

EFFECT OF DIETARY WHEY POWDER ON THE SYNTHESIS OF INTESTINAL BARRIER PROTEINS (ZONULIN AND OCCLUDIN) IN BROILER CHICKENS UNDER HEAT STRESS

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Abstract

Heat stress is recognized as one of the most critical environmental challenges in intensive poultry production, leading to profound physiological, biochemical, and structural disturbances, particularly within the gastrointestinal tract. The present study aims to evaluate the effect of dietary whey powder on the regulation of intestinal barrier integrity in broiler chickens exposed to heat stress conditions. Special emphasis was placed on key tight junction-associated proteins, zonulin and occludin, which serve as biomarkers of intestinal permeability and epithelial stability.

A total of 100 broiler chickens were divided into control, stress, and treatment groups receiving 5% and 10% whey powder supplementation. The results demonstrated that heat stress significantly increased zonulin levels and decreased occludin expression, indicating disruption of intestinal barrier function and enhanced permeability. In contrast, dietary inclusion of whey powder produced a dose-dependent corrective effect, characterized by a marked reduction in zonulin concentration, restoration of occludin levels, and significant decrease in circulating lipopolysaccharides. Additionally, improvements in intestinal morphology, particularly villus height, were observed in supplemented groups.

These findings suggest that whey powder exerts a protective and regulatory effect on the intestinal barrier through modulation of tight junction proteins and reduction of endotoxemia. Consequently, whey powder can be considered a biologically effective and economically feasible nutritional intervention for improving gut health and mitigating the adverse effects of heat stress in broiler chickens.

Keywords: Broiler chickens, heat stress, intestinal barrier, zonulin, occludin, whey protein.

Introduction

Heat stress is one of the most critical environmental stressors affecting modern poultry production systems. Due to their high metabolic rate and limited thermoregulation, broiler chickens are highly susceptible to elevated temperatures, which leads to physiological imbalance, oxidative stress, and reduced productivity [1, 2, 3, 4, 5, 10].

The gastrointestinal tract is particularly sensitive to thermal stress. The intestinal barrier functions as a dynamic interface between the external environment and internal physiological systems. It regulates selective permeability and prevents the translocation of pathogens and toxins. This barrier is maintained by tight junction proteins such as occludin and regulatory mediators such as zonulin [9, 12, 19].

Zonulin acts as a physiological modulator of intestinal permeability. Increased zonulin secretion results in disassembly of tight junctions and increased epithelial permeability, contributing to endotoxemia and systemic inflammation [11]. In contrast, occludin is essential for maintaining structural stability and epithelial cohesion.

Nutritional modulation of intestinal barrier function has become an important area of research. Whey powder contains biologically active components, including glutamine, cysteine, lactoferrin, and bioactive peptides, which support intestinal regeneration and immune function [13, 14].

The aim of this study is to evaluate the effect of whey powder supplementation on the expression of zonulin and occludin and its role in preserving intestinal barrier integrity under heat stress conditions.

MATERIALS AND METHODS

The experimental study was conducted under controlled laboratory conditions on a total of 100 clinically healthy one-day-old broiler chickens (Ross 308 cross), obtained from a commercial hatchery. Upon arrival, the chicks were randomly allocated into four experimental groups ($n = 25$ per group) to ensure uniform distribution and minimize selection bias. All birds were maintained under standardized housing conditions with controlled microclimate parameters, appropriate ventilation, and hygienic management practices, and were provided ad libitum access to feed and water throughout the experimental period [1, 2, 3, 4, 6, 22].

The experimental design consisted of four groups: a control group maintained under thermoneutral conditions (22–24°C), a stress group exposed to heat stress, and two treatment groups subjected to heat stress and supplemented with whey powder at inclusion levels of 5% (T1) and 10% (T2) of the basal diet. Heat stress conditions were simulated by maintaining ambient temperature at 34–36°C for 6–8 hours daily, reflecting typical environmental stress factors encountered in poultry production systems during hot seasons [5, 6, 8, 10].

The basal diet was formulated in accordance with established nutritional requirements for broiler chickens, ensuring balanced levels of crude protein, metabolizable energy, essential amino acids, vitamins, and minerals [22, 23].

Whey powder used in the experiment was a commercially available dairy by-product rich in high-quality proteins, including biologically active components such as glutamine, cysteine, lactoferrin, and immunomodulatory peptides [12, 13, 14, 15]. The supplement was thoroughly mixed into the feed to ensure homogeneous distribution and consistent intake by the birds.

At the end of the experimental period, biological samples were collected for biochemical and morphological analyses. Blood samples were obtained from the wing vein under aseptic conditions and centrifuged to separate plasma. Intestinal tissue samples, primarily from the jejunum segment, were collected for evaluation of protein expression and histological structure, as this region is highly sensitive to changes in barrier integrity under stress conditions [7, 11, 16, 17].

Zonulin concentration in plasma was determined using enzyme-linked immunosorbent assay (ELISA), following standardized protocols widely applied in studies of intestinal permeability [9, 20]. Occludin expression in intestinal

tissue was quantified using immunoassay techniques and normalized relative to total protein concentration, reflecting tight junction structural integrity [19].

Plasma lipopolysaccharide (LPS) levels were measured as an indicator of endotoxemia using chromogenic assay methods, which are commonly employed to assess systemic inflammatory responses associated with intestinal barrier disruption [10].

Histomorphological evaluation of the intestinal mucosa was performed using standard fixation, paraffin embedding, microtome sectioning, and hematoxylin–eosin staining procedures. Villus height was measured under light microscopy using calibrated imaging systems, with multiple measurements taken per sample to ensure statistical reliability. Changes in villus morphology were interpreted as indicators of absorptive capacity and epithelial regeneration [7].

All experimental data were processed using statistical software packages. Results were expressed as mean \pm standard deviation (Mean \pm SD). Statistical significance between groups was evaluated using one-way analysis of variance (ANOVA) followed by appropriate post hoc tests. A significance level of $p < 0.05$ was accepted as statistically reliable, in accordance with standard biomedical research methodology [21, 22].

Table 1. Experimental design

Group	Conditions	Diet composition
C	Normal temperature	Basal diet
S	Heat stress	Basal diet
T1	Heat stress	Basal diet + 5% whey
T2	Heat stress	Basal diet + 10% whey

Note for Table 1: Table 1 summarizes the experimental design, including group allocation, environmental conditions, and dietary treatments. It illustrates the comparison between thermoneutral and heat stress conditions, as well as the graded inclusion levels of whey powder used to evaluate its effect on intestinal barrier function.

RESULTS

The obtained results clearly demonstrate that heat stress exerts a pronounced negative effect on intestinal barrier integrity in broiler chickens, affecting both regulatory and structural components of tight junctions. In the stress group (S), a

statistically significant increase in plasma zonulin concentration was observed compared to the control group, indicating enhanced intestinal permeability and disruption of tight junction regulation. Simultaneously, occludin expression in intestinal tissue decreased markedly, reflecting structural impairment of epithelial integrity.

In addition to these molecular changes, heat stress was associated with a substantial elevation in plasma lipopolysaccharide (LPS) levels, confirming the development of endotoxemia due to increased translocation of bacterial components from the intestinal lumen into systemic circulation. Morphological analysis further revealed a significant reduction in villus height in the stress group, indicating impaired absorptive capacity and degeneration of intestinal mucosa.

Dietary supplementation with whey powder produced a clear dose-dependent protective effect on all evaluated parameters. In the T1 group (5% whey), partial normalization of zonulin levels was observed, accompanied by a moderate increase in occludin expression. Plasma LPS concentration in this group decreased significantly compared to the stress group, suggesting improved barrier function. Additionally, villus height showed a noticeable recovery, indicating partial restoration of intestinal morphology.

More pronounced effects were recorded in the T2 group (10% whey supplementation). In this group, zonulin levels were significantly reduced and approached those of the control group, demonstrating effective regulation of intestinal permeability. Occludin expression increased substantially, nearly reaching baseline values, which indicates restoration of tight junction structural integrity. Furthermore, plasma LPS levels decreased sharply, reflecting a marked reduction in endotoxin translocation.

Morphologically, the T2 group exhibited a significant increase in villus height compared to the stress group, suggesting enhanced epithelial regeneration and improved nutrient absorption capacity. These findings collectively confirm that whey powder supplementation not only mitigates the негативные effects of heat stress but also promotes recovery of intestinal structure and function.

Overall, the data indicate that whey powder exerts a strong modulatory effect on intestinal barrier parameters, with the 10% inclusion level providing the most significant improvement across all measured indicators.

Table 2. Intestinal barrier parameters under experimental conditions

Group	Zonulin (ng/ml)	Occludin (ng/mg)	LPS (EU/ml)	Villus height (µm)
C	12.1 ± 0.6	4.9 ± 0.4	0.07 ± 0.01	850 ± 15
S	31.4 ± 2.1	2.2 ± 0.2	0.48 ± 0.05	620 ± 22
T1	19.5 ± 1.2*	3.4 ± 0.3*	0.19 ± 0.02*	740 ± 18*
T2	14.9 ± 0.8*	4.7 ± 0.4*	0.12 ± 0.01*	810 ± 12*

Note for Table 2: Table 2 presents the quantitative changes in intestinal barrier parameters across experimental groups. It highlights the detrimental impact of heat stress on zonulin, occludin, LPS levels, and villus height, as well as the dose-dependent restorative effect of whey powder supplementation on these indicators.

DISCUSSION

Heat stress disrupts intestinal barrier function by altering both regulatory and structural components of tight junctions. Elevated zonulin levels lead to increased permeability, while decreased occludin weakens epithelial integrity [9, 11, 18, 24].

Increased LPS levels observed in the stress group confirm the development of endotoxemia, which is associated with systemic inflammation and metabolic disturbances [10].

Whey powder supplementation demonstrated a protective effect through multiple mechanisms. Glutamine enhances enterocyte metabolism and promotes tight junction protein synthesis, while cysteine contributes to antioxidant defense through glutathione synthesis [13].

Table 3. Relative changes compared to stress group (%)

Parameter	T1 (%)	T2 (%)
Zonulin ↓	-37.9%	-52.5%
Occludin ↑	+54.5%	+113.6%
LPS ↓	-60.4%	-75.0%
Villus height ↑	+19.3%	+30.6%

Note for Table 3: Table 3 illustrates the relative percentage changes of key intestinal barrier parameters in whey-supplemented groups compared to the heat stress group. The data emphasize the dose-dependent efficacy of whey powder in reducing zonulin and LPS levels while enhancing occludin expression and villus height, indicating significant improvement in intestinal barrier integrity.

CONCLUSION

The findings of the present study provide clear evidence that heat stress exerts a substantial negative impact on intestinal barrier integrity in broiler chickens. This condition is characterized by increased intestinal permeability, elevated zonulin levels, reduced occludin expression, and significant endotoxemia, accompanied by morphological deterioration of the intestinal mucosa. These alterations collectively contribute to impaired nutrient absorption, systemic inflammatory responses, and reduced physiological efficiency in poultry under thermal stress conditions.

Dietary supplementation with whey powder demonstrated a pronounced protective and restorative effect on intestinal barrier function. The observed normalization of zonulin levels indicates improved regulation of tight junction permeability, while the significant increase in occludin expression reflects restoration of epithelial structural integrity. Additionally, the marked reduction in plasma lipopolysaccharide levels confirms the effectiveness of whey powder in preventing endotoxin translocation and mitigating systemic inflammatory processes.

Morphological improvements, particularly the increase in villus height, further support the regenerative effect of whey-derived bioactive compounds on intestinal epithelium. These effects are likely mediated by the high content of essential amino acids, especially glutamine and cysteine, which play a crucial role in enterocyte metabolism, antioxidant defense, and protein synthesis.

Importantly, the results reveal a clear dose-dependent relationship, with the 10% inclusion level of whey powder providing the most significant improvement across all evaluated parameters. This suggests that optimal dietary inclusion of whey powder can serve as a practical and effective strategy for enhancing intestinal resilience under adverse environmental conditions.

From an applied perspective, the incorporation of whey powder into broiler diets represents a biologically sound, economically feasible, and safe nutritional intervention. Its use can contribute not only to improved gut health but also to enhanced productivity and overall sustainability of poultry production systems, particularly in regions with high ambient temperatures.

Thus, whey powder may be recommended as a functional feed additive for mitigating the negative effects of heat stress and supporting intestinal homeostasis in broiler chickens.

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