

Supplementary Information (SI)

Oral delivery of electrodynamically encapsulated *Lactiplantibacillus plantarum* CRD7 modulates gut health, antioxidant activity, and cytokines related inflammation and immunity in mice

Vinay Venkatesh Varada¹, Sachin Kumar^{1*}, Sravani Balaga¹, Antony Johnson Thanippilly¹, Heartwin A. Pushpadass², Rashmi, H. M³, Babu Lal Jangir³, Nitin Tyagi¹, Ashish Kumar Samanta¹

¹Rumen Biotechnology Lab., Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India

²Dairy Engineering Section, ICAR-National Dairy Research Institute, Southern Regional Station, Bengaluru, India

³Department of Veterinary Pathology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004, India

*Corresponding author: email: arensachin@gmail.com (Sachin Kumar); Phone number: +91-1842259069

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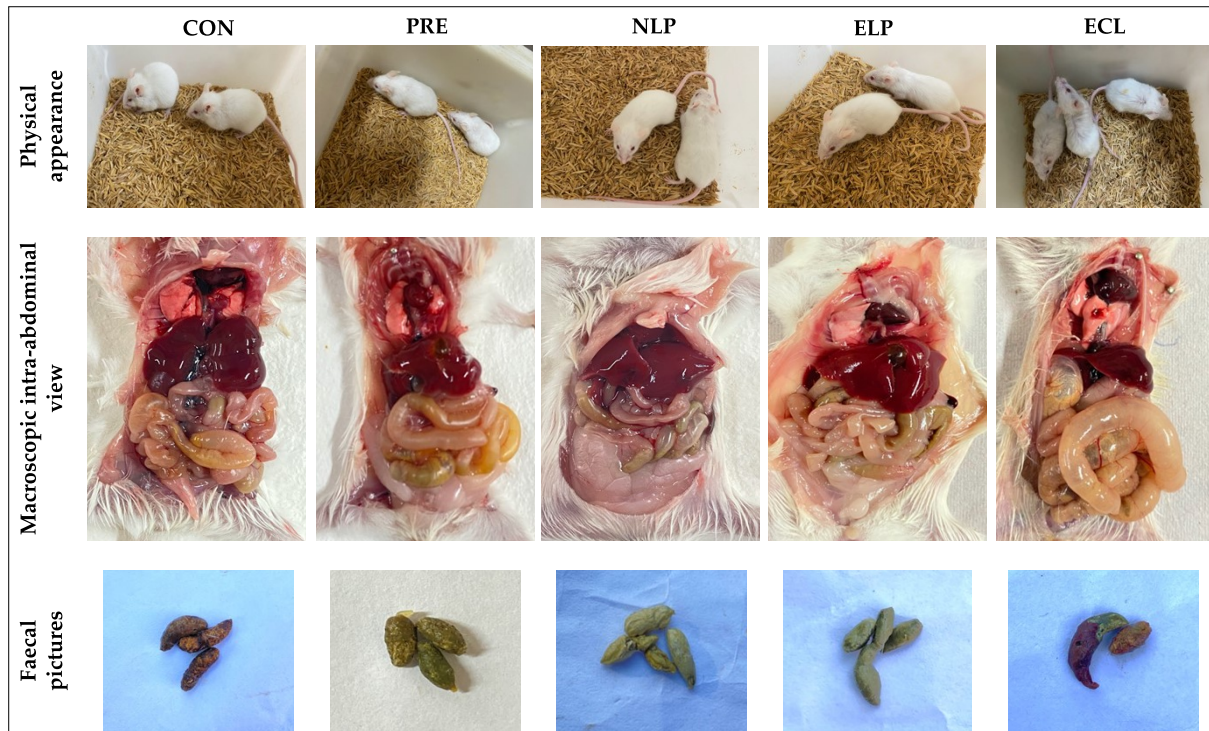
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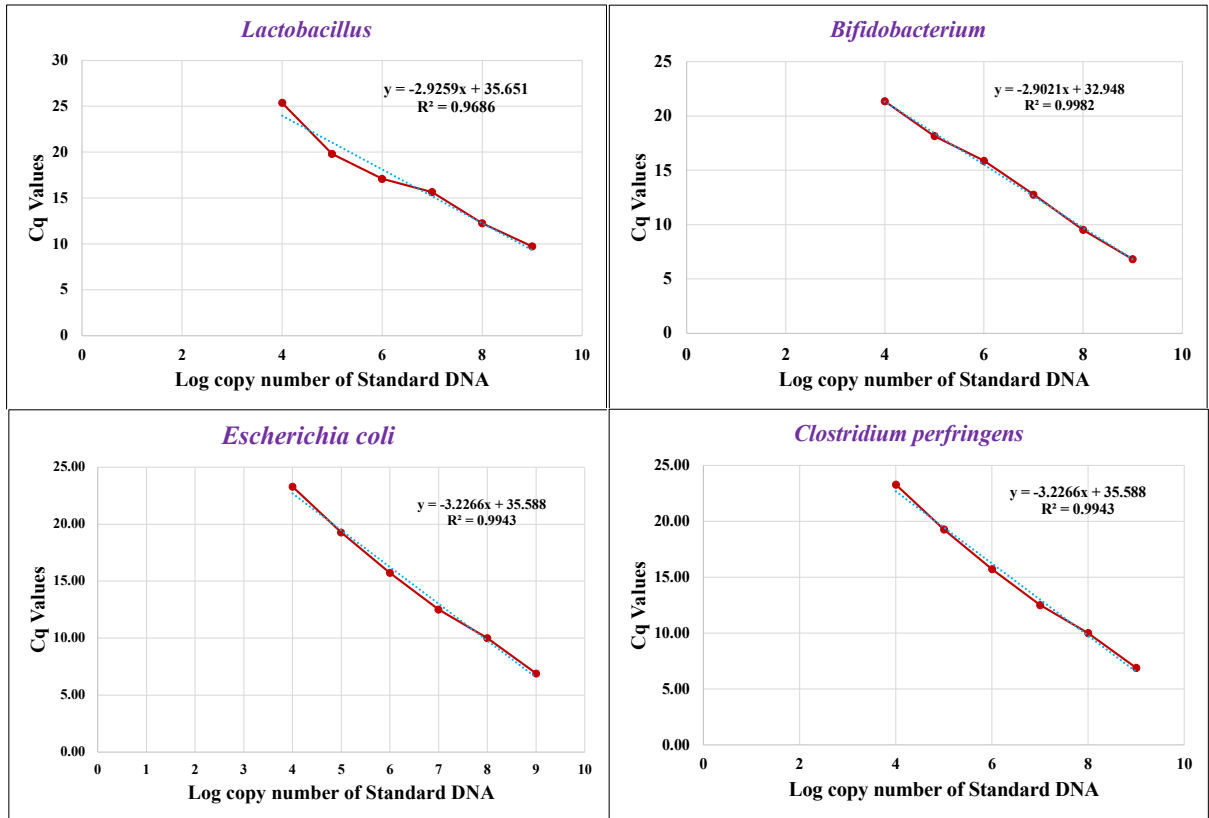
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Supplementary Fig. 1. Digital photography of physical appearance, macroscopic intraabdominal view, and stool pictures of mice among different dietary treatment groups. Mice in CON, PRE, NLP, and ELP appears to be healthy with improved lustrous hair coat and had normal stool. Whereas, ECL group mice were having piloerected hairs with inflamed visceral organs and bloody stool.



Supplementary Fig. 2. Standard curve of *Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, and *Clostridia perfringens* constructed by absolute quantitative real time PCR (qRT-PCR)

Supplementary Table 1 Composition of the mineral and vitamin mixture of purified diet fed to mice during the experiment

Mineral mixture (AIN-93G)		Vitamin mixture (AIN-93-VX)	
Ingredients	Levels (%)	Ingredients	Levels(g/kg)
Calcium carbonate	35.7	Nicotinic acid	3.00
Monopotassium phosphate	19.6	D-Calcium pantothenate	1.60
Potassium citrate monohydrate	7.078	Pyridoxine HCl	0.70
Sodium chloride	7.4	Thiamine HCl	0.60
Potassium sulfate	4.66	Riboflavin	0.60
Magnesium oxide	2.4	Folic acid	0.20
Ferric citrate	0.606	D-Biotin	0.02
Zinc carbonate	0.165	Vitamin B ₁₂ (0.1% triturated in mannitol)	2.50
Manganese carbonate	0.063	a-Tocopherol powder (250 U/gm)	30.00
Copper carbonate	0.03	Vitamin A palmitate (250,000 U/gm)	1.60
Sodium selenate, Anhydrous	0.001	Vitamin D3 (400,000 U/gm)	0.25
Potassium iodate	0.00103	Phylloquinone	0.0075
Ammonium molybdate.4H ₂ O	0.000795	Powdered sucrose	959.655
Sodium metasilicate.9H ₂ O	0.145		
Chromium potassium sulfate.12H ₂ O	0.0275		
Lithium chloride	0.00174		
Boric acid	0.008145		
Sodium fluoride	0.00635		
Nickel carbonate	0.00318		
Ammonium vanadate	0.00066		
Powdered sugar	22.1		

Supplementary Table 2 Details of primers and protocol used in the quantification of bacteria DNA present in faeces and caecal digesta of mice by quantitative real time PCR technique

Bacterial group	Primers Sequence (5' -3')	Product size (bp)	Annealing temperature (°C)	Reference
<i>Lactobacillus</i>	F:GAGGCAGCAGTAGGGAATCTTC R:GGCCAGTTACTACCTCTATCCTTCTTC	126	55	Martin et al., 2020
<i>Bifidobacterium</i>	F:TACACCGGAATAGCTCCTGG R:CGTCAAGCTGATAGGACGC	115	58	
<i>Escherichia coli</i>	F:AGAAGCTTGCTCTTTGCTGA R:CTTTGGTCTTGCGACGTTAT	120	57	
<i>Clostridium perfringens</i>	F:TCATCATTCAACCAAGGAGCAATCC R: CCTTGGTAGGCCGTTACCC	105	55	

F, Forward; R, Reverse

Reference:

Martin, C.C., de Oliveira, S.M.F.N., dos Reis Costa, J.F., Baccili, C.C., Silva, B.T., Hurley, D.J., Gomes, V., 2020. Influence of feeding fresh colostrum from the dam or frozen colostrum from a pool on indicator gut microbes and the inflammatory response in neonatal calves. Res. Vet. Sci. 135, 355–365. <https://doi.org/10.1016/j.rvsc.2020.10.017>.

Supplementary Table 3 Details of primers used for relative expression of gut permeability-associated genes by qRT-PCR

Gene	Primer Sequence (5' -3')	Amplicon length (bp)	Reference
Claudin-1	F: CCCTTCAGCAGAGCAAGGTT R: TAGGGCAACCAAGTGCCTTT	123	Santiya et al., 2020
Zona occluden-1	F: CCTAAGACCTGTAACCATCT R: CTGATAGATATCTGGCTCCT	82	
Occludin	F: ATGTCCGGCCGATGCTCTC R: TTTGGCTGCTCTTGGGTCTGTAT	308	
GAPDH	F: GCACCGTCAAGGCTGAGAAC R: TGGTGAAGACGCCAGTGGA	138	

F, Forward; R, Reverse

Reference:

Santiya, M., Bhat, M.I., Gupta, T., Kapila, S., Kapila, R., 2020. Safety assessment of potential probiotic *Lactobacillus fermentum* MTCC-5898 in murine model after repetitive dose for 28 days (Sub-Acute Exposure). *Probiotics Antimicrob. Proteins* 12 (1), 259-270. <https://doi.org/10.1007/s12602-019-09529-6>.

Supplementary Table 4 Effects of non-encapsulated and encapsulated *L. plantarum* CRD7 on blood parameters of mice

Attributes	Dietary groups [†]					SEM	P value
	CON	PRE	NLP	ELP	ECL		
Hb (g/dL)	11.04 ^b	11.24 ^b	12.55 ^c	12.61 ^c	9.49 ^a	0.37	0.032
HCT (%)	46.12	46.29	46.07	46.02	44.23	0.30	0.170
RBC (millions/ μ L)	8.98 ^b	9.00 ^b	9.04 ^b	9.06 ^b	6.43 ^a	0.30	0.005
MCV (fl)	50.32 ^b	50.23 ^b	50.73 ^b	51.12 ^b	45.75 ^a	0.56	0.003
MCH (pg)	13.56	13.52	13.46	13.39	12.52	0.24	0.683
MPV (fl)	8.14	8.16	8.12	8.10	7.53	0.18	0.821
MCHC (%)	27.14	27.11	27.07	26.93	26.12	0.20	0.538
WBC ($10^3/\mu$ L)	10.22 ^a	10.15 ^a	10.08 ^a	10.09 ^a	13.37 ^b	0.36	0.001
Neutrophils (%)	6.64 ^a	6.58 ^a	6.40 ^a	6.22 ^a	8.53 ^b	0.30	0.093
Lymphocytes (%)	90.40 ^a	90.02 ^a	90.14 ^a	90.26 ^a	93.53 ^b	0.46	0.060

Dietary groups[†]: Basal diet with no supplementation (CON), supplemented with encapsulated material without probiotic (PRE; 100 mg), Supplemented with non-encapsulated *L. plantarum* CRD7 (NLP; 100 μ l/d/mouse), Supplemented with encapsulated *L. plantarum* CRD7 (ELP; 100 mg/d/mouse) having 1×10^9 CFU/g of CRD7; Supplemented with *E. coli* (ECL; 10^9 CFU/mouse/d). SEM, Standard Error of Mean. ^{a,b,c}Means bearing different superscripts in a row (P < 0.05)

Supplementary Table 5 Effects of non-encapsulated and encapsulated *L. plantarum* CRD7 on biochemical parameters of mice

Attributes	Dietary groups [†]					SEM	P value
	CON	PRE	NLP	ELP	ECL		
Total protein (g/dL)	7.24 ^a	7.81 ^{ab}	8.12 ^b	8.25 ^b	12.11 ^c	1.84	<0.001
Albumin (g/dL)	2.72 ^a	2.80 ^a	2.61 ^a	2.91 ^a	9.89 ^b	2.94	<0.001
Globulin (g/dL)	4.52 ^b	5.00 ^{bc}	5.50 ^c	5.33 ^c	2.23 ^a	1.29	<0.001
A/G	0.60 ^a	0.56 ^a	0.47 ^a	0.54 ^a	5.44 ^b	2.32	<0.001
Calcium (mg/dL)	9.32	9.44	9.22	9.77	10.02	0.68	0.473
Phosphorous (mg/dL)	12.80	13.00	12.40	13.10	13.43	1.04	0.796
SGOT (Unit/L)	51.30 ^a	50.40 ^a	52.40 ^a	51.20 ^a	90.74 ^b	16.2	<0.001
SGPT (Unit/L)	35.30 ^a	34.90 ^a	35.0 ^a	35.60 ^a	92.12 ^b	23.4	<0.001
Urea (mg/dL)	53.20 ^a	54.40 ^a	53.60 ^a	54.70 ^a	82.04 ^b	11.9	<0.001
Creatinine (mg/dL)	0.51 ^a	0.60 ^a	0.48 ^a	0.37 ^a	1.52 ^b	0.46	<0.001
Total cholesterol (mg/dL)	110.0 ^a	109.0 ^a	108.0 ^a	109.0 ^a	120.04 ^b	5.42	<0.001
Triglycerides (mg/dL)	94.00 ^a	94.40 ^a	94.60 ^a	94.20 ^a	98.22 ^b	2.42	0.050
LDL- cholesterol (mg/dL)	26.00 ^a	26.90 ^a	25.30 ^a	25.10 ^a	43.16 ^b	7.75	<0.001
HDL- cholesterol (mg/dL)	65.30 ^b	63.30 ^b	64.20 ^b	65.20 ^b	57.50 ^a	3.32	<0.001
VLDL- cholesterol (mg/dL)	18.80 ^a	18.83 ^a	18.92 ^a	18.87 ^a	19.64 ^b	0.48	0.048
Atherogenic index	0.15 ^a	0.17 ^a	0.16 ^a	0.16 ^a	0.23 ^b	0.02	<0.001

Dietary groups[†]: Basal diet with no supplementation (CON), supplemented with encapsulated material without probiotic (PRE; 100 mg), Supplemented with non-encapsulated and *L. plantarum* CRD7 (NLP; 100 µl/d/mouse), Supplemented with encapsulated *L. plantarum* CRD7 (ELP; 100 mg/d/mouse) having 1x10⁹ CFU/g of CRD7; Supplemented with *E. coli* (ECL; 10⁹ CFU/mouse/d). SEM, Standard Error of Mean. ^{a,b,c}Means bearing different superscripts in a row (P < 0.05).

$$VLDL = \frac{Triglycerides}{5}, LDL = Total\ cholesterol - (HDL + VLDL), Atherogenic\ index = \frac{LDL}{HDL}$$

Supplementary Table 6 The incidence of non-encapsulated and encapsulated *L. plantarum* CRD7 translocation during subacute exposure to mice

Attributes	Dietary groups [†]					No. of confirmed colonies by Gram staining	No. of confirmed colonies by PCR	% Translocation
	CON	PRE	NLP	ELP	ECL			
Blood								
MRS	0/8	0/8	0/8	0/8	8/8 (>50)	0	0	0
BHI	0/8	0/8	0/8	0/8	8/8 (>50)	0	0	0
Liver								
MRS	0/8	0/8	0/8	0/8	8/8 (>50)	0	0	0
BHI	0/8	0/8	0/8	0/8	8/8 (>50)	0	0	0
Kidney								
MRS	0/8	0/8	0/8	0/8	8/8 (>50)	0	0	0
BHI	0/8	0/8	0/8	0/8	8/8 (>50)	0	0	0
Spleen								
MRS	0/8	0/8	0/8	0/8	8/8 (6-13)	0	0	0
BHI	0/8	0/8	0/8	0/8	8/8 (32)	0	0	0
Lungs								
MRS	0/8	0/8	0/8	0/8	8/8 (9-12)	0	0	0
BHI	0/8	0/8	0/8	0/8	8/8 (20)	0	0	0

Values are presented as the number of animals that were positive for bacterial translocation/total number of animals tested per group.

Numbers in brackets represents the number of colonies counted (CFU/g of tissue).

MRS, De Man Rogosa and Sharpe agar; BHI, brain heart infusion agar.

Dietary groups[†]: Basal diet with no supplementation (CON), supplemented with encapsulated material without probiotic (PRE; 100 mg), Supplemented with non-encapsulated *L. plantarum* CRD7 (NLP; 100 µl/d/mouse), Supplemented with encapsulated *L. plantarum* CRD7 (ELP; 100 mg/d/mouse) having 1x10⁹ CFU/g of CRD7; Supplemented with *E. coli* (ECL; 10⁹ CFU/mouse/d).

Supplementary Table 7 Pearson's correlation matrix of various parameters assessed in this study

	ABW	AFI	Faecal pH	Lactate	Ammonia	Acetate	Propionate	Butyrate	TSCFA	LAB	BIF	<i>E. coli</i>	<i>C. perfringens</i>	SOD	CAT	GpX	IgA	IgG	FITC	IL-6	IL-10	TNF-alpha	TGF-beta	MCP-1
ABW	1	.961**	-.922*	.937*	-.980**	.962**	.970**	.960**	.969**	.955*	.909*	-.907*	-.904*	.914*	.985**	.798	.977**	.958*	-.928*	-.931*	.979**	-.981**	.926*	-.928*
AFI	.961**	1	-.810	.831	-.928*	.878	.933*	.907*	.903*	.978**	.869	-.943*	-.830	.902*	.977**	.915*	.998**	.997**	-.978**	-.911*	.982**	-.953*	.985**	-.982**
Faecal pH	-.922*	-.810	1	-.968**	.959*	-.985**	-.960**	-.971**	-.980**	-.877	-.956*	.855	.982**	-.927*	-.913*	-.587	-.844	-.825	.811	.938*	-.900*	.945*	-.788	.801
Lactate	.937*	.831	-.968**	1	-.974**	.989**	.938*	.968**	.975**	.860	.924*	-.809	-.943*	.873	.921*	.556	.861	.826	-.782	-.884*	.892*	-.943*	.770	-.776
Ammonia	-.980**	-.928*	.959*	-.974**	1	-.992**	-.987**	-.995**	-.995**	-.952*	-.963**	.916*	.959**	-.945*	-.985**	-.723	-.947*	-.928*	.901*	.953*	-.970**	.993**	-.892*	.897*
Acetate	.962**	.878	-.985**	.989**	-.992**	1	.978**	.992**	.997**	.917*	.962**	-.881*	-.973**	.933*	.958*	.648	.904*	.882*	-.854	-.943*	.940*	-.977**	.840	-.848
Propionate	.970**	.933*	-.960**	.938*	-.987**	.978**	1	.992**	.991**	.976**	.981**	-.959**	-.973**	.982**	.986**	.776	.950*	.946*	-.938*	-.989**	.984**	-.997**	.925*	-.932*
Butyrate	.960**	.907*	-.971**	.968**	-.995**	.992**	.992**	1	.998**	.952*	.985**	-.929*	-.982**	.966**	.976**	.706	.926*	.914*	-.897*	-.970**	.963**	-.991**	.882*	-.890*
TSCFA	.969**	.903*	-.980**	.975**	-.995**	.997**	.991**	.998**	1	.944*	.975**	-.916*	-.979**	.957*	.973**	.699	.926*	.910*	-.890*	-.965**	.961**	-.990**	.876	-.883*
LAB	.955*	.978**	-.877	.860	-.952*	.917*	.976**	.952*	.944*	1	.944*	-.989**	-.912*	.972**	.988**	.886*	.981**	.988**	-.988**	-.975**	.995**	-.980**	.983**	-.986**
BIF	.909*	.869	-.956*	.924*	-.963**	.962**	.981**	.985**	.975**	.944*	1	-.945*	-.992**	.986**	.948*	.693	.886*	.886*	-.889*	-.982**	.941*	-.970**	.867	-.878*
<i>E. coli</i>	-.907*	-.943*	.855	-.809	.916*	-.881*	-.959**	-.929*	-.916*	-.989**	-.945*	1	.909*	-.983**	-.958*	-.884*	-.944*	-.964**	.982**	.980**	-.972**	.955*	-.971**	.977**
<i>C. perfringens</i>	-.904*	-.830	.982**	-.943*	.959**	-.973**	-.973**	-.982**	-.979**	-.912*	-.992**	.909*	1	-.969**	-.927*	-.631	-.855	-.849	.849	.970**	-.918*	.958*	-.823	.837
SOD	.914*	.902*	-.927*	.873	-.945*	.933*	.982**	.966**	.957*	.972**	.986**	-.983**	-.969**	1	.956*	.789	.915*	.926*	-.942*	-.998**	.963**	-.970**	.922*	-.932*
CAT	.985**	.977**	-.913*	.921*	-.985**	.958*	.986**	.976**	.973**	.988**	.948*	-.958*	-.927*	.956*	1	.832	.986**	.979**	-.961**	-.963**	.996**	-.996**	.957*	-.959**
GpX	.798	.915*	-.587	.556	-.723	.648	.776	.706	.699	.886*	.693	-.884*	-.631	.789	.832	1	.897*	.927*	-.945*	-.797	.868	-.789	.956*	-.951*
IgA	.977**	.998**	-.844	.861	-.947*	.904*	.950*	.926*	.926*	.981**	.886*	-.944*	-.855	.915*	.986**	.897*	1	.996**	-.976**	-.926*	.989**	-.967**	.980**	-.978**
IgG	.958*	.997**	-.825	.826	-.928*	.882*	.946*	.914*	.910*	.988**	.886*	-.964**	-.849	.926*	.979**	.927*	.996**	1	-.991**	-.934*	.988**	-.959*	.994**	-.993**
FITC	-.928*	-.978**	.811	-.782	.901*	-.854	-.938*	-.897*	-.890*	-.988**	-.889*	.982**	.849	-.942*	-.961**	-.945*	-.976**	-.991**	1	.947*	-.979**	.943*	-.998**	1.00**
IL-6	-.931*	-.911*	.938*	-.884*	.953*	-.943*	-.989**	-.970**	-.965**	-.975**	-.982**	.980**	.970**	-.998**	-.963**	-.797	-.926*	-.934*	.947*	1	-.971**	.977**	-.928*	.938*
IL-10	.979**	.982**	-.900*	.892*	-.970**	.940*	.984**	.963**	.961**	.995**	.941*	-.972**	-.918*	.963**	.996**	.868	.989**	.988**	-.979**	-.971**	1	-.990**	.975**	-.977**
TNF-alpha	-.981**	-.953*	.945*	-.943*	.993**	-.977**	-.997**	-.991**	-.990**	-.980**	-.970**	.955*	.958*	-.970**	-.996**	-.789	-.967**	-.959*	.943*	.977**	-.990**	1	-.934*	.939*
TGF-beta	.926*	.985**	-.788	.770	-.892*	.840	.925*	.882*	.876	.983**	.867	-.971**	-.823	.922*	.957*	.956*	.980**	.994**	-.998**	-.928*	.975**	-.934*	1	-.999**
MCP-1	-.928*	-.982**	.801	-.776	.897*	-.848	-.932*	-.890*	-.883*	-.986**	-.878*	.977**	.837	-.932*	-.959**	-.951*	-.978**	-.993**	1.00**	.938*	-.977**	.939*	-.999**	1

ABW – average body weight; AFI – average feed intake; TSCFA – total short chain fatty acids; LAB – *Lactobacillus*; BIF – *Bifidobacterium*; SOD – superoxide dismutase; CAT – catalase; GpX – glutathione peroxidase; IgA – immunoglobulin A; IgG – immunoglobulin G; FITC – fluorescein isothiocyanate-dextran; IL-6 – interleukin 6; IL-10 – interleukin 10; TNF-alpha - tumour necrosis factor-alpha; TGF-beta – transforming growth factor; MCP-1 - Monocyte chemoattractant protein-1.