



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

Efecto del nano acetato de α -tocoferol dietético más selenio sobre la productividad, estado inmunitario, 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 with three pens (n=20 per pen). Four doses of nano α -tocopherol acetate and selenium (0, 5, 7, and 10 mg/kg) were included in the basal diet for 42 days. **Results:** Final body weight, cumulative body weight gain, and final conversion ratio significantly improved with increasing NTS levels (p<0.05). Carcass traits and weight of internal organs increased in the groups fed 7 and 10 mg/kg NTS. High NTS doses significantly increased immune vaccine titers against Newcastle disease, avian influenza virus, infectious bursal disease, and infectious bronchitis. Moreover, dietary addition of NTS improved intestinal morphometry. **Conclusion:** Thus, NTS (7 and 10 mg/kg) can be added to poultry rations to improve production, carcass characteristics, and immune status of broilers. Further studies are needed to evaluate NTS residues in meat.

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

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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 por corral). Se incluyeron cuatro dosis de nano α -tocoferol acetato y selenio (0, 5, 7 y 10 mg/kg) en la dieta basal durante 42 días. **Resultados:** El peso corporal total, la ganancia de peso acumulada y la tasa de conversión alimenticia final 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, el virus de la influenza aviar, la enfermedad infecciosa de la bursa y la bronquitis infecciosa. Además, la adición dietaria de NTS mejoró algunas características morfológicas 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; pollo de engorde; 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 baia). Quatro doses de acetato de nano α -tocoferol e selênio (0, 5, 7 e 10 mg/kg) foram incluídas na dieta basal por 42 dias. **Resultados:** O peso corporal total, ganho de peso cumulativo e a conversão alimentar final 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, vírus da gripe aviária, doença infecciosa da bolsa e bronquite infecciosa. Além disso, a adição dietética de NTS melhorou algumas propriedades morfológicas 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; frangos de corte; imunidade; minerais vestigiais; morfologia intestinal; nanominerais; selênio; tocoferol; vitamina E.

Introduction

Natural and synthetic growth promoters are used in poultry diets to improve growth efficiency. Growth promoting antibiotics have been used 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). Nevertheless, bacterial resistance to antibiotics in humans is a problem that demands good planning and comprehensive research (Chattopadhyay, 2014). Many countries have banned the use of antibiotic growth promoters (AGP) in animal feeds, which highlights the complexity of the issue (Cuong *et al.*, 2021). Numerous feed additives, such as herbal plants, beneficial bacteria, oligosaccharides, essential oils (Al-Sultan *et al.*, 2016; Sabah-Abdulameer *et al.*, 2022), and nanoparticles (Saad Ibrahim *et al.*, 2022) are used aiming to replace AGP. Nanoparticles are used as feed additives for farm animals, affecting the physiological status of some organs (Saad Ibrahim *et al.*, 2022). Furthermore, nanovitamins and minerals are used in both animal nutrition and the medical field (Swain *et al.*, 2000). The use of antimicrobial, antifungal, and immune induction methods can increase growth efficiency in broiler chickens (Hassan-Pour *et al.*, 2015; Patra and Lalhriatpuii, 2020).

It is also known that selenium (SE) can improve the immune status of avian and animal species (Ebeid *et al.*, 2013; Rayman, 2004). Pečjak *et al.* (2022) reported that SE enhances the growth factor and meat characteristics, while reducing stress and mortality in poultry. However, the use of SE in poultry depends on its chemical form and combination with other compounds (Surai *et al.*, 2018). The mixture of SE and tocopherol (vitamin E) is commonly used in poultry feed (Surai, 2002a). Recently, the interest in nano-SE has increased because of its 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 tocopherol on growth performance, less attention

has been paid to immune induction in broilers fed nano selenium.

Tocopherol protects biological membranes and heals injured cells (Sharifi-Rad *et al.*, 2020). The mixture of tocopherol and SE can stimulate growth production and immunity (Singh *et al.*, 2006). Moreover, the mixture of tocopherol and SE can increase blood IgG and IgM levels (Et-Shenawy *et al.*, 2015). Tocopherol (150 IU/kg) and SE (0.06 ppm) seem to increase antibody response in broiler chickens, with cell-mediated immunity response when fed 300 IU/kg tocopherol and 1 mg/kg SE (Lara *et al.*, 2013). Although it is well-known that the interaction between SE and tocopherol can enhance growth and the antioxidant system, few studies have evaluated the effect of the interaction between nano tocopherol and SE in broilers. Therefore, the present study aimed to evaluate the effects of incremental levels of a 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 (act: Alqas-rec.2021-Jun-EA98737).

Nanoparticles

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

Animal and dietary management

A total of 240 (50% males, 50% females) one-day-old Ross 308 broiler chickens were studied during a 42-days trial. The chickens (45 g \pm 5 average weight) were randomly distributed into four treatment groups, each containing three pens (n=20 per 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

feeding program consisted of starter feed from 1 to 21 d, and grower feed from 22 to 42 d. The experimental diet formulations were formulated according to the nutritional requirements of broiler chickens based on the 1994 Nutrient Requirements of Poultry (NRC; Table 1).

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

Grower 22–42 d	Starter 1–21 d	Ingredients (%)
Corn	65.5	69.5
Soybean meal (48% protein)	32.0	38.0
Soybean oil (8900 kcal/kg)	1.0	1.0
NaCl	0.3	0.3
Methionine	0.1	0.1
Lysine	0.1	0.1
Premix*	1.0	1.0
Total	100	100
Chemical analysis		
ME (kcal/kg)	2900	2850
Crude protein (%)	20.2	22.4
Calcium (%)	0.2	0.1
Phosphorus (%)	0.1	0.2
Methionine + cysteine	0.8	0.8
Lysine	1.1	1.2

*Premix 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.

Growth performance

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 the experimental period by dividing FI by BWG (Mohseni *et al.*, 2021).

Vaccination program

Each group received ocular vaccination against Newcastle disease (ND; Nobilis® ND

LaSota, Intervet Co, Millsboro, USA) and IBV (Hitchner IB) at the first and tenth days of age. Infectious bursal disease (IBD) vaccine was given by eye-drop on day 14. Avian influenza (AI) and ND vaccines were intramuscularly administered on the first day of life (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. Mean antibody titers were read according to Fanar *et al.* (2020).

Carcass traits

Internal organs and dressing percentage. On day 42, two chickens (one female and 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 the birds was recorded before slaughtering and again after evisceration. Carcass parts (breast and thigh of warm carcass), immune organs (bursa of Fabricius and spleen), and other organs (heart, liver, and gizzard) were weighted. Organ weight was calculated as percentage of carcass weight (Attia *et al.*, 2020).

Intestinal histomorphometry. To assess intestinal histomorphometry, 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, jejunum samples were dehydrated using a tissue processing machine (Leica, ASP300, Tokyo, Japan). Intestinal samples were cleared with xylene and embedded in a Leica EG 1160 paraffin embedding station

(Leica EG, 1160, Bensheim, Germany). The hematoxylin (Gurrs, London, UK) and eosin (Chroma Gesellschaft, Münster, Germany) was used to stain the samples (4–5 mm thick) and then examined under light microscope (100X). Depth of invagination between adjacent villi (crypt depth) and 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 protocol reported 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 $p < 0.05$ value was considered as a significant difference (Steel and Torrie, 1980). The results were presented as mean \pm standard errors (MSE).

Results

Growth performance

Table 2 shows growth performance results. Body weight (BW) and cumulative 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. Final feed intake (g/bird) did not change ($p > 0.05$) with 5 and 7 mg/kg NTS during the different phases of the experiment, but it significantly increased with

10 mg/kg NTS compared with the control group ($p < 0.05$). Cumulative FCR was improved by 5 and 7 mg/kg NTS compared to the control group. Moreover, chickens receiving 10 mg/kg NTS showed lower FCR value (1.61) compared to the control (1.81) during the study ($p < 0.05$; Table 2).

Effect of NTS on carcass traits and internal organs

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

Immune response

At day 34, a significant increase ($p < 0.05$) was observed in 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).

Intestinal histomorphometry

No changes were observed in villi height and width in the jejunum of chickens receiving NTS diet compared to the control. The most significant histomorphometry changes were related to villi height (VH): crypt depth (CD) ratio ($p \leq 0.05$; Table 6 and Figure 2).

Table 2. Growth performance of broilers fed α -tocopherol acetate and selenium.

Treatment	Final feed intake (g)	Final body weight(g)	Final body weight gain (g)	Final feed conversion ratio
T1	4249.8 ^b \pm 27.5	2391.7 ^b \pm 73.7	2350.8 ^b \pm 73.3	1.81 ^a \pm 0.1
T2	4301.3 ^{ab} \pm 34.1	2580 ^a ^b \pm 116.8	2539.5 ^a ^b \pm 116.6	1.70 ^{ab} \pm 0.1
T3	4315.7 ^{ab} \pm 5.6	2666.7 ^a \pm 33.3	2623.7 ^a \pm 33.0	1.64 ^{ab} \pm 0.0
T4	4328.4 ^a \pm 5.4	2725 ^a \pm 14.4	2683.1 ^a \pm 14.5	1.61 ^b \pm 0.0

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 are indicated by different superscript letters (^{a, b}). All values are presented as mean values \pm SEM (n=4).

Table 3. Effect of NTS on carcass traits and internal organs of broilers.

Group	Items	Warm carcass weight (g)	Breast muscle (g)	Thigh muscle (g)	Liver (g)	Heart (g)	Gizzard (g)
T1		1775 ^b ±134	929 ^b ±100	595 ^b ±83	45 ^b ±3	10 ^b ±0	28 ^b ±4
T2		1937 ^b ±99	1005 ^{ab} ±58	713 ^b ±32	50 ^{ab} ±0	12 ^{ab} ±1	35 ^{ab} ±0
T3		2180 ^a ±73	1148 ^{ab} ±41	897 ^a ±21	57 ^{ab} ±6	12 ^{ab} ±0	37 ^{ab} ±3
T4		2267 ^a ±95	1172 ^a ±38	928 ^a ±11	58 ^a ±2	14 ^a ±1	40 ^a ±0

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 are indicated by different superscript letters (^{a, b}). All values are presented as mean values \pm SEM (n=4).

Table 4. Effect of NTS diet on weight of immune organs of broilers fed α -tocopherol acetate and selenium.

Group	Items	Bursa of Fabricius (g)	Spleen (g)
T1		2.13	2.43
T2		2.03	2.03
T3		2.33	3.33
T4		3.00	4.00
SEM		0.5	0.47
p-value		0.33	0.23

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 are presented as means and standard error of the mean (SEM; n=4).

Table 5. Effect of NTS diet on immune titers of broiler chickens after 34 days.

Parameters	Dietary treatment				SEM	p-value
	T1	T2	T3	T4		
Infectious bronchitis (IU)	2,94 ^d	3,92 ^c	4,98 ^b	7,62 ^a	3,36	0.001
Infectious bursal diseases (IU)	4,44 ^d	5,63 ^c	7,19 ^b	8,72 ^a	2,61	0.001
Newcastle disease (IU)	6,06 ^d	8,43 ^c	10,37 ^b	12,66 ^a	548.7	0.001
Avian influenza (%)	11.33 ^c	12.67 ^c	32.33 ^b	49.33 ^a	2.36	0.001

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 are indicated by different superscript letters (^{a, b, c, d}). All values are represented as mean and standard error of the mean (SEM; n=4).

Table 6. Effect of NTS diet on jejunum histomorphometry of broilers after 42 days.

Group	Items	VH (μ)	VW (μ)	CD (μ)	VH/CD
T1		1,330	133.9	311.8 ^a	4.00 ^b
T2		1,270	126.1	212.4 ^c	5.47 ^a
T3		1,282	147.2	265.4 ^b	4.56 ^b
T4		1,230	140.5	283.8 ^{ab}	4.25 ^b
SEM		39.8	8.6	11.0	0.25

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 are indicated by different superscript letters (^{a, b, c}). All values are presented as mean and standard error of the mean (SEM; n=4).

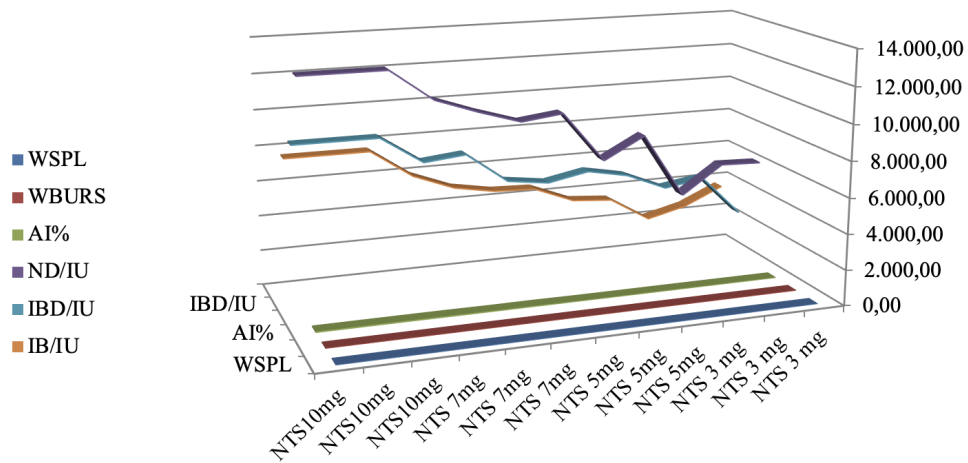


Figure 1. Effect of NTS diet on immune titers of broiler chickens after 34 days.

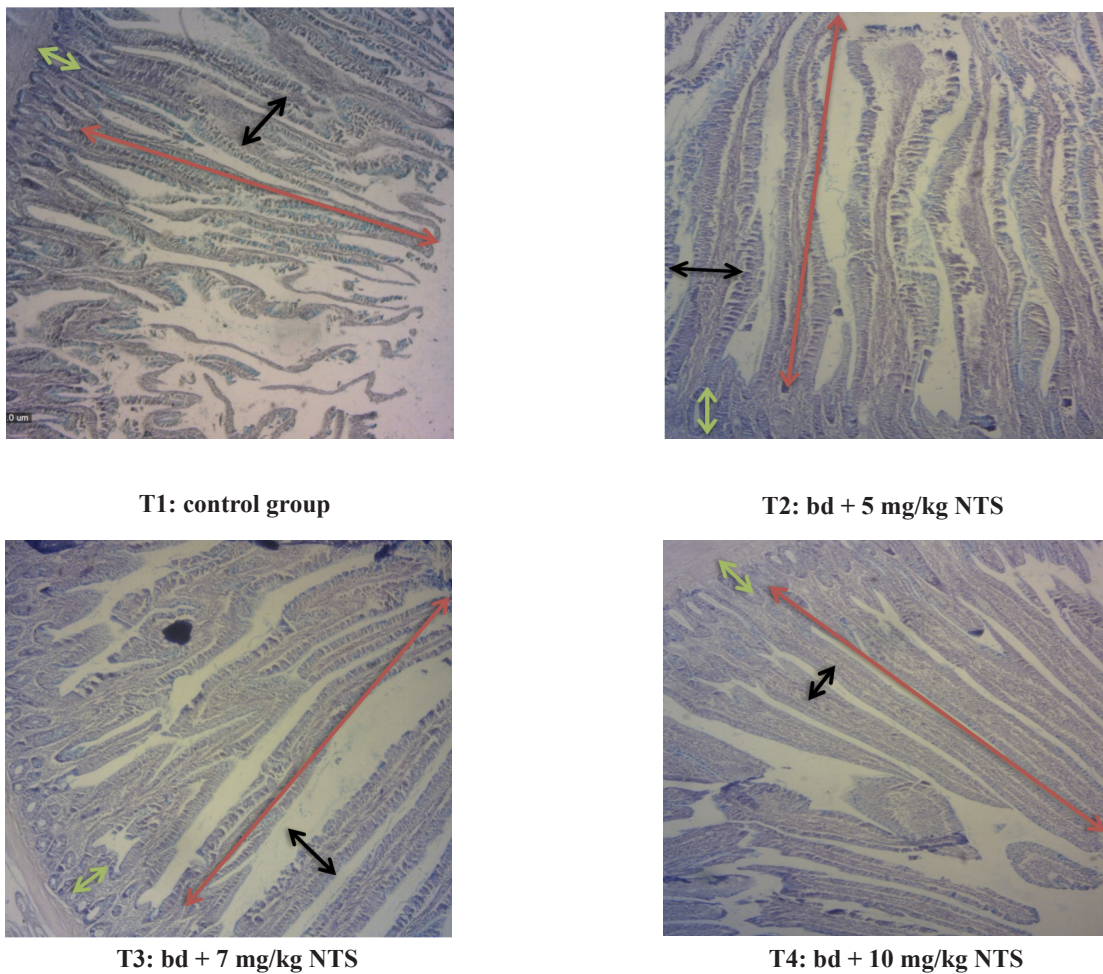


Figure 2. Histological features in jejunum of broiler chickens stained with haematoxylin and eosin (H&E) 100X. Control, birds fed basal diet (T1), basal diet containing 5 mg/kg of NTS (T2, basal diet containing 7 mg/kg NTS feed (T3); basal diet containing 10 mg/kg of NTS (T4). Red line = villus height;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

Discussion

This study showed that NTS had a positive synergistic effect on growth (BW, BWG, FI, and carcass traits) and health (intestinal histomorphometry, immune response, and blood profile) of broilers. The NTS feed additives have antioxidant compounds that protect chickens from oxidative deterioration (Cui Zhu *et al.*, 2022). Several studies have suggested that NTS can inhibit (Ahmadi and Kurdistani 2010; Mancapecjak *et al.*, 2022) or stimulate growth (Harsij *et al.*, 2020; Olla *et al.*, 2021). In the present study, growth factors improved with 7 and 10 mg/kg NTS. These findings are consistent with the results of Salahuddin *et al.* (2017), reporting that vitamin E (100 mg) and SE powder (0.22 mg/kg) improved live BW and BWG. The results of Dosoky *et al.* (2021) were also in line with the previous studies (2, 4, and 8 ppm of SE). This improvement may be due to the antioxidant effect of NTS on intestinal status, improved gut health, and absorption, because a healthy gut increases 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 affecting 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 effect of NTS. The improvement in health status and immune responses of chickens fed NTS might be attributed to improved cellular functions and productivity with little use of nutrients (Fondevila, 2010). The best growth observed can be attributed to lower energy expenditure on feather growth (Edens *et al.*, 2001; Roozbeh-Shabani *et al.*, 2019) since growth of feathers is highly demanding in energy (Yoon *et al.*, 2007).

In contrast, some researchers reported that NTS supplementation did not affect broiler production (Swain *et al.*, 2000; Habibian *et al.*, 2014; Pompeu *et al.*, 2018). These inconsistent results are possibly attributable to the structure of SE used in the study, where SE activity depends on its biochemical form. Surai *et al.*

(2002b) showed that SE nanoparticles is better than organic SE; the effect of SE depends on its synthesis, doses, and the route of administration. The increase in feed consumption of broilers fed high NTS doses (7 and 10 mg/kg) may be attributed to the health status of chicken's gut because of the antioxidant properties of vitamin E and/or SE.

In contrast, Salahuddin *et al.* (2017) revealed that 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) reported that vitamin E and/or SE did not affect feed consumption.

The highest FCR was observed 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 by Choct *et al.* (2004), who increased SE doses improving the FCR response of chickens. Ziaei *et al.* (2013) reported that FCR of broilers improved with the addition of vitamin E and SE. These findings differ from those reported by Habibian *et al.* (2014) in which vitamin E and SE did not affect FCR during the whole experimental period. Ryu *et al.* (2005) and Edens (2000) discussed that vitamin E and SE are less palatable, which decreases feed consumption and FCR.

In our study, the enhancement in dressing percentages, carcass parts, and weight of the internal organs in chickens receiving several levels of NTS agreed with the study by Mahmoud H El-Deep *et al.* (2016). Furthermore, dietary addition of nano SE increased muscle and plasma vitamin E content under hot environmental conditions. This finding is congruent with the study by Ahmadi *et al.* (2018), in which NTS (4, 8, and 12 mg/kg) increased the relative weight of organs and carcass with no effect 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 using several SE doses. The

improvement observed in carcass parts (pectoral and thigh muscle) of chickens receiving nano SE agrees with previous findings (Konieczka *et al.*, 2015; Ahmadi *et al.*, 2018).

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

We have seen how dietary NTS minimizes common diseases in Iraq. Typically, the addition of NTS (5, 7, and 10 mg/kg) stimulates 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 significantly affected. Several studies showed that nano SE affect 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 the immune cells. The increase of toxic compounds in neutrophils leads to low immune function (Wen *et al.*, 1998). Furthermore, a lack of SE interferes with the presence of nutrients in the bloodstream that are responsible for improving the immune system (Cao *et al.*, 2002). Therefore, the combination of SE and vitamin E induces the immune response and cell

proliferation. The oxidizing and peroxidative effects or the biological and toxic potential of SE depend on its chemical composition and structure. It has also been reported that SE nanoparticles are more efficient in terms of immune response, especially when combined with vitamin E (Zhang *et al.*, 2008; Ahmadi *et al.*, 2018).

Tocopherol and SE significantly increase antibodies titers 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 researchers 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 the present study is also congruent with the studies by Payne and Southern (2005), and Funari Junior *et al.* (2012), who reported that nano SE in poultry feed enhance cytokine activity and immune response. Cytokines and immune complexes are increased with better nutrient and cell growth due to SE and vitamin E supplementation (Grivennikov *et al.*, 2010). Vitamin E can increase antibody titers and cellular immune response in broilers; it can also be used as an immunostimulant in broiler chickens at a dose of 100 mg/kg (Babak Darabighane *et al.*, 2017). In addition, vitamin E can improve the immune response against several viral diseases (Abdulwahid *et al.*, 2016).

In conclusion, according to the results of this study, NTS (5, 7, 10 mg/kg) has a significant effect on growth, FCR value, carcass traits, and intestinal histomorphometry. Besides, NTS has a potential to improve the immune response of broiler chickens against common viral diseases. However, the existing data regarding NTS residues in meat are contradictory and need further research.

Declarations

Conflict of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

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Author contributions

Study concept and experimental design: Yahya SA. Acquisition of data: Raed Rabea. Sample analysis and interpretation of data: Majeed Ajafar. Manuscript Drafting: Raed Rabea. Statistical analysis: Yahya SA. Laboratory Administration, technical, and support on research proposal: Majeed Ajafar.

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. *Global Vet* 2010; 5(6):312–317. <https://www.researchgate.net/publication/216528277>
- Ahmadi M, Ahmadian A, Seidavi AR. Effect of different levels of nano-selenium on performance, blood parameters, immunity and carcass characteristics of broiler chickens. *Poult Sci J* 2018; 6(1):99–108. <https://doi.org/10.22069/psj.2018.13815.1276>
- Ahmed Z, Malhi M, Soomro SA, Gandahi JA, Arijio A, Bhutto B, Qureshi TA. Dietary selenium yeast supplementation improved some villi morphological characteristics in duodenum and jejunum of young goats. *J Anim Plant Sci* 2016; 26:382–387. <https://doi.org/10.22069/psj.2018.13815.1276>
- Aliyu Ibrahim Muhammad, Dalia Alla Mohamed, Loh Teck Chwen, Henny Akit, Anjas Asmara Samsudin. Effect of selenium sources on laying performance, egg quality characteristics, intestinal morphology, microbial population and digesta volatile fatty acids in laying hens. *Animals* 2021; 11(6):1681. <https://doi.org/10.3390/ani11061681>
- Al-Sultan SI, Abdel-Raheem SM, El-Ghareeb WR, Mohamed MH. Comparative effects of using prebiotic, probiotic, synbiotic and acidifier on growth performance, intestinal microbiology and histomorphology of broiler chicks. *JPN J Vet Res* 2016;64(Supplement2): S187–S95. https://www.aun.edu.eg/veterinary_medicine/article/33307
- Aravind KL, Gowdh CV, Manjunath BP, Rajendera AY, Ganpule SP. Influence of dietary level of selenium and vitamin E on growth immunity and carcass traits in broiler chickens. *Indian J Poult Sci* 2001; 36(1):58–62.
- Arrieta Acevedo JM, Díaz Cruz A, Ávila González E, Guinzberg Perrusquia R, Piña Garza E. Oxidative hepatic status and productive behaviour of broilers, fed on two sources of selenium and high levels of vitamins E and C. *Vet Méx* 2000; 31(2):113–119.
- Attia YA, Al-Harhi MA, Abo-El-Maaty HM. The effects of different oil sources on performance, digestive enzymes, carcass traits, biochemical, immunological, anti-oxidant, and morphometric responses of broiler chicks. *Front Vet Sci* 2020; 7:181.
- Babak Darabighane, Ali Mahdavi, Farzad Mirzaei, Aghjehgheshlagh Abolfazl Zarei. Effect of Aloe vera and vitamin E supplementation on the immune response of broilers. *Rev Colomb Cienc Pecu* 2017; 30(2):23–45. <http://dx.doi.org/10.17533/udea.rccp.v30n2a07>
- Bobade SP, Sarag AN, Rekhate DH, Dhok AP, Joge SV. Efficacy of vitamin E and selenium on growth performance of broilers. *Vet World* 2009; 2(1):20. <https://doi.org/10.1016%2Fj.psj.2022.101857>

- Burton RM, Higgins PJ, McConnell KP. Reaction of selenium with immunoglobulin molecules. *Biochimica et Biophysica Acta* 1977; 493:323–331. [https://doi.org/10.1016/0005-2795\(77\)90188-X](https://doi.org/10.1016/0005-2795(77)90188-X)
- Calik A, Emami NK, White MB, Walsh MC, Romero LF, Dalloul RA. Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part I: Growth performance, body composition and intestinal nutrient transporters. *Poult Sci* 2022; 101(6):101857. <https://doi.org/doi:10.1016/j.psj.2022.101857>
- Cao YZ, Weaver JA, Reddy CC, Sordillo LM. Selenium deficiency alters the formation of eicosanoids and signal transduction in rat lymphocytes. *Prostaglandins Other Lipid Mediat* 2002; 70 (1-2):131–143. [https://doi.org/10.1016/S0090-6980\(02\)00018-7](https://doi.org/10.1016/S0090-6980(02)00018-7)
- Chattopadhyay MK. Use of antibiotics as feed additives: a burning question. *Front Microbiol* 2014; 5:334.
- 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>
- Cuong NV, Kiet BT, Hien VB, Truong BD, Phu DH, Thwaites G, Choisy M, Carrique-Mas J. Antimicrobial use through consumption of medicated feeds in chicken flocks in the Mekong Delta of Vietnam: A three-year study before a ban on antimicrobial growth promoters. *PLoS One* 2021; 16(4):e0250082. <https://doi.org/10.1371/journal.pone.0250082>
- Dosoky WM, Fouda MM, Alwan AB, Abdelsalam NR, Taha AE, Ghareeb RY, El-Aassar MR, Khafaga AF. Dietary supplementation of silver-silica nanoparticles promotes histological, immunological, ultrastructural, and performance parameters of broiler chickens. *Sci Rep* 2021; 11(1):1–15. <https://doi.org/10.1038/s41598-021-83753-5>
- Ebeid TA, Zeweil HS, Basyony MM, Dosoky WM, Badry HF. Fortification of rabbit diets with vitamin E or selenium affects growth performance, lipid peroxidation, oxidative status and immune response in growing rabbits. *Livestock Sci* 2013; 155:323–331. <https://doi.org/10.1016/j.livsci.2013.05.011>
- Edens FW. Involvement of sel-Plex in physiological stability and performance of broiler chickens. *Biotechnology in Feed Industry. Proc. 17th, Alltech Ann. Symp.* 2001:349–376. (Nottingham, Nottingham University Press).
- Ege G, Bozkurt M, Koçer B, Tüzün A E, Uygun M, Alkan G. Influence of feed particle size and feed form on productive performance, egg quality, gastrointestinal tract traits, digestive enzymes, intestinal morphology, and nutrient digestibility of laying hens reared in enriched cages. *Poult Sci* 2019; 98(9):3787–3801. <https://doi.org/10.3382/ps/pez082>
- El-Shenawy NS, AL-Harbi MS, Hamza RZ. Effect of vitamin E and selenium separately and in combination on biochemical, immunological and histological changes induced by sodium azide in male mice. *Exp Toxicol Pathol* 2015; 67:65–76. <https://doi.org/10.1016/j.etp.2014.10.005>
- Fanar AI, Salah M, Balqees Z, Yasir A. Follow up the antibodies titer against Newcastle disease virus in broiler breeders using ELISA test. *Iraqi J Vet Sci* 2020; 34(2):295–299. <https://doi.org/10.33899/ijvs.2019.125931.1189>
- 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 starter period. *IJB* 2013; 3(5):95–100. <https://doi.org/10.1016/j.etp.2014.10.005>
- Felehgari K, Ahmadi F, Rokhzadi A, Kurdestany AH, Khah MM. The effect of dietary silver nanoparticles and inorganic selenium supplementation on performance and digestive organs of broilers during starter period. *Bull Env Pharmacol Life Sci* 2013; 2(8):104–108.

Fondevila M. Potential use of silver nanoparticles as an additive in animal feeding. In: David Pozo (Eds.), Silver Nanoparticles. Vol 1. Rijeka, Croatia: InTech. 2010:325–334.

Funari Junior P, Albuquerque R, Murarolli VDA, Raspantini LER, Cardoso ALS, Tessari ENC, Alves FR. Diferentes fontes e níveis de selênio sobre a imunidade humoral de frangos de corte. *Ciência Rural* 2012; 42:154–159. <https://doi.org/10.1590/S010384782011005000150>

Ghazi S, Moeini MM. Effects of dietary selenium and vitamin E on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. *Biol Trace Elem Res* 2016;169(1):142–152. <https://doi.org/10.1007/s12011-015-0404-6>

Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; 140(6) 883–899. <https://doi.org/10.1016/j.cell.2010.01.025>

Habibian M, Ghazi S, Moeini MM. Effects of dietary selenium and vitamin E on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. *Biol Trace Elem Res* 2016; 169:142–152. <https://doi.org/10.1007/s12011-015-0404-6>

Habibian M, Ghazi S, Moeini MM, Abdolmohammadi A. Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. *Int J Biometeorol* 2014; 58:741–52. <http://dx.doi.org/10.1007/s00484-013-0654-y>

Harsij M, Gholipour Kanani H, Adineh H. Effects of antioxidant supplementation (nano-selenium, vitamin C and E) on growth performance, blood biochemistry, immune status and body composition of rainbow trout (*Oncorhynchus mykiss*) under sub-lethal ammonia exposure. *Aquaculture* 2020; 521:734–9742. <https://doi.org/10.1016/j.aquaculture.2020.734942>

Hassanpour H, Mirshokraei P, Sadrabad EK, Dehkordi AE, Layeghi S, Afzali A, Mohebbi A. In vitro effect of nanosilver on gene expression of

superoxide dismutases and nitric oxide synthases in chicken Sertoli cells. *Animals* 2015; 9(2):295–300. <https://doi.org/10.1017/s1751731114002262>

Jia H, Hou W, Wei L, Xu B, Liu X. The structures and antibacterial properties of nano-SiO₂ dietary fat, vitamin E and selenium concentrations on the fatty acid profile and oxidative stability of frozen stored broiler meat. *J Anim Feed Sci* 2008; 24:244–251. <https://doi.org/10.1016/j.dental.2007.04.015>

Konieczka P, Czauderna M, Rozbicka-Wieczorek A, Smulikowska S. The effect of Kumar N, Garg AK, Dass RS, Chaturvedi VK, Mudgal V, Varshney VP. Selenium supplementation influences growth performance, antioxidant status and immune response in lambs. *Anim Feed Sci Tech* 2015; 153:7787. <https://doi.org/10.1016/j.anifeedsci.2009.06.007>

Kumbhar S, Khan AZ, Parveen F, Nizamani ZA, Siyal FA, El-Hack MEA, Huang K. Impacts of selenium and vitamin E supplementation on mRNA of heat shock proteins, selenoproteins and antioxidants in broilers exposed to high temperature. *Amb Express* 2018; 8(1):1–10. <https://doi.org/10.1186%2Fs13568-018-0641-0>

Lara LJ, Rostagno MH. Impact of heat stress on poultry production. *Animals* 2013; 3:356–369. <https://doi.org/10.3390/ani3020356>

Liao X, Lu L, Li S, Liu S, Zhang L, Wang G, Li A, Luo X. Effects of selenium source and level on growth performance, tissue selenium concentrations, antioxidation, and immune functions of heat-stressed broilers. *Biological Trace Element Research*. 2012; 150:158–165. <https://doi.org/10.1007/s12011-012-9517-3>

Mahmoud H El-Deep, Daichi Ijiri, Tarek A Ebeid, Akira O Htsuka. Effects of dietary nano-selenium supplementation on growth performance, antioxidative status, and immunity in broiler chickens under thermoneutral and high ambient temperature conditions. *J Poult Sci* 2016; 53: 274–283. <https://doi.org/10.2141/jpsa.0150133>

- Pečjak M, Leskovec J, Levart A, Salobir J, Rezar V. Effects of dietary vitamin E, vitamin C, selenium and their combination on carcass characteristics, oxidative stability and breast meat quality of broiler chickens exposed to cyclic heat stress. *Animals* 2022; 12:1789. <https://doi.org/10.3390/ani12141789>
- Mohseni Ghacchpoghm Fakeraei J, Hasseini SA, Lotfollah H. Performance immune response and blood biochemistry of broiler chicken fed with plant growth compound. *Trop Anim J* 2021; 44(1):62–70. <https://doi.org/10.5398/tasj.2021.44.1.62>
- Nadworny PL, Wang J, Tredget EE, Burrell RE. Anti-inflammatory activity of nanocrystalline silver-derived solutions in porcine contact dermatitis. *J Inflamm* 2010; 7(1):1–20. <https://doi.org/10.1186%2F1476-9255-7-13>
- National Research Council. Nutrient Requirements for Poultry, 9th ed; National Academy of Sciences. 1994. Washington, DC, USA.
- Olla A, Khalifa Rasha A, Al Wakeel Shabaan A, Hemed Mohamed M, Abdel-Daim Ghadeer M, Albadrani Ahmad El Askary, Sabreen E. Fad Fatma Elgendey. The impact of vitamin E and/or selenium dietary supplementation on growth parameters and expression levels of the growth-related genes in broilers. *BMC Vet Res* 2021; 17:251. <https://doi.org/10.1186/s12917-021-02963-1>
- Patra A, Lalhriatpuii M. Progress and prospect of essential mineral nanoparticles in poultry nutrition and feeding—A review. *Biol Trace Elem Res* 2020; 197(1):233–253. <https://doi.org/10.1186/s12917-021-02963-1>
- Payne RL, Southern LL. Comparison of inorganic and organic selenium sources for broilers. *Poult Sci* 2005; 84:898–902. <https://doi.org/10.1093/ps/84.6.898>
- Peng X, Cui Y, Cui W, Deng J, Cui H. The decrease of relative weight, lesions, and apoptosis of bursa of Fabricius induced by excess dietary selenium in chickens. *Biol Trace Elem Res* 2009; 131:33–42. <http://dx.doi.org/10.1007/s12011-009-8345-6>
- Pompeu MA, Cavalcanti LF, Toral FL. Effect of vitamin E supplementation on growth performance, meat quality, and immune response of male broiler chickens: a meta-analysis. *Livest Sci* 2018; 208:5–13. <https://doi.org/10.1016/j.livsci.2017.11.021>
- Rama Rao SV, Prakash B, Raju M VL N, Panda A K, Poonam S, Murthy OK. Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. *Asian Austral J Anim Sci* 2013; 26:247–252. <https://doi.org/10.5713/ajas.2012.12299>
- Rayman MP. The use of high-selenium yeast to raise selenium status. How does it measure up? *BJN* 2004; 92:557–573. <https://doi.org/10.1079/bjn20041251>
- Roozbeh Shabani, Jafar Fakhraei, Hossein Mansoori Yarahmad, Alireza Seidavi. Effect of different sources of selenium on performance and characteristics of immune system of broiler chickens. *R Bras Zootec* 2019; 48:e20180256. <https://doi.org/10.1590/rbz4820180256>
- Ryu YC, Rhee MS, Lee KM, Kim, BC. Effects of different levels of dietary supplemental selenium on performance, lipid oxidation, and color stability of broiler chicks. *Poult Sci* 2005; 84:809–815. <https://doi.org/10.1093/ps/84.5.809>
- Saad Ibrahim Al-Sultana, Abdel Rahman Taha Herebab, Khaled MA. Hassaneinc, Sherief M S, Abd-Allah Usama T, Mahmoud Sherief M, Abdel-Raheem AF. The impact of dietary inclusion of silver nanoparticles on growth performance, intestinal morphology, caecal microflora, carcass traits and blood parameters of broiler chickens. *Italian J Ani Sci* 2022; 21(1):967–978. <https://doi.org/10.1080/1828051X.2022.2083528>
- Sabah Abdulameer Y, Abbas Alwan I. Improvement of growth performance, biochemical blood profiles, and meat peroxidation by the inclusion of mustard seed extract in broilers' drinking water. *Arch Razi Inst* 2022; 77(1):429–437. <http://dx.doi.org/10.22092/ARI.2021.356803.1912>

- Sabah Abdulameer Y, Hamzah Ajeel H, Bakir Al-Hilli Z. Effects of supplementation of brassica juncea seed extract in drinking water on intestinal histomorphometry, bacteriology, and serum biochemistry parameters of broiler chicken. *Arch Razi Inst* 2021; 76 (4):837–846. <https://doi.org/10.22092%2Fari.2021.355948.1746>
- Salahuddin M, Khandaker ZH, Mohiuddin M, Billah MM. Effect of supplementation of vitamin E and selenium from Lolyvit-ES on the performance of broiler. *Asian J Med Biol Res* 2017; 3(4):521–528. <http://dx.doi.org/10.3329/ajmbr.v3i4.35344>
- Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho WC, Sharifi-Rad J. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Front Physiol* 2020; 11:694. <http://dx.doi.org/10.3389/fphys.2020.00694>
- Shi LG, Xun WJ, Yue WB, Zhang CX, Ren YS, Shi L, Wang Q, Yang RJ, Lei FLE. Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. *Small Rumin Res* 2011b; 96:49–52.
- Shi LG, Xun WJ, Yue WB, Zhang CX, Ren YS, Liu Q, Wang Q, Shi L. Effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep. *Anim Feed Sci Tech* 2011a; 163:136–142. <https://doi.org/10.1016/j.anifeedsci.2010.10.016>
- Singh H, Sodhi S, Kaur R. Effects of dietary supplements of selenium, vitamin E or combinations of the two on antibody responses of broilers. *Br Poult Sci* 2006; 47:714–719. <https://doi.org/10.1080/00071660601040079>
- Steel RG, Torrie JH. Principles and procedures of statistics a biometrical approach. 2nd ed. New York, NY: Mc-Grow-Hill Book Co. supported silver/zinc-silver materials, *Dentist Materials* 1980; 24:244–249. <http://dx.doi.org/10.1016/j.dental.2007.04.015>
- Surai PF. Selenium in poultry nutrition. 1. Antioxidant properties, deficiency and toxicity. *World's Poult Sci J* 2002a; 58: 333–347. <http://dx.doi.org/10.1079/WPS20020026>
- Surai PF, Kochish II, Fisinin VI, Velichko OA. Selenium in Poultry Nutrition: from Sodium Selenite to Organic Selenium Sources. *J Poult Sci* 2018; 55(2):79–93. <http://doi.org/10.2141/jpsa.0170132>
- Surai PF. Selenium in poultry nutrition 2. Reproduction, egg and meat quality and practical applications. *World's Poult Sci J* 2002b; 58:431–450. <https://doi.org/10.1079/WPS20020032>
- Swain BK, Johri TS, Majumdar S. Effect of supplementation of vitamin E, selenium and their different combinations on the performance and immune response of broilers. *Br Poult Sci* 2000; 41:287–292. <https://doi.org/10.1080/713654938>
- Tong C, Li Pm, Yu LH, Li L, Li Km, Chen Y, Yang SH, Mio L. Selenium-rich yeast attenuates ochratoxin A-induced small intestinal injury in broiler chickens by activating the Nrf2 pathway and inhibiting NF-KB activation. *J Funct Foods* 2020; 66:103784. <https://doi.org/10.1016/j.jff.2020.103784>
- Uni Z, Noy Y, Sklan D. Posthatch changes in morphology and function of the small intestines in heavy and light-strain chicks. *Poult Sci* 1995; 74(10):1622–1629. <https://doi.org/10.3382/ps.0741622>
- Wang D, Zhou X, She R, Xiong J, Sun Q, Peng K, Liu L, Liu Y. Impaired intestinal mucosal immunity in specific-pathogen-free chickens after infection with very virulent infectious bursal disease virus. *Poult Sci* 2009; 88:1623–1628. <https://doi.org/10.3382/ps.2009-00124>

- Wang H, Zhang J, Yu HQ. Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. *Free Radic Biol Med* 2007; 42:1524–1533. <https://doi.org/10.1016/j.freeradbiomed.2007.02.013>
- Wen W, Weiss SL, Sunde RA. UGA codon position affects the efficiency of selenocysteine incorporation into glutathione peroxidase-1. *Chem Biol* 1998; 273:28533. <https://doi.org/10.3382/ps.2009-00124>
- Xu ZR, Hu CH, Xia M, Zhan XA, Wang MQ. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult Sci* 2003; 82:1030–1036. <https://doi.org/10.1093/ps/82.6.1030>
- Yoon L, Werner TM, Butler J. M. Effect of source and concentration of selenium on growth performance and selenium retention in broiler chicken. *Poult Sci* 2007; 86:727–730. <https://doi.org/10.1093/ps/86.4.727>
- Yuming G, Qing T, Jianmin Y, Zhirong J. Supplemental effect of vitamin E on poultry performance. *Anim Feed Sci Technol* 2000; 89:165–173. <https://doi.org/10.1017/S0043933911000511>
- Zhang J, Wang X, Xu T. Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with Se-methylselenocysteine in mice. *Toxicol Sci* 2008; 101:22–31. <https://doi.org/10.1093/toxsci/kfm221>
- Zhou X, Wang Y. Influence of dietary nano elemental selenium on growth performance, tissue selenium distribution, meat quality, and glutathione peroxidase activity in Guangxi Yellow chicken. *Poult Sci* 2011; 90:680–686. <https://doi.org/10.3382/ps.2010-00977>
- Ziaei N, Moradi-Kor N, Pour EE. The effects of different levels of vitamin-E and organic selenium on performance and immune response of laying hens. *Afr J Biotech* 2013; 12(24):3884. <http://dx.doi.org/10.5897/AJB13.12278>