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Original Research

Effects of *Lactobacillus rhamnosus* Supplementation on Growth Performance, Immune Function, and Antioxidant Capacity of Newborn Foals

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a r t i c l e i n f o

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A B S T R A C T

This study aimed to explore the effects of *Lactobacillus rhamnosus* GG (LGG) supplementation on the growth performance, immune function, and antioxidant capacity of foals. Fifteen newborn foals with similar birth weight (51.67 \pm 6.07 kg) and good health were randomly assigned to three groups: control group and test groups I and II, which were supplemented with 5.0×10^9 CFU/day and 1.0×10^{10} CFU/day 100211 0

increase the number of neutrophils, improve phagocytosis, and increase immunoglobulin expression [\[16\].](#page-6-0)

Lactobacillus rhamnosus GG (LGG) is a gram-positive facultative anaerobic bacterium first

Fig. 1. Effect of supplementary LGG feeding on foal growth. (A) is the daily average body weight increase, (B) is the daily average body height increase, (C) is the daily average body length increase, and

J. Shi, G. Zhao, X. Huang et al. Journal of Equine Veterinary Science 129 (2023) 104501

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bacterial structure of LGG can also enhance intestinal development in animals. In the present study, newborn foals were supplemented with LGG. LGG did not significantly alter the growth of young foals initially. However, the daily gains in weight, height, and chest circumference significantly increased with time. This finding may be attributed to the LGG count and duration of LGG colonization and function in the animals' gastrointestinal tract.

Newborn foals obtain their immunoglobulins mainly through breast milk, which contains a high concentration of IgA and IgG. Most of the IgA remains in the intestinal mucosa to strengthen its immunological barrier function, whereas IgG enters the bloodstream through the small intestinal wall to participate in humoral immunity. Studies have shown that adding LGG to infant food can effectively improve the immunoglobulin content in blood. Yan et al. [\[27\]](#page-6-0) found that LGG gavage can effectively promote mouse growth and significantly improve IgA production. In the present study, supplementary LGG feeding could effectively increase plasma IgA and IgG levels in foals, particularly at 30 and 150 days. High dose LGG could more effectively increase IgA and IgG plasma levels and improve the humoral immunity of foals.

Grabig et al. [\[28\]](#page-6-0) found that supplementation with probiotics can effectively increase the expression of TLR4, which activates MyD88 and NF-κB signaling to increase the expression of proin-flammatory factors. Yoo et al. [\[29\]](#page-6-0) reported that some bacteria produce SCFAs by fermenting carbohydrates to regulate host immune cells and provide a carbon source for colon cells. Studies have shown that lactic acid bacteria and their cell wall components can act on human peripheral blood mononuclear cells and promote TNF- α , IL-6, and IL-10 secretion, thereby enhancing immunity. For instance, Miettinen et al. [\[30\]](#page-6-0) showed that LGG can act on cytokines in animal blood and promote TNF- α , IL-6, and IL-10 production, which can alleviate immune system disorders caused by the intake of pathogenic bacteria. Other studies have shown that LGG can inhibit inflammatory responses. For example, LGG can inhibit the signal transduction of lipopolysaccharide receptor TLR4, p65/NF-kB, p38/MAPK, and ERK1/2 and downregulate TNF- α and IL-6 through TLR4 and TLR9 expression to reduce the inflammatory response $[31-34]$. Zhang et al. $[35]$ showed that adding LGG to the diet of weaned piglets can inhibit the increase in IL-6, IL-1β, and TNF-α expression caused by *Escherichia coli* and reduce the inflammatory response of piglets. Additionally, Pena et al. [\[36\]](#page-6-0) cultivated intestinal mouse microorganisms in vitro and showed that LGG can act on macrophages and inhibit TNF- α secretion to alleviate and prevent intestinal inflammation; however, the effect on IL-10 was not significant, and the mechanism by which LGG acts on macrophages remains unclear. In the present study, LGG promoted the inflammatory response of foals in the early stage of the study, increasing IL-6, IL-1 β , and TNF- α secretion. In later stages, LGG inhibited the expression of proinflammatory factors and upregulated IFN- γ to reduce the inflammatory damage to cells. Wu [\[37\]](#page-6-0) proposed that probiotics may act as microbial antigens in the underdeveloped digestive tract of young animals, stimulate the regulation of intestinal mucosal immunity, promote the expression of TLRs, and stimulate the production of downstream cytokines. Wu [\[37\]](#page-6-0) demonstrated that LGG stimulated the innate immunity of foals when they were young, improves their defense against pathogens, and inhibited the inflammatory response caused by pathogens at the age of 150 days.

Animals contain a high concentration of unsaturated fatty acids, which are susceptible to free radical damage, thus producing cytotoxic peroxides [\[38\].](#page-6-0) In the normal state, the free radicals in the body are balanced, but when stimulated by drugs, inflammation, and emotional tension, the level of free radicals increases markedly, causing damage to animal cell structure and organs. T-AOC reflects the body's ability to compensate for external stimuli and the strength of the body's free radical metabolism [\[39\].](#page-6-0) SOD

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eliminates the toxicity of superoxide anion, protecting cells from oxidative damage [\[40\].](#page-6-0) CAT can decompose hydrogen peroxide in the body and prevent the formation of free radicals. GSH-Px is an important enzyme for scavenging organic hydroperoxides that replaces catalase and scavenges hydrogen peroxide in tissues with low catalase concentration. MDA, a product of free radical-induced lipid peroxidation, exhibits cytotoxicity and genotoxicity

- [2] Quercia S, Freccero F, Castagnetti C, Soverini M, Turroni S, Biagi E, Rampelli S, Lanci A, Mariella J, Chinellato E, Brigidi P, Candela M. Early colonisation and temporal dynamics of the gut microbial ecosystem in Standardbred foals. Equine Vet J 2019;51(2):231–7. doi[:10.1111/evj.12983.](https://doi.org/10.1111/evj.12983)
- [3] Frizzo LS, Zbrun MV, Soto LP, Signorini ML. Effects of probiotics on growth performance in young calves: a meta-analysis of randomized controlled trials. Anim Feed Sci Technol 2011;169(3-4):147–56. [doi:10.1016/j.anifeedsci.2011.06.](https://doi.org/10.1016/j.anifeedsci.2011.06.009) 009
- [4] Morley PS, Townsend H. A survey of reproductive performance in thoroughbred mares and morbidity, mortality and athletic potential of their foals. Equine Vet J 1997;29(4):290-7. doi:10.1111/j.2042-3306.1997.tb03126.x
- [5] Steiner N, Lindner A. Reproduction data in breeding mares, diseases and losses among suckling foals and preventive husbandry in German stud farms. Tierarztl Prax [1993;21\(4\):316–22.](http://refhub.elsevier.com/S0737-0806(23)00291-5/sbref0005)
- [6] Frederick J, Giguere S, Sanchez LC. Infectious agents detected in the feces of diarrheic foals: a retrospective study of 233 cases (2003–2008). J Vet Intern Med 2009:23(6):1254-60. doi:10.1111/j.1939-1676.2009.0383.x
- [7] Schiffrin EJ, Blum S. Interactions between the microbiota and the intestinal mucosa. Eur J Clin Nutr 2002;56(3):S60–4. doi[:10.1038/sj.ejcn.1601489.](https://doi.org/10.1038/sj.ejcn.1601489)
- [8] Lewis BB, Buffie CG, Carter RA, Leiner I, Toussaint NC, Miller LC, Gobourne A, Ling L, Pamer EG. Loss of microbiota-mediated colonization resistance to Clostridium difficile infection with oral vancomycin compared with metronidazole. J Infect Dis 2015;212(10):1656–65. doi[:10.1093/infdis/jiv256.](https://doi.org/10.1093/infdis/jiv256)
- [9] Pérez-Cobas AE, Moya A, Gosalbes MJ, Latorre A. Colonization resistance of the gut microbiota against Clostridium difficile. Antibiotics 2015;4(3):337–57. doi[:10.3390/antibiotics4030337.](https://doi.org/10.3390/antibiotics4030337)
- [10] Barrow PA, Brooker BE, Fuller R, Newport MJ. The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine. J Appl Bacteriol 1980;48(1):147–54. [doi:10.1111/j.1365-2672.1980.](https://doi.org/10.1111/j.1365-2672.1980.tb05216.x) tb05216.x.
- [11] Fuller R, Turvey A. Bacteria associated with the intestinal wall of the fowl (Gallus domesticus). J Appl Bacteriol 1971;34(3):617–22. [doi:10.1111/j.1365-2672.](https://doi.org/10.1111/j.1365-2672.1971.tb02325.x) 1971.th02325.x
- [12] Tejero-Sariñena S, Barlow J, Costabile A, Gibson GR, Rowland I. Antipathogenic activity of probiotics against Salmonella Typhimurium and Clostridium difficile in anaerobic batch culture systems: is it due to synergies in probiotic mixtures or the specificity of single strains? Anaerobe 2013;24:60–5. doi[:10.1016/j.anaerobe.2013.09.011.](https://doi.org/10.1016/j.anaerobe.2013.09.011)
- [13] Kareem KY, Hooi LF, Teck CL, May FO, Anjas AS. Inhibitory activity of postbiotic produced by strains of Lactobacillus plantarum using reconstituted media supplemented with inulin. Gut Pathogens 2014;6(1):1–7. doi:10.1186/ [1757-4749-6-23.](https://doi.org/10.1186/1757-4749-6-23)
- [14] Fuller R. Probiotics in man and animals. J Appl Bacteriol 1989;66(5):365-78. doi[:10.1111/j.1365-2672.1989.tb05105.x.](https://doi.org/10.1111/j.1365-2672.1989.tb05105.x)
- [15] Bogere P, Choi YJ, Heo J. Probiotics as alternatives to antibiotics in treating post-weaning diarrhoea in pigs: review paper. S Afr J Anim Sci 2019;49(3):403–16. doi[:10.4314/sajas.v49i3.1.](https://doi.org/10.4314/sajas.v49i3.1)
- [16] Scharek L, Guth J, Reiter K, Weyrauch KD, Taras D, Schwerk P, Schmidt MFG, Wieler LH, Tedin K. Influence of a probiotic Enterococcus faecium strain on development of the immune system of sows and piglets. Vet Immunol Immunopathol 2005;105(1-2):151–61. doi[:10.1016/j.vetimm.2004.12.022.](https://doi.org/10.1016/j.vetimm.2004.12.022)
- [17] Goldstein EJ, Tyrrell KL, Citron DM. Lactobacillus species: taxonomic complexity and controversial susceptibilities. Clin Infect Dis 2015;60(suppl_2):S98– S107. doi[:10.1093/cid/civ072.](https://doi.org/10.1093/cid/civ072)
- [18] Tsai CC, Chan CC, Huang WY, Lin JS, Chan P, Liu HY, Lin YS. Applications of Lactobacillus rhamnosus spent culture supernatant in cosmetic antioxidation, whitening and moisture retention applications. Molecules 2013;18(11):14161– 71. doi[:10.3390/molecules181114161.](https://doi.org/10.3390/molecules181114161)
- [19] Lam EK, Yu L, Wong HP, Wu WK, Shin VY, Tai EK, So WH, Woo PC, Cho CH. Probiotic Lactobacillus rhamnosus GG enhances gastric ulcer healing in rats. Eur J Pharmacol 2007;565(1-3):171–9. doi[:10.1016/j.ejphar.2007.02.050.](https://doi.org/10.1016/j.ejphar.2007.02.050)
- [20] Collado MC, Meriluoto J, Salminen S. In vitro analysis of probiotic strain combinations to inhibit pathogen adhesion to human intestinal mucus. Food Res Int 2007;40(5):629–36. doi[:10.1007/s00284-007-0144-8.](https://doi.org/10.1007/s00284-007-0144-8)
- [21] Martin-Gallausiaux C, Béguet-Crespel F, Marinelli L, Jamet A, Ledue F, Blottière HM, Lapaque N. Butyrate produced by gut commensal bacteria activates TGF-beta1 expression through the transcription factor SP1 in human intestinal epithelial cells. Sci Rep 2018;8(1):1–13. doi[:10.1038/s41598-018-28048-y.](https://doi.org/10.1038/s41598-018-28048-y)
- [22] Macfarlane GT, Steed H, Macfarlane S. Bacterial metabolism and healthrelated effects of galacto-oligosaccharides and other prebiotics. J Appl Microbiol 2008;104(2):305-44. doi:10.1111/j.1365-2672.2007.03520.x
- [23] Zhang L, Jiang X, Liu X, Zhao X, Liu S, Li Y, Zhang Y, Growth, health, rumen fermentation, and bacterial community of Holstein calves fed Lactobacillus rhamnosus GG during the preweaning stage. J Anim Sci 2019;97(6):2598– 608. doi[:10.1093/jas/skz126.](https://doi.org/10.1093/jas/skz126)
- [24] Luise D, Spinelli E, Correa F, Nicodemo A, Bosi P, Trevisi P. The effect of a single, early-life administration of a probiotic on piglet growth performance and faecal microbiota until weaning. Ital J Anim Sci 2021;20(1):1373–85. doi[:10.1080/1828051x.2021.1952909.](https://doi.org/10.1080/1828051x.2021.1952909)
- [25] Zapata O, Cervantes A, Barreras A, Monge-Navarro F, González-Vizcarra VM, Estrada-Angulo A, Urías-Estrada JD, Corona L, Zinn RA, Martínez-Alvarez IG, Plascencia A. Effects of single or combined supplementation of probiotics and prebiotics on ruminal fermentation, ruminal bacteria and total tract digestion in lambs. Small Rumin Res 2021;204:106538. [doi:10.1016/j.smallrumres.2021.](https://doi.org/10.1016/j.smallrumres.2021.106538) 106538.
- [26] Kang J, Lee JJ, Cho JH, Choe J, Kyoung H, Kim SH, Kim HB, Song M. Effects of dietary inactivated probiotics on growth performance and immune responses of weaned pigs. J Anim Sci Technol 2021;63(3):520. doi[:10.5187/jast.2021.e44.](https://doi.org/10.5187/jast.2021.e44)
- [27] Yan F, Liu L, Cao H, Moore DJ, Washington MK, Wang B, Peek RM, Acra SA, Polk DB. Neonatal colonization of mice with LGG promotes intestinal development and decreases susceptibility to colitis in adulthood. Mucosal Immunol 2017;10(1):117–27. doi[:10.1038/mi.2016.43.](https://doi.org/10.1038/mi.2016.43)
- [28] Grabig A, Paclik D, Guzy C, Dankof A, Baumgart DC, Erckenbrecht J, Raupach B, Sonnenborn U, Eckert J, Schuman RR, Wiedenmann B, Dignass AU, Sturm A. Escherichia coli strain Nissle 1917 ameliorates experimental colitis via tolllike receptor 2-and toll-like receptor 4-dependent pathways. Infect Immun 2006;74(7):4075–82. doi[:10.1128/iai.01449-05.](https://doi.org/10.1128/iai.01449-05)
- [29] Yoo JY, Groer M, Dutra SVO, Sarkar A, McSkimming DI. Gut microbiota and immune system interactions. Microorganisms 2020;8(10):1587. doi:10.3390/ [microorganisms8101587.](https://doi.org/10.3390/microorganisms8101587)
- [30] Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect Immun 1996;64(12):5403–5. doi[:10.1128/iai.64.12.5403-5405.1996.](https://doi.org/10.1128/iai.64.12.5403-5405.1996)
- [31] El-Nezami HS, Chrevatidis A, Auriola S, Salminen S, Mykkänen H. Removal of common Fusarium toxins in vitro by strains of Lactobacillus and Propionibacterium. Food Addit Contam 2002;19(7):680–6. doi:10.1080/ [02652030210134236.](https://doi.org/10.1080/02652030210134236)
- [32] Gribar SC, Sodhi CP, Richardson WM, Anand RJ, Gittes GK, Branca MF, Jakub A, Shi X, Shah S, Ozolek JA, Hackam DJ. Reciprocal expression and signaling of TLR4 and TLR9 in the pathogenesis and treatment of necrotizing enterocolitis. J Immunol 2009;182(1):636–46. doi[:10.4049/jimmunol.182.1.636.](https://doi.org/10.4049/jimmunol.182.1.636)
- [33] Guo S, Nighot M, Al-Sadi R, Alhmoud T, Nighot P, Ma TY. Lipopolysaccharide regulation of intestinal tight junction permeability is mediated by TLR4 signal transduction pathway activation of FAK and MyD88. J Immunol 2015;195(10):4999–5010. doi[:10.4049/jimmunol.1402598.](https://doi.org/10.4049/jimmunol.1402598)
- [34] Mao X, Gu C, Hu H, Tang J, Chen D, Yu B, He J, Luo J, Tian G. Dietary Lactobacillus rhamnosus GG supplementation improves the mucosal barrier function in the intestine of weaned piglets challenged by porcine rotavirus. PLoS One 2016;11(1):e0146312. doi[:10.1371/journal.pone.0146312.](https://doi.org/10.1371/journal.pone.0146312)
- [35] Zhang L, Xu YQ, Liu HY, Lai T, Ma JL, Wang JF, Zhu YH. Evaluation of Lactobacillus rhamnosus GG using an Escherichia coli K88 model of piglet diarrhoea: Effects on diarrhoea incidence, faecal microflora and immune responses. Vet Microbiol 2010;141(1-2):142–8. doi[:10.1016/j.vetmic.2009.09.003.](https://doi.org/10.1016/j.vetmic.2009.09.003)
- [36] Pena JA, Versalovic J. Lactobacillus rhamnosus GG decreases TNF-α production in lipopolysaccharide-activated murine macrophages by a contact-independent mechanism. Cell Microbiol 2003;5(4):277–85. [doi:10.1046/j.1462-5822.2003.](https://doi.org/10.1046/j.1462-5822.2003.t01-1-00275.x) t01-1-00275.x.
- [37] Wu TT. The Effects of supplement with ruminal fluid, probiotics on the gas[trointestinal](http://refhub.elsevier.com/S0737-0806(23)00291-5/sbref0037) microbiota and immunity of lambs aged 28 days. Xinjiang Agricultural University; 2016. in china.
- [38] Ratcliffe N, Wieczorek T, Drabińska N, Gould O, Osborne A, Costello BDL. A mechanistic study and review of volatile products from peroxidation of unsaturated fatty acids: an aid to understanding the origins of volatile organic compounds from the human body. J Breath Res 2020;14(3):34001–17. doi[:10.1088/1752-7163/ab7f9d.](https://doi.org/10.1088/1752-7163/ab7f9d)
- [39] Ahmad H, Tian J, Wang J, Khan MA, Wang Y, Zhang L, Wang T. Effects of dietary sodium selenite and selenium yeast on antioxidant enzyme activities and oxidative stability of chicken breast meat. J Agric Food Chem 2012;60(29):7111–20. doi[:10.1021/jf3017207.](https://doi.org/10.1021/jf3017207)
- [40] Holdom MD, Lechenne B, Hay RJ, Hamilton AJ, Monod M. Production and characterization of recombinant Aspergillus fumigatus Cu, Zn superoxide dismutase and its recognition by immune human sera. J Clin Microbiol 2000;38(2):558– 62. doi[:10.1128/jcm.38.2.558-562.2000.](https://doi.org/10.1128/jcm.38.2.558-562.2000)
- [41] Espinoza CL, Madrid VA, Taborga LL, Villena GJ, Cuellar FM, Carrasco AH. Synthesis of nine safrole derivatives and their antiproliferative activity towards human cancer cells. J Chil Chem Soc 2010;55(2):219–22. doi:10.4067/ [s0717-97072010000200016.](https://doi.org/10.4067/s0717-97072010000200016)
- [42] Das D, Goyal A. Antioxidant activity and γ -aminobutyric acid (GABA) producing ability of probiotic Lactobacillus plantarum DM5 isolated from Marcha of Sikkim. LWT Food Sci Technol 2015;61(1):263–8. doi[:10.1016/j.lwt.2014.11.013.](https://doi.org/10.1016/j.lwt.2014.11.013)
- [43] Kuda T, Kawahara M, Nemoto M, Takahashi H, Kimura B. In vitro antioxidant and anti-inflammation properties of lactic acid bacteria isolated from fish intestines and fermented fish from the Sanriku Satoumi region in Japan. Food Res Int 2014;64:248–55. doi[:10.1016/j.foodres.2014.06.028.](https://doi.org/10.1016/j.foodres.2014.06.028)
- [44] Li J, Li Q, Gao N, Wang Z, Li F, Li J, Shan A. Exopolysaccharides produced by Lactobacillus rhamnosus GG alleviate hydrogen peroxide-induced intestinal oxidative damage and apoptosis through the Keap1/Nrf2 and Bax/Bcl-2 pathways in vitro. Food Funct 2021;12(20):9632–41. doi[:10.1039/d1fo00277e.](https://doi.org/10.1039/d1fo00277e)