# Supplementary Materials: Aflatoxin B1 Impairs Bone Mineralization in Broiler Chickens

**Supplementary Table S1.** Effect of increasing dosage of Aflatoxin B1 contaminated diet on microstructure of femoral metaphysis on day 8 [1].

| Bone Region | Parameters[2] | Unit | Treatment Groups[3] | | | | | SEM | P-values[4] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T1 | T2 | T3 | T4 | T5 | | $P_{Model}$ | $P_{Linear}$ | $P_{Quadratic}$ |
| **Total** | BMC | g | 0.016 | 0.019 | 0.018 | 0.018 | 0.020 | 0.001 | 0.5944 | 0.2700 | 0.81380 |
| | BMD | g/mm³ | 0.28 | 0.33 | 0.32 | 0.31 | 0.34 | 0.02 | 0.1163 | 0.0907 | 0.4325 |
| | TV | mm³ | 58.2 | 56.2 | 56.5 | 56.7 | 57.9 | 2.8 | 0.9817 | 0.9934 | 0.5577 |
| | BV | mm³ | 25.8 | 29.9 | 29.0 | 27.4 | 31.3 | 2.5 | 0.5777 | 0.2999 | 0.8889 |
| | BV/TV | - | 44.2 | 52.6 | 51.2 | 47.7 | 53.9 | 2.7 | 0.1104 | 0.1042 | 0.5284 |
| | TS | mm² | 94 | 92 | 94 | 93 | 94 | 3 | 0.9887 | 0.9255 | 0.6830 |
| | BS | mm² | 165 | 155 | 159 | 163 | 153 | 10 | 0.8961 | 0.6146 | 0.9804 |
| | BS/BV | mm⁻¹ | 6.6 | 5.4 | 5.6 | 6.1 | 5.0 | 0.4 | 0.0619 | 0.0484 | 0.6849 |
| | BS/TV | mm⁻¹ | 2.84 | 2.77 | 2.81 | 2.86 | 2.65 | 0.09 | 0.4817 | 0.3110 | 0.4111 |
| **Cortical** | BMC | g | 0.014 | 0.017 | 0.016 | 0.015 | 0.017 | 0.001 | 0.3981 | 0.2452 | 0.6860 |
| | BMD | g/mm³ | 0.506 | 0.544 | 0.531 | 0.530 | 0.538 | 0.013 | 0.3768 | 0.2750 | 0.3784 |
| | TV | mm³ | 26.6 | 30.9 | 30.2 | 28.2 | 32.1 | 2.6 | 0.6387 | 0.3439 | 0.8409 |
| | BV | mm³ | 25.2 | 29.5 | 28.7 | 26.8 | 30.9 | 2.4 | 0.5313 | 0.2820 | 0.8853 |
| | BV/TV | - | 94.8 | 95.8 | 95.1 | 95.3 | 96.5 | 0.7 | 0.5683 | 0.2634 | 0.6404 |
| | TS | mm² | 135.2 | 129.3 | 131.2 | 132.4 | 131.3 | 3.8 | 0.8718 | 0.7110 | 0.5491 |
| | BS | mm² | 154.7 | 147.5 | 152.5 | 153.4 | 147.4 | 8.5 | 0.9614 | 0.7562 | 0.9571 |
| | BS/BV | mm⁻¹ | 6.36 | 5.19 | 5.40 | 5.84 | 4.82 | 0.38 | 0.0850 | 0.0616 | 0.7310 |
| | BS/TV | mm⁻¹ | 6.03 | 4.97 | 5.13 | 5.57 | 4.64 | 0.35 | 0.0954 | 0.0700 | 0.6872 |
| **Trabecular** | BMC | g | 0.0021 | 0.0018 | 0.0017 | 0.0020 | 0.0017 | 0.0001 | 0.3461 | 0.3300 | 0.4285 |
| | BMD | g/mm³ | 0.0736 | 0.0810 | 0.0745 | 0.0770 | 0.0761 | 0.0036 | 0.6877 | 0.9246 | 0.5888 |
| | TV | mm³ | 28.12 | 21.96 | 23.17 | 25.20 | 22.76 | 1.42 | 0.0621 | 0.1334 | 0.1597 |

| Parameter | Unit | T1 | T2 | T3 | T4 | T5 | SEM | $P_{Model}$ | $P_{Linear}$ | $P_{Quadratic}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **BV** | mm³ | 0.222 | 0.181 | 0.144 | 0.244 | 0.128 | 0.048 | 0.4560 | 0.4510 | 0.9460 |
| **BV/TV** | - | 0.786 | 0.847 | 0.623 | 0.927 | 0.509 | 0.181 | 0.5473 | 0.4436 | 0.5545 |
| **TS** | mm² | 63.2 | 53.6 | 55.5 | 58.6 | 54.4 | 2.1 | 0.0373 | 0.0898 | 0.1657 |
| **BS** | mm² | 10.5 | 7.4 | 6.6 | 10.0 | 5.6 | 1.7 | 0.2382 | 0.2152 | 0.8169 |
| **BS/BV** | mm⁻¹ | 50.0 | 45.7 | 46.7 | 44.7 | 48.3 | 3.4 | 0.8270 | 0.6861 | 0.3298 |
| **BS/TV** | mm⁻¹ | 0.379 | 0.346 | 0.287 | 0.385 | 0.227 | 0.061 | 0.3690 | 0.2021 | 0.6971 |

[1] Data represents mean values of six femur bone samples per treatment. SEM represents the pooled standard error of the mean for each parameter across all treatment groups.

[2] Parameters: BMC, bone mineral content; BMD, bone mineral density; TV, tissue volume; BV, bone volume; BV/TV, bone volume/ tissue volume; TS, tissue surface area; BS, bone surface area; BS/BV, bone surface area/ bone volume; BS/ TV, bone surface area/ tissue volume.

[3] Treatment groups: T1 (< 2 ppb AFB1), T2 (75-79 ppb AFB1), T3 (150 ppb AFB1), T4 (230-260 ppb AFB1), T5 (520-560 ppb AFB1).

[4] P-values: $P_{Model}$, p-value for the whole model; $P_{Linear}$, p-value for the linear regression; $P_{Quadratic}$, p-value for the quadratic regression

**Supplementary Table S2.** Effect of increasing dosage of Aflatoxin B1 contaminated diet on tibial bone parameters on day 8 [1].

| Parameters[2] | Treatment Groups[3] | | | | | SEM | P-values[4] | | |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | | P$_{Model}$ | P$_{Linear}$ | P$_{Quadratic}$ |
| FBW (g) | 0.983 | 0.982 | 1.031 | 1.018 | 1.053 | 0.055 | 0.8879 | 0.3466 | 0.96515 |
| DBW (g) | 0.327 | 0.329 | 0.339 | 0.342 | 0.353 | 0.018 | 0.8516 | 0.2704 | 0.8540 |
| FFDW (g) | 0.322 | 0.330 | 0.335 | 0.333 | 0.356 | 0.020 | 0.8419 | 0.3077 | 0.7721 |
| AW (g) | 0.1454 | 0.1525 | 0.1548 | 0.1512 | 0.1656 | 0.0108 | 0.7745 | 0.2750 | 0.8342 |
| Ash % | 44.92 | 46.11 | 46.06 | 45.38 | 46.47 | 0.64 | 0.5247 | 0.2892 | 0.7509 |

[1] Data represents mean values of six tibia bone samples per treatment. SEM represents the pooled standard error of the mean for each parameter across all treatment groups.

[2] Parameters: FBW, fresh bone weight; DBW, dry bone weight; FFDW, fat free dry weight; AW, ash weight.

[3] Treatment groups: T1 (< 2 ppb AFB1), T2 (75-79 ppb AFB1), T3 (150 ppb AFB1), T4 (230-260 ppb AFB1), T5 (520-560 ppb AFB1).

[4] P$_{Model}$, p-value for the whole model; P$_{Linear}$, p-value for the linear regression; P$_{Quadratic}$, p-value for the quadratic regression.

**Supplementary Table S3.** Effect of increasing dosage of Aflatoxin B1 contaminated diet on mRNA levels of calcium and phosphorus transporter genes on day 8 [1].

| Genes[1] | Treatment Groups[3] | | | | | SEM | P-values[4] | | |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | | $P_{Model}$ | $P_{Linear}$ | $P_{Quadratic}$ |
| CALB1 | 1.00 | 1.07 | 1.80 | 1.62 | 0.87 | 0.39 | 0.4116 | 0.8166 | 0.1109 |
| CASR | 1.00 | 0.71 | 0.75 | 0.68 | 0.63 | 0.22 | 0.7679 | 0.2665 | 0.6418 |
| NCX1 | 1.00 | 0.66 | 0.72 | 0.76 | 1.02 | 0.23 | 0.6989 | 0.8495 | 0.1713 |
| NaPi-IIb | 1.00 | 1.25 | 1.20 | 1.53 | 1.10 | 0.21 | 0.4980 | 0.4868 | 0.2370 |
| PMCA1 | 1.00[ab] | 1.40[a] | 0.94[ab] | 0.48[ab] | 0.33[b] | 0.25 | 0.0395 | 0.0086 | 0.2407 |
| VDR | 1.00 | 1.89 | 2.16 | 1.41 | 2.26 | 0.38 | 0.1530 | 0.1044 | 0.4585 |

[a-b] Means within a column not sharing a common letter differ significantly ($P < 0.05$).

[1] Data are presented as mean values of fold change in mRNA expression of calcium and phosphorus transporter genes using $2^{-\Delta\Delta Ct}$ method. Data relates to 6 jejunum samples per treatment.

[2] Genes: CALB1, calbindin 1; CaSR, calcium-sensing receptor; NCX1, sodium-calcium exchanger 1; NaPi-IIB, sodium-phosphate cotransporter; PMCA1b, plasma membrane calcium ATPase 1b; VDR, vitamin D receptor.

[3] Treatment groups: T1 (< 2 ppb AFB1), T2 (75-79 ppb AFB1), T3 (150 ppb AFB1), T4 (230-260 ppb AFB1), T5 (520-560 ppb AFB1).

[4] $P_{Model}$, p-value for the whole model; $P_{Linear}$, p-value for the linear regression; $P_{Quadratic}$, p-value for the quadratic regression.

**Supplementary Table S4.** Effect of increasing dosage of Aflatoxin B1 contaminated diet on mRNA levels of tight junction proteins on day 8 [1].

| Genes[2] | Treatment Groups[3] | | | | | SEM | P-values[4] | | |
|---|---|---|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | | $P_{Model}$ | $P_{Linear}$ | $P_{Quadratic}$ |
| MUC2 | 1.00 | 0.91 | 1.14 | 0.80 | 1.28 | 0.20 | 0.6294 | 0.5558 | 0.5192 |
| CLDN1 | 1.00 | 0.48 | 1.21 | 0.57 | 0.95 | 0.26 | 0.3939 | 0.9944 | 0.6982 |
| ZO1 | 1.00 | 0.72 | 0.85 | 0.57 | 0.75 | 0.12 | 0.1842 | 0.1092 | 0.284 |
| OCLN | 1.00 | 1.00 | 1.09 | 1.06 | 0.99 | 0.11 | 0.9715 | 0.9103 | 0.5989 |

[1] Data are presented as mean values of fold change in mRNA expression of tight junction related genes using $2^{-\Delta\Delta Ct}$ method. Data relates to 6 jejunum samples per treatment.

[2] Genes: MUC2, mucin 2; CLDN1, claudin 1; ZO1, zonula occludens; and OCLN, occluding.

[3] Treatment groups: T1 (< 2 ppb AFB1), T2 (75-79 ppb AFB1), T3 (150 ppb AFB1), T4 (230-260 ppb AFB1), T5 (520-560 ppb AFB1).

[4] $P_{Model}$, p-value for the whole model; $P_{Linear}$, p-value for the linear regression; $P_{Quadratic}$, p-value for the quadratic regression.