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Dietary acidification as a strategy to modulate gut microbiota, improve intestinal integrity, and enhance zootechnical performance of broiler chickens subjected to avian pathogenic *Escherichia coli* (APEC) challenge and heat stress

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Introduction

Modern poultry production faces significant challenges in sustaining productive performance under adverse conditions, particularly under high sanitary and environmental pressure. Infection with avian pathogenic *Escherichia coli* (APEC) and heat stress are known to compromise intestinal homeostasis, promoting dysbiosis, increasing intestinal permeability, and triggering inflam-

matory responses (Ringseis & Eder, 2022; Wei et al., 2025). This process results in impaired nutrient absorption and, consequently, reduced production efficiency in broilers. In parallel with this challenging scenario, increasing restrictions on the use of antimicrobial growth promoters have intensified the need for nutritional strategies that functionally target the intestinal environ-

ment (MAPA, 2026). Among the main alternatives, dietary acidification through the use of blends of organic acids and non-acid compounds has shown relevant potential to modulate the gut microbiota, particularly by controlling enterobacteria, and to support the maintenance of intestinal mucosal integrity (Vera-Álava et al., 2023). Although the effects of this strategy are well documented under sanitary challenge conditions, the efficacy of dietary acidifiers under extreme challenge scenarios characterized by the simultaneous interaction of sanitary and environmental stressors remains poorly explored. In this context, the objective of this study was to evaluate the effect of including the acidifier pHPerfect Platinum® in broiler diets on productive performance, gut microbiota, and intestinal integrity of broiler chickens subjected to an extreme challenge model involving APEC inoculation combined with heat stress.

Materials and Methods

A total of 660 male Cobb 500™ broilers were allocated in a completely randomized design with two treatments, 10 replicates, and 33 birds per experimental unit. Birds were fed diets according to breeder recommendations with ad libitum access to water. The study was approved by the institutional ethics committee (protocol no. 045.01.25). Treatments included: T1, challenged control with APEC inoculation (1.0×10^7 CFU/mL) in 7-day-old chicks, followed by heat stress in 37-day-old birds (32°C for 2 h); and T2, the same conditions as T1 with dietary inclusion of an acidifier (500

g/t; pHPerfect Platinum®). The additive acidifier consists of a blend of organic acids combined with a glycerol matrix and ammonium salts, containing lactic acid (30 g/kg), citric acid (25 g/kg), and ascorbic acid (25 g/kg). Zootechnical performance parameters were evaluated, including final body weight, average daily gain, feed conversion ratio, production efficiency index (PEI), and viability. Ileal content samples (two birds per experimental unit) were collected at 42 days for quantification of total enterobacteria and *Bacillus* spp. Jejunal samples were also collected for intestinal morphometry and calculation of the intestinal absorptive index (IAI) according to Kisiński et al. (2002). Data were analyzed using linear models with one-tailed planned contrasts ($\alpha = 0.05$).

Results and Discussion

As shown in Table 1, treatment with pHPerfect Platinum® increased final body weight ($p = 0.028$) and showed a trend toward improved average daily gain ($p = 0.065$). Significant effects on intestinal health were observed, including increased villus height ($p = 0.0046$), villus-to-crypt ratio ($p = 0.0118$), and absorptive index ($p = 0.0094$). Additionally, a significant reduction in Enterobacteriaceae ($p = 0.0004$) and a reduction in the Enterobacteriaceae/*Bacillus* ratio ($p = 0.0008$) were detected, indicating a positive modulation of the gut microbiota. Overall, these results suggest that the treatment improves productive performance primarily through enhanced intestinal integrity and microbiota balance.

Table 1 - Effect of pHPerfect Platinum® on zootechnical performance, intestinal morphometry, and microbiota profile of broiler chickens subjected to high challenge conditions with APEC and heat stress

Parameter	T1	T2	p value
Final body weight (grams)	3,057.05	3,098.34	0.028
Daily weight gain (grams)	71.60	72.68	0.065
Adjusted feed conversion ratio (FCR)	1.872	1.930	0.968
Production Efficiency Index (PEI)	337.20	345.17	0.242
Viability (%)	82.30	84.00	0.268
Villus height (µm)	7,161.965	8,110.664	0.0046
Crypt depth (µm)	550.834	526.180	0.4021
Villus-to-crypt ratio	13.390	15.587	0.0118
Intestinal Absorptive Index (IAI)	4.832	5.479	0.0094
<i>Bacillus</i> spp. count (log ₁₀ CFU/g)	3.218	3.194	0.554
Enterobacteriaceae count (log ₁₀ CFU/g)	5.043	3.133	0.0004
Enterobacteriaceae/ <i>Bacillus</i> spp. ratio	1.634	1.009	0.0008



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Conclusions

The inclusion of pHPerfect Platinum® in broiler diets significantly improved growth performance, intestinal integrity, and microbiota balance under high-challenge conditions, demonstrating its

potential as an effective strategy to mitigate sanitary and environmental challenges in commercial production systems.

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