Effect of dietary nano α-tocopherol acetate plus selenium on productivity, immune status, intestinal morphometry and carcass traits of broiler chicken

Efecto del nano acetato de α-tocoferol dietético más selenio sobre la productividad, estado inmunológico, morfometría intestinal y características de la canal en pollos de engorde

Efeito do acetato de nano α-tocoferol mais selênio na produtividade, estado imunológico, morfometria intestinal e características de carcaça de frangos de corte

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Abstract

**Background:** Nano-vitamins and minerals are used in animal nutrition to improve performance and health of broiler chickens as a potential alternative to antibiotic growth promoters. Objective: To evaluate the effect of several levels of a dietary nano α-tocopherol acetate plus selenium compound (NTS) on growth, intestinal histomorphometry, carcass traits, and immune status of broiler chickens.

**Methods:** A total of 240 one-day-old Ross 308 chickens were randomly divided into four groups, each one containing three pens (n=20 in each pen). Four doses of nano α-tocopherol acetate and SE (0, 5, 7, and 10 mg/kg) were included in the basal diet for 42 days. **Results:** The final body weight (BW), cumulative body weight gain (BWG), and final feed conversion ratio (FCR) significantly improved when NTS levels were increased (P<0.05). The carcass traits and weight of internal organs enhanced in the groups fed with 7 and 10 mg/kg NTS. With high doses of NTS significantly increased the immune vaccine titers against Newcastle disease (ND), avian influenza (AI) virus, infectious Bursal disease (IBD), and infectious bronchitis (IB). Moreover, dietary addition of NTS improved some intestinal morphometric properties. **Conclusion:** Thus, NTS (7 and 10 mg/kg) can be added in poultry rations to improve production, carcass characteristics, and immune status of broilers. Further studies are needed to evaluate NTS residues in meat.

**Keywords:** immunity; intestinal morphology; nanominerals; nanovitamins; poultry; selenium; tocopherol; trace minerals; vitamin E.

Resumen

**Antecedentes:** Las nano-vitaminas y minerales se utilizan en nutrición animal para mejorar el rendimiento y la salud de los pollos de engorde como una posible alternativa al uso de antibióticos promotores del crecimiento. **Objetivo:** Evaluar el efecto de varios niveles dietarios de un acetato de nano α-tocoferol más un compuesto de selenio (NTS) en el crecimiento, histomorfometría intestinal, características de la canal y estado inmunológico de pollos de engorde. **Métodos:** Un total de 240 pollos Ross 308 de un día de edad se dividieron aleatoriamente en cuatro tratamientos, cada grupo con tres corrales (n=20 en cada corral). Se incluyeron cuatro dosis de nano α-tocoferol acetato y SE (0, 5, 7 y 10 mg/kg) en
la dieta basal durante 42 días. **Resultados:** El peso corporal total (BW), la ganancia de peso acumulada (BWG) y la tasa de conversión alimenticia final (FCR) mejoraron significativamente al aumentar el nivel de NTS ($P<0.05$). Las características de la canal y el peso de los órganos internos mejoraron en los grupos alimentados con 7 y 10 mg/kg de NTS. El incremento del nivel de NTS aumentó significativamente los títulos de vacunas inmunitarias contra la enfermedad de Newcastle (ND), el virus de la influenza aviar (AI), la enfermedad infecciosa de Bursal (IBD) y la bronquitis infecciosa (IB). Ademá, la adición dietaria de NTS mejoró algunas propiedades morfométricas intestinales. **Conclusión:** Es viable agregar NTS (7 y 10 mg/kg) en la ración para mejorar la producción, las características de la canal y el estado inmunológico de pollos de engorde. Se necesitan más estudios para evaluar los residuos de NTS en la carne.

**Palabras clave:** aves de corral; inmunidad; minerales traza; morfología intestinal; nanominerales, nanovitaminas; selenio; tocoferol; vitamina E.

**Resumo**

**Antecedentes:** Nanovitaminas e minerais são utilizados na nutrição animal para melhorar o desempenho e a saúde de frangos de corte como uma possível alternativa ao uso de antibióticos promotores de crescimento. **Objetivo:** Avaliar o efeito de vários níveis dietéticos de um acetato de nano α-tocoferol mais um composto de selênio (NTS) no crescimento, histomorfometria intestinal, características de carcaça e estado imunológico de frangos de corte. **Métodos:** Um total de 240 pintos Ross 308 de um dia de idade foram divididos aleatoriamente em quatro tratamentos, cada grupo com três baias (n=20 em cada baía). Quatro doses de acetato de nano α-tocoferol e SE (0, 5, 7 e 10 mg/kg) foram incluídas na dieta basal por 42 dias. **Resultados:** O peso corporal total (PC), o ganho de peso cumulativo (BWG) e a conversão alimentar final (FCR) melhoraram significativamente com o aumento do nível de NTS ($P<0.05$). As características da carcaça e o peso dos órgãos internos melhoraram nos grupos alimentados com 7 e 10 mg/kg de NTS. O incremento do nível de NTS aumentou significativamente os títulos de vacinas imunes contra a doença de Newcastle (ND), vírus da gripe aviária (AI), doença infecciosa da bolsa (IBD) e bronquite infecciosa (IB). Além
disso, a adição dietética de NTS melhorou algumas propriedades morfométricas intestinais. **Conclusão:** É viável a adição de NTS (7 e 10 mg/kg) na ração para melhorar a produção, as características de carcaça e o estado imunológico de frangos de corte. Mais estudos são necessários para avaliar os resíduos de NTS em carnes.

**Palavras-chave:** aves de criação; imunidade; minerais vestigiais; morfologia intestinal; nanominerais; selênio; tocoferol; Vitamina E.

**Introduction**

Natural and synthetic growth promoters can be used in poultry feed to improve growth efficiency. Antibiotics, as growth promoters, have been applied to increase growth efficiency and minimize mortality by altering the microbiota in the intestinal tract of healthy chickens, resulting in improved growth efficiency (Sabah Abdulameer *et al.*, 2022). The occurrence of bacterial resistance to some antibiotics in bacterial populations isolated from animal fields is a potential problem, which needs good planning and comprehensive research (Chattopadhyay, 2014). Many countries have banned the use of antibiotic growth promoters (AGP) in animal feed, which highlights the complexity of the issue (Cuong *et al.*, 2021). Currently, there are numerous feed additives available in the market, including herbal plants, beneficial bacteria, oligosaccharides, essential oils (Al-Sultan *et al.*, 2016; Sabah Abdulameer *et al.*, 2022), and nanoparticles (Saad Ibrahim *et al.*, 2022). Nanoparticles are applied as feed additives in farm animals and poultry, affecting the physiological status of some animal organs (Saad Ibrahim *et al.*, 2022). Furthermore, nanovitamins and minerals are applied in animal nutrition and medical field (Swain *et al.*, 2000). It has been noted that using antimicrobial, antifungal, and immune induction methods can increase the growth efficiency of broiler chickens (Hassan pour *et al.* 2015; Patra and Lalhriatpuii, 2020). Selenium (SE) could improve the immune status of avian and animal species (Ebeid *et al.*, 2013; Rayman, 2004). Pečjak *et al.*, (2022) revealed that SE element could enhance the growth factor and meat characteristics, while reducing stress conditions and mortality rate in poultry. However, the utilization of SE in poultry depends on its shape and combination with another compound.
(Surai et al., 2018). The mixture of SE and tocopherol (Vit E) is commonly used in poultry feed (Surai, 2002a). Recently, nano-SE has taken more interest because of its high utilization, good catabolic efficiency, high metabolic ability, and low toxic effects compared with selenite in poultry (Wang et al., 2009), mice, rabbit (Wang et al., 2007), rat (Jia et al., 2005), and ovine (Shi et al., 2011a; Shi et al., 2011b). While there is enough evidence on the role of SE and vitamin E on growth performance, less attention has been paid to immune induction in broilers fed nano selenium. Alpha-tocopherol (Vit E) is a lipid-soluble element that protects biological membranes and heals injured cells (Sharifi-Rad et al., 2020). The mixture of tocopherol and SE can stimulate growth production and immune condition (Singh et al., 2006). Moreover, in an experimental study, the mixture of tocopherol and SE significantly enhanced blood IgG and IgM levels (Et-Shenawy et al., 2015). The feed additives of tocopherol (150 IU/kg) and SE (0.06 ppm) in broiler chickens increased the antibody response, while the best cell-mediated immunity was observed in the feed containing tocopherol (300 IU/kg) and SE (1 mg/kg) (Lara et al., 2013). Although it is well-known that the interaction between SE and tocopherol can enhance the growth production and antioxidant system, limited studies have evaluated the effect of interaction between nano tocopherol and SE on broiler chickens. Therefore, the present study aimed to evaluate the effects of the mixture of nano α-tocopherol acetate and SE on growth performance, immune response, carcass traits, and intestinal histomorphometry of broiler chickens.

**Materials and methods**

**Ethical considerations**

The Ethics Committee for Poultry Research at Al-Qasim Green University in Iraq approved the study protocol (code: Alqas-rec.2021-Jun-EA98737).

**Nanoparticles**

The powder mixture of nano α-tocopherol acetate (vitamin E) 10% and sodium selenite 0.1% (SE) was used (Rheinvet Animal Health GmbH®, Neuwied, Germany) (www.rheinvet.de).

**Animal and dietary management**
A total of 240 (50 % males vs. 50 % females via using wing feather sexing, one-day-old Ross 308 broiler chickens were studied after 42 days of treatment. The chickens were randomly distributed (average weight per chick was 45 g ± 5) into four treatment groups, each group containing three pens (n=20 in each pen). Four doses of nano α-tocopherol acetate and SE (0, 5, 7, and 10 mg/kg) were included in the basal diet for 42 days. The feed procedure included starter feed from 1:21 d and grower feed from 22:42 d. The experimental diet formulations were designed according to the nutritional requirements of broiler chickens based on the 1994 Nutrient Requirements of Poultry (NRC) (Table 1).

The chickens were randomly divided into four groups as follows: control group (treatment1) (basal diet without any feed additive); treatment 2 (basal diet containing 5 mg/kg of nano α-tocopherol acetate and selenium (NTS); treatment 3 (basal diet containing 7 mg/kg of NTS; and treatment 4 (basal diet containing 10 mg/kg of NTS). The litter system was used for keeping the birds using standard conditions according to the Ross broiler guidelines. Water and feed were provided ad libitum. In the first week, the temperature was 33±1 °C, which was reduced to 27 °C and 24 °C in the second and third weeks, respectively. From the beginning of fourth week until the final day of the experiment, the temperature was kept at 23±1 °C.

Table 1. Feed constituents and chemical analysis (g/kg) of basal feed in broiler chickens supplemented with NTS for 42 days.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Starter 1-21 d</th>
<th>Grower 22-42 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>69.5</td>
<td>65.5</td>
</tr>
<tr>
<td>Soy meal (48% pr)</td>
<td>38.0</td>
<td>32.0</td>
</tr>
<tr>
<td>Soy bean oil (8900 kcal/kg)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>(Nacl)</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Methionine (Me)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Premix ≠</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Chemical analysis

| ME (kcal/kg) | 2850 | 2900 |
Growth factors, collection, and processing of intestinal samples

The feed intake (FI), average body weight (ABW), and body weight gain (BWG), in each pen were measured weekly. Feed conversion ratio (FCR) was measured for all period by dividing FI by BWG (Mohseni et al., 2021).

Vaccination programs

Each group was administered Newcastle disease (ND) (Nobilis® ND LaSota, Intervet Co, Millsboro, USA) and IBV (Hitchner IB) by an ocular route at the first and tenth days of age. Infectious bursal disease (IBD) vaccine was given by eye-drop route on day 14. Avian influenza (AI) and ND vaccines were administered on the first day of life intramuscularly (Jordan Bio Industries Co, Amman, Jordan) according to the Ross broiler recommendation.

Immune response

On day 34, blood was drawn from the jugular vein of two chickens per replicate (one male and one female) and centrifuged at 4500 rpm for 5 min. The sera were isolated and stored at −20°C until immunological analysis. The titers were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit "(ID.VET.BASELINES-MENA)" according to the manufacturer’s protocol. The mean antibodies titer was accounted according to Fanar et al., (2020).

Carcass traits

Internal organs and dressing percentage

<table>
<thead>
<tr>
<th></th>
<th>22.4</th>
<th>20.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Methionine + cysteine</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Premix mixture per kg of diet: Vit D3 3,500 IU; Vit B6 (riboflavin) 3.44 mg; Menadione 2.29 mg; Niacin 40.17 mg; Vit E (α-tocopherol) 44.7 IU; Iron 120 mg; Zinc 120 mg; Pantothenic acid 6.46 mg; Pyridoxine 2.29 mg; Biotin 0.05 mg; Folic acid 0.56 mg; Cyanocobalamin 0.05 mg; Vit A 12000 IU; Thiamine 1.43 mg; Copper 15 mg; Manganese 150 mg; Cobalt 0.4 mg; Selenium 0.3 mg; Iodine 1.5 mg.
On day 42, two chickens (one female vs. one male) were randomly selected from each replicate and prevented from feeding for 6 hours. The chickens were weighed, slaughtered, scalded, plucked, eviscerated, and washed. The weight of bird was balanced before slaughtering and again after evisceration. The carcass parts (breast and thigh of warm carcass), immune organs (bursa of Fabricius and spleen), and other organs (heart, liver, and gizzard) were picked and assessed separately to measure the weight of these organs. Carcass organs were calculated as percentage of carcass weight (Attia et al., 2020).

Intestinal histomorphometry studies
To assess intestinal histomorphometric parameters, about 2.5 cm of the middle part of the jejunum (one bird/pen) was excised and washed with normal saline. The samples were fixed in a 10% solution of neutral buffered formalin (NBF). Increased concentrations of ethyl alcohol were used to dry the samples. Next, dehydration technique was applied to jejunum samples using a tissue processing machine (Leica, ASP300, Tokyo, Japan). The intestinal samples were cleared with xylene and embedded in Leica EG 1160 paraffin embedding station (Leica EG, 1160, Bensheim, Germany). The hematoxylin (Gurrs, London, UK) and eosin (Chroma Gesellschaft, Münster, Germany), (H&E) method was used to stain the samples (4–5 mm thick) and the samples were examined under light microscope (100X). The depth of invagination between the adjacent villi (crypt depth) and the height from the tip of the villi to the villi crypt junction (villi height) was measured with an image analyzer (Diagnostic Instruments Inc, Sterling Heights, Michigan USA) according to the protocol presented in our previous study (Abdulameer et al., 2021).

Statistical analysis
Data were evaluated by analysis of variance (ANOVA) in SPSS 19. Duncan's multiple range (DMR) post-hoc test was applied. A value $P <0.05$ was considered as the significant difference (Steel and Torrie, 1980). The results were presented as mean ± standard errors (MSE).

Results
Effect of NTS on growth factors
Growth factors of chicks is revealed in Table 2. Body weight (BW) and accumulative body weight gain (BWG) significantly increased with increasing NTS levels from 5 to 10 mg/kg ($P<0.05$). The highest BW (2,725 g) and BWG (2,666 g) were recorded on day 42 in the group receiving 7 and 10 mg/kg NTS.

The final feed intake (g/bird) did not change ($P>0.05$) by 5 and 7 mg/kg NTS during different phases of the experiment, but it significantly increased by 10 mg/kg NTS in comparison with the control group ($P<0.05$). However, the accumulative FCR enhanced by feed additive of 5 and 7 mg/kg NTS compared to the control group. Moreover, chickens receiving 10 mg/kg NTS diet showed a lower FCR value (1.61) compared to the control (1.81) during the study period ($P<0.05$) (Table 2).

**Table 2.** Growth performance after addition of NTS for 42 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Final Feed Intake (g)</th>
<th>Final Body Weight (g)</th>
<th>Final Body Weight Gain (g)</th>
<th>Final Feed Conversion Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>4249.83$^{b}$±27.49</td>
<td>2391.67$^{b}$±73.72</td>
<td>2350.78$^{b}$±73.28</td>
<td>1.81$^{a}$±0.05</td>
</tr>
<tr>
<td>T2</td>
<td>4301.3$^{ab}$±34.13</td>
<td>2580$^{ab}$±116.76</td>
<td>2539.45$^{ab}$±116.57</td>
<td>1.70$^{ab}$±0.08</td>
</tr>
<tr>
<td>T3</td>
<td>4315.66$^{ab}$±5.60</td>
<td>2666.67$^{a}$±33.33</td>
<td>2623.67$^{a}$±33.00</td>
<td>1.64$^{ab}$±0.02</td>
</tr>
<tr>
<td>T4</td>
<td>4328.35$^{a}$±5.40</td>
<td>2725$^{a}$±14.43</td>
<td>2683.11$^{a}$±14.53</td>
<td>1.61$^{b}$±0.01</td>
</tr>
</tbody>
</table>

T1: basal diet (bd) without additives; T2: bd + 5 mg/kg NTS; T3: bd + 7 mg/kg NTS; and T4: bd + 10 mg/kg NTS. Statistical differences ($P<0.05$) between experimental groups within columns were indicated by different superscript letters ($^a$-$^b$). All values were represented as mean values ± SEM (n=4).

**Effect of NTS diet on carcass traits and internal organs**

NTS diet (7 and 10 mg/kg) significantly increased the warm carcass weight and organ weight compared to the control ($P<0.05$) (Table 3). The weight of liver, gizzard, and heart significantly increased by using the NTS diet at 10 mg/kg ($P<0.05$). Also, the weight of thigh and pectoral muscle significantly increased.
with increasing the NTS dosage compared to the control ($P<0.05$). However, the weight of bursa of Fabricius and spleen did not change significantly ($P >0.05$) (Table 4).

**Table 3.** Effect of NTS diet on carcass traits and internal organs of broiler chickens after 42 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Worm Carcass Weight (g)</th>
<th>Breast Muscle (g)</th>
<th>Thigh Muscle (g)</th>
<th>Liver (g)</th>
<th>Heart (g)</th>
<th>Gizzard (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1775b±134.25</td>
<td>929.26b±100.17</td>
<td>595b±82.51</td>
<td>45b±2.8</td>
<td>10b±0</td>
<td>28.33b±4.4</td>
</tr>
<tr>
<td>T2</td>
<td>1936.66b±99.38</td>
<td>1005ab±58.3</td>
<td>713.33b±32.4</td>
<td>50ab±0</td>
<td>11.67ab±0.8</td>
<td>35ab±0</td>
</tr>
<tr>
<td>T3</td>
<td>2180a±72.51</td>
<td>1148.33ab±41.06</td>
<td>896.66a±20.8</td>
<td>56.66ab±6.0</td>
<td>12.33ab±0.3</td>
<td>36.66ab±3.3</td>
</tr>
<tr>
<td>T4</td>
<td>2266.66a±95.27</td>
<td>1171.66a±38.4</td>
<td>928.33a±10.9</td>
<td>58.33a±1.6</td>
<td>14a±1.0</td>
<td>40a±0</td>
</tr>
</tbody>
</table>

T1: basal diet (bd) without additives; T2: bd + 5 mg/kg NTS; T3: bd + 7 mg/kg NTS; and T4: bd + 10 mg/kg NTS. Statistical differences ($P<0.05$) between experimental groups within columns were indicated by different superscript letters (a, b). All values were represented as mean values ± SEM (n=4).

**Table 4.** The effect of NTS diet on the weight of immune organs after 42 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Items</th>
<th>Bursa of Fabricius (g)</th>
<th>Spleen (g)</th>
</tr>
</thead>
</table>


T1: basal diet (bd) without additives; T2: bd + 5 mg/kg NTS; T3: bd + 7 mg/kg NTS; and T4: bd + 10 mg/kg NTS. All values were represented as mean values and standard error of the means (SEM) (n=4).

**Immune response**

At day 34, there was a significant increase \((P<0.05)\) in the antibody titers against IBV, IBD, ND, and AI in the group receiving NTS diet (5, 7, and 10 mg/kg) (Table 5 and Figure 1).

**Table 5.** The effect of NTS diet on immune titers of broiler chickens after 34 days.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary treatment</th>
<th>SEM</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious bronchitis/IU</td>
<td>T1 (2.944^{d})</td>
<td>T2 (3.921^{c})</td>
<td>T3 (4.984^{b})</td>
</tr>
<tr>
<td>Infectious bursal diseases/IU</td>
<td>4.442(^{d})</td>
<td>5.634(^{c})</td>
<td>7.185(^{b})</td>
</tr>
<tr>
<td>Newcastle disease/IU</td>
<td>6.062(^{d})</td>
<td>8.432(^{c})</td>
<td>10.372(^{b})</td>
</tr>
<tr>
<td></td>
<td>11.33(^{c})</td>
<td>12.67(^{c})</td>
<td>32.33(^{b})</td>
</tr>
</tbody>
</table>
T1: basal diet (bd) without additives; T2: bd + 5 mg/kg NTS; T3: bd + 7 mg/kg NTS; and T4: bd + 10 mg/kg NTS. Statistical differences ($P<0.05$) between experimental groups within rows were indicated by different superscript letters (\(^a, b, c, d\)). All values were represented as mean values and values and standard error of the means (SEM) (n=4).

**Figure 1.** Effect of NTS diet on immune titers after 34 days.

**Intestinal histomorphometry**

There were no histomorphometric changes in villi height and width of the jejunum in chickens receiving NTS diet compared to the control. The most significant histomorphometric changes were related to villi height (VH):crypt depth (CD) ratio ($P<0.05$) (Table 6, Figure 2).
Table 6. The effect of NTS diet on histomorphometric properties of jejunum of small intestine after 42 days.

<table>
<thead>
<tr>
<th>Items</th>
<th>VH (µ)</th>
<th>VW (µ)</th>
<th>CD(µ)</th>
<th>VH/CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1,330</td>
<td>133.9</td>
<td>311.8a</td>
<td>4.00b</td>
</tr>
<tr>
<td>T2</td>
<td>1,270</td>
<td>126.1</td>
<td>212.4c</td>
<td>5.47a</td>
</tr>
<tr>
<td>T3</td>
<td>1,282</td>
<td>147.2</td>
<td>265.4b</td>
<td>4.56b</td>
</tr>
<tr>
<td>T4</td>
<td>1,230</td>
<td>140.5</td>
<td>283.8ab</td>
<td>4.25b</td>
</tr>
<tr>
<td>SEM</td>
<td>39.8</td>
<td>8.6</td>
<td>11.0</td>
<td>0.25</td>
</tr>
</tbody>
</table>

T1: basal diet (bd) without additives; T2: bd + 5 mg/kg NTS; T3: bd + 7 mg/kg NTS; and T4: bd + 10 mg/kg NTS. VH: Villi height, VW: Villi width, CD: Crypt depth. Statistical differences (P<0.05) between experimental groups within columns were indicated by different superscript letters (a, b, c). All values were represented as mean values and standard error of the means (SEM) (n=4).
T3: bd + 7 mg/kg NTS  
T4: bd + 10 mg/kg NTS

**Figure 2.** Histological features in the jejunum of broiler chickens stained with haematoxylin and eosin (H&E) 100X. Control, birds fed basal diet (T1), basal diet containing 5 mg/kg of NTS (T2), basal diet containing 7 mg/kg NTS feed (T3); basal diet containing 10 mg/kg of NTS (T4). Red line = Villus height, black line = Villus Width, green line = Crept depth.

**Discussion**

This study showed that the NTS had a synergistic effect, which led to positive effect on growth factors (BW, BWG, FI, and carcass traits) and health (intestinal histomorphometry, immune responses and blood profiles) of commercial chickens. The NTS feed additives have antioxidant compounds that protect chickens from oxidative deterioration (Cui Zhu *et al.*, 2022). Several studies suggested that the NTS feed additives can inhibit (Ahmadi and Kurdistani 2010; Mancapecjak *et al.*, 2022) or stimulate growth factors (Harsij *et al.*, 2020; Olla *et al.*, 2021). In our study, growth factors improved with 7,10 mg/kg of NTS. These findings were consistent with the results of Salahuddin *et al.* (2017), reporting that feed additives of dietary vitamin E (100 mg) and SE powder (0.22 mg/kg) improved the live BW and BWG. The results of Dosoky *et al.* (2021) were also in line with the results of some previous studies (2, 4, and 8 ppm of SE).
improvement may be due to the antioxidant effect of NTS on intestinal status, improved gut health, and element absorption, because a healthy gut increases the nutrient absorption, weight gain, and feed consumption in NTS-fed broilers (Ghazi et al., 2016). Also, NTS has anti-inflammatory effects because it acts as a free radical scavenger and stimulates many enzymes that affect the mechanism of inflammation and healing (Alicalik et al., 2022). Furthermore, vitamin E and SE stimulate gut enzymes, which is another possible reason for the growth stimulating property of NTS. The improvement in the health status and immune responses of chickens fed NTS might be attributed to an increase in the physiological state of the cells, allowing them to achieve physiological functions and productivity with little use of nutrients (Fondevila, 2010). The best growth in the experimental groups can be attributed to the lower energy expenditure on feather growth (Edens et al., 2001; Roozbeh Shabani et al., 2019) since the growth of feathers needs higher energy to achieve this function (Yoon et al., 2007).

In contrast, some scientists reported that NTS supplementation did not affect broiler production (Habibian et al., 2014; Swain et al., 2000; Pompeu et al., 2018). These inconsistent results are possibly attributable to the structure of SE used in the study where the activity of SE depends on its biochemical form. Surai et al., (2002b) showed that nanoparticle size in natural SE is better than organic SE; the effect of SE depends on its synthesis ways, the dosage, and the route of administration. The increase in feed consumption of broilers to high dose of NTS (7 and 10 mg/kg) may be attributed to the health status of chickens’ gut as a result of the antioxidant property of vitamin E and/or SE.

In contrast, Salahuddin et al. (2017) revealed that the broiler feed intake did not change by feed additive of vitamin E and SE. Also, Yuming et al. (2000) stated that feed consumption was not affected by vitamin E and SE supplements. Likewise, Arrieta et al. (2002) revealed that vitamin E and/or SE, as feed additives, did not affect feed consumption.

The highest FCR value was seen in birds that received NTS, which can be attributed to the significant increase in feed intake and weight gain. Our results are also in line with the study carried out by Choct et al. (2004) in which increasing the SE dosage enhanced the FCR value of chickens. Ziaei et al. (2013) revealed that FCR value enhanced with addition of vitamin E and SE to the broilers’ feed. These findings are different from those reported by Habibian et al. (2014) in which...
vitamin E and SE levels did not affect FCR value during the whole experimental period. Ryu et al., (2005) and Edens (2000) discussed that vitamin E and SE feed additives are less palatable, which decreases feed consumption and FCR.

**Weight of internal organs and carcass traits**

In our study, the enhancement in dressing percentages, carcass parts, and weight of the internal organs in chickens receiving different levels of NTS agreed with the study by Mahmoud H El-Deep et al., (2016). Furthermore, adding nano SE in the diet increased the muscle and plasma vitamin E content in hot environmental conditions. This finding was congruent with the study by Ahmadi et al., (2018), in which NTS diet (4, 8, and 12 mg/kg) increased the relative weight of organs and carcass parts; this had insignificant effects on immune organs. Similar to our results, Felehgari et al., (2013) reported that the weight of internal organs increased, but the weight of some organs did not change in different SE dosages. The improvement in the carcass parts (pectoral and thigh muscle) in chickens receiving nano SE is in agreement with some previous findings (Konieczka et al., 2015; Ahmadi et al., 2018).

**Histomorphology of small intestine**

The crypt depth is a major index of health in chickens (Uni et al., 1995). The increased ratio of villi height: crypt depth (VH/CD) can enhance the nutrient absorption and improve gastrointestinal tract secretion and production (Ege et al., 2019). Also, the VH/CD ratio is positively related with an enhanced turnover of epithelial cells. In our study, the mixture of SE and vitamin E did not affect the villus height and its width. In a similar vein, Aliyu Ibrahim Muhammad (2021) indicated that feed additive of SE from yeast origin significantly increased the VH/CD ratio in the small intestine ($P<0.05$), but it reduced crypt depth in the small intestine. Similarly, Ahmed et al., (2016) showed no effect on villi height in the ileum of chickens receiving dietary organic SE from yeast origin. In addition, VH/CD ratio enhanced by SE supplementation, and antioxidants and immune properties significantly improved (Tong et al., 2020).

**Immune response**

In our study, we witnessed that the dose of dietary NTS minimized the common diseases in Iraq. Typically, the addition of NTS (5, 7, and 10 mg/kg) stimulated
the immune response. High doses of NTS were often more effective. In addition, NTS supplementation increased the immune response against AI, IB, IBD, and ND, but the immune organs (bursa of Fabricius and spleen) in the NTS group were not affected significantly. Several studies showed that nano SE affected antibody titers (Zhou and Wang, 2011; Liao et al., 2012). Since SE acts as an immunostimulant, SE deficiency leads to suppression of immune function due to reduced division of neutrophils and macrophages. This depression in cell division may be attributed to increased lipid peroxidation, which is followed by the accumulation of toxic compounds within immune cells. The increase of accumulated toxic compounds in neutrophils leads to low immune function (Wen et al., 1998). Furthermore, a lack of SE in the body interferes with the presence of nutrients in the bloodstream that are responsible for improving the immune system (Cao et al., 2002). Therefore, SE and vitamin E mixture induces the immune responses and cell proliferation. Studies indicate that the oxidizing and peroxidative effects or the biological and toxic potential of SE depend on its chemical composition and structural framework. It has also been reported that SE nanoparticles are more efficient in the immune response, especially when combined with vitamin E (Zhang et al., 2008; Ahmadi et al., 2018) our findings confirmed this issue.

Tocopherol and SE treatment significantly increased the titers of antibodies after vaccination against ND, IB, IA, and IBD (Wang et al., 2007; Kumar et al., 2009). The ability of lymphocytes to divide is reduced due to the lack of SE in the feed. Therefore, lymphocyte production decreases, leading to decreased immune function. Peng et al. (2009) argued that bioactive SE is more effective in inducing Lymphocyte cell division. Most scholars have argued that SE improves immune function via enhancing T helper cells and improving cytokine secretion (Burton et al., 1977).

The high immune titers after NTS treatment in this study was also congruent with the studies by Payne and Southern (2005), Funari Junior et al (2012) who reported that nano SE in poultry feeds enhanced cytokine activities and immune response. High cytokines and immune complexes are increased with better nutrient introduction and cell growth due to SE and vitamin E supplementation (Grivennikov et al., 2010). Vitamin E can increase antibody titers and cellular immune responses of broilers; it can also be used as an immunostimulant in broiler
chickens at a dosage of 100 mg/kg (Babak Darabighane et al., 2017). In a same
vein, the addition of vitamin E as the feed additive can improve immune response
against several viral diseases (Abdulwahid et al., 2016).

In conclusion according to the results of this study, NTS diet (5, 7, 10 mg/kg) had
a significant effect on production rate, FCR value, carcass traits, and intestinal
histomorphometry. Besides, NTS had a potential efficacy in improving the
immune responses of broiler chickens against common viral diseases. However,
the existing data regarding NTS residues in meat are contradictory and need
further studies.

Declarations

Conflict of interest
The authors declare they have no conflicts of interest with regard to the work
presented in this report.

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References
Abdulwahid MT, Zahid AH, Kadum MJ. Effect of vitamin E and cod liver oil
supplement with bivalent oil based vaccine of Newcastle disease and infectious
bronchitis disease on immune response of the broiler. Iraqi J Agri Sci 2016;
47(3): 892-899.
Ahmadi F, Hafsi-Kurdestani A. The Impact of silver nanoparticles on growth
performance, lymphoid organs, and oxidative stress indicators in broiler chicks.


Cui Zhu, Jingsen Yang, Xiaoyan Nie, Qiwen Wu, Li Wang, Zongyong Jiang. Influences of dietary vitamin E, selenium-enriched yeast, and soy isoflavone supplementation on growth performance, antioxidant capacity, carcass traits, meat quality and gut microbiota in finishing pigs. Antioxidants 2022; 11: 1510. https://doi.org/10.3390/antiox11081510


Farhad Ahmadi, Mehran Mohammadi Khah, Saman Javid, Ayoub Zarneshan, Loghman Akradi, Pezhman Salehifar. The effect of dietary silver nanoparticles on performance, immune organs, and lipid serum of broiler chickens during


https://doi.org/10.1007/s12011-015-0404-6


Shi LG, Xun WJ, Yue WB, Zhang CX, Ren YS, Shi L, Wang Q, Yang RJ, Lei FLE. Effect of sodium selenite, Se-yeastand nano-elemental selenium on growth
performance, Se concentration and antioxidant status in growing male goats. Small Ruminant Research 2011b; 96: 49-52.


