Supplementary Materials: Biological System Responses of Dairy Cows to Aflatoxin B1 Exposure Revealed with Metabolomic Changes in Multiple Biofluids

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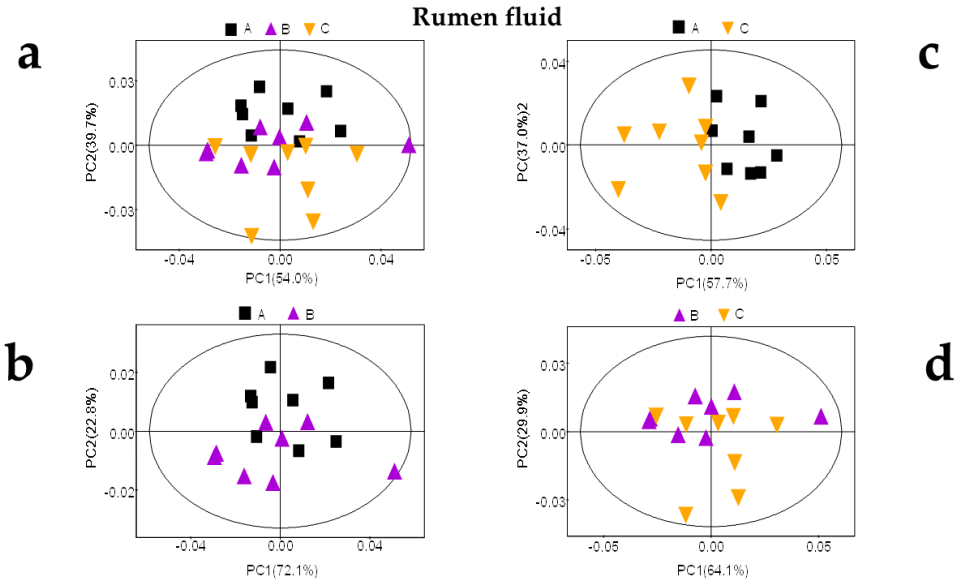

Figure S1. PCA analysis based on 1H NMR spectra of *rumen fluid* obtained from different groups. (**a**) PCA profiles among A, B and C; (**b**) PCA profiles between A and B; (**c**) PCA profiles between A and C; (**d**) PCA profiles between B and C. Each point on the score chart represents a sample. Control (A): black squares; AFB20 group (B): purple triangles; AFB40 group (C): yellow triangles.

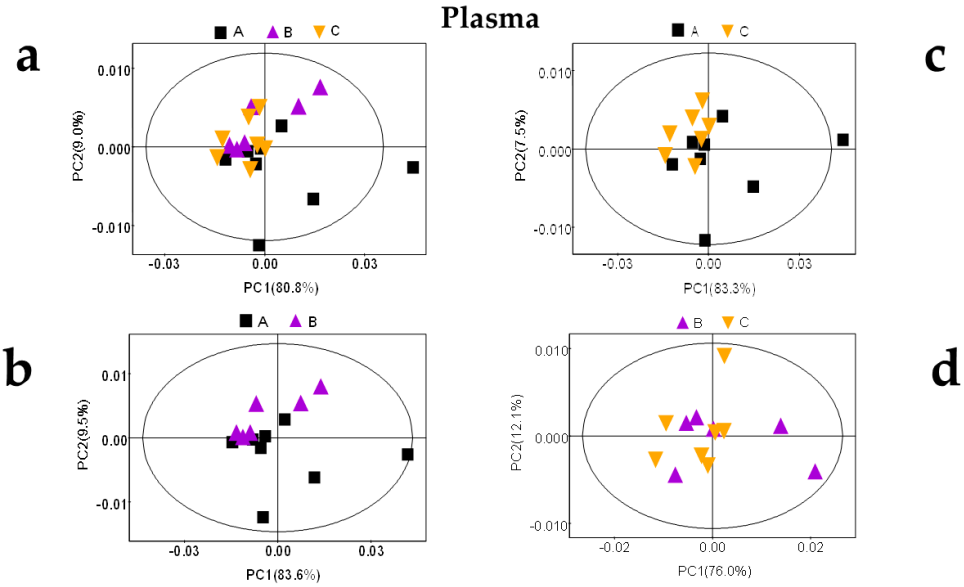

Figure S2. PCA analysis based on 1H NMR spectra of *plasma* obtained from different groups. (**a**) PCA profiles among A, B and C; (**b**) PCA profiles between A and B; (**c**) PCA profiles between A and C; (**d**) PCA profiles

between B and C. Each point on the score chart represents a sample. Control (A): black squares; AFB20 group (B): purple triangles; AFB40 group (C): yellow triangles.

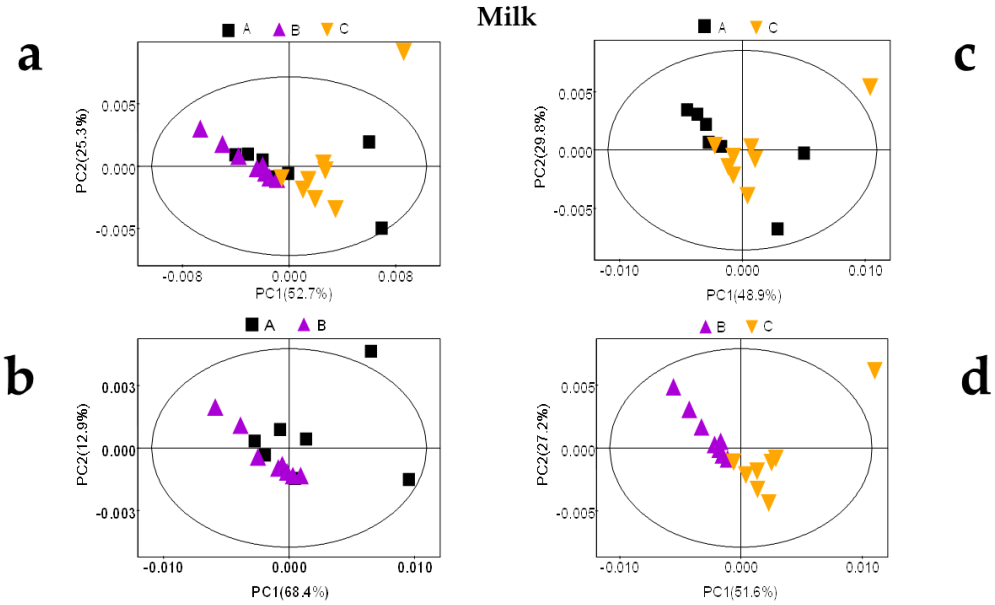

Figure S3. PCA analysis based on 1H NMR spectra of *milk* obtained from different groups. (**a**) PCA profiles among A, B and C; (**b**) PCA profiles between A and B; (**c**) PCA profiles between A and C; (**d**) PCA profiles between B and C. Each point of the score chart represents a sample. Control (A): black squares; AFB20 group (B): purple triangles; AFB40 group (C): yellow triangles.

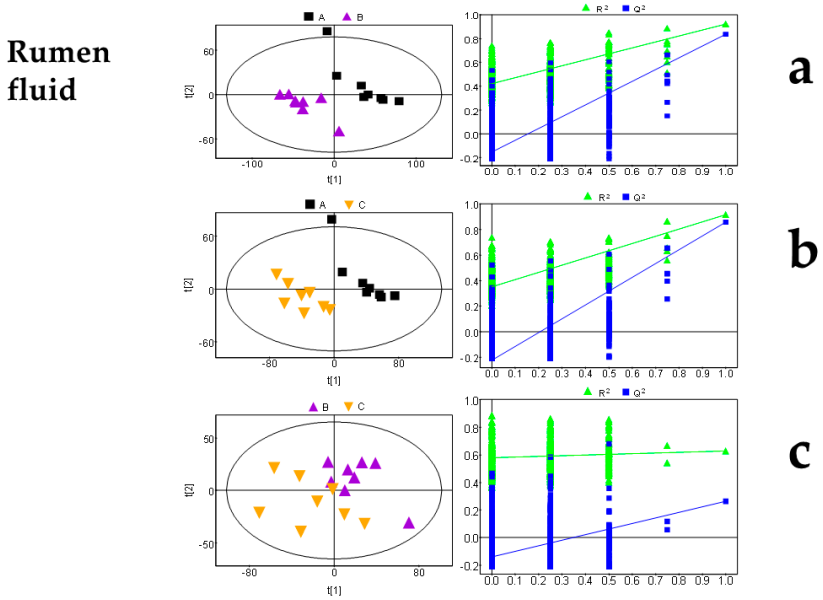

Figure S4. PLS-DA scores plots based on 1H NMR spectra of *rumen fluid* obtained from different groups. (**a**) PLS-DA profiles between A and B [R^2X = 72.9%, R^2Y = 0.923, Q^2 = 0.836; intercept: R^2 = (0.0, 0.43), Q^2 = (0.0, −0.015)]; (**b**) PLS-DA profiles between A and C [R^2X = 71%, R^2Y = 0.916, Q^2 = 0.858; intercept: R^2 = (0.0, 0.359), Q^2 = (0.0, −0.235)]; (**c**) PLS-DA profiles between B and C [R^2X = 57.9%, R^2Y = 0.627, Q^2 = 0.262; intercept: R^2 = (0.0, 0.591), Q^2 = (0.0, −0.134)]. Each point on the score chart represents a sample. Control (A): black squares; AFB20 group (B): purple triangles; AFB40 group (C): yellow triangles.

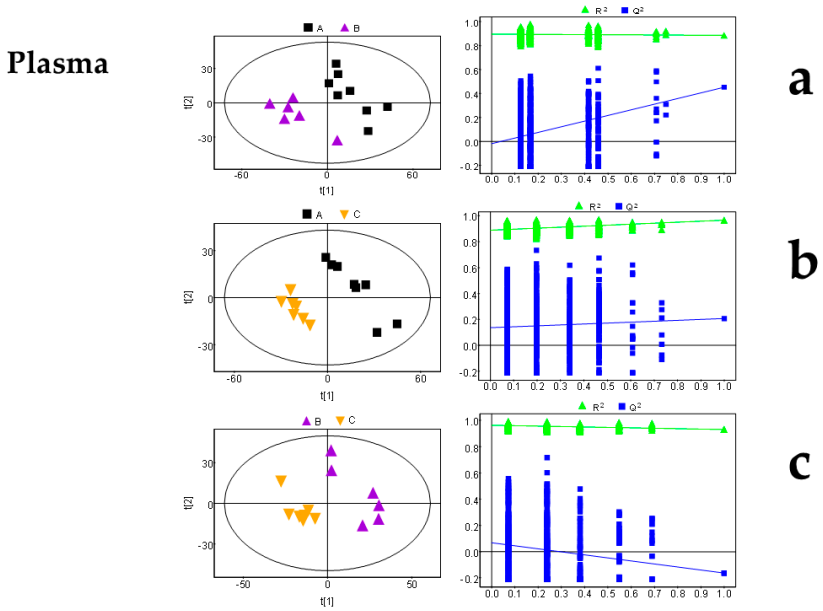

Figure S5. PLS-DA scores plots based on 1H NMR spectra of *plasma* obtained from different groups. (**a**) PLS-DA profiles between A and B [R^2X = 33%, R^2Y = 0.886, Q^2 = 0.452; intercept: R^2 = (0.0, 0.894), Q^2 = (0.0, −0.0377)]; (**b**) PLS-DA profiles between A and C [R^2X = 24.5%, R^2Y = 0.965, Q^2 = 0.205; intercept: R^2 = (0.0, 0.895), Q2 = (0.0, 0.17)]; (**c**) PLS-DA profiles between B and C [R^2X = 23%, R^2Y = 0.931, Q^2 = 0.164; intercept: R^2 = (0.0, 0.962), Q^2 = (0.0, 0.0559)]. Each point on the score chart represents a sample. Control (A): black squares; AFB20 group (B): purple triangles; AFB40 group (C): yellow triangles.

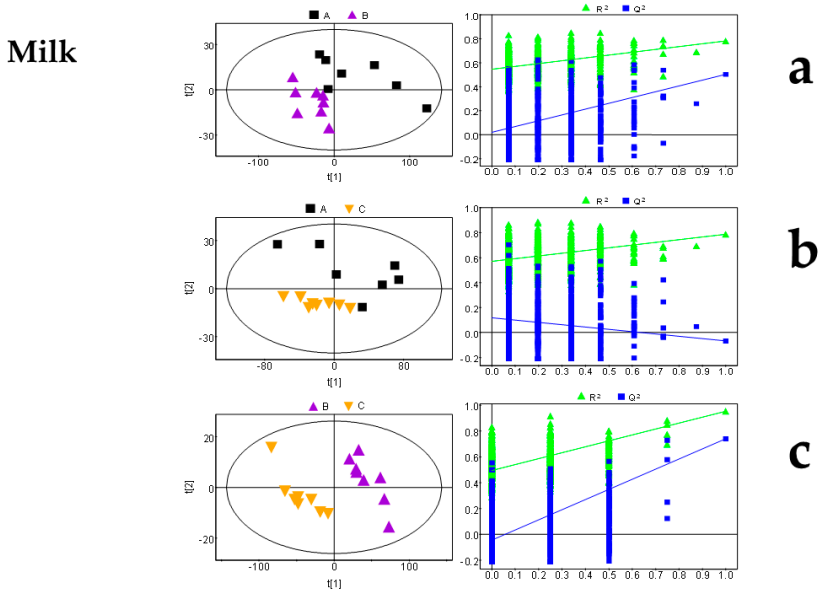

Figure S6. PLS-DA scores plots based on 1H NMR spectra of *milk* obtained from different groups. (**a**) PLS-DA profiles between A and B [R^2X = 70%, R^2Y = 0.781, Q^2 = 0.503; intercept: R^2 = (0.0, 0.553), Q^2 = (0.0, 0.0165)]; (**b**) PLS-DA profiles between A and C [R^2X = 62.5%, R^2Y = 0.787, Q^2 = (0.0, −0.0666); intercept: R^2 = (0.0, 0.587), Q2 = (0.0, 0.16)]; (**c**) PLS-DA profiles between B and C [R^2X = 65.4%, R^2Y = 0.952, Q^2 = 0. 0.738; intercept: R^2 = (0.0, 0.506), Q^2 = (0.0, −0.0519)]. Each point on the score chart represents a sample. Control (A): black squares; AFB20 group (B): purple triangles; AFB40 group (C): yellow triangles.

Table S1. Ingredients of the total mixed ration [1].

Item	Amount (%)
Oat hay	2.24
Corn silage	45.57
Alfalfa hay	8.07
Soybean meal	4.86
DDGS[2]	5.58
Corn meal	11.88
Wheat bran	2.24
Extruded soy flour	0.56
Corn bran shotcrete	4.59
Cottonseed	4.48
Mineral meal	0.22
Diamond XP	0.13
$NaHCO_3$	0.54
NaCl	0.07
Premix	1.12
Water	7.84
Nutrients, % of DM[3]	
Crude protein	17.65
Fat	4.15
Nonfiber carbohydrate	39.5
Neutral detergent fiber	29.49
Calcium	0.8
Phosphorus	0.48
Ash	9.27
Energy (mcal/Kg)	
Metabolic energy	2.88
Net Energy	1.67

Notes: [1] Control group (AFB1 null); AFB20 group (20μg/kg in the total mixed ration); and AFB40 group (40μg/kg in the total mixed ration). [2] Dried distillers' grains with soluble. [3] Dry matter.

Table S2. Rumen fluid metabolites that differed significantly between groups and their correlation coefficients.

Metabolites [b]	r [a]		
	A vs. B	A vs. C	B vs. C
Val (valine): 0.98 (d), 1.02 (d)	0.870	0.897	-
Leu (leucine): 0.96 (d), 0.97 (d)	0.888	0.911	-
Ile (isoleucine): 0.94 (t); 1.00 (d)	0.808	0.957	-
Butyrate: 0.90 (t), 1.56 (m)	-	−0.681	−0.792
Ethanol: 1.18 (t)	-	0.748	0.743
Lac (lactate): 1.32 (d); 4.11 (q)	0.707	0.679	0.785
Ala (alanine): 1.48 (d)	0.862	0.893	-
TMA (trimethylamine): 2.88 (s)	0.673	0.733	-
Raffinose: 5.41 (d), 3.56 (m), 3.67 (d), 3.76 (m), 3.82 (m), 3.89 (m), 3.94 (t), 4.21 (d)	−0.843	−0.794	-
Succ (succinate): 2.41 (s)	0.800	0.669	0.704
MMA (methylamine): 2.61 (s)	−0.802	−0.842	-
Choline: 3.20 (s)	0.850	0.847	0.794
Scy (scyllitol): 3.36 (s)	0.902	0.817	0.702
Gly (glycine): 3.56 (s)	0.925	0.878	0.870
α-KG (α-ketoglutarate): 2.49 (t), 3.02 (t)	0.904	0.936	-
Uracil: 5.80 (d), 7.54 (d)	0.822	0.869	-
Tyr (Tyrosine): 6.91 (d), 7.18 (d)	0.864	0.920	0.717
Phe (phenylalanine): 7.32 (d), 7.37 (t), 7.42 (dd)	0.843	0.908	0.839
Hypoxanthine: 8.22 (s), 8.20 (s)	0.885	0.850	-

[a] Correlation coefficients: positive and negative signs indicate positive and negative correlations between concentrations, respectively. Correlation coefficient $|r| > 0.666$ was used as the cutoff value for statistical significance based on the discrimination significance at the level of $p = 0.05$ and df (degree of freedom) = 7. ''-'' means the correlation coefficient $|r| < 0.666$. [b] Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet; br, broad resonance.

Table S3. Plasma metabolites that differed significantly between groups and their correlation coefficients.

Metabolites [b]	r [a]		
	A vs. B	A vs. C	B vs. C
Val (Valine): 0.98 (d), 1.04 (d)	−0.859	-	0.775
Leu (Leucine): 0.96 (d), 0.97 (d)	−0.848	-	0.881
Ile (Isoleucine): 0.94 (t); 1.00 (d)	−0.873	-	0.786
Ace (Acetate): 1.91 (s)	−0.778	-	-
Choline: 3.19 (s)	0.786	-	-
PC/GPC: 3.22 (s)	0.759	0.734	-
Gly (Glycine): 3.56 (s)	0.773	-	−0.776
Phe (Phenylalanine): 7.32 (d), 7.37 (t), 7.42 (dd)	-	−0.708	-
L1: Lipid, $C\underline{H}_3$-$(CH_2)_n$-(LDL): 0.86 (br)	-	0.708	-
L3: Lipid, CH_3-$(C\underline{H}_2)_n$- (LDL): 1.27 (br)	-	0.710	-
L6: Lipid, -$C\underline{H}_2$-CH = CH-: 2.01 (br)	-	0.726	-
L9: Lipid, -CH = CH-: 5.27 (br)	-	0.732	-

[a] Correlation coefficients: positive and negative signs indicate positive and negative correlations in the concentrations, respectively. Correlation coefficient $|r| > 0.666$, 0.707 or 0.755 was used as the cutoff value for statistical significance based on the discrimination significance at the level of $p = 0.05$ and df (degree of freedom) = 7, 6, or 5, respectively. ''-'' means correlation coefficient $|r| < 0.666$, 0.707, or 0.755, respectively. [b] Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet; br, broad resonance.

Table S4. Milk metabolites that differed significantly between groups and their correlation coefficients.

Metabolites [b]	r [a]		
	A vs. B	**A vs. C**	**A vs. B**
OAG (O-acetyl-glycoprotein): 2.05 (s)	-	0.779	-
Cr (Creatine): 3.03 (s), 3.93 (s)	-	-	0.691
Orotate: 6.20 (s)	-	0.669	-
Phe (Phenylalanine): 7.27 (d), 7.30 (t), 7.38 (dd)	-	−0.758	-
L1: Lipid, CH3-(CH2)n-(LDL): 0.86 (br)	-	−0.741	0.875
L2: Lipid, CH3-(CH2)n-(VLDL): 0.88 (br)	-	−0.766	0.895
L5: Lipid, -CH2-CH2-C = O (VLDL): 1.58 (br)	-	−0.689	0.902
L6: Lipid, -CH2-CH = CH-: 2.01 (br)	-	−0.719	0.891
L7: Lipid, -CH2-C = O: 2.23 (br)	-	−0.694	0.912

[a] Correlation coefficients: positive and negative signs indicate positive and negative correlations in the concentrations, respectively. Correlation coefficient $|r| > 0.602$ was used as the cutoff value for the statistical significance based on the discrimination significance at the level of $p = 0.05$ and *df* (degree of freedom) = 7. ''-'' means correlation coefficient $|r| < 0.666$. [b] Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet; br, broad resonance.