

Supplementary Materials: Aflatoxin B₁ Exposure in Sheep: Insights into Hepatotoxicity Based on Oxidative Stress, Inflammatory Injury, Apoptosis, and Gut Microbiota Analysis

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Table S1. Primer sequences for qPCR analysis.

Gene	Gene ID	Sequence (5'-3')	Fragment length (bp)
β-actin	NM_001009784.3	F:GCTCTCTTCCAGCCTTCCTT R:AGGTCTTTGCGGATGTCG	101
TNF-α	XM_012100437.3	F:CCCAACTCCCTCTGTTTATGTT R:GGACACCTTGACCTCCTGAATA	102
IL-1β	NM_001009465.2	F:TCTCCCTAAAGAAGCCATAC R:AGCGTCTCAGCACGAATA	151
IL-6	NM_001009392.1	F:ACGAGTGGGTAAAGAACGCAAAGG R:GCCGAGCTACTTCATCCGAATAG	99
IL-10	NM_001009327.1	F:GGTGATGCCACAGGCTGAGAAC R:GCTCCACCGCCTTGCTCTTG	143
SOD-1	NM_001145185.2	F:TTCTGCGGCGTTTCCTTGTCTG R:CGAAGCGGATGGTGCCTTGC	138
SOD-2	NM_001280703.1	F:GGCAGCGTCCACCATGTTGTC R:GCGTTGATGTGCGGCTCCAG	144
Caspase-3	XM_027962551.1	F:CCCCTGTCAGCAACATTAATCC R:CCGGAGTCCACTGATTTGCT	150
Bax	XM_027978594.1	F:CGCATTGGAGATGAATTGGACAGTAAC R:CAGTTGAAGTTGCCGTCGGAAAAC	128
Bcl-2	XM_027960877.1	F:TGTGGATGACCGAGTACCTGAACC R:AGAGACAGCCAGGAGAAATCAAACAG	127

Note: F, Forward primer; R, Reverse primer. β-actin, reference gene.

Table S2. Effects of AFB₁ exposure on body temperature, breathing, and heart rate of sheep.

Factors	Item	Pro-test	Post-test	D-value
Body temperature	Control group	39.25±0.39 ^a	39.20±0.55 ^a	-0.05±0.29
	AFB ₁ group	38.88±0.50 ^a	39.53±0.78 ^a	0.65±0.23
	<i>P</i> -value	0.280	0.519	0.043
Breathing	Control group	17.75±2.99 ^a	18.25±2.98 ^a	0.5±2.52
	AFB ₁ group	13.25±4.65 ^a	12.75±4.03 ^a	-0.5±1.0
	<i>P</i> -value	0.280	0.071	0.488
Heart rate	Control group	56.75±5.56 ^a	73.50±12.79 ^a	16.75±13.25
	AFB ₁ group	67.25±11.30 ^a	57.50±8.70 ^a	-9.75±11.45
	<i>P</i> -value	0.146	0.084	0.022

Note: Mean values with same superscripts in a row have no significant ($P > 0.05$), compared pro-test to post-test ($n = 6/\text{group}$); D-value: The differences between post-intervention and pre-intervention.

Table S3. Effects of AFB1 exposure on serum biochemistry of sheep.

Item	Control group	AFB1 group	P-value
TP (g/L)	69.03±5.87	63.17±9.10	0.214
ALB (g/L)	24.72±2.88	21.18±3.66	0.093
GLO (g/L)	44.30±4.53	41.97±7.07	0.512
A/G	0.56±0.08	0.51±0.01	0.397
TBIL (umol/L)	1.6±0.3 ^a	2.85±0.47 ^b	0.002
AST (U/L)	126.83±22.38	152±37.67	0.190
ALT (U/L)	14.83±10.80	20.33±6.28	0.582
AMY (U/L)	15.83±10.21	12.75±10.90	0.660
TG (mmol/L)	0.44±0.15	1.32±1.47	0.470
CRE (umol/L)	57.20±7.36	63.20±11.45	0.306
BUN (mmol/L)	8.37±2.78	9.57±4.64	0.599
BUN/CRE	149.97±55.98	152.77±65.03	0.938
Ca (mmol/L)	2.10±0.063 ^a	1.96±0.083 ^b	0.006
P (mmol/L)	2.56±0.31	2.14±0.52	0.119
GLU (mmol/L)	4.67±3.18	3.30±2.05	0.513
CK (U/L)	262±262.46	512.83±648.79	0.411

Note: ^{a,b}Mean values with different superscripts in a row differ significantly ($P < 0.05$, $n = 6/\text{group}$).

Conjunctival color

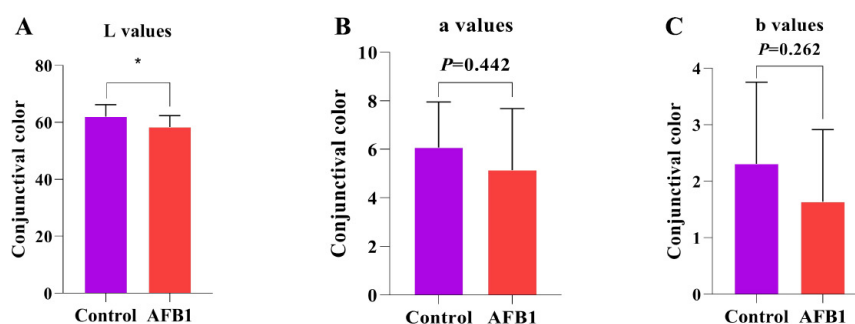


Figure S1. Effects of AFB1 exposure on conjunctival color. (A–C) Conjunctival color [lightness (L values), redness (a values), yellowness (b values)]. Data represent means±s.d., Student's t-test. Statistics were considered significant at $P < 0.05$. * $P < 0.05$.