supplementation with pine needle powder and vitamin E on the growth performance and serum biochemical profile of Japanese quail (*Coturnix coturnix japonica*). A total of 160 day-old quail chicks were randomly assigned to four dietary treatment groups with four replicates of 10 birds each. The treatments included: AS1 (control, basal diet), AS2 (basal diet + 200 mg/kg pine needle powder), AS3 (basal diet + 200 mg/kg vitamin E), and AS4 (basal diet + 200 mg/kg pine needle powder + 200 mg/kg vitamin E). The feeding trial lasted for six weeks. Results showed that birds in the AS4 group exhibited significantly higher ($p < 0.05$) body weight gain and improved feed conversion ratio (FCR) compared to the control and other treatment groups. Supplementation with pine needles and vitamin E, both individually and in combination, did not affect feed intake significantly but enhanced growth efficiency. The serum biochemical analysis revealed that total protein, albumin, and globulin levels were significantly increased ($p < 0.05$) in the supplemented groups, particularly in AS4. Moreover, serum cholesterol and triglycerides were markedly reduced, while liver enzyme activities (ALT and AST) were significantly lower in treated groups, indicating improved liver function and reduced oxidative stress. In conclusion, the combined supplementation of pine needle powder and vitamin E positively influenced growth performance, protein metabolism, lipid profile, and liver enzyme activity in Japanese quail. These findings suggest that this natural antioxidant blend could serve as an effective alternative to synthetic growth promoters for enhancing the productivity and health status of poultry.

1. Introduction

Feed additives can be natural or synthetic sources that are added to the diet to perform one or more functions that may enhance growth performance, improve health condition, and ensure the safety of animal products. Japanese quail (*Coturnix coturnix japonica*) has emerged as a model bird in avian research and a commercially valuable species due to its rapid growth rate, early sexual maturity, high egg production, and short generation interval [1]. Optimizing the nutrition of Japanese quail through supplementation with natural ingredients such as pine needles and vitamin E is a promising strategy to boost productivity and health, particularly in the face of increasing consumer demand for residue-free poultry products [2]. Pine needles, especially from species like *Pinus roxburghii* and *Pinus sylvestris*, are rich in bioactive compounds including polyphenols, flavonoids, essential oils, and antioxidants [3]. These natural compounds are known to exhibit antimicrobial, anti-inflammatory, and immunomodulatory properties. Traditionally used in herbal medicine, pine needles have gained attention as potential feed

additives for livestock and poultry. Their antioxidant activity may reduce oxidative stress in birds, thereby supporting better nutrient utilization and growth [4]. Studies have shown that dietary supplementation of pine needle powder can enhance feed efficiency, body weight gain, and general health in poultry by improving intestinal health and immunity [5]. Vitamin E (α-tocopherol), a fat-soluble antioxidant, plays a vital role in protecting cellular membranes from oxidative damage, enhancing immune responses, and improving overall performance in poultry [2, 6]. It scavenges free radicals and stabilizes polyunsaturated fatty acids in the cell membranes, thereby reducing lipid peroxidation and maintaining cellular integrity [7]. In Japanese quail, vitamin E supplementation has been reported to improve growth rate, reproductive performance, and serum biochemical parameters such as total protein, cholesterol, triglycerides, and liver enzymes [8]. It also modulates the oxidative status and immune function, thereby contributing to enhanced disease resistance and better meat quality.

Combining pine needles and vitamin E may provide synergistic effects by amplifying their individual benefits. The antioxidant components in pine needles, such as chlorophyll, proanthocyanidins, and vitamin C, may work in concert with vitamin E to further reduce oxidative stress and support metabolic functions in growing quail [9]. Additionally, the phytochemicals in pine needles may modulate the gut microbiota and enhance nutrient absorption, leading to improved growth performance and serum biochemical profiles. Despite the promising attributes of both pine needles and vitamin E, studies investigating their combined effects on Japanese quail are limited. Serum biochemical parameters serve as critical indicators of health, metabolism, and organ function in poultry. Parameters such as total protein, albumin, globulin, cholesterol, triglycerides, glucose, and liver enzymes (ALT, AST) reflect the physiological and metabolic status of birds under different dietary treatments [10]. Evaluating these biomarkers in response to dietary supplementation with pine needles and vitamin E provides valuable insights into their efficacy and safety as functional feed ingredients. Given the need for safe, natural growth promoters in the poultry industry, and the increasing scrutiny over the use of synthetic additives and antibiotics, exploring alternative feed additives like pine needles and vitamin E is both timely and relevant. Such additives not only enhance animal performance but also contribute to sustainable and organic poultry farming practices [2, 7]. Moreover, with the rising cost of commercial feed additives, exploring the potential of readily available natural resources like pine needles could be economically advantageous, especially in regions where these trees are abundant. The aim of this study is to determine the separate and combined effects of pine needle powder and vitamin E powder on the growth performance and serum biochemical profile of Japanese quail. The study builds on previous research by examining how a natural feed supplement affects body weight gain, feed conversion ratio, and important serum metabolites, with the aim of offering practical guidance on its use in quail production.

2. Materials and methods

2.1. *Site and Duration of Experiment*

The experiment was performed at the Hazara University in 2016-2017 at Biochemistry Lab under controlled environmental conditions. The experiment consisted of a 1-week adaptation period and a 5-week experimental feeding period. Basal diet

Table 1: Ingredients in basal diet.

Ingredient	**(%)**
Rice	14.00
Soybean meal	17.00 (46%)
Corn	41.00
Rice polish	5.00
Gluten meal	3.00 (27%)
Fish meal	3.00
Canola meal	7.00
Molasses	4.00
Marble chips	0.50
Guar meal	3.00
DL-methionine	0.1
Dicalcium phosphate	1.70
Vitamin + mineral remix	10.24
Salt	0.16
L-lysine HCl	0.3
Analyzed composition	
Lysine	1.32
Crude protein	24.00
DL-methionine	0.51
Metabolizable energy	(kcal kg−1) 2900

Provided per kg of diet: vitamin A (retinol) - 12,000 IU; vitamin D3 (cholecalciferol) - 2400 IU; vitamin E (DL-α-tocopherol) - 50 IU; vitamin K (menadione) - 4 mg; vitamin B1 (thiamine) - 3 mg; vitamin B2 (riboflavin) - 6 mg; vitamin B5 Fe - 60 mg; choline chloride - 200 mg; Cu - 5 mg; Co - 0.2 mg; Mn - 80 mg; Se - 0.15 mg; Zn - 60 mg; I - 1 mg; (pantothenic acid) - 25 mg; vitamin B6 (pyridoxine) - 5 mg; vitamin B12 (cyanocobalamin) - 0.03 mg; folic acid - 1 mg

2.2 Animals and the Management of Experiments

A 160 Japanese quail (*Coturnix coturnix japonica*) chicks purchased from a commercial hatchery, all of them healthy and one-day old. Upon arrival, the birds were weighed and divided into four nutritional treatment groups at random. Each treatment group included four duplicates, with 10 birds per replicate (a total of 40 birds per treatment group). These birds were housed in identically sized cages that were maintained clean and ventilated, and they had unlimited access to feed and water. Standard brooding management practices (23L:1D lighting program and vaccination schedule) were implemented.

2.3 Experimental Design and Diets

A completely randomized design (CRD) was employed to assess the effects of pine needle powder and vitamin E powder. The birds were allocated to the following dietary treatments: AS1 (Control): Basal diet without supplementation; AS2: Basal diet + 200 mg/kg pine needle powder; AS3: Basal diet + 200 mg/kg vitamin E powder; AS4: Basal diet + 100 mg/kg pine needle powder + 100 mg/kg vitamin E powder. The basal diet was formulated to meet the nutritional requirements of growing Japanese quail according to NRC (1994) guidelines. Pine needles (*Pinus roxburghii*) were collected from mature trees, washed, shade-dried, ground into fine powder, and stored in airtight containers. The vitamin E used in the study was DL-α-tocopheryl acetate (98% purity). (Table 1).

2.4 Growth Performance

At the beginning and end of the experiment, each bird was weighed, and the increase in body weight was used to calculate body weight gain. The total feed supplied was subtracted from the residual feed to determine the weekly feed consumption. The following is the feed-conversion ratio (FCR):

$$\text{FCR} = \frac{\text{Total feed intake (g)}}{\text{Body weight gain (g)}}$$

Mortality, if any, was recorded daily, and growth data were adjusted accordingly.

2.5 Blood Sample Collection

Three birds per duplicate (n = 12 birds per treatment) were chosen at random and fasted for the whole night at the conclusion of the 6-week period. Sterile syringes were used to draw 2–3 mL of blood from the wing vein. After being moved into standard centrifuge tubes, the samples were left to coagulate at room temperature. Centrifugation at 3,000 rpm for 10 minutes was used to separate the serum, which was then kept at -20°C until additional biochemical examination.

2.6 Serum Biochemical Analysis

Using an automated biochemistry analyzer and commercial diagnostic kits (e.g., Randox, Merck), serum samples were examined for the following biochemical parameters: Alanine aminotransferase (ALT; U/L), aspartate aminotransferase (AST; U/L), total cholesterol (mg/dL), triglycerides (mg/dL), glucose (mg/dL), total protein (g/dL), albumin (g/dL), and globulin (g/dL; determined by deducting albumin from total protein). To guarantee accuracy, every measurement was carried out three times.

2.7 Data Analysis

One-way analysis of variance (ANOVA) was used to analyze the data using SPSS version (version 12.0). Duncan's Multiple Range Test (DMRT) was used to separate means if differences were found ($p < 0.05$). Means ± standard error are used to display the results.

3 Results

The inclusion of pine needle powder and vitamin E, either individually or in combination, had a significant effect ($p < 0.05$) on the growth performance of Japanese quail over the 6-week feeding period (Table 2 and Figure 1). Birds in the AS4 group (200 mg/kg pine needles + 200 mg/kg vitamin E) recorded the highest final body weight (182.5 ± 2.3 g) and total body weight gain (138.4 ± 2.1 g), followed by the AS3 group supplemented with vitamin E alone (174.2 ± 1.9 g). The AS2 group (pine needles only) also showed a significant improvement in body weight gain (167.6 ± 2.0 g) compared to the control group (AS1), which had the lowest final body weight (159.3 ± 2.2 g). Feed intake did not differ significantly ($p > 0.05$) among the treatment groups, indicating that dietary supplementation did not adversely affect feed palatability. However, feed conversion ratio (FCR) was significantly improved ($p < 0.05$) in the supplemented groups. The best FCR (2.44 ± 0.04) was observed in the AS4 group, indicating improved feed efficiency, while the control group showed the poorest FCR (2.85 ± 0.05). The effects of dietary treatments on serum biochemical parameters are presented in Table 3 and Figure 2. Supplementation with pine needles and vitamin E significantly ($p < 0.05$) improved several serum health indicators. Total Protein and Albumin: Birds in AS4 showed the highest serum total protein (5.21 ± 0.12 g/dL) and albumin levels (2.83 ± 0.09 g/dL), suggesting improved protein metabolism and liver function. These values were significantly higher than those in the control group (4.46 ± 0.10 and 2.34 ± 0.08 g/dL, respectively). Globulin: Calculated globulin levels were also elevated in the AS4 group, indicating enhanced immune response, possibly due to the synergistic antioxidant effects of pine needles and vitamin E. Cholesterol and Triglycerides**:** A significant reduction ($p < 0.05$) in serum total cholesterol and triglycerides was observed in the AS2, AS3, and AS4 groups compared to the control. The lowest cholesterol level (122.5 ± 3.4 mg/dL) was observed in the AS4 group, followed by AS3 (129.7 ± 3.6 mg/dL), whereas the control had the highest value (147.2 ± 4.2 mg/dL). Similar trends were observed in triglyceride levels, indicating improved lipid metabolism due to antioxidant supplementation. Glucose: Serum glucose concentrations were moderately reduced in all supplemented groups, with the lowest value recorded in T4 (192.3 ± 3.1 mg/dL) compared to the control (208.7 ± 3.9 mg/dL), although the differences were not statistically significant ($p > 0.05$). Liver Enzymes (ALT and AST): The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), important markers of liver health, were significantly ($p < 0.05$) lower in the supplemented groups, particularly in T4. Birds in the control group showed elevated ALT (42.5 ± 1.6 U/L) and AST (195.4 ± 5.7 U/L) levels, whereas AS4 had the lowest levels (35.1 ±

1.2 and 168.2 ± 4.9 U/L, respectively), indicating better liver integrity and reduced oxidative stress.

Table 2: Impact of Vitamin E and Pine Needles on Japanese Quail Growth Performance

Treatment	Weight Gain (g)	Feed Intake (g)	FCR
AS1 (Control)	115.6 ± 2.1	329.5 ± 4.6	2.85 ± 0.05
AS2 (Pine Needles)	123.8 ± 2.0	325.7 ± 4.3	2.63 ± 0.04
AS3 (Vitamin E)	129.7 ± 1.8	328.2 ± 3.9	2.53 ± 0.04
AS4 (Pine + Vit E)	138.4 ± 2.1	337.2 ± 4.8	2.44 ± 0.04

Table 3. Impact of Vitamin E and Pine Needles on Japanese Quail Serum Biochemical Parameters

Parameter	AS1 (Control)	AS2 (Pine)	AS3 (Vit E)	AS4 (Pine + Vit E)
Total Protein (g/dL)	4.46 ± 0.10	4.79 ± 0.11	5.06 ± 0.10	5.21 ± 0.12
Albumin (g/dL)	2.34 ± 0.08	2.56 ± 0.07	2.74 ± 0.08	2.83 ± 0.09
Globulin (g/dL)	2.12 ± 0.09	2.23 ± 0.10	2.32 ± 0.09	2.38 ± 0.11
ALT (U/L)	42.5 ± 1.6	38.7 ± 1.3	36.4 ± 1.2	35.1 ± 1.2
AST (U/L)	195.4 ± 5.7	182.9 ± 5.1	174.6 ± 5.0	168.2 ± 4.9

Figure 1. Impact of Vitamin E and Pine Needles on Japanese Quail Growth Performance

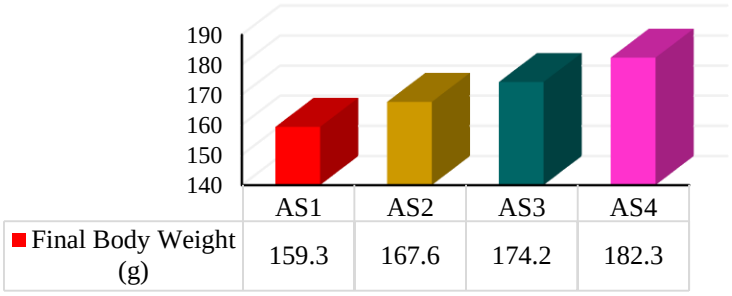
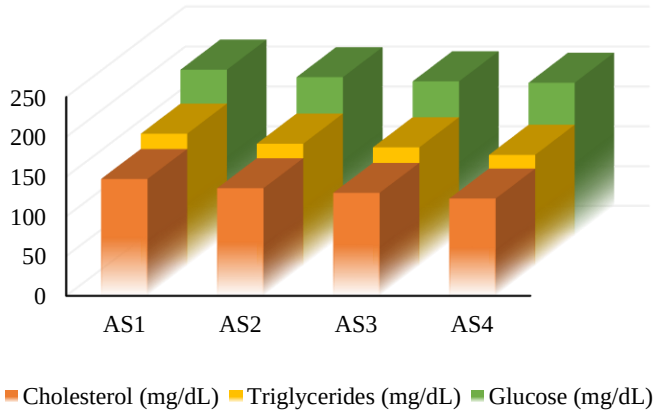


Figure 2. Impact of Vitamin E and Pine Needles on Japanese Quail Serum Biochemical Parameters.



4 Discussion

The effects of vitamin E supplementation and pine needle powder on Japanese quail growth performance and blood biochemical profile were investigated in this study. The findings clearly indicate that dietary supplementation with both pine needles and vitamin E positively influenced the overall health and productivity of the birds. The improvement in body weight gain and feed conversion ratio (FCR) observed in quails supplemented with pine needles and vitamin E, particularly in the AS4 group (combination of both), suggests a synergistic effect on nutrient utilization and metabolism. Pine needles, being rich in bioactive compounds such as flavonoids, terpenoids, and essential oils, may enhance digestion, stimulate appetite, and support gut microbial balance [12]. These compounds also exhibit antimicrobial and anti-inflammatory properties, which can contribute to better gut health and nutrient absorption. Strong lipid-soluble antioxidant vitamin E is essential for enhancing immunological response and shielding cell membranes from oxidative damage [6]. Its inclusion in the diet likely helped reduce oxidative stress in growing quails, thereby enhancing metabolic efficiency and promoting tissue development. The observed improvement in FCR is consistent with findings reported by Panda et al. [13], who noted enhanced feed efficiency in broilers with vitamin E supplementation. Furthermore, the no significant difference in feed intake among the treatment groups indicates that the improvements in weight gain and FCR were not due to increased feed consumption but rather to better nutrient assimilation and metabolic utilization facilitated by the antioxidants.

The serum biochemical parameters reflected the positive physiological impact of the dietary treatments. Birds receiving the combined supplementation (AS4) exhibited the highest

levels of serum total protein and albumin, which are indicators of improved protein synthesis and liver function. This suggests that pine needle bioactives, along with vitamin E, may enhance hepatic activity and support protein metabolism. Similar increases in serum protein and albumin levels have been documented in poultry supplemented with herbal antioxidants and vitamin E [14]. The significant reductions in serum cholesterol and triglyceride levels in the AS2, AS3, and AS4 groups may be attributed to the hypolipidemic properties of pine needles and the lipid-protective effects of vitamin E. Pine needles contain polyphenols and sterols that may inhibit lipid synthesis or promote lipid catabolism, as reported by Lee et al. [15]. Vitamin E, on the other hand, protects lipids from peroxidation and supports lipid transport mechanisms, leading to better lipid profile regulation [7, 15]. Although serum glucose levels were not significantly different among treatments, the numerical reduction observed in the supplemented groups may indicate improved glucose utilization and reduced stress. Pine needle antioxidants might enhance insulin sensitivity or modulate carbohydrate metabolism, although further mechanistic studies are required to confirm this effect.

The liver enzyme activities (ALT and AST) were significantly lower in supplemented groups, especially in AS4, indicating reduced hepatic stress and better liver integrity. Elevated liver enzymes are typically associated with liver damage or inflammation. The protective effect observed here is in line with the hepatoprotective roles of pine phytochemicals and vitamin E reported in earlier studies [16]. This supports the hypothesis that antioxidant supplementation helps maintain hepatic cellular function and reduces oxidative damage [17].

The combined supplementation of pine needle powder and vitamin E (AS4) consistently produced the most favorable results across all growth and biochemical parameters. This indicates a synergistic interaction between natural plant bioactives and a known antioxidant, which may enhance the physiological and immunological resilience of quail. Such synergistic effects have been demonstrated in other studies involving combinations of herbal additives and vitamins [2,7,17]. These results are encouraging from both animal welfare and production perspectives. They suggest that natural feed additives, when used in combination, may reduce the need for synthetic growth promoters or antibiotics while supporting healthy growth and metabolic function.

5 Conclusions

Overall, the findings from this study underscore the potential benefits of supplementing Japanese quail diets with pine needle powder and vitamin E, either individually or in combination. The observed improvements in growth performance, serum protein profiles, lipid metabolism, and liver function highlight the value of natural antioxidants in promoting health and productivity in poultry systems. Future research should focus on elucidating the molecular mechanisms behind these effects and evaluating the long-term safety and economic viability of such feed interventions.

Conflicts of interest

There are no conflicts of interest.

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