ABSTRACT

**Background:** Functional constipation is a chronic gastrointestinal disease in children. Pharmacological treatment of constipation is mostly based on the prescription of laxatives. In addition to this treatment, complementary therapies were also proposed to treat constipation. In this study, the effect of whey protein in the treatment of constipation was investigated.

**Method and Materials:** A total of 56 children with functional constipation within the age range of 1 to 16 years were included in the study. Patients were randomly divided into the intervention (n=28) and control (n=28) groups. The intervention group received 15 g whey protein and the control group received 15 g placebo. Both groups received 0.4 - 0.8 gr/kg PEG powder. The amount of PEG was adjusted every four days based on having soft stools. Demographic information were collected and recorded. Furthermore, fecal consistency, stool frequency, symptoms of stool retention, fecal

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incontinence, and abdominal pain were recorded prior to the intervention and at the second and fourth weeks of the intervention.

**Results**: All patients had soft daily stools at the end of the intervention. At the end of the fourth week, the mean required amounts of PEG powder were respectively 0.62 and 0.28 g/kg in the control and intervention groups, which showed a significant difference (p value ≤0.001).

**Conclusion**: Based on these findings, whey protein is safe and can improve constipation. However, more studies with larger sample size and longer follow-up period are needed to confirm the results of this study.

**Keywords**: Functional constipation; children; whey protein.

**ABBREVIATIONS**

*Polyethylene glycol (PEG)*

**1. INTRODUCTION**

Constipation is a common disorder in children that manifests itself in a range of symptoms such as decreased stool frequency, hard and thick stools, stools with pain, fecal incontinence, and abdominal pain [1]. Constipation is a symptom of large intestine dysfunction. Constipation can be caused by impaired intestinal movement or impaired rectal function and fecal control mechanisms [2,3].

The muscular and nervous systems of the intestine are the two important parts of the intestinal motility. Intestinal motility is also affected by factors such as bile acid metabolism, mucus secretion, intestinal microorganisms, immune system, and composition of its contents. The imbalance between these factors can lead to intestinal motility disorders [4]. A large number of microorganisms live in the colon that have positive effects on their host. These living organisms play a role in the evolution and stimulation of the intestinal nervous system and the brain [5].

The Lactobacillus and Bifidobacterium species in animal models normalize the large intestine movements [6]. These organisms ferment undigested oligosaccharides, as prebiotics, that reach the colon [7]. Some foods can play a prebiotic role, such as milk lactose and protein [8]. Milk, as a functional food, plays an important role in human health. Cow’s milk contains positive physiological effects and has long been consumed by humans as a part of the food pyramid [9]. The main proteins in milk are casein and whey (20%), which contains alphalactalbumin, beta-lactoglobulin, albumin, and immunoglobulins, lactoferrin [10]. Whey protein is usually obtained as a by-product of the cheese production from milk. During the cheese production process, the milk whey protein, lactose, and oligosaccharides are separated. The isolated fluid contains 1% whey and 5% lactose [11].

Whey protein plays a role in the long intestine function [12]. The literature suggested its role in regulating the immune system, increasing muscle strength, and improving antimicrobial activity [13-15]. Studies also reported the positive effect of whey protein on human health, considered its prebiotic role [16-17]. Laxatives such as polyethylene glycol (PEG) are common medicines in treating constipation [18]. PEG has osmotic properties and softens the stool, but it should be consumed for more than six months or longer and has a treatment response of about 50 to 60% [19]. Long-term duration of treatment and incomplete response have necessitated finding another treatment that address mechanism of this disorder. more appropriate effectiveness. Given the physiological roles of whey protein, it was hypothesized that this supplement may play a role in treating constipation.

**2. METHODS AND MATERIALS**

**2.1 Participants**

In this study, we investigated 1-16 year-old children with functional constipation who referred to a pediatric gastrointestinal clinic in 2018-2019. Functional constipation was diagnosed based on Roman IV criteria. Patients’ information including demographic characteristics, age of disease onset, associated symptoms including abdominal pain, fecal incontinence, fecal retention, consistency of feces, medications, milk intake, and history of bovine protein allergy were recorded in the checklists. Feces consistency was determined based on the Bristol stool scale.
and the pain intensity was determined from 0 to 100 based on visual criteria.

Exclusion criteria included finding a sign of organic disease in the history or examination of the patient, having neurological and psychological disorders, having a history of anorectal surgery, being treated for constipation for more than six months, and having a history of cow milk protein allergy and lactose intolerance.

This study was conducted in accordance with Helsinki principles. Consent forms were obtained from parents and children.

2.2 Study Material

2.2.1 Whey protein powder

The whey powder made by Nik Pharmaceutical Company was prepared in packages of 300 g. The powder composition contained 9% whey protein, 60% lactose, 28% maltodextrin, and ph 4.7. Because whey protein contains maltodextrin we used maltodextrin powder as a placebo that prepared from the School of Pharmacy and packaged in similar packages.

2.2.2 Study design

In this single-blind clinical trial that adheres to CONSORT guidelines, after obtaining a history and examination of children with chronic functional constipation, the participants were divided into the control and intervention groups according to the random number table. Enrollment was done by the pediatric gastroenterologist. Disimpaction was performed in the case that fecal impaction was diagnosed in patients. All participants were provided with toilet training. If any child consumed more than 750 cc of cow’s milk in 24 hours, the value was reduced to lower than 750 cc. The control group members were asked to consume 0.4-0.8 g PEG 3350 powder per kg of body weight daily. They were also instructed to dissolve all 15 g of the powder in 200 g of water. Furthermore, they were required to dissolve 15 g of the placebo in 150 cc of water and consume it before breakfast.

The intervention group received 0.4-0.8 g PEG powder per kg of body weight. Furthermore, they were supposed to dissolve 15 g whey powder in 150 cc water and consume it before breakfast. The recommended amount of powder was prescribed based on personal experiences of the physicians. Parents were instructed to adjust the amount of PEG powder every four days, so that the children had soft daily stools. The Persian traditional medicine students collected the study informations from parents or patients on days 15 and 30 of the study by telephone call. To this end, a checklist was used including the following information: stool frequency, feces consistency (based on the Bristol stool scale), fecal incontinence (Yes/ No), abdominal pain (Yes/ No, severity based on visual criteria), adherence to medications, and drug side effects including abdominal pain, nausea, vomiting, skin manifestations, irritation, etc.

Furthermore, patients’ and parents’ acceptance rates regarding consumption of PEG and whey powder were asked based on the following criteria.

1. The child eats easily and eagerly; 2. The child eats without resistance; 3. The child eats with protest; 4. The child eats with a lure; 5. The child eats by force and coercion; 6. The child resists eating even by force and coercion, but in the case of eating, s/he tolerates; 7. If the child eats anyway, s/he vomits.

Patients with an adherence rate of less than 75%, those who consumed other laxatives or drugs with motility effects, and participants who could not be followed up on were removed and their data were discarded.

In case of drug complications, the patient was asked to stop using the drugs and alternative therapy was started. In this case, information related to the drug side effects was included in the statistical analysis.

2.2.3 Outcome

The primary outcome of the study was the patients in the intervention group have soft stool with a 50% reduction in PEG consumption.

2.2.4 Sample size

Considering the significant level of 5%, test power of 80%, and results of the pilot study, the approximate standard deviation of PEG dose was estimated at $s = 5$. Furthermore, 50% decrease of drug dose was considered significant clinically and the total sample size was calculated as 25 people in each group. Randomization was performed using the Random Allocation Software.
2.2.5 Statistical analysis

Statistical analysis was performed using SPSS 23. Descriptive statistics were reported in terms of mean ± standard deviation, median, and range. In order to determine the difference between the two groups, the T-test and Mann-Whitney test were run for continuous data and the Chi-square test was used for categorical data. The Repeated Measures test was also applied for in-group analysis.

3. RESULTS

A total of 56 patients were included in the study, and they were randomly categorized into the intervention (n=28) and control (n=28) groups. During the study, four patients were excluded from the analysis due to lack of follow-up. Fig. 1 illustrates the participants' information. Fig. 1.

No significant difference was observed between the two groups in terms of age, gender, height, and body mass index. The average frequency of stools in these 52 patients was twice a week, their feces consistency was 1.4 based on the Bristol stool scale, 82.1% of the patients had abdominal pain, and 38.35% reported fecal incontinence (Table 1).

3.1 PEG Dose

At the beginning of intervention, the mean starting dose of PEG was 0.65 and 0.67 gr / kg in the control and intervention groups, respectively. In the second week of intervention, the PEG dose decreased in both groups, but no statistically significant difference was found between the two groups (P-value= 0.141). In the fourth week, the amount of PEG decreased significantly in the intervention group (0.28gr/kg) and showed a significant difference compared to the control group (0.62gr/kg) (P-value <0.001). According to Fig. 2, repeated measures test, and P-value < 0.001, change in the mean dose of PEG over time was significant between the intervention and control groups. The amount of PEG was decreased to zero in 11 patients of the intervention group in the fourth week, while no patient in the control group could stop PEG until the fourth week. The treatment results are presented in Table 2.

3.2 Feces Consistency

Based on the Bristol stool scale, most children with constipation had feces consistency of type one or two and no difference was observed between the two groups in this regard. After two weeks of treatment, feces consistency decreased and became softer. The mean Bristol scores were 3.8 and 4 in the control and intervention groups, respectively; no significant difference was observed between the two groups (P-value = 0.531). In the fourth week, no significant change was found between the two groups in terms of fecal consistency (P = 0.266). Moreover, the mean feces consistency did not change significantly between the second and fourth weeks of intervention.

3.3 Stool Frequency

At the beginning of the study, the mean stool frequency was not significantly different between the two groups (twice a week in the control group and 1.9 times a week in the intervention group). In the second week of intervention, the frequency of stools increased to 6.5 and 7.5 times per week in the control and intervention groups, respectively. In the fourth week of intervention, the average frequency of stools per week increased to 6.7 and 7.9 in the control and intervention groups, respectively. No significant difference was found between the two groups in the second and fourth weeks (P-value =0.131).

3.4 Fecal Incontinence

At the beginning of the study, 40.7% of the intervention group and 36% of the control group members had fecal incontinence; no significant difference was found between the two groups (P-value = 0.781). In the second week, fecal incontinence was completely controlled in both groups.

3.5 Abdominal Pain

Prior to the intervention, 82.1% of the patients reported abdominal pain; 68% in the control group and 96.3% in the intervention group. The abdominal pain complaint was significantly higher in the intervention group (P-value = 0.01). In the second week of intervention, the abdominal pain complaint decreased in the control (12%) and intervention (7.4%) groups, but the two groups were not significantly different in this regard (P-value =0.662). In the fourth week, no change was reported in the abdominal pain complaints in the intervention group, but abdominal pain complaints decreased to 4% in the control group. However, no significant difference was observed between the two study
groups (P-value=1). In terms of pain intensity, the mean pain intensity rates were respectively 53.33 and 72.38 in the control and intervention groups at the beginning of the study, which showed no significant difference. In the second and fourth weeks, no significant difference was found between the two groups. The mean changes of pain intensity over time were significant in both groups based on repeated measures test (P≤0.001).

### CONSORT 2010 Flow Diagram

**Fig. 1. The participants’ information**

| Table 1. Patients characteristic at baseline |
|---------------------------------------------|
| Sex                          | Peg(n=25) | Peg & Whey (n=27) | p  |
| Male (%)                     | 11(44)    | 9(33.3)            | 0.75 |
| Female (%)                   | 14(56)    | 18(66.7)           |     |
| Age at enrollment (Y)        | 4.96±2.14 | 5.7±2.76          | 0.253 |
| Weight (kg)                  | 17.36±4.93| 19.81±9.09        | 0.230 |
| Height (cm)                  | 105.76±14.82| 112.20±16.39  | 0.145 |
| Mean body mass (kg)          | 15.33±1.71| 15.05±2.81        | 0.673 |
| Number of bowel movement per week | 2±1.24 | 1.96±1.28        | 0.915 |
| Stool consistency according to Bristol scale | 1.44±0.5 | 1.44±0.5         | 0.975 |
| Abdominal pain (%)           | 17(68)    | 26(96.3)           | 0.01 |
| Severity of abdominal pain   | 53.33±48.62| 55.78±36.45    | 0.245 |
| fecal incontinency(%)        | 9(36)     | 11(40.7)          | 0.781 |
| Retentive posturing (%)      | 17(68)    | 21(77.8)          | 0.536 |
decreased significantly in the intervention received whey powder and PEG powder. The and placebo, while the intervention group study. The control group received PEG powder constipation in children. To this end, 52 children In this study, we investigated the effect of whey powder in the intervention group was lower than the control group, this difference was not acceptance rate was higher in the intervention than the control group, this difference was not significant (P-value= 0.69). Acceptance of the whey powder in the intervention group was lower than PEG in the control group, but the difference was not significant (P-value= 0.830).

3.6 Complications and Acceptance of the Drug

During the first week of the intervention, two patients developed complications. In the control group, one patient had 3.5% abdominal pain and in the intervention group, one patient reported 3.5% anal irritation. Acceptance of PEG was appropriate in both groups, as 84.6% of patients accepted the drug easily. Although the acceptance rate was higher in the intervention than the control group, this difference was not significant (P-value= 0.69). Acceptance of the whey powder in the intervention group was lower than PEG in the control group, but the difference was not significant (P-value= 0.830).

4. DISCUSSION

In a study of children with constipation, 63% had hard stools, 30.6% had fecal incontinence, and 64% complained about abdominal pain [21]. In another study among 222 children with functional constipation, 41.4% complained about abdominal pain and 33.8% had fecal incontinence [22]. In our research, abdominal pain was more frequent in the studied patients, which can be due to the differences in the sample size, participants' age, and tertiary nature of the study center.

According to the latest guidelines provided by ESPGHAN, PEG is considered as a first-line treatment for constipation. According to this guideline, the starting dose of PEG is 0.2-0.8 gr / kg, which is adjusted based on the patient's response [18-23]. The optimal dose can vary depending on the condition of each patient [24]. A study reported the optimal dose of 0.84 g / kg for PEG to control constipation in children. In this study, PEG acceptance was at an appropriate level and no serious side effects were reported for this drug [25]. In another study, the appropriate dose of PEG was 0.63 mg / kg for controlling constipation [26]. In the present study, the optimal dose of PEG was 0.62 mg / kg to control constipation in the fourth week of intervention, but this amount decreased to 0.28 mg / kg in the group receiving whey protein. The amount of PEG was zero in 40% of the patients.

Fig. 2. Mean dose of PEG in the intervention and control groups. repeated measures test showed a significant difference between groups (p<0.001) over time.

Fig. 2. Effect of whey protein powder on PEG dose in children with constipation over time. Intervention group (red line) received PEG and whey protein powder and control group (blue line) received PEG and Placebo. After a fourth week mean dose of PEG in the intervention group was 0.28±0.31 g/kg and in the control group was 0.62±0.42 g/kg. The repeated measures test showed a significant difference between groups (p<0.001) over time.
### Table 2. Treatment results

|                               | Peg        | Peg & whey | Peg vs Peg & whey |
|-------------------------------|------------|------------|-------------------|
| Peg dose (gr/kg)              | Week 1     | Week 2     | Week 4            | Week 1     | Week 2     | Week 4     | P      |
|                               |            |            |                   |            |            |            |        |
| Peg dose (gr/kg)              | 0.65±0.23  | 0.61±0.19  | 0.62±0.42         | 0.67±0.22  | 0.43±0.43  | 0.28±0.31  | 0.141  | 0.000   |
| Number of bowel movement per week | 2±1.24     | 6.58±1.16  | 6.7±0.96          | 1.96±1.28  | 7.58±3.97  | 7.99±4.03  | 0.230  | 0.131   |
| Stool consistency according to Bristol scale | 1.44±0.50  | 4±0.28     | 3.9±0.20          | 1.44±0.50  | 4±0.78     | 4.03±0.58  | 0.531  | 0.266   |
| Abdominal pain (%)            | 17(68)     | 3(12)      | 1(4)              | 26(96.3)   | 2(7.4)     | 2(7.4)     | 0.662  | 1.000   |
| Severity of abdominal pain    | 53.33±48.62| 8.88±22.98 | 1.11±4.71         | 72.38±36.45| 9.52±30.07 | 9.52±30.37 | 0.654  | 0.571   |
| fecal incontinency(%)         | 9(36)      | 3(12)      | 1(4)              | 26(96.3)   | 2(7.4)     | 2(7.4)     | 0.662  | 1.000   |
| Retentive posturing (%)       | 53.33±48.62| 8.88±22.98 | 1.11±4.71         | 72.38±36.45| 9.52±30.07 | 9.52±30.37 | 0.654  | 0.571   |
The whey protein, a healthy and nutritious source of protein, is obtained from the coagulation reaction in producing cheese from milk [27]. Separation of whey proteins of cow milk is accompanied by separation of lactose and oligosaccharides [8]. After enzymatic digestion, the whey lactose can be converted to GOS (Galacto-oligosaccharides), which is consumed as a substrate by intestinal microbes [11]. These studies investigated the role of whey protein in gastrointestinal function and showed that it can increase Lactobacillus and Bifidobacterium, two organisms effective in intestinal colonization of the intestine by some intestine and normal mice. In other words, between mice with no microbes in the large bowel, showed that bowel movements were different [36]. Administration of Lactobacillus microorganisms normalizes the bowel movements [36]. Food-induced changes in the gastrointestinal microbiota in animal models have confirmed their effects on gastrointestinal motility [37]. Propionate and butyrate stimulate colonic muscle contraction in rats [38]. In human studies, SCFA also increased ilium motility [33]. In patients with constipation, prescription of probiotics had positive effects on its treatment, although some studies did not confirm this effect [39-40]. Moreover, some studies showed that constipation improved with SCFA changes [41].

This study showed the beneficial effect of whey protein powder in treating constipation. This result can be attributed to various factors, such as the role of probiotics in immune regulation and motility of whey protein as well as its oligosaccharides and lactose. This study was single blind due to the taste of whey protein powder and was conducted using a small sample size with a four-week follow up period. A study with a larger sample size and longer follow up over the changes in probiotics and SCFA can be more helpful in determining the effect of whey protein on constipation.

5. CONCLUSION

Functional constipation is a chronic disorder that requires long-term treatment. Considering the beneficial effects of whey protein, as a supplement, on gastrointestinal tract and mechanisms of constipation, such as changes in motility and gastrointestinal flora, it can be considered as a suitable treatment along with conventional drugs.

CONSENT AND ETHICS APPROVAL

This study was conducted in accordance with Helsinki principles. Consent forms were obtained from parents and children. This study was also approved by the Ethics Committee of Yazd University of Medical Sciences (IR.SSU.REC.1397.115) and was registered on the clinical trial website (IRCTID: IRCT20190122042459N1).

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and
producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rahhal R, Us A. functional constipation. In: kleinman re, goulet o-j, mieli-vergani g, sanderson ir, sherman pm, shneider ml, editors. walker's pediatric gastrointestinal disease sixth ed. USA: PMPH-USA; 2018; 991-1005.
2. Dinning P, Wiklendt L, Maslen L, Patton V, Lewis H, Arkwright J, et al. Colonic motor abnormalities in slow transit constipation defined by high resolution, fibre-optic manometry. Neurogastroenterology & Motility. 2015;27(3):379-88.
3. Wald A. Motility disorders of the colon and rectum. Current opinion in gastroenterology. 2012;28(1):52-6.
4. Dimidi E, Christodoulides S, Scott SM, Whelan K. Mechanisms of action of probiotics and the gastrointestinal microbiota on gut motility and constipation. Advances in Nutrition. 2017;8(3):484-94.
5. Bercik P, Collins S, Verdu E. Microbes and the gut-brain axis. Neurogastroenterology & Motility. 2012;24(5):405-13.
6. Kunze WA, Mao YK, Wang B, Huizinga JD, Ma X, Forsythe P, et al. Lactobacillus reuteri enhances excitability of colonic AH neurons by inhibiting calcium-dependent potassium channel opening. Journal of cellular and molecular medicine. 2009;13(8b):2261-70.
7. Carbonero F, Benefiel AC, Gaskins HR. Contributions of the microbial hydrogen economy to colonic homeostasis. Nature Reviews Gastroenterology & Hepatology. 2012;9(9):504.
8. Oliveira DL, Costabile A, Wilbey RA, Grandison AS, Duarte LC, Roseiro LB. In vitro evaluation of the fermentation properties and potential prebiotic activity of caprine cheese whey oligosaccharides in batch culture systems. Biofactors. 2012;38(6):440-9.
9. Bhat Z, Bhat H. Milk and dairy products as functional foods: a review. International Journal of Dairy Science. 2011;6(1):1-12.
10. Whey protein. Monograph. Altern Med Rev. 2008;13(4):341-7.
11. Sánchez-Moya T, López-Nicolás R, Planes D, González-Bermúdez C, Ros-Berruezo G, Frontela-Saseta C. In vitro modulation of gut microbiota by whey protein to preserve intestinal health. Food & function. 2017;8(9):3053-63.
12. Dalziel JE, Young W, McKenzie CM, Haggarty NW, Roy NC. Gastric emptying and gastrointestinal transit compared among native and hydrolyzed whey and casein milk proteins in an aged rat model. Nutrients. 2017;9(12):1351.
13. Ha E, Zemel MB. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. The Journal of nutritional biochemistry. 2003;14(5):251-8.
14. Boutrou R, Henry G, Sanchez-Rivera L. On the trail of milk bioactive peptides in human and animal intestinal tracts during digestion: A review. Dairy science & technology. 2015;95(6):815-29.
15. Madureira A, Tavares T, Gomes AM, Pintado M, Malcata FX. Invited review: physiological properties of bioactive peptides obtained from whey proteins. Journal of dairy science. 2010;93 (2): 437-55.
16. McAllan L, Skuse P, Cotter PD, O'Connor P, Cryan JF, Ross RP, et al. Protein quality and the protein to carbohydrate ratio within a high fat diet influences energy balance and the gut microbiota in C57BL/6J mice. PLoS One. 2014;9(2):889.
17. Gomez E, Tuohy K, Gibson G, Klinder A, Costabile A. In vitro evaluation of the
fermentation properties and potential prebiotic activity of Agave fructans. Journal of Applied Microbiology. 2010;108(6):2114-21.
18. Tabbers M, DiLorenzo C, Berger M, Faure C, Langendam M, Nurko S, et al. Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPGHAN. Journal of pediatric gastroenterology and nutrition. 2014;58(2):258-74.
19. Pijpers MA, Bongers ME, Benninga MA, Berger MY. Functional constipation in children: a systematic review on prognosis and predictive factors. J Pediatr Gastroenterol Nutr. 2010;50(3):256-68.
20. Chumpitazi BP, Self MM, Czyzewski DI, Cejka S, Swank PR, Shulman RJ. Bristol Stool Form Scale reliability and agreement decreases when determining Rome III stool form constipation. Neurogastroenterology & Motility. 2016;28(3):443-8.
21. Oswari H, Alatas FS, Hagar B, Cheng W, Pramadyani A, Benninga MA, et al. Epidemiology of Paediatric constipation in Indonesia and its association with exposure to stressful life events. BMC gastroenterology. 2018;18(1):146.
22. Dehghani SM, Kuloee N, Honar N, Imanieh M-H, Haghighat M, Javaherizadeh H. Clinical manifestations among children with chronic functional constipation. Middle East journal of digestive diseases. 2015;7(1):31.
23. Koppen IJ, Broekaert IJ, Wilschanski M, Papadopoulou A, Ribes-Koninckx C, Thapar N, et al. Role of polyethylene glycol in the treatment of functional constipation in children. Journal of Pediatric Gastroenterology and Nutrition. 2017;65(4):361-3.
24. Lee SH, Bae SH. Maintenance dose of electrolyte free polyethylene glycol (PEG) 4000 in Korean children with chronic functional constipation. Korean Journal of Pediatrics. 2007;50(12):1212-6.
25. Pashankar DS, Bishop WP. Efficacy and optimal dose of daily polyethylene glycol 3350 for treatment of constipation and encopresis in children. The Journal of pediatrics. 2001;139(3):428-32.
26. Afzal NA, Tighe MP, Thomson MA. Constipation in children. Italian journal of pediatrics. 2011;37(1):28.
27. Patel S. Functional food relevance of whey protein: A review of recent findings and scopes ahead. Journal of Functional Foods. 2015;19:308-19.
28. Sanchon J, Fernandez-Tome S, Miralles B, Hernández-Ledesma B, Tomé D, Gaudichon C, et al. Protein degradation and peptide release from milk proteins in human jejunal. Comparison with in vitro gastrointestinal simulation. Food Chemistry. 2018;239:486-94.
29. Mahe S, Roos N, Benamouzig R, Davin L, Luengo C, Gagnon L, et al. Gastrojejunal kinetics and the digestion of [15N] beta-lactoglobulin and casein in humans: the influence of the nature and quantity of the protein. The American journal of clinical nutrition. 1996;63(4):546-52.
30. Dalziel JE, Anderson RC, Bassett SA, Lloyd-West CM, Haggarty NW, Roy NC. Influence of bovine whey protein concentrate and hydrolysate preparation methods on motility in the isolated rat distal colon. Nutrients. 2016;8(12):809.
31. Indrio F, Riezzo G, Giordano P, Ficarella M, Miolla MP, Martini S, et al. Effect of a partially hydrolysed whey infant formula supplemented with starch and Lactobacillus reuteri DSM 17938 on regurgitation and gastric motility. Nutrients. 2017;9(11):1181.
32. Wu S-L, Ding D, Fang A-P, Chen P-Y, Chen S, Jing L-P, et al. Growth, gastrointestinal tolerance and stool characteristics of healthy term infants fed an infant formula containing hydrolyzed whey protein (63%) and intact casein (37%): A randomized clinical trial. Nutrients. 2017;9(11):1254.
33. Kamath P, Phillips S, Zinsmeister A. Short chain fatty acids stimulate ileal motility in humans. Gastroenterology. 1988;95(6):1496-502.
34. Parthasarathy G, Chen J, Chen X, Chia N, O’Connor HM, Wolf PG, et al. Relationship between microbiota of the colonic mucosa vs feces and symptoms, colonic transit, and methane production in female patients with chronic constipation. Gastroenterology. 2016;150(2):367-79.
35. Chassard C, Dapoigny M, Scott KP, Crouzet L, Del’Homme C, Marquet P, et al. Functional dysbiosis within the gut microbiota of patients with...
constipated-irritable bowel syndrome. Alimentary pharmacology & therapeutics. 2012;35(7):828-38.

36. Husebye E, Hellström PM, Sundler F, Chen J, Midtvedt T. Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2001;280(3): 368-80.

37. Kashyap PC, Marcobal A, Ursell LK, Larauche M, Duboc H, Earle KA, et al. Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. Gastroenterology. 2013;144(5):967-77.

38. Fukumoto S, Tatewaki M, Yamada T, Fujiyama M, Mantyh C, Voss M, et al. Short-chain fatty acids stimulate colonic transit via intraluminal 5-HT release in rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2003;284(5):R1269-R76.

39. Kim S. Constipation Research group of Korean Society of Neurogastroenterology and Motility. Change of fecal flora and effectiveness of the short-term VSL# 3 probiotic treatment in patients with functional constipation. 2015.

40. Dimidi E, Christodoulides S, Fragkos KC, Scott SM, Whelan K. The effect of probiotics on functional constipation in adults: a systematic review and meta-analysis of randomized controlled trials. The American journal of clinical nutrition. 2014;100(4):1075-84.

41. Barbara G, Stanghellini V, Brandi G, Cremon C, Di Nardo G, De Giorgio R, et al. Interactions between commensal bacteria and gut sensorimotor function in health and disease. American journal of gastroenterology. 2005;100(11):2560-8.