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Research Article

Effects of a Short-term Supplementation with Liquid Oligofructose-enriched Inulin on Faecal Characteristics and Selected Serum Metabolites of Healthy Saanen Kids

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Abstract

The objective of this study was to evaluate the effects of a short-term supplementation with liquid oligofructose-enriched inulin on faecal characteristics and selected serum metabolites of healthy Saanen kids. Twenty-four kids (44 days of age) were allotted to a control (CG) or an experimental (EG) group. Each group consisted of 12 kids. Each kid in EG was supplemented with 0.8 and 1.6 g/d of oligofructose-enriched inulin from day 1 to 5 and from day 6 to 15, respectively. Liquid oligofructose-enriched inulin supplementation did not affect faecal score and pH (P>0.05). Faecal acetate, propionate and total SCFA concentrations did not differ (P>0.05) between CG and EG, whereas faecal butyrate concentration was higher (P<0.05) in kids supplemented with liquid oligofructose-enriched inulin. Due to trophic and antiinflammatory effects of butyrate, we hypothesize that oligofructose-enriched inulin supplementation may be useful to help tissue repair and regeneration, particularly during an intestinal infection. Faecal *Lactobacillus, Bifidobacterium* and *Clostridium perfringens* concentrations were not affected by oligofructose-enriched inulin supplementation (P>0.05). Daily dose of oligofructose-enriched inulin tended to increase serum glucose concentrations (P<0.09, P<0.08). Serum urea and albumin concentrations were similar between groups (P>0.05). Serum total protein and globulin levels were lower in EG compared with CG (P<0.05). During the experimental period lasting for 15 days, there were no differences in growth performance parameters between groups (P>0.05).

Keywords: *Liquid oligofructose-enriched inulin, Faecal characteristics, Serum metabolites, Saanen kids*

Sıvı Formdaki Oligofruktoz ile Zenginleştirilmiş İnulin Katkısının Kısa Süreli Kullanımının Sağlıklı Saanen Irkı Oğlaklarda Dışkı Özellikleri ve Bazı Serum Parametreleri Üzerine Etkisi

Özet

Bu çalışmanın amacı, sıvı formdaki oligofruktoz ile zenginleştirilmiş inulin katkısının kısa süreli kullanımının sağlıklı Saanen Irkı oğlaklarda dışkı özellikleri ve bazı serum parametreleri üzerine etkisini değerlendirmekti. Kırk dört günlük yaştaki 24 oğlak kontrol (KG) ve deneme (DG) olmak üzere 2 gruba ayrıldı. Her bir grupta 12 oğlak yer aldı. DG'deki her bir oğlağa deneme periyodunun ilk 5 günü 0.8 g/gün ve sonraki 10 gün boyunca da 1.6 g/gün oligofruktozla zenginleştirilmiş inulin verildi. Sıvı haldeki oligofruktozla zenginleştirilmiş inulin katkısının dışkı skoru ve pH'sı üzerine bir etkisi görülmedi (P>0.05). Dışkıdaki asetat, propiyonat ve toplam uçucu yağ asidi düzeyleri KG ve DG arasında farklılık göstermezken (P>0.05) dışkıdaki bütirat miktarı sıvı formdaki oligofruktozla zenginleştirilmiş inulin katkısını alan oğlaklarda daha yüksekti (P<0.05). Bütirik asidin besleyici ve antiinflamatuar etkilerinin olması sebebiyle, oligofruktozla zenginleştirilmiş inulin kullanımının özellikle intestinal enfeksiyonlar sırasında doku onarımı ve yenilenmesine yardım etmek için yararlı olabileceği ön görülmüştür. Dışkıdaki *Lactobacillus, Bifidobacterium* ve *Clostridium perfringens* konsantrasyonları oligofruktozla zenginleştirilmiş inulin uygulaması ile değişmedi (P>0.05). Oligofruktozla zenginleştirilmiş inulin katkısının günlük dozu serum glikoz konsantrasyonlarını arttırma eğilimindeydi (P<0.09, P<0.08). Serum üre ve albümin konsantrasyonları gruplar arasında benzerdi (P>0.05). DG'deki serum total protein ve globülin seviyeleri KG'dekinden daha düşüktü (P<0.05). On beş gün süren deneme periyodu sırasında gelişim performansı parametreleri bakımından gruplar arasında farklılık bulunmadı (P>0.05).

Anahtar sözcükler: *Oligofruktozla zenginleştirilmiş inulin, Dışkı özellikleri, Serum metabolitleri, Saanen Irkı oğlaklar*

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INTRODUCTION

Prebiotics are selectively fermented ingredients which can be able to modulate the composition and activity of the intestinal bacterial populations towards a healthier microflora [1,2]. Fructans are a class of the non-digestible oligosaccharides defined as a prebiotic and include short-chain fructooligosaccharides (scFOS), oligofructose and inulin [1,3].

Fructans are fermented by beneficial types of colonic bacteria *(Lactobacillus* and *Bifidobacterium)* [1,4]. *Lactobacillus* and *Bifidobacterium* are desirable colonic bacteria due to their health benefits such as inhibitory effect on the growth of pathogenic bacteria *(Escherichia coli*, *Clostridium* species and *Salmonella* species) in the colon and improving host immunity [5]. Colonic fermentation of fructans increases the production of short-chain fatty acids (SCFA), especially acetate, propionate and butyrate [6,7]. SCFA are bactericidal substances [8] and increased SCFA production reduces colonic pH, which may suppress the proliferation of potential pathogenic bacterial species [1,9]. In addition to potential effects of SCFA on the intestinal pH and pathogens, especially butyrate is associated with a trophic effect on the colonic epithelium [5,10]. Butyrate is the major energy source of colonocytes and seems to make the greatest contribution to the integrity of the colon [11,12].

Some of the previous studies in animals have shown that fructans can alter the intestinal pH [13,14], SCFA concentrations [6,7,13] and bacterial populations [15-17], as well as blood glucose [18,19] and urea [20,21] levels. In addition, it has been reported that fructans may improve the growth performance of animals [15,17,22]. But, on the other hand, a satiety effect of prebiotic supplementation can jeopardize the attempt to increase body weight gain in livestock [23].

Inulin and oligofructose are the most studied prebiotic supplements [2,24]. In addition to these fructans, a specific mixture (Synergy1, Orafti®, BENEO-Orafti S.A., Tienen, Belgium) has been developed. Synergy1 known as oligo-fructose-enriched inulin is a combination of chicory inulin molecules with selected chain lengths, enriched by a specific fraction of oligofructose. The unique chain length distribution of oligofructose-enriched inulin provides a sustained fermentation activity throughout the entire colon [24].

Although some information obtained from previous studies is available on the effects of feeding prebiotics in dogs [4], cats [16], rabbits [13], horses [7], pigs [21,25,26], calves [27,28] and lambs [29], no information has been reported, except for the results of our previous study [14], on the effects of prebiotic supplementation in kids. The objective of this study was to evaluate the effects of a short-term supplementation with liquid oligofructose-enriched inulin on the faecal responses of score, pH, SCFA concentrations and selected bacterial populations as indicators of intestinal health and serum urea and glucose levels in healthy Saanen kids.

MATERIAL and METHODS

Experimental Unit and Animals: The study was carried out at Uludag University Applied Research Center for Veterinary Faculty Unit in Bursa, Turkey. All animals were handled according to the EU directive number 86/609/EEC concerning the protection of animals used for experimental and scientific purposes. In addition, this study was conducted under an approved protocol by Animal Care and Use Committee of University of Uludag (approval number: 22.02.2012, 24/B2).

Twenty-four healthy Saanen kids with 44 days of age (12 male and 12 female) were used in the current study. Healthy kids were selected by clinical examination and monitoring in respect to general appearance and diarrhoea. The kids were sorted by parity of their dams and body weight at the beginning of the study and assigned to one of the two groups (control; CG and experimental; EG). Each group consisted of 12 kids (6 male and 6 female).

Management and Experimental Design: At 44 days of age, the kids were removed from their dams and housed in individual pens equipped with feeders and waterers throughout the experimental period lasting for 15 days. During the first 5 days of the experimental period, the kids were allowed to stay with their dams for 45 min in the morning (08.30 h to 09.15 h). The kids were weaned at 48 days of age. Water was offered *ad libitum* during the experimental period. Pelleted starter concentrate (Saf Feed Industry, Eskişehir, Turkey) was given 400 and 500 g once a day during the period from day 1 to 5 and from day 6 to 15 at 09.15 h, respectively. Nutrient analyses of pelleted starter concentrate were performed according to the AOAC [30]. Nutrient compositions of pelleted starter concentrate were presented in *Table 1*.

Each kid in EG was supplemented with 0.8 and 1.6 g/d of oligofructose-enriched inulin (Orafti®Synergy1, BENEO-Orafti S.A., Tienen, Belgium) from day 1 to 5 and from day 6 to 15 at 09.15 h, respectively, whereas the kids in CG did not receive oligofructose-enriched inulin. Synergy1 contained 93.5% of oligofructose-enriched inulin according to the certificate of analysis reported by BENEO-Orafti S.A. The daily dose of oligofructose-enriched inulin was dissolved in 10 ml of distilled water, and then administered to the kids in EG by sucking using a syringe. The kids in CG received orally only 10 ml of distilled water.

Faecal Score, the Occurrence of Diarrhoea and Faecal pH: Faecal samples were collected from each kid by retrieval from the rectum on day 1, 5 and 15 at 11.15 h. Faecal samples were scored in respect to consistency on all collection days according to the following system: 1 = watery, diarrhoea; 2 = soft, unformed; 3 = soft, formed;

Table 1. *Nutrient compositions of starter concentrate on dry matter basis*
Tablo 1. *Başlangıç yeminin kuru maddedeki besin maddesi kompozisyonu*

Item	Starter Concentrate[a, b]
Dry matter, %	92.95
CP, %	21.24
Ether extract, %	4.44
NDF, %	27.98
ADF, %	14.15
ADL, %	4.31
NFC[c], %	38.94
Ash, %	7.40

[a] Saf Feed Industry, Eskişehir, Turkey; [b] Contained the main ingredients: ground corn grain, ground barley grain, wheat bran, soybean oil, soybean meal, corn gluten meal, sunflower meal, molasses, mineral and vitamin mix, limestone, salt; [c] NFC (Nonfiber carbohydrate) = 100 - (NDF% + CP% + Ether extract% + Ash%)

4 = hard, formed; and 5 = hard, dry pellets. During the study, the kids were closely monitored daily in respect to diarrhoea. Faecal score of 1 was considered to be diarrhoea. The kids treated for diarrhoea were recorded. On day 5 and 15, each faecal sample was diluted 10-fold with distilled water as described by Verlinden et al.[31]. The mixture of faecal sample and distilled water was homogenized and faecal pH was immediately measured using an electronic pH meter (PT-10, Sartorius AG, Goettingen, Germany).

Concentrations of Faecal SCFA: After faeces collections on day 15, 2.0 g of fresh faecal samples were immediately placed in plastic tubes, and then acidified and diluted with 2 ml of 25% metaphosphoric acid and 6 ml of distilled water as described by Flickinger et al.[3]. The samples were centrifuged at 25.000 x *g* for 20 min (Eba-21, Hettich GmbH & Co.KG, Tuttlingen, Germany) within 1 h after faeces collections. The supernatants obtained were frozen at –20°C until the analysis. Before the analysis, the supernatant was thawed, centrifuged at 13.000 x *g* for 10 min and transferred into a gas chromatograph sample vial. Concentrations of acetate, propionate, butyrate and total SCFA (acetate + propionate + butyrate) were determined using a Perkin Elmer Auto System gas chromatograph (Hewlett Packard Agilent Technologies 6890N Network GC System, Serial CN10447002, China) and a glass column (30 m x 0.32 mm i.d.) packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb (Supelco Inc., Bellefonte, PA, USA).

Selected Faecal Bacterial Populations: Sterile faecal samples could be collected from 8 of 12 and 7 of 12 kids in CG and EG, respectively, on day 15 by retrieval from rectum using sterile gloves. Faecal samples were placed in sterile sampling bags and immediately transported to the laboratory. One g of each faecal sample was homogenized with 9 ml of saline peptone water. Subsequently, serial 10-fold dilutions were made in saline peptone water and plated onto relevant selective media. *Lactobacillus* was grown on Man Rogosa Sharpe agar (MRS agar, Hypet Media, Diatek, Istanbul, Turkey) Each plate was incubated at 37°C for 48 h in anaerobic jar (Oxoid AN0035A, Basingstoke, Hampshire, UK) with gas generating sachet (Oxoid CN0020C, Basingstoke, Hampshire, UK). Non-spore-former rods, gram-positive and catalase-negative isolates were regarded as *Lactobacillus*. *Bifidobacterium* was grown on Bifidobacterium Selective Medium agar (BSM agar, Hypet Media, Diatek, Istanbul, Turkey). The plates were incubated at 37°C for 48 to 72 h in anaerobic jar with gas generating sachet. The selected bacterial colonies were investigated with regard to cell morphology by Gram staining. The colonies with gram-positive rods and characteristic bifurcated "Y" and "V" shapes were recorded as *Bifidobacterium*. *Clostridium perfringens* was grown on 4-Methylumbelliferyl phosphate-supplemented (MUP, Merck 1.00888, Darmstadt, Germany) Tryptose Sulfite Cycloserine agar (TSC agar, Hypet Media, Diatek, Istanbul, Turkey) containing egg yolk emulsion and selective supplement. Each plate was incubated at 37°C for 24 h in anaerobic jar with gas generating sachet. Each presumptive black colony were added to 10 ml of Thioglycolate Broth (Merck 1.08190, Darmstadt, Germany) and then incubated at 37°C for 16 to 18 h. Activated cultures were prepared for identifying by Gram staining and biochemical tests. The colonies with gram-positive and nonmotile rods, lactose positive, nitrate reduction positive, gelatine positive and motility negative were considered to be *Clostridium perfringens*. The bacterial counts were expressed as log 10 cfu per gram of faecal samples.

Serum Glucose, Urea, Total Protein, Albumin and Globulin Measurement: On day 15 at 2 h and 4 h after feeding, blood samples were collected from the jugular vein into serum separator tubes. Serum glucose and urea concentrations were determined using a reflotron analyzer (The Boehringer Mannheim Reflotron, Roche Diagnostics, Mannheim, Germany) with Reflotron®Glucose REF 10744948 diagnostic kit and Reflotron®Urea REF 11200666 diagnostic kit, respectively. Serum total protein, albumin and globulin levels were determined using a VetScan analyzer (Abaxis Inc., Union City, USA) with large animal profiles 500-0023 rotor.

Body Weight, Feed Intake and Feed Efficiency: The kids were weighed before feeding at day 1, 5 and 15. Daily starter concentrate feed intake was individually measured. Average daily weight gain (ADG), average daily feed intake (ADFI), and feed efficiency (ADG/ADFI) were calculated for each kid.

Statistical Analysis: Statistical analyses were conducted by using SPSS software [32]. Data for faecal score and pH were tested to determine normal distribution by Kolmogorov-Smirnov test and F-test, respectively. Faecal score and pH were analysed by independent sample T-test. Data for the amounts of individual and total SCFA in faeces and serum metabolites were tested to determine normal distribution by F-test. The analyses for faecal SCFA concentrations

and serum metabolites were performed by independent sample T-test. Faecal bacterial populations were analysed by the Mann–Whitney test. Data for growth performance parameters were tested to determine normal distribution by Kolmogorov-Smirnov test. Growth performance parameters were analysed by independent sample T-test. Differences between groups were considered significant at P≤0.05. Statistical trends were indicated as P<0.1.

RESULTS

Faecal score and faecal pH were not different (P>0.05) between groups *(Table 2)*. Diarrhoea developed in 1 of 12 and 2 of 12 kids in CG and EG, respectively, during the experimental period. The number of kids treated for diarrhoea was one in both CG and EG *(Table 2)*. The amounts of acetate, propionate and total SCFA (acetate + propionate + butyrate) in faeces did not differ (P>0.05) between groups, whereas faecal butyrate concentration was higher (P<0.05) on day 15 in EG compared with CG *(Table 2)*. No differences (P>0.05) in faecal concentrations of *Lactobacillus*, *Bifidobacterium* and *Clostridium perfringens* were found between CG and EG *(Table 3)*. Serum glucose concentrations tended to be higher (P<0.09, P<0.08) on day 15 in EG compared with CG *(Table 4)*. Serum urea concentrations were not different (P>0.05) on day 15 between groups *(Table 4)*. Serum total protein and globulin levels were lower (P<0.05) on day 15 in EG compared with CG while serum albumin levels and albumin/globulin ratios did not differ (P>0.05) between groups *(Table 4)*. During the experimental period lasting for 15 days, there were no differences (P>0.05) in body weight, ADG, ADFI and feed efficiency between groups *(Table 5)*.

DISCUSSION

***Faecal Score, the Occurrence of Diarrhoea and Faecal pH*:** Faecal scores were similar between groups in this study, which was in agreement with the results reported by Hill et al.[27], who added inulin (4 or 8 g/d) or mannanoligosaccharides (MOS; 6 g/d) to milk replacer of calves, and by Kara et al.[14], who supplemented inulin (0.6 g/d) to kids through oral gavage. In the current study, a lower faecal score would indicate formation of softer faeces. We observed that faecal score was decreased on day 5 and 15 in comparison to day 1 in EG *(Table 2)*. However, the decrease in faecal score had no clinical importance since it remained in an acceptable range and was not associated with diarrhoea. Potential adverse side effects such as loose faeces and diarrhoea may occur at high doses of fructans

Table 2. *Effect of liquid oligofructose-enriched inulin supplementation on faecal score, the occurrence of diarrhoea, faecal pH and faecal SCFA concentrations*
Tablo 2. *Sıvı haldeki oligofruktozla zenginleştirilmiş inulinin dışkı skoru, diyare oluşumu, dışkı pH'sı ve uçucu yağ asidi konsantrasyonu üzerine etkisi*

Item		Groups		SEM	P-Values
		CG[a] (n = 12)	EG[b] (n = 12)		
Faecal score[c]	day 1	2.83	3.33	0.32	0.09
	day 5	2.83	2.83	0.21	1.00
	day 15	3.00	2.83	0.24	0.50
The number of kids with diarrhoea/total kids		1/12	2/12	-	
The number of kids treated for diarrhoea/total kids		1/12	1/12	-	
Faecal pH	day 5	7.62	7.76	0.11	0.16
	day 15	7.75	7.63	0.13	0.31
Faecal SCFA concentrations (mmol/l; day 15)	Acetate	4.67	5.03	0.44	0.45
	Propionate	1.59	1.88	0.19	0.14
	Butyrate	1.42	1.71	0.14	0.05
	Total SCFA[d]	7.67	8.62	0.76	0.23

*[a] Control group; [b] Group supplemented with oligofructose-enriched inulin; [c] Faecal scoring system: **1** = watery, diarrhoea; **2** = soft, unformed; **3** = soft, formed; **4** = hard, formed; **5** = hard, dry pellets; [d] Acetate + Propionate + Butyrate*

Table 3. *Effect of liquid oligofructose-enriched inulin supplementation on faecal bacterial populations*
Tablo 3. *Sıvı haldeki oligofruktozla zenginleştirilmiş inulinin dışkıdaki bakteriyel popülasyon üzerine etkisi*

Item		Groups		SEM	P-Values
		CG[a] (n = 8)	EG[b] (n = 7)		
Faecal bacterial populations (cfu $\log_{10}$/g fresh faeces; day 15)	*Lactobacillus*	4.31	4.19	0.27	0.78
	Bifidobacterium	4.26	4.40	0.45	0.78
	Clostridium perfringens	2.24	1.74	0.35	0.64

[a] Control group; [b] Group supplemented with oligofructose-enriched inulin

Table 4. *Effect of liquid oligofructose-enriched inulin supplementation on serum glucose, urea, total protein, albumin and globulin levels and albumin/globulin ratio*

Tablo 4. *Sıvı haldeki oligofruktozla zenginleştirilmiş inulinin serum glikoz, üre, total protein, albümin ve globülin düzeyleri ve albümin/globülin oranı üzerine etkisi*

Item		Groups		SEM	P-Values
		CG[a] (n = 12)	EG[b] (n = 12)		
Glucose, mg/dl	2 h after feeding	80.87	87.15	2.53	0.09
	4 h after feeding	71.31	78.24	2.21	0.08
Urea, mg/dl	2 h after feeding	49.26	47.88	1.61	0.52
	4 h after feeding	49.73	48.39	1.70	0.61
Total protein, g/dl	2 h after feeding	6.70	6.47	0.07	0.04
	4 h after feeding	6.79	6.57	0.06	0.04
Albumin, g/dl	2 h after feeding	3.36	3.35	0.07	0.93
	4 h after feeding	3.55	3.52	0.07	0.91
Globulin, g/dl	2 h after feeding	3.34	3.12	0.06	0.02
	4 h after feeding	3.24	3.05	0.06	0.03
Albumin/globulin ratio	2 h after feeding	1.01	1.09	0.04	0.17
	4 h after feeding	1.10	1.18	0.04	0.17

[a] Control group; [b] Group supplemented with oligofructose-enriched inulin

Table 5. *Effect of liquid oligofructose-enriched inulin supplementation on growth performance*

Tablo 5. *Sıvı haldeki oligofruktozla zenginleştirilmiş inulinin büyüme performansı üzerine etkisi*

Item		Groups		SEM	P-Values
		CG[a] (n = 12)	EG[b] (n = 12)		
Body weight, kg	day 1	10.46	10.41	0.53	0.96
	day 5	10.67	11.12	0.55	0.57
	day 15	12.20	12.31	0.54	0.89
ADG[c], kg (day 1 to 15)		0.12	0.13	0.01	0.60
ADFI[c], g	day 1 to 5	218.52	228.67	18.46	0.70
	day 5 to 15	370.18	347.47	30.54	0.61
	day 1 to 15	332.03	305.26	28.46	0.52
Feed efficiency[c], g/g (day 1 to 15)		0.39	0.41	0.04	0.78

*[a] Control group; [b] Group supplemented with oligofructose-enriched inulin; [c] **ADG:** average daily weight gain; **ADFI:** average daily feed intake; Feed efficiency: ADG/ADFI*

or at moderate levels of ingestion in unadapted animals, which is due to excessive amount of fermentation of fructans by colonic bacteria [1,6]. In our study, daily dose of oligofructose-enriched inulin did not adversely affect faecal score of the kids in EG.

During the experimental period, diarrhoea occurred in 1 of 12 and 2 of 12 kids in CG and EG, respectively *(Table 2)*. The kid with diarrhoea in CG was treated with antibiotics for 5 days. While one of the kids that developed diarrhoea in EG was treated with antibiotics for 5 days, the other kid with diarrhoea in EG recovered without antibiotic treatment within 2 day. In general, the kids used in our study were healthy. Heinrichs et al.[33] reported that potential health effects of prebiotics might not be observed in healthy animals. We also observed that inulin supplementation did not affect the incidence of diarrhoea in healthy kids [14]. A previous study using piglets demonstrated that supplementation of scFOS decreased the incidence of diarrhoea during a pathogen challenge [34]. Halas et al.[26] also reported that 8% inulin added to the diet reduced the incidence of diarrhoea in pigs infected with *E. coli*. In this respect, different results of fructan supplementation on the incidence of diarrhoea may be observed in kids facing a diarrhoeal disease challenge.

Faecal pH was similar between groups in our study *(Table 2)*. The result of faecal pH was not in agreement with our previous data [14]. However, despite the fact that inulin supplementation decreased faecal pH in our previous study using kids [14], this effect of inulin was not consistent. Barry et al.[4] reported that faecal pH was not decreased in adult dogs when 0.2% or 0.4% inulin or scFOS was added to the diet. Conversely, Berg et al.[7] observed that faecal pH was lower for yearling horses supplemented with 8 or 24 g/d of scFOS in comparison to control horses.

Kanakupt et al.[16] reported that faecal pH was less for cats fed the diet supplemented with 0.5% scFOS plus 0.5% galactooligosaccharides compared with the control group. In the current study, our previous study [14] and the studies mentioned above [4,7,16], these different results for faecal pH may be caused by differences in the type of prebiotics and diets used and different dose and duration of prebiotic supplementation.

Concentrations of Faecal SCFA: Butyrate plays an essential role in maintaining colonic epithelium integrity [11,12] and exerts antiinflammatory effects [35], which may help tissue repair and regeneration [36]. Greater butyrate concentrations are thought to have an important role in intestinal health [10]. Based on the increase in faecal butyrate concentration of EG *(Table 2)*, we hypothesize that oligofructose-enriched inulin supplementation may be useful to help tissue repair and regeneration, particularly during the intestinal infections causing epithelial damage and colonic inflammation. Faecal SCFA concentrations can be used as an indicator of fermentation patterns in the colon and the amount of SCFA in faeces can provide relevant information with respect to changes in SCFA production [7], as was demonstrated in our study. Flickinger et al.[3] observed that supplementation of 1, 2 or 3 g/d scFOS did not alter concentrations of faecal acetate, propionate, butyrate and total SCFA. Xu et al.[25] reported that 0.4% fructooligosaccharides (FOS) added to the diet of piglets increased the amount of acetate in faeces while there were no significant changes in faecal propionate and butyrate concentrations of the piglets supplemented with FOS. Propst et al.[6] observed that 0.3%, 0.6% or 0.9% oligofructose and 0.3%, 0.6% or 0.9% inulin added to the diet increased the amounts of faecal acetate, propionate, butyrate and total SCFA in dogs. Berg et al.[7] observed that concentrations of faecal acetate, propionate, butyrate and total SCFA were increased in yearling horses supplemented with 8 or 24 g/d of scFOS in comparison to control horses. In the current study with oligofructose-enriched inulin and aforementioned studies [3,6,7,25], the results for faecal SCFA were different and variable, which may have been due to fermentation characteristic (the rapid or the slow fermentation) of fructans used, the amount of fructan fermentation in the colon and the type of basal diet used [1,3].

Selected Faecal Bacterial Populations: Faecal concentrations of *Lactobacillus*, *Bifidobacterium* and *Clostridium perfringens* were not affected by oligo fructose-enriched inulin supplementation *(Table 3)*. Increased SCFA concentrations and lowered pH are generally associated with a reduction in the growth of pathogenic bacteria in the intestine [1,5,12]. In this study, the result of faecal SCFA concentrations and no decrease in faecal pH supported the conclusion that oligofructose enriched inulin did not affect faecal concentration of *Clostridium perfringens* in healthy kids. In our previous study [14], it was found that supplementation of 0.6 g/d inulin did not decrease faecal concentrations of total *Clostridium* and *Escherichia coli* in healthy kids. Barry et al.[4] demonstrated that faecal concentrations of *Clostridium perfringens, Lactobacillus* and *Bifidobacterium* were not affected in healthy dogs when 0.2% or 0.4% inulin or scFOS was added to the diet. The results of prebiotic supplementation on intestinal microflora may be better during a disease challenge when the numbers of beneficial bacteria in the colon are decreased [37]. Heinrichs et al.[33] reported that the reason for the lack effect of prebiotic supplementation on faecal bacterial populations might be that calves used in their study were healthy. Since the kids used in our study were generally healthy during the experimental period, faecal bacterial populations may have not been affected by oligofructose-enriched inulin supplementation. Kanakupt et al.[16] reported that faecal concentrations of *Bifidobacterium* were increased in healthy cats supplemented with 0.5% scFOS, 0.5% galactooligosaccharides or 0.5% scFOS plus 0.5% galactooligosaccharides. Zhao et al.[17] observed that dietary supplementation with 1% and 2% fructan decreased *Escherichia coli* and increased *Lactobacillus* concentrations in faeces of healthy pigs. In contrast to ineffectiveness of fructans on colonic bacteria of healthy animals as observed in our study and some previous studies [4,14,33], it has been shown that fructans may alter faecal bacterial populations of healthy animals in the studies by Kanakupt et al.[16] and by Zhao et al.[17]. Thus, the effects of fructans on faecal microflora in healthy animals warrant further scrutiny.

Concentrations of Serum Glucose, Urea, Total Protein, Albumin and Globulin: It has been reported that fructans have the potential to decrease blood glucose level [19,20]. Conversely, in our study, serum glucose concentration tended to be higher when oligofructose-enriched inulin was supplemented to kids at the level of 0.46% (1.6 g/d) of average daily starter concentrate consumption *(Table 4)*. Similar to our result, 1% oligofructose or 1% inulin added to the diet of laying hens increased serum glucose levels [18]. In addition, Xu et al.[25] also observed that 0.4% FOS added to the diet increased serum glucose concentration in pigs. Diez et al.[38] reported that the inclusion of 7% inulin in the diet did not affect plasma glucose concentration in dogs. Diez et al.[20] observed that 2% sugar beet fiber plus 8% inulin added to the diet of dogs reduced plasma glucose level compared with the diets without additional fibre or with 1% sugar beet fiber plus 4% inulin. As observed in our study and aforementioned studies [18,20,25,38], it is likely that higher levels of dietary fibres such as fructans and beet fiber may play a role in lowering blood glucose concentration [1,20]. Nevertheless, the effect of liquid oligofructose-enriched inulin on serum glucose concentration remains unclear in kids.

The reduced pH as a result of colonic fermentation of prebiotics enhances the conversion of absorbable

ammonia into less absorbable ammonium. In addition, prebiotics serving as an energy source for intestinal bacteria may induce the trapping of nitrogen in the form of bacterial protein for extra bacterial growth, thus decreasing ammonia absorption. These effects are associated with a reduction in blood urea concentration [39,40]. Younes et al.[39] reported that FOS added to the diet decreased both pH of caecal content and plasma urea level in rats. Samal et al.[41] also demonstrated that supplementation of Jerusalem artichoke as a source of inulin reduced both pH of caecal, colonic and rectal contents and blood urea concentration in rats. In our study, serum urea concentrations were similar for both groups *(Table 4)*. The absent effect of oligofructose-enriched inulin on serum urea concentration agreed with no decrease in faecal pH and no change in faecal bacterial populations in EG.

In our study, serum total protein, albumin and globulin concentrations and albumin/globulin ratio were within reference ranges [42] in both CG and EG. These data suggest that there were no major metabolic problems at the end of the study in any group. Serum albumin concentrations and albumin/globulin ratios were similar between groups. Serum total protein and globulin levels were lower for EG in comparison to CG *(Table 4)*. Globulins are subdivided into α-, β- and γ-globulins. The γ globulins are largely composed of immunoglobulins and the α- and β-globulin fractions contain a great variety of different proteins [42]. Due to the presence of different globulin fractions identified as α, β and γ, the reason of difference in serum globulin concentration between CG and EG remained unclear.

Growth performance: A satiety effect of prebiotic supplementation can jeopardize the attempt to increase body weight gain in livestock [23]. However, in our study, liquid oligofructose-enriched inulin supplementation did not lead to a negative effect on body weight, ADG and ADFI *(Table 5)*. In contrast to a satiety effect of prebiotics, it has been shown that fructan supplementation may improve feed efficiency and growth performance of young animals [17,22,43]. Mul [44] observed an improvement in ADG and feed efficiency when 2 to 5 g/kg of oligofructose was added to milk replacer of calves. Xu et al.[15] observed that supplementation of 4.0 g/kg FOS to the diet increased ADG of broilers while 2 or 8 g/kg of FOS added to the diet had no effect on ADG. Hill et al.[27] demonstrated no improvement in ADG, ADFI and feed efficiency of calves supplemented with 4 and 8 g/d inulin. Grand et al.[28] reported that the addition of 3 or 6 g/d scFOS in the milk replacer had no effect on body weight, ADG, ADFI and feed efficiency in calves. Based on the current and previous studies, it can be seen that the effects of fructan supplementation on growth performance are inconsistent. These different results for growth performance may be related to amount of fructan compounds in the basal diet, the type of fructans used, the dose and the duration of fructan supplementation, as well as stress status of animals [17,23]. In our study, no effect of liquid oligofructose-enriched inulin on growth performance of kids may be due to the supplementation period (15 days) being short.

In conclusion, liquid oligofructose-enriched inulin supplemented to kids did not negatively affect faecal score. Supplementation of oligofructose-enriched inulin did not alter faecal pH and faecal *Lactobacillus*, *Bifidobacterium* and *Clostridium perfringens* concentrations. Higher doses than 1.6 g oligofructose-enriched inulin/d may be necessary to alter faecal pH and faecal bacterial populations of healthy kids. Faecal butyrate concentration was higher in kids supplemented with oligofructose-enriched inulin. Since butyrate exerts antiinflammatory effects and plays an essential role in maintaining colonic epithelium integrity, liquid oligofructose-enriched inulin supplementation may be useful to help tissue repair and regeneration, particularly during an intestinal infection. Daily dose of oligofructose-enriched inulin tended to increase serum glucose concentrations. Short-term supplementation with liquid oligofructose-enriched inulin did not alter growth performance parameters. The results of our study will be useful to determine the dose and duration of fructan supplementation in future studies investigating the effects of fructans on faecal characteristics, serum metabolites, health parameters and growth performance in young small ruminants.

Conflict of Interest: There are no conflicts of interest issues concerning this submission.

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