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Short Communication

Assessing Factors that Shape Neonatal Gut Microbiota in Erbil Province/Iraq

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Abstract

Bifidobacteria and Lactobacilli are considered the initial microbial species to colonize the gastrointestinal tract in the human host. In this study, we investigated the influence of delivery mode, feeding style, infant sex, mother's age, number of siblings, and birthplace on the prevalence of Lactobacillus and Bifidobacteria in the gut of 20 newborns aged 1-3 weeks in Erbil. Fecal samples from infants and breast milk from mothers were examined for the presence of Bifidobacteria and Lactobacilli using selective culture media (BSM and MRS respectively). The results showed that breast milk samples (95%) contained the two bacterial genera, but only 30% of the stool samples contained Lactobacillus ($p = 0.\ 0.037$) and 50% of them contained Bifidobacteria ($p > 0.\ 0.05$). Babies who were strictly on breast milk had a significantly higher chance of harboring Bifidobacteria ($p= 0.\ 0003$) and Lactobacilli ($p = 0.\ 0034$) in their feces than those who were given both breast milk formula milk. Babies born by caesarian section had a lesser chance to have Lactobacillus in their gut compared to those who were naturally born via the birth canal ($p = 0.\ 009$). Baby sex, mother's age, number of siblings, and birthplace did not affect the prevalence of Bifidobacteria. Our results suggest that the feeding type and delivery mode are the most crucial factors affecting the composition of both Lactobacillus and Bifidobacteria in the newborn's gut.

Keywords: Bifidobacteria, intestinal flora, Lactobacilli, microbe-host interaction, probiotic.

1. Introduction

Gut microbiota involves numerous microbial populations that are associated with the human host in a symbiotic relationship. Microbiota applies a substantial influence on host health through its role in the regulation of physiology, immune responses, and nutritional status (Fraher, O'Toole, & Quigley, 2012). Disturbance in the gut microbiome is thought to be associated with several diseases including obesity, diabetes, cancers, inflammatory bowel diseases, gout, depression, arthritis, and others (Ding *et al.*, 2019). It has been found that the diversity of gut microbiota among ethnic groups is associated with eco-geographical factors including lifestyle and dietary habit (Nakayama *et al.*, 2015).

Initial establishment of the gut microbiota starts straight after a baby's birth (Soto *et al.*, 2014; West, Dzidic, Prescott, & Jenmalm, 2017). Later, a complex microbiota composition will develop depending on the dietary nature. It is estimated that over 1000 species of bacteria exists in the intestine of an adult human (Qin *et al.*, 2010). The influence of intestinal bacteria can be harmful, beneficial, or neutral on human health (Liang, Leung, Guan, & Au, 2018; Tlaskalova-Hogenova *et al.*, 2011). Bifidobacterium and Lactobacillus are thought to be the most beneficial bacteria to human health and are expected to contribute to physiological functions (Soto *et al.*, 2014; Tanaka & Nakayama, 2017). Recently published studies of metagenomics on the functionality and composition of the microbiome in infant gut suggest that there is a link between compositional features of the infant gut microbiota and intestinal diseases in infants or other illnesses such as metabolic disorders, inflammatory bowel disease and asthma which could manifest during adulthood (Milani, Duranti, & Bottacini, 2017).

Factors that contribute to shaping the compositions of Bifidobacteria and Lactobacillus in the neonatal gut include delivery mode, feeding type, gestational time, birthplace, antibiotic use, farm residence, number of siblings, and presence of furry pets at home (Penders *et al.*, 2006; Vandenplas *et al.*, 2020). Vaginally delivered infants are expected to acquire their mother's vaginal microflora, but the passing mechanism is still not clear. After delivery, the diversity of infants' gut microflora depends on feeding style and breast feeding is expected to promote colonization of a distinctive microbial profile (Hoang, Levy, & Vandenplas, 2021; Madan *et al.*, 2016).

Infants born through vagina delivery are colonized with gut microbiota immediately after birth, while babies born through Cesarean section might require a long time to acquire these intestinal bacteria (Dunn, Jordan, Baker, & Carlson, 2017). The development of microbiota is modulated and driven by dietary compounds in breast milk which support the colonization of certain microbes (Rodriguez *et al.*, 2015; Tanaka & Nakayama, 2017). Breastfeeding is thought to enhance colonization of gut Bifidobacteria and Lactobacillus, while formula-milk promotes high diversity of gut microbiota leading to a strong competition among different microbial taxa (Backhed *et al.*, 2015; Elsen, Garssen, Burcelin, &

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Verhasselt, 2019). Globally, there is a rising interest in the role of gut microbiota in neonatal health and in Iraq; there is a lack of empirical studies focusing on neonatal gut microbiota. The private health sector particularly in the Kurdistan Region is increasingly expanding, but this is associated with a high caesarean delivery rate (25.4%) which is to be higher than the WHO's recommended rate (15%) (Shabila, 2017). In the current study, the role of delivery mode, feeding type, baby's gender, mother's age, number of previous birth, and birthplace were examined to determine the prevalence of Lactobacillus and Bifidobacteria in the gut. Breast milk, infant stool sample, and five types of commercially available probiotic supplements were thoroughly examined to investigate the possibility of transferring probiotic quality from mothers to newborns. The study will provide a better understanding of factors that may affect the development of the gastrointestinal microbiome in newborns.

2. Methodology

2.1. Ethical approval

This study was approved by the Clinical Research Ethics Committee of the Erbil General Directorate of Health/ the Ministry of Health KRG-Iraq, and their regulations were properly followed. Mothers of newborn participants were provided with written informed consent and the research guidelines (consent form attached).

2.2. Bacterial isolation and identification

Quality control strains of Lactobacillus and Bifidobacteria were prepared using commercially available probiotics including; Entero Junior (Italy), Lactoflor- Kids (Bulgaria), ProIBS (Germany), Advanced Probiotic (USA), and Probiodex (Italy).

During one month (between December 2020 and January 2021), fecal samples from 20 newborns aged 3 weeks and approximately 10 ml of their mothers' breast milk were collected using sterile Eppendorf tubes at the Maternity Teaching Hospital-Erbil. Samples were properly labeled and quickly transferred on ice to the lab for culturing and identification. Briefly, quality control strains of Lactobacillus and Bifidobacteria were prepared by transferring 5 grams from each of the above mentioned probiotic sources separately to 45 ml of sterilized BHI broth (Brain Heart Infusion Broth, Thermo Scientific, USA) in order to promote the growth of lyophilized bacteria. Simultaneously, 5 grams from newborns' fecal sample and 5 ml from mothers' breast milk sample were separately transferred to 45 ml of sterilized BHI (Brain Heart Infusion Broth, Thermo Scientific, USA). Tubes containing inoculated BHI were incubated under anaerobic conditions using an anaerobic jar (Oxoid AnaeroJar 2.5L, UK) at 37°C for 48 hours. After the incubation time, homogenate was prepared from each BHI tube and diluted using tenfold dilution. From appropriate dilutions, an amount of 0.1 ml was taken and spread on sterile MRS agar (de Man, Rogosa, and Sharpe, Oxoid-UK) for Lactobacillus, and on BSM agar (Bifidus Selective Medium, Merck- Germany) for Bifidobacteria. After inoculation, plates were kept under anaerobic conditions using an anaerobic jar at 37°C for 48 hours.

To identify colonies of Lactobacillus and Bifidobacteria, we followed the guidelines of manufacturers of the two selective medias (MRS and BSM). Colonies of Lactobacillus appeared white, large and embedded in MRS Agar, while Bifidobacteria appeared purple/brown on BSM. For additional purification, 10–15 colonies which showed characteristics of Lactobacillus and Bifidobacteria were sub cultured on MRS and BSM respectively. Gram stain and KOH test, Catalase Test and Spore Staining were carried out for all the Lactobacillus and Bifidobacterium suspected colonies. Briefly, a drop of KOH (3%) solution was added on a glass slide and part of a suspected colony from freshly cultured bacteria was thoroughly mixed to make a dense suspension. After constant stirring, isolates which have not become thick and stringy products were selected since Bifidobacterium and Lactobacillus species are Gram-positive cells. This was followed by a routine gram stain procedure (Gram stain kit-010221, Diapath- Italy) using cells from the same colony. The catalase test was conducted by adding one drop of 3% Hydrogen Peroxide (3% H2O2) on 48 hours old cultures on a clean glass slide. When the formation of oxygen bubbles happened, the catalase test was considered a positive reaction. Spore-staining was performed using Schaeffer and Fulton Spore Stain Kit (Merck- Germany). Only non-endospore forming isolates were considered for subsequent examination.

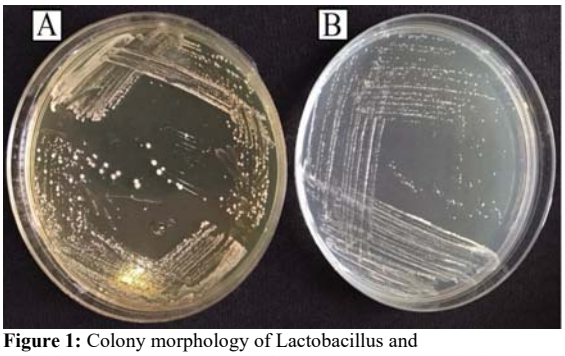

Figure 1: Colony morphology of Lactobacillus and Bifidobacterium on MRS (A) and BSM (B) agar respectively after 48 hours of incubation at 37C under anaerobic conditions.

2.3. Statistical analysis

Association between the abundance of Lactobacillus and Bifidobacteria in mothers' breast milk and their newborns' gut was examined using Chi-square test. Regression analysis was used to examine the influence of independent variables including delivery mode, feeding type, baby's gender, mother's age, number of previous birth, and birthplace on the abundance of the two bacterial genera in newborns' feces. Statistical tests were performed in SPSS (SPSS Statistics V22-IBM) using a p-value that is less than 0.05 considered as the level of significance.

3. Results

Quality control strains of Lactobacillus and Bifidobacterium species were successfully isolated from four probiotic sources; Lactoflor- Kids (Bulgaria), ProIBS (Germany), Advanced Probiotic (USA), and Probiodex (Italy). One of the sources (Entero Junior, Italy) did not harbor any of the two bacterial genera.

Twenty mothers participated in this study, and their babies included 9 females (45%) and 11 males (55%). Twelve of the babies were delivered by SC (60%), while 8

(40%) of them were delivered through the birth canal. Apart from one baby born at home, the rest (95%) were born in the hospital. All the babies were on breast milk, but 10 of them (50%) were on both breast and formula milk. Among mothers who contributed to the study, 11

(55%) had zero or one more pregnancy in the past while the other 9 (45%) had 3 or more pregnancies in the past. All the mothers and babies were neither on antibiotics nor on probiotic/food supplements. Most of the babies (95%) were born at no lesser than 37 weeks, but only one of them was born at 36 weeks of pregnancy and its medical history was showing no abnormality. Premature babies who were born at lesser than 36 weeks of pregnancy and those who have been identified with congenital birth defects were excluded from the study.

There were statistically significant differences in the prevalence rate of Lactobacillus and Bifidobacteria between breast milk and stool samples; stool samples of babies who were born via birth canal and C-section; stool samples of babies who were strictly on breast milk and babies who were fed both breast milk and formula milk (table 1).

Generally, most of breast milk samples contained both Lactobacillus and Bifidobacteria, but only one of the stool samples found to contain these bacterial genera. It was found that 19 (95%) of the breast milk samples were positive for both Lactobacillus and Bifidobacteria except one of the breast milk samples neither supported the growth of Lactobacillus nor Bifidobacteria. Only six (30%) of the stool samples contained Lactobacillus.

Delivery mode had influence on the prevalence of the two bacterial genera in the babies' stool. There was a remarkable difference in the prevalence of Lactobacillus between naturally born babies and those were delivered via C-section, but the difference in the prevalence of Bifidobacteria was not found to be significant. Babies who were born via the birth canal had a higher prevalence of Lactobacillus (65%) and Bifidobacteria (75%) compared to other babies who were born via C-section (8.3% and 33.3% respectively).

Stool samples from babies who were strictly on breast milk showed a higher prevalence of Lactobacillus (60%) and Bifidobacteria (90%) compared to babies who were fed the two milk types (0% and 10% respectively).

There were no differences in the prevalence of Bifidobacteria in the stool content between male and female babies. Although stool samples from female babies had slightly lesser prevalence of Lactobacillus species (11.1%) compared to male babies (45.5), the difference was statistically not significant.

Table 1. The Chi square (X^2) was set at 95% confidence interval and one degree of freedom. Two-tailed P value was selected and stated in four digits.

Characteristics	For Lactobacillus					For Bifidobacteria				
	Positive	Negative	N	X2	P value	Positive	Negative	N	X2	P value
Breast milk	19	1	20	4.33	0.037	19	1	20	1.647	0.199
Baby stool sample	6	14	20	4.33	0.037	10	10	20	1.647	0.199
Strictly on breast milk	6	4	20	8.571	0.003	9	1	20	12.8	0.000
Breast milk plus formula milk	0	10	20	-	-	1	9	20	12.8	0.000
Birth canal	5	3	20	6.706	0.010	6	2	20	3.33	0.068
C-Section	1	11	20	6.706	0.010	8	4	20	3.33	0.068
Female	1	8	20	2.78	0.095	5	4	20	0.202	0.653
Male	5	6	20	2.78	0.095	5	6	20	0.202	0.653

4. Discussions

A good understanding of the initial development of the neonatal intestinal ecosystem can be the key to the prevention or modification of several important diseases including Necrotizing enterocolitis (NEC), hematopoietic abnormalities, intraventricular hemorrhage and chronic lung diseases (Ding *et al.*, 2019; Elsen *et al.*, 2019; George Kerry *et al.*, 2018; Tanaka & Nakayama, 2017). Adhesion and colonization of probiotic bacteria, such as Lactobacilli and Bifidobacteria in the gastrointestinal tract of the host, is believed to be one of the essential features required for delivering their health benefits (Costa & Weese, 2019; George Kerry *et al.*, 2018; Rinninella *et al.*, 2019). Probiotics of the human gut start to establish at a very early stage of life (Dunn *et al.*, 2017; Tanaka & Nakayama, 2017). The majority of mothers (95%) who contributed to the study have possessed the two bacterial species in their breast milk; one of the milk samples did not contain any of the two bacterial species. Although it was not clear what could have contributed to the absence of essential probiotic bacteria in breast milk, the bacteria could have become inactivated during the transportation.

Five commercial probiotic supplements were used in the study to prepare quality control strains of Bifidobacteria and Lactobacillus. Four out of five contained the bacterial species just as the manufactural description. Although all the products used in this study had at least 6 months of validity or longer (some up to 2 years) left before the expiry date, the reason for the absence of Lactobacillus in Entero Junior (Italy) could be due to improper storage.

Although most of breast milk samples were rich in the two bacterial species, only 30% of the fecal samples from their babies supported the growth of Lactobacillus and only 50% of them contained viable Bifidobacteria. The prevalence of both bacterial species was generally low in the fecal samples, and this is not in agreement with what was found by Martin *et al.* (2012) that breastfeeding is expected to transfer bacteria particularly bile salt resistant anaerobes such as Bifidobacteria and Lactobacillus from the mother to babies; and there they can colonize neonatal gut.

Various factors can affect the early establishment of gut microbiota including delivery mode (vaginal or cesarean delivery), feeding type (breast milk and/or formula milk), cessation of milk feeding, antibiotic usage, timing, and type of the introduction of solid foods, location and other mother's related factors (Tanaka & Nakayama, 2017). The prevalence of both Lactobacillus and Bifidobacteria can also be influenced indirectly through the ecosystem changes of intestinal flora due to intrinsic factors such as genetics and infections or extrinsic factors such as dietary intake and medications (Abrahamsson, Sinkiewicz, Jakobsson, Fredrikson, & Björkstén, 2009). For example, half of the newborn participants (50%) who participated in the study were taking both breast milk and formula milk. Lactobacillus prevalence was significantly higher in the infants who were solely fed on breast milk than those who were taking both breast and formula milk while Bifidobacterium prevalence did not differ significantly between the two feeding regimes. Studies suggest that breastfeeding infants are expected to receive bacterial flora which exists in their mothers' milk (Abrahamsson *et al.*, 2009) particularly during the first month of lactation (Collado, Laitinen, Salminen, & Isolauri, 2012). Some studies suggest that formula milk induces the growth of various bacterial species in infants' guts (Ho *et al.*, 2018), and this might explain strong competition with Lactobacillus and Bifidobacteria.

Interestingly, the prevalence of both Bifidobacteria and Lactobacillus was significantly higher in naturally born infants than what was found in those who were born via C-section. Only 40% of the babies were born via birth canal while the rest were born through C-section. Babies who were delivered via birth canal generally their stool samples were rich with the two studied bacteria, but only a few sample (8.3% and 33.3% respectively) of stool samples of babies who were born via C-section contained Lactobacillus and Bifidobacteria. It has been well established that babies are free of probiotic bacteria in the mother's womb and their microbial flora starts to establish only during and after the birth (Sanz, 2011; Tanaka & Nakayama, 2017). Women's vagina contains a large number of normal flora including Lactobacillus species (Dunn *et al.*, 2017) which can help passing many of these bacteria to a newborn's body and hence their gastrointestinal tracts (Martin *et al.*, 2012). Although vaginal swab is recommended for babies who are born via CS (Dominguez-Bello *et al.*, 2016), this procedure was not followed in the studied hospital. These results support the results of previous studies in this field that CS-born babies have poorer flora in their GIT (Hoang *et al.*, 2021).

Other factors such as maternal age, surgical history, social history, obstetrical history, and the number of pregnancies did not associate with the prevalence of Bifidobacteria and Lactobacillus in the infant stool sample. Female infants had a slightly lesser prevalence rate (11.1%) of Lactobacillus compared to male babies (45.5%), but the difference was not (table 1). Although sex differences in gut microbiota have been found in adults (Kim, Unno, Kim, & Park, 2020), the current study did not find a significant influence of gender on compositions of the neonatal gut Lactobacillus and Bifidobacteria.

5. Conclusion

In conclusion, these findings suggest that environmental factors, particularly feeding type and delivery mode, can greatly impact the microbiota composition of newborns. Infants who are born via CS have little chance to obtain Lactobacillus in their gut. Although breast milk is rich in many important microbiotas it becomes less useful when is used along with artificial formula milk. Because of the important roles are played by probiotics in the infants' health, our results support previous studies that administration of probiotic supplements might be recommended for infants with poor gut microbiota which could be due to the mode of delivery and feeding practices.

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References

Abrahamsson, T. R., Sinkiewicz, G., Jakobsson, T., *et al.* 2009. Probiotic lactobacilli in breast milk and infant stool in relation to oral intake during the first year of life. *J Pediatr Gastroenterol Nutr.,* **49 (3)**, pp. 349-354.

Backhed, F., Roswall, J., Peng, Y., *et al.* 2015. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe,* **17 (5)**, pp. 690-703.

Collado, M. C., Laitinen, K., Salminen, S., *et al.* 2012. Maternal weight and excessive weight gain during pregnancy modify the immunomodulatory potential of breast milk. *Pediatr Res,* **72 (1)**, pp. 77-85.

Costa, M., & Weese, J. S. 2019. Methods and basic concepts for microbiota assessment. *Vet J,* **249** pp. 10-15.

Ding, R. X., Goh, W. R., Wu, R. N., *et al.* 2019. Revisit gut microbiota and its impact on human health and disease. *J Food Drug Anal,* **27 (3)**, pp. 623-631.

Dominguez-Bello, M. G., De Jesus-Laboy, K. M., Shen, N., *et al.* 2016. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med,* **22 (3)**, pp. 250-253.

Dunn, A. B., Jordan, S., Baker, B. J., *et al.* 2017. The Maternal Infant Microbiome: Considerations for Labor and Birth. *MCN Am J Matern Child Nurs,* **42 (6)**, pp. 318-325.

Elsen, L. W. J. v. d., Garssen, J., Burcelin, R., *et al.* 2019. Shaping the Gut Microbiota by Breastfeeding: The Gateway to Allergy Prevention? *Front Pediatr,* **7** pp. 47.

Fraher, M. H., O'Toole, P. W., & Quigley, E. M. 2012. Techniques used to characterize the gut microbiota: a guide for the clinician. *Nat Rev Gastroenterol Hepatol,* **9 (6)**, pp. 312-322.

George Kerry, R., Patra, J. K., Gouda, S., *et al.* 2018. Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis,* **26 (3)**, pp. 927-939.

Ho, N. T., Li, F., Lee-Sarwar, K. A., *et al.* 2018. Meta-analysis of effects of exclusive breastfeeding on infant gut microbiota across populations. *Nat Commun,* **9 (1)**, pp. 4169.

Hoang, D. M., Levy, E. I., & Vandenplas, Y. 2021. The impact of Caesarean section on the infant gut microbiome. *Acta Paediatr,* **110 (1)**, pp. 60-67.

Kim, Y. S., Unno, T., Kim, B. Y., *et al.* 2020. Sex Differences in Gut Microbiota. *World J Mens Health,* **38 (1)**, pp. 48-60.

Liang, D., Leung, R. K., Guan, W., *et al.* 2018. Involvement of gut microbiome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut Pathog,* **10** pp. 3.

Madan, J. C., Hoen, A. G., Lundgren, S. N., *et al.* 2016. Association of Cesarean Delivery and Formula Supplementation With the Intestinal Microbiome of 6-Week-Old Infants. *JAMA Pediatr,* **170 (3)**, pp. 212-219.

Martin, V., Maldonado-Barragan, A., Moles, L., *et al.* 2012. Sharing of bacterial strains between breast milk and infant feces. *J Hum Lact,* **28 (1)**, pp. 36-44.

Milani, C., Duranti, S., & Bottacini, F. 2017. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. *Microbiology and molecular biology reviews : MMBR,* **81 (4)**, pp. e00036-00017.

Nakayama, J., Watanabe, K., Jiang, J., *et al.* 2015. Diversity in gut bacterial community of school-age children in Asia. *Sci Rep,* **5** pp. 8397.

Penders, J., Thijs, C., Vink, C., *et al.* 2006. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics,* **118 (2)**, pp. 511-521.

Qin, J., Li, R., Raes, J., *et al.* 2010. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature,* **464 (7285)**, pp. 59-65.

Rinninella, E., Raoul, P., Cintoni, M., *et al.* 2019. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms,* **7 (1)**.

Rodriguez, J. M., Murphy, K., Stanton, C., *et al.* 2015. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis,* **26** pp. 26050.

Sanz, Y. 2011. Gut microbiota and probiotics in maternal and infant health. *Am J Clin Nutr,* **94 (6 Suppl)**, pp. 2000S-2005S.

Shabila, N. P. 2017. Rates and trends in cesarean sections between 2008 and 2012 in Iraq. *BMC Pregnancy Childbirth,* **17 (1)**, pp. 22.

Soto, A., Martin, V., Jimenez, E., *et al.* 2014. Lactobacilli and bifidobacteria in human breast milk: influence of antibiotherapy and other host and clinical factors. *J Pediatr Gastroenterol Nutr,* **59 (1)**, pp. 78-88.

Tanaka, M., & Nakayama, J. 2017. Development of the gut microbiota in infancy and its impact on health in later life. *Allergol Int,* **66 (4)**, pp. 515-522.

Tlaskalova-Hogenova, H., Stepankova, R., Kozakova, H., *et al.* 2011. The role of gut microbiota (commensal bacteria) and the mucosal barrier in the pathogenesis of inflammatory and autoimmune diseases and cancer: contribution of germ-free and gnotobiotic animal models of human diseases. *Cell Mol Immunol,* **8 (2)**, pp. 110-120.

Vandenplas, Y., Carnielli, V. P., Ksiazyk, J., *et al.* 2020. Factors affecting early-life intestinal microbiota development. *Nutrition,* **78** pp. 110812.

West, C. E., Dzidic, M., Prescott, S. L., *et al.* 2017. Bugging allergy; role of pre-, pro- and synbiotics in allergy prevention. *Allergol Int,* **66 (4)**, pp. 529-538.