Clinical Nutrition 35 (2016) 399–407

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Supplementation of tributyrin improves the growth and intestinal digestive and barrier functions in intrauterine growth-restricted piglets

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ARTICLE INFO

Article history:
Received 14 January 2015
Accepted 5 March 2015

Keywords:
Tributyrin
IUGR
Suckling period
Small intestine
Piglets

SUMMARY

Background & aims: Intrauterine growth restriction (IUGR) neonates suffer from growth restriction. Tributyrin (TB), a pro-drug of butyrate, can facilitate the growth of animals. This study was to investigate the effects of TB supplementation on the growth of IUGR neonatal piglets.

Methods: Sixteen IUGR and 8 NBW (normal body weight) neonatal piglets were chosen, weaned at 7th day and fed basic milk diets (NBW and IUGR group) or the basic diets supplemented with 0.1% tributyrin (IT group, IUGR piglets fed with tributyrin) until day 21 (n = 8). The body weights of the piglets on days 0, 7, 10, 14, 17, and 20 were measured. The digestive enzyme activity, intestinal morphology, immunoglobulin levels and gene expression of IgG, FcRn and GPR41 in the small intestines were analyzed.

Results: The body weights of the piglets in the IUGR and IT group were similar, and both were lower than the NBW group on days 10 and 14. However, after day 17, the IT group exhibited improved ($P < 0.05$) body weights compared to that of the IUGR group. The piglets were sacrificed on day 21. Compared with the NBW piglets, IUGR impaired the development of immune organs and small intestines, impaired the intestinal villus morphology, decreased ($P < 0.05$) most of the tested intestinal digestive enzyme activities, decreased ($P < 0.05$) the ileal sIgA and IgG levels, and down-regulated ($P < 0.05$) the intestinal IgG and GPR41 expression. Piglets in the IT group exhibited a better-developed ($P < 0.05$) spleen and small intestines, improved intestinal villus morphology, increased ($P < 0.05$) intestinal villus surface areas, enhanced ($P < 0.05$) digestive enzyme activities, and up-regulated ($P < 0.05$) expression of IgG and GPR41 mRNA compared to those of the IUGR group.

Conclusions: TB supplementation improves the growth and the intestinal digestive and barrier functions in IUGR piglets during the suckling period.

1. Introduction

Intrauterine growth restriction (IUGR) is a serious problem in human infants and animals. Pigs are multifetal animals and exhibit serious IUGR occurrence [1]. IUGR significantly affects the neonatal survival, postnatal growth, immunity, and life-long health of the animals [2]. Although better management techniques and mammalian nutrient regulation have substantially helped improve complications, IUGR continues to be a significant problem because the knowledge of the effect of nutrition on the mechanisms modulating neonatal infants' growth is still deficient [3]. IUGR can significantly affect the development and barrier functions of the intestine in neonates and may cause long-term growth impairment [4]. Studies on the effects of new nutrients on IUGR may be helpful in solving the complications that are associated with IUGR.

Tributyrin (TB), a triglyceride with 3 butyrate glyceryl-tributyrates, can act as a butyrate pro-drug [5]. Butyrate, a kind of

Abbreviations: NBW, normal body weight; IUGR, intrauterine growth restriction; TB, tributyrin; IT, IUGR piglets that were fed TB-supplemented milk diets; SCFA, short-chain fatty acid; SI, small intestine; MLN, mesenteric lymph nodes; sIgA, secretory Immunoglobulin A; IgG, Immunoglobulin G; FcRn, Fc receptor; GPR41, G-protein-coupled receptor 41 (also called free fatty acid receptor 3 or FFAR3).

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http://dx.doi.org/10.1016/j.clnu.2015.03.002
0261-5614/

short-chain fatty acid (SCFA), can protect the intestine by inhibiting the growth of bacteria, increasing mucosal cell proliferation, and improving intestinal cell development and colonic defense work [6]. Butyrate can also has an effect on the immunity and has anti-inflammatory effects [7]. In addition, pre-weaning supplementation with sodium butyrate is more efficient in stimulating feed intake and body growth compared with post-weaning supplementation [8]. A study on weaned piglets showed that the supplementation of sodium butyrate only improved the beginning phase of the piglets' adaption to the solid diets [9]. Another study of weaned piglets also showed that sodium butyrate addition to the diets does not increase the body growth but may modulate their immunity [7]. The use of TB can reduce the drawbacks of butyrate; TB is better orally tolerated than the butyrate and has been accepted as a food additive [10,11]. The role of TB for suckling piglets is not clear at present, and the effects of TB on IUGR for both humans and livestock have not been studied. The aim of the study was to explore the effects of suckling period TB addition on the body growth and the intestinal digestive and barrier functions of IUGR neonatal piglets.

2. Materials and methods

2.1. Piglets and diets

The experiment was conducted on a trial pig farm that was owned by the Anyou Company (Xuancheng, Anhui Province, China). Neonatal Duroc × (Landrace × Yorkshire) piglets were chosen from eight sows. The sows were all with the birth order of the 3rd or 4th and were fed the same gestating diet, which met the National Research Council (NRC, 2012) nutrient requirements [12]. In each litter, two IUGR and one normal-body-weight piglets were obtained. Piglets with birth body weights of 0.95 kg (SD 0.08) and 1.56 kg (SD 0.07) were defined as IUGR and NBW piglets, respectively [13]. The NBW piglets were allocated to the NBW (normal birth weight) group, and the two IUGR piglets from one litter were randomly assigned to the IUGR and IT (IUGR with TB supplementation) groups (n = 8/group, 4 males and 4 females in each group). The piglets in the NBW and IUGR group were fed basic milk diets, while the piglets in the IT group were fed basic milk diets supplemented with 0.1% tributyrin from 7 to 21 days after birth (the composition of the diets and the nutrient content are shown in Table 1). Tributyrin (casein-coated; the content of TB was 50%) was supplied for free by the Xin' ao Company (Xia Men, China). Six piglets (n = 6/group, 3 males and 3 females) in each group were selected randomly and sacrificed for further study.

All procedures of the animal experiment were accepted by the Nanjing Agricultural University's Institutional Animal Care and Use Committee, People's Republic of China. The piglets were weighed on days 0, 7, 11, 14, 17, and 21. The piglets were fed a warm milk substitute (milk bubbled with warm water, 100 g of milk in 600 ml of water) ad libitum every 2–3 h (calculated according to the total body weight of the piglets in each treatment, about 75 ml per kg of body weight) approximately 9–10 times every day. We measured the milk that was supplied and the milk that remained in each group with a plastic volumetric cylinder and calculated the absolute intake (intake = supplied-remaining) at each feeding time from days 11–20. We further converted this volume to the weight of the milk substitute taken by the formula: milk density = weight/volume. We calculated the ADG (average body weight gain of a day), ADFI (average feed intake of a day), and the gain-to-feed ratio from days 11–20; however, we obtained the data of the means of each group but no SEM (standard error mean). Because we intended to imitate the natural feeding conditions for the piglets, we fed them approximately 10 times each day for 14 days. To save time and to imitate the natural feeding conditions more effectively, we did not use bottle feeding, which requires that each piglet be fed individually. Instead, we taught the piglets how to drink the warm milk from plates and fed all of the piglets at the same time. The disadvantage of this was that we could only obtain the average feed intake in one group but not the feed intake for each piglet.

Table 1
Composition and nutrient content of the diets (DM basis).

Items	Control	TB[a]
Ingredients (g/kg)		
Whey protein concentrate (34% CP)	300	300
Milk fat powder (11% CP)	350	350
Whole milk powder	200	200
α-Casein	50	50
Glucose	60	60
Pre-mixed mixture[b]	40	40
TB	0	1
Nutrient content[c]		
CP (%)	23.31	23.28
Digestible energy (MJ/kg)	14.88	14.88
Lysine (%)	1.51	1.51
Methionine (%)	0.43	0.43
Threonine (%)	0.89	0.89
Tryptophan (%)	0.25	0.25
Ca (%)	0.85	0.85
Total P (%)	0.70	0.71

CP, crude protein; TB, tributyrin; Ca calcium; P phosphorus.
[a] In the TB diets, 0.1% TB was added to the control diet. Tributyrin (casein coated; net content of TB is 50%) was supplied for free by the Xin' ao Company in Xia Men. The two diets were isonitrogenous and isoenergetic.
[b] The main contents of the pre-mixed mixture (per kg of the pre-mixed mixture): Cu (as $CuSO_4 \cdot 5H_2O$) 600 mg; Fe ($FeSO_4 \cdot 7H_2O$) 8400 mg; Mn ($MnSO_4 \cdot H_2O$) 315 mg; Se (as Na_2SeO_3) 17 mg; Zn ($ZnSO_4 \cdot 7H_2O$) 12,500 mg; Vitamin A 55,000 IU; Vitamin D 5500 IU; Vitamin E 400 IU; Vitamin K 12.5 mg; Biotin 2 mg; Choline 15 mg; Folacin 7.5 mg; Riboflavin 100 mg; Thiamin 317.5 mg; Vitamin B_6 175 mg; Vitamin B_{12} 500 mg.
[c] The nutrient contents of CP, Ca, and P were the examined values, the contents of other nutrient were calculated according to the contents of the nutrient of the ingredients referring to NRC (2012) [15].

The number of piglets who had diarrhea was recorded during the experimental period (from days 11–20), and the diarrhea rates were calculated using the following formula: diarrhea rate = the sum of diarrhea piglets on each day during the experiment ÷ (the number of the experimental piglets × the number of experimental days) × 100%. The piglets in the three groups (NBW, IUGR and IT group) were raised in three separate little plastic houses (1.5 m × 0.7 m × 0.7 m; environmentally controlled; the ambient temperature is 33 °C) and could have water freely. The piglets that were selected for slaughter were stunned by electric shock and killed by jugular bloodletting at the end of the experimental period.

2.2. Sample collections

The 18 piglets (from the NBW, IUGR, and IT groups; 6 piglets per group) were weighed prior to euthanization. The SI was taken out from the abdominal cavity immediately after the animal died and was divided into the duodenum (about first 10-cm segment after the stomach), the jejunum (about half of the small intestine below the duodenum), and the ileum (the left part of the small intestine). The weight and length of the emptied SI with its mesenteric attachments removed were measured. Two samples of about 1 cm in length from each section were collected. One sample was fixed in 4% buffered formaldehyde; the other sample was fixed in 2.5% buffered glutaraldehyde. The contents from the three segments of the SI were collected and stored in a −20 °C freezer for digestive enzyme activity testing. The mucosa scrapings of the small