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The Interplay of Litter Types, Blends of L-selenomethionine, and Vitamin E in Broiler Chicken's Performance and Health

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ABSTRACT

The choice of litter type (LT) in broiler chicken production can significantly influence overall performance and health. Adding specific combinations of L-selenomethionine and Vitamin E (L-SeMet + V.E) to drinking water can impact broiler chickens' health. This study aimed to explore the effects of two LT types, wood shavings (WS) and perforated plastic litter (PL) as well as water supplementation with L-SeMet + V.E, on growth performance, blood parameters, antioxidant capacity, and thyroid hormone levels in broiler chickens. A total of 312 one-day-old male broiler chickens were randomly assigned to two different LT groups (WS and PL), with 156 chicks in each. Within each LT group, the chickens were further divided into four subgroups: a control group (T0) that received plain water, and three treatment groups that received drinking water supplements. Each subgroup consisted of three replicates, with 13 broiler chickens per replicate. The water treatments involved varying levels of supplementation with L-SeMet and vitamin E: T1 (0.5 mg/L + 250 mg/L), T2 (1 mg/L + 250 mg/L), and T3 (1.5 mg/L + 250 mg/L). Chickens were assessed for growth performance, feed efficiency, and blood biochemical parameters including lipids, liver enzymes, antioxidants, and thyroid hormones. Chickens raised on PL and receiving T3 exhibited significantly enhanced performance and feed efficiency, outperforming those raised on WS and given different supplementation levels. Significant enhancements were observed in the blood lipid profile and liver enzyme levels across the different LT groups, with the highest values recorded in the PL group. Additionally, broiler chicken in the T2 group, along with the WS × T2 and PL × T3 interactions, showed a notable improvement in blood biochemical parameters. Similarly, chickens raised on PL and given T3-supplemented water, along with the interactions WS × T2 and PL × T3, showed a significant improvement in antioxidant status and thyroid hormone levels in comparison to chickens raised on WS and the other treatment combinations. The use of PL and supplemenation with L-SeMet + V.E in drinking water were found to enhance growth performance, improve blood parameters, increase antioxidant capacity, and influence thyroid hormone levels in broiler chickens.

Keywords: Antioxidant, Broiler chicken, L-selenomethionine and Vitamin E, Productivity, Thyroid hormone

INTRODUCTION

To enhance poultry health, it is important to concentrate on both management environment and dietary approaches in order to achieve optimal results. The success of broiler chicken production is influenced by a range of environmental factors, one of the most crucial being the type and proper management of litter material (Pepper and Dunlop, 2021). The use of high-quality bedding materials in commercial poultry production has attracted considerable attention due to its impact on productive performance (Bilgili et al., 2009). The performance and well-being of broiler chickens is greatly influenced by the type of litter employed in their production (Okasha et al., 2021a; b). It is common knowledge that litter is utilized to

reduce birds' exposure to excrement and to absorb excess moisture (Farghly et al., 2018). However, poor litter quality can lead to the development of pododermatitis in broiler chickens, resulting in discomfort and welfare concerns (Shepherd and Fairchild, 2010), as well as influencing immunological responses, performance, and vulnerability to diseases (De Jong et al., 2014; Wei et al., 2015).

Although litter is traditionally employed in poultry farming, particularly in broiler production, improvements in management practices can enhance both the environmental factors and the well-being of the poultry. One option is to utilize raised plastic flooring, commonly found in broiler chicken barns (Li et al., 2017). Previous study has indicated that broiler chicken productivity can be maintained by utilizing perforated plastic flooring (Almeida et al., 2017), while also improving broiler chicken health (Okasha et al., 2021b). Plastic litter offers durability and cost-effectiveness, as it does not degrade, require replacement, or involve high maintenance costs. It is easy to install and clean plastic litter types, with the potential to reduce occurrences of foot pad injuries (Farghly et al., 2018). Maintaining ideal litter conditions is essential for broiler chicken performance, as excessive moisture, ammonia levels, and the presence of pathogenic organisms can have negative consequences (De Toledo et al., 2020). These include footpad dermatitis, respiratory disorders, and reduced performance such as an unfavorable feed conversion ratio (FCR), decreased body weight (BW), and body weight gain (BWG) (Abougabal and Taboosha, 2023). Therefore, it is essential to meticulously choose and oversee the appropriate litter type in order to ensure optimal broiler chicken health, welfare, and overall performance.

Selenium (Se) is a vital element found in at least 25 selenoproteins that regulate various biological functions in the body (Wang et al., 2017). Selenium's main biological roles include antioxidant defense, adjustment of the immune system, and control of the body's inflammatory reaction (Rayman and Stranges, 2013; Selim et al., 2015). These roles may positively impact the efficiency and metabolic function of broiler chickens. Poultry feed supplements can include both types of selenium, specifically sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3) (Wickramasuriya et al., 2023). A major advantage of supplying selenomethionine (SeMet) over utilizing inorganic selenium or alternative organic compounds is that SeMet is metabolized with methionine, creating a reserve of selenium in broiler chicken tissues (Surai et al., 2018). According to study of Gangadoo et al. (2020), organic selenium is absorbed and retained more effectively as compared to synthetic forms, with higher concentrations in the duodenum, ileum, and spleen, indicating increased uptake, while lower levels are found in the brain, liver, and breast tissues. To prevent Se deficiency and meet the poultry demands, dietary enrichment with natural selenium is widely practiced due to its superior bioavailability (Surai et al., 2018).

Supplementing broiler chicken diets with selenium has demonstrated favorable impacts on growth, feed markers, immunity, and decreased mortality (Habibian et al., 2016; Calik et al., 2022a). Organic selenium compounds such as hydroxyselenomethionine (OH-SeMet) and L-selenomethionine (L-SeMet) improve selenium reserves in birds, enhance antioxidant protection, and boost resilience to stress in breeder nutrition (Surai and Fisinin, 2014; Wang et al., 2021a; Babazadeh and Ahmadi Simab, 2022). The enhancement of antioxidant capacity has been found to be associated with the activation of selenium-dependent antioxidant enzymes (Liu et al., 2021). In addition, selenium plays a vital role as a component of glutathione peroxidases (Huang et al., 2019). These enzymes, together with vitamin E, contribute to the protection of cells from free radicals (Yoon et al., 2007). Vitamin E is the primary antioxidant that is soluble in lipids, and it holds significant importance in cell membranes (Packer et al., 2001; Gao et al., 2010; Traber and Stevens, 2011). It acts as a crucial lipid antioxidant by interrupting chain reactions and scavenging free radicals within cell membranes and subcellular structures (Young et al., 2003). The studies has shown that adding vitamin E to broiler chicken diets can provide notable benefits due to its potent antimicrobial properties and its capacity to bolster the immune functions (Dalia et al., 2018; Calik et al., 2022a). It can be hypothesized that providing preeminent levels of vitamin E as a supplement can offer benefits in preventing the negative impact of husbandry-related stressors such as transportation, handling, and vaccination, all leading to reduced broiler chicken performance (Perez-Carbajal et al., 2010; Calik et al., 2022b).

The study aimed to evaluate the effects of adding L-selenomethionine and vitamin E (L-SeMet + V.E) supplementation in drinking water on growth performance, feed efficiency, blood profiles, antioxidant enzymes, and T3 and T4 levels. It also explored the impact of this supplementation on broiler chickens raised on either wood shavings or perforated plastic litter, focusing on how it may improve performance and health and mitigate stress effects.

MATERIALS AND METHODS

Ethical approval

The animal care and experimental protocols were approved by the Research Ethics Committee at the Faculty of Agriculture, Moshtohor, Benha University (REC-FOABU/No.16000/3), were observed. The experimental broiler chickens were subjected to the utmost care, and all required actions were implemented to minimize discomfort.

Key features of the different litter types

The study utilized a washable perforated plastic floor (Al-Ekhlass for Plastics Industry and Trading, Qalyubia, Egypt), measuring 100 × 100 × 15 mm (length × width × height), and weighing 1.75 kg with a resistance of up to 200 kg/m2 per unit. The wood shavings (beech) used for the poultry litter were obtained from a nearby tree processing facility. These soft, dry, and non-toxic shavings were spread at a depth of 7 cm. The materials exhibited high absorbency and were resistant to clumping.

Animals and study design

All broiler chicks (Ross 308) were obtained from a commercial hatchery (Association of Al-Tanmia for Hatching and Poultry Production, Qalyubia, Egypt). The chicks were transported in a temperature-controlled vehicle for about one hour to the experimental farm located at the Poultry Research Unit, Faculty of Agriculture, Benha University, Egypt. Upon arrival, the chicks were housed in research enclosures within a controlled-geographical area, where stringent measures were implemented to maintain optimal experimental and hygienic conditions. All pens, each measuring 1 × 1 meter, were placed in the same location to ensure consistency. The stocking density in each pen was maintained at 13 chicks per square meter. Continuous electric lighting was provided for the first five days, after which the lighting schedule was adjusted to 20 hours of light and 4 hours of darkness for the following 30 days. Lighting intensity was applied in accordance with Directive 2007/43/EC in order to enhance the welfare of broiler chickens. Gas-powered space heaters were used in this experiment. The air temperature and relative humidity were kept at 33°C and 63%, respectively, for as long as three days before the chicks were placed in the pens. Throughout the initial week of the trial, the temperature was maintained at 33°C. Following this, the temperature was gradually reduced by 2-3°C per week until it reached the target range of 25°C by day 28. The broiler chickens were vaccinated according to a specified schedule: On day 8, they were administered the Newcastle Disease Virus (NDV) Clone 30 vaccine via eye drop, in addition to a bivalent NDV vaccine administered through injection under the neck membrane. The Gumboro intermediate vaccine was given at 12 days of age, followed by the NDV Clone 30 vaccine again at 21 days of age. The inactivated Avian Influenza (AI) H5N2 vaccine was administered at 10 days of age (Nobilis, Intervet, Boxmeer, The Netherlands). The immunization process followed established guidelines and protocols, and a licensed veterinarian supervised its implementation.

For a 35-day experiment, a total of 312 one-day-old male Ross 308 broiler chicks with an average initial body weight of 46.22 ± 0.1 g were used. The study employed a 2 × 4 factorial arrangement of treatments with three replicates, resulting in 24 experimental groups of chickens (n = 13 per group). Each group served as an experimental unit for the study. The chicks were randomly divided into two primary groups based on litter types: wood shavings and perforated plastic litter (n = 156 chicks per litter type). The chicks from each litter type were randomly allocated into a control group and three experimental groups (n = 39 chicks per group), which received different levels of L-SeMet + V.E into their drinking water. The experimental sets were labeled as follows: T1, (0.5 mg L-SeMet + 250 mg V.E); T2, (1 mg L-SeMet + 250 mg V.E); and T3, (1.5 mg L-SeMet + 250 mg V.E) mg/L, respectively. In this study, the supplementation level of vitamin E was increased in alignment with the guidelines provided by Aviagen (Aviagen, 2019). This finding aligns with earlier study, where an average supplementation of 200 mg/kg of vitamin E was shown to enhance oxidative status, improve performance parameters, and positively influence meat quality in broiler chickens (Mazur-Kuśnirek et al., 2019). In the current study, the organic selenium (Se) content in the diet was adjusted to the maximum permissible level, ranging from 1 to 3 mg/kg, consistent with the levels utilized in prior studies (Calik et al., 2022b; Kazem and Al-Khafaji, 2023). The vitamin E product added to the water was a misallied liquid solution that could disperse in water. It contained 250 mg of α-tocopherol per liter (L-selenomethionine® and vitamin E®, Alcon Biosciences Pvt. Ltd, Mumbai, India). Table 1 details the nutritional makeup of the experimental diets meticulously computed and analyzed, and then utilized in various stages of the experiment. The broiler chickens were fed a pelleted basal diet formulated to meet and slightly exceed the nutrient requirements specified by the National Research Council (NRC, 1994). Both feed and water were provided *ad libitum* for the entire duration of the study.

Table 1. The composition of ingredients and nutrients in the basal starter and grower diets in broiler chickens (Ross 308)

Ingredients (%)	Starter diet (0-3 weeks)	Gower diet (3-5 weeks)
Yellow Corn	60.56	65.4
Soybean meal (40%)	22	16.1
Corn gluten meal (60%)	12.6	13.2
Calcium hydrogen phosphate	1.5	1.7
Limestone	2.01	2.32
Salt (NaCl)	0.42	0.42
VIT and Min premix [1]	0.3	0.3
Choline chloride (50%)	0.2	0.2
L-lysine (78%)	0.24	0.24
Methionine	0.17	0.12
Total	100	100
Energy and nutrient composition		
ME (kcal/kg)	3000	3053
Crude protein (%)	22.29	20.77
EE (%)	2.80	3.00
Crude fiber,%	3.00	2.77
Ca (%)	1.21	1.19
T. phosphorus (%)	0.65	0.62
Av. phosphorus (%)	0.42	0.40
Lys (%)	1.11	0.95
Met (%)	0.50	0.40
Met + Cys (%)	0.95	0.80

[1]This feed formulation comprises a mineral and vitamin premix, providing the following concentrations (mg/3kg): Iron 80, Manganese 100, Copper 8, Zinc 75, Selenium 0.15, and Iodine 0.35. Additionally, it includes vitamin A (12,500 IU), vitamin D3 (2,500 IU), vitamin E (30 IU), vitamin K3 (2.65 mg), vitamin B1 (2 mg), vitamin B2 (6 mg), vitamin B3 (50 mg), vitamin (B512 mg), vitamin B7 (0.0325 mg), vitamin B9 (1.25 mg), and vitamin B12 (0.025 mg) per kilogram. These values were calculated based on feedstuff data as shown in the NRC (1994). Abbreviations: ME (kcal/kg): Metabolisable Energy (kcal/kg), EE (%): Ether Extract (%), T. phosphorus (%): Total phosphorus (%), Av. phosphorus (%): Available phosphorus (%), Met (%): Methionine, Met + Cys (%) Methionine and Cystine (%)

Taking samples and measurements

Growth performance and feed efficiency

Broiler chickens were weighed weekly to individually assess their growth performance, including body weight (BW) and body weight gain (BWG). Additionally, daily feed intake (FI) was recorded in replicates to calculate the ratio between (FI/g: BWG/g) expressed as feed conversion efficiency ratio (FCR). The European Production Efficiency Factor (EPEF) was calculated using the following equation: EPFE = (Livability[%] × BW [kg])/(FCR × Trial duration) × 100.

Blood samples

Twelve broiler chickens were chosen at random from each main group at the end of the 35-day trial (four per replicate). After a 12-hour period of fasting, 5 mL of blood was extracted from the wing vein. The samples were carefully placed into sterile tubes containing heparin and serum separator for subsequent analysis of thyroid hormones. The tubes were centrifuged for ten minutes at 4°C at 3500 × g to isolate the plasma. The plasma lipid profile, including total lipids, cholesterol (CHO), triglycerides (TRIG), low-density lipoproteins (LDL), and high-density lipoproteins (HDL), was analyzed using commercially available kits (Meikang Incorporated, Ningbo, China) following the methodology outlined by Gornall et al. (1949). Additionally, liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using a colorimetric method as described by Sirois (2019). The enzymes were measured using kits procured from the Nanjing Jiancheng Institute of Bioengineering (Jiangsu, China).

Evaluating antioxidant capacity

To assess the concentration of total antioxidants (T-AOC) in plasma, a commercially available kit (Randox, UK) was utilized, which was based on the method developed by Miller et al. (1993). The activity of blood glutathione peroxidase (GPx, EC 1.11.1.9) was assessed using commercially available GPx kits from Randox (Crumlin, UK), adhering to the manufacturer's instructions. The method for GPx measurement was based on the procedure established by Paglia and Valentine (1967). Additionally, superoxide dismutase (SOD, EC 1.1.5.1) activity in erythrocyte lysates was measured using kits provided by Randox Laboratories Ltd. (Crumlin, UK), following the protocols outlined by Woolliams et al. (1983). Moreover, plasma malondialdehyde (MDA) levels were measured using a modified fluorometric technique as detailed by Jo and Ahn (1998).

Measuring thyroid hormone levels

Serum samples were used to assess the levels of thyroid hormones (T3 and T4) using radioimmunoassay (RIA) kits, following the manufacturer's instructions. The techniques outlined by Renden et al. (1994) were employed for this analysis.

Statistical analysis

The pen served as the experimental unit (n = 24), and group means were subjected to statistical analysis. The data underwent analysis utilizing the General Linear Models (GLM) procedure of two-way ANOVA in SPSS (IBM SPSS, Version 27.0. Armonk, NY). Tukey's test was utilized to differentiate between treatments and assess the differences. The linear model was specified as:

$$Yijk = \mu + LTi + Tj + (LTT)\,ij + eijk$$

where Yijk represents the kth observation, and μ stands for the overall mean. The term LTi refers to the effect of the ith litter type (wood shavings and plastic litter), whereas Tj represents the effect of the jth L-SeMet + V.E treatment level (0, 0.5, 1, 1.5 + 250 V.E per mg/L). Additionally, the term (LTT) ij represents the interaction effect between the ith litter type and the jth L-SeMet + V.E treatment. The term eijk refers to the experimental error, assuming a mean of zero ($\bar{X} = 0$) and a variance of $\sigma^2 e$. Statistical significance was assessed using a P-value threshold of 0.001, unless otherwise specified.

RESULTS

Growth performance and feed efficiency

Table 2 highlights the effects of litter type and L-SeMet + V.E supplementation on broiler chickens' growth and feed efficiency up to 35 days. Significant improvements ($p < 0.001$) were observed in growth performance (BW and BWG) and feed efficiency (CFI, CFCR, and EPEF) due to both litter type and L-SeMet + V.E inclusion. While initial body weights showed no differences ($p = 0.648$), supplementation had a notable impact on growth traits during later periods. The addition of of 1.5 + 250 mg/l L-SeMet + V.E to the drinking water resulted in a significant increase in BW, BWG EPEF and decreased CFCR ($p < 0.001$). Interactions between WS with T2 and PL with T3 showed the most significant improvements in growth, feed efficiency, and European Production Efficiency Factor (EPEF) ($p < 0.001$).

Blood biochemical analysis

Table 3 summarizes the findings of the blood lipid profile and liver enzyme analysis. Statistically significant differences ($p < 0.001$) were detected in the plasma concentrations of CHO, TRIG, LDL, HDL, and AST among broiler chickens reared on WS and PL litters. The incorporation of 1 + 250 mg/l L-SeMet + V.E into the drinking water significantly altered all blood parameters ($p < 0.001$), with increased HDL levels and reduced lipid profiles and liver enzymes. Some interactions such as WS × T2 increased HDL, while others such as PL × T3 reduced CHO, TRIG, and LDL levels. Both PL × T3 and WS × T2 significantly decreased AST and ALT levels, respectively, as compared to other interactions ($p < 0.001$).

Antioxidants and thyroid hormones

Table 4 and Graph 1 show significant differences ($p < 0.001$) in plasma antioxidants (TAOC, GPX, MDA) and serum thyroid hormones (T3, T4) among broiler chickens raised on different litter types. Chickens on PL litter exhibited higher antioxidant levels and thyroid hormones with lower MDA, as compared to those on WS litter. Supplementation with L-SeMet + V.E at 1.5 + 250 mg/L significantly improved antioxidant status and thyroid hormone levels across all parameters ($p < 0.001$). Interaction analysis revealed that chickens raised on both litter types and given T2 or T3 had the highest antioxidant capacity (TAOC, GPX, SOD) and thyroid hormones (T3, T4), with the lowest MDA levels ($p < 0.001$).

Table 2. Effects of different litter types, L-Selenomethionine + Vitamin E, and their interactions on broiler chickens' growth and feed efficiency

Items		BW (g)		BWG (g)	CFI (g/bird)	CFCR	EPEF
		0	5 WKS	0-5 WKS	0-5 WKS	0-5 WKS	0-5 WKS
Litter types (LT)	WS	46.26	2175.47^{b}	2129.0.5^{b}	3094.50^{b}	1.46^{a}	425.72^{b}
	PL	46.49	2307.66^{a}	2261.48^{a}	3137.67^{a}	1.39^{b}	453.72^{a}
	SEM	0.342	3.930	16.604	6.057	0.014	6.593
L-SeMet + V.E (T) mg/l	T0	47.22	2218.66^{c}	2171.44^{b}	3132.79^{b}	1.44^{b}	428.44^{b}
	T1	46.34	2048.00^{d}	2001.65^{c}	3072.73^{c}	1.53^{a}	398.52^{c}
	T2	46.29	2282.52^{b}	2236.23^{b}	3100.05^{bc}	1.38^{bc}	464.30^{a}
	T3	45.66	2417.09^{a}	2371.74^{a}	3165.80^{a}	1.35^{c}	484.84^{a}
	SEM	0.484	5.558	23.481	9.464	0.20	8.847
LT × T	WS × T0	47.56	2149.33^{e}	2101.77^{cde}	3156.73^{b}	1.50^{ab}	408.86^{cd}
	WS × T1	45.86	2084.00^{f}	2038.14^{de}	3050.87^{d}	1.50^{ab}	406.77^{cd}
	WS × T2	46.52	2335.71^{b}	2289.17^{b}	3090.50^{cd}	1.35^{cd}	483.32^{b}
	WS × T3	46.00	2132.86^{e}	2087.14^{cde}	3104.93^{bcd}	1.49^{abc}	409.63^{cd}
	PL × T0	46.88	2288.00^{c}	2241.12^{bc}	3104.53^{bcd}	1.39^{bc}	451.28^{bc}
	PL × T1	46.83	2012.00^{g}	1965.17^{e}	3094.60^{cd}	1.57^{a}	390.27^{d}
	PL × T2	46.05	2229.33^{d}	2183.29^{bcd}	3109.60^{bcd}	1.42^{bc}	445..23^{bc}
	PL × T3	45.31	2701.33^{a}	2656.35^{a}	3226.67^{a}	1.21^{d}	560.05^{a}
	SEM	0.684	7.861	33.207	13.382	0.280	10.339
p-value	LT	0.648	<.001	<.001	<.001	0.007	<.001
	T	0.193	<.001	<.001	<.001	<.001	<.001
	LT × T	0.572	<.001	<.001	<.001	<.001	<.001

The data sets reflect the mean values obtained from three replications per treatment. Different letters denote significant differences ($p < 0.001$) among means within the same column for each factor and interactions. Abbreviations: BW: Body weight, BWG: Body weight gain, CFI: Cumulative feed intake, CFCR: Cumulative feed conversion ratio, EPEF: European production efficiency factor, L: Litter types, WS: Wood shavings, PL: Perforated plastic litter, T: Treatments, WKS: Weeks, L-SeMET: L-selenomethionine, V.E: Vitamin E, SEM: Standard erorr mean, T0: Control group (plain water), T1: 0.5 + 250 mg/l L-SeMET + V.E, T2: 1 + 250 mg/l L-SeMET + V.E, T3: 1.5 + 250 mg/l L-SeMet + V.E

Table 3. Effects of different litter types, L-Selenomethionine + Vitamin E, and their interactions on broiler chickens' lipid profile and liver enzymes

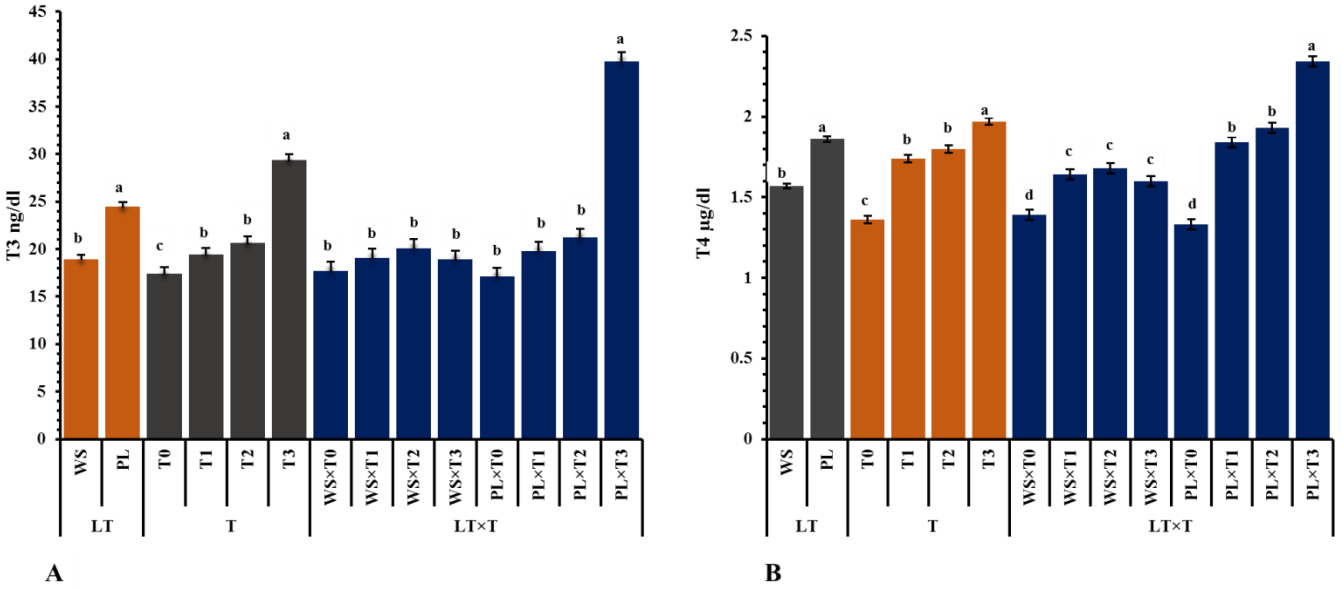
Items		TL (mg/dl)	CHO (mg/dl)	TRIG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AST (U/l)	ALT (U/l)
Litter Types (LT)	WS	851.98	216.66^{a}	74.07^{a}	156.92^{a}	44.92	152.64^{a}	57.96
	PL	779.76	123.21^{b}	58.02^{b}	70.14^{b}	41.46	144.40^{b}	58.29
	SEM	7.208	2.699	2.430	2.083	0.768	1.067	1.578
L-SeMet + V.E (T) mg/l	T0	1130.95^{a}	198.81^{a}	101.23^{a}	140.56^{a}	35.06^{b}	1.66.79^{a}	72.42^{a}
	T1	821.42^{b}	173.81^{ab}	77.775^{b}	112.70^{b}	39.47^{b}	143.59^{b}	57.11^{b}
	T2	549.21^{c}	146.43^{c}	42.590^{c}	98.445^{d}	49.75^{a}	141.26^{b}	44.91^{c}
	T3	761.90^{b}	160.71^{bc}	42.595^{c}	102.44^{bc}	48.49^{a}	142.45^{b}	58.06^{b}
	SEM	10.312	3.862	3.436	2.981	1.084	1.526	2.231
LT × T	WS × T0	1071.43^{ab}	242.86^{a}	106.17^{a}	183.42^{a}	32.33^{d}	178.52^{a}	74.30^{a}
	WS × T1	857.14^{bcd}	228.57^{a}	87.65^{ab}	160.66^{ab}	38.21^{cd}	140.28^{bcd}	44.63^{b}
	WS × T2	550.81^{e}	176.19^{b}	49.38^{cd}	133.24^{b}	58.78^{a}	139.26^{cd}	42.56^{b}
	WS × T3	928.57^{abc}	219.05^{a}	53.09^{cd}	150.39^{b}	50.38^{ab}	152.52^{bc}	70.36^{a}
	PL × T0	1190.48^{a}	154.76^{bc}	96.30^{a}	97.71^{c}	37.79^{cd}	155.07^{b}	70.54^{a}
	PL × T1	785.71^{cde}	119.05^{cd}	67.90^{bc}	64.74^{d}	40.73^{bcd}	146.91^{bcd}	69.60^{a}
	PL × T2	547.62^{e}	116.67^{cd}	35.80^{d}	63.65^{d}	40.73^{bcd}	143.26^{bcd}	47.26^{b}
	PL × T3	595.24^{de}	102.38^{d}	32.10^{d}	54.94^{d}	46.60^{bc}	132.38^{d}	45.76^{b}
	SEM	14.583	5.461	4.860	4.215	1.533	2.16	3.156
Two-way ANOVA								
p-value	LT	0.086	<.001	<.001	<.001	0.054	0.001	0.885
	T	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	LT × T	0.007	0.01	<.001	0.054	<.001	<.001	<.001

The data sets reflect the mean values obtained from three replications (4 chickens in each replicate) per treatment. Different letters denote significant differences ($p < 0.001$) among means within the same column for each factor and interactions. Abbreviations: L: Litter types, WS: Wood shavings, PL: Perforated plastic litter, T: Treatments, L-SeMET: L-selenomethionine, V.E: Vitamin E, T0: Control group (plain water), T1: 0.5 + 250 mg/l L-SeMET + V.E, T2: 1 + 250 mg/l L-SeMET + V.E, T3: 1.5 + 250 mg/l L-SeMET + V.E, SEM: Standard error mean, TL: Total lipids, CHO: Cholesterol, TRIG: Triglycerides, LDL: Low density lipoproteins, HDL: High density lipoproteins, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 4. Effects of different litter types, L-Selenomethionine + Vitamin E, and their interactions on broiler chickens' antioxidant levels

Items		TAOC (mM/L)	GPX (U/GT)	SOD (U/ml)	MDA (nmol/mL)
Litter Types (LT)	WS	0.77^{b}	43.67^{b}	3.48	3.65^{a}
	PL	0.89^{a}	48.62^{a}	3.52	2.37^{b}
	SEM	0.011	0.452	0.112	0.052
L-SeMet + V.E (T) mg/l	T0	0.74^{b}	19.21^{d}	2.81^{b}	5.28^{a}
	T1	0.77^{b}	29.22^{c}	3.27^{b}	3.25^{b}
	T2	0.83^{b}	58.35^{b}	3.94^{a}	2.05^{c}
	T3	0.97^{a}	77.81^{a}	3.99^{a}	1.43^{c}
	SEM	0.016	0.414	0.158	0.074
LT × T	WS × T0	0.67^{c}	19.45^{e}	2.88^{c}	6.30^{a}
	WS × T1	0.77^{bc}	38.90^{d}	3.30^{bc}	1.96^{c}
	WS × T2	0.88^{b}	97.26^{a}	4.38^{ab}	1.15^{c}
	WS × T3	0.77^{bc}	38.90^{d}	3.38^{bc}	5.20^{ab}
	PL × T0	0.82^{bc}	18.98^{e}	2.75^{c}	4.26^{b}
	PL × T1	0.77^{bc}	19.55^{e}	3.25^{c}	2.21^{c}
	PL × T2	0.79^{bc}	58.36^{c}	3.50^{bc}	1.72^{c}
	PL × T3	1.18^{a}	77.80^{b}	4.60^{a}	1.31^{c}
	SEM	0.023	0.586	0.224	0.105
Two-way ANOVA					
p-value	LT	<.001	<.001	0.804	<.001
	T	<.001	<.001	<.001	<.001
	LT × T	<.001	<.001	0.002	<.001

The data sets reflect the mean values obtained from three replications (4 chickens in each replicate) per treatment. Different letters denote significant differences ($p < 0.001$) among means within the same column for each factor and interactions. Abbreviations**:** L: Litter types, WS: Wood shavings, PL: Perforated plastic litter, T: Treatments, L-SeMET: L-selenomethionine, V.E: Vitamin E, SEM: standard error mean, T0: Control group (plain water), T1: 0.5 + 250 mg/l L-SeMET + V.E, T2: 1 + 250 mg/l L-SeMET + V.E, T3: 1.5 + 250 mg/l L-SeMet + V.E, TAOC: Total antioxidants (mM/L), GPX: Glutathione peroxidase (U/GT), SOD: Superoxide dismutase (U/ml), MDA: Malondialdehyde (nmol/ml)



Graph 1. Effects of litter types, L-Selenomethionine + Vitamin E, and their interactions on serum levels of T3 (A) and T4 (B) in broiler chickens. The data represent mean values from three replicates (4 chickens in each replicate) per treatment. The use of distinct letters placed above the columns indicates significant variances, established by Tukey's test with a significance level of $p < 0.001$. Abbreviations: L: Litter types, WS: Wood shavings, PL: Perforated plastic litter, T: Treatments, L-SeMET: L-selenomethionine, V.E: Vitamin E, T0: Control group (plain water), T1: 0.5 + 250 mg/l L-SeMET + V.E, T2: 1 + 250 mg/l L-SeMET + V.E, T3: 1.5 + 250 mg/l L-SeMet + V.E, T3: Triiodothyronine hormone (ng/dl), T4: Thyroxin hormone (µg/dl)

DISCUSSION

Growth performance and feed efficiency

There is a notable emphasis on enhancing the productive performance of broiler chickens. Achieving better performance requires the implementation of efficient management practices, ensuring optimal housing conditions, and carefully formulating their diet (Bist et al., 2024). The findings of the current investigation unveiled that broiler chickens raised on PL litter type exhibited significantly superior growth performance compared to those housed on other WS litter systems. This was evident in parameters of growth performance (BW and BWG), and feed efficiency (CFI, FCR, and EPEF). These results are attributed to the fact that broiler chickens reared on perforated plastic litter types consumed a higher amount of feed. This is likely due to a reduced likelihood of pecking in the litter, which leads to increased feed intake. Consequently, this positively affects body weight (BW) and leads to an increase in body weight gain (BWG). Chickens raised on plastic flooring exhibited comparatively greater weight gains but had a lower FCR compared to chickens reared solely on wood shavings (Almeida et al., 2017; Çavuşoğlu et al., 2018). This improvement may be attributed to the fact that WS bedding absorbs broiler chicken feces, resulting in increased moisture and interaction with ammonia. Consequently, the surface temperature of the bedding is somewhat elevated, which affects the environmental comfort for the broiler chickens. This leads to a decrease in feed intake and adversely affects feed conversion efficiency. However, this is not the case with PL litter type, where broiler chickens kept in deep litter systems exhibited the lowest FI and BW (Abd El-Wahab et al., 2020). Broiler chickens raised on plastic litter exhibit a lower pecking at litter, which results in increased feed intake. The higher observed feed intake could account for the variations in growth performance and feed efficiency among different litter types. Reducing contact with fecal matter improves the overall health of broiler chickens, leading to enhanced growth performance (Wang et al., 2015). In the present study, broiler chickens reared on PL litter exhibited significantly higher values for body weight gain, feed intake, and feed conversion ratio compared to those raised on WS litter. Without a doubt, the factors encompassed within the European Production Efficiency Factor (EPEF), including bird livability percentage, body weight (kg), FCR, and days until slaughter, as discussed and examined earlier, demonstrate superior performance in broiler chickens housed in PL systems, resulting in a higher EPEF score.

The findings of this study show that supplementing broiler chickens' drinking water with organic L-SeMet combined with vitamin E is more effective in preserving growth performance and improving feed efficiency (Table 2). The higher supplementation rates (1 and 1.5 mg/L L-SeMet + 250 mg/L V.E) yielded more pronounced positive effects. This finding indicates that the combination of organic L-SeMet was effective in enhancing energy utilization of feed. The findings from this study align with the results of Calik et al. (2022b), suggesting that incorporating organic selenium + V.E into broiler chickens' diet at a level of 1:250 mg/kg can significantly enhance both BWG and FI. The observed enhancement in productive performance in this investigation can be attributed to antioxidant properties of organic L-SeMet and V.E, which help protect against potential stressors encountered during standard husbandry practices. Selenium plays crucial roles in the body, primarily as a key component of selenoproteins (Wang et al., 2021a). These selenoproteins participate in various functions, comprising the metabolism of thyroid hormones. Specifically, they facilitate the transformation of T4 into its active state T3 (Schomburg, 2012), which has a crucial function in controlling energy and protein assimilation in the body (Wickramasuriya et al., 2023). The current study suggests that increased thyroid hormone levels significantly enhance growth performance and feed efficiency in broiler chickens. However, limited published studies exists on the effects of L-SeMet + V.E supplementation via drinking water under standard husbandry practices, indicating a need for further investigation.

Blood biochemical analysis

The assessment of blood biochemical factors serves as a valuable tool for identifying metabolic and nutritional changes, which are crucial for assessing the overall health condition in poultry (Ghasemi et al., 2013). The study revealed significant effects of litter type, L-SeMet + V.E supplementation, and their interaction on blood biochemical factors. Broiler chickens raised on both litter types and given T2 or T3 showed increased HDL levels and reduced plasma lipids and liver enzymes. The findings of the current studyare in line with a former experiment conducted by Okasha et al. (2021b) on broiler chickens. Similarly, broiler chickens reared on wood shavings and sand showed significantly lower levels of CHO, TRIG, LDL compared to those reared on plastic litter. Additionally, higher levels of HDL were obverved in broiler chickens reared on WS litter compared to those raised on sand litter (Lasheen et al., 2023). The study found higher AST activity in broiler chickens raised on wood shavings compared to perforated plastic litter, though it did not impact broiler chickens' well-being. These results are consistent with previous findings, with AST activity highest in the avian heart, followed by the liver and skeletal muscle (Ognik and Krauze, 2016; Costa et al., 2021).

Incorporation of L-SeMet + V.E into drinking water led to decreased plasma lipid profile and liver enzymes, alongside increased plasma HDL levels in broiler chickens raised on both types of litter, as indicated in Table 3. These findings align with Kang et al. (2000) who proposed that selenium has a cholesterol-lowering effect, decreasing both cholesterol and triglycerides levels. Additionally, Amer et al. (2019) discovered that incorporating Se, irrespective of its origin, resulted in a reduction in TRIG levels in rabbits. Selenium plays a role in metabolism of lipids (Zhang et al., 2018). The study suggests that L-SeMet + V.E supplementation improves lipid metabolism by increasing HDL levels and reducing LDL levels, which is beneficial as lower LDL minimizes the risk of oxidase-low-density lipoproteins (ox-LDL) formation and cholesterol buildup in arterial walls (Toth, 2005). Therefore, selenium has a crucial function in safeguarding cells against oxidative stress by boosting the activity of glutathione peroxidase (Negis et al., 2006). Incorporation of L-SeMet + V.E had a significant impact on the serum ALT and AST levels in broiler chickens. This is reinforced by the discovery of Dalia et al. (2017)**,** who observed a notable reduction in the liver enzymes in chickens fed organic selenium. Moreover, Biswas et al. (2010) documented comparable results, noting a reduction in ALT and AST concentrations in broiler chickens that received a diet enriched with Se at a level of 0.5 and 1 mg/kg. A decrease in lipid profile and liver enzyme levels indicates improved protection against oxidative damage by enhancing the redox state. These findings are supported by the antioxidant status results obtained in this study.

Antioxidants and thyroid hormones

In avian physiology, monitoring oxidative status plays a crucial role in assessing health changes (Surai et al., 2019). Assessing antioxidant levels in broiler chickens typically involves analyzing parameters such as T-AOC and MDA in the bloodstream, as well as the activity of enzymatic scavengers SOD and GPx (Ghasemi et al., 2020). Broiler chickens raised on PL litter and supplemented with 1.5 mg/L of L-SeMet + V.E showed improved serum antioxidant status and thyroid hormones,

highlighting its protective role against oxidative stress. In line with the present results, Ghanima et al. (2020) and El-Maaty et al. (2023) demonstrated that broiler chickens reared on perforated PL exhibited improved antioxidant status in contrast to those reared on floor litter or in batteries. According to Okasha et al. (2021b), substantial differences were noted in the plasma GPX and MDA levels of broiler chickens as a result of the litter type. Antioxidant and thyroid hormone levels in broiler chickens raised on wood shavings may be influenced by stress factors such as mold, iodine interference, and ammonia exposure (Şen et al., 2023). Further research is needed to fully understand the effects of different litter types on broiler chicken production under standard practices.

Chickens' antioxidant system is regulated by numerous crucial enzymes, comprising T-AOC, GPx, SOD, and MDA (Wang et al., 2021b). Antioxidant properties in selenium stem from its role in GSH-Px, where it forms part of the enzyme's active center, aiding in the removal of unstable molecules generated during metabolism (Huang et al., 2019; Deng et al., 2022). The findings suggest reduced lipid oxidation, likely due to the higher bioavailability of organic selenium combined with V.E., which enhances selenium retention in the body. Consequently, this increased retention contributes to elevated levels of GSH-Px (Zhang et al., 2014). Likewise, Dalia et al. (2017) found that bacterial organic selenium (BOSe) is a superior selenium source for broiler chickens, enhancing antioxidant levels and glutathione peroxidase gene activation more effectively than inorganic selenium. Thyroid hormones (T3 and T4) play a vital role in gluconeogenesis and glycogen synthesis in poultry, facilitating the transformation of non-carbohydrate substrates into glucose and promoting the production and storage of glycogen in the liver (Duntas and Brenta, 2018). The current study revealed that L-SeMet + V.E supplementation elevated T3 and T4 levels in broiler chickens, likely attributed to the functions of selenium in promoting the conversion of T4 to T3 and sustaining selenium proteins. Similarly, Lin et al. (2014) demonstrated that selenium deficiency hinders the conversion of T4 to T3 in chickens by decreasing the activity of critical metabolic enzymes (Iodothyronine Deiodinase Type-1, Iodothyronine Deiodinase Type-2, and Iodothyronine Deiodinase Type-3) and selenoproteins (Thioredoxin Reductase 2, Selenoprotein I, Selenoprotein U, Glutathione peroxidase 1, and Glutathione Peroxidases 2), thereby indirectly disrupting thyroid hormone metabolism. Enhancing antioxidant response and regulating T3 and T4 through nutrition is of growing interest. The study hypothesizes that supplementing L-SeMet and Vitamin E in drinking water may improve antioxidant and thyroid hormone levels, boosting broiler chickens' performance and feed efficiency. Supplementing L-SeMet and Vitamin E in drinking water could lead to cost savings through improved feed efficiency, reduced health issues, and enhanced broiler chickens' welfare due to improved health and growth. These benefits make it a promising strategy for poultry farming.

CONCLUSION

Broiler chickens raised on perforated plastic litter and supplemented with L-SeMet + Vitamin E in drinking water showed improved growth, higher feed efficiency, enhanced antioxidant status, and elevated T3 and T4 levels. The supplementation also improved blood parameters, increasing HDL and reducing lipid profile and liver enzyme levels. This approach demonstrates significant benefits for broiler chicken productivity and health under standard husbandry practices. By enhancing strategies for organic supplementation, assessing their long-term impacts, and investigating the interactions between L-SeMet and other nutrients, broiler chicken farming practices could be significantly advanced. These initiatives would contribute to sustainable, efficient, and welfare-focused poultry production in the future.

DECLARATIONS

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Authors' contributions

Hamada Okasha, Eman Rady, Osama El-Garhy, and Gaafar EL-Gendi authored the original text, contributed to the experimental design, carried out the experiments, and conducted the statistical analysis. Hamada Okasha and Eman Rady contributed to both the statistical analysis and the design of the experiments. Hamada Okasha, Eman Rady, Osama El-Garhy, and Gaafar EL-Gendi analyzed and reviewed the results and then composed the final essay. All authors have reviewed and approved the final version of the manuscript for publication.

Competing interests

The authors declare no conflicts of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors.

Availability of data and materials

The data obtained in this study can be obtained from the corresponding author upon reasonable request.

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