Lactobacillus_rhamnosus GG reverses zearalenone-induced pathological macrophage polarization to restore placental angiogenesis and efficiency

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Supplemental Table S1 Ingredients and nutritional levels of diet for pregnant sows

Ingredients	Ration, %	Nutritional levels	Content
Corn	63.15	Crude protein, %	14.58
Soybean meal	14.5	Digestible energy, Kcal/kg	3063
Wheat bran	18	Standard ileal digestible lysine, %	0.60
Fish meal	1	Calcium, %	0.81
Methionine	0.02	Total phosphorus, %	0.69
Threonine	0.01	Standardized total tract digestible phosphorus, %	0.41
Limestone	1.01		
Dicalcium phosphate	1.21		
Sodium chloride	0.45		
Choline Chloride	0.15		
*Premix	0.50		
Total	100	□	□

*Each kilogram of feed contains: Vitamin A, 8 000IU; Vitamin D3, 600IU; Vitamin E, 60IU; Vitamin K, 4mg; Vitamin B1, 5mg; Vitamin B2, 8mg; Vitamin B6, 7.5mg; Vitamin B12, 0.02mg; D-biotin, 0.2mg; D-pantothenic acid, 20mg; Folic acid, 1.5mg; Niacinamide, 30mg; Copper, 16mg; Iron, 165mg; Iodine, 0.3mg; Zinc, 165mg; Manganese, 30mg; Selenium, 0.3mg.

Supplemental table S2 AIN-93M diet formulated for maintenance of adult rodents

Ingredient	g/kg diet
Cornstarch	465.692
Casein (>85% protein)	140
Dextrinized cornstarch (90-94% tetrasaccharides)[1]	155
Sucrose	100
Soybean oil (no additives)	40
Fiber[2]	50
Mineral mix (AIN-93M-MX)	35
Vitamin mix (AIN-93-VX)	10
L-Cystine	1.8
Choline bitartrate (41.1% choline)[3]	2.5
Tert-butylhydroquinone	0.008

Supplemental table S3 Estimated minimal nutrient composition of AIN-93M rodent diets

Nutrient	AIN-93M, /kg diet
Total energy, kcal	3601
% as protein	14.1
% as CHO	75.9
% as fat	10
Moisture, g	68
Total fat,g	40
Saturated, g	6.2
Monounsaturated, g	9.3
Polyunsaturated, g	23.1
Linoleic acid, g	10.4
Linolenic acid, g	2.7
Total carbohydrate, g	727.3
Complex carbohydrates, g	421.9
Simple sugars, g	257.9
Cellulose, g	47.5
Total protein, g	125.8
Amino acids (typical analysis)	
Alanine, g	3.3
Arginine, g	4.5

Aspartic acid, g	8
Cystine, g	2.4
Glutamic acid, g	25.5
Glycine, g	2.3
Histidine, g	3.3
Isoleucine, g	5.9
Leucine, g	10.9
Lysine, g	9.2
Methionine, g	3.3
Phenylalanine, g	6.2
Proline, g	14.3
Serine, g	6.7
Threonine, g	4.7
Tryptophan, g	1.6
Tyrosine, g	6.6
Valine, g	7
Total ash, g	38.9
Minerals	
Calcium, mg	5000
Phosphorus, mg	3000
Magnesium, mg	511
Sodium, mg	1033

Potassium, mg	3600
Chloride, mg	1613
Sulfur (inorganic), mg	300
Iron, mg	45
Zinc, mg	35
Manganese, mg	10
Copper, mg	6
Iodine, mg	0.2
Molybdenum, mg	0.15
Selenium, mg	0.17
Silicon, mg	5
Chromium, mg	1
Fluoride, mg	1
Nickel, mg	0.5
Boron, mg	0.5
Lithium, mg	0.1
Vanadium, mg	0.1
Vitamin	
Nicotinic acid, mg	30
Ca pantothenate, mg	15
Pyridoxine, mg	6
Thiamin, mg	5

Riboflavin, mg	6
Folie acid, mg	2
Biotin, mg	0.2
Vitamin B-12, μg	25
Vitamin K, μg	860
Vitamin E, IU	75
Vitamin A, IU	4000
Vitamin D, IU	1000
Choline, mg	1000

Supplemental table S4 Primer sequences for the target and reference genes

Gene	GenBank No.	Sequence (5'-3')	Length
Rat			
iNOS	NM_012611.3	F: CCAAGGTGACCTGAAAGAGGAA	22
		R: ATCCTGTGTTGTTGGGCTGG	20
Arg-1	NM_017134.3	F: GGTGGAGACCACAGTATGGC	20
		R: GCAGATTCCCAGAGCTGGTT	20
Mrc-1	NM_001106123.2	F: TCTCCCTCAATGGAACACACAC	22
		R: AAATTGCCGTGAGTCCAAGAGT	22
CD86	NM_020081.2	F: AAGACATGTGTAACCTGCACCA	22
		R: AAGCTTGCCTCTTCACAGGA	20
β-Actin	NM_031144.3	F: ACAACCTTCTTGCAGCTCCTC	21
		R: CTGACCCATACCCACCATCAC	21
GAPDH	NM_017008.4	F: TCTCTGCTCCTCCCTGTTCT	20
		R: CGATACGGCCAAATCCGTTC	20
Pig			
iNOS	NM_001143690.1	F: AGCCCAGAGGGCTTTATCAC	20
		R: GGTGGGAGCACATCTAGCTT	20
Arg-1	NM_214048.2	F: TTTCTCCAAGGGTCAGCCAC	20
		R: AGGGACATCAGCAAAGCACA	20
GADPH	NM_001206359.1	F: CCAAGGAGTAAGAGCCCCTG	20
		R: AAGTCAGGAGATGCTCGGTG	20

β-actin	XM_003124280.4	F: CTCCAGAGCGCAAGTACTCC	20
		R: AATGCAACTAACAGTCCGCC	20

Abbreviations: iNOS, nitric oxide synthase 2; Arg-1, arginase 1; CD86, CD86 molecule; MRC-1, mannose receptor C-type 1.

Supplemental table S5 The table of RNA-Seq data statistics

Samples	Clean reads	Clean bases	GC Content	≥Q30%
CONA1	30,039,724	8,991,233,740	49.45%	95.27%
CONA2	21,803,861	6,528,555,488	49.28%	95.22%
CONA3	20,325,689	6,084,468,594	49.85%	95.22%
LGGB1	27,674,602	8,283,567,640	49.65%	94.97%
LGGB2	25,300,177	7,572,464,272	49.73%	94.87%
LGGB3	28,038,660	8,394,408,484	49.82%	95.12%
Z1C1	20,843,461	6,240,087,442	49.70%	95.62%
Z1C2	20,506,416	6,138,710,262	49.66%	95.47%
Z1C3	29,865,321	8,938,388,192	49.42%	95.08%
Z1LE1	28,474,544	8,524,387,496	49.72%	95.14%
Z1LE2	21,869,974	6,547,998,628	49.53%	95.34%
Z1LE3	25,608,016	7,668,075,754	49.77%	95.40%
Z2D1	21,770,589	6,517,328,478	49.57%	95.45%
Z2D2	21,725,665	6,500,975,158	49.67%	95.25%
Z2D3	29,261,223	8,758,763,260	49.25%	94.95%
Z2LF1	29,501,810	8,829,708,756	49.83%	95.22%
Z2LF2	21,357,973	6,393,895,912	49.50%	94.88%
Z2LF3	28,876,621	8,648,176,720	49.62%	95.25%

Note:

(1) Samples: sample analysis ID.

(2) Clean reads: Total number of pair-end reads in the Clean Data.

(3) Clean bases: Total number of bases in the Clean Data.

(4) GC content: The GC content of the Clean Data, i.e., the percentage of G and C bases among the total bases in the Clean Data.

(5) ≥Q30%: The percentage of bases with a quality score ≥ 30 in the Clean Data.

Supplemental table S6 Statistical table of processing results of sample sequencing data

Sample ID	Raw CCS	Clean CCS	Effective CCS	AvgLen (bp)	Effective (%)
CON1	59,963	59,825	49,555	1,455	82.64
CON10	63,071	62,937	49,766	1,459	78.9
CON2	56,142	56,013	45,432	1,456	80.92
CON3	60,356	60,259	46,566	1,458	77.15
CON4	67,744	67,581	56,448	1,457	83.33
CON5	66,476	66,322	53,156	1,460	79.96
CON6	67,969	67,815	55,515	1,459	81.68
CON7	59,149	58,985	50,133	1,456	84.76
CON8	54,332	54,237	44,256	1,454	81.45
CON9	56,436	56,289	46,885	1,454	83.08
ZEN1	66,095	65,982	55,670	1,453	84.23
ZEN10	59,773	59,611	48,588	1,455	81.29
ZEN2	56,239	56,047	46,458	1,458	82.61
ZEN3	60,666	60,481	49,865	1,460	82.2
ZEN4	66,281	66,146	53,176	1,460	80.23
ZEN5	61,327	61,182	49,236	1,456	80.28
ZEN6	64,949	64,836	54,135	1,456	83.35
ZEN7	60,498	60,350	50,550	1,458	83.56

ZEN8	68,932	68,823	55,891	1,457	81.08
ZEN9	64,621	64,465	54,085	1,457	83.7
ZENLGG1	68,423	68,254	55,955	1,455	81.78
ZENLGG10	65,084	64,968	53,950	1,455	82.89
ZENLGG2	54,768	54,626	44,455	1,455	81.17
ZENLGG3	68,177	67,986	57,100	1,455	83.75
ZENLGG4	61,702	61,430	51,964	1,455	84.22
ZENLGG5	54,058	53,906	46,617	1,455	86.24
ZENLGG6	62,358	62,188	50,909	1,455	81.64
ZENLGG7	56,856	56,652	48,550	1,453	85.39
ZENLGG8	40,106	39,936	36,710	1,454	91.53
ZENLGG9	56,152	55,990	43,994	1,457	78.35

Note:

Sample ID: Sample name.

Raw-CCS: Number of Circular Consensus Sequence reads identified for the sample.

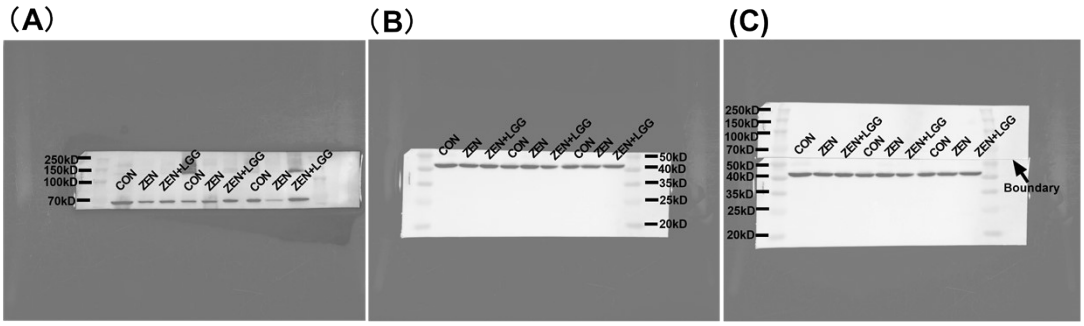
Clean CCS: Number of sequences after identification and primer removal.

Effective-CCS: Number of sequences used for subsequent analysis after length filtering and chimera removal.

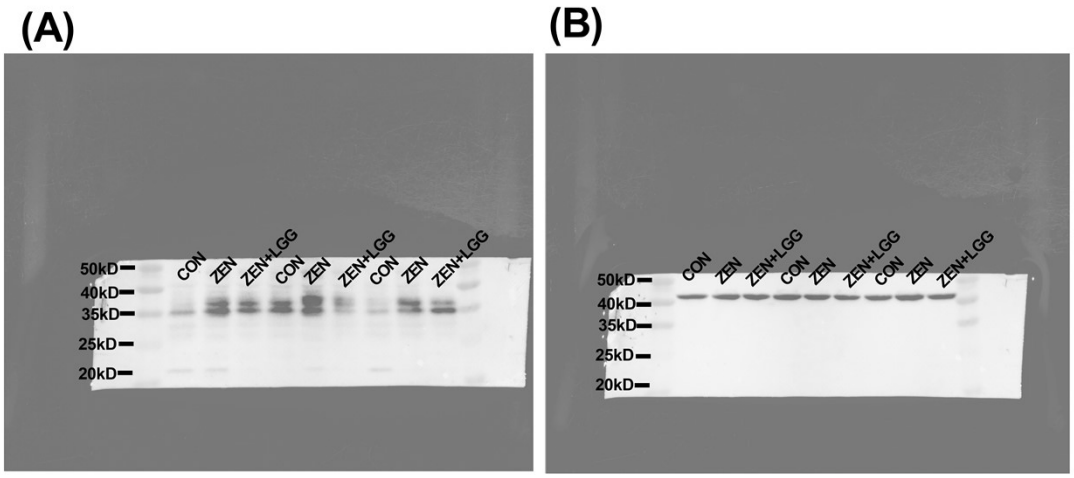
AvgLen (bp): Average sequence length of the sample.

Effective (%): Percentage of Effective-CCS relative to Raw-CCS.

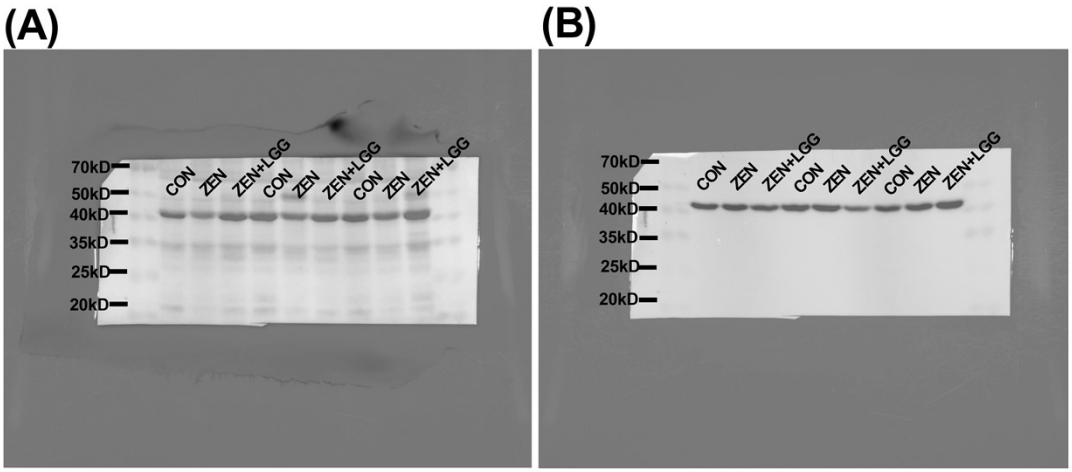
Below are the original data of the Western blot in the manuscript.



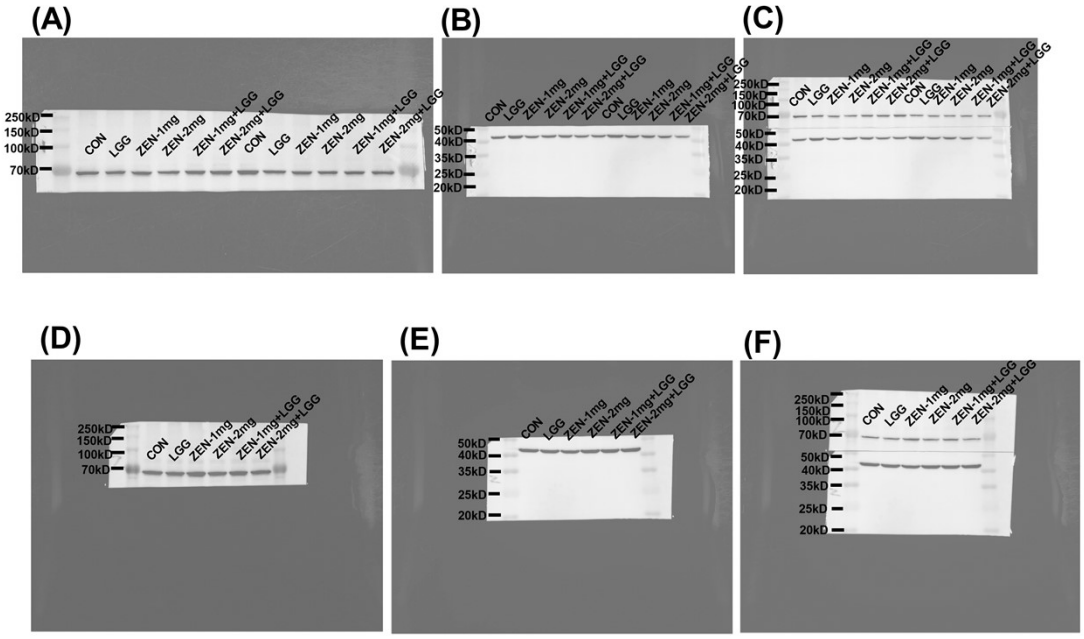
The raw data of iNOS protein expression in the placenta from Figure 2. (A) iNOS protein expression; (B) β-actin protein expression; (C) Co-expression of iNOS and β-actin on the same membrane. Abbreviations: CON, the control group; ZEN, the zearalenone group; ZEN+LGG, the zearalenone plus *Lactobacillus_rhamnosus* GG group.



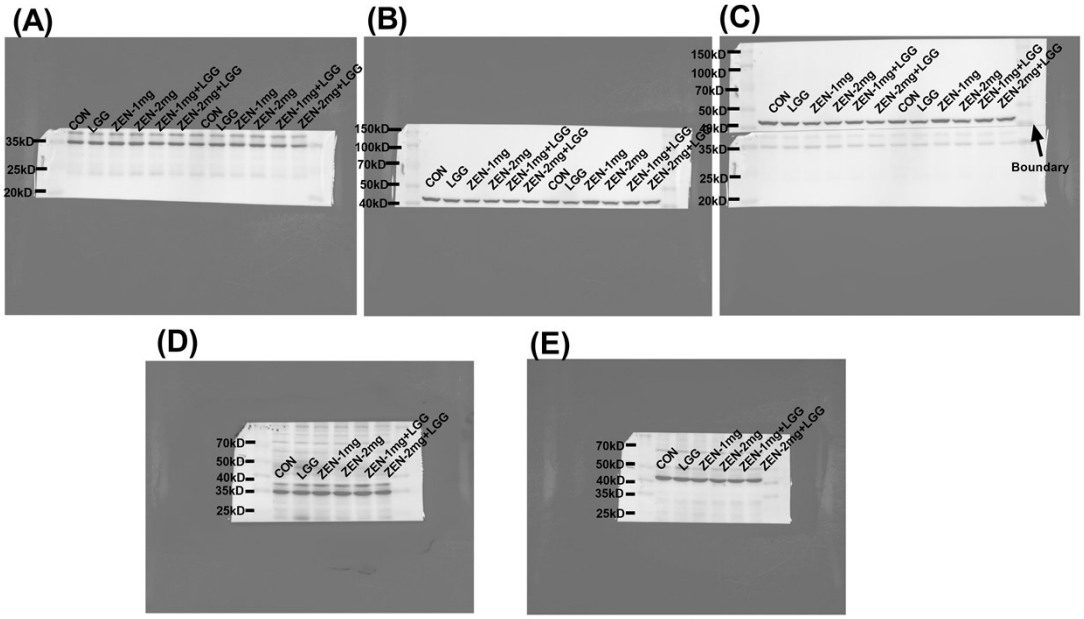
The raw data of Arg-1 protein expression in the placenta from Figure 2. (A) Arg-1 protein expression; (B) β-actin protein expression. Arg-1 and β-actin are expressed on the same membrane. Abbreviations: CON, the control group; ZEN, the zearalenone group; ZEN+LGG, the zearalenone plus *Lactobacillus_rhamnosus* GG group.



The raw data of VEGF protein expression in the placenta from Figure 2. (A) VEGF protein expression; (B) β-actin protein expression. VEGF and β-actin are expressed on the same membrane. Abbreviations: CON, the control group; ZEN, the zearalenone group; ZEN+LGG, the zearalenone plus *Lactobacillus_rhamnosus* GG group.



The raw data of iNOS protein expression in the liver from Figures 5. (A) iNOS protein expression; (B) β-actin protein expression; (C) Co-expression of iNOS and β-actin on the same membrane. (D) iNOS protein expression; (E) β-actin protein expression; (F) Co-expression of iNOS and β-actin on the same membrane. Abbreviations: CON, the control group; LGG, the *Lactobacillus_rhamnosus* GG group; ZEN-1mg, zearalenone at 1 mg/kg body weight; ZEN-2mg, zearalenone at 2 mg/kg body weight; ZEN-1mg+LGG, zearalenone at 1 mg/kg body weight plus *Lactobacillus_rhamnosus* GG; ZEN-2mg+LGG, zearalenone at 1 mg/kg body weight plus *Lactobacillus_rhamnosus* GG.



The raw data of Arg-1 protein expression in the liver from Figures 5. (A) Arg-1 protein expression; (B) β-actin protein expression; (C) Co-expression of Arg-1 and β-actin on the same membrane. (D) Arg-1 protein expression; (E) β-actin protein expression. Abbreviations: CON, the control group; LGG, the *Lactobacillus_rhamnosus* GG group; ZEN-1mg, zearalenone at 1 mg/kg body weight; ZEN-2mg, zearalenone at 2 mg/kg body weight; ZEN-1mg+LGG, zearalenone at 1 mg/kg body weight plus *Lactobacillus_rhamnosus* GG; ZEN-2mg+LGG, zearalenone at 1 mg/kg body weight plus *Lactobacillus_rhamnosus* GG.