

Effects of Dietary Tributyrin and Nucleotide Supplementation on Growth Performance, Serum Biochemical Indices, and Intestinal Morphology in Weaned Piglets (Postprint)

Authors: Zhu Rongsheng, Wang Huaizhong, Qi Bo, Huang Baohua, Sun Shouli, Wang Jiancai, Wei Bin, Hu Hongmei

Date: 2018-12-20T00:00:00+00:00

Abstract

This experiment was conducted to investigate the effects of dietary supplementation of tributyrin and nucleotides on growth performance, serum biochemical indices, and intestinal morphology in piglets. One hundred sixty healthy 21-day-old Duroc × Landrace × Yorkshire (DLY) crossbred weaned piglets weighing approximately 8.4 kg were selected and randomly allocated into 2 groups, with 10 replicates per group and 8 piglets per replicate. The control group was fed a basal diet, while the experimental group was fed a test diet supplemented with 0.20% tributyrin and 0.15% nucleotides. The experimental period lasted 38 days. The results showed that: 1) Compared with the control group, dietary supplementation of tributyrin and nucleotides significantly increased the average daily gain of weaned piglets ($P < 0.05$), extremely significantly decreased the diarrhea rate ($P < 0.01$), significantly increased serum immunoglobulin A, immunoglobulin G, immunoglobulin M, and globulin contents ($p < 0.05$), and significantly decreased serum diamine oxidase activity and glucose content ($P < 0.05$); 2) Compared with the control group, dietary supplementation of tributyrin and nucleotides significantly increased villus height and villus height/crypt depth ratio in the duodenum and jejunum of weaned piglets ($P < 0.05$), and significantly increased trypsin and maltase activities in jejunal contents ($P < 0.05$). These results indicate that dietary supplementation with 0.20% tributyrin and 0.15% nucleotides can promote piglet growth, enhance immune function, reduce intestinal damage caused by weaning stress, increase digestive enzyme activities, and improve nutrient digestion and absorption in piglets.

Full Text

Effects of Dietary Tributyrin and Nucleotide on Growth Performance, Serum Biochemical Indices, and Intestinal Morphology of Weaned Piglets

ZHU Rongsheng^{1,2}, WANG Huaizhong^{1,2*}, QI Bo³, HUANG Baohua^{1,2}, SUN Shouli^{1,2}, WANG Jiancai^{1,2}, WEI Bin, HU Hongmei^{1,2}

¹Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan 250100, China

²Key Laboratory of Disease Control and Animal Breeding of Shandong Province, Jinan 250100, China

³College of Animal Science and Technology, Qingdao Agricultural University, Qingdao 266109, China

Jinan Haihua Biological Technology Co., Ltd., Jinan 250108, China

Abstract

This experiment was conducted to investigate the effects of dietary tributyrin and nucleotide supplementation on growth performance, serum biochemical indices, and intestinal morphology of weaned piglets. One hundred and sixty healthy 21-day-old Duroc × Landrace × Yorkshire (DLY) crossbred piglets with an average body weight of 8.4 kg were randomly allocated into two groups, each consisting of 10 replicates with 8 piglets per replicate. The control group was fed a basal diet, while the experimental group received the basal diet supplemented with 0.20% tributyrin and 0.15% nucleotide. The trial lasted for 38 days. The results showed that: (1) compared with the control group, dietary supplementation with tributyrin and nucleotide significantly increased average daily gain ($P < 0.05$), extremely significantly reduced diarrhea rate ($P < 0.01$), significantly elevated serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), and globulin concentrations ($P < 0.05$), and significantly decreased serum diamine oxidase activity and glucose concentration ($P < 0.05$); (2) supplementation significantly increased villus height and the villus height/crypt depth ratio in the duodenum and jejunum ($P < 0.05$), and significantly enhanced trypsin and maltase activities in jejunal contents ($P < 0.05$). These findings indicate that dietary supplementation with 0.20% tributyrin and 0.15% nucleotide can promote growth, enhance immunity, mitigate intestinal damage caused by weaning stress, increase digestive enzyme activity, and improve nutrient digestion and absorption in weaned piglets.

Keywords: tributyrin; nucleotide; weaned piglets; growth performance; serum biochemical indices; intestinal morphology

Weaning is ideally a gradual, progressive process that should extend beyond 17 weeks [1]. However, with the intensification and scaling-up of swine production,

most farms have advanced weaning age to 7–28 days to improve sow reproductive efficiency. This abrupt transition subjects piglets to sudden changes in environment and diet, causing weaning stress that damages intestinal barrier function, reduces feed intake [2], induces villus atrophy [3–5], and increases susceptibility to infectious diseases [3].

Tributylin serves as an excellent source of butyric acid that reaches the intestine directly, providing energy and nutrients for intestinal development, promoting intestinal epithelial cell proliferation, and enhancing nutrient absorption and utilization [6–8]. Previous studies have demonstrated that dietary supplementation with 0.08%–0.30% tributyrin increased average daily gain in weaned piglets by 5.79%–11.75%, decreased feed-to-gain ratio by 11.75%, significantly increased jejunal villus height by 15.9%, significantly reduced crypt depth by 8.9%, and increased intestinal contents of cadaverine, putrescine, spermine, and histamine by 1.5–10.0-fold. Moreover, tributyrin supplementation significantly alleviated acetic acid-induced increases in creatinine and prostaglandin E2 levels in the rectum [8–12]. The optimal supplementation level of tributyrin has been reported to be 0.1%–0.3% [8,10,12–13].

Nucleotides are biologically active compounds that are normally synthesized endogenously. However, dietary supplementation becomes beneficial for piglet growth performance and health during periods of rapid growth and stress [14]. While suckling piglets consume nucleotide-rich sow milk, post-weaning diets contain minimal nucleotides. Therefore, nucleotide supplementation in weaned piglet diets can mitigate weaning stress and increase feed intake [15–16]. Studies have shown that adding 0.5–2.0 g/kg nucleotides to weaned piglet diets significantly increased average daily gain and average daily feed intake by 3.14%–11.07% and 5.58%–15.50%, respectively, and reduced feed-to-gain ratio by 3.16%–4.24% [17]. Additionally, nucleotide supplementation decreased diarrhea rate from 15.63% to 1.53% and mortality from 4.69% to 1.56% [16], while playing important roles in immune function maintenance and oxidative stress reduction [18–19].

Based on the established benefits of tributyrin for intestinal development and nutrient absorption [8,10] and nucleotides for improving feed intake and growth performance [14–17], combined supplementation of both compounds in weaned piglets has not been reported. Most studies have focused on single-additive applications. Therefore, this experiment was designed to investigate the combined effects of dietary tributyrin and nucleotide supplementation on growth performance, serum biochemical indices, and intestinal morphology of weaned piglets, providing a foundation for their combined application.

Materials and Methods

Experimental Design

The experiment was conducted at Heze Hongxing Original Pig Breeding Co., Ltd. One hundred and sixty healthy 21-day-old Duroc × Landrace × Yorkshire

(DLY) crossbred piglets with an average body weight of 8.4 kg were randomly divided into two groups, each comprising 10 replicates of 8 piglets. The control group received a basal diet, while the experimental group received the basal diet supplemented with 0.20% tributyrin and 0.15% nucleotide. The trial lasted 38 days, during which piglets had ad libitum access to feed and water, and were managed according to routine farm protocols and normal immunization procedures. The composition and nutrient levels of the basal diet are presented in Table 1 .

Experimental Materials

Tributyrin and nucleotides were provided by Jinan Haihua Biological Technology Co., Ltd. The tributyrin product contained 45% purity, while the nucleotide product contained 50% purity, with the remainder being carrier.

Growth Performance Measurements

Piglets were individually weighed on days 1 and 38 before morning feeding. During the trial, fecal consistency was observed daily between 9:00-10:00 and 17:00-18:00, and individual diarrhea cases were recorded. Mortality and culling events were recorded individually, with feed remaining and wastage weighed promptly when deaths or culls occurred. Average daily feed intake and feed-to-gain ratio were calculated per replicate, while average daily gain and diarrhea rate were calculated per individual.

Serum Biochemical Indices

On day 38, piglets were fasted and weighed before morning feeding, then 10 mL of blood was collected from the anterior vena cava of each piglet into non-anticoagulant tubes. After standing for 15 minutes, serum was separated by centrifugation at 3,000 r/min for 10 minutes and stored at -80°C. Serum concentrations of urea nitrogen, uric acid, glucose, total cholesterol, total protein, globulin, immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), and diamine oxidase activity were measured using a Hitachi 7180 biochemical analyzer. Reagent kits were purchased from Sichuan Mike Biological Technology Co., Ltd., and procedures followed the manufacturer' s instructions.

Intestinal Morphology and Mucosal Immune Cell Counting

On day 38, three piglets from each group were randomly selected for slaughter after fasting and weighing. Tissue samples from the duodenum, jejunum, and ileum were collected for paraffin sectioning. After hematoxylin-eosin (HE), Alcian blue-periodic acid-Schiff (AB-PAS), and toluidine blue staining, sections were photographed at 10×20 magnification. Villus height, crypt depth, mucosal thickness, and intestinal wall thickness were measured using Image-Pro Plus 6.0 software. Goblet cells, columnar cells, lymphocytes, and mast cells were counted using the following methods: (1) five longest and most regularly

arranged villi per cross-section were examined to count columnar cell density; (2) five longest and most regularly arranged villi per cross-section were examined to count lymphocytes and goblet cells per 100 intestinal epithelial columnar cells; (3) the entire intestinal wall cross-section, including mucosa, submucosa, muscularis, and serosa, was examined to count mast cells and lymphocytes per 100 columnar cells.

Digestive Enzyme Activity in Jejunal Contents

On day 38, three piglets from each group were randomly selected for slaughter after fasting and weighing. Jejunal contents were collected to measure lipase, trypsin, amylase, maltase, lactase, and sucrase activities using colorimetric methods. Reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute, and procedures followed the manufacturer's instructions.

Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 19.0 software. When significant differences were detected, multiple comparisons were performed using the LSD method. $P < 0.05$ was considered significant, and $P < 0.01$ was considered extremely significant. Results are expressed as "mean \pm standard error."

Results

Effects on Growth Performance

As shown in Table 2, initial body weight was similar between groups with no significant difference ($P > 0.05$). After 38 days, final body weight in the experimental group was numerically 4.86% higher than the control group, but the difference was not significant ($P > 0.05$). Compared with the control group, the experimental group showed 5.83% higher average daily feed intake ($P > 0.05$) and 7.76% higher average daily gain ($P < 0.05$), while diarrhea rate was extremely significantly reduced by 61.90% ($P < 0.01$). Feed-to-gain ratio did not differ significantly between groups ($P > 0.05$). These results indicate that dietary supplementation with tributyrin and nucleotide increased average daily feed intake, significantly improved average daily gain, and extremely significantly reduced diarrhea rate in weaned piglets.

Effects on Serum Biochemical Indices

As shown in Table 3, compared with the control group, the experimental group exhibited a significant 10.34% reduction in serum glucose concentration ($P < 0.05$), significant or extremely significant increases in serum IgA, IgG, and IgM concentrations by 82.03% ($P < 0.01$), 15.65% ($P < 0.05$), and 15.69% ($P < 0.05$), respectively, a significant 15.82% increase in serum globulin concentration ($P < 0.05$), and an extremely significant 34.04% decrease in serum diamine oxidase activity ($P < 0.01$). No significant differences were observed in serum

urea nitrogen, uric acid, total cholesterol, or total protein concentrations between groups ($P > 0.05$). These findings demonstrate that dietary tributyrin and nucleotide supplementation significantly increased serum globulin and immunoglobulin concentrations while significantly reducing serum glucose concentration and diamine oxidase activity.

Effects on Intestinal Morphology

As shown in Table 4, villus height decreased progressively from duodenum to jejunum to ileum, while crypt depth was greater in jejunum and ileum than in duodenum. The villus height/crypt depth (V/C) ratio followed the same trend as villus height. Compared with the control group, the experimental group showed varying degrees of increased villus height and V/C ratio, and decreased crypt depth across all intestinal segments. Specifically, villus height in duodenum and jejunum significantly increased by 25.58% and 26.98% ($P < 0.05$), respectively. The V/C ratio in duodenum and jejunum increased significantly or extremely significantly by 45.55% ($P < 0.01$) and 32.51% ($P < 0.05$), respectively. Crypt depth decreased in all segments but differences were not significant ($P > 0.05$). Ileal villus height and V/C ratio increased by 10.39% and 8.44%, respectively, but these differences were not significant ($P > 0.05$).

Intestinal wall thickness and mucosal thickness also decreased progressively from duodenum to jejunum to ileum. Compared with the control group, the experimental group showed increased intestinal wall thickness by 5.16%, 4.88%, and 9.02% in duodenum, jejunum, and ileum, respectively ($P > 0.05$), and increased mucosal thickness by 3.30% ($P > 0.05$), 10.91% ($P > 0.05$), and 35.89% ($P < 0.01$), respectively.

No significant differences were observed in goblet cell, columnar cell, lymphocyte, or mast cell counts among duodenum, jejunum, and ileum between groups ($P > 0.05$). However, numerically, the experimental group showed 32.82% and 22.69% increases in duodenal goblet cells and lymphocytes, respectively; 25.37%, 10.10%, and 22.66% increases in jejunal goblet cells, columnar cells, and mast cells, respectively; and 28.09%, 19.21%, 11.28%, and 25.35% increases in ileal goblet cells, columnar cells, lymphocytes, and mast cells, respectively.

These results indicate that dietary tributyrin and nucleotide supplementation increased villus height, V/C ratio, intestinal wall thickness, and mucosal thickness while reducing crypt depth in weaned piglets.

Effects on Intestinal Enzyme Activities

As shown in Table 5, the experimental group exhibited higher activities of lipase, trypsin, amylase, maltase, lactase, and sucrase in intestinal contents, with increases of 18.08%, 75.00%, 9.69%, 78.21%, 8.66%, and 17.16%, respectively. Notably, trypsin and maltase activities increased significantly ($P < 0.05$), while other enzyme activities showed non-significant improvements ($P > 0.05$).

Discussion

Effects on Growth Performance

Previous studies have reported that dietary supplementation with 0.1% tributyrin did not significantly affect average daily gain, average daily feed intake, or feed-to-gain ratio, but significantly reduced diarrhea rate by 6.83% and decreased mortality from 18.75% to 6.25% [10,12]. Supplementation with 0.15%-0.20% tributyrin significantly increased average daily gain by 10.24%-11.79%, reduced feed-to-gain ratio by 4.14%-6.62%, and decreased diarrhea rate by 49.35%-66.67%, while increasing average daily feed intake by 3.10%-7.02% and significantly improving apparent digestibility of crude fat, crude protein, and energy by 4.09%-12.38% [11,13]. Combined supplementation of tributyrin with lactitol or oregano oil has shown superior effects on piglet growth performance compared to individual additives [5,10,12]. Dietary nucleotide supplementation at 0.5%-2.0% significantly increased average daily gain and average daily feed intake by 3.14%-11.07% and 5.58%-15.50%, respectively, and reduced feed-to-gain ratio by 3.16%-4.24% [17], while decreasing diarrhea rate from 15.63% to 1.53% and mortality from 4.69% to 1.56% [16].

Tributyrin reaches the distal intestine where it is rapidly hydrolyzed by lipase into glycerol and butyric acid, which is absorbed by intestinal epithelial cells to provide energy for cell development [20]. Butyric acid not only supplies carbohydrates and energy for intestinal cells but also prevents osmotic diarrhea [21]. During intestinal absorption, butyric acid promotes sodium, potassium, and water absorption, thereby exerting anti-diarrheal effects [20]. Dietary butyrate supplementation has been shown to increase plasma cholecystokinin levels four-fold, which promotes growth [22]. Bioactive nucleotides play important roles in metabolism, structural functions, surveillance, immune system maintenance, and oxidative stress reduction [18-19], and can increase feed intake during rapid growth and stress periods [14].

When tributyrin was combined with lactitol, the tributyrin inclusion level was 3,000 mg/kg [8,10], while combination with oregano oil used 1,000 mg/kg tributyrin [12], with combined supplementation showing significantly greater effects than individual additives [8,10,12]. Piva et al. [10] found that combined tributyrin and lactitol supplementation increased average daily gain by 78.99% or 5.97% compared to individual supplementation, with 0% mortality in the combined group versus 6.25% in individual additive groups and 18.75% in the control group. Zhang et al. [12] reported that combined tributyrin and oregano oil supplementation reduced diarrhea rate to 3.96% and increased average daily gain by 8.86% and 6.65% compared to individual tributyrin or oregano oil supplementation, respectively.

In the current study, dietary supplementation with 0.20% tributyrin and 0.15% nucleotide increased average daily feed intake, significantly improved average daily gain by 7.76%, and extremely significantly reduced diarrhea rate from 4.96% to 1.89% (a 61.90% reduction). While the improvement in average daily

gain was smaller than reported by Piva et al. [10] and Zhang et al. [12], the reduction in diarrhea rate was more pronounced, possibly due to differences in additive levels or ratios.

Effects on Serum Biochemical Indices

Reduced feed intake after weaning causes intestinal villus atrophy [3-5], intestinal barrier dysfunction, and increased susceptibility to infectious diseases, severely affecting growth performance and health [3] and damaging tissue and organ cell membranes, leading to altered enzyme activities. Serum total protein is an important protein source for tissue repair and energy provision, reflecting dietary crude protein levels and protein absorption and metabolism. High total protein concentration indicates rapid protein anabolism. Globulin, secreted by plasma cells, reflects anti-stress capacity. IgA, IgG, and IgM are immunoglobulins secreted by bone marrow-derived lymphocytes that participate in humoral immunity, with serum concentrations reflecting immune status [23].

Tributylin improves immune function primarily by controlling inflammatory responses and regulating anti-inflammatory cytokine expression [24], while nucleotides enhance humoral immune responses to T lymphocyte-dependent antigens, increasing IgG and IgM production [17,25]. Diamine oxidase is a highly active intracellular enzyme in mammalian small intestinal mucosal villi that participates in histamine and polyamine metabolism. When intestinal mucosa is damaged, diamine oxidase enters the bloodstream, making serum diamine oxidase activity an indicator of intestinal mechanical barrier integrity and damage severity [26].

Previous studies have shown that 0.1% tributyrin supplementation increased serum IgA, IgG, and IgM concentrations by 24.42%, 13.68%, and 15.00%, respectively, while combined tributyrin and oregano oil supplementation increased these immunoglobulins by 24.42%, 17.31%, and 8.75%, respectively, without affecting total protein or globulin concentrations [12]. Tributyrin supplementation at 0.10%–0.15% increased serum total protein and globulin concentrations in a dose-dependent manner [11]. The current study found that combined tributyrin and nucleotide supplementation significantly increased serum globulin and immunoglobulin concentrations and extremely significantly reduced serum diamine oxidase activity by 34.04%, consistent with Yang [11] and Zhang et al. [12], indicating enhanced immune function and improved intestinal development with reduced weaning stress.

Serum urea nitrogen, the primary end product of protein metabolism, accurately reflects protein metabolism and amino acid balance [27]. The observed 2.31% reduction in serum urea nitrogen and significant 10.34% reduction in serum glucose suggest improved protein utilization and enhanced carbohydrate metabolism, likely due to tributyrin providing energy for intestinal development and nucleotides supplying essential nucleosides for cell proliferation and differentiation, thereby promoting intestinal development, improving nutrient

absorption, and enhancing protein metabolism.

Effects on Intestinal Morphology

The current study demonstrated that dietary supplementation with 0.20% tributyrin and 0.15% nucleotide increased villus height by 10.39%-26.98% and reduced crypt depth by 3.13%-9.44% in all intestinal segments. These findings align with Piva et al. [8], who reported increased mucosal thickness and jejunal villus height with reduced crypt depth following tributyrin and lactitol supplementation. The increased absorptive surface area may result from enhanced intestinal epithelial cell mitosis, reduced apoptosis, or a combination of both [22,28-29].

The progressive decrease in intestinal wall and mucosal thickness from duodenum to ileum was observed in both groups, with the experimental group showing increases of 4.88%-9.02% in wall thickness and 3.30%-35.89% in mucosal thickness. These results are consistent with Piva et al. [8] and likely reflect rapid proliferation of intestinal epithelial, mucosal, and immune cells, which increases wall and mucosal thickness while enhancing villus vitality and nutrient digestion [30].

Intestinal epithelial cells form the first line of defense against feed toxins and pathogenic bacteria, containing receptors for toxins and bacteria and housing immune cells such as lymphocytes and goblet cells [31-34]. Goblet cells regulate intestinal immune function through specific and non-specific immune mechanisms, secreting acidic substances that form a protective barrier on the mucosal surface [35]. Mast cells provide anti-infective immune function through cytokine secretion and participate in acquired immunity [30,36-37], making lymphocytes, goblet cells, and mast cells crucial for intestinal immune regulation. The numerical increases in these cell populations following tributyrin and nucleotide supplementation suggest enhanced intestinal immune function, possibly through inhibited apoptosis and stimulated proliferation of these immune cells.

Effects on Intestinal Enzyme Activities

Dong et al. [38] reported that tributyrin supplementation significantly increased digestive enzyme activities in intrauterine growth-restricted piglets, with lactase activity increasing 3.66-fold, 1.35-fold, and 2.63-fold in duodenum, jejunum, and ileum, respectively, and sucrase, maltase, and lipase activities in ileum increasing 2.30-fold, 2.43-fold, and 4.15-fold, respectively. Trypsin activity in jejunum and ileum increased 2.82-fold and 10.52-fold, respectively. The current study similarly found that tributyrin and nucleotide supplementation increased activities of lipase, trypsin, amylase, maltase, lactase, and sucrase, with trypsin and maltase activities significantly increasing by 75.00% and 78.21%, respectively. These improvements likely result from tributyrin and nucleotides providing energy and nucleosides for intestinal development, increasing villus height and absorptive surface area, and promoting digestive enzyme secretion. Thus, trib-

utyryn supplementation enhances intestinal digestion and absorption capacity [39], while increased enzyme activities strengthen intestinal defense against bacterial invasion [38].

Conclusion

Dietary supplementation with 0.20% tributyrin and 0.15% nucleotide significantly increased average daily gain by 7.76% ($P < 0.05$) and extremely significantly reduced diarrhea rate by 61.90% ($P < 0.01$) in weaned piglets. Supplementation numerically increased intestinal villus height, V/C ratio, intestinal wall thickness, and mucosal thickness ($P > 0.05$), significantly increased trypsin and maltase activities in ileal contents ($P < 0.05$), significantly elevated serum immunoglobulin concentrations by 15.65%–82.03% ($P < 0.05$), and extremely significantly reduced serum diamine oxidase activity by 34.04% ($P < 0.01$).

References

- [1] VAN DER MEULEN J, KOOPMANS S J, DEKKER R A, et al. Increasing weaning age of piglets from 4 to 7 weeks reduces stress, increases post-weaning intake improve intestinal functionality[J]. *Animal*, 2010, 4(10): 1653-1661.
- [2] WIJTTEEN P J, VAN DER MEULEN J, VERSTEGEN M W. Intestinal barrier function and absorption in pigs after weaning: a review[J]. *British Journal of Nutrition*, 2011, 105(7): 967-981.
- [3] LALLÈSA J-P, BOUDRYA G, FAVIERA C, et al. Gut function dysfunction young pigs: physiology[J]. *Animal Research*, 2004, 53(4): 301-316.
- [4] MOESER A J, KLOK C V, RYAN K A, et al. Stress signaling pathways activated by weaning mediate intestinal dysfunction pig[J]. *American Journal Physiology: Gastrointestinal Liver Physiology*, 2007, 292(1): G173-G181.
- [5] PLUSKE J R, WILLIAMS I H, AHERNE F X. Villous height and crypt depth in piglets in response to increases in the intake of cows milk after weaning[J]. *Animal Science Journal*, 1996, 62(1): 145-158.
- [6] GASCHOTT T, STEINHILBER D, MILOVIC V, et al. Tributyrin, a stable and rapidly absorbed prodrug of butyric acid, enhances antiproliferative effects of dihydroxycholecalciferol in human colon cancer cells[J]. *The Journal of Nutrition*, 2001, 131(6): 1839-1843.
- [7] KUEFER R, HOFER M D, ALTUG V, et al. Sodium butyrate and tributyrin induce in vivo growth inhibition and apoptosis in human prostate cancer[J]. *British Journal of Cancer*, 2004, 90(2): 535-541.
- [8] PIVA A, GRILLI E, FABBRI L, et al. Intestinal metabolism of weaned piglets fed a typical United States or European diet with or without supplementation tributyrin lactitol[J]. *Journal of Animal Science*, 2008, 86(11): 2952-2961.
- [9] HOU Y Q, WANG L, YI D, et al. Dietary supplementation with tributyrin alleviates intestinal injury in piglets challenged with intrarectal administration of acetic acid[J]. *British Journal of Nutrition*, 2014, 111(10): 1748-1759.
- [10] PIVA A, PRANDINI A, FIORENTINI L, et al. Tributyrin and lactitol syn-

- ergistically enhanced the trophic status of the intestinal mucosa and reduced histamine levels in the gut of nursery pigs[J]. *Journal of Animal Science*, 2002, 80(3): 670-680.
- [11] YANG L. Effects of tributyrin on growth performance, intestinal structure and blood biochemical indices of weaned piglets[J]. *Feed Industry*, 2012(Suppl 1): 46-49.
- [12] ZHANG Y, WANG M, LI F F, et al. Effects of tributyrin and oregano oil on growth performance, serum biochemical indices and nutrient apparent digestibility in weaned piglets[J]. *Chinese Journal of Animal Nutrition*, 2016, 28(9): 2786-2794.
- [13] HOU G F, LI R, WANG H J, et al. Effects of tributyrin on growth performance and nutrient digestibility in weaned piglets[J]. *Swine Production*, 2014(5): 4-6.
- [14] FRANKIČ T, PAJK T, REZAR V, et al. The role of dietary nucleotides in reduction of DNA damage induced by T-2 toxin and deoxynivalenol in chicken leukocytes[J]. *Food and Chemical Toxicology*, 2006, 44(11): 1838-1844.
- [15] MATEO C D, PETERS D N, STEIN H H. Nucleotides in sow colostrum and milk at different stages of lactation[J]. *Journal of Animal Science*, 2004, 82(5): 1339-1342.
- [16] MARTINEZ-PUIG D, MANZANILLA E G, MORALES J, et al. Dietary nucleotide supplementation reduces occurrence of diarrhoea in early weaned pigs[J]. *Livestock Science*, 2007, 108(1/2/3): 276-279.
- [17] WEAVER A C, KIM S W. Supplemental nucleotides high in inosine 5' - monophosphate to improve the growth and health of nursery pigs[J]. *Journal of Animal Science*, 2014, 92(2): 645-651.
- [18] SAUER N, EKLUND M, BAUER E, et al. The effects of pure nucleotides on performance, humoral immunity, gut structure numbers intestinal bacteria newly weaned pigs[J]. *Journal of Animal Science*, 2012, 90(9): 3126-3134.
- [19] SALOBIR J, REZAR V, PAJK T, et al. Effect of nucleotide supplementation on lymphocyte DNA damage induced by dietary oxidative stress in pigs[J]. *Animal Science*, 2005, 81(1): 135-140.
- [20] LEONEL A J, ALVAREZ-LEITE JI. Butyrate: implications for intestinal function[J]. *Current Opinion in Clinical Nutrition and Metabolic Care*, 2012, 15(5): 474-479.
- [21] CRESCI G, NAGY L E, GANAPATHY V. Lactobacillus GG and tributyrin supplementation reduce antibiotic-induced intestinal injury[J]. *Journal of Parenteral and Enteral Nutrition*, 2013, 37(6): 763-774.
- [22] KOTUNIA A, WOLIŃSKI J, LAUBITZ D, et al. Effect of sodium butyrate on the small intestine development in neonatal piglets fed [correction of feed] by artificial sow[J]. *Journal of Physiology and Pharmacology Supplement*, 2004, 55(Suppl 2): 59-68.
- [23] HE Y, ZHAO W, QIN Y W, et al. Effects of NS compound lactic acid bacteria preparation on immune level of weaned piglets[J]. *Progress in Veterinary Medicine*, 2011, 32(12): 51-56.
- [24] LEONEL A J, TEIXEIRA L G, OLIVEIRA R P, et al. Antioxidative and immunomodulatory effects of tributyrin supplementation on experimental coli-

- tis[J]. *British Journal of Nutrition*, 2013, 109(8): 1396-1407.
- [25] SAUER N, MOSENTHIN R, BAUER E. The role of dietary nucleotides in single-stomached animals[J]. *Nutrition Research Reviews*, 2011, 24(1): 46-59.
- [26] ZHAO J K, ZHANG X P. Current status and research progress of intestinal barrier function assessment methods[J]. *Hebei Medical Journal*, 2009, 31(22): 3134-3136.
- [27] MALMLOF K. Amino acid in farm animal nutrition metabolism, partition and consequences of imbalance[J]. *Journal of Agricultural Research*, 1988, 18(4): 191-193.
- [28] GODLEWSKI M M, SLUPECKA M, WOLIŃSKI J, et al. Into the unknown—the death pathways in the neonatal gut epithelium[J]. *Journal of Physiology and Pharmacology*, 2005, 56(Suppl 3): 7-24.
- [29] GODLEWSKI M M, HALLAY N, BIERLA J B, et al. Molecular mechanism of programmed cell death in the gut epithelium of neonatal piglets[J]. *Journal of Physiology and Pharmacology*, 2007, 58(Suppl 3): 97-113.
- [30] ZIEGLER T R, EVANS M E, FERNÁNDEZ-ESTÍVARIZ C, et al. Trophic and cytoprotective nutrition for intestinal adaptation, mucosal repair, and barrier function[J]. *Annual Review of Nutrition*, 2003, 23(1): 229-261.
- [31] GU X H, ZHANG H F, SHE R P, et al. Effects of weaning age on intestinal mucosal hydrolase activities and numbers of intraepithelial lymphocytes and goblet cells in piglets[J]. *Chinese Journal of Animal Nutrition*, 2005, 17(1): 35-39.
- [32] KOU T, LÜ J Q, LI W, et al. Effects of dietary arginine supplementation for sows on immune cell numbers in piglet intestine[J]. *Chinese Journal of Animal Nutrition*, 2014, 26(8): 2077-2084.
- [33] QIAO S Y, HOU C L, ZENG X F. Regulatory effects and mechanisms of lactic acid bacteria on intestinal barrier function in pigs[J]. *Chinese Journal of Animal Nutrition*, 2014, 26(10): 3052-3063.
- [34] XU Z W. Nutritional regulation of intestinal injury repair and its mechanism and application in piglets[J]. *Chinese Journal of Animal Nutrition*, 2014, 26(10): 3033-3045.
- [35] TANG X Y, CHEN Z L, LUO Q H, et al. Effects of soybean isoflavones on intestinal intraepithelial lymphocytes, goblet cells and leptin long-form receptor in rats[J]. *Journal of Zhejiang University (Agriculture and Life Sciences)*, 2013, 39(3): 343-350.
- [36] HAO Z R, ZHU Z N, WANG M, et al. Effects of soybean isoflavones on lactation performance, immune function and interleukin-4 level of mammary gland mast cells in dairy cows during late lactation[J]. *Chinese Journal of Animal Nutrition*, 2010, 22(6): 1679-1686.
- [37] ZHU Z N, HAO Z R, WANG M, et al. Effects of soybean isoflavones on tumor necrosis factor- secretion and surface immunoglobulin A level of mammary gland mast cells in high-yielding dairy cows during late lactation[J]. *Chinese Journal of Animal Nutrition*, 2011, 23(1): 112-121.
- [38] DONG L, ZHONG X, HE J T, et al. Supplementation of tributyrin improves the growth and intestinal digestive and barrier functions in intrauterine growth-restricted piglets[J]. *Clinical Nutrition*, 2016, 35(2): 399-407.

[39] CLAUS R, GÜNTNER D, LETZGU H. Effects of feeding fat-coated butyrate on mucosal morphology and function small intestine pig[J]. Journal of Animal Physiology and Animal Nutrition, 2007, 91(7/8): 312-318.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv –Machine translation. Verify with original.