Probiotics decrease the stress response and intestinal permeability of term neonates with low Apgar scores

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Abstract. To observe the effect of probiotics on the stress responses and intestinal permeability of term neonates with low Apgar scores, the present study retrospectively analyzed the clinical data of 78 term neonates (42 males and 36 females). In the control group (n=38), total parenteral nutrition and comprehensive treatment (anti-infection therapy) were provided. In the observation group (n=40), the neonates were administered *Lactobacillu*s Complex Capsules in addition to the control group treatment. The corticotropin-releasing factor level was determined using ELISA; cortisol levels were determined using a radioimmunoprecipitation assay; D-lactate and diamine oxidase levels were determined using ultraviolet spectrometry; procalcitonin levels were determined using ECL; and C-reactive protein levels were determined using a protein analyzer. Following treatment, the levels of all parameters were lower in the observation group compared with the control group, and the differences were statistically significant (P<0.05). In the observation group, the daily milk intake was 16.57±2.58 ml, which was significantly higher than that of the control group (13.26±1.87 ml), while the length of hospital stay and total parenteral nutrition time, which were 12.31±2.02 and 6.21±1.26 days, respectively, in the observation group, were significantly shorter than those of the control group (14.86±2.58 and 8.86±1.78 days, respectively), and the differences were statistically significant (P<0.001). The results of the present study suggested that probiotics can ameliorate the stress response and intestinal permeability of term neonates with low Apgar scores, thereby, facilitating gastrointestinal function recovery.

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Introduction

Neonatal asphyxia refers to an anomaly in the respiration of neonates, caused by various factors, such as inadequate oxygen levels in the mother's blood or lowered respiration rates, before, during or after birth, that can damage multiple systems, and involves severe sequelae or can even cause mortality (1). Neonatal asphyxia usually arises as hypoxia, ischemia and abnormal metabolism; respiratory failure blocks alveolar gas exchange, which causes hypoxia (2). In neonates, the systemic and organ damage severity is proportionately associated with the severity of hypoxia (3). Out of all neonates, ~4,000,000 succumb to neonatal asphyxia, accounting for nearly 23% of cases of mortality in developing countries in 2013 (4,5). A previous study demonstrated that neonatal asphyxia is a major cause of mortality within 1 week after birth, and a factor contributing to perinatal mortality, neurologic handicap and developmental dysfunction (6). The Apgar scale is a convenient and widely accepted method that is frequently used to evaluate neonatal asphyxia; a 1-min Apgar score <7 suggests mild asphyxia, while a score <3 suggests severe asphyxia (7,8).

Perinatal hypoxia and infection may result in potent postnatal stress responses, impacting the neuroendocrine and gastrointestinal systems (9,10). The decrease in gastrointestinal permeability and increased intestinal inflammation further contribute to systemic organ dysfunction, since inflammatory factors pass through the intestinal mucosa (11). Probiotics are characterized by their ability to sustain the microecological balance within the intestines, and regulate the neuroendocrine functions in stress responses. They can protect the intestinal mucosa and ameliorate intestinal barrier function by facilitating cell proliferation and migration, which prevents cell apoptosis and enhance sprotein synthesis (12). A study demonstrated that probiotics can serve as an auxiliary treatment for stress-induced intestinal responses in neonatal mice, with significant improvements noted in gastrointestinal function (13).

Since, to the best of our knowledge, no studies have reported the stress response and intestinal permeability of term neonates with low Apgar scores, the present study aimed to elucidate the effect of probiotics on the stress responses and intestinal permeability of term neonates with low Apgar scores to provide a theoretical basis for clinical treatment.

Materials and methods

General materials. The present study retrospectively analyzed the clinical data of 78 term neonates (42 males and 36 females) who were admitted to the Department of Neonatology at The Second Hospital of Lanzhou University (Lanzhou, China) for treatment between March 2017 and March 2018. Neonates born to mothers with a history of smoking or drug abuse, metabolic depression, hypertension during pregnancy, and acute infectious diseases were excluded from the present study. Additionally, all neonates with congenital heart disease, early-stage infection, aspiration pneumonia, gastrointestinal deformity or bleeding were excluded from the present study. Neonates were born at 37-41 weeks' gestation, weighed 2-4 kg and had Apgar scores of <7 points (14). In the control group (n=38), total parenteral nutrition and comprehensive treatment (anti-infection therapy) were provided; in the observation group (n=40), *Lactobacillus* Complex Capsules were administered to the neonates in addition to the control group treatment. Due to ethical reasons, a placebo only treatment was not approved. The present study was approved by the Ethics Committee of The Second Hospital of Lanzhou University, and the family of each neonate provided written informed consent.

Treatment methods. Following resuscitation, neonates in the control group received total parenteral nutrition and anti-infection therapy, in addition to regular nursing, which is standard treatment. Breast feeding was prioritized, but formula milk (Mead Johnson & Company, LLC) was considered, and the milk intake was increased according to the condition of the neonate. During the treatment period, a number of neonates were infected and then received anti-infection therapy. The anti-infection therapy regimen was: Neonates were given ampicillin (100 mg/kg/day) for 3 days. Neonates that had a positive penicillin skin test were given cefazolin (50 mg/kg/day) but not ampicillin for 3 days. On the third day, neonates with normal peripheral blood C-reactive protein (CRP) levels stopped antibiotics treatment. When the intake was ≥120 ml/kg/day, the feeds were gradually transitioned to total parenteral nutrition. In addition to the treatment provided in the control group, neonates in the observation group were given probiotics treatment and were administered with *Bifidobacterium* triple live capsules (Shanghai Shanyao Xinyi Pharmaceutical Co., Ltd.; Guoyao Zhunzi approval no. S10950032). Each capsule contained 210 mg powder, and the number of live bacteria was $>1.0x10^7$ CFU. The capsule was dissolved in warm water (<40˚C), and half a capsule was administered twice/day for 7 consecutive days.

Detection of indices of stress response and intestinal permeability. At 1 day before treatment and 7 days after treatment, 2 ml of venous blood was drawn from the neonates prior to the intravenous infusion and breast feeding, and the serum was isolated via centrifugation at 1,500 x g for 5 min at 25-30˚C for later use. Corticotropin-releasing factor (CRF) levels were determined using ELISA (cat. no. DECO0324; Beijing Zhongke Quality Inspection Biotechnology Co., Ltd.), cortisol using a radioimmunoprecipitation assay (cat. no. 2114-500; Biovision, Inc.), D-lactate and diamine oxidase (DAO) using an ultraviolet spectrometer (UVS-99; Avans Biotechnology Inc.), procalcitonin (PCT) using ECL (cat. no. 1705060; Bio-Rad Laboratories, Inc.), and CRP using a protein analyzer (cat. no. HC01001319; Gerhardt GmbH). All procedures were conducted in strict accordance with the kit instructions, and each experiment was conducted in triplicate and the results were averaged. Additionally, the mean daily milk intake during 7 days of treatment, length of hospital stay (LOS) and total parenteral nutrition duration were recorded.

Radioimmunoprecipitation assay was performed as follows: A total of 10 μl cortisol blank serum and 10 μl serum samples were added to two coating tubes respectively. Each tube was added with 1.0 ml labelling buffer solution and mixed by low speed vortex at 37˚C for 46 min, following which excess liquid was removed. ^{125}Iodine-labeled cortisol in the remaining liquid was then counted in the γ-counter for 1 min and cortisol concentration was calculated using a standard cortisol curve at 1, 3, 10, 25 and 60 μg/dl with repetitions of the steps described above. ECL was performed using a fully automated chemiluminescence immunoassay analyzer (SMART 300; Chongqing Keysmile Biotechnology Co., Ltd.)

Statistical analysis. SPSS software (version 17.0; SPSS, Inc.) was utilized for the data analyses. Enumeration data were compared using a χ^2 test. Measurement data are presented as the means ± SD and were compared using a Kolmogorov-Smirnov test. All data was normally distributed and analyzed using at-test. The intragroup comparisons before and after treatment were conducted with a paired t-test. Repeated measures ANOVA with Bonferroni post hoc test was used for multiple comparisons. All data were calculated from three experiments. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Comparison of general data. There were no significant differences identified in gestational age, sex, weight, Apgar score, type of birth and intrauterine hypoxia history between the observation and control groups ($P>0.05$; Table I).

Stress response of neonates before vs. after treatment. The cortisol, CRF and CRP levels were significantly lower after treatment compared with before treatment in the two groups ($P<0.001$). Before treatment, there were no significant intergroup differences ($P>0.05$), but after treatment, the levels in the observation group were all significantly lower than those in the control group ($P<0.05$; Fig. 1; Table II).

Intestinal permeability of neonates before vs. after treatment. The D-lactate, PCT and DAO levels of the observation and control groups before treatment were 1.59±0.28 and 1.57±0.26 mM, 1.08±0.15 and 1.05±0.13 ng/l, and 18.62±3.14 and 18.12±2.98 U/l, respectively. The D-lactate, PCT and DAO levels of the observation and control groups after treatment were 0.68±0.12 and 1.09±0.18 mM, 0.24±0.05 and 0.62±0.08 ng/l, and 5.63±1.35 and 9.82±2.46 U/l, respectively. The D-lactate, PCT and DAO levels were significantly lower

Table I. Comparison of the general data of the observation and control groups.

Factors	Observation group (n=40), n (%)	Control group (n=38), n (%)	t or χ^2	P-value
Sex			1.251	0.364
Male	24 (60.00)	18 (47.37)		
Female	16 (40.00)	20 (52.63)		
Gestational age (weeks)	38.96±1.02	38.73±0.98	1.015	0.314
Height (cm)	48.63±3.56	49.04±2.97	0.551	0.583
Weight (kg)	3.86±0.38	3.77±0.34	1.100	0.275
1-min Apgar score	5.26±0.81	5.41±0.89	0.779	0.438
5-min Apgar score	6.41±1.26	6.58±1.12	0.629	0.532
Type of birth			0.425	0.607
Natural labor	9 (22.50)	11 (28.95)		
Cesarean section	31 (77.50)	27 (71.05)		
Intrauterine hypoxia			0.167	0.815
Yes	26 (65.00)	23 (60.53)		
No	14 (35.00)	15 (39.47)		

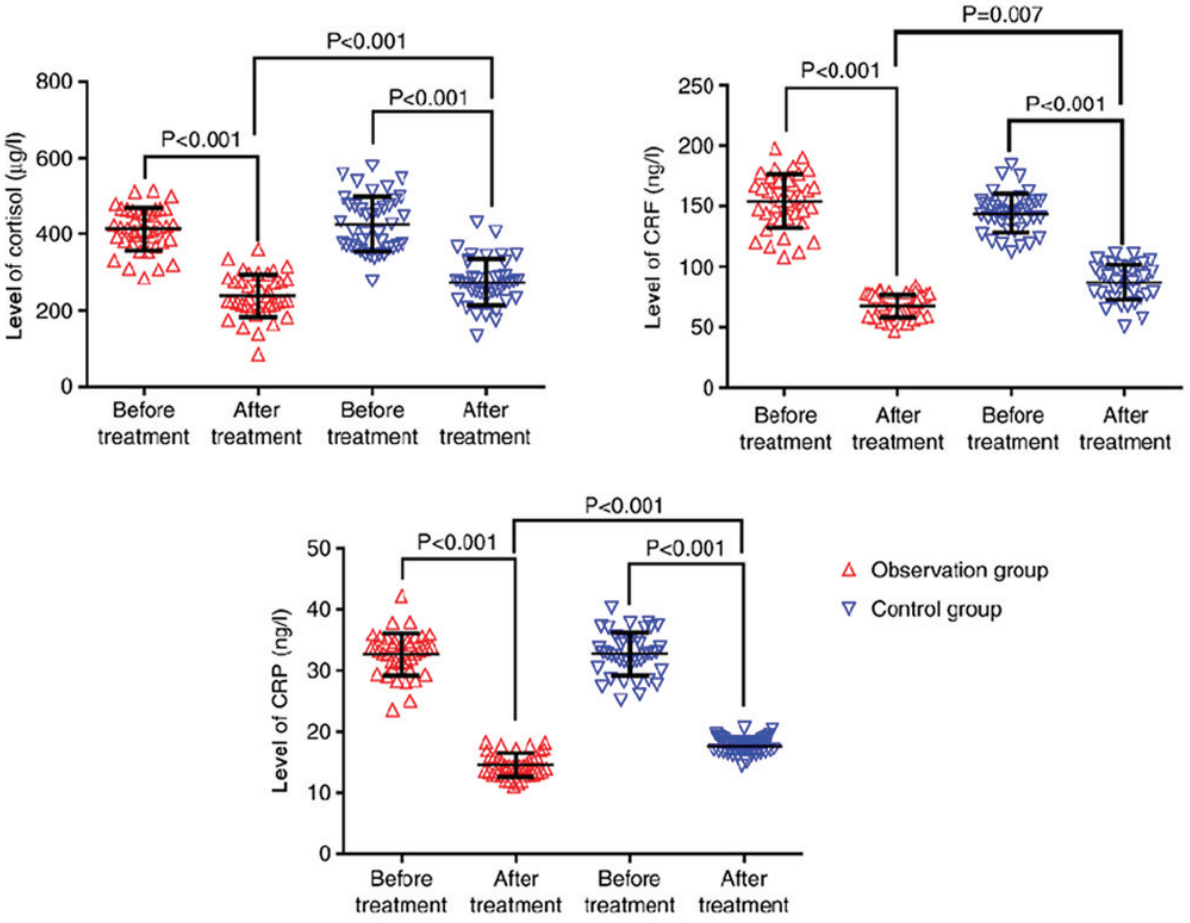

Figure 1. Comparison of stress indexes in the observation and control groups before and after treatment. CRF, corticotropin-releasing factor; CRP, C-reactive protein.

after treatment compared with before treatment in the two groups (P<0.001). Before treatment, there were no significant intergroup differences (P>0.05); however, after treatment, the levels in the observation group were all significantly lower than those in the control group (P<0.001; Fig. 2; Table III).

Gastrointestinal function recovery of neonates. The mean daily milk intake was significantly higher in the observation group (16.57±2.58 ml) than in the control group (13.26±1.87 ml), while the mean LOS and total parenteral nutrition duration of the observation group (12.31±2.02 and 6.21±1.26 days)

Table II. Comparison of stress responses of neonates before vs. after treatment in the observation and control groups.

Group	Number (n)	Time point	Cortisol (μg/l)	CRF (ng/l)	CRP (ng/l)
Observation	40	Before treatment	147.56±21.05	421.65±63.75	32.53±3.16
		After treatment	68.63±11.58[a]	235.82±42.32[a]	14.64±1.65[a]
		t	20.78	15.36	31.74
		P-value	<0.001	<0.001	<0.001
Control	38	Before treatment	144.67±19.68	416.58±76.15	31.78±3.56
		After treatment	84.62±14.53	265.37±51.26	17.35±1.52
		t	15.13	10.15	22.98
		P-value	<0.001	<0.001	<0.001

[a]P<001 vs. control group. CRF, corticotropin-releasing factor; CRP, C-reactive protein.

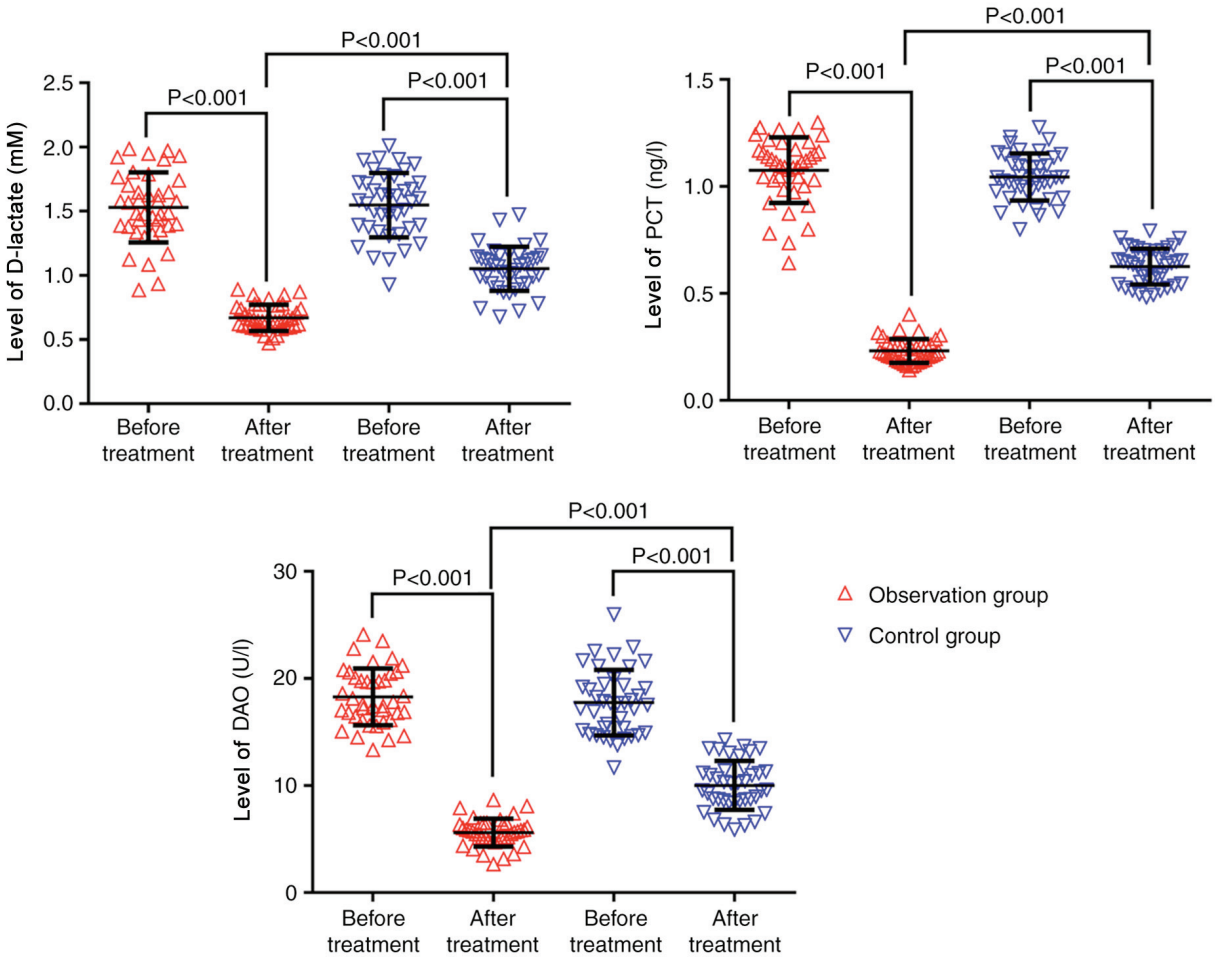

Figure 2. Comparison of intestinal permeability in the observation and control groups of neonates before and after treatment. DAO, diamine oxidase; PCT, procalcitonin.

were significantly shorter than those of the control group (14.86±2.58 and 8.86±1.78 days; P<0.001; Fig. 3; Table IV).

Infections during treatment. In the observation and control groups, there were 4 cases (10.0%) and 11 cases (28.95%) with infections during the treatment. The occurrence of infection in the observation group was significantly lower than that in the control group (P<0.05). In the observation group, one case of umbilical inflammation and three cases of bacterial pneumonia occurred. In the control group, two cases of umbilical inflammation, one case of scleredema, one case of impetigo and seven cases of bacterial pneumonia occurred (Table V). No sepsis and other infectious diseases, including bacterial meningitis, occurred in either group. All infections were controlled after treatment with the anti-infective regimen.

Table III. Comparison of intestinal permeability of neonates before vs. after treatment in the observation and control groups.

Group	Number (n)	Time point	D-lactate (mM)	PCT (ng/l)	DAO (U/l)
Observation	40	Before treatment	1.59±0.28	1.08±0.15	18.62±3.14
		After treatment	0.68±0.12[a]	0.24±0.05[a]	5.63±1.35[a]
		t	18.89	33.60	24.04
		P-value	<0.001	<0.001	<0.001
Control	38	Before treatment	1.57±0.26	1.05±0.13	18.12±2.98
		After treatment	1.09±0.18	0.62±0.08	9.82±2.46
		t	9.433	17.48	13.24
		P-value	<0.001	<0.001	<0.001

[a]P<001 vs. control group. DAO, diamine oxidase; PCT, procalcitonin.

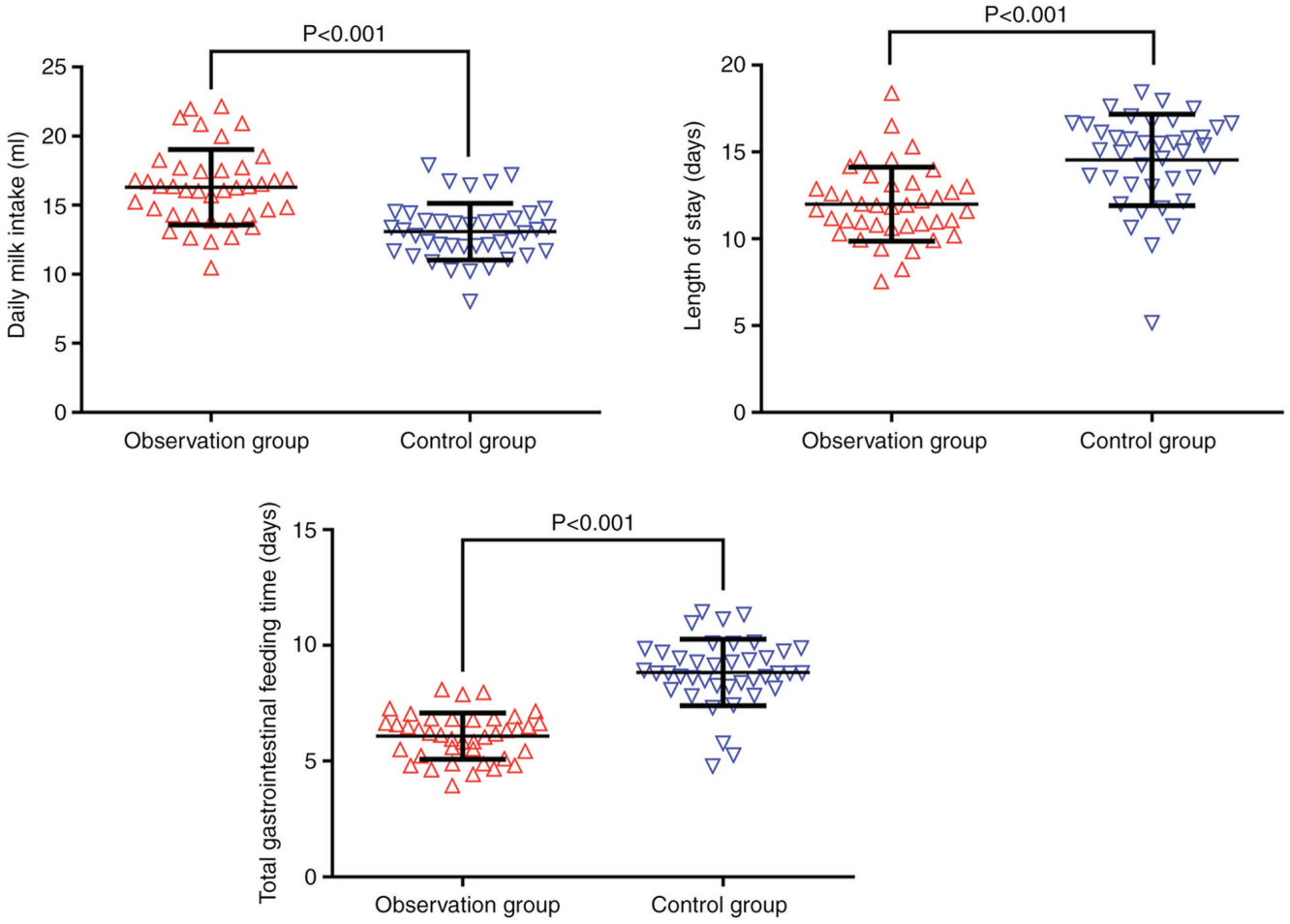

Figure 3. Comparison of intestinal tract recovery in the observation and control groups of neonates.

Discussion

Neonates with low Apgar scores are more susceptible to hypoxia and ischemia, which cause organ damage and functional anomalies (15). Due to the vulnerability of the neonatal gastrointestinal tract mucosa this can result in abdominal distension, milk regurgitation or even necrotic colitis (16). The neonatal gastrointestinal tract is developing, which makes it more vulnerable to bacterial invasion via the gut due to decreased amounts of bile acids and gastric acid, particularly in the presence of mucosal damage (17). An animal experiment revealed that probiotics can regulate intestinal barrier function long after the stress responses (18). Additionally, a clinical trial demonstrated that probiotics can improve epithelial barrier function (19). Therefore, it is rational to use probiotics in the treatment of stress responses and intestinal permeability in cases of neonatal asphyxia.

Table IV. Comparison of gastrointestinal function recovery of neonates before vs. after treatment in the observation and control groups.

Group	Number (n)	Daily milk intake (ml)	Length of stay in hospital (days)	Total gastrointestinal feeding time (days)
Observation	40	16.57±2.58	12.31±2.02	6.21±1.26
Control	38	13.26±1.87	14.86±2.58	8.86±1.78
T		6.459	4.874	7.619
P-value		<0.001	<0.001	<0.001

Table V. Comparison of the infections in the observation and control groups.

Group	Number (n)	Umbilical inflammation (n)	Bacterial pneumonia (n)	Scleredema (n)	Impetigo (n)	Total infection, n (%)
Observation	40	1	3	0	0	4 (10.00)
Control	38	2	7	1	1	11 (28.95)
t						4.504
P-value						0.034

The severity of neonatal asphyxia is associated with the circulating cortisol level (20). CRF, a neuroendocrine peptide composed of several amino acids, can regulate stress responses in humans and animals (21). In neonatal blood, CRP levels are regulated by multiple factors, including neonatal asphyxia, intracranial infection, premature rupture of the fetal membranes and marked alterations in humoral immune responses after birth (22). Therefore, in the present study, cortisol, CRF and CRP levels served as indicators of the degree of the stress response.

Damage to the intestinal mucosa also affects the microecological balance in the intestinal bacterial community due to the production of large amounts of D-lactate which can be rapidly delivered into the blood circulation due to increased intestinal permeability (23). Additionally, damage to the intestinal mucosa and an increase in intestinal permeability enhance DAO activity (24). A study on PCT that mainly focused on acute infections suggested that PCT levels are physiologically increased following premature birth, primarily due to neonatal asphyxia (25). Thus, D-lactate, PCT and DAO were selected as indicators of intestinal permeability in the present study.

In the present study, the levels of cortisol, CRF, CRP, D-lactate, PCT and DAO in the observation and control groups were significantly lower after treatment compared with before treatment (P<0.001). Prior to treatment, intergroup comparisons exhibited no statistically significant differences in the levels of cortisol, CRF, CRP, D-lactate, PCT and DAO (P>0.05), whereas after treatment, these levels in the observation group were all significantly lower than those in the control group (P<0.05). These results suggested that treatment in combination with probiotics can mitigate the stress response and intestinal permeability of term neonates with low Apgar scores.

Among the few studies reporting on asphyxia in term neonates, some reported that probiotics can sustain the balance of intestinal bacteria and promote maturation of the gastrointestinal function, while reducing the incidence of feeding intolerance in premature neonates; thus, probiotics have been widely used in the treatment of gastrointestinal diseases in infants (26,27). In the present study, the daily mean milk intake was significantly higher in the observation group (16.57±2.58 ml) than in the control group (13.26±1.87 ml; P<0.001), which suggested that probiotics can facilitate the recovery of gastrointestinal function of term neonates with low Apgar scores. Probiotics treatment could reduce the stress response and accelerated the recovery of gastrointestinal function. Probiotics interact with other anaerobic bacteria to form a natural barrier, which can efficiently reduce or even block the contact of intestinal mucosa with pathogenic microorganisms (28). Additionally, probiotics can induce the immune response, enhance cellular immunity and mitigate stress responses (29,30).

In summary, the administration of probiotics in combination with standard treatment could mitigate the stress response and decreased the intestinal permeability of term neonates with low Apgar scores to protect and facilitate gastrointestinal function recovery. Therefore, the results of the present study provided a theoretical basis for clinical treatment and contribute to the wide application of probiotics in clinical practice.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JW conceived and designed the study and interpreted the results of the experiments. JZ and YH contributed to the design of the study and the interpretation of experimental results. JC performed experiments, analyzed data, prepared figures and drafted the manuscript. JW and YH approved final version of manuscript. YH edited and revised manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Second Hospital of Lanzhou University, and the family of each neonate provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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