Effects of Prebiotic Supplementation in Formula Powder on Growth Performance, Blood Biochemical Indices, and Nutrient Apparent Digestibility in SD Rats: Postprint

Authors: Zhou Shuiyue, Hang Yuanxin, Zhang Yanchun, Dai Zhiyong, Pan Lina, Wang Jianwu, Fang Rejun

Date: 2018-12-25T00:00:00+00:00

Abstract

This study aimed to investigate the effects of dietary prebiotic supplementation in formula diet on growth performance, blood biochemical parameters, and nutrient apparent digestibility in SD rats. Forty-eight healthy 15-day-old SD rats (one week before weaning) with similar body weight were selected and, after a 3-day acclimatization period, randomly divided into three groups (n=16): Group A, Group B, and Group C. Group A served as the control group and was fed a basal formula diet, while Groups B and C were experimental groups fed formula diets supplemented with oligosaccharide prebiotic or polysaccharide prebiotic, respectively. The feeding period lasted 28 days. The results showed: 1) Compared with the control group, the average daily gain of SD rats in Groups B and C increased by 3.55% and 8.33%, respectively, and the feed-to-gain ratio decreased by 9.80% and 13.06%, respectively, but the differences were not significant (P>0.05). 2) Compared with the control group, serum high-density lipoprotein cholesterol (HDL-C) content in Group B SD rats was significantly increased (P 0.05), while serum urea nitrogen (UN) content was significantly decreased (P 0.05); serum insulin (Ins), insulin-like growth factor-I (IGF-I), and HDL-C contents in Group C SD rats were significantly increased (P 0.05). 3) Compared with the control group, the contents of arginine (Arg), serine (Ser), and alanine (Ala) in plasma non-essential amino acids of Group B SD rats were significantly increased (P 0.05); the contents of Arg, Ser, glycine (Gly), Ala, and tyrosine (Tyr) in plasma non-essential amino acids of Group C SD rats were significantly increased (P 0.05). 4) Compared with the control group, the apparent digestibility of crude protein and crude fat in Group B increased by 2.42% and 5.57%, respectively, but the differences were not significant (P>0.05); the apparent digestibility of crude protein and crude fat in Group C increased by 7.39%

and 8.20%, respectively, with significant differences (P 0.05). It was concluded that under the conditions of this experiment, supplementation of either oligosaccharide prebiotic or polysaccharide prebiotic in formula diet could improve blood biochemical parameters and enhance nutrient apparent digestibility in SD rats, thereby promoting growth to a certain extent, with the polysaccharide prebiotic showing superior efficacy.

Full Text

Effects of Prebiotics Supplemented in Infant Formula on Growth Performance, Blood Biochemical Indices and Nutrient Apparent Digestibility of SD Rats

ZHOU Shuiyue[1], HANG Yuanxin[2], ZHANG Yanchun[3], DAI Zhiyong[3], PAN Lina[3], WANG Jianwu[2], *FANG Rejun[1]*

[1]College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China
[2]Xiangya School of Public Health, Central South University, Changsha 410128, China
[3]Ausnutria Dairy (China) Co., Ltd., Changsha 410005, China

Abstract: This experiment was conducted to investigate the effects of prebiotics supplemented in infant formula on growth performance, blood biochemical indices, and nutrient apparent digestibility in SD rats. Forty-eight healthy 15-day-old SD rats (one week before weaning) with similar body weights were selected and, after a 3-day adaptation period, randomly divided into three groups: group A (control), group B, and group C, with 16 rats per group. Group A was fed a common infant formula, while groups B and C were fed infant formulas supplemented with oligosaccharide prebiotics or polysaccharide prebiotics, respectively. The feeding trial lasted for 28 days. The results showed that: 1) Compared with the control group, the average daily gain (ADG) of groups B and C increased by 3.55% and 8.33%, respectively, while the feed-to-gain ratio (F/G) decreased by 9.80% and 13.06%, respectively, but these differences were not significant (P>0.05). 2) Compared with the control group, serum high-density lipoprotein cholesterol (HDL-C) content in group B was significantly increased (P 0.05), while serum urea nitrogen (UN) content was significantly decreased (P 0.05). In group C, serum insulin (Ins), insulin-like growth factor-I (IGF-I), and HDL-C contents were significantly increased (P 0.05). 3) Compared with the control group, plasma non-essential amino acids including arginine (Arg), serine (Ser), and alanine (Ala) in group B were significantly increased (P 0.05); in group C, plasma non-essential amino acids including Arg, Ser, glycine (Gly), Ala, and tyrosine (Tyr) were significantly increased (P 0.05). 4) Compared with the control group, the apparent digestibility of crude protein and crude fat in group B increased by 2.42% and 5.57%, respectively, but the differences were not significant (P>0.05); in group C, the apparent digestibility of crude protein and crude fat increased by 7.39% and 8.20%, respectively, with significant differences

(P 0.05). In conclusion, under the conditions of this experiment, supplementation of infant formula with either oligosaccharide or polysaccharide prebiotics can improve blood biochemical indices and nutrient apparent digestibility in SD rats, thereby promoting their growth to a certain extent, with polysaccharide prebiotics showing better effects.

Keywords: infant formula; prebiotics; growth performance; blood biochemical indices; nutrient apparent digestibility

Introduction

Breast milk is the most important food source for mammals after birth, containing abundant proteins, fats, carbohydrates, and bioactive substances that provide sufficient energy and nutrition for healthy infant growth and development [**?**]. As the third most abundant bioactive nutrient component in breast milk, prebiotics are actually lacking in infant formulas [**?**]. Research has found that the gut microbiota of breastfed infants differs from that of infants fed conventional infant formulas. Since breast milk is rich in prebiotics and small amounts of probiotics, adding prebiotics to conventional infant formulas can enhance their nutritional value [**?**]. Prebiotics are substances that cannot be degraded by digestive enzymes secreted by animals in the digestive tract but can selectively stimulate the growth of beneficial intestinal bacteria, including oligosaccharides, polysaccharides, polyols, plant extracts, protein hydrolysates, etc. [**?**, **?**]. Among them, polydextrose, as a polysaccharide, has prebiotic effects [**?**]. Currently, prebiotics widely used in human foods include lactulose, galactooligosaccharides, fructooligosaccharides, inulin and its hydrolysates, maltooligosaccharides, and resistant starch [**?**]. Studies have shown that in clinical trials, inulin-type fructooligosaccharides and galactooligosaccharides have beneficial effects on human digestive and immune health [**?**]. The combination of galactooligosaccharides and polydextrose can improve nutrient absorption in pigs and SD rats and may enhance memory through non-humoral regulatory mechanisms [**?**, **?**]. Polydextrose also affects nutrient absorption, immune regulation, and intestinal function [**?**].

In 2011, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition confirmed through scientific data that adding prebiotics to infant formulas generally does not cause side effects in terms of safety and growth in healthy infants [**?**, **?**]. However, researchers have not yet reached a consensus on the safety and efficacy of adding prebiotics to infant formulas [**?**]. Therefore, investigating the safety and efficacy of prebiotic-supplemented infant formulas has become an important responsibility for nutrition researchers. This study aimed to investigate the effects of supplementing infant formulas with oligosaccharide prebiotics and polysaccharide prebiotics on growth performance, blood biochemical indices, and nutrient apparent digestibility in SD rats, providing scientific evidence for further simulating breast milk and better meeting the

nutritional needs of healthy growth and development in Chinese infants.

1. Materials and Methods

1.1 Experimental Materials **Infant formulas:** Common infant formula, infant formula supplemented with oligosaccharide prebiotics, and infant formula supplemented with polysaccharide prebiotics were all provided by Ausnutria Dairy (China) Co., Ltd. The oligosaccharide-supplemented formula contained 2.13% oligosaccharides (1.85% fructooligosaccharides and 0.28% galactooligosaccharides), while the polysaccharide-supplemented formula contained 2.20% polydextrose.

Milk solutions: Milk solution 1 was prepared by mixing common infant formula with water at a ratio of 1:3; milk solution 2 was prepared by mixing oligosaccharide-supplemented formula with water at 1:3; and milk solution 3 was prepared by mixing polysaccharide-supplemented formula with water at 1:3. All solutions were prepared fresh before feeding.

Diets: Diet 1 was formulated with corn, wheat bran, and common infant formula at a ratio of 7:2:1; diet 2 contained corn, wheat bran, and oligosaccharide-supplemented formula at 7:2:1; and diet 3 contained corn, wheat bran, and polysaccharide-supplemented formula at 7:2:1. All diets were produced by an experimental animal company in Hunan Province.

1.2 Experimental Animals and Grouping The experimental animals consisted of 48 specific pathogen-free (SPF) grade SD rats, 15 days old (one week before weaning), with an average body weight of (29.39±1.89) g, provided by Changsha Tianqin Biotechnology Co., Ltd. (production license No. SCXK (Xiang) 2014-0011). The rats were housed in the SPF animal laboratory of Central South University at a temperature of 20-26 °C and relative humidity of 40%-70% with a 12 h light/12 h dark cycle.

After a 3-day adaptation period, the SD rats were randomly divided into three groups based on body weight: control group (group A), oligosaccharide group (group B), and polysaccharide group (group C), with 16 rats per group. All rats were housed individually for a 28-day feeding period. The feeding protocols are detailed in Table 1 .

Table 1 Feeding status and methods of SD rats

Groups	Phase 1 (Days 1-7)	Phase 2 (Days 8-28)
A	Milk solution 1 + Diet 1	Milk solution 1 + Diet 1
B	Milk solution 2 + Diet 2	Milk solution 2 + Diet 2
C	Milk solution 3 + Diet 3	Milk solution 3 + Diet 3

Note: 1) In phase 2, milk and feed were mixed in a ratio of 7:3.

1.3 Sample Collection Starting from day 8 of the experimental period, fresh feces (approximately 5 g) were collected from each cage twice daily (morning and afternoon) for 14 consecutive days. After removing impurities such as hair, the feces were mixed uniformly, 0.5 mL of 10% sulfuric acid was added, and samples were stored at -20 °C. The fecal samples were then dried at 65 °C, pulverized, and passed through a 40-mesh sieve for determination of nutrient apparent digestibility.

At the end of the feeding trial, after 24 h of fasting, rats were weighed on the morning of day 29. Blood was collected by orbital enucleation, and rats were euthanized by cervical dislocation. The abdominal cavity was opened to examine the stomach, liver, spleen, kidneys, pancreas, and small intestine for lesions. The stomach (after washing contents with saline), liver, spleen, and kidneys were weighed. Fresh blood samples collected in EDTA anticoagulant tubes were left at room temperature for 30 min, then centrifuged at 3,000 r/min for 15 min to obtain plasma, which was stored at -20 °C for determination of free amino acid content. Blood samples collected in potassium oxalate/sodium fluoride tubes were stored at -20 °C for blood glucose determination. Blood samples collected in ordinary serum tubes were left for 30 min, centrifuged at 3,000 r/min for 15 min to obtain serum, which was stored at -20 °C for serum biochemical analysis.

1.4 Analytical Methods

1.4.1 Growth Performance Daily feed intake, initial body weight, and final body weight (morning of day 29) were recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

- ADG (g/d) = (final weight - initial weight)/28
- ADFI (g/d) = total feed intake/28
- F/G = total feed consumption/total weight gain

1.4.2 Organ Indices Body weight and organ weights were recorded on the morning of day 29 to calculate organ indices using the formula: Organ index = organ weight (g)/body weight (g).

1.4.3 Blood Biochemical Indices Plasma free amino acids: Plasma free amino acid content was determined by RP-HPLC-FMOC-CL pre-column derivatization liquid chromatography.

Chromatographic conditions: Silversil C18 column (5 m, 4.6 mm × 150 mm, Dikma); mobile phase A, acetonitrile:water = 10:90 (v/v, containing 0.05% formic acid); mobile phase B, acetonitrile:water = 90:10 (v/v); flow rate 1.0

mL/min. Gradient program: 0-8 min, mobile phase B 24%-35%; 8-15 min, 35%-40%; 15-22 min, 40%-48%; 22-25 min, 48%-64%; 25-28 min, 64%-82%; 28-30 min, 82%-82%; 30-35 min, 82%-24%; stop at 38 min. Detection wavelength: excitation (ex) 260 nm, emission (em) 305 nm; injection volume 20 L; column temperature 37 °C.

Derivatization reaction: Accurately pipette 10 L of standard solution or sample (10 mol/L), add 100 L borate buffer (pH=9.0) and 20 L 9-fluorenylmethyl chloroformate (5 mmol/L in acetone), react in a 60 °C water bath for 1 h, add 20 L 1 mol/L HCl, centrifuge, and inject 20 L.

Sample preparation and measurement: Accurately measure 100 L sample, add 100 L methanol, mix well, stand at room temperature for 10 min, centrifuge at 15,000 r/min for 10 min, take 10 L supernatant for derivatization as with standard samples, and quantify by peak area.

Serum insulin and IGF-I: Serum insulin (Ins) and insulin-like growth factor-I (IGF-I) contents were determined by enzyme-linked immunosorbent assay (ELISA) using an MB-530 microplate reader (Shenzhen Huisong Technology Development Co., Ltd.). The procedure was as follows: Prepare standard solutions, wash buffer, biotin-labeled antibody working solution, and horseradish peroxidase-labeled avidin working solution according to instructions; equilibrate all reagents to room temperature (18-25 °C) for at least 30 min; set up standard and sample wells, add 100 L standard or sample to each well, mix gently, cover with plate sealer, incubate at 37 °C for 2 h; discard liquid, shake dry, no washing needed; add 100 L biotin-labeled antibody working solution to each well, cover with new plate sealer, incubate at 37 °C for 1 h; discard liquid, shake dry, wash plate 3 times (2 min soak, 200 L per well), shake dry; add 100 L horseradish peroxidase-labeled avidin working solution, cover with new plate sealer, incubate at 37 °C for 1 h; discard liquid, shake dry, wash plate 5 times (2 min soak, 200 L per well), shake dry; add 90 L substrate solution to each well in sequence, develop color at 37 °C for 15-30 min protected from light; add 50 L stop solution to each well in sequence to terminate reaction; measure optical density (OD) values at 450 nm within 5 min after termination using the MB-530 microplate reader.

Other serum parameters: Serum total protein (TP), albumin (ALB), total bilirubin (T-BIL), direct bilirubin (D-BIL), urea nitrogen (UN), total cholesterol (CHO), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) activities, and blood glucose content were determined using a CS-T300 automatic biochemical analyzer (Changchun Dirui Industrial Co., Ltd.) with detection kits provided by Changchun Dirui Medical Technology Co., Ltd. Serum alkaline phosphatase (AKP) was determined by micro-enzymatic method using detection kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.4.4 Nutrient Apparent Digestibility Dry matter, crude protein, and crude fat contents in diets and feces were determined according to conventional analysis methods in *Feed Analysis and Detection* [**?**]. Acid-insoluble ash (AIA) was used as an indicator, with AIA content determined according to GB/T 23743-2009. Nutrient apparent digestibility was calculated as follows [**?**, **?**]:

Nutrient apparent digestibility (%) = 100 - (M2n/M1n) × (M1m/M2m) × 100

Where: M1m = AIA content in diet; M2m = AIA content in feces; M1n = nutrient content in diet; M2n = nutrient content in feces.

1.5 Statistical Analysis Experimental data were analyzed by one-way ANOVA using SPSS 18.0 statistical software, with Duncan' s multiple comparison test used for post-hoc comparisons.

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2. Results

2.1 Effects of Prebiotics on Growth Performance of SD Rats As shown in Table 2 , supplementation with either oligosaccharide or polysaccharide prebiotics in infant formula had no significant effects on average daily feed intake (ADFI), average daily gain (ADG), or feed-to-gain ratio (F/G) in SD rats (P>0.05). However, compared with the control group, ADG in group B increased by 3.55% and F/G decreased by 9.80%, while in group C, ADG increased by 8.33% and F/G decreased by 13.06%.

Table 2 Effects of prebiotics supplemented in infant formula on growth performance of SD rats

Groups	ADG (g/d)	ADFI (g/d)	F/G
A	5.64±0.85	18.32±1.51	2.45±0.32
B	5.84±0.28	18.65±0.97	2.21±0.07
C	6.11±0.77	18.63±1.04	2.13±0.24

In the same row, values with the same or no letter superscripts indicate no significant difference (P>0.05), while different lowercase letters indicate significant difference (P 0.05). The same applies below.

2.2 Effects of Prebiotics on Organ Indices of SD Rats As shown in Table 3 , there were no significant differences in stomach, liver, spleen, or kidney indices among the three groups of SD rats (P>0.05).

Table 3 Effects of prebiotics supplemented in infant formula on organ indexes of SD rats

Items	Group A	Group B	Group C
Stomach	0.0207±0.0036	0.0189±0.0013	0.0171±0.0019
Liver	0.0440±0.0048	0.0458±0.0031	0.0427±0.0015
Spleen	0.0046±0.0031	0.0039±0.0024	0.0023±0.0004
Kidney	0.0121±0.0007	0.0107±0.0013	0.0105±0.0008

2.3 Effects of Prebiotics on Blood Biochemical Indices of SD Rats As shown in Table 4 , compared with the control group, serum HDL-C content in group B was significantly increased (P 0.05) and serum UN content was significantly decreased (P 0.05). In group C, serum Ins, IGF-I, and HDL-C contents were significantly increased (P 0.05).

Table 4 Effects of prebiotics supplemented in infant formula on serum biochemical indices and blood glucose content of SD rats

Items	Group A	Group B	Group C
Ins (nIU/mL)	15,972.53±4,550.03	15,847.37±3,831.51	19,262.05±5,686.57
IGF-I (ng/mL)	141.46±43.58	142.03±31.67	208.85±43.93
AKP (U/L)	529.32±65.50	586.32±114.44	482.12±114.98
GLU (mmol/L)	6.12±1.13	7.25±0.57	6.99±1.05
TP (g/L)	56.33±4.23	56.15±3.08	55.30±2.78
ALB (g/L)	35.15±2.68	34.90±1.61	33.48±0.74
T-BIL (mol/L)	4.06±1.93	2.81±0.92	2.09±0.34
D-BIL (mol/L)	2.51±1.51	1.65±0.90	0.90±0.26
ALT (U/L)	82.50±8.35	86.75±11.44	89.00±27.80
AST (U/L)	265.25±74.03	258.25±17.63	278.75±26.20
UN (mmol/L)	4.89±2.91	1.73±0.75	2.63±0.83
CHO (mmol/L)	3.23±2.03	2.58±0.44	2.38±0.30
TG (mmol/L)	0.89±0.18	0.59±0.38	0.50±0.21
HDL-C (mmol/L)	0.90±0.49	1.64±0.17	1.51±0.21
LDL-C (mmol/L)	0.70±0.19	0.75±0.19	0.63±0.13
CK (U/L)	2,244.00±467.46	1,710.00±232.77	1,664.00±465.59

As shown in Table 5 , compared with the control group, plasma non-essential amino acids including arginine (Arg), serine (Ser), and alanine (Ala) in group B were significantly increased (P 0.05). In group C, plasma non-essential amino

acids including Arg, Ser, glycine (Gly), Ala, and tyrosine (Tyr) were significantly increased (P 0.05). Groups B and C showed varying degrees of increase in essential amino acids including lysine (Lys), tryptophan (Trp), threonine (Thr), methionine (Met), valine (Val), phenylalanine (Phe), and leucine (Leu), but these differences were not significant (P>0.05).

Table 5 Effects of prebiotics supplemented in infant formula on free amino acid contents in plasma of SD rats (mol/L)

Items	Group A	Group B	Group C
Lys	183.20±81.89	223.17±92.97	284.90±57.45
Trp	206.30±78.65	248.16±81.91	302.67±64.05
Thr	141.27±36.78	179.21±40.61	207.16±59.32
Met	26.35±9.69	36.08±5.65	40.56±8.28
Val	103.47±31.25	127.62±5.47	137.08±39.60
Phe	27.45±13.32	37.68±14.75	42.50±16.08
Leu	55.82±16.09	61.37±7.07	73.50±20.29
Ile	212.17±70.17	189.64±25.89	203.70±60.04
His	231.60±86.09	195.86±36.63	188.72±92.18
Asn	187.50±89.17	133.19±84.53	183.85±130.82
Arg	73.50±12.44	238.15±71.31	213.80±49.92
Tau	1,007.29±502.08	1,596.84±846.75	1,919.31±671.88
Gln	392.24±108.34	397.81±80.28	436.47±176.92
Orn	1,567.19±281.69	1,788.38±416.46	1,819.24±677.58
Ser	159.00±32.89	233.51±44.26	289.29±73.42
Asp	18.43±15.95	23.34±9.38	30.15±23.82
Glu	128.17±40.32	146.32±19.11	185.26±26.65
Gly	214.05±29.89	240.73±35.19	319.22±47.55
Ala	434.21±65.71	817.06±36.73	892.99±122.26
Tyr	171.41±42.99	249.14±26.25	283.59±53.94
Pro	448.39±67.53	533.79±112.30	538.37±161.44
Cys	392.42±107.05	368.28±142.99	313.11±69.36

2.4 Effects of Prebiotics on Nutrient Apparent Digestibility of SD Rats As shown in Table 6 , compared with the control group, the apparent digestibility of crude protein and crude fat in group B increased by 2.42% and 5.57%, respectively, but the differences were not significant (P>0.05). In group C, the apparent digestibility of crude protein and crude fat increased by 7.39% and 8.20%, respectively, with significant differences (P 0.05).

Table 6 Effects of prebiotics supplemented in infant formula on nutrient apparent digestibility of SD rats

Items	Group A	Group B	Group C
Crude protein	74.36±3.07	76.21±1.73	79.88±3.02
Ether extract	64.62±3.33	68.24±3.42	69.93±2.21
Dry matter	85.33±0.54	85.94±0.61	85.95±0.57

3. Discussion

3.1 Effects of Prebiotics on Growth Performance and Organ Indices Research results on the effects of prebiotic supplementation in infant formulas on growth performance in young animals have been inconsistent. Costalos et al. [**?**] reported that infant formula enriched with fructooligosaccharides and galactooligosaccharides had no significant effect on growth rate in infants aged 6-12 weeks. Ashley et al. [**?**] conducted a 120-day trial with 419 infants and found that prebiotic supplementation in infant formula had no significant effect on growth rate from 14-120 days of age. Huang et al. [**?**] showed that dietary fructooligosaccharides had no significant effect on feed-to-gain ratio in weaned SD rats. Hang et al. [**?**] found that dietary mannan oligosaccharides had no significant effect on growth performance in piglets during the first week after weaning but significantly affected growth performance after the second week. In contrast, some studies have found that prebiotic supplementation in infant formulas can increase infant weight without affecting height and brain development [**?**, **?**]. Differences among studies may be related to prebiotic type and dosage, diet composition, animal species, physiological state, and experimental housing conditions. In this experiment, prebiotic supplementation in infant formulas could increase ADG and ADFI and decrease F/G in SD rats to some extent, but these effects were not significant, possibly due to large variations between replicates, feed change frequency during the experimental period, and changes in physiological status of SD rats, which is consistent with some previous findings.

Healthy animals have organ indices within normal ranges, and excessively large or small internal organs may indicate potential lesions [**?**]. In this experiment, there were no significant differences in stomach, liver, spleen, or kidney indices among the three groups of SD rats, and all values were within the standard range for normal physiological status [**?**, **?**], indicating that the various infant formulas did not alter organ structure or function.

3.2 Effects of Prebiotics on Blood Biochemical Indices Prebiotics have been shown to reduce blood lipids and regulate fat metabolism, though the specific mechanisms remain unclear [**?**]. Serum Ins and IGF-I contents reflect the metabolism of the three major nutrients in animals, but their metabolic mechanisms and pathways differ, as do their functions. Ins may primarily inhibit protein catabolism, while IGF-I may primarily promote protein anabolism [**?**]. Studies have shown that high-protein infant formulas can increase plasma and

tissue levels of amino acids that promote Ins production (such as Leu, isoleucine (Ile), Arg, Ala, and Phe [**?**]), thereby stimulating Ins and IGF-I secretion, promoting fat deposition and weight gain, but potentially causing obesity [**?**]. Yang et al. [**?**] suggested that other amino acids regulate Ins secretion mainly through: 1) acute responses, such as increasing glutamate dehydrogenase activity; and 2) chronic responses, such as gene transcription and regulation of -cell metabolism. Hogg et al. [**?**] found that in fetal rat islets, amino acids stimulated IGF-I more strongly than glucose, and during early animal growth, amino acids could significantly affect Ins secretion by regulating anabolic pathways and fat deposition [**?**, **?**]. In this experiment, compared with the control group, oligosaccharide prebiotic supplementation significantly increased serum HDL-C content and significantly increased serum Arg, Ser, and Ala contents. Polysaccharide prebiotic supplementation significantly increased serum Ins, IGF-I, and HDL-C contents and significantly increased serum Arg, Ser, Gly, Ala, and Tyr contents. Additionally, oligosaccharide prebiotic supplementation significantly decreased serum UN content. These results are consistent with previous studies. Possible reasons for prebiotic regulation of serum nutrient metabolism include: 1) Oligosaccharides and some polydextrose can promote bifidobacteria proliferation in the animal intestine, and bifidobacteria can mediate fat metabolism by affecting the activity of -hydroxy- -methylglutaryl-CoA reductase in the cholesterol synthesis enzyme system [**?**]; and 2) Increased protein utilization efficiency and elevated plasma levels of amino acids related to Ins and IGF-I production stimulate Ins and IGF-I secretion, thereby accelerating nutrient metabolism.

Prebiotics can also reduce the incidence of cancer and cardiovascular diseases. Studies have shown that high-density lipoprotein can participate in reverse cholesterol transport by regulating ATP-binding cassette proteins A1 and G1, reduce macrophage cholesterol content, and has important anti-inflammatory and antioxidant functions for maintaining health [**?**]. HDL metabolism is related to serum TG content, with higher serum TG leading to easier HDL degradation [**?**], which is basically consistent with this experiment' s results. Prebiotics may regulate blood pressure by modulating serum CK activity. Studies have found that CK may lower blood pressure through: 1) reducing local ATP levels in contractile proteins and myosin ATPase activity; 2) promoting nitric oxide production; and 3) reducing renal water and sodium retention capacity [**?**, **?**].

3.3 Effects of Prebiotics on Nutrient Apparent Digestibility Nutrient apparent digestibility measurement can directly reflect diet digestibility and animal digestive capacity [**?**]. This experiment showed that polysaccharide prebiotic supplementation in infant formula significantly improved the apparent digestibility of crude protein and crude fat in SD rats, while oligosaccharide prebiotic supplementation improved digestibility but not significantly. Kawasaki et al. [**?**] reported that dietary fructooligosaccharides had no significant effect on nitrogen apparent digestibility in guinea pigs but significantly improved nitrogen retention. Lu et al. [**?**] showed that infant formula supplementation with fructooligosaccharides and galactooligosaccharides could improve protein

utilization in SD rats. Martinelli et al. [?] found that puddings containing whey protein and polydextrose could reduce appetite and hunger in healthy adults. Ibarra et al. [?] found that adding polydextrose to breakfast could reduce Ins secretion, while adding it to lunch could reduce hunger, possibly because polydextrose improved nutrient digestibility and utilization. Additionally, Quan et al. [?] reported that dietary fiber could promote intestinal digestion and absorption. These results are basically consistent with our findings. Prebiotics may improve nutrient digestibility by promoting intestinal probiotic proliferation, as probiotics can secrete galactosidase, which animals cannot secrete themselves, and galactosidase can improve the digestion and absorption efficiency of crude protein, crude fat, and polysaccharides [?].

4. Conclusion

Under the conditions of this experiment, supplementation of infant formula with either oligosaccharide or polysaccharide prebiotics can improve blood biochemical indices and nutrient apparent digestibility in SD rats, thereby promoting their growth to a certain extent, with polysaccharide prebiotics showing better effects.

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