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ORIGINAL PAPER

Effect of lactic acid bacterial probiotic on piglet performance and serum biochemical parameters in Xiangcun Black lactating sows

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ABSTRACT. The present study was conducted to evaluate the effect of lactic acid bacterial probiotic (LABP) supplementation on piglet performance and serum biochemical parameters. Gestating Xiangcun Black sows (n = 50, day 7 ante partum) were randomly divided into 5 groups: C, L50, L100, L200, L300. These groups were administered daily 0, 50, 100, 200, and 300 ml of LABP (live *Lactobacillus plantarum* CCFM8610 ≥ 1×10^9 CFU/ml), respectively. The results showed that litter weight at weaning ($P < 0.01$) increased linearly with raising dietary LABP levels. Sows that received LABP from day 107 of gestation (G107) until weaning (L21) had the highest piglet survival rate over the three week period compared to the control group ($P < 0.05$). Serum alkaline phosphatase ($P < 0.01$) activity and total cholesterol ($P < 0.05$) level decreased but triglyceride concentration increased linearly with the increasing dietary LABP levels. Furthermore, serum sarcosine and carnosine concentrations were significantly higher in the L200 group compared to the C, L100, and L300 groups ($P < 0.05$). Sows in the L50 group had the highest serum levels of leucine, lysine, valine, and arginine compared to the other groups ($P < 0.05$). In conclusion, our findings demonstrated that increasing dietary LABP levels in Xiangcun Black sows enhanced litter weight at weaning and reduced piglet mortality by influencing lipid and amino acid metabolism.

Introduction

Sow health plays an important role in producing healthy offspring, healthy sows tend to have more litters and a higher number of piglets per litter (Andersson et al., 2020). Oral administration of recombinant *Lactobacillus johnsonii* protein was shown to activate humoral immunity against porcine epidemic diarrhoea (PED), leading to increased levels of SIgA and IgG antibodies in maternal milk, which in turn protected piglets against PED virus (PEDV) infection (Zheng et al., 2021). Piglets preweaning mortality decreased and faecal *Lactobacillus* and *Enterococci* counts increased as a result of *Enterococcus faecium* DSM 7134 supplementation in sow's diet (Lan and Kim, 2020).

Moreover, supplementation with co-fermented feed containing *Bacillus subtilis* and *Enterococcus faecium* in the sows' diet reduced the abundance of *Enterobacteriaceae* but increased the levels of

Lactobacillus and *Succiniclasticum* in the intestinal microbiota (Wang et al., 2021a). The mobilisation of the gut microbiome was reported to be associated with shifts in serum metabolites (Zhang et al., 2021). There is a positive correlation between *Lactobacillus* and serum bile acid metabolites, which are associated with lipid metabolism (Huang et al., 2019). Reported showed that, the relative abundance of *Lactobacillus* significantly increased from the late stages of pregnancy (5 days before parturition, LP) to the postpartum period (within 6 h after delivery, PO), and predicted functional capacities of these gut microbiome indicated that serum amino acid metabolism and glucan biosynthesis decreased, while carbohydrate and lipid metabolism increased during this stage (Huang et al., 2019). Administration of *Lactobacillus johnsonii* XS4 in diets at the end of pregnancy and during lactation had a positive effect on litter weight at birth and weaning (Wang et al., 2014). Milk is the main source of nutrients for nursing piglets, and probiotics in the sows' diet showed beneficial a beneficial influence on milk composition and, subsequently, the growth of their offspring.

Xiangcun Black pig, a cross of Duroc local × Taoyuan Black, exhibits distinctive traits,, such as black hair, high resilience, high productivity, strong maternal instinct, multiple births, and superior meat quality, though they show slower growth rates. Faecal bacterial communities vary significantly between pig breeds. For instance, the gut microbiota of Chinese Jinhua pigs was shown to be composed of 70.4% *Firmicutes* and 14.4% *Bacteroidetes* (Yang et al., 2018), while those of Yorkshire, and Landrace pigs contained 42.0% and 45.6% *Firmicutes* and 51.4%, 47.6% *Bacteroidetes*, respectively (Pajarillo et al., 2014). These differences in microbial composition can be maternally transmitted, as confirmed by studies of the umbilical cord and adult pigs (Patil et al., 2020). Even when maintained on the same diet, significant differences were observed in the composition of short chain fatty acids (SCFAs) and secondary bile acids in Meihua piglets (a fatty, slow-growing Chines breed) and Landrace piglets (a lean, fast-growing European breed) (Ajouz et al., 2014). It is still unclear whether the application of a lactic acid bacterial probiotic (LABP) in Xiangcun Black sows, known for their strong resilience, has a beneficial effect on piglet performance by affecting the sow's metabolism. The purpose of this study was to evaluate the effects and determine optimal LABP dose in terms of piglet growth, survival rates during lactation, and serum biochemical parameters related to amino acid and lipid metabolism in Xiangcun Black sows. We hypothesised that supplementing the sows' diet with LABP would improve offspring growth by enhancing amino acid metabolism of the sows.

Material and methods

Animal care

The experimental design and procedures in this study were reviewed and approved by the Animal Care and Use Committee of the Hengyang Normal University and Xiangcun high-technology agricultural company under ethical approval number HN-UACUC-S201911058.

Animals and experimental design

Xiangcun Black sows with a parity 3 or 4, were randomly assigned to one of five experimental diets, with 10 sows per replicate, housed in farrowing pens (2.20 × 1.80 m^2) from 7 days before farrowing until 21 days post-delivery. Sows from the control group were fed a control diet (C, Table 1) based on maize and soybean meal providing 3302 kcal/kg of digestible energy and 16.5% crude protein. The experimental groups were fed with a control diet supplemented daily with 50, 100, 200, or 300 ml of LABP, brown liquid, *Lactobacillus plantarum* CCFM8610, 1 × 10^9 CFU/ml, Lifeng Biotechnology, Changsha, Hunan, China) corresponding to the L50, L100, L200, and L300 groups, respectively. LABP was mixed with 300 g of feed and administered to sows before 8:00 a.m. each day. Sows were fed three times a day at 08:00, 12:00 and 18:00. If any feed remained 60 min after the first feeding, no additional feed was provided at that time. Sows had *ad libitum* access to water throughout the experiment, which ended at day 28 of lactation. The animal experiment was carried out at the commercial pig farm of Xiangcun High-Tech Agriculture Co., Ltd. in Loudi, Hunan province, with feeding and immunisation procedures following the company's standard breeding practices.

Sample collection

Feed intake was recorded daily. On days 7 and 21 of lactation, blood samples from the sows were collected via jugular vein puncture into 10 ml tubes after an overnight fast. Blood was centrifuged at 3000 *g* for 10 min at 4 °C to separate the serum, which was then stored at −80 °C until analysis.

Table 1. Calculated ingredient composition of diets for lactating sows (as fed basis)

Ingredients[1], %	Groups				
	C	L50	L100	L200	L300
Maize	59.00	59.00	59.00	59.00	59.00
Soya bean meal (CP 43%)	10.00	10.00	10.00	10.00	10.00
Wheat bran	8.00	8.00	8.00	8.00	8.00
Extruded full-fat soybean	16.00	16.00	16.00	16.00	16.00
Limestone	0.96	0.96	0.96	0.96	0.96
Dicalcium phosphate	0.88	0.88	0.88	0.88	0.88
Premix[2]	0.48	0.48	0.48	0.48	0.48
Choline chloride	0.14	0.14	0.14	0.14	0.14
Milk powder	3.00	3.00	3.00	3.00	3.00
Glucose	0.80	0.80	0.80	0.80	0.80
Salt	0.32	0.32	0.32	0.32	0.32
Fermented soybean meal (CP 50%)	0.24	0.24	0.24	0.24	0.24
L-lysine HCl	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100
Lactic acid probiotic supplement, ml/sow/day	0	50	100	200	300
Nutrient[1]					
crude protein, %	16.40	16.40	16.40	16.40	16.40
calcium, %	0.64	0.64	0.64	0.64	0.64
phosphorus, %	0.53	0.53	0.53	0.53	0.53
digestible P, %	0.31	0.31	0.31	0.31	0.31
salt, %	0.54	0.54	0.54	0.54	0.54
lysine[3], %	0.95	0.95	0.95	0.95	0.95
methionine + cystine, %	0.70	0.70	0.70	0.70	0.70
threonine, %	0.65	0.65	0.65	0.65	0.65
tryptophan, %	0.19	0.19	0.19	0.19	0.19
digestive energy, kcal/kg	13.8	13.8	13.8	13.8	13.8
crude fibre, %	2.20	2.20	2.20	2.20	2.20
crude fat, %	5.20	5.20	5.20	5.20	5.20

[1] levels of dietary lactic acid bacterial probiotic supplementation for C, L50, L100, L200, and L300 were 0, 50, 100, 200, and 300 ml 1 ×10^9 CFU/ml per sow per day, respectively; [2] nutritional level of the diets were calculated using ingredient values obtained from the National Research Council (NRC, 2012); the following quantities of vitamins and trace minerals were provided per kilogram of complete diet: IU: vitamin A as retinyl acetate 10000, vitamin D_3 as cholecalciferol 1400, vitamin E as DL-alpha tocopheryl acetate 4; mg: vitamin K as menadione dimethylpyrimidinol bisulphite 1.28, thiamine as thiamine mononitrate 1.00, riboflavin 3.85, pyridoxine as pyridoxine hydrochloride 1.00, D-pantothenic acid as D-calcium pantothenate 12.0, niacin 10.25, folic acid 1.35, biotin 0.21, Cu as copper sulphate 20, Fe as ferrous sulphate 80, Mn as manganese sulphate 44.0, Zn as zinc sulphate 88.0, Se as sodium selenite 0.15, I as ethylenediamine dihydrochloride 0.12; [3] amino acids are presented as standardised ileal digestible AA

Piglet growth performance

The number of piglets born alive or dead was recorded. Body weight of all piglets was recorded on days 1, 7, 14, and 21. Cross-fostering was performed within 3 days of parturition among sows receiving the same treatment. Creep feed was not provided, and sow milk was the sole source of nutrition for the piglets during the first 21 days of lactation.

Diarrhoea score and incidence

From day 3 to 21, the faecal score of piglets was recorded twice daily (at 10:00 and 16:00) by the same person. The diarrhoea scoring system was as follows: 1 – hard stool, 2 – moulding, 3 – soft stool, 4 – runny faeces, 5 – liquid stool. The diarrhoea score was calculated by counting the number of pigs with diarrhoea per sow twice a day. The diarrhoea rate was calculated by dividing the number of piglets with diarrhoea by the total number of experimental piglets throughout the lactation period.

Chemical analysis

Serum activity of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and lactic dehydrogenase (LDH), as well as the levels of total protein (TP), albumin (ALB), globulins (GLB), creatinine (CREA), urea nitrogen (UREA), triglycerides (TG), total cholesterol (TC), glucose (GLU), low density lipoprotein cholesterol (LDLC), uric acid (UA), and high density lipoprotein cholesterol (HDLC) were measured using a Beckman CX4 automatic biochemistry analyser (Biochemical Analytical Instrument, Beckman Coulter Inc., Brea, CA, USA). After treatment with 10% sulphosalicylic acid, the serum supernatant was obtained by centrifugation at 15000 rpm for 20 min. The supernatant was then diluted with 0.02 mol/l hydrochloric acid (HCl). Serum samples collected on day 21 of lactation were subsequently analysed for amino acid (AA) concentrations using an oxidation analysis method on a3200 Q TRAP LC/MS/MS system (Applied Biosystems, Waltham, MA, USA) equipped with an RP-C18 column (150 mm length, 4.6 mm diameter, 5 mm particle size).

Statistical analysis

The impact of LABP on sow and piglet performance was analysed using one-way ANOVA and a general linear model (GLM) implemented in the SAS 8.2 software package (SAS Institute Inc., Cary, NC, USA), Duncan's multiple test was used to compare the differences between the supplemented groups and the control group. Sows or a litter of piglets were considered the experimental unit. For metabolites measured in serum samples from day 7 to day 21, a mixed effects analysis REML was performed in SAS, with LABP treatment, time, and their interaction as the main factors, and sow as a random factor. The significance of the relationship between LABP level and sow/piglet performance was tested using the regression

analysis (REG) procedure of SAS, and a linear model was selected using the R stepwise function at $P < 0.15$. Data were expressed as least-squares means ± SEM. Means were considered significantly different at $P < 0.05$ and trends were noted when $0.05 \leq P \leq 0.10$.

Results

Reproductive performance

No statistically significant differences in feed intake were observed between the individual treatments during the first, second, third and fourth week of lactation (Table 2; $P > 0.05$). The treatment did not affect litter size, including the total number of piglets born, piglets born alive, weak piglets, and the number of weaned piglets ($P > 0.05$). There were also no significant differences in litter weight at farrowing or in average piglet weight at farrowing and weaning between the different groups ($P > 0.05$). However, litter weight at weaning was significantly higher in sows that consumed more than 100 ml of LABP per day during lactation compared to the control group ($P < 0.05$). Additionally, litter weight at weaning ($P < 0.01$) increased linearly with increasing levels of dietary LABP.

Piglet growth performance

There were no significant differences in piglet survival rates in the first, second and third week of lactation between the groups ($P > 0.05$; Table 3). However, sows that received LABP from day 107 of gestation (G107) until weaning (L21) had higher piglet survival rate over the three weeks compared to the control group ($P < 0.05$). No differences were observed in litter weight gain per sow or average daily weight gain per piglet (ADG) in individual groups during the first and second weeks of lactation ($P > 0.05$). Litter weight gain per sow and ADG during third week were higher in the L200 group compared to the other treatments ($P < 0.05$). Over the entire 21-day lactation period, litter weight gain per sow increased in the L200 group compared to the control and L50 groups ($P < 0.05$). Piglet ADG in L200 tended to be higher than in the control and L50 groups during lactation ($P = 0.077$). A significant positive correlation was identified between LABP levels in the sow's diet, piglet survival rate, and litter weight gain per sow during the third week and throughout the three weeks of lactation ($P < 0.05$).

Incidence of diarrhoea

There were no significant differences in diarrhoea rate or diarrhoea score between the groups during the first, second, third, and fourth week of

Table 2. Effects of dietary levels of lactic acid bacterial probiotic on reproductive performance in Xiangcun Black sows

Item[a]	Groups					SEM	*P*-value	P_{linear}	$P_{quadratic}$
	C	L50	L100	L200	L300				
Average feed intake per sow, kg/day									
days 1–7	2.61	2.38	2.68	3.00	2.40	0.13	0.527	NS	NS
days 8–14	3.77	3.89	4.58	4.20	3.80	0.15	0.382	NS	NS
days 15–21	4.42	4.77	4.68	4.82	4.40	0.13	0.776	NS	NS
days 22–28	4.64	4.83	5.02	4.80	4.45	0.12	0.679	NS	NS
days 1–28	3.83	3.97	4.27	4.12	3.67	0.12	0.482	NS	NS
Litter size, n/sow									
total born	11.33	10.17	9.67	9.50	10.67	0.33	0.400	NS	NS
born alive	10.83	10.00	9.67	9.50	10.33	0.33	0.715	NS	NS
born weak[a]	0.50	0.50	0.33	0.17	0.67	0.17	0.913	NS	NS
weaned	7.83	8.83	9.33	9.00	9.83	0.27	0.225	+0.045	+0.078
Litter weight, kg/sow									
at farrowing	12.44	14.78	11.73	12.61	13.68	0.42	0.210	NS	NS
at weaning	24.33^{B}	30.39^{AB}	34.43^{A}	38.16^{A}	35.99^{A}	1.36	0.030	+0.007	+0.042
Average piglet weight, kg/piglet									
liveborn	1.19	1.48	1.23	1.33	1.36	0.05	0.301	NS	NS
weaned[b]	3.14	3.45	3.76	4.28	3.61	0.13	0.112	+0.127	NS

SEM – standard error of the mean; dietary levels of lactic acid bacterial probiotic supplementation for C, L50, L100, L200, and L300 were 0, 50, 100, 200, and 300 ml 1 × 10^9 CFU/ml per sow per day, respectively. NS indicates that no variable met the 0.15 significance level for entry into the model selected by the stepwise method in the REG procedure; '+' indicates a positive effect; '−' indicates a negative effect; [a] – weak piglets are defined as those with a body weight below 0.5 kg; [b] due to the low weight of the piglets, they were still sucking after 21 days, and feed was provided for the piglets concurrently, so we did not record piglet weight data from day 21 of lactation; [AB] – values in a row without common superscripts differ significantly at $P < 0.05$

Table 3. Effects of dietary levels of lactic acid bacterial probiotic on piglet growth performance in Xiangcun Black sows

Item[a]	Groups					SEM	*P*-value	P_{linear}	$P_{quadratic}$
	C	L50	L100	L200	L300				
Survival rate, %									
days 1–7	93.88	98.49	100.00	100.00	95.77	1.00	0.236	NS	NS
days 8–14	92.59	95.00	98.15	96.67	100.00	1.26	0.417	0.082	NS
days 15–21	90.28	100.00	100.00	98.15	98.33	1.34	0.157	NS	NS
days 1–21	77.59^{B}	93.64^{A}	98.15^{A}	94.82^{A}	94.10^{A}	1.59	0.004	0.047	NS
Total litter weight gain, kg/sow									
days 1–7	5.73	4.61	7.32	6.72	6.70	0.41	0.284	NS	NS
days 8–14	4.86	5.47	7.25	4.93	8.77	0.77	0.432	NS	NS
days 15–21	1.70^{C}	6.09^{BC}	8.33^{B}	13.86^{A}	6.98^{BC}	0.77	0.001	+0.027	NS
days 1–21	12.29^{C}	16.17^{BC}	22.90^{AB}	25.51^{A}	22.83^{AB}	1.30	0.021	+0.010	+0.059
Average piglet weight gain, g/day									
days 1–7	102.21	77.14	114.16	102.01	97.67	6.52	0.503	NS	NS
days 8–14	95.06	107.62	118.97	84.60	119.08	9.31	0.716	NS	NS
days 15–21	81.53^{B}	96.57^{B}	128.41^{B}	234.41^{A}	104.73^{B}	13.38	0.010	NS	NS
days 1–21	278.80	281.33	361.54	421.01	321.48	17.26	0.077	NS	NS

SEM − standard error of the mean; [a] – dietary lactic acid bacterial probiotic supplementation levels for C, L50, L100, L200, and L300 were 0, 50, 100, 200, and 300 ml 1 × 10^9 CFU/ml per sow per day, respectively; NS means that no variable met the 0.15 significance level for entry into the model selected by the stepwise method in the REG procedure; '+' indicates a positive effect; '−' indicates a negative effect; [ABC] – values in a row without common superscripts differ significantly at $P < 0.05$

Table 4. Effects of dietary levels of lactic acid bacterial probiotic on offspring diarrhoea incidence in Xiangcun Black sows

Item[a]	Groups					SEM	*P*-value	P_{linear}	$P_{quadratic}$
	C	L50	L100	L200	L300				
Number of diarrhoea observations									
day 1–7	1.17	0.50	1.67	0.00	0.17	0.25	0.227	NS	NS
day 8–14	4.50	2.50	5.00	2.67	4.50	0.73	0.732	NS	NS
day 15–21	1.83	1.83	1.33	1.50	2.83	0.30	0.564	NS	NS
day 22–28	0.33	0.33	1.00	0.33	1.33	0.27	0.664	NS	NS
day 1–28	7.83	5.17	9.00	4.50	8.83	0.83	0.304	NS	NS
Diarrhoea rate[b], %									
day 1–7	3.17	2.00	4.67	0.00	0.50	0.90	0.476	NS	-0.149
day 8–14	14.33	10.50	17.50	8.67	15.00	2.60	0.823	NS	NS
day 15–21	5.83	6.50	4.33	4.67	10.33	1.19	0.532	NS	NS
day 22–28	1.33	1.33	3.17	1.33	4.50	0.95	0.761	NS	NS
day 1–28	12.45	8.25	13.39	7.34	12.59	1.24	0.419	NS	NS
Diarrhoea score									
day 1–7	1.72	0.71	2.53	0.00	0.24	0.39	0.242	NS	NS
day 8–14	6.93	3.78	7.20	4.38	6.69	1.11	0.800	NS	NS
day 15–21	3.20	3.32	2.04	2.50	3.88	0.50	0.797	NS	NS
day 22–28	0.60	0.43	1.62	0.45	1.78	0.38	0.623	NS	NS
day 1–28	24.67	20.33	29.67	14.67	30.33	3.04	0.460	NS	NS

[a] – dietary lactic acid bacterial probiotic supplementation levels for C, L50, L100, L200, and L300 were 0, 50, 100, 200, and 300 ml 1 × 10^9 CFU/ml per sow per day, respectively; [b] – diarrhoea rate = number of piglets with diarrhoea × 100% / (total number of piglets × total number of experimental days); diarrhoea score standard: 1 – hard stool, 2 – moulding, 3 – soft stool, 4 – runny faeces, 5 – liquid stool; diarrhoea score was calculated for each piglet twice daily; NS indicates that no variable met the 0.15 significance level for entry into the model selected by the stepwise method in the REG procedure; '+' indicates a positive effect; '−' indicates a negative effect

lactation, as well as across the entire four-week lactation period ($P > 0.05$; Table 4).

Serum metabolites

No treatment effects were observed regarding serum AST, LDH, TP, ALB, GLB, UREA, CREA and LDL-C concentrations (Table 5). However, lactation time significantly affected serum activity of ALT, AST, and ALP, as well as LDH, UREA, TG, TC, and HDL-C levels, with concentrations being higher on day 21 compared to day 7 ($P < 0.05$). In the L50 group, serum ALT activity was lower than those in the C, L200 and L300 groups ($P < 0.05$), but not significantly different from L100 ($P < 0.05$).

Table 5. Effects of dietary levels of lactic acid bacterial probiotic on serum indices in Xiangcun Black sow

Item[a]	Groups										SEM	*P*-value			P_{linear}	$P_{quadratic}$
	C		L50		L100		L200		L300							
	D_7	D_{21}	D_7	D_{21}	D_7	D_{21}	D_7	D_{21}	D_7	D_{21}		D	T	D * T		
ALT, U/l	33.75^{A}	51.35	37.74^{B}	26.63	30.60^{AB}	46.08	35.92^{A}	49.12	44.24^{A}	43.07	1.12	0.012	0.022	0.004	NS	NS
AST, U/l	35.13	58.78	44.32	49.08	35.80	71.18	40.60	73.22	42.12	73.28	2.40	0.440	<0.0001	0.334	NS	NS
ALP, U/l	41.08^{A}	52.33	30.14^{A}	60.85	36.30^{AB}	47.03	25.98^{B}	39.28	34.52^{B}	32.65	1.19	0.003	<0.0001	0.010	(−)<0.0001	(−)<0.0001
LDH, U/l	370.80	536.20	611.60	514.10	405.96	629.60	489.18	776.60	520.52	674.76	22.28	0.235	0.007	0.184	+0.135	NS
TP, g/l	82.10	81.80	79.22	72.93	77.64	82.20	77.16	87.76	80.56	80.70	1.42	0.278	0.279	0.271	NS	NS
ALB, g/l	41.23	43.75	45.58	43.05	44.16	47.30	47.86	46.20	43.04	39.97	0.64	0.084	0.891	0.429	NS	NS
GLB, g/l	40.90	38.25	34.06	34.25	33.50	36.00	29.28	31.60	37.38	37.00	0.91	0.088	0.822	0.938	NS	NS
UREA, mmol/l	4.75	6.49	5.25	6.93	4.98	6.16	5.49	5.30	5.96	6.84	0.11	0.215	0.001	0.159	NS	NS
CREA, µmol/l	189.40	209.08	213.34	191.80	200.56	213.88	219.56	186.58	200.74	248.85	3.58	0.265	0.519	0.016	+0.099	+0.030
GLU, mmol/l	2.71^{A}	2.53	1.97^{B}	1.80	2.89^{A}	2.50	3.32^{A}	2.42	2.06^{A}	3.20	0.09	0.030	0.620	0.017	+0.068	+0.035
TG, mmol/l	0.25^{B}	0.27	0.25^{B}	0.37	0.25^{B}	0.42	0.37^{A}	0.64	0.37^{A}	0.54	0.01	<0.0001	<0.0001	0.055	+0.0001	+0.002
TC, mmol/l	2.51^{AB}	3.42	3.19^{AB}	2.91	2.62^{A}	4.02	2.93^{BC}	2.56	2.40^{C}	2.54	0.06	0.004	0.014	0.001	−0.017	−0.014
HDL-C, mmol/l	0.66^{AB}	1.14	0.86^{AB}	1.02	0.84^{A}	1.40	0.87^{AB}	0.99	0.69^{B}	0.95	0.02	0.025	<0.0001	0.059	−0.122	−0.076
LDL-C, mmol/l	1.16^{AB}	1.11	1.16^{AB}	1.02	1.22^{A}	1.35	1.10^{AB}	0.96	1.00^{B}	0.96	0.03	0.094	0.537	0.770	NS	−0.130

ALT – alanine transaminase, AST – aspartate transaminase, ALP – alkaline phosphatase, LDH – lactic dehydrogenase, TP – total protein, ALB – serum albumin, GLB – serum globulin, UREA – urea nitrogen, CREA – creatinine, GLU – glucose, TG – triglycerides, TC – total cholesterol, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, D – diet, T – time; D_7 and D_{21} indicate days 7 and 21 of lactation; [a] – dietary lactic acid bacterial probiotic supplementation levels for C, L50, L100, L200, and L300 were 0, 50, 100, 200, and 300 ml 1 × 10^9 CFU/ml, respectively; NS indicates that no variable met the 0.15 significance level for entry into the model selected by the stepwise method in the REG procedure; '+' indicates a positive effect; '−' indicates a negative effect; [ABC] – values in a row without common superscripts differ significantly at $P < 0.05$

Sows from the L50 and C groups had higher serum ALP activity than those from the L200 and L300 groups ($P < 0.05$). Serum GLU concentration was lower in the L50 group compared to the other groups ($P < 0.05$). Serum TG concentrations were lower in the C, L50, and L100 groups compared to the L200 and L300 groups ($P < 0.05$). Sows supplemented with 100 ml of LABP during lactation showed higher serum TC and HDL-C concentrations than those in L300 ($P < 0.05$). There was a significant interaction effect between LABP and lactation time on the activity of ALT and ALP, as well as serum CREA, GLU, and TC concentrations ($P < 0.05$). Additionally, serum TG and HDL-C levels tended to be affected by this interaction ($P = 0.055$ and 0.059, respectively). Serum ALP activity ($P < 0.01$) and TC levels ($P < 0.05$) decreased, while TG increased linearly with increasing dietary LABP levels. Serum CREA and GLU levels increased quadratically with rising dietary LABP doses ($P < 0.05$).

Serum amino acids

Serum amino acid profiles of sows fed diets with different levels of LABP are summarised in Table 6. There were no significant differences in the concentrations of histidine, isoleucine, methionine,

Table 6. Effects of dietary levels of lactic acid bacterial probiotic on serum amino acid content at D21 of lactation in Xiangcun Black sow, μg/ml

Item[a]	Groups					SEM	*P*-value	P_{linear}	$P_{quadratic}$
	C	L50	L100	L200	L300				
EAA									
histidine	8.47	11.37	13.12	15.00	10.63	0.81	0.331	NS	NS
isoleucine	10.70	15.21	11.63	12.26	10.99	0.65	0.422	NS	NS
leucine	20.81^{B}	29.59^{A}	23.50^{B}	19.09^{B}	21.47^{B}	0.64	0.007	NS	NS
lysine	16.09^{B}	30.93^{A}	20.76^{B}	18.26^{B}	19.09^{B}	0.89	0.005	NS	NS
methionine	4.50	5.89	6.16	5.37	5.60	0.14	0.078	NS	NS
phenylalanine	9.76	13.21	11.43	10.47	10.66	0.34	0.158	NS	NS
threonine	13.72	18.06	13.87	16.21	14.22	0.58	0.277	NS	NS
valine	22.35^{B}	31.59^{A}	23.22^{B}	20.07^{B}	20.42^{B}	0.79	0.011	−0.069	−0.064
NEAA									
alanine	37.14	43.81	51.39	42.46	37.11	1.59	0.175	NS	NS
arginine	10.47^{B}	26.43^{A}	12.01^{B}	13.84^{B}	13.76^{B}	0.80	0.001	NS	NS
aspartic acid	5.50^{C}	9.24^{A}	7.66^{B}	4.99^{C}	8.09^{AB}	0.17	<0.0001	NS	NS
glutamic acid	40.36^{B}	66.02^{A}	64.89^{A}	42.15^{B}	47.37^{B}	1.09	<0.0001	NS	NS
glycine	47.64	56.22	59.40	64.22	48.72	1.96	0.195	NS	NS
serine	10.81	14.15	14.77	12.66	12.97	0.48	0.303	NS	NS
tyrosine	13.41	17.04	13.90	18.12	15.09	0.99	0.702	NS	NS
cysteine	1.54^{B}	6.73^{A}	1.05^{B}	2.63^{B}	1.20^{B}	0.19	<0.0001	NS	−0.137
proline	33.98	39.69	29.06	34.13	26.72	1.12	0.070	−0.074	−0.061
Other amino acids									
P-serine	2.51^{ABC}	3.70^{A}	3.50^{AB}	2.27^{C}	2.37^{BC}	0.14	0.042	−0.133	−0.077
taurine	13.84^{B}	16.51^{B}	22.44^{A}	13.78^{B}	12.29^{B}	0.54	0.002	NS	−0.057
urea	143.16	124.75	136.76	134.94	138.55	3.33	0.697	NS	NS
sarcosine	1.09^{C}	1.89^{AB}	1.54^{BC}	2.26^{A}	1.39^{BC}	0.07	0.004	NS	NS
α-aminoadipate	3.62	6.06	3.60	5.44	4.11	0.28	0.118	NS	NS
citrulline	12.46	9.87	10.58	10.22	12.07	0.55	0.681	NS	−0.144
α-aminobutyric acid	0.88	0.90	1.16	1.42	1.02	0.05	0.110	NS	NS
cystathionine	0.40	0.48	0.37	0.44	0.31	0.02	0.289	NS	NS
β-alanine	0.83	1.09	1.05	1.06	0.76	0.04	0.176	NS	NS
β-aminoisobutyric acid	0.16	0.09	0.11	0.12	0.11	0.01	0.368	NS	NS
γ-aminobutyric acid	0.10^{AB}	0.09^{ABC}	0.11^{A}	0.07^{BC}	0.07^{C}	0.00	0.042	−0.009	−0.006
ehanolamine	0.14^{BC}	0.26^{A}	0.18^{B}	0.11^{C}	0.11^{C}	0.01	<0.0001	−0.022	−0.013
hydroxylysine	1.17	0.67	0.90	1.14	1.09	0.07	0.386	NS	NS
ornithine	11.18^{B}	18.29^{A}	11.74^{B}	12.67^{B}	10.93^{B}	0.60	0.037	NS	NS
1-methylhistidine	0.40	0.45	0.69	0.81	0.62	0.04	0.077	+0.067	NS
3-methylhistidine	2.97	2.59	4.72	5.00	4.71	0.26	0.084	+0.030	+0.080
carnosine	1.58^{B}	1.38^{B}	0.87^{B}	2.78^{A}	1.13^{B}	0.10	0.002	NS	NS
hydroxyproline	3.24	4.85	4.39	5.98	4.10	0.21	0.052	NS	NS

SEM – standard error of the mean, EAA – essential amino acids, NEAA – non-essential amino acids; [a] – dietary lactic acid bacterial probiotic supplementation levels for C, L50, L100, L200, and L300 were 0, 50, 100, 200, and 300 ml 1 × 10^9 CFU/ml per sow per day, respectively; NS indicates that no variable met the 0.15 significance level for entry into the model selected by the stepwise method in the REG procedure; '+' indicates a positive effect; '−' indicates a negative effect; [ABC] – values in a row without common superscripts differ significantly at $P < 0.05$

phenylalanine, threonine, alanine, glycine, serine, tyrosine, proline, urea, α-aminoadipate, citrulline, α-aminobutyric acid, cystathinonine, β-alanine, β-aminoisobutyric acid, hydroxylysine, 1-methylhistidine and 3-methylhistidine between the groups ($P > 0.05$). In contrast, the concentrations of leucine, lysine, valine, arginine, cysteine, ethanolamine, and ornithine were all significantly higher in sows from the L50 group compared to the other groups ($P < 0.05$). Sows in the L50 group also had higher serum glutamic acid and p-serine concentrations than those from the L200 and L300 groups ($P < 0.05$). Serum sarcosine and carnosine concentrations were higher in the L200 group than in the C, L100, and L300 groups ($P < 0.05$). Sows in the L100 group had higher serum taurine but lower aspartic acid levels compared to the L50 group ($P < 0.05$). Serum γ-aminobutyric acid ($P < 0.01$) and ethanolamine ($P < 0.05$) concentrations decreased, while 3-methylhistidine ($P < 0.05$) concentrations increased linearly with rising dietary LABP levels.

Discussion

LABP supplementation, particularly at a dosage of 200 ml during late pregnancy and lactation, exerted beneficial effects on weaned piglet weight gain per sow. Previous studies have shown similar outcomes; for instance, piglet weaning weight increased when *Lactobacillus johnsonii* XS4 was supplemented (Wang et al., 2014), or when a 6% mixed selected LABP was included in the sow diet from day 90 of pregnancy until weaning (Wang et al., 2021b). *Lactobacillus* has been detected in sow milk (Martín et al., 2009) and oral fluid (Hattab et al., 2021) after supplementation in the sow's diet. Lactic acid bacteria can transfer from the maternal gut to the mammary gland during late pregnancy and lactation in mice (Perez et al., 2007). In this study, serum TG increased, while total protein levels remained stable as lactation progressed. Similar findings were reported by Lingaas et al. (1992), where total protein and its fractions decreased, while lipid metabolite concentrations increased during the first month of lactation compared to the third month. Protein metabolites (total protein and urea) and lipid metabolites (triglycerides and cholesterol) are key components of milk. Both lipogenesis and lipolysis are crucial in determining plasma TG levels under normal dietary conditions in mice (Zhang et al., 2012). Since the feed intake of sows remained consistent during lactation, the higher serum TG levels observed in the L200 group suggested reduced maternal fat mobilisation or increased hepatic lipogenesis. Plasma glucose is a major precursor for synthesis of milk constituents. The conversion of aspartate to glucose and its subsequent oxidation to carbon dioxide can affect the activity of alkaline phosphatase (ALP). Consistent with previous findings (Zhou et al., 2017), ALP activity increased as lactation progressed. However, the lower serum ALP activity in the L200 group implied a decrease in glucose oxidation in this group compared to the control group. Carnosine is mainly synthesised and stored in skeletal muscle and consists of histidine and β-alanine. However, both histidine and β-alanine concentrations were not affected by LABP treatment in the present study. The elevated serum carnosine levels in L200 could be linked to muscle metabolism or antioxidant function, though further investigation is required to clarify this association.

In the present study, sows from the L50 group had elevated serum concentrations of leucine, valine, cysteine, lysine, glutamic acid, arginine and its intermediary metabolite ornithine. Since blood was sampled at least 8 h post-feeding, free amino acids reflect the availability of amino acids that were removed from the blood and metabolised by the cells (Reverter et al., 2000). There are three main sources of free amino acids in the blood, breakdown of tissue proteins, *in vivo* synthesis of non-essential amino acids, and the digestion and absorption of dietary protein. These free amino acids can be utilised by the lactating mammary gland for milk protein synthesis. Previous research had shown that mammary protein breakdown significantly decreased without affecting milk protein synthesis when sows were fed a diet with excess valine (Trottier et al., 2002). Furthermore, high valine concentrations can antagonise the transport of isoleucine and leucine (Hruby Weston et al., 2024). Oxidation of branched-chain keto acids, such as valine and leucine, results in the formation of acetyl-CoA and succinyl-CoA (Hamaya et al., 2021), which ultimately leads to energy production (Shimomura et al., 2001). Glutamate could be generated through transaminase activity. Sows in the L50 group had higher glutamine levels but lower ALT activity compared to the other groups. Given that feed intake was comparable across groups, the lower ALT activity in L50 suggests that more body reserves may have been mobilised for milk synthesis to meet the nutrient demands of piglets. These findings imply that lactic acid probiotic

supplementation may positively influence piglet growth by affecting host metabolism.

Supplementing lactating sows with *Lactobacilli* has been shown to improve piglet survival. For instance, maternal diets fermented with *Bacillus subtilis* and *Enterococcus faecium* have been found to reduce offspring susceptibility to colonic inflammation by increasing the abundance of gut *Lactobacillus* population and enhancing microbial metabolic functions (Wang et al., 2022a). Additionally, *Lactobacillus* supplementation in the diets of sows or piglets has been linked to increased activity of antioxidant enzymes and improved serum immune indices (Wang et al., 2021b). A similar antioxidant effect was observed for *Lactiplantibacillus plantarum* Inducia in an overweight volunteer, where it reduced oxidative stress and total peroxide levels (Štšepetova et al., 2023). In the present study, LABP supplementation in the L50 and L100 groups significantly increased glutamic acid concentrations compared to the control group. There was a significant positive correlation between L-glutamine in milk and gut *Lactobacilli* in piglets (Wang et al., 2022a). Lactobacilli are recognised as beneficial microbiota due to their ability to lower environmental pH (Alakomi et al., 2000) and produce health-promoting factors such as bacteriocins or conjugated linoleic acid (O'Shea et al., 2012). Moreover, supplementing weaned piglets' diets with *L. reuteri* P7, *L. amylovorus* P8, and *L. johnsonii* P15 has been shown to reduce diarrhoea incidence by inhibiting enterotoxigenic *Escherichia coli* K88 (Wang et al., 2021b). Cholesterol levels decreased linearly with increasing dietary LABP doses, a similar effect to that previously observed in mice administered *Lactobacillus acidophilus* ATCC43121 and *L. fermentum* MF27 (Oh et al., 2021). Cholesterol is involved in maintaining homeostasis in response to stress (Wang et al., 2022b). Therefore, the improved piglet survival rate linked to LABP supplementation has been attributed to its influence on maternal amino acid or cholesterol metabolism, indicating a metabolic adaptation that supports better reproductive outcomes.

Conclusions

The result demonstrated that the application of lactic acid bacterial probiotic (LABP) during late pregnancy and lactation in Xiangcun Black sows had a beneficial effect on weaned piglet weight gain and survival. Serum alkaline phosphatase activity and total cholesterol levels decreased, while triglyceride concentrations increased linearly with rising dietary LABP supplementation. Additionally, serum amino acid concentrations such as carnosine and glutamic acid were modulated by dietary LABP addition during lactation. This study expands our knowledge regarding strategies promoting health and growth of piglets through the maternal administration of lactic acid bacterial probiotic.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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