EFFECTS OF POLYAMINE IN BREASTMILK ON THE MATURATION OF THE SMALL INTESTINE: A SYSTEMATIC REVIEW

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ABSTRACT

Introduction: Food allergy and food hypersensitivity prevalence may be reduced if infants are breastfed during their early life. Certain components of breastmilk promote the development and maturation of the small intestine and lower the uptake of allergens that may cause an allergic reaction. Among those, polyamine has also been proposed to have an essential role in the small intestine maturation. However, how polyamine works on the intestine has not yet been established. Therefore, this study aimed to review the effects of polyamine in breastmilk on the maturation of the small intestine. **Methods:** A systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Studies were identified through five electronic database based on seven keywords and they were included if the following inclusion criteria were met; evaluated the effects of polyamine on the maturation of small intestine, published in English between the year of 1998 and 2020, as well as involved either animal or human experiments. **Results:** A total of 1,121 relevant abstracts were screened, and finally four full text articles were evaluated. All the studies showed that polyamine significantly induced the small intestine maturation in rats and piglets. This was characterized by positive morphological changes such as increased intestinal weight, more developed intestinal villus and enzymatic changes, increased maltase and sucrose specific activity (SA) and decreased lactose SA that appeared close to the natural phenomena. It was suggested that 0.4umol/g bodyweight and 463nmol/ml bodyweight of supplementation of polyamine exert maturation in rat and piglet small intestine, respectively. **Conclusions:** This study provides evidence that polyamine in breastmilk induces the maturation of small intestine proposing its importance in reducing allergy incidence among infants. However, further research is needed on infant small intestine to determine whether polyamine can exert the same effect as in animals.

KEYWORDS: Polyamine, Breastmilk, Small intestine maturation

INTRODUCTION

In humans, the maturation of organs is particularly rapid during the perinatal period. The development of the gastrointestinal tract (GIT) is stimulated by the transition from parenteral feeding (umbilical cord) to enteral feeding after birth via breastmilk or formula milk. However, the GIT still undergoes postnatal growth and tries to adapt in response to the feeding even though the digestive and absorptive processes are developed sufficiently during this transition (Berin & Sampson, 2013). Infants face a greater challenge in which there will be interactions between gastrointestinal mucosal surfaces and environmental stimuli served by commensal and pathogenic microbiota and dietary agents. During term delivery, the structural and cellular components of the gastrointestinal immune system are present. However, developmentally, the system is still immature and may interact inappropriately to environmental stimuli that can trigger allergy reactions (Berin & Sampson, 2013; Sheard &Walker, 2009). Breast milk is composed of complex and a variety of nutrients such as macronutrients and micronutrients, hormones, as well as bioactive components that protect the infant from any infections and inflammations and facilitate the establishment of microbiota leading to their growth, development, and health status (Mazzocchi et al, 2019). Among the breastmilk compounds, there is polyamine, which is also characterized as spermine, spermidine, and putrescine. Polyamine is an inorganic compound synthesized in cells and known to be essential for the proliferation and development of mammalian cells. It can be found abundantly in foods such as internal organs, fermented foods, mushroom species, soybean products and also in human milk, rat's milk, and cow's milk that become the main source for infants (Timmons et al., 2012). Emerging studies have scientifically proven that polyamine ingestion exerts effects on an individual's health. Due to its antioxidant and anti-inflammatory properties, an increased intake of polyamine can decrease the risk of age-associated disease, cardiovascular disease (CVD), heart failure, and maintain healthy blood pressure. In addition, in cancer, restriction of polyamine intake may suppress tumour where polyamine metabolism is targeted as chemoprevention in cancer. Moreover, in infant health, it is proposed that polyamine ingestion might lower the incidence of infant allergy due to its role in the maturation of the small intestine (Dandrifosse et al., 2000). However, evidence on how ingestion of polyamine induces the maturation of the small intestine is yet to be established. Therefore, this study was conducted to review ~~available information on~~ the effects of polyamine on the small intestine maturation as well as the quantity of polyamine in breastmilk that can induce such effects.

METHODS

The review process was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Articles related to the evaluation of polyamine on the maturation of small intestine was gathered from five databases: SCOPUS, PubMed, Science Direct and SpringerLink. The following search terms were used; Effect* OR Outcome* AND Polyamine OR Spermine OR Spermidine OR Putrescine AND small intestine. The criteria for papers to be included in the review were

that papers focusing on the study of polyamine effects on the maturation of the small intestine. Journals that were written in English; published between 1998 and 2020, and involved animals or humans as participants, were also included. The selected studies were reviewed if they fulfilled the inclusion criteria. The studies that match the exclusion criteria were omitted to prevent bias in the results. The study selection was important to avoid misinterpretation, misjudgment, and misleading of the information in the paper. Cochrane Risk of Bias for randomized study was used as a tool in assessing the risk of bias of the study. The assessment of the risk of bias was done at various levels. Firstly, the risk of bias for study across outcome was summarized since all studies did not have the same risk of bias. Secondly, the risk of bias for outcome within a study was summarized as a different outcome might have different risks of bias. The judgement was described as 'low risk', 'high risk ', or 'unclear risk'. The risk of bias for the whole review was summarized to evaluate the value of judgement of the outcomes. After all the process of data charting was completed, all information pertaining to the study were analysed using descriptive analysis. Information regarding the number of studies, type of study design, sources of information and year of study, were presented in a table adopted from Guideline for Systematic Review from *The American Journal of Occupational Therapy (AJOT)*. This study did not involve any human or animal participation. Therefore, neither ethical approval nor participant's consent was needed to conduct this study.

RESULTS

A total of 1,584 articles were identified. After removing duplicates by using Mendeley, 1,121 articles were identified. These articles were screened for their titles and abstracts. A total of 58 articles were chosen for further screening based on inclusion and exclusion criteria. From the 58-full text articles, 54 were rejected. These included 21 purely irrelevant articles, 12 review papers, 20 articles that did not discuss the effects of polyamine on the maturation of small intestine, and 3 articles that discussed the effects of polyamine in infant formulas. Finally, four intervention studies were included in this review. All the four studies were conducted on animals (3 on rats and 1 on piglets). All the studies examined the effect of polyamine towards the maturation of the small intestine which was characterized by the changes in morphological and enzymatic parameters. All these studies were experimental with the presence of different concentrations of polyamine spermine as interventions and the absence of polyamine as controls. Three studies (Studies 1, 2, 3) used polyamine spermine meanwhile one study (Study 4) used both spermine and spermidine to evaluate the effects on the maturation of the small intestine (Table 1).

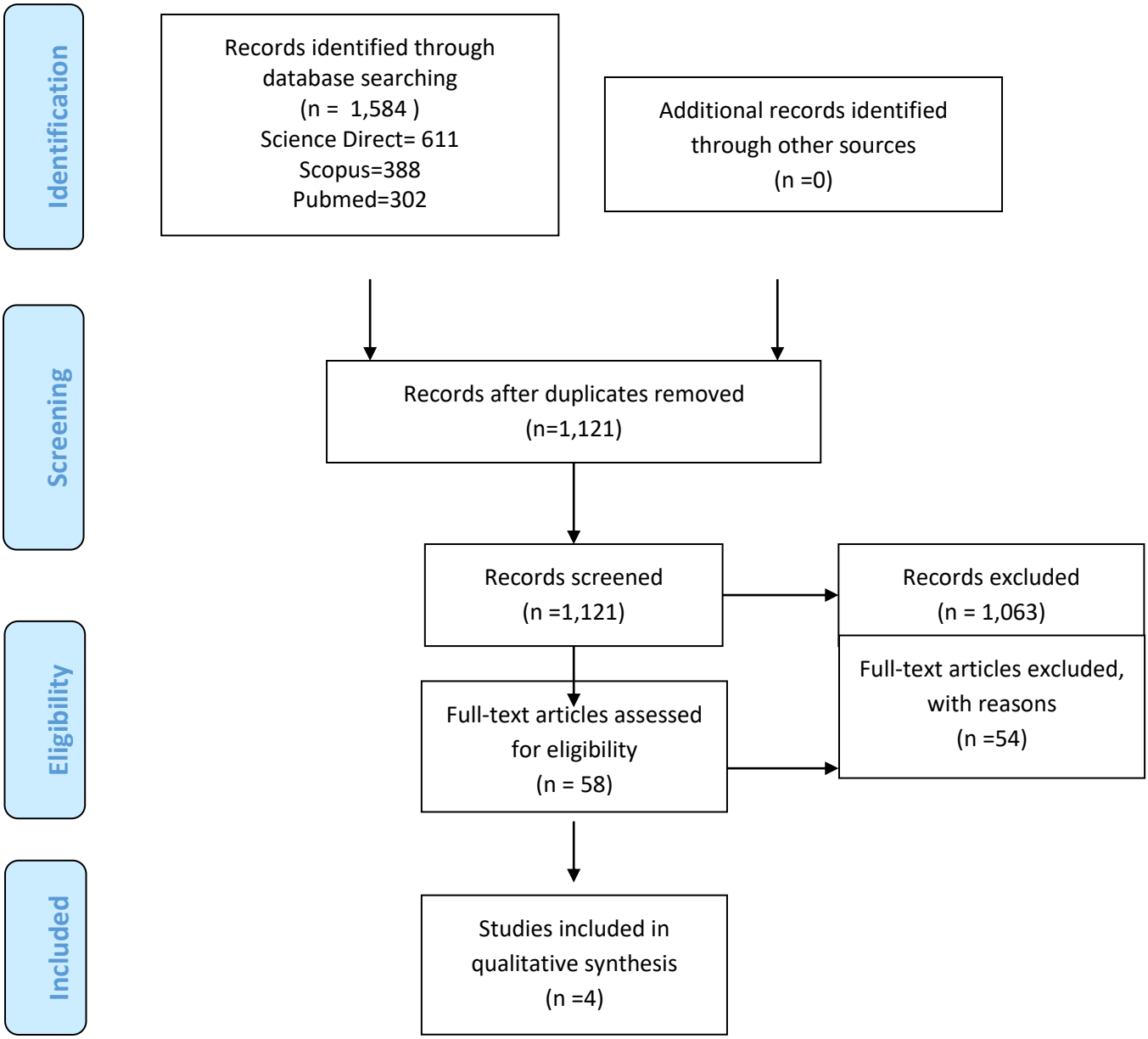

Figure 1 PRISMA 2009 Flow Guidelines

Table 1 Summary of findings on the effects of polyamine on the maturation of the small intestine in animals

Author/Year	Level of evidence, Study Design, Sample	Intervention and Control Groups	Results
STUDY 1	**Level II**	*Intervention* 8 days old pup received spermine with (0.4 µmol/g body weight) daily for 7 days *Control* Absence of spermine	Effect on intestinal weight In the treated group with spermine for 3-7 days, higher intestinal weight in both jejunum (P < 0.01) and ileum (P< 0.001) was observed compared to the control group. This indicates trophic effect of spermine Enzymatic Analysis In control group, at any time, high lactase specific activity (SA), low maltase and undetected sucrose SA were observed. In the treated group reduced lactase SA (P<0.001) was observed at any time. Increased of sucrose and maltase SA were observed after 3 days of treatment (P<0.001). But, after 7 days, sucrose SA and maltase SA reduced (p<0.001). This indicates spermine could not retain its function for long time. Morphological Analysis In treated group with spermine for 3-7 days, jejunum more developed (large diameter, thicker and with numerous villi) than in the treated group. Results were not shown. In ileum, after 3 days of spermine supplementation, large supranuclear vacuoles (LSV) in enterocytes disappeared but reappeared at 4-7 days and became obvious after 7 days of treatment. *Effects of polyamine on intestinal maturation:* Polyamine significantly induced maturation of small intestine but only retained its function in short term period
Deloyer, Peulen & Dandrifosse (2005)	Experimental Design Wistar rats N=10		
STUDY 2	***Level II***	***Intervention***	**Enzymatic Analysis**
Peulen, Gharbi, Powroznik &	*Experimental Design*	*11 days old pup received spermine with (0.4 µmol/g body weight in 50*	Lactase SA reduced (p<0.05), maltase and sucrose SA increased (p<0.01) in the treated group. This indicates successful maturation of small

Dandrifosse (2004)	*N= Wistar rats*	*µl water) daily for 3 days* *Control* *Absence of spermine*	intestine ***In situ* alkaline phosphatase (IAP) analysis** Alkaline phosphatase activity increased (twofold) in jejunum (P<0.01) and decreased in ileum (fivefold) (P<0.001) in the treated group Expansion of IAP activity on the whole length of villus was observed in jejunum and in ileum, IAP became weak after 3 days of spermine ingestion. This indicates spermine induce maturation of duodenum *Effects of polyamine on intestinal maturation:* Polyamine induced maturation of small intestine by modifying IAP activity
STUDY 3 Peulen, Pirlet, Klimek Goffinet & Dandrifosee (1998)	Level II Experimental Design Wistar rats N=11	*Intervention* 11 days old pup received spermine with (0.4 µmol/g body weight in 50 µl water) daily, for 3 days *Control* Absence of spermine	**Effect on intestinal weight** Weight of the proximal small intestine was (p<0.05) lower in the controls than in the treated group. This indicates successful maturation from tropic effects. **Morphological Analysis** After 8 hours ingestion of spermine, increased swelling in villi in proximal small intestine was observed and after 3 days, the villi returned to normal size as similarly observed in the control group. This indicates polyamine did not modify proximal part of small intestine due to apoptosis from polyamine After 8 hours ingestion of spermine, shortening of villi in distal small intestine with missing supranucleur vesicle was observed compared to the control group. After 3 days, the villi has recovered to normal height but still with the absence of supranucleur vesicles. **Protein analysis** After 3 days supplementation of spermine, protein content increased (P<0.01), DNA decreased in both proximal and distal small intestine (P<0.001) compared to the control group. A decreased RNA content (P<0.001) was observed in proximal and an increased RNA content (P<0.001) in distal small intestine was observed compared to the control group **Enzymatic Analysis** Lactase SA decreased (P<0.001) 4 hrs after spermine ingestion. Maltase (P<0.05) and sucrose SA (P<0.001) increased in both proximal small intestine and distal small intestine 3 days after spermine ingestion. This indicates successful maturation *Effects of polyamine on intestinal maturation:*

Polyamine induced maturation of small intestine			
STUDY 4 Van Wettere, Willson , Pain &Forder (2016**)**	Level II Experimental Design **Experiment 1** Piglet from first and third lactation sow N=6 **Experiment 2** Piglet suckling 18 first and third lactation sows N=54	**Experiment 1** *Intervention* Piglet received spermine with (463nmol/ml in 2 ml water) every 2nd day from day 14 to days 22 *Control* Absence of spermine **Experiment 2** *Intervention* Piglet received spermine with (463nmol/ml in 2 ml water) or spermidine 2013nmol/ml every 2nd day from day 14 to days 22 *Control* Absence of spermine	**Morphological analysis** **Experiment 1** Oral supplementation of spermine resulted in 41% increase in villus height, 21% decrease in crypt depth (P<0.01) compared to the control group **Experiment 2** Spermine and spermidine-supplemented piglets suckling first lactation sows grew faster (P<0.05) between days 14 and 18 postpartum compared to the control group *Effects of polyamine on intestinal maturation:* Polyamine improved maturation of small intestine by increasing the absorption surface area and increased villus height

Maturation of small intestine due to the supplementation polyamine towards rats and piglet was reported in all four experimental studies. This was characterized by the positive morphological and enzymatic changes in the small intestine; weight of intestine, disaccharide SA, amount and height of villus, and protein activity that imitated matured intestine. Out of the four studies, only Study 1 analysed both short- and long-term effects of polyamine on small intestine maturation meanwhile the rest (Study 2, 3 and 4) only analysed the short-term effects of polyamine on small intestine. The quality of the included studies was assessed in terms of the risk of bias in this systematic review. Overall, all four included studies showed an equal distribution of bias. Low risk of bias was reported more than the unclear in all bias except for performance bias and other bias. The low risk of bias was due to most of the studies were randomized (selection bias) and the outcome of studies (attrition bias) and the reporting results (reporting bias) were adequate and sufficient. Thus, all studies showed a fair distribution of bias.

DISCUSSION

This study reviewed how polyamine induced the maturation of the small intestine. A positive outcome was reviewed in all four studies with regards to the roles of polyamine as a bioactive compound that influences the postnatal maturation of intestine. Positive morphological and biochemical modifications that characterized maturation of intestines can be observed in the rats and piglets supplemented with polyamine compared to the non-supplemented ones. From the results obtained from the review papers, the polyamine induced the maturation of small intestine through the changes in their weight, height and amount of villus as well as changes in disaccharides specific activities. As early as after three days of spermine administration, a significant increase in the intestinal weight of rats was observed. The increase in intestinal weight indicated the trophic effects of polyamine that lead to hyperplasia and/or hypertrophy were ~~being~~ maintained during the supplementation days of polyamine of the rats. This was possibly due to the role of polyamine in cell proliferation, as well as its effects on the appearance of Na+, K+ and ATPase in the small intestine that involves in cell volume activities (Deloyer, 2005; Peulen,2004). Moreover, some findings reported that polyamine exerts effects on the amount of villus in the small intestine and its structure (Deloyer, 2005, Peulen, 1998 & Van Wettere, 2016). In a study by Deloyer (2005), numerous amounts of villi were observed in jejunum thus contribute to a better-developed jejunum. Meanwhile, in a study by Van Wettere (2016), increased villus height and reduction of crypt death were observed in pigs. Sherd and Walker (2009) emphasized that undifferentiated villus inside the crypt that proliferates and migrates up to the villus is vital as nutrients such as carbohydrate, protein, fat, vitamin and minerals are absorbed along the villus in the intestine into the bloodstream. The more peak the villus, the higher the surface area for nutrient absorption will occur. On the other hand, in Study 3 (Peulen, 1998), swelling in villi was observed after eight hours of polyamine ingestion indicates polyamine induce maturation of the intestine. Deloyer, Peulen and Dandrifosse (2005) explained that the ingestion of polyamine activates the hypothalamic-pituitary-adrenal (HPA) thus inducing an increase of ACTH as well as plasma corticosterone concentration. It

is well known that corticosterone induces the intestinal maturation when there is a high level of sucrose and maltase specific activities. In consequence, three studies (Deloyer, 2005, Peulen, 2004 & Peulen, 1998) showed that there was a significant increase in maltase SA and sucrose SA and a significant decrease in lactose SA. This indicates successful maturation of the intestine could occur with polyamine supplementation. With regards to the changes in protein content of the intestine, Peulen (1998) demonstrated that the findings were unclear and further analysis was needed. Thus, the polyamine mechanism on the protein changes is still not fully understood. Therefore, all the positive morphological and biochemical parameters except for protein content, indicates that spermine induced maturation of the intestine appears close to natural phenomena. However, long supplementation of polyamine does not prevent some parameters such as sucrose and maltase SA to turn into typical 'immature' level while others such as mucosa and lactase SA levels retain its typical 'mature' level (Deloyer, 2005). The disaccharide activities observed were poor after more than three days of spermine supplementation. This indicates that long-lasting spermine treatment could not retain the adult enzymatic pattern. The reduction of the SA might suggest that there was an interruption of intestinal cell stimulation by corticosteroid. This might be due to: (1) epithelium become impermeable to polyamine; spermine unable to induce production of soluble factors that stimulate HPA axis, (2) the mucosa might become insensitive compared to in immature cells thus would not stimulate secretion of HPA-activating factor, (3) age of rat; HPA- axis become unresponsive, respond to of polyamine to SA decreasing with age or (4) the 'mature' state of mucosa might make it unresponsive to corticosterone. Thus, it is suggested that in weaning, the maturation of epithelium can be maintained by a corticosterone-independent mechanism (Deloyer, 2005). Meanwhile, the lactase SA level remained low even after long-lasting treatment might be influenced by the interleukin-2 or gastrointestinal hormones such as bombesin. These two could be secreted without interruption during the maturation of the intestine by spermine. After all, this was reported in an animal study, thus more research is needed to know whether long-lasting spermine treatment in humans will exert the same effects on the disaccharide SA level (Deloyer, 2005). In all the animal studies, the dose of the spermine used on rats was 0.4 µmol/g body weight (Deloyer, 2005, Peulen, 2004 & Peulen 1998) before weaning and demonstrated that the maturation of small intestine could be induced. Although the concentration used was considered toxic, it could be rejected. Peulen et al. (1998) discussed that a high concentration of polyamine ingested in bolus is quickly reduced by dilution of gastric content. Moreover, the amount of polyamine did not exceed the amount found in the daily food of adult rats and was still below toxicity level. If the concentration were toxic, the animal would have died. Meanwhile, in a piglet study (Van Wettere, 2016), the spermine used was calculated 20% more than normally received in cow's milk which was at 463nmol/ml. In the piglet study, it was reported that even if the same concentration of polyamine is used, all studies have a different outcome from each other. Therefore, it was suggested that other than the dose, the timing of supplementation might also be responsible for the differences in intestinal alterations observed in the studies conducted. Therefore, consideration can be taken to indicate that 0.4umol/g body weight of polyamine supplementation exerts maturation of rat small intestine and 463nmol/ml for piglets. Nevertheless, more study is needed to clarify the potential toxicity of polyamine concentration at a certain level as

explained in animal studies and the optimal amount of polyamine needed by human infants. Finally, the physiological of polyamine induced the maturation of the small intestine can be supported by the concentration of polyamine in the milk throughout the lactation. In rat's milk, the concentration of putrescine and spermine is much lower than in human milk, with less than 2.5 μm and less than 1 μm respectively. The amount of putrescine increases throughout the lactation. Moreover, the concentration of polyamine in all human food is up to 300 nmol/g, although the concentration in different types of food components is variable. Therefore, in all studies conducted on rats (Deloyer, 2005, Peulen, 2004 & Peulen 1998) , the amount of polyamine (spermine) used to analyze the effects on small intestine was 0.4umol/g body weight is lower than in human food and significantly showed maturation as observed in matured one suggesting that polyamine seems to be efficient in inducing small intestine maturation.

CONCLUSION(S)

This study aimed to review the effects of polyamine milk on the maturation of the small intestine with the potential in immune system maturation. The ingestion of polyamine induced maturation of small intestine can be characterized by the positive changes in morphological and enzymatic that matched with matured intestine such as intestinal weight, villus amount and structure, disaccharide SA, enzyme content. Based on the evidence, the amount of polyamine that could induce the maturation of small intestine in rats and piglets were 0.40 umol/g bodyweight and 463nmol/ml respectively. However, there are still limited studies on the effects of polyamine on human small intestine. Thus, it is suggested for extensive research to be done on the effects of polyamine in breastmilk on the human small intestine. It is hoped that this review can demonstrate that polyamine is essential in the maturation of the small intestine and reduction of allergy incidence among infants.

REFERENCES

Berin, M. C., & Sampson, H. A. (2013). Mucosal immunology of food allergy. *Current biology*, *23*(9), R389-R400.

Dandrifosse, G., Peulen, O., El Khefif, N., Deloyer, P., Dandrifosse, A. C., & Grandfils, C. (2000). Are milk polyamines preventive agents against food allergy?. *Proceedings of the Nutrition Society*, *59*(1), 81-86.

Deloyer, P., Peulen, O., & Dandrifosse, G. (2005). Intestinal effects of long-lasting spermine ingestion by suckling rats. *Experimental physiology*, *90*(6), 901-908.

Mazzocchi, A., Giannì, M. L., Morniroli, D., Leone, L., Roggero, P., Agostoni, C., ... & Mosca, F. (2019). Hormones in breast milk and effect on infants' growth: a systematic review. *Nutrients*, *11*(8), 1845.

Peulen, O., Deloyer, P., & Dandrifosse, G. (2004). Short-term effects of spermine ingestion on the small intestine: a comparison of suckling and weaned rats. *Reproduction Nutrition Development*, *44*(4), 353-364.

Peulen, O., Dewé, W., Dandrifosse, G., Henrotay, I., & Romain, N. (1998). The relationship between spermine content of human milk during the first postnatal month and allergy in children. *Public health nutrition*, *1*(3), 181-184.

Peulen, O., Gharbi, M., Powroznik, B., & Dandrifosse, G. (2004). Differential effect of dietary spermine on alkaline phosphatase activity in jejunum and ileum of unweaned rats. *Biochimie*, *86*(7), 487-493.

Sheard, N. F., & Walker, W. A. (1988). The role of breast milk in the development of the gastrointestinal tract. *Nutrition reviews (USA)*.

Van Wettere, W. H. E. J., Willson, N. L., Pain, S. J., & Forder, R. E. A. (2016). Effect of oral polyamine supplementation pre-weaning on piglet growth and intestinal characteristics. *animal*, *10*(10), 1655-1659.