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L-carnitine and Emulsifier Change the Intestinal Morphology in Chickens Under High-altitude Stress

Fathollah Farzam Hasani[1], Behnam Ahmadipour[1], Hossein Hassanpour[2,3*], Samira Abaszadeh[1]

1. Department of Animal Sciences, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
3. Department of Health Equity Research Center, Shahed University, Tehran, Iran

Abstract

Background and Objective: This study aimed to investigate the effect of L-carnitine and emulsifiers on the intestinal morphology of chickens subjected to high-altitude stress. It focused on parameters including villus height, width, surface area, and lamina propria thickness in the duodenum, jejunum, and ileum to assess their potential for enhancing nutrient absorption and promoting gut health.

Materials and Methods: Ninety-one-day-old broiler chicks were raised at 2100 m above sea level for 42 days. They were divided into a control group and eight treatment groups receiving L-carnitine (0.005% or 0.01% of diet) and emulsifiers (0.1% or 0.2% of diet), individually or in combination. Intestinal segments (duodenum, jejunum, and ileum) were collected, fixed, stained, and analyzed to measure villus dimensions, lamina propria thickness, and surface area.

Results: Supplementation with L-carnitine and emulsifiers increased the height and width of villi in the duodenum, jejunum, and ileum ($P < 0.05$). Higher doses of both additives demonstrated partial effectiveness in enhancing these parameters. There was a significant increase in intestinal surface area across all segments. However, lamina propria thickness decreased in all treated groups compared to the control group ($P < 0.05$). The weight of birds increased in all treated groups compared to the control group ($P < 0.05$).

Conclusion: L-carnitine and emulsifiers positively influenced intestinal morphology under high-altitude stress, leading to enhanced villus dimensions and increased surface area. Nonetheless, the observed reduction in lamina propria thickness raises important questions about its implications for intestinal health and warrants further investigation.

Keywords: L-carnitine, Emulsifiers, Intestinal Morphology, High-altitude, Nutrient Absorption

1. Introduction

High-altitude environments present unique physiological challenges to poultry, significantly impacting their growth, health, and overall productivity (1). At high altitudes, chickens are exposed to reduced atmospheric oxygen (hypoxia), causing increased oxidative stress (2, 3). This factor can disrupt normal physiological processes, particularly affecting the gastrointestinal tract, which plays a pivotal role in nutrient absorption (4, 5), immune

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***Corresponding Author:** Hossein Hassanpour **E-mail:** Hassanpour-h@sku.ac.ir

function (6, 7), and overall metabolic health (8, 9). The intestinal morphology, including the structure and function of the villi, is especially vulnerable to high-altitude stress, as hypoxia and oxidative damage can impair intestinal barrier integrity, reduce nutrient absorption efficiency, and compromise gut health (5, 10, 11).

In recent years, dietary interventions have gained attention as a practical approach to mitigate the adverse effects of high-altitude stress in poultry. Among these, L-carnitine and emulsifiers have emerged as promising candidates due to their distinct yet complementary roles in supporting metabolic and digestive processes (12-15). L-carnitine, a quaternary ammonium compound synthesized from lysine and methionine, is well-known for its role in facilitating the transport of long-chain fatty acids into the mitochondria for β-oxidation, thereby enhancing energy production (16). Additionally, L-carnitine exhibits antioxidant properties, helping to neutralize reactive oxygen species (ROS) and reduce oxidative stress, which is particularly beneficial under high-altitude conditions where ROS production is elevated (17-19).

Emulsifiers, on the other hand, are food additives that improve the digestion and absorption of dietary fats by enhancing the emulsification of lipids in the gastrointestinal tract (20). Emulsifiers can provide a more efficient energy source through promoting better lipid utilization, which is crucial for animals under stress (21, 22). Furthermore, emulsifiers have been shown to support gut health by stabilizing the intestinal environment and potentially improving the integrity of the intestinal mucosa (23).

The combination of L-carnitine and emulsifiers may offer synergistic benefits (24) in alleviating high-altitude stress in chickens. While L-carnitine supports energy metabolism and reduces oxidative damage, emulsifiers ensure optimal nutrient absorption and intestinal health. Together, these additives could help maintain intestinal morphology, including the height and structure of villi, and the overall surface area available for nutrient absorption, all of which are critical for sustaining growth and performance under stressful conditions.

This study aimed to investigate the effects of dietary supplementation with L-carnitine and emulsifiers on intestinal morphology in chickens exposed to high-altitude stress. By measuring dimensions of villus height, width, surface area, and lamina propria, the research evaluated the rate of intestinal absorption and secretion following the use of these additives

2. Materials and Methods

2.1. Rearing of birds at high altitudes, treatments, and sampling

A total of 90 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) under standard conditions. The chicks were divided into a control group and eight treated groups including L-carnitine (Carniking, Lohmann Co. Ltd., Germany), 0.005% of diet (C0.005), L-carnitine 0.01% of diet (C0.01), emulsifier (Lysoforte, Kemin Co. Ltd., USA) 0.1% of diet (E0.1), emulsifier 0.2% of diet (E0.2), C0.005 + E0.2, C0.01 + E0.1, and C0.01+E0.2 with 10 chicks per group.

2.2. Sampling of the intestine and fixation

At the end of the 42-day experimental period, all chickens were fasted for 6 hours with free access to water to ensure empty digestive tracts and minimize variability in intestinal measurements. Chickens were euthanized by decapitation using a sharp blade, following approved protocols to ensure instantaneous and humane death. Immediately after euthanasia, the abdominal cavity was opened, and the entire small intestine was carefully excised. The duodenum (from the gizzard to the bile duct entry), jejunum (between the bile duct entry and Meckel's diverticulum), and ileum (distal end to the ileocecal junction) were identified and separated. From each segment, a 2.00 cm section was collected at standardized locations: the midpoint of the duodenal loop, the midpoint of the jejunum, and 5 cm proximal to the ileocecal junction for the ileum. These segments were immediately rinsed with ice-cold phosphate-buffered saline (PBS, pH 7.4) to remove luminal contents and then processed for fixation within 2 minutes of collection to preserve tissue integrity. The samples were immediately fixed in Clark's solution (25.00% acetic acid and 75.00% ethyl alcohol) for 45 minutes. For long-term preservation, the samples were subsequently transferred to 55.00% ethyl alcohol (25).

2.3. Measurement of intestinal villus dimensions and surface area

Following fixation and staining with periodic acid-Schiff (PAS) reagent, the intestinal segments were

carefully dissected to isolate the mucosal layer. Villus height and width were measured using a calibrated light microscope (Olympus CX23) equipped with an ocular micrometer (eyepiece graticule) at 10× magnification. For each intestinal segment (duodenum, jejunum, and ileum), 3 intact, well-oriented villi were randomly selected per sample. Villus height was defined as the vertical distance from the tip of the villus to the base at the villus-crypt junction, excluding the crypt depth. Measurements were taken along the central axis of each villus. Villus width was measured at the widest part of the villus, perpendicular to the height axis. To ensure accuracy, three independent measurements were taken for each villus, and the average value was recorded. Only vertically oriented villi with intact tips and clearly visible lamina propria were included to avoid artifacts from oblique sectioning. The final values were expressed in millimeters (mm) using the microscope's calibration scale. Additionally, the thickness of the lamina propria was measured at the base of the villus. The surface area of the villi was calculated using the formula: Villus Surface Area = $\pi \times VW \times VL$, where VW represents villus width and VL represents villus height (25, 26).

2.4. Statistical analysis

Data are presented as means and pooled standard error of the mean (SEM). The pooled SEM is a statistical measure used to estimate the variability or uncertainty in the mean of a combined dataset when comparing two or more groups. It is particularly useful in situations where the groups have similar variances. The pooled SEM accounts for the variability within each group and combines it into a single estimate, providing a more accurate measure of the standard error for the overall dataset. 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 software (1997) in a completely randomized design.

3. Results

3.1. Intestinal villus height and width

Table 1 indicates the effect of L-carnitine and emulsifier on the villus height and width of duodenum, jejunum, and ileum of chickens raised at high altitude after 42 days.

The highest villus heights were observed in the C0.01 + E0.1, C0.01, and C0.01 + E0.2 groups, which were significantly greater than the control group ($P < 0.05$). The C0.005 + E0.1 and C0.005 + E0.2 groups exhibited moderate improvements, while the C0.005, E0.1, and E0.2 groups showed smaller but still significant increases compared to the control group ($P < 0.05$). Duodenal villus width increased in all treated groups compared to the control group ($P < 0.05$). The E0.1 showed the lowest duodenal villus width compared to the control and C0.01 + E0.1 ($P < 0.05$).

Table 1. Effect of L-carnitine and emulsifier on the intestinal parameters of chickens raised at high altitude

Intestinal parameters (mm)	Control	C0.005	C0.01	E0.1	E0.2	C0.005 +E0.1	C0.005+ E0.2	C0.01 +E0.1	C0.01 +E0.2	Pooled SEM	p-value
Duodenum											
Villus height	0.46^{d}	0.60^{bc}	0.76^{a}	0.56^{c}	0.63^{bc}	0.64^{b}	0.66^{b}	0.77^{a}	0.75^{a}	0.008	<0.001
Villus width	0.28^{c}	0.40^{ab}	0.43^{ab}	0.40^{b}	0.42^{ab}	0.45^{ab}	0.44^{ab}	0.45^{a}	0.44^{ab}	0.005	<0.001
Jejunum											
Villus height	0.43^{e}	0.48^{cde}	0.56^{a}	0.47^{de}	0.48^{cde}	0.49^{bcd}	0.52^{abc}	0.54^{ab}	0.55^{a}	0.005	<0.001
Villus width	0.32^{c}	0.36^{b}	0.41^{a}	0.37^{b}	0.38^{ab}	0.39^{ab}	0.39^{ab}	0.38^{ab}	0.40^{ab}	0.004	<0.001
Ileum											
Villus height	0.38^{c}	0.50^{b}	0.58^{a}	0.49^{b}	0.53^{ab}	0.54^{ab}	0.52^{b}	0.55^{ab}	0.54^{ab}	0.006	<0.001
Villus width	0.29^{d}	0.35^{bc}	0.40^{a}	0.33^{cd}	0.38^{ab}	0.39^{ab}	0.38^{ab}	0.40^{a}	0.40^{a}	0.004	<0.001

C0.005, L-carnitine-0.005%; C0.01, L-carnitine-0.01%; E0.1, emulsifier-0.1%; E0.2, emulsifier-0.2%; SEM, standard error of mean; Data presented as mean; ^{a,b,c,d,e} significant differences between groups in each raw ($P < 0.05$).

The jejunal villus height in different groups is presented in Table 1. The C0.01, C0.005 + E0.1, C0.005 + E0.2, C0.01 + E0.1, and C0.01 + E0.2 groups showed higher villus heights than the control group ($P < 0.05$), while it did not change in C0.005, E0.1, and E0.2 groups compared to the control group ($P > 0.05$). The jejunal villus width was greater in all treatment groups than in the control group ($P < 0.05$). There were no significant changes between treatment groups except for the C0.01 group. The ileal villus height was greater in all treatment groups than in the control group ($P < 0.05$). The ileal villus width was also significantly greater in all treatment groups than in the control group except for the E0.1 group.

3.2. Intestinal surface area

Figure 1 represents the effect of L-carnitine and emulsifier on the duodenal, jejunal, and ileal surface area in chickens raised at high altitudes. The duodenal, jejunal, and ileal surface area increased in all treatment groups compared to the control group ($P < 0.05$). The C0.005, E0.1, and E0.2 had lower duodenal and jejunal surface area than other treatment groups ($P < 0.05$). The C0.005 and E0.1 had also lower ileal surface area than other treatment groups ($P < 0.05$).

3.3. Intestinal lamina propria thickness

Figure 2 represents the effect of L-carnitine and emulsifier on the duodenal, jejunal, and ileal lamina propria thickness in chickens raised at high altitudes. The duodenal, jejunal, and ileal lamina propria thickness decreased in all treatment groups compared to the control group ($P < 0.05$). No significant differences in lamina propria thickness were observed among most treatment groups in the duodenum, jejunum, or ileum.

3.4. Weights of chickens at the end of experiment

Figure 3 illustrates the impact of L-carnitine and emulsifier on the body weights of chickens reared at high altitudes over a 42-day period. Compared to the control group, all treatment groups showed a significant increase in mean weight ($P < 0.05$), though no notable differences were detected among the treatment groups themselves.

4. Discussion

The study investigated the effects of L-carnitine and emulsifier supplementation on intestinal morphology (in three segments: duodenum, jejunum, and ileum) in chickens raised at high altitudes. The significant increases in villus height and width observed in the duodenum, jejunum, and ileum of treated groups, particularly in the C0.01 + E0.1 and C0.01 + E0.2 groups, suggest that L-carnitine and emulsifiers synergistically may enhance intestinal morphology. As mentioned, L-carnitine, known for its role in energy metabolism and antioxidant properties, likely contributed to reducing oxidative stress and improving cellular repair mechanisms in the intestinal epithelium. Our earlier research also found that L-carnitine reduces lipid peroxidation by lowering malondialdehyde (MDA) levels in chickens reared at high altitudes (27). Emulsifiers, by enhancing lipid digestion and absorption, may have provided additional energy resources necessary for maintaining and repairing intestinal structures under stress conditions. These findings align with previous studies showing that dietary interventions targeting energy metabolism and oxidative stress can improve intestinal health in poultry (28). The moderate improvements in villus height and width in the C0.005 + E0.1 and C0.005 + E0.2 groups, as well as the smaller but significant increases in the C0.005, E0.1, and E0.2 groups, further support partially the dose-dependent benefits of these additives.

The increased surface area in the duodenum, jejunum, and ileum across all treatment groups underscores the potential of L-carnitine and emulsifiers to enhance nutrient absorption capacity. The greater duodenal surface area in the combination of C0.01 with E0.1 and E0.2 groups indicates that these additives may have a synergistic effect on intestinal morphology. However, this effect is limited to the duodenum and does not extend to the jejunum and ileum. A study investigated the impact of hypobaric hypoxia on gut morphology, focusing on parameters such as duodenum and ileum villus height, villus surface area, lamina propria thickness, and villus crypt depth in two strains of broilers: ascites-resistant (RES) and ascites-susceptible (SUS) (29). Their findings revealed that under high-altitude hypoxic conditions, the duodenal villus surface area was significantly lower in the SUS line compared to the RES line. They proposed that this reduction in surface area may indicate impaired enteric function, potentially explaining the higher incidence of ascites in these chickens. Additionally, the decreased nutrient absorption associated with reduced gut efficiency

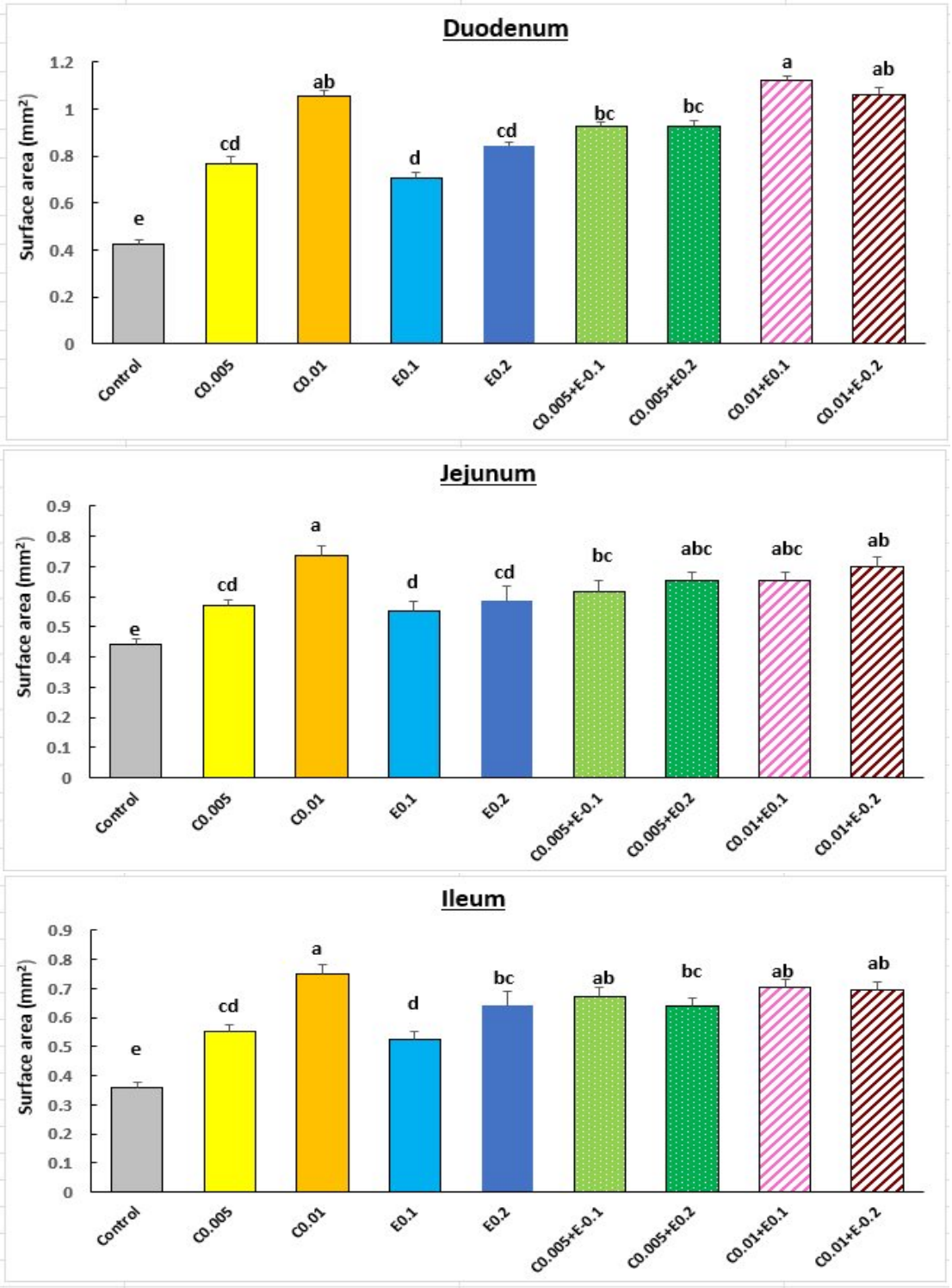

Fig. 1. Effect of L-carnitine (C) and emulsifier (E) on intestinal surface area in chickens raised in high altitude. C0.005, L-carnitine-0.005%; C0.01, L-carnitine-0.01%; E0.1, emulsifier-0.1%; E0.2, emulsifier-0.2%; Data as mean ± standard error (SE); a,b,c,d significant differences between groups ($P < 0.05$).

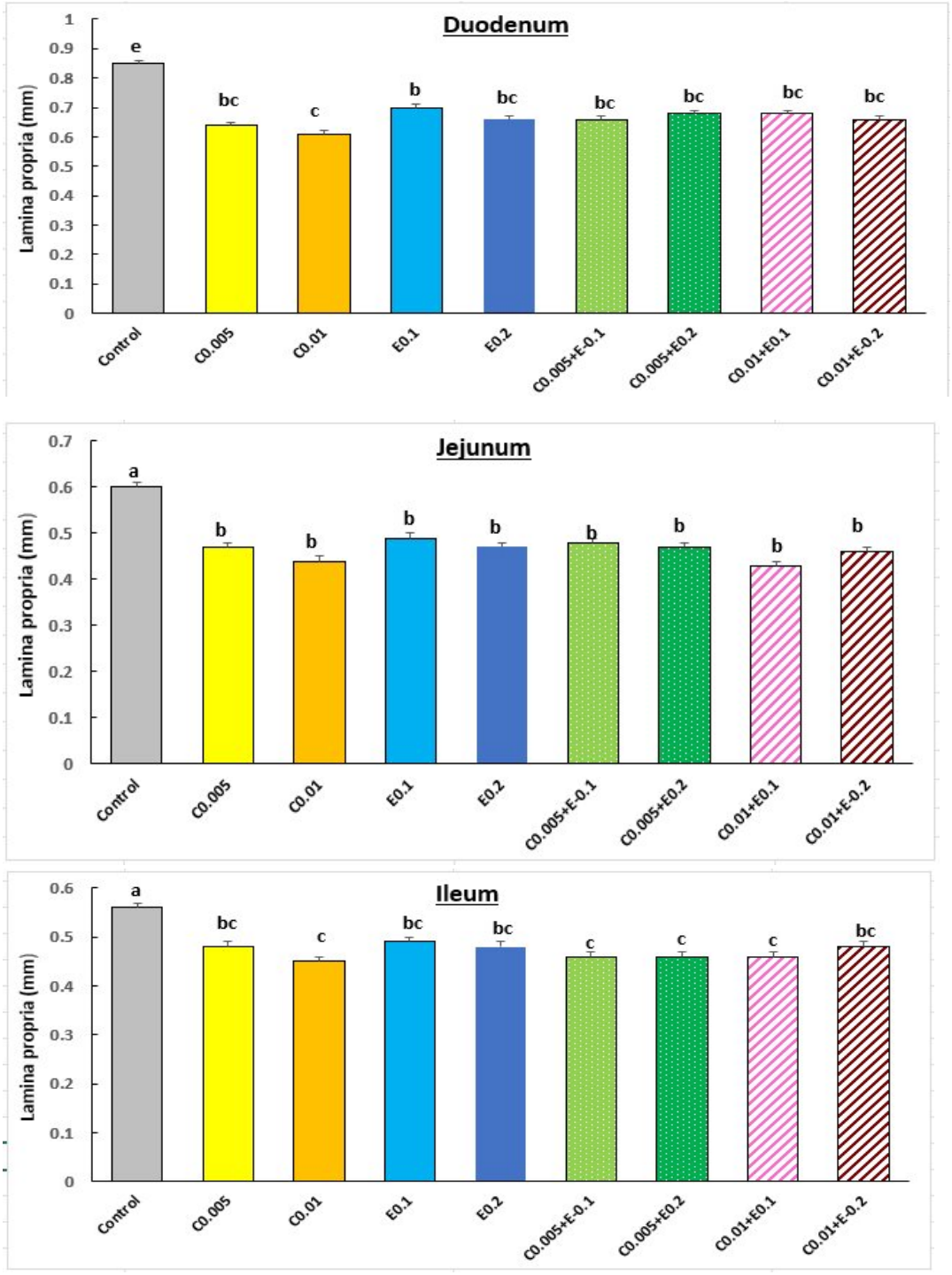

Fig. 2. Effect of L-carnitine (C) and emulsifier (E) on intestinal lamina propria thickness in chickens raised at high altitude. C0.005, L-carnitine-0.005%; C0.01, L-carnitine-0.01%; E0.1, emulsifier-0.1%; E0.2, emulsifier-0.2%; Data as mean ± standard error (SE); a,b,c,d significant differences between groups ($P < 0.05$).

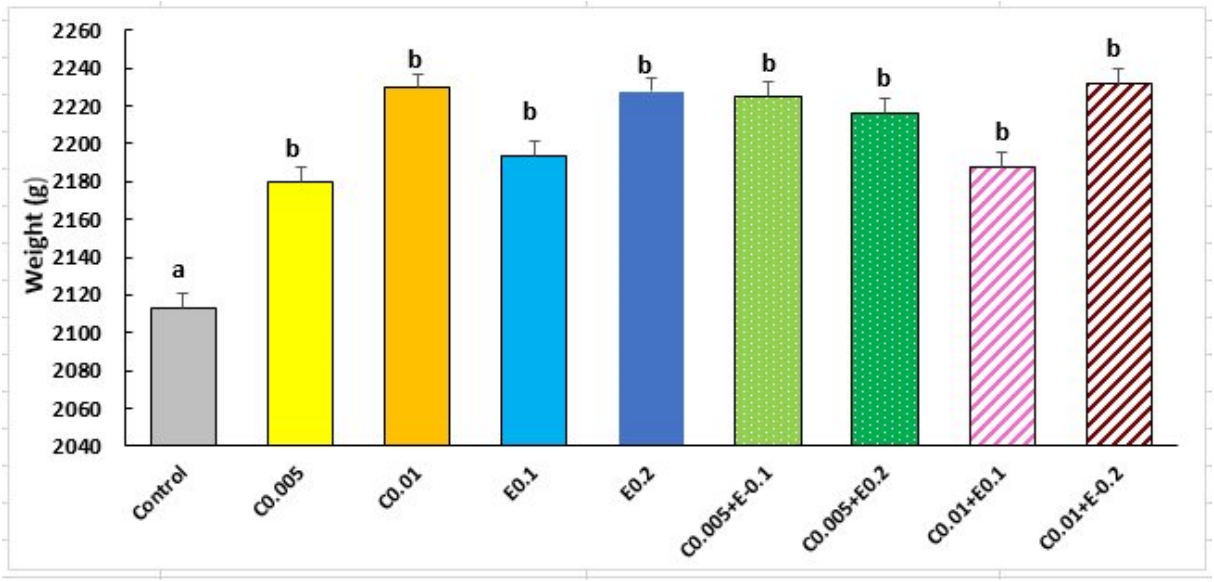

Fig. 3. Effect of L-carnitine (C) and emulsifier (E) on weight of chickens raised at high altitude after 42 days. C0.005, L-carnitine-0.005%; C0.01, L-carnitine-0.01%; E0.1, emulsifier-0.1%; E0.2, emulsifier-0.2%; Data as mean ± standard error (SE); a,b, significant differences between groups ($P < 0.05$).

could account for the increased relative gut weights previously noted in the SUS lines (30). It is important to note that the gastrointestinal tract demands substantial oxygen, and hypoxia can hinder gut development in commercial broilers, leading to a deterioration of overall gut architecture (31). In our study, L-carnitine and emulsifiers positively influenced various aspects of intestinal morphology, particularly enhancing villus dimensions (height and width) and surface area (a key indicator of intestinal absorption) in broilers subjected to high-altitude stress.

Notably, the improvements in intestinal morphology were paralleled by significant increases in body weight across all treatment groups, despite the absence of differences among the supplemented groups. This suggests that the enhanced villus dimensions and surface area likely contributed to better nutrient absorption and utilization, ultimately supporting growth performance under high-altitude stress. However, the lack of variation in weight gain among treatment groups implies that the synergistic effects of L-carnitine and emulsifiers on intestinal structure may not translate into further growth benefits beyond a certain threshold.

The lamina propria in the intestines of chickens is a critical layer of connective tissue located beneath the epithelial lining. It plays several important roles, including supporting the intestinal epithelium and containing various immune cells and glands. The lamina propria contains glands, specifically Lieberkühn's glands, which secrete substances such as mucus. (32). These glands play a vital role in sustaining the intestinal environment and facilitating digestion. This layer is also rich in lymphoid tissue, contributing to the gut-associated lymphoid tissue. This plays a vital role in protecting against pathogens and maintaining gut health (33, 34). The significant reduction in lamina propria thickness observed in all treatment groups raises concerns about its potential negative effects on secretion and digestive function. The lamina propria is vital for maintaining gut health and immune responses, and thinning this layer may compromise intestinal immunity. While previous studies have indicated some positive effects of L-carnitine (35, 36), it has also been reported that L-carnitine consumption for four weeks can lead to a decrease in thymus weight (37). Therefore, further research is needed to clarify the effects of carnitine and emulsifiers on intestinal immunity. The improvements in intestinal morphology observed in this study have important implications for poultry production in high-altitude regions. By enhancing villus height, width, and surface

area, L-carnitine and emulsifiers can potentially improve nutrient absorption, growth performance, and overall health in chickens exposed to high-altitude stress. These findings suggest that dietary supplementation with L-carnitine and emulsifiers could be a practical strategy to mitigate the challenges of high-altitude environments and enhance productivity in poultry farming.

Conclusion

This study reveals that dietary supplementation with L-carnitine and emulsifiers partially improves intestinal morphology in chickens exposed to high-altitude stress. While the combination of L-carnitine and emulsifiers showed promising effects on certain parameters, its efficacy was not consistent across all segments of the intestine (duodenum, jejunum, and ileum) or all measured morphological indicators. The observed reduction in lamina propria thickness across all treatment groups raises concerns about potential impacts on intestinal immunity and secretory functions, warranting further investigation.

Overall, the study highlights the potential of L-carnitine and emulsifiers as effective dietary interventions to mitigate the adverse effects of high-altitude stress in poultry, thereby improving growth performance in challenging environments.

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Compliance with ethical standards

All procedures in this study were approved by the Institutional Animal Care and Use Committee of Shahrekord University (Code: IR.SKU.REC.1396.088).

Conflicts of interest

The authors declare that they have no competing interests.

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