**Supplementary material\_Animal journal**

Effect of functional oils on the immune response of broilers challenged with *Eimeria* spp.

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Table S1. The “I See Inside” (ISI) histological analysis scores, presented as means ± standard error, of duodenum samples of broilers at 21 days of age

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Self-blade thickness | Epithelial thickness | Proliferation of enterocytes | Epithelial plasma infiltration | Mixed inflammatory infiltration of lamina propria | Goblets cells | Congestion | Necrosis (apical karyolysis) | Presence of oocysts | Score |
| *Challenge status* | | | | | | | | | | |
| Unchallenged | 1.76±0.14 b | 2.67±0.10 b | 2.47±0.10 b | 0.77±0.03 a | 1.63±0.18 | 1.91±0.15 | 0.90±0.12 b | 0.05±0.03 | 0.13±0.06 | 12.33±0.46 b |
| Challenged | 3.65±0.15 a | 3.22±0.09 a | 3.11±0.10 a | 0.61±0.04 b | 1.55±0.17 | 1.75±0.16 | 1.46±0.17 a | 0.05±0.039 | 3.20±0.33 | 18.62±0.57 a |
| *Additive* | | | | | | | | | | |
| Control | 2.37±0.19 b | 2.76±0.12 b | 2.51±0.13 b | 0.61±0.04 | 1.29±0.20 | 1.37±0.18 | 1.00±0.17 | 0.08±0.05 | 2.53±0.40 | 14.55±0.70 b |
| Monensin | 2.37±0.19 b | 2.81±0.13 b | 2.65±0.13 b | 0.75±0.04 | 1.83±0.22 | 1.52±0.19 | 0.97±0.17 | 0±0 | 1.20±0.25 | 14.12±0.69 b |
| CNSL - castor oil | 3.37±0.20 a | 3.26±0.11 a | 3.21±0.11 a | 0.70±0.05 | 1.66±0.22 | 2.60±0.191 | 1.57±0.20 | 0.08±0.05 | 1.26±0.28 | 17.75±0.64 a |
| *Association between challenge status and additive* | | | | | | | | | | |
| Control\*Un | 1.60±0.23 | 2.56±0.17 | 2.23±0.17 | 0.68±0.06 | 1.08±0.26 | 2.10±0.29 ab | 0.70±0.20 | 0.08±0.083 | 0.00±0.00 c | 11.05±0.66 |
| Monensin\*Un | 1.50±0.24 | 2.53±0.19 | 2.30±0.17 | 0.80±0.057 | 0.58±0.07 | 1.20±0,24 bc | 0.90±0.21 | 0±0 | 0.00±0.00 c | 11.48±0.81 |
| Control\*CH | 3.15±0.28 | 2.96±0.18 | 2.80±0.18 | 0.55±0.073 | 1.50±0.29 | 0.65±0.18 c | 1.30±0.27 | 0.083±0.08 | 5.06±0.66 a | 18.06±1.08 |
| Monensin\*CH | 3.25±0.26 | 3.10±0.16 | 3.00±0.18 | 0.70±0.064 | 1.41±0.29 | 1.85±0.28 ab | 1.05±0.28 | 0±0 | 2.40±0.45 b | 16.76±1.02 |
| CNSL - CO\*Un | 2.20±0.28 | 2.93±0.16 | 2.90±0.16 | 0.83±0.072 | 1.58±0.32 | 2.45±0.24 a | 1.10±0.21 | 0.08±0.08 | 0.40±0.20 c | 14.48±0.85 |
| CNSL - CO\*CH | 4.55±0.20 | 3.60±0.14 | 3.53±0.14 | 0.58±0.07 | 1.75±0.31 | 2.75±0.29 a | 2/05±0.33 | 0.08±0.08 | 2.13±0.50 b | 21.03±0.77 |
| *Probabilities* | | | | | | | | | | |
| Challenge status | <0.001 | <0.001 | <0.001 | 0.004 | 0.740 | 0.437 | 0.080 | 1.000 | <0.001 | <0.001 |
| Additive | <0.001 | 0.006 | <0.001 | 0.132 | 0.198 | <0.001 | 0.340 | 0.368 | <0.001 | <0.001 |
| Additive\*Challenge | 0.257 | 0.735 | 0.927 | 0.507 | 0.101 | 0.001 | 0.303 | 1.000 | <0.001 | 0.598 |

a,b,c Different letters in the same column indicate a statistically significant difference, *P* ≤ 0.05 according to LSMEANS test.

Un= unchallenged; CH = challenged (CH) with pool *Eimeria spp.* at 14 days age of broilers; CNSL-CO = Commercial blend of cashew nut shell liquid and castor oil.

Table S2. The “I See Inside” (ISI) histological analysis scores, presented as means ± standard error, of jejunum samples of broilers at 21 days of age

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Self-blade thickness | Epithelial thickness | Proliferation of enterocytes | Epithelial plasma infiltration | Mixed inflammatory infiltration of lamina propria | Goblets cells | Congestion | Necrosis (apical karyolysis) | Presence of oocysts | Score |
| *Challenge status* | | | | | | | | | | |
| Unchallenged | 1.83±0.13 b | 2.50±0.07 a | 1.03±0.08 b | 0.38±0.03 b | 3.75±0.18 | 3.78±0.11 a | 1.31±0.13 | 1.47±0.19 b | 0.04±0.03 | 16.11±0.60 b |
| Challenge | 3.13±0.13 a | 2.16±0.08 b | 1.55±0.10 a | 0.61±0.03 a | 4.86±0.15 | 3.13±0.12 b | 1.15±0.13 | 3.41±0.25 a | 3.02±0.23 | 23.05±0.68 a |
| *Additive* | | | | | | | | | | |
| Control | 2.65±0.17 a | 2.21±0.10 | 1.48±0.12 a | 0.37±0.04 b | 4.16±0.20 | 3.37±0.16 | 1.05±0.14 | 2.12±0.27 b | 1.33±0.20 | 18.77±0.75 b |
| Monensin | 2.025±0.17 b | 2.26±0.08 | 0.98±0.10 b | 0.55±0.04 a | 4.25±0.21 | 3.47±0.13 | 1.07±0.16 | 1.20±0.21 c | 0.90±0.17 | 16.74±0.73 b |
| CNSL - castor oil | 2.77±0.15 a | 2.51±0.09 | 1.41±0.12 a | 0.55±0.04 a | 4.50±0.23 | 3.52±0.13 | 1.57±0.18 | 4.00±0.31 a | 2.36±0.31 | 23.23±0.94 a |
| *Association between challenge status and additive* | | | | | | | | | | |
| Control\*Un | 2.05±0.21 | 2.40±0.10 | 1.30±0.14 | 0.26±0.05 | 4.00±0.28 bc | 3.75±0.19 | 1.15±0.22 bc | 1.08±0.26 | 0±0 c | 16.00±0.80 |
| Monensin\*Un | 1.30±0.20 | 2.46±0.11 | 0.83±0.13 | 0.43±0.06 | 3.25±0.31 c | 3.65±0.17 | 0.50±0.16 c | 0.66±0.22 | 0.06±0.06 c | 13.16±.,81 |
| Control\*CH | 3.25±0.25 | 2.03±0.17 | 1.66±0.21 | 0.48±0.06 | 4.33±0.30 abc | 3.00±0.26 | 0.95±0.19 bc | 3.16±0.44 | 2.66±0.33 b | 21.55±1.17 |
| Monensin\*CH | 2.75±0.25 | 2.06±0.12 | 1.13±0.14 | 0.68±0.06 | 5.25±0.21 a | 3.30±0.21 | 1.65±0.26 ab | 1.75±0.35 | 1.73±0.30 b | 20.31±1.03 |
| CNSL – CO\*Un | 2.15±0.23 | 2.63±0.15 | 0.96±0.18 | 0.45±0.06 | 4.00±0.35 bc | 3.95±0.21 | 2.30±0.25 a | 2.66±0.41 | 0.06±0.06c | 19.18±1.30 |
| CNSL – CO\*CH | 3.40±0.16 | 2.40±0.11 | 1.86±0.14 | 0.66±0.06 | 5.00±0.29 ab | 3.10±0.14 | 0.85±0.22 bc | 5.33±0.40 | 4.66±0.47 a | 27.28±1.14 |
| *Probabilities* | | | | | | | | | | |
| Challenge status | <0.001 | 0.002 | <0.001 | <0.001 | <0.001 | <0.001 | 0.360 | <0.001 | <0.001 | <0.001 |
| Additive | 0.002 | 0.055 | 0.004 | 0.005 | 0.505 | 0.759 | 0.030 | <0.001 | <0.001 | <0.001 |
| Additive\*Challenge | 0.840 | 0.803 | 0.134 | 0.956 | 0.019 | 0.438 | <0.001 | 0.088 | <0.001 | 0.481 |

a,b,c Different letters in the same column indicate a statistically significant difference, *P* ≤ 0.05 according to LSMEANS test.

Un= unchallenged; CH = challenged (CH) with pool *Eimeria spp.* at 14 days age of broilers; CNSL-CO = Commercial blend of cashew nut shell liquid and castor oil.

Table S3. The “I See Inside” (ISI) histological analysis scores, presented as means ± standard error, of ileum samples of broilers at 21 days of age

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Self-blade thickness | Epithelial thickness | Proliferation of enterocytes | Epithelial plasma infiltration | Mixed inflammatory infiltration of lamina propria | Goblets cells | Congestion | Necrosis (apical karyolysis) | Presence of oocysts | Score |
| *Challenge* | | | | | | | | | | |
| Unchallenged | 4.73±0.18 | 3.35±0.12 b | 2.72±0.14 | 0.50±0.04 b | 3.33±0.26 | 5.15±0.19 | 2.11±0.20 | 2.63±0.23 | 0±0 | 24.55±0.79 |
| Challenge | 5.48±0.17 | 3.92±0.11 a | 3.44±0.12 | 0.91±0.05 a | 5.75±0.30 | 4.98±0.20 | 1.90±0.17 | 4.05±0.28 | 0.95±0.13 | 31.40±0.84 |
| *Additive* | | | | | | | | | | |
| Control | 4.75±0.22 | 3.43±0.14 b | 2.83±0.16 | 0.54±0.05 b | 4.58±0.36 | 4.30±0.21 b | 1.85±0.21 | 2.95±0.32 | 0.66±0.13 | 25.99±1.09 b |
| Monensin | 4.65±0.22 | 3.46±0.14 b | 2.98±0.15 | 0.58±0.05 b | 3.91±0.33 | 5.42±0.21 a | 2.20±0.26 | 3.00±0.29 | 0±0 | 26.22±0.87 b |
| CNSL - castor oil | 5.90±0.21 | 4.01±0.15 a | 3.38±0.17 | 1.00±0.07 a | 5.12±0.39 | 5.47±0.27 a | 1.97±0.22 | 4.08±0.34 | 0.76±0.15 | 31.72±1.10 a |
| *Association between challenge status and additive* | | | | | | | | | | |
| Control\*Un | 3.90±0.31 d | 2.96±0.19 | 2.16±0.22 b | 0.43±0.07 | 2.50±0.42 b | 4.55±0.33 | 1.85±0.31 | 1.91±0.37 c | 0±0 b | 20.28±.,25 |
| Monensin\*Un | 4.90±0.30 bcd | 3.23±0.21 | 2.86±0.23 ab | 0.36±0.06 | 3.58±0.44 b | 5.05±0.24 | 2.35±0.39 | 3.00±0.41bc | 0±0 b | 25.35±1.32 |
| Control\*CH | 5.65±0.28 ab | 3.90±0.18 | 3.60±0.20 a | 0.65±0.07 | 6.66±0.47 a | 4.05±0.28 | 1.85±0.28 | 4.00±0.48ab | 1.33±0.24 a | 31.70±1.45 |
| Monensin\*CH | 4.40±0.33 cd | 3.70±0.17 | 3.10±0.19 a | 0.80±0.07 | 4.25±0.48 b | 5.80±0.34 | 2.05±0.34 | 3.00±0.43bc | 0±0 b | 27.10±1.139 |
| CNSL - CO\*Un | 5.40±0.32 abc | 3.86±0.22 | 3.13±0.26 a | 0.71±0.08 | 3.91±0.47 b | 5.85±0.39 | 2.15±0.34 | 3.00±0.43bc | 0±0 b | 28.03±1.38 |
| CNSL - CO\*CH | 6.40±0.26 a | 4.16±0.20 | 3.63±0.22 a | 1.28±0.10 | 6.33±0.59 a | 5.10±0.38 | 1.80±0.29 | 5.16±0.50 a | 1.53±0.28 a | 35.41±1.58 |
| *Probabilities* | | | | | | | | | | |
| Challenge | 0.003 | 0.001 | <0.001 | <0.001 | <0.001 | 0.544 | 0.425 | <0.001 | <0.001 | <0.001 |
| Additive | <0.001 | 0.006 | 0.066 | <0.001 | 0.046 | 0.001 | 0.566 | 0.017 | <0.001 | <0.001 |
| Additive\*Challenge | 0.001 | 0.270 | 0.022 | 0.096 | 0.002 | 0.059 | 0.850 | 0.023 | <0.001 | 0.202 |

a,b,c,d Different letters in the same column indicate a statistically significant difference, *P* ≤ 0.05 according to LSMEANS test.

Un = unchallenged; CH = challenged (CH) with pool *Eimeria spp.* at 14 days age of broilers; CNSL-CO = Commercial blend of cashew nut shell liquid and castor oil.