Amol University of Special Modern Technologies

Caspian Journal of Veterinary Sciences

doi: 10.22034/cjvs.2026.592645.1086

Journal homepage: https://cjvs.ausmt.ac.ir/

Effects of dietary propylene glycol with or without B-complex vitamins on serum biochemical and hematological responses in broiler chickens

Hossein Vakili[1], Yahya Ebrahimnezhad[1*], Naser Maheri-Sis[1], Jamshid Ghiasi Ghalehkandi[2], Abolfazl Aghajanzadeh-Golshani[1]

[1]Department of Animal Science, Shab. C., Islamic Azad University, Shabestar, Iran.
[2]Department of Veterinary Medicine, Shab. C., Islamic Azad University, Shabestar, Iran.

(*) Corresponding Author: ebrahimnezhad@gmail.com

Article Info

Article history:

Submit Date: 19 July 2026

Accept Date: 28 August 2026

Online Date: 15 November 2026

Keywords:

B-complex vitamins

Broiler chicken

Hematology

Propylene glycol

Serum biochemistry

Abstract

Propylene glycol (PG) has been proposed as an alternative glucogenic energy source in poultry nutrition, but its metabolic effects with B-complex vitamins remain unclear. A total of 660 one-day-old Ross 308 broiler chicks were allocated to a completely randomized 3 × 4 factorial design comprising four dietary PG levels (0, 4, 8, and 12%) and three B-complex vitamin levels (0, 0.10, and 0.20%). Twelve treatments included five replicates of 11 birds each. Serum biochemical and hematological responses of male and female broilers were evaluated. Dietary PG did not significantly affect serum glucose, lactate, pyruvate, urea, uric acid, lactate dehydrogenase (LDH), or high-density lipoprotein (HDL), although uric acid and LDH increased numerically at higher inclusion levels. White blood cell and platelet counts were unaffected. In contrast, triglyceride, total cholesterol, and low-density lipoprotein (LDL) concentrations increased significantly at the highest PG level ($p < 0.05$). Red blood cell count, hemoglobin, and hematocrit increased significantly in males ($p < 0.05$), while females showed similar nonsignificant numerical trends. B-complex supplementation produced no measurable additional effects or interactions with PG. Overall, PG modified lipid metabolism and erythrocyte-related hematological characteristics without significantly affecting most carbohydrate or nitrogen metabolism indicators. Dietary inclusion of 4–8% PG appears more appropriate than 12% under the conditions of this study.

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Introduction

The poultry industry is under increasing pressure to improve production efficiency while reducing reliance on conventional feed ingredients, particularly cereal grains, which are increasingly required for human consumption. The expanding competition between human food and livestock feed resources has encouraged the search for alternative dietary energy sources capable of partially replacing cereal grains without compromising animal health, metabolic homeostasis or productive performance (EFSA, 2018; FAO, 2023; OECD–FAO, 2024).

Among the proposed alternatives, propylene glycol (PG; 1,2-propanediol) has attracted considerable attention because of its glucogenic properties and rapid hepatic metabolism. Following intestinal absorption, PG is readily metabolized to lactate and pyruvate before entering gluconeogenic pathways, thereby providing a rapidly available source of metabolic energy (Huff, 1961; Ruddick, 1972; ATSDR, 2008). These metabolic characteristics have resulted in extensive investigation of PG as an alternative glucogenic energy source in both ruminant and non-ruminant animals.

The nutritional value of PG in poultry was first demonstrated more than five decades ago. Early studies reported that partial replacement of dietary cereal energy with PG maintained satisfactory growth performance when moderate inclusion levels were used, whereas excessive dietary supplementation impaired productive responses and altered normal physiological function (Persons *et al.,* 1968; Waldroup and Bowen, 1968; Bayley *et al.,* 1969). These pioneering studies established PG as a potential alternative dietary energy source for broiler chickens and provided the basis for subsequent investigations of its metabolic and physiological effects.

Beyond poultry, PG has been evaluated in several animal species. Long-term toxicological studies indicated that PG is generally well tolerated under appropriate exposure conditions in experimental animals (Weil *et al.,* 1971; Ema *et al.,* 2014), whereas studies in rabbits demonstrated its close association with lactate metabolism (Morshed *et al.,* 1991). In transition dairy cows, PG has been widely investigated as a glucogenic substrate capable of modifying energy balance and intermediary metabolism (Nielsen and Ingvartsen, 2004; Tan *et al.,* 2024). More recently, Yakan *et al*. (2023) demonstrated that dietary PG influenced several metabolic variables and hepatic expression of genes and proteins associated with lipid metabolism, including PPARγ, DGAT1, ChREBP, and SREBP-1c. Similar metabolic responses have also been reported in Nile tilapia, where dietary PG affected physiological responses and transcriptional regulation without marked adverse effects (Soaudy *et al.,* 2021; Saleh *et al.,* 2026).

Because PG is metabolized through intermediary carbohydrate metabolism, its physiological effects may extend beyond simple energy supply. The conversion of PG to lactate and pyruvate links this compound directly to gluconeogenesis and cellular energy production, making circulating concentrations of glucose, lactate, pyruvate and lactate dehydrogenase (LDH) useful indicators of its metabolic utilization (Ruddick, 1972; Nielsen and Ingvartsen, 2004). In addition, alterations in lipid metabolism following PG supplementation may be reflected by changes in serum triglycerides, cholesterol and lipoprotein fractions, whereas urea and uric acid provide information regarding nitrogen metabolism and protein utilization. Recent research in broilers has also demonstrated that dietary interventions can substantially modify serum lipid profiles and blood biochemical characteristics, highlighting the importance of evaluating metabolic responses alongside productive traits (Ai *et al.,* 2025; Ahmadipour *et al.,* 2025).

The metabolic consequences of dietary interventions may also be reflected in hematological characteristics. Erythropoiesis and hemoglobin synthesis are metabolically demanding processes requiring adequate energy supply and enzyme activity. Consequently, alterations in energy metabolism may influence erythrocyte count, hemoglobin concentration and hematocrit, while leukocyte and platelet profiles provide additional information regarding physiological adaptation and general health status (Maxwell and Robertson, 1998; Campbell, 2015). Nevertheless, compared to productive performance and biochemical responses, the influence of dietary PG on hematological variables has received relatively little attention in broiler chickens.

The efficiency of PG utilization may also depend on the availability of vitamin-dependent metabolic pathways. B-complex vitamins function as essential cofactors for numerous enzymes involved in carbohydrate oxidation, gluconeogenesis, and cellular energy metabolism (McDowell, 2000; Combs, 2012). Adequate dietary B-vitamin supply supports normal metabolic function and physiological adaptation in poultry (Alagawany *et al.,* 2021; Kidd, 2022). However, it remains unclear whether supplemental B-complex vitamins can improve the metabolic utilization of PG or modify physiological responses associated with increasing PG inclusion in broiler diets.

Despite increasing interest in PG as an alternative glucogenic energy source, limited information is available regarding its dose-dependent effects on the hematological profile and serum biochemical characteristics of broiler chickens, particularly when combined with supplemental B-complex vitamins. Clarifying these responses is important for understanding the metabolic utilization of PG and evaluating its suitability as a partial substitute for cereal-derived dietary energy. Therefore, the present study was designed to determine whether B-complex vitamin supplementation could modify or mitigate the metabolic and hematological responses to relatively high dietary PG levels. Our hypothesis was that increased PG intake may increase the demand on metabolic pathways involved in its utilization, while B-vitamins, as important metabolic cofactors, may influence the capacity of these pathways to respond to this metabolic load. The specific interaction between relatively high PG levels and B-complex vitamin supplementation has received limited attention in broiler chickens. Therefore, the main contribution of this study was the evaluation of PG responses under different levels of B-vitamin availability rather than the evaluation of PG or B-vitamins independently.

Materials and Methods

Experimental design and birds

The experiment was conducted at the poultry research facilities of Islamic Azad University, Shabestar Branch, Iran. All experimental procedures involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011) and the institutional animal care requirements of Amol University of Special Modern Technologies.

A total of 660 one-day-old Ross 308 broiler chickens were used in a completely randomized design with a 3 × 4 factorial arrangement. Experimental factors consisted of four dietary levels of propylene glycol (0, 4, 8 and 12%) and three dietary levels of B-complex vitamins (0, 0.10 and 0.20%). Twelve experimental treatments were established with five replicates per treatment and 11 birds per replicate. Male and female birds were reared together throughout the experimental period under standard commercial management conditions from day 1 to day 42. The inclusion levels of PG were selected to evaluate both practical dietary incorporation and the physiological responses associated with increasing glycogenic substrate availability. The 12% PG inclusion level was selected as a high-dose treatment to investigate potential metabolic and hematological responses to excessive dietary PG and to determine whether supplemental B-complex vitamins could attenuate possible adverse effects. This level was not intended to represent a recommended or presumed safe dietary inclusion level. Growth performance responses to the dietary PG treatments were evaluated separately and were reported in a companion manuscript currently under consideration for publication. The present manuscript focused specifically on serum biochemical and hematological responses.

Experimental diets

Starter (1–10 days), grower (11–24 days) and finisher (25–42 days) diets were formulated to meet or exceed the nutrient requirements recommended for Ross 308 broiler chickens. Ingredient composition and calculated nutrient contents were presented in Tables 1–3. PG (USP grade; Lot No, F02641UP) was supplied by SK Pic global Co., Ltd., Ulsan, Republic of Korea and partially replaced cereal-derived energy sources while maintaining similar calculated nutrient densities among experimental diets. The commercial B-complex vitamin premix (Golbar Shimi Dane Co., Iran) contained the principal water-soluble B vitamins, including thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, cyanocobalamin, biotin, and choline.

Bird management

Birds were housed in floor pens under

conventional commercial management practices. Feed and water were provided ad libitum throughout the experiment. Ambient temperature, ventilation and lighting programs were managed according to Ross 308 management recommendations. Vaccination schedules and routine health management practices were performed according to regional commercial standards.

Blood sampling

At 42 days of age, one male and one female broiler were randomly selected from each replicate pen. Before blood collection, feed was withdrawn for 5 hours, while birds had free access to water. Blood sampling began at 06:00 hours, following the 5 hours feed withdrawal period. The environmental temperature and relative humidity during sampling were maintained according to the recommended conditions for 42-day-old Ross 308 broilers. Blood samples were collected aseptically from the brachial (wing) vein using sterile disposable syringes. For serum biochemical analyses, blood was transferred into plain vacuum tubes and allowed to clot at room temperature. Samples were subsequently centrifuged at 3000 rpm for 10 minutes, and serum was harvested and stored at −20°C until laboratory analyses. For hematological analyses, blood samples were immediately transferred into EDTA-containing tubes and analyzed shortly after collection.

Biochemical analyses

Serum glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL), uric acid, urea, lactate, pyruvate, and lactate dehydrogenase (LDH) activity were measured using commercial diagnostic kits (Pars Azmoon Co., Tehran, Iran), according to themanufacturer's instructions. LDL-cholesterol was directly measured using a commercial enzymatic kit (Pars Azmoon, Iran). All biochemical measurements were performed using an automated clinical chemistry analyzer (BT 3000 Plus, Biotecnica Instruments S.P.A., Rome, Italy) according to the manufacturer's recommendations and quality-control procedures.

Hematological analyses

Hematological determinations included: Red blood cell count (RBC), Hemoglobin concentration (Hb), Hematocrit (Hct), White blood cell count (WBC), Platelet count (PLT). Measurements were performed using standard hematological laboratory procedures routinely employed in avian clinical pathology with an automated analyzer (Mindray BC-3000plus).

Statistical analysis

Before statistical analysis, the data were evaluated for normality and homogeneity of variance to verify the assumptions of parametric analysis. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Data were analyzed using the general linear model (GLM) procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). The following statistical model was applied:

$$Y_{ijk} = \mu + P_i + B_j + (PB)_{ij} + e_{ijk}$$

Where Y_{ijk} is the observed variable, μ is the overall mean, P_i is the effect of PG level, B_j is the effect of B-complex vitamin level, $(PB)_{ij}$ is the interaction effect between PG and B-complex vitamins and e_{ijk} is the residual error. When treatment effects were significant ($p < 0.05$), means were separated using Duncan's multiple range test (Duncan, 1955).

Results

Serum biochemical characteristics of male broiler chickens

The effects of dietary PG, B-complex vitamin supplementation and their interaction on serum biochemical variables of male broiler chickens were presented in Table 4.

Dietary PG had no significant effect on serum glucose, lactate, pyruvate, urea, HDL cholesterol, uric acid or LDH activity ($p > 0.05$). In contrast, serum triglyceride, total cholesterol, and LDL cholesterol were significantly affected by dietary PG ($p < 0.05$). Birds receiving the highest dietary PG level exhibited significantly greater serum triglyceride, total cholesterol and LDL concentrations than the control group, whereas no significant differences were observed among the lower PG inclusion levels. Dietary supplementation with B-complex vitamins did not significantly affect any of the evaluated serum biochemical variables ($p > 0.05$). Likewise, no significant interaction between dietary PG and B-

complex vitamin supplementation was detected for any serum biochemical parameter ($p > 0.05$).

Serum biochemical characteristics of female broiler chickens

The effects of dietary PG, B-complex vitamin supplementation and their interaction on serum biochemical characteristics of female broiler chickens were presented in Table 5.

Dietary PG did not significantly influence serum glucose, HDL cholesterol, uric acid, urea, lactate, LDH activity or pyruvate concentration ($p > 0.05$). However, serum triglyceride, total cholesterol, and LDL cholesterol were significantly affected by dietary PG ($p < 0.05$). Increasing dietary PG resulted in significantly greater serum triglyceride, total cholesterol, and LDL concentrations, with the highest values observed in birds fed the greatest PG inclusion level. B-complex vitamin supplementation had no significant effect on any serum biochemical variable evaluated ($p > 0.05$). Similarly, no significant interaction between dietary PG and B-complex vitamin supplementation was observed for any serum biochemical parameter ($p > 0.05$).

Hematological characteristics of male broiler chickens

The effects of dietary PG, B-complex vitamin supplementation and their interaction on hematological characteristics of male broiler chickens were presented in Table 6.

Dietary PG significantly affected erythrocyte-related hematological indices, including RBC count, Hb concentration, and Hct ($p < 0.05$). Compared to the control treatment, dietary supplementation with PG significantly increased RBC count, Hb concentration, and Hct, whereas no significant differences were detected among the PG-supplemented groups. In contrast, total WBC count and platelet count were not significantly influenced by dietary PG ($p > 0.05$). B-complex vitamin supplementation did not significantly affect any hematological variable evaluated ($p > 0.05$). Likewise, no significant interaction between dietary PG and B-complex vitamin supplementation was observed for any hematological characteristic ($p > 0.05$).

Hematological characteristics of female broiler chickens

The effects of dietary PG, B-complex vitamin supplementation and their interaction on hematological characteristics of female broiler chickens were presented in Table 7.

Unlike the responses observed in male broilers, dietary PG did not significantly affect RBC count, Hb concentration, Hct, total WBC count or platelet count in female birds ($p > 0.05$). Similarly, dietary supplementation with B-complex vitamins had no significant effect on any hematological parameter evaluated ($p > 0.05$). Furthermore, no significant interaction between dietary PG and B-complex vitamin supplementation was detected for any hematological characteristic ($p > 0.05$).

Discussion

Dietary PG primarily affected lipid-related biochemical variables and erythrocyte-associated hematological indices, whereas most indicators of carbohydrate and nitrogen metabolism remained stable. In contrast, supplemental B-complex vitamins did not significantly affect any measured variable or interact with dietary PG. These findings suggest that the physiological response to PG was mainly related to energy partitioning and erythrocyte homeostasis rather than generalized metabolic disturbance. The significant increases in serum triglycerides, total cholesterol and LDL cholesterol with increasing PG inclusion suggest that PG altered lipid metabolism, particularly at higher dietary concentrations. PG is rapidly absorbed and metabolized through lactate and pyruvate intermediates before entering gluconeogenic pathways (Huff, 1961; Ruddick, 1972). When glucose homeostasis is adequately maintained, additional carbon supplied by PG may influence hepatic energy partitioning and lipid synthesis (Leeson and Summers, 2009; Scanes, 2022; Xie *et al.,* 2023). Recent molecular evidence supports this interpretation. Yakan *et al*. (2023) reported that PG supplementation altered hepatic expression of PPARγ, DGAT1, ChREBP, and SREBP-1c, indicating that PG can influence regulatory pathways involved in lipid synthesis and energy metabolism. Their subsequent study also demonstrated dose- and duration-dependent changes in lipid-metabolism-related genes, proteins and fatty-acid profiles following PG supplementation (Yakan *et al.,* 2025). Thus, the

increase in circulating lipid fractions observed in the present study may reflect altered metabolic partitioning of PG-derived carbon rather than impaired carbohydrate utilization.

Comparable metabolic responses have been reported across animal species. In dairy cattle, PG modifies intermediary energy metabolism according to physiological state and dietary conditions (Nielsen and Ingvartsen, 2004; Tan *et al.,* 2024). In Nile tilapia, dietary PG has also been associated with changes in lipid-metabolism-related gene expression (Soaudy *et al.,* 2021; Saleh *et al.,* 2026). Importantly, the classical poultry studies of Persons *et al*. (1968), Waldroup and Bowen (1968), and Bayley *et al*. (1969) primarily evaluated growth performance, feed utilization, and the energetic value of PG rather than detailed serum biochemical responses. The present study therefore extends those early nutritional observations by demonstrating that increasing PG inclusion can also influence circulating lipid fractions in broilers.

The absence of a significant effect on HDL cholesterol, despite its numerical decline with increasing PG, suggests that not all lipoprotein fractions respond equally to dietary PG. Such differential regulation of serum lipid fractions is consistent with recent broiler studies demonstrating that dietary energy and lipid sources can modify LDL and HDL concentrations independently (Ai *et al.,* 2025). Therefore, the principal lipid response to PG in the present study appears to involve triglycerides, total cholesterol and LDL rather than a generalized alteration of all circulating lipoproteins.

In contrast to lipid metabolism, glucose, lactate and pyruvate concentrations were not significantly affected by PG. The maintenance of stable glucose concentrations indicates that increasing PG inclusion did not disrupt systemic glucose homeostasis. Likewise, the absence of lactate and pyruvate accumulation suggests efficient utilization of PG-derived intermediates and effective metabolic regulation (Ruddick, 1972; Scanes, 2022). Similar stability of glucose and intermediary metabolites has been reported in other animal species receiving PG (Morshed *et al.,* 1991; Nielsen and Ingvartsen, 2004). The findings of Yakan *et al*. (2023), which demonstrated dose- and duration-dependent biochemical responses without evidence of major metabolic impairment, further support the concept that PG can be efficiently incorporated into intermediary metabolism .

Serum LDH activity was also not significantly affected by PG, although a numerical increase occurred in males at the highest PG inclusion level. Because LDH participates in the reversible conversion of lactate and pyruvate and may increase with tissue injury or metabolic stress, the absence of a significant response, together with stable lactate and pyruvate concentrations, does not indicate detectable tissue damage under the conditions of the present experiment (Thrall *et al.,* 2012; Campbell, 2015). Thus, the highest PG level appeared to influence lipid-related variables more strongly than carbohydrate-related indicators.

Similarly, serum urea and uric acid concentrations remained unchanged in both sexes. Uric acid is the principal end product of nitrogen metabolism in birds, and its stability suggests that increasing PG inclusion did not substantially alter protein catabolism or amino-acid utilization for energy (Kaneko *et al.,* 2008; Campbell, 2015). Comparable stability of nitrogen-related biochemical variables has been reported in rabbits and other animal models receiving PG (Morshed *et al.,* 1991). These findings further indicate that the major metabolic effect of PG in the present study was associated with lipid partitioning rather than protein metabolism.

The hematological findings demonstrated a more specific response. In males, RBC count, Hb concentration and Hct increased significantly with increasing PG inclusion, while females showed a similar numerical dose-dependent pattern without statistical significance. The consistency of the direction of response between sexes suggests a biological association with increasing PG exposure, although the greater magnitude in males indicates possible sex-related differences in the response.Information concerning PG-induced hematological responses in poultry remains limited. Previous toxicological studies reported that PG is generally well tolerated under appropriate exposure conditions, without evidence of marked hematological toxicity (Weil *et al.,* 1971; Ema *et al.,* 2014). Species-specific responses have nevertheless been described, particularly in cats, in which erythrocytes are susceptible to oxidative injury following prolonged PG exposure (Ema *et al.,* 2014).

Therefore, the increases in RBC count, Hb and Hct observed in the present study should not be interpreted as direct evidence of toxicity or as unequivocal evidence of a beneficial effect.

Although increased erythrocyte indices can theoretically improve oxygen-carrying capacity, excessive increases may also increase blood viscosity. In the present study, the values remained within reported physiological ranges for broilers (Meluzzi *et al.,* 1992; Maxwell and Robertson, 1998; Campbell, 2015). Consequently, the response may represent a physiological adaptation to altered energy metabolism rather than pathological erythrocytosis. The greater response in males may also reflect sex-related differences in erythropoietic activity or metabolic demand, although the underlying mechanism cannot be established from the measured variables alone.

In contrast, leukocyte and platelet counts remained unchanged. Because these variables are sensitive to inflammatory, immune and hematopoietic disturbances, their stability suggests that PG did not produce a generalized hematological or inflammatory response under the experimental conditions (Maxwell and Robertson, 1998; Campbell, 2015). Thus, the hematological effect of PG was primarily restricted to erythrocyte-associated variables.

Supplemental B-complex vitamins did not significantly affect any biochemical or hematological variable and did not interact with PG. B-complex vitamins function as cofactors in numerous metabolic pathways, including glycolysis, the tricarboxylic acid cycle, and gluconeogenesis (McDowell, 2000; Combs, 2012). Nevertheless, the absence of an additional response suggests that the basal diet provided sufficient vitamin concentrations to support normal intermediary metabolism. Recent broiler research similarly indicated that dietary supplementation with vitamin-containing bioactive complexes can influence serum biochemical and antioxidant responses under specific nutritional or environmental conditions, but responses depend strongly on the physiological challenge and dietary background (Azzam *et al.,* 2023). Under the relatively adequate nutritional conditions of the present study, additional B-complex supplementation therefore did not provide a measurable advantage. The absence of significant PG × B-complex interactions further indicated that the metabolic responses to PG occurred largely independently of supplemental B-vitamin concentration. This finding is practically relevant because increasing vitamin supplementation beyond nutritional requirements may not necessarily improve the utilization of PG when basal dietary vitamin supply is already adequate (Alagawany *et al.,* 2021; Kidd, 2022).

Recent broiler research has also demonstrated that changes in lipid metabolism may be accompanied by alterations in oxidative status and expression of genes involved in lipid utilization. For example, Ahmadipour *et al*. (2025) reported that dietary interventions affecting energy and lipid metabolism modified serum cholesterol, triglycerides, LDL and HDL as well as oxidative-stress-related variables and genes in broilers. Although oxidative-stress biomarkers were not measured in the present study, these findings provide a mechanistic context for interpreting the lipid responses observed following PG supplementation and indicate a useful direction for future research.

Overall, the present findings indicated that increasing dietary PG primarily modified circulating lipid fractions and erythrocyte-related hematological variables, while glucose, lactate, pyruvate, LDH, urea, and uric acid remained largely stable. The response was more pronounced at the highest PG inclusion level, whereas the lower inclusion levels produced fewer statistically significant alterations. The absence of significant effects from B-complex supplementation suggests that additional B vitamins did not modify PG utilization when the basal diet was nutritionally adequate. Therefore, under the conditions of the present experiment, PG inclusion at 4–8% appears to represent a more appropriate practical range, whereas the 12% inclusion level should be applied cautiously because of the observed changes in serum lipid and erythrocyte-related variables.

It was concluded that dietary PG primarily affected serum lipid fractions and erythrocyte-related hematological variables, while most indicators of carbohydrate and nitrogen metabolism remained unchanged. The effects were more evident at the highest inclusion level. Supplemental B-complex vitamins did not significantly modify biochemical or hematological

responses or interact with PG. Under the conditions of this study, dietary PG at 4–8% appears to be a more appropriate inclusion range, whereas the 12% level should be used cautiously. Further research is warranted to clarify the molecular mechanisms underlying the lipid and hematological responses to PG.

Acknowledgment

This manuscript was extracted from the Ph.D dissertation of the first author in Animal Nutrition at the Shab. C., Islamic Azad University. The authors gratefully acknowledge the financial and technical support provided by the Shab. C., Islamic Azad University.

Conflict of Interest

All authors declare that they have no conflicts of interest.

References

Alagawany, M; Abd El-Hack, ME; Farag, MR; Elnesr, SS and El-Kholy, MS (2021). Nutritional significance and health benefits of vitamins in poultry nutrition: A review. Worlds Poult. Sci. J., 77: 253–276.

Agency for Toxic Substances and Disease Registry (ATSDR) (2008). Toxicological profile for propylene glycol. U.S. Department of Health and Human Services, Atlanta.

Ahmadipour, B; Hasani, FF; Hassanpour, H and Khajali, F (2025). Effect of L-carnitine and emulsifier on growth performance, lipid profile, lipogenesis, and oxidative stress of broiler chickens raised at high altitude. Poult. Sci., 104: 105157.

Ai, H; Lee, YY; Lu, Y; Tan, CP; Lai, OM; Li, A; Zhang, Y; Wang, Y and Zhang, Z (2025). Effect of structured lipids as dietary supplements on the fatty acid profile, carcass yield, blood chemistry, and abdominal fat deposition of female broilers. Poult. Sci., 104: 104579.

Azzam, MMM; Al-Abdullatif, AA; Akasha, ME; Alhotan, RA; Suliman, GM; Lahaye, L and Santin, E (2023). Effects of protected complex of biofactors and antioxidants on growth performance, serum biochemistry, meat quality, and intestinal antioxidant and immunomodulatory-related gene expressions of broiler chickens. Poult. Sci., 102: 102666.

Bayley, HS; Slinger, SJ and Summers, JD (1969). The use of propylene as a source of energy for the chick. Poult. Sci., 48: 19–22.

Campbell, TW (2015). *Exotic Animal Hematology and Cytology*. 4th ed. Wiley-Blackwell, Ames.

Combs, GF (2012). *The Vitamins: Fundamental Aspects in Nutrition and Health*. 4th ed. Academic Press, San Diego.

Duncan, DB (1955). Multiple range and multiple F tests. Biometrics, 11: 1–42.

EFSA Panel on Food Additives and Nutrient Sources Added to Food (2018). Re-evaluation of propane-1, 2-diol (E1520) as a food additive. EFSA. J., 16: 5235–5240.

Ema, M.; Gharbi, N and Nohmi, T (2014). Safety evaluation of propylene glycol: A review of toxicological studies in experimental animals and humans. Food Chem. Toxicol., 72: 132–148.

Food and Agriculture Organization of the United Nations (FAO) (2023). The State of Food and Agriculture. Rome: FAO.

Huff, E (1961). The metabolism of 1, 2-propanediol. Biochim. Biophys. Acta., 48: 506-517.

Kaneko, JJ; Harvey, JW and Bruss, ML (2008). *Clinical Biochemistry of Domestic Animals*. 6th ed. Academic Press, San Diego.

Kidd, MT (2022). Nutritional modulation of immune function in poultry. Worlds Poult. Sci. J., 78: 41–56.

Leeson, S and Summers, JD (2009). *Commercial Poultry Nutrition*. 3rd ed. Nottingham University Press, Nottingham.

Maxwell, MH and Robertson, GW (1998). The avian heterophil leucocyte: A review. Worlds Poult. Sci. J., 54: 155–178.

McDowell, LR (2000). *Vitamins in Animal and Human Nutrition*. 2nd ed. Iowa State University Press, Ames.

Meluzzi, A; Primiceri, G; Giordani, R and Fabris, G (1992). Determination of blood constituents reference values in broilers. Poult. Sci., 71: 337–345.

Morshed, KM; Nagpaul, JP; Amma, MKP and Desjeux, JF (1991). The role of propylene glycol metabolism in lactatemia in the rabbit. Biochem. Med. Metab. Biol., 46: 145–151.

National Research Council (NRC) (2011). *Guide for the Care and Use of Laboratory Animals*. 8th ed., Washington, DC, USA, National Academies Press. PP: 1–220.

Nielsen, NI and Ingvartsen, KL (2004). Propylene glycol for dairy cows: a review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. Anim. Feed Sci. Technol., 115: 191–213.

Organization for Economic Cooperation and Development (OECD) (2024). *Food and Agriculture Organization of the United Nations* (FAO). OECD–FAO Agricultural Outlook 2024–2033: OECD Publishing; Rome: FAO.

Persons, JN; Damron, BL; Waldroup, PW and Harms, RH (1968). Propylene glycol as an energy source for poultry. Poult. Sci., 47: 351–353.

Ruddick, JA (1972). Toxicology, metabolism and biochemistry of 1, 2-propanediol. Toxicol. Appl. Pharmacol., 21: 102–111.

Saleh, DR; El-Nahas, AF; Abd El Naby, WSH; Aly, HA; El-Haroun, E and Khatab, SA (2026). Evaluation of propylene glycol and essential oil supplementation on growth performance, feed efficiency, serum

biochemical indices, hematological parameters, and the expression of antifreeze IV and lipid metabolism-related genes in Nile tilapia. Animals, 16: 615.

Scanes, CG (2022). *Sturkie's Avian Physiology*. 7th ed. Academic Press, San Diego.

Soaudy, MR; Mohammady, EY; Elashry, MA; Ali, MM; Ahmed, NM; Hegab, MH; El-Garhy, HAS; El-Haroun, ER and Hassaan, MS (2021). Possibility mitigation of cold stress in Nile tilapia under biofloc system by dietary propylene glycol: Performance, feeding status, immune, physiological responses and transcriptional response of delta-9-desaturase gene. Aquaculture, 538: 736519.

Tan, J; Zhao, H; Li, L; Wang, Y; Pan, Y; Fang, L; Zhao, Y and Jiang, L (2024). Propylene glycol alleviates oxidative stress and enhances immunity in ketotic cows through modulating amino acid and lipid metabolism. Antioxidants, 13: 1146.

Thrall, MA; Weiser, G; Allison, RW and Campbell, TW (2012). *Veterinary Hematology and Clinical Chemistry*. 2nd ed. Wiley-Blackwell, Ames.

Waldroup, PW and Bowen, TE (1968). Evaluation of propylene glycol asan energy source in broiler diets. Poult Sci., 47: 1911–1917.

Weil, CS; Smyth, HF; Carpenter, CP and, Knaak, JB (1971). Results of chronic feeding of propylene glycol in dogs. Food Cosmet. Toxicol., 9: 479–490.

Xie, C; Zhang, Y; Liu, H; Wang, J and Zhao, G (2023). Dietary energy sources regulate lipid metabolism and serum biochemical indices in broiler chickens. Poult. Sci., 102: 102437.

Yakan, A; Özkan, H; Kaya, U; Keçeli, HH; Dalkiran, S; Karaaslan, I; Ünal, N; Akçay, A; Arslan, K; Akyüz, B; Güngör, G; Çamdeviren, B; Küçükoflaz, M; Sariözkan, S and Özbeyaz, C (2023). Effects of propylene glycol used at different doses in Akkaraman lambs rations on metabolism-related parameters and liver gene and protein expression during different feeding periods. Anim. Sci. J., 94: e13886.

Yakan, A; Özkan, H; Keçeli, HH; Kaya, U; Karaaslan, I; Dalkiran, S; Ünal, N; Çamdeviren, B; Güngör, G; Küçükoflaz, M; Sariözkan, S; Arslan, K; Akyüz, B; Akçay, A and Özbeyaz, C (2025). Dose- and duration-dependent effects of propylene glycol on lipid metabolism-related mRNAs, proteins, and fatty acids in the adipose tissue of fattening Akkaraman lambs. Food Sci. Nutr., 13: e71336.

Table 1. Ingredient and calculated nutrient composition of the experimental diets fed to broiler chickens from 1–10 days.

	Dietary treatment											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Ingredients (%)												
Corn grain	56.43	56.43	56.43	48.56	48.56	48.56	40.93	40.93	40.93	33.15	33.15	33.15
Soybean meal (44% CP)	36.87	36.87	36.87	38.45	38.45	38.45	39.98	39.98	39.98	41.52	41.52	41.52
Vegetable oil	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Dicalcium phosphate	2.47	2.47	2.47	2.47	2.47	2.47	2.50	2.50	2.50	2.55	2.55	2.55
Calcium CO3	0.96	0.96	0.96	0.96	0.96	0.96	0.92	0.92	0.92	0.90	0.90	0.90
Bicarbonate Na	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine†	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.23	0.23	0.23
L-lysine HCl‡	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-threonine§	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix¶	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix◊	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Propylene glycol	0	0	0	4.00	4.00	4.00	8.00	8.00	8.00	12.00	12.00	12.00
Vitamin B-complex	0	0.10	0.20	0	0.10	0.20	0	0.10	0.20	0	0.10	0.20
Common salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Inert	0.20	0.10	0	2.49	2.39	2.29	4.60	4.50	4.40	6.80	6.70	6.60
Calculated chemical composition												
Metabolizable energy (kcal/kg)	2880	2880	2880	2880	2880	2880	2880	2880	2880	2880	2880	2880
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Available phosphorus (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chloride (%)	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26
Potassium (%)	0.92	0.92	0.92	0.93	0.93	0.93	0.93	0.93	0.93	0.94	0.94	0.94
Linoleic acid (%)	2.26	2.26	2.26	2.09	2.09	2.09	1.93	1.93	1.93	1.76	1.76	1.76
Lysine (%)	1.31	1.31	1.31	1.34	1.34	1.34	1.36	1.36	1.36	1.39	1.39	1.39
Methionine(%)	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Methionine + cysteine (%)	0.93	0.93	0.93	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
Threonine (%)	0.85	0.85	0.85	0.86	0.86	0.86	0.87	0.87	0.87	0.87	0.87	0.87
Tryptophan (%)	0.31	0.31	0.31	0.31	0.31	0.31	0.32	0.32	0.32	0.33	0.33	0.33
Analyzed composition												
Crude protein (%)	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50	21.50
Ether extract (%)	4.21	4.21	4.21	3.93	3.93	3.93	3.66	3.66	3.66	3.37	3.37	3.37
Crude fiber (%)	2.68	2.68	2.68	2.57	2.57	2.57	2.46	2.46	2.46	2.35	2.35	2.35

†DL-methionine, feed grade 99% (Evonik), ‡L-lysine feed grade 98.5% (AlborzGostar Darou Co), §L-threonine, feed grade 98.5% (Evonik); ¶provided per kilogram of diet: 15,000 IU vitamin A (retinol), 3,750 IU vitamin D3 (cholecalciferol), 20.2 mg vitamin E (alpha-tocopherol), 3.7 mg vitamin K3 (menadione), 3 mg vitamin B1 (thiamine), 7.5 mg vitamin B2 (riboflavin), 55 mg niacin, 4.5 mg of vitamin B6 (pyridoxine), 1 mg biotin, 15 mg vitamin B12 (cobalamin), and 11.5 pantothenic acid; ◊provided per kilogram of diet: 50 mg Mn, 47 mg Zn (zinc oxide), 25 mg Fe, 7.5 mg Cu (copper sulphate), 2.6 mg I, and 0.40 mg Se.

Table 2. Ingredient and calculated nutrient composition of the experimental diets fed to broiler chickens from 11–24 days.

	Dietary treatment											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Ingredients (%)												
Corn grain	57.00	57.00	57.00	49.20	49.20	49.20	41.50	41.50	41.50	33.75	33.75	33.75
Soybean meal (44% CP)	33.34	33.34	33.34	34.94	34.94	34.94	36.45	36.45	36.45	38.02	38.02	38.02
Vegetable oil	4.80	4.80	4.80	4.80	4.80	4.80	4.80	4.80	4.80	4.80	4.80	4.80
Dicalcium phosphate	2.16	2.16	2.16	2.20	2.20	2.20	2.22	2.22	2.22	2.25	2.25	2.25
Calcium CO3	0.88	0.88	0.88	0.85	0.85	0.85	0.83	0.83	0.83	0.81	0.81	0.81
Bicarbonate Na	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine†	0.22	0.22	0.22	0.22	0.22	0.22	0.23	0.23	0.23	0.23	0.23	0.23
L-lysine HCl‡	0.10	0.10	0.10	0.09	0.09	0.09	0.10	0.10	0.10	0.09	0.09	0.09
L-threonine§	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix¶	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix◊	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Propylene glycol	0	0	0	4.00	4.00	4.00	8.00	8.00	8.00	12.00	12.00	12.00
Vitamin B-complex	0	0.10	0.20	0	0.10	0.20	0	0.10	0.20	0	0.10	0.20
Common salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.35	0.35	0.35
Inert	0.43	0.33	0.23	2.64	2.54	2.44	4.81	4.71	4.61	6.99	6.89	6.79
Calculated chemical composition												
Metabolizable energy (kcal/kg)	3090	3090	3090	3090	3090	3090	3090	3090	3090	3090	3090	3090
Calcium (%)	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chloride (%)	0.28	0.28	0.28	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Potassium (%)	0.85	0.85	0.85	0.86	0.86	0.86	0.86	0.86	0.86	0.87	0.87	0.87
Linoleic acid (%)	3.84	3.84	3.84	3.67	3.67	3.67	3.51	3.51	3.51	3.34	3.34	3.34
Lysine (%)	1.22	1.22	1.22	1.23	1.23	1.23	1.26	1.26	1.26	1.28	1.28	1.28
Methionine(%)	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Methionine + cysteine (%)	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Threonine (%)	0.79	0.79	0.79	0.80	0.80	0.80	0.80	0.80	0.80	0.81	0.81	0.81
Tryptophan(%)	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.29	0.29	0.30	0.30	0.30
Analyzed composition												
Crude protein (%)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Ether extract (%)	7.30	7.30	7.30	7.02	7.02	7.02	6.74	6.74	6.74	6.46	6.46	6.46
Crude fiber (%)	2.55	2.55	2.55	2.45	2.45	2.45	2.33	2.33	2.33	2.23	2.23	2.23

†DL-methionine, feed grade 99% (Evonik), ‡L-lysine feed grade 98.5% (AlborzGostar Darou Co), §L-threonine, feed grade 98.5% (Evonik); ¶provided per kilogram of diet: 15,000 IU vitamin A (retinol), 3,750 IU vitamin D3 (cholecalciferol), 20.2 mg vitamin E (alpha-tocopherol), 3.7 mg vitamin K3 (menadione), 3 mg vitamin B1 (thiamine), 7.5 mg vitamin B2 (riboflavin), 55 mg niacin, 4.5 mg of vitamin B6 (pyridoxine), 1 mg biotin, 15 mg vitamin B12 (cobalamin), and 11.5 pantothenic acid; ◊provided per kilogram of diet: 50 mg Mn, 47 mg Zn (zinc oxide), 25 mg Fe, 7.5 mg Cu (copper sulphate), 2.6 mg I, and 0.40 mg Se.

Table 3. Ingredient and calculated nutrient composition of the experimental diets fed to broiler chickens from 25–42 days.

	Dietary treatment											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
												Ingredients [%]
Corn grain	61.00	61.00	61.00	53.10	53.10	53.10	45.25	45.25	45.25	37.45	37.45	37.45
Soybean meal (44% CP)	29.20	29.20	29.20	30.75	30.75	30.75	32.32	32.32	32.32	33.89	33.89	33.89
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	2.05	2.05	2.05	2.08	2.08	2.08	2.10	2.10	2.10	2.15	2.15	2.15
Calcium CO3	0.85	0.85	0.85	0.83	0.83	0.83	0.81	0.81	0.81	0.77	0.77	0.77
Bicarbonate Na	0.10	0.10	0.10	0.09	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10
DL-methionine[†]	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.20	0.21	0.21	0.21
L-lysine HCl[‡]	0.09	0.09	0.09	0.10	0.10	0.10	0.10	0.10	0.10	0.09	0.09	0.09
L-threonine[§]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[¶]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[◊]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Propylene glycol	0	0	0	4.00	4.00	4.00	8.00	8.00	8.00	12.00	12.00	12.00
Vitamin B-complex	0	0.10	0.20	0	0.10	0.20	0	0.10	0.20	0	0.10	0.20
Common salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.37	0.37	0.37
Inert	0.56	0.46	0.36	2.89	2.79	2.69	5.16	5.06	4.96	7.37	7.27	7.17
Calculated chemical composition												
Metabolizable energy (kcal/kg)	3150	3150	3150	3150	3150	3150	3150	3150	3150	3150	3150	3150
Calcium (%)	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Available phosphorus (%)	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
Chloride (%)	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Potassium (%)	0.78	0.78	0.78	0.78	0.78	0.78	0.79	0.79	0.79	0.80	0.80	0.80
Linoleic acid (%)	4.01	4.01	4.01	3.84	3.84	3.84	3.67	3.67	3.67	3.51	3.51	3.51
Lysine (%)	1.22	1.22	1.22	1.23	1.23	1.23	1.26	1.26	1.26	1.28	1.28	1.28
Methionine(%)	0.49	0.49	0.49	0.50	0.50	0.50	0.49	0.49	0.49	0.50	0.50	0.50
Methionine + cysteine (%)	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
Threonine (%)	0.72	0.72	0.72	0.73	0.73	0.73	0.74	0.74	0.74	0.74	0.74	0.74
Tryptophan (%)	0.25	0.25	0.25	0.26	0.26	0.26	0.27	0.27	0.27	0.27	0.27	0.27
Analyzed composition												
Crude protein (%)	18.50	18.50	18.50	18.50	18.50	18.50	18.50	18.50	18.50	18.50	18.50	18.50
Ether extract (%)	7.61	7.61	7.61	7.33	7.33	7.33	7.04	7.04	7.04	6.76	6.76	6.76
Crude fiber (%)	2.48	2.48	2.48	2.37	2.37	2.37	2.26	2.26	2.26	2.15	2.15	2.15

[†]DL-methionine, feed grade 99% (Evonik), [‡]L-lysine feed grade 98.5% (AlborzGostar Darou Co), [§]L-threonine, feed grade 98.5% (Evonik); [¶]provided per kilogram of diet: 15,000 IU vitamin A (retinol), 3,750 IU vitamin D3 (cholecalciferol), 20.2 mg vitamin E (alpha-tocopherol), 3.7 mg vitamin K3 (menadione), 3 mg vitamin B1 (thiamine), 7.5 mg vitamin B2 (riboflavin), 55 mg niacin, 4.5 mg of vitamin B6 (pyridoxine), 1 mg biotin, 15 mg vitamin B12 (cobalamin), and 11.5 pantothenic acid; [◊]provided per kilogram of diet: 50 mg Mn, 47 mg Zn (zinc oxide), 25 mg Fe, 7.5 mg Cu (copper sulphate), 2.6 mg I, and 0.40 mg Se.

Table 4. Effects of dietary propylene glycol and B-complex vitamins supplementation on serum biochemical parameters of male broiler chickens.

Treatment		Traits									
		Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Uric Acid (mg/dl)	Urea (mg/dl)	Lactate (mmol/l)	LDH (U/l)	Pyruvate (mmol/l)
Main effect											
Propylene glycol (%)											
0		248.13	49.60[b]	153.60[b]	78.60	64.60[b]	3.57	8.93	1.03	3157.8	0.56
4		242.13	49.87[b]	161.67[b]	77.80	71.60[b]	3.45	9.47	1.05	3301.1	0.59
8		237.93	50.53[b]	163.40[b]	76.33	76.93[b]	3.35	10.00	0.98	3869.1	0.57
12		233.87	66.60[a]	199.60[a]	73.27	112.80[a]	4.21	10.53	1.11	4516.1	0.57
SEM[1]		0.79	1.07	2.64	0.30	2.77	0.07	0.09	0.007	79.79	0.0014
B-complex vitamins (%)											
0		243.45	54.45	164.35	78.70	74.30	3.75	9.80	1.05	3888.4	0.57
0.1		239.25	54.85	168.75	78.25	77.65	4.34	10.40	1.07	4083.9	0.59
0.2		238.85	53.15	165.60	72.55	79.50	3.44	9.00	1.01	3660.8	0.56
SEM		0.33	0.11	1.03	0. 44	1.08	0.06	0.09	0.004	62.77	0.0019
Interaction effects											
Propylene glycol × B-complex vitamins											
0	0	249.60	48.20	151.20	78.40	63.00	3.78	9.40	1.14	4035.40	0.61
0	0.1	251.60	45.20	164.40	82.00	73.20	4.32	9.20	0.96	2609.00	0.54
0	0.2	243.20	56.20	145.20	73.00	57.60	2.90	9.80	1.00	2829.00	0.54
4	0	243.60	58.40	163.40	80.60	71.20	4.14	9.60	1.05	3402.60	0.59
4	0.1	239.80	44.20	151.40	78.20	67.60	2.24	12.00	1.14	3866.00	0.62
4	0.2	243.00	46.20	170.20	77.00	76.00	4.28	8.40	0.97	2634.60	0.55
8	0	239.00	53.80	155.20	81.20	61.80	2.90	9.40	0.93	3863.00	0.55
8	0.1	238.60	49.40	175.40	78.00	89.20	4.54	9.60	1.14	4631.00	0.62
8	0.2	236.60	48.40	159.60	69.80	79.80	2.92	7.80	0.88	3113.40	0.53
12	0	241.60	57.40	187.60	74.60	101.20	4.20	10.80	1.10	4252.60	0.53
12	0.1	227.00	65.60	189.80	74.80	102.60	4.26	10.80	1.05	5229.60	0.57
12	0.2	233.00	61.80	187.40	70.40	104.60	3.68	10.00	1.20	4066.00	0.61
SEM		0.54	1.17	1.64	0.31	1.27	0.14	0.12	0.014	74.36	0.0057
p-value											
Propylene glycol		0.3287	0.0209	0.0001	0.4982	0.0001	0.1033	0.3036	0.4459	0.0854	0.8283
B-complex vitamins		0.7616	0.9472	0.1020	0.1135	0.1093	0.2766	0.1884	0.6895	0.1565	0.4812
Propylene glycol × B-complex vitamins		0.9787	0.2254	0.1700	0.9415	0.1190	0.0858	0.5908	0.2903	0.6691	0.1618

Table 5. Effects of dietary propylene glycol and B-complex vitamins supplementation on serum biochemical parameters of female broiler chickens.

Treatment		Traits									
		Glucose (mg/ dl)	Triglyceride (mg/ dl)	Cholesterol (mg/ dl)	HDL (mg/ dl)	LDL (mg/dl)	Uric acid (mg/ dl)	Urea (mg/ dl)	Lactate (mmol/l)	LDH (U/l)	Pyruvate (mmol/l)
	Main effect										
	Propylene glycol (%)										
0		242.33	47.47[b]	136.33[b]	75.80	60.33[b]	3.23	10.93	1.12	3559.2	0.61
4		239.53	49.47[b]	143.00[b]	74.27	60.80[b]	3.42	11.20	1.12	4113.2	0.58
8		241.60	50.00[b]	146.07[b]	74.87	61.47[b]	3.57	10.13	1.08	4123.7	0.55
12		230.00	64.67[a]	179.47[a]	71.87	89.47[a]	4.05	10.40	1.08	4528.9	0.57
SEM[1]		0.73	1.02	2.47	0.46	1.85	0.087	0.063	0.003	51.38	0.003
	B-complex vitamins (%)										
0		243.00	53.45	152.20	74.35	65.00	3.51	10.70	1.10	4363.2	0.58
0.1		238.15	52.90	149.05	70.85	69.40	3.36	10.30	1.07	3643.6	0.59
0.2		233.95	52.35	152.40	71.40	69.65	3.22	11.00	1.13	4237.0	0.57
SEM		0.58	0.07	0.24	0.71	0.34	0.018	0.045	0.004	49.58	0.001
	Interaction effects										
	Propylene glycol × B-complex vitamins										
0	0	246.20	46.20	135.60	77.80	53.60	3.10	12.40	1.11	4973.40	0.60
0	0.1	245.40	50.00	145.40	74.60	60.60	2.84	10.40	1.09	3718.00	0.63
0	0.2	235.40	52.20	148.00	70.40	66.80	2.96	10.00	1.17	36.79.80	0.60
4	0	248.60	48.40	148.80	75.60	69.00	3.92	10.80	1.17	4967.40	0.64
4	0.1	235.80	51.60	125.20	61.80	53.00	3.68	11.00	1.07	3814.40	0.54
4	0.2	234.20	42.40	135.00	66.20	60.40	3.86	11.80	1.13	4802.00	0.56
8	0	226.00	52.80	148.00	79.60	58.60	2.54	10.20	1.12	3705.40	0.55
8	0.1	227.80	47.80	154.40	74.40	70.20	2.98	10.20	1.08	3373.40	0.59
8	0.2	236.20	49.40	135.80	70.60	55.60	2.38	10.00	1.05	3598.80	0.52
12	0	251.20	66.40	176.40	84.40	78.80	4.48	9.40	1.01	3806.60	0.53
12	0.1	243.60	62.20	171.20	64.60	93.80	3.96	9.60	1.06	3665.60	0.59
12	0.2	230.00	65.40	190.80	78.40	95.80	3.70	12.20	1.18	4867.40	0.61
SEM		0.95	0.48	1.42	0.61	1.12	0.033	0.15	0.007	66.55	0.006
					P-value						
Propylene glycol		0.1681	0.0155	0.0001	0.1412	0.0001	0.1059	0.8229	0.9483	0.6351	0.3171
B-complex vitamins		0.2385	0.9757	0.8529	0.1053	0.5058	0.7578	0.8110	0.7825	0.4957	0.8221
Propylene glycol × B-complex vitamins		0.4562	0.9442	0.2490	0.3830	0.0993	0.9628	0.7649	0.9572	0.9230	0.2348

Values were presented as mean ± SEM. Means within the same row with different superscript letters (a and b) differ significantly ($p < 0.05$). SEM= Standard error of the means.

Table 6. Effects of dietary propylene glycol and B-complex vitamins supplementation on hematological parameters of male broiler chickens.

Treatment	Blood analysis[2]				
	RBC	**Hb**	**Hct**	**WBC**	**PLT**
Main effect					
Propylene glycol (%)					
0	2.78[b]	12.95[b]	39.95[b]	0.72	15.6
4	3.05[a]	14.01[a]	43.56[a]	0.77	19.0
8	3.08[a]	14.11[a]	44.73[a]	0.69	16.8
12	3.17[a]	14.32[a]	45.71[a]	0.80	14.2
SEM[1]	0.021	0.079	0.32	0.006	0.26
B-complex vitamins (%)					
0	2.98	13.85	43.72	0.78	16.5
0.1	3.09	14.01	43.64	0.76	17.7
0.2	2.98	13.68	43.09	0.70	15.0
SEM	0.008	0.021	0.045	0.006	0.17
Interaction effects					
Propylene glycol × B-complex vitamins					
0 0	2.70	13.30	41.36	0.85	15.0
0 0.1	2.81	13.08	40.38	0.72	13.8
0 0.2	2.82	12.46	38.12	0.61	18.0
4 0	3.15	14.26	44.50	0.81	19.2
4 0.1	2.99	14.04	42.78	0.81	22.8
4 0.2	3.03	13.74	43.40	0.70	15.0
8 0	2.98	13.64	43.80	0.70	16.8
8 0.1	3.17	14.16	44.38	0.72	18.6
8 0.2	3.09	14.54	46.00	0.64	15.0
12 0.1	3.08	14.20	45.24	0.78	15.0
12 0.2	3.40	14.76	47.04	0.77	15.6
12 0.2	3.01	14.00	44.84	0.85	12.0
SE	0.009				0.33
M		0.053	0.18	0.009	
P-value					
Propylene glycol	0.0021	0.0126	0.0013	0.4668	0.2012
B-complex vitamins	0.3309	0.6900	0.8564	0.4159	0.3955
Propylene glycol × B-complex vitamins	0.3994	0.7259	0.7140	0.7433	0.5436

Values were presented as mean ± SEM. SEM= Standard error of the means. Means within the same row with different superscript letters (a and b) differ significantly ($p < 0.05$). [2]RBC = Red blood cell (10^6/µl); WBC = White blood cell (10^3/µl); Hct = Hematocrit value (%). Hb = Hemoglobin (g/dl); Platelets 10^3/µl.

Table 7. Effects of dietary propylene glycol and B-complex vitamins supplementation on hematological parameters of female broiler chickens.

Treatment		Blood analysis[2]				
		RBC	Hb	Hct	WBC	Platelet
Main effect						
Propylene glycol (%)						
0		2.96	13.56	42.04	0.87	20.2
4		2.95	13.49	41.42	0.85	19.2
8		2.97	13.43	41.44	0.74	18.8
12		3.08	13.86	43.48	0.83	18.4
SEM[1]		0.008	0.025	0.12	0.008	0.1
B-complex vitamins (%)						
0		2.96	13.55	41.80	0.91	18.9
0.1		3.02	13.58	42.17	0.79	19.1
0.2		2.98	13.61	42.31	0.77	19.5
SEM		0.004	0.004	0.034	0.001	0.04
Interaction effects						
Propylene glycol × B-complex vitamins						
0	0	2.94	13.34	41.26	0.99	18.0
0	0.1	3.03	14.02	43.62	0.92	25.2
0	0.2	2.92	13.32	41.24	0.70	17.4
4	0	2.91	13.46	41.08	1.14	17.4
4	0.1	2.95	13.32	40.74	0.65	16.2
4	0.2	2.99	13.70	42.44	0.75	24.0
8	0	2.90	13.32	41.42	0.71	16.2
8	0.1	2.92	13.04	39.78	0.75	19.8
8	0.2	3.08	13.92	43.12	0.75	20.4
12	0.1	3.10	14.10	43.44	0.79	24.0
12	0.2	3.20	13.96	44.54	0.85	15.0
12	0.2	2.94	13.52	42.46	0.86	16.2
SEM		0.012	0.052	0.19	0.020	0.6
p-value						
Propylene glycol		0.5228	0.7017	0.3464	0.5290	0.9588
B-complex vitamins		0.7704	0.9846	0.8929	0.2149	0.9781
Propylene glycol × B-complex vitamins		0.6937	0.6510	0.5440	0.1501	0.3074

Values were presented as mean ± SEM. SEM= Standard error of the means. [2]RBC = Red blood cell (10^6/µl); WBC = White blood cell (10^3/µl); Hct = Hematocrit value (%); Hb = Hemoglobin (g/dl); Platelets 10^3/µl.