**Effects of L-leucine *in ovo* feeding on thermotolerance, growth and amino acid metabolism under heat stress in broilers**

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**Table S1** *Effects of L-leucine (L-Leu) in ovo feeding on organ weight and plasma metabolites in male broilers exposed to acute heat stress in 29 or 30 d of age1*.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Control | | | L-Leu | | | *P*-value |
| Organ or body weight |  |  |  |  |  |  |  |
| Body (g) | 1515 | ± | 21 | 1548 | ± | 47 | 0.507 |
| Liver (g) | 45.5 | ± | 2.7 | 43.4 | ± | 2.5 | 0.577 |
| Liver/ body (%) | 3.04 | ± | 0.19 | 2.63 | ± | 0.05 | 0.111 |
| Heart (g) | 8.49 | ± | 0.27 | 9.13 | ± | 0.34 | 0.160 |
| Heart/ body (%) | 0.55 | ± | 0.017 | 0.60 | ± | 0.026 | 0.124 |
| Plasma metabolites2 |  |  |  |  |  |  |  |
| Glucose (mg/dl) | 258.9 | ± | 2.9 | 260.7 | ± | 7.9 | 0.839 |
| NEFA (mEq/l) | 210.9 | ± | 9.3 | 243.5 | ± | 34.2 | 0.345 |
| TG (mg/dl) | 22.9 | ± | 2.4 | 24.6 | ± | 1.5 | 0.558 |
| Ketone body (mmol/l) | 376.9 | ± | 20.2 | 360.0 | ± | 41.9 | 0.723 |
| LA (mg/dl) | 49.0 | ± | 4.3 | 42.8 | ± | 4.5 | 0.340 |
| CORT (ng/ml) | 4.78 | ± | 2.0 | 3.86 | ± | 0.72 | 0.648 |
| T3 (ng/ml) | 3.89 | ± | 0.18 | 3.62 | ± | 0.12 | 0.221 |
| T4 (ng/ml) | 21.0 | ± | 2.3 | 25.7 | ± | 4.6 | 0.412 |
| T3/T4 | 0.190 | ± | 0.031 | 0.167 | ± | 0.026 | 0.569 |

1The number of chicks used in each group was n = 7. Values of results are means ± SEM

2NEFA, non-esterified fatty acid; TG, triacylglycerol; LA, lactic acid; CORT, corticosterone; T3, triiodothyronine; T4, thyroxine.

**Table S2** *Effects of L-leucine (L-Leu) in ovo feeding on organ weight and plasma metabolites in 44-d-old male broilers exposed to chronic heat stress1*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Control | | | L-Leu | | | *P*-value |
| Organ or body weight |  |  |  |  |  |  |  |
| Body (g) | 2009 | ± | 55 | 2044 | ± | 33 | 0.611 |
| Liver (g) | 55.4 | ± | 4.7 | 49.4 | ± | 2.6 | 0.318 |
| Liver/ body (%) | 2.74 | ± | 0.21 | 2.43 | ± | 0.16 | 0.284 |
| Heart (g) | 8.49 | ± | 0.27 | 9.13 | ± | 0.34 | 0.160 |
| Heart/ body (%) | 0.32 | ± | 0.011 | 0.31 | ± | 0.003 | 0.734 |
| Plasma metabolites2 |  |  |  |  |  |  |  |
| Glucose (mg/dl) | 263.9 | ± | 9.5 | 261.7 | ± | 8.1 | 0.868 |
| TG (mg/dl) | 47.1 | ± | 7.2 | 54.4 | ± | 8.1 | 0.512 |
| GOT (U/l) | 193.9 | ± | 8.0 | 213.3 | ± | 8.4 | 0.120 |
| UA (mg/dl) | 3.94 | ± | 0.24 | 3.46 | ± | 0.20 | 0.157 |

1The number of chicks used in each group was as follows: Control = 9; L-Leu = 7. Values of results are means ± SEM

2TG, triacylglycerol; GOT, glutamate oxaloacetate transaminase; UA, uric acid.

**Table S3** *Effects of L-leucine (L-Leu) in ovo feeding on meat quality in 44-d-old male broilers exposed to chronic heat stress1*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Control | | | L-Leu | | | *P*-value |
| Shear force value (N) | 14.2 | ± | 0.35 | 13.6 | ± | 1.11 | 0.594 |
| Moisture content (%) | 73.3 | ± | 0.19 | 73.3 | ± | 0.20 | 0.887 |
| Cooking loss (%) | 25.4 | ± | 0.64 | 25.4 | ± | 0.54 | 0.991 |
| Drip loss of centrifuged meat (%) | 20.7 | ± | 1.05 | 21.9 | ± | 1.08 | 0.444 |
| Drip loss of thawed meat (%) | 19.2 | ± | 0.82 | 19.5 | ± | 0.81 | 0.835 |
| pH | 5.54 | ± | 0.05 | 5.54 | ± | 0.04 | 0.934 |
| Lightness (L\*) | 51.4 | ± | 0.96 | 50.2 | ± | 1.6 | 0.521 |
| Redness (a\*) | 1.58 | ± | 0.23 | 1.95 | ± | 0.36 | 0.392 |
| Yellowness (b\*) | 14.5 | ± | 0.43 | 14.1 | ± | 0.23 | 0.485 |

1The number of chicks used in each group was as follows: Control = 9; L-Leu = 7. Values of results are means ± SEM