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Effect of L-carnitine, emulsifier, and their combination on lipid peroxidation, nitric oxide production, and heterophil/lymphocyte ratio in chickens under high altitude

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Abstract

Background and Objective: High-altitude environments pose challenges for broiler chickens, causing hypoxia, reduced growth rates, and increased disease susceptibility. This stress affects their cardiovascular and respiratory systems. This study explores the impact of L-carnitine and emulsifiers on lipid peroxidation, nitrite production (as an indicator of nitric oxide), and the heterophil/lymphocyte ratio in chickens experiencing high-altitude stress.

Materials and Methods: Broiler chicks were raised at high altitudes (2100 m) for 42 days, divided into a control and three treatment groups receiving L-carnitine, emulsifier, or both. Blood samples were collected to analyze nitrite and malondialdehyde (MDA) levels. The heterophil/lymphocyte (H/L) ratio was determined from blood smears

Results: Nitrite levels were increased in the L-carnitine and L-carnitine + emulsifier groups compared to controls, while MDA levels decreased in both treatment groups compared to controls ($P<0.05$). Additionally, the H/L ratio was significantly reduced in all treatment groups compared to the control group ($P<0.05$).

Conclusion: This study determined the significant benefits of L-carnitine and emulsifiers in improving the adverse effects of high-altitude conditions such as oxidative stress, vasoconstriction, and hypertension in broiler chickens.

Keywords: Carnitine, Broiler, Hypoxia, Oxidative stress, Immunity

1. Introduction

High-altitude environments present unique physiological challenges for poultry [1]. These conditions can adversely affect the health and performance of chickens, leading to compromised immune function and altered metabolic processes. The effects of high altitude on broiler chickens are multifaceted. Hypoxia is a primary concern, resulting in physiological stress that manifests as decreased growth rates, poor feed conversion, and increased susceptibility to diseases [2]. Broiler chickens' cardiovascular and respiratory systems are particularly affected, as they must work harder to deliver adequate oxygen to tissues. This increased workload can result in metabolic disorders such as ascites, characterized by fluid accumulation in the abdominal cavity, which is often linked to the stress of high-altitude conditions [3, 4]. When exposed to high altitude, the body experiences increased reactive oxygen species (ROS) production due to enhanced metabolic activity and mitochondrial respiration under low oxygen conditions. This overproduction of ROS can overwhelm the body's antioxidant defenses, leading to oxidative stress. Studies have shown that during acute exposure to hypobaric hypoxia, there is a marked

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increase in oxidative stress biomarkers, such as malondialdehyde (MDA), a product of lipid peroxidation, indicating cellular damage [5, 6].

Among the various strategies to mitigate these effects, nutritional interventions have gained attention, particularly using L-carnitine and emulsifiers. L-carnitine, a naturally occurring compound, plays a crucial role in fatty acid metabolism and energy production, while emulsifiers enhance nutrient absorption and improve feed efficiency [7, 8].

Recent studies have indicated that L-carnitine may possess antioxidant properties, potentially reducing lipid peroxidation, a process that can lead to cellular damage and impaired function [9]. Additionally, emulsifiers have been shown to influence the gut microbiota and enhance the bioavailability of nutrients, which may further support the immune system and overall health of chickens [10, 11]. The interplay between these two additives could provide a synergistic effect, particularly during high-altitude stress.

Furthermore, the production of nitric oxide (NO), a critical signaling molecule involved in various physiological processes, is often altered under stress conditions. NO plays a significant role in immune response and vascular regulation, making it an important factor to consider in poultry health [12, 13]. One of the primary functions of NO is vasodilation, where it relaxes the smooth muscles of blood vessels, leading to increased blood flow and reduced blood pressure. This action is essential for maintaining vascular tone and overall cardiovascular health [14]. Additionally, NO inhibits platelet aggregation, preventing the clumping of platelets and reducing the risk of thrombosis, which promotes smooth blood flow and helps regulate blood pressure levels [15]. Within the immune system, NO is produced by neutrophils and macrophages to help kill pathogens, playing a vital role in the immune response against infections. Furthermore, it modulates inflammatory responses, contributing to the balance between pro-inflammatory and anti-inflammatory signals [16].

The heterophil/lymphocyte (H/L) ratio is a well-established indicator of stress in birds, reflecting the balance between innate and adaptive immune responses. An elevated H/L ratio is often associated with increased stress levels, which can be exacerbated in high-altitude conditions [17, 18].

This study aims to investigate the effects of L-carnitine, emulsifiers, and their combination on lipid peroxidation, nitrite (as a NO indicator) nitric oxide production, and the heterophil/lymphocyte ratio in chickens subjected to high-altitude stress. By elucidating the potential benefits of these nutritional interventions, this research seeks to contribute to the development of effective strategies for enhancing poultry health and performance in challenging environments.

2. Materials and Methods

2.1. Breeding of birds at high altitudes and sampling

A total of 40 one-day-old Ross 308 broiler chicks were randomly assigned to floor pens and raised for 42 days at high altitudes (2100 m above sea level). The chicks were divided into a control group and three treated groups including L-carnitine (Carniking®, Lohmann Co. Ltd., Germany), 50 mg/kg diet; emulsifier (Lysoforte®, Kemin Co. Ltd., USA), 1 g/kg diet; L-carnitine + emulsifier) with 10 chicks per group.
At 42 days of age, 5 chickens from each group were randomly selected. The blood samples were collected from the brachial vein before euthanasia. The serum of each blood sample was separated and prepared for nitric oxide metabolite (nitrite) and MDA measurements.

2.2. Nitrite measurement

In this method, nitrate is first reduced to nitrite using cadmium, after which the total nitrite is quantified through the Griess reaction. Briefly, the serum samples were deproteinized by adding zinc sulfate (75 mmol/l) and sodium hydroxide (55 mmol/l) solutions. After centrifuging, the supernatant was recovered and diluted in glycine buffer (45 g/l, pH 9.7). Cadmium granules (2 - 2.5 g) were rinsed three times with deionized distilled water and swirled in a CuSO4 solution (5 mmol/l) in glycine-NaOH buffer (15 g/l, pH 9.7) for 5 min to become activated. Freshly activated cadmium granules were added to pretreated deproteinized serum. After continuous stirring for 10 min, the samples were transferred to appropriately labeled tubes for nitrite determination by Griess reaction. Griess reagent 1 (1% sulfanilamide in 5% phosphoric acid) was added to the sample tubes and then incubated for 10 min at room temperature, protected from light. Griess reagent 2 was added (0.1% N-napthylethylenediamine dihydrochloride in water) to all samples and absorbance was measured within 10 min in a spectrophotometer at a wavelength of 540 nm. Data were calculated from standard curves and expressed as µM (19, 20).

2.3. *Thiobarbituric acid reactive substances (TBARS) assay*

The reagents employed in the assay were sourced from Sigma-Aldrich (St. Louis, MO, USA). The MDA, a well-established biomarker for lipid peroxidation, was quantified in serum samples

utilizing the TBARS assay. Trichloroacetic acid was introduced to the serum samples to facilitate protein precipitation, after which the resulting supernatants underwent centrifugation. Following centrifugation, the samples were incubated for 10 minutes in a boiling water bath after adding an equivalent volume of thiobarbituric acid (TBA). Absorbance was then measured at 532 nm using a spectrophotometer (Corning 480, USA) after the samples were cooled. The data generated from the TBARS assay were subsequently converted to micromolar units (µM) (21).

2.4. Estimation of heterophil/lymphocyte ratio

An aliquot of blood was obtained on glass slides to prepare the blood smear to determine differential leucocyte count. The smears were stained using May-Grunwald and Giemsa stains, approximately 2 to 4 h after methyl alcohol fixation. One hundred leucocytes, including granular (heterophils) and nongranular (lymphocytes), were counted and the heterophil to lymphocyte ratio (H:L) was calculated (17). All chemical reagents were obtained from Sigma-Aldrich Co. (Sigma-Aldrich Co., St. Louis, MO, USA).

2.5. Statistical analysis

Data are presented as means ± standard error (SE). The Kolmogorov–Smirnov test was conducted to assess the normality of the data. Parametric tests were employed for comparisons of normally distributed data. Statistical comparisons between experimental groups were performed by one-way ANOVA using SAS (1997) software in a completely randomized design.

3. Results

Figure 1 illustrates the nitrite levels across different experimental groups. Nitrite levels significantly increased in the L-carnitine and L-carnitine + emulsifier groups compared to the control and emulsifier groups ($P < 0.05$). There were no significant differences between L-carnitine and L-carnitine + emulsifier groups or between control and emulsifier groups in the nitrite levels ($P > 0.05$).

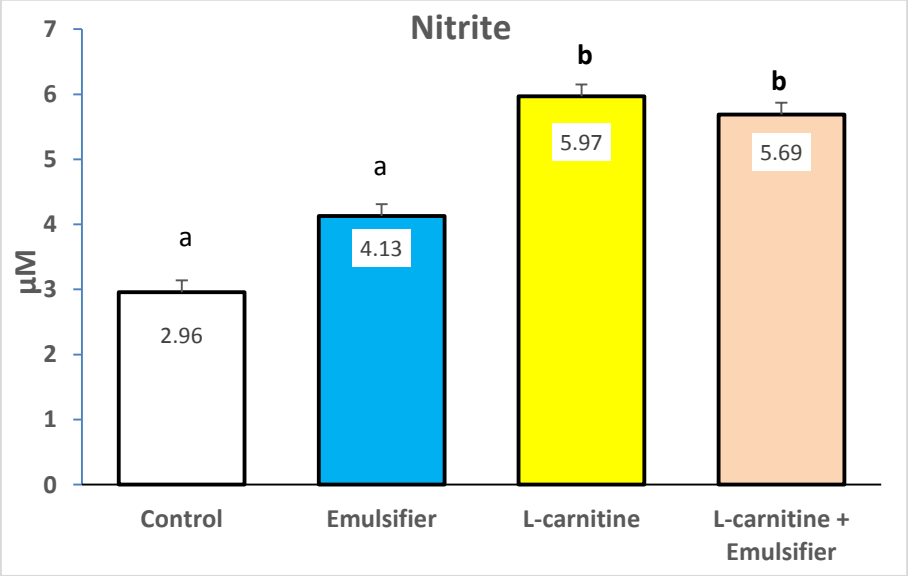

Fig. 1. Comparison of nitrite (as an indicator of nitric oxide) levels between different experimental groups of chickens after 42 days. Values are means ± standard error. a and b significant difference between treatments ($P < 0.05$).

Figure 2 indicates the MDA levels in different experimental groups. MDA levels significantly decreased in the L-carnitine and L-carnitine + emulsifier groups compared to the control group (P < 0.05). No significant differences existed between L-carnitine, emulsifier, and L-carnitine + emulsifier groups in the MDA levels (P > 0.05).

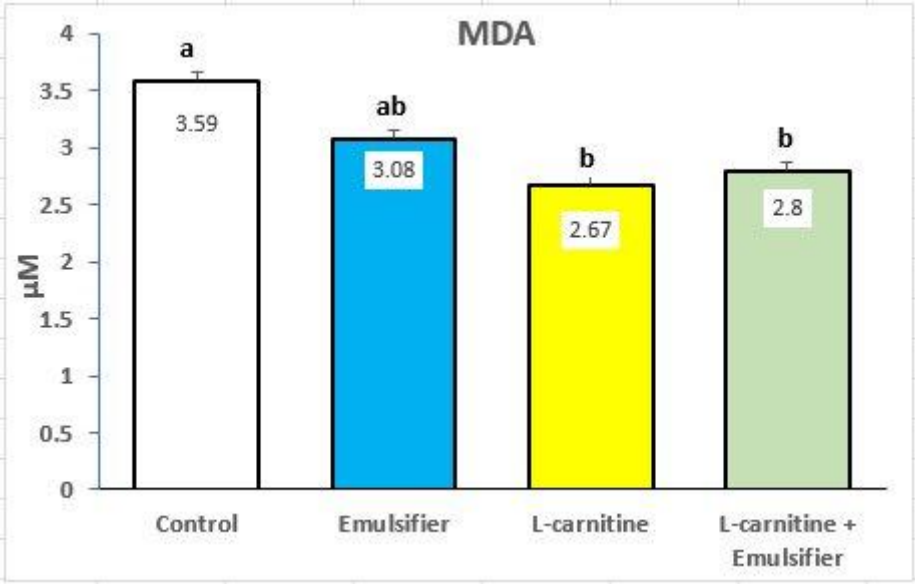

Fig. 2. Comparison of malondialdehyde (MDA) levels between different experimental groups of chickens after 42 days. Values are means ± standard error. a and b significant difference between treatments (P < 0.05).

Figure 3 expresses the heterophil/lymphocyte ratio in different experimental groups. This ratio significantly decreased in the L-carnitine, emulsifier, and L-carnitine + emulsifier groups compared to the control group (P < 0.05). No significant differences existed between the L-carnitine, emulsifier, and L-carnitine + emulsifier groups in the H/L ratio (P > 0.05).

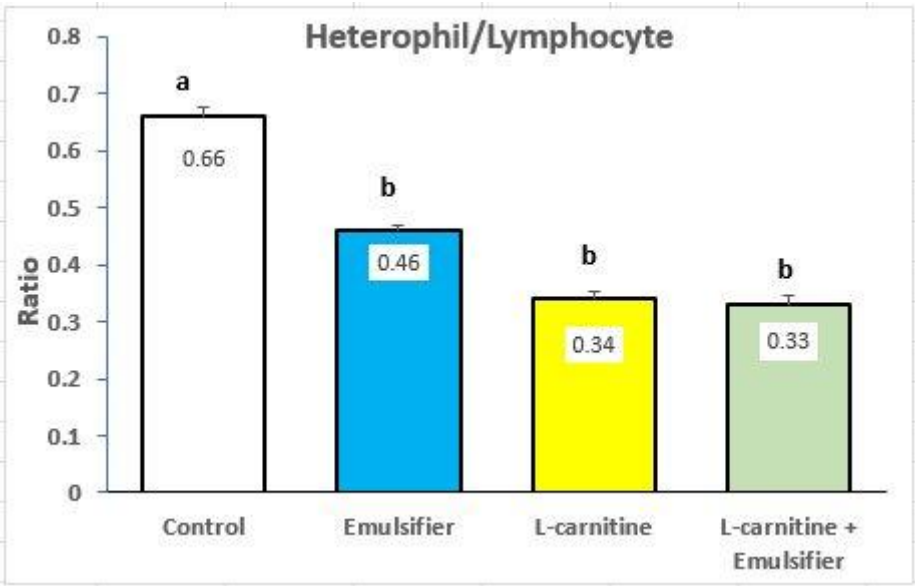

Fig.3. Comparison of heterophil to lymphocyte ratios between different experimental groups of chickens after 42 days. Values are means ± standard error. a and b significant difference between treatments (P < 0.05).

4. Discussion

This study provides important insights into the effects of L-carnitine, emulsifiers, and their combination on lipid peroxidation, nitric oxide production, and the heterophil/lymphocyte (H/L) ratio in chickens exposed to high-altitude conditions. The results indicate that both L-carnitine and emulsifiers significantly reduced oxidative stress markers, as evidenced by decreased MDA levels, a key indicator of lipid peroxidation. These findings suggest that nutritional interventions may effectively mitigate oxidative damage in chickens subjected to hypoxia.

One of the primary ways L-carnitine exerts its effects is by facilitating the transport of long-chain fatty acids into the mitochondria for β-oxidation, which is crucial for energy production. This process not only generates ATP but also helps to reduce the accumulation of fatty acids that can contribute to oxidative stress when they are oxidized outside the mitochondria (22).

Additionally, L-carnitine has been associated with enhancing the activity of various antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, which play critical roles in neutralizing ROS (23, 24). Furthermore, L-carnitine may help stabilize mitochondrial membranes, reducing the leakage of electrons and subsequent ROS generation during electron transport. This stabilization is particularly important under conditions of metabolic stress, such as those experienced at high altitudes, where hypoxia can lead to increased mitochondrial dysfunction and oxidative stress (25).

However, it is essential to consider the conflicting findings from previous studies regarding the efficacy of L-carnitine in poultry. While some research supports its antioxidant properties and benefits in reducing oxidative stress (9, 26), other studies have reported minimal or no significant effects on performance or health parameters in chickens (27, 28). These discrepancies may stem from differences in experimental designs, such as variations in dosage, duration of supplementation, or the specific strains of chickens used.

The role of emulsifiers in enhancing nutrient absorption and improving gut health has been determined (29, 30). While this study demonstrated a positive impact on improving the H/L ratio in hypoxic conditions, many studies also suggested that emulsifiers may improve many specific immunities in chickens (31, 32).

This study showed an increase in nitrite levels in both the L-carnitine and L-carnitine + emulsifier groups, indicating a corresponding rise in nitric oxide levels. As mentioned, one of the primary positive effects of nitric oxide in high-altitude conditions is its role in vasodilation. NO facilitates the relaxation of smooth muscle cells in blood vessels, leading to increased blood flow and improved oxygen delivery to tissues (33, 34). This is crucial for broiler chickens, as their cardiovascular and respiratory systems must work harder to compensate for lower oxygen levels at high altitudes (35). Enhanced blood flow can help alleviate some of the stress associated with hypoxia, supporting better overall metabolic function and growth performance (36). NO is also known to play a significant role in the immune response (37). In high-altitude environments, where chickens may be more susceptible to respiratory infections due to stress and compromised immune function, increased NO production can enhance the ability of these immune cells to combat infections effectively (38). This immune-enhancing effect is particularly important for maintaining health and reducing mortality rates among broiler chickens raised at high altitudes (39). Additionally, nitric oxide has been shown to have antioxidant properties. By modulating oxidative stress levels, NO can help protect cells from damage caused by reactive oxygen species (ROS), often elevated under hypoxic conditions. The balance between NO production and ROS is critical; while excessive ROS can lead to cellular damage and metabolic disorders, adequate NO levels can mitigate these effects by promoting antioxidant defences (40, 41).

Conclusion

This study highlights the significant benefits of L-carnitine and emulsifiers in mitigating the adverse effects of high-altitude conditions on broiler chickens. L-carnitine treatment effectively reduced oxidative stress, as indicated by lower malondialdehyde levels, a marker of lipid peroxidation. Additionally, the increase in nitric oxide production in the L-carnitine and L-carnitine + emulsifier groups suggests enhanced vasodilation and improved blood flow, which are crucial for maintaining metabolic function and supporting immune responses under hypoxic stress. Moreover, the reduction in the heterophil/lymphocyte ratio across treatment groups indicates a potential alleviation of stress and an enhancement of immune function. These results highlight the importance of nutritional interventions in poultry management, particularly in challenging environments like high altitudes.

Acknowledgment

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Compliance with ethical standards

All the procedures in this study were approved by the Institutional Animal Care and Use Committee of Shahrekord University, based on the welfare standard of the 1964 Declaration of Helsinki.

Conflicts of interest

The authors declare that they have no competing interests.

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