



# Effects of compound organic acid calcium on growth performance, hepatic antioxidation and intestinal barrier of male broilers under heat stress

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**OBJECTIVE:** To investigate the effects of compound organic acid calcium (COAC) on growth performance, hepatic antioxidation and intestinal barrier of male broilers under heat stress.

**MEASUREMENTS AND MAIN RESULTS:** The experiment was conducted in a randomized controlled trial. The broilers were divided into four groups: control (0.4% COAC), heat stress (0.4% COAC), heat stress + COAC (0.4% COAC), and heat stress + COAC + vitamin E (0.4% COAC). The results showed that the heat stress group had significantly lower body weight gain (BWG) and feed conversion ratio (FCR) compared to the control group. The addition of COAC significantly improved BWG and FCR in the heat stress group. The heat stress + COAC + vitamin E group showed the highest BWG and the lowest FCR. The heat stress group had significantly higher levels of malondialdehyde (MDA) and lower levels of superoxide dismutase (SOD) and catalase (CAT) in the liver compared to the control group. The addition of COAC significantly reduced MDA levels and increased SOD and CAT levels in the liver. The heat stress + COAC + vitamin E group showed the lowest MDA levels and the highest SOD and CAT levels. The heat stress group had significantly higher levels of D-lactate in the jejunum compared to the control group. The addition of COAC significantly reduced D-lactate levels in the jejunum. The heat stress + COAC + vitamin E group showed the lowest D-lactate levels.

**CONCLUSIONS:** The addition of COAC significantly improved growth performance, hepatic antioxidation and intestinal barrier of male broilers under heat stress. The addition of vitamin E further improved growth performance, hepatic antioxidation and intestinal barrier of male broilers under heat stress.

**KEY WORDS:** Broilers, Heat stress, Compound organic acid calcium, Growth performance, Hepatic antioxidation, Intestinal barrier.

## INTRODUCTION

Heat stress is a major environmental stressor for broilers, which can lead to reduced growth performance, increased mortality, and economic losses (1, 2). Heat stress can also lead to oxidative stress and intestinal barrier dysfunction, which can further exacerbate the negative effects of heat stress (3, 4). Therefore, it is important to find effective strategies to mitigate the effects of heat stress on broilers.

Compound organic acid calcium (COAC) is a natural feed additive that has been shown to have various beneficial effects on broilers, including improved growth performance, enhanced immune response, and improved intestinal health (5, 6). COAC has been shown to have antioxidant properties, which may help to reduce oxidative stress and improve intestinal barrier function (7, 8). Therefore, the addition of COAC to broiler diets may be a promising strategy to mitigate the effects of heat stress.

The present study was conducted to investigate the effects of COAC on growth performance, hepatic antioxidation and intestinal barrier of male broilers under heat stress. The results showed that the addition of COAC significantly improved growth performance, hepatic antioxidation and intestinal barrier of male broilers under heat stress. The addition of vitamin E further improved growth performance, hepatic antioxidation and intestinal barrier of male broilers under heat stress.

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**Table 3.** Gene names and primer sequences

Gene	Primer sequence 5'-3'
<i>MUC-2</i>	F: GCCTGCCAGGAAATCAAG R: CGACAAGTTTGCTGGCACAT
<i>Ocln</i>	F: GAGCCCAGACTACCAAAGCAA R: GCTTGATGTGGAAGAGCTTGTTG
<i>Cldn1</i>	F: TGGCCACGTCATGGTATGG R: AACGGGTGTGAAAGGGTCATAG
<i>Cldn3</i>	F: AATGCGCCATCTCTGCAAAC R: GTTCTCCGCCAGACTCTCC
<i>TLR2</i>	F: TGTTCTGTTTCATCCTCATCCT R: AGTTGGAGTCGTTCTCACTGT
<i>-actin</i>	F: TATGTGCAAGGCCGGTTTC R: TGCTTTCTGGCCATACCAA
<i>TLR4</i>	F: GAATGACACGGACACTCTT R: ACATAGGAACCTCTGACAAC
<i>TLR15</i>	F: CTGTGCTTCTGGTGCTAA R: ATCGTGCTCGTGTATGA
<i>IL-1</i>	F: CGACATCAACCAGAAGTGCTT R: GTCCAGGCGGTAGAAGATGA
<i>iNOS</i>	F: TACTCTGGCGTCATTACTC R: GCATAGATCACAGTCACCTT
<i>TGF- 2</i>	F: TCTCGGAGCAGCGGATAGA R: AATCCAAGTTCCTGTCTCTGT

F, forward; R, reverse; *MUC-2*, mucin 2; *Ocln*, occluding; *Cldn*, claudin; *TLR*, toll-like receptor; *IL*, interleukin; *iNOS*, inducible nitric oxide synthase; *TGF- 2*, transforming growth factor-beta 2.

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**Table 4.** Effects of dietary compound organic acid calcium on growth performance of broilers

Items	21 days of age <sup>1)</sup>			SEM	p-value
	Control	0.4% COAC	0.8% COAC		
Body weight (g)					
1 day of age	45.80	45.19	45.40	0.35	0.52
21 days of age	563.45 <sup>a</sup>	600.15 <sup>b</sup>	594.71 <sup>b</sup>	9.91	0.04
42 days of age	1,498.89 <sup>a</sup>	1,608.86 <sup>b</sup>	1,603.45 <sup>b</sup>	35.31	0.01
1 to 21 days of age					
ADFI (g)	42.68	44.02	43.43	0.74	0.50
ADG (g)	24.65 <sup>a</sup>	26.43 <sup>b</sup>	26.16 <sup>b</sup>	0.48	0.04
F/G	1.73 <sup>b</sup>	1.67 <sup>a</sup>	1.66 <sup>b</sup>	0.01	0.01
22 to 42 days of age					
ADFI (g)	93.43	95.59	95.98	1.62	0.55
ADG (g)	44.54 <sup>a</sup>	48.03 <sup>b</sup>	48.04 <sup>b</sup>	1.51	0.02
F/G	2.10 <sup>b</sup>	1.99 <sup>a</sup>	2.00 <sup>a</sup>	0.04	0.01
1 to 42 days of age					
ADFI (g)	67.99	69.77	69.71	0.95	0.37
ADG (g)	34.60 <sup>a</sup>	37.23 <sup>b</sup>	37.10 <sup>b</sup>	0.85	0.01
F/G	1.97 <sup>b</sup>	1.87 <sup>a</sup>	1.88 <sup>a</sup>	0.03	0.02

Values reported as means (n = 6).

COAC, compound organic acid calcium; SEM, standard error of means for 6 broilers each; ADFI, the average daily feed intake; ADG, the average daily gain; F/G, the ratio of feed gain.

<sup>1)</sup> Control = basal diet without any feed additive; 0.4% COAC = basal diet + 0.4% compound organic acid calcium; 0.8% COAC = basal diet + 0.8% compound organic acid calcium.

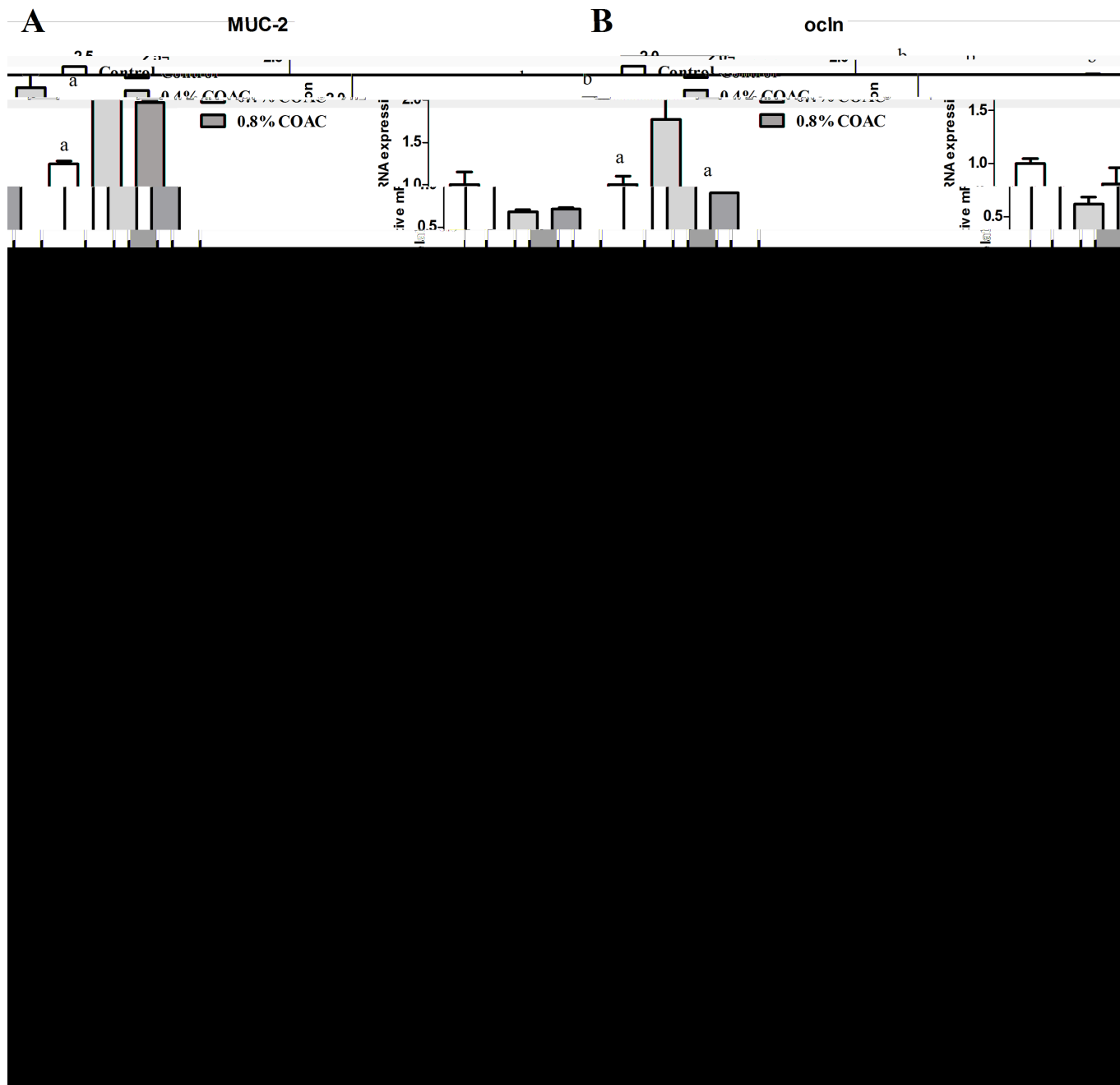
<sup>a,b</sup> Means in the same row with different superscripts differ statistically (p < 0.05).

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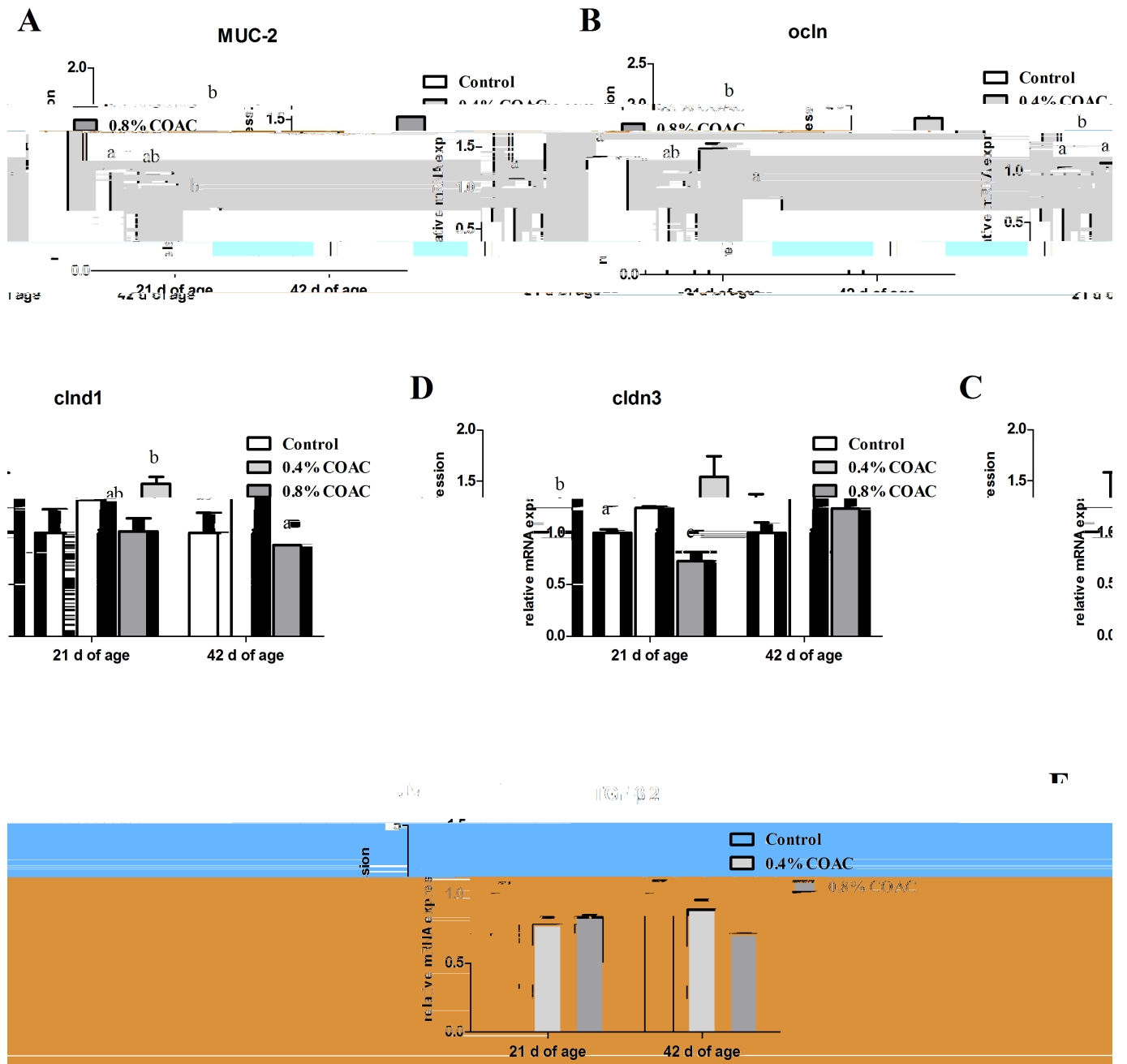
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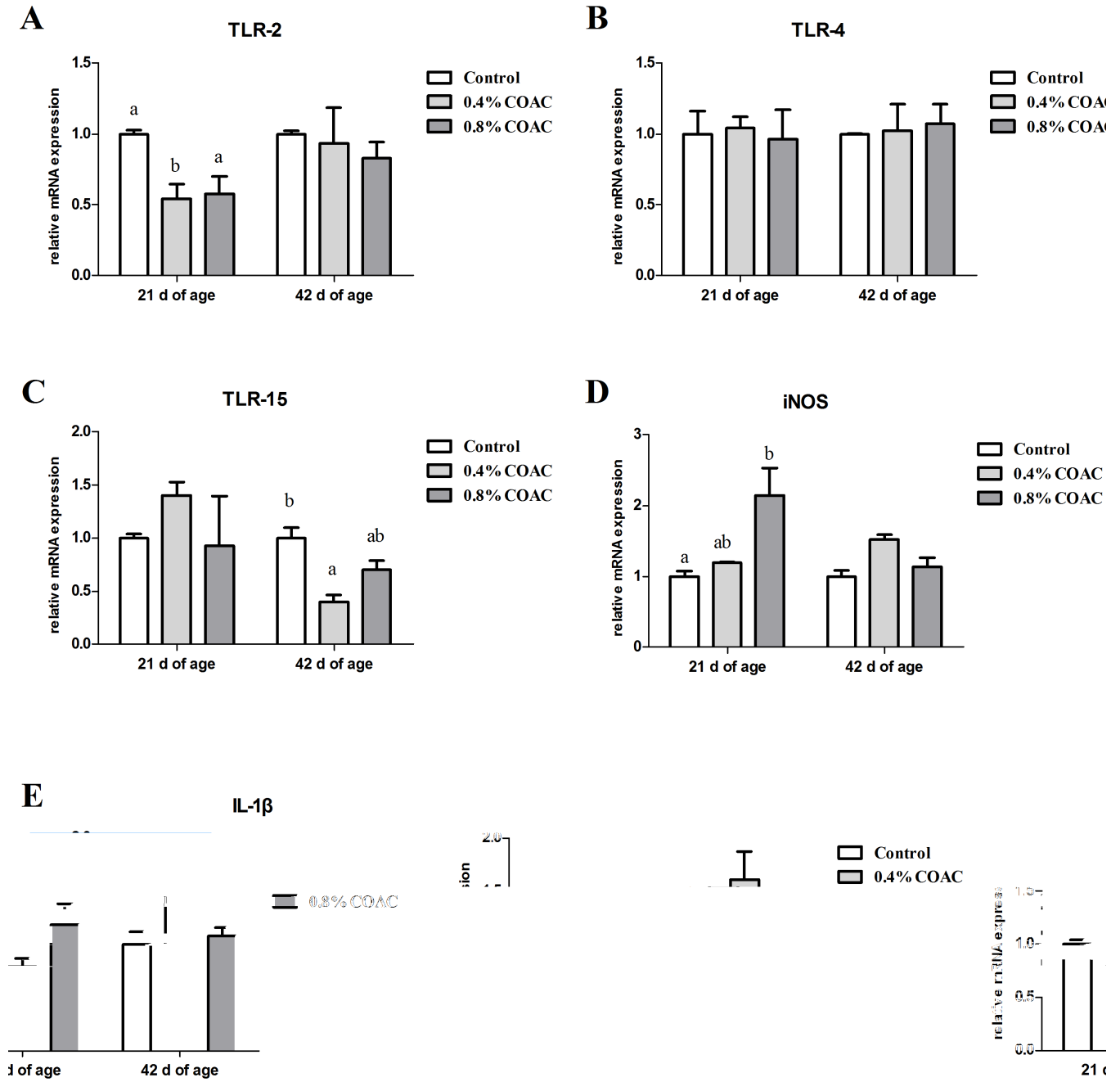


**Figure 1.** Effects of compound organic acid calcium on MUC-2, ocln, cldn1, cldn3 and TGF- 2 mRNA expression in jejunum of broilers. At 21 and 42 days of age, the expression of MUC-2 (A), ocln (B), cldn1 (C), cldn3 (D) and TGF- 2 (E) were measured by real-time polymerase chain reaction. MUC-2, mucin 2; Ocln, occluding; Cldn, claudin; TGF- 2, transforming growth factor-beta 2. Different letters (a-c) denote a statistical difference (p<0.05).

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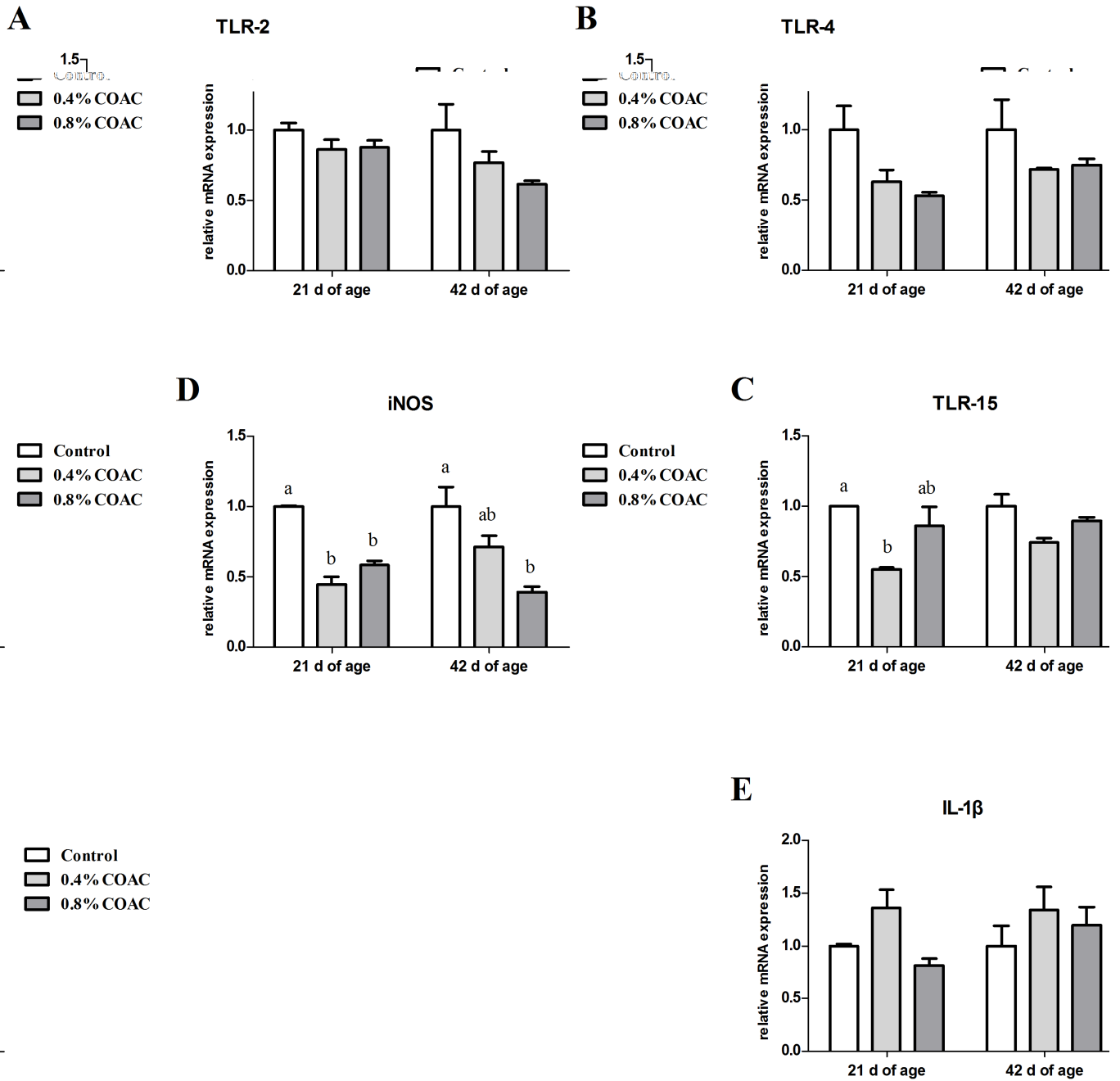
**Figure 2.** Effects of compound organic acid calcium on ocln, cldn1, cldn3 and TGF- 2 mRNA expression in ileum of broilers. At 21 and 42 days of age, the expression of ocln (A), cldn1 (B), cldn3 (C) and TGF- 2 (D) were measured by real-time polymerase chain reaction. Ocln, occluding; Cldn, claudin; TGF- 2, transforming growth factor-beta 2. Different letters (a-c) denote a statistical difference ( $p < 0.05$ ).



**Figure 3.** Effects of compound organic acid calcium on TLR, iNOS and IL-1 mRNA expression in jejunum of broilers. At 21 and 42 days of age, the expression of TLR-2 (A), TLR-4 (B), TLR-15 (C), iNOS (D) and IL-1 (E) were measured by real-time polymerase chain reaction. TLR, toll-like receptor; iNOS, inducible nitric oxide synthase; IL-1, interleukin 1. Different letters (a, b) denote a statistical difference ( $p < 0.05$ ).

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**Figure 4.** Effects of compound organic acid calcium on TLR, iNOS, and IL-1 mRNA expression in ileum of broilers. At 21 and 42 days of age, the expression of TLR-2 (A), TLR-4 (B), TLR-15 (C), iNOS (D), and IL-1 (E) were measured by real-time polymerase chain reaction. TLR, toll-like receptor; iNOS, inducible nitric oxide synthase; IL, interleukin. Different letters (a, b) denote a statistical difference ( $p < 0.05$ ).

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21. 2010 05-63. // . /10.4141/ 0 04
22. 2015 31-163- .
23. 200-16-555-62. // . / 10.33 2/ .2006-00116
24. 1 6 :353-66.
25. B 1 32:5 5-603. // /10.1016/ 000 - 120( )000-5-2
26. B 2003 44 545-50. // . /10.10 0/000-166031000161 334
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29. B, , , ,
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32. B 200 -5- 3. // . /10.2-54/ 200 -0100-5
33. 2014 12-3401-13. // . /10.1242/ .14502
34. 2014 14-141-53. // . /10.103 / 360
35. B, B B C B 201 . // . /10. 11 6/ 40104-01-0220-2
36. B , , -2
37. 200 120-2462- . // . /10.1161/ .10 . 51 1
38. 2014 13- 602-10. // . /10.3 23/ .2014.602.610