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Effects of dietary polyamines at physiologic doses in early-weaned piglets

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Abstract

Objective: Polyamines are essential for many cell functions, and they form part of the composition of maternal milk; despite this, their addition to infant formulas is currently under evaluation. The aim of the present study was to evaluate the effects of milk formulas designed to resemble sow milk supplemented with polyamines at maternal physiologic milk doses on the gut maturation of early-weaned piglets.

Methods: We fed 30 newborn piglets with maternal milk ($n = 10$), a control milk formula ($n = 10$), or a milk formula supplemented with polyamines (5 nmol/mL of spermine and 20 nmol/mL of spermidine, $n = 10$) for 13 d (day 2 after birth through day 15). Several growth and intestinal development parameters were measured.

Results: The piglets fed the formula containing polyamine at physiologic doses showed significantly increased crypt depth in the small intestine compared with those fed with the control formula. Villus length was correlated to crypt depth. Although there were no differences in the disaccharidase activities between the animals fed the two formulas, alkaline phosphatase and γ-glutamyl transferase activities tended to be higher in the jejunum of those fed the polyamine-supplemented diet. Dietary polyamines did not significantly modify the gut mucosal concentrations of putrescine, spermine, or spermidine.

Conclusion: Milk formulas supplemented with polyamines at maternal milk physiologic doses slightly enhanced gut growth and maturation in neonatal piglets.

Keywords: Polyamines; Human milk; Small intestine; Gut development; Piglets

Introduction

Neonatal life, when only one type of food is the source of nutrition, is a very vulnerable period. Although several factors are involved in infants' health, breast milk is considered the optimal source of nutrition during the first 6 mo of life. The nutritional components of human milk may influence intestinal maturation in neonates [1,2], and milk polyamines have been related to gut maturation and development in several studies [2–5]. Nevertheless, these components have not yet been incorporated into infant formulas because more studies are needed to clarify their role during this early stage of life.

Polyamines (putrescine, spermidine, and spermine) are aliphatic molecules present in the cells of all organisms [6,7]. Dietary polyamines contribute significantly to the luminal gastrointestinal polyamine pool [8–10]. Physiologic intracellular polyamine levels are essential for many cell functions, such as protein, RNA, and DNA synthesis, and the stabilization of DNA and chromatin structure [11]. In addition, polyamines may modify the immune response, block calcium ion channels, and regulate apoptosis [12] and are involved in cell differentiation and proliferation [13]. In suckling rats, the oral administration of high doses of polyamines was correlated with the early appearance of morphologic and biochemical modifications typical of the mature intestine [3,5,10].

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In contrast, polyamine-deficient diets resulted in a significant hypoplasia of the small intestinal and colonic mucosa [14]. Although the importance of luminal gastrointestinal polyamines is well documented, little is known about their effects at physiologic levels.

Polyamines are present in appreciable amounts in human milk [15–18]. Maternal milk polyamine levels are higher during the first week of lactation [15], which raises a question of the physiologic effect of these substances on neonates. At present, infant formulas are not supplemented with any exogenous source of polyamines, and their polyamine levels are 10 times lower than in human milk [19]. Because milk polyamines could exert stimulatory effects on postnatal growth and gut maturation in lactation [15–18], their addition to infant formulas could be of major importance for the newborn.

The purpose of the present study was to determine the effects of neonatal milk formulas supplemented with polyamines, at the physiologic levels found in maternal milk, on gut development, by evaluating mucosal morphology, enzymatic activities, and polyamine levels in the small intestine of early-weaned piglets.

Materials and methods

Animals and diets

Thirty newborn piglets (Landrace by Large White) were provided by the veterinary farm of the University of Murcia (Murcia, Spain). They were matched by gender. Twenty piglets were nursed by sows until 2 d of age, after which they were randomly allocated into one of two groups (10 animals/group): control formula group ($n = 10$) and polyamine-supplemented formula group ($n = 10$). The piglets for these two groups came from the same litter as those from the maternal milk group. The piglets were housed in cages provided with attached spot heat lamps and fed ad libitum every 3 h for 13 d. The rest of the animals ($n = 10$) continued to be nursed by sows until 15 d of age (sow milk group). The study was approved by the animal care committee at the University of Murcia and conformed to the European Union Regulation of Animal Care for the care and use of animals for research.

The milk formula was designed to resemble sow milk in its macronutrient composition and to meet National Research Council nutrient requirements for the growing piglet [20]. The ingredients for the preparation of the milk formula were 118 g of cow's milk solids (3.6% fat), 137 g of calcium caseinate, 153 g of demineralized milk whey, 451 g of cream (35% fat), 116 g of olive oil, 182 g of coconut oil, 36 g of soybean oil, 3.5 g of lecithin, 206 g of lactose, 37 g of a mineral complex, and 3.3 g of a vitamin complex (Piensos CARN, Murcia, Spain) per kilogram. The nutrient composition of the milk formula is presented in Table 1. The formulas were dissolved in warm water at a concentration of 200 g/L. No detectable polyamine levels were found in the control milk formula for three replicates. The polyamine-supplemented formula contained 5 nmol/mL of spermine (Sigma Chemical Co., St. Louis, MO, USA), and 20 nmol/mL of spermidine (Sigma Chemical Co.), reflecting the polyamine levels detected in sow milk. The polyamine dose used was calculated from the mean concentration of polyamines in 10 milk samples from all the different sows (Table 2). Numerous factors modify the amount of milk polyamines, such as genetic factors, the preweaning period, environmental factors and, especially, the nutritional state and diet [2]. It was therefore important that the sows used in the present study were subjected to the same conditions of feeding, light, and water availability as those used for the quantification of milk polyamine concentration.

Table 1
Nutrient composition of milk formula in percentage of dry mass

Composition (%)	
Raw protein	27.05
Lysine	2.14
Fat	33.22
Lactose	29.96
Vitamins*	5.0
Minerals*	4.76
Fatty acids (% total weight)	
4:0	0.0319
6:0	0.0159
8:0	1.0781
10:0	1.0863
12:0	8.9509
14:0	3.7651
16:0	31.0650
16:1	0.0262
18:0	4.8008
18:1	35.1280
18:2	12.5001
18:3	0.988
C20	0.1183

* The mineral and vitamin mixture contained (milligrams per kilogram of diet): dibasic calcium phosphate 22,240, calcium citrate tetrahydrate 5070, sodium phosphate dibasic dodecahydrate 5060, manganese sulfate 1530, ferrous lactate 1380, potassium sulfate 920, copper sulfate 10.7, manganese sulfate 32.6, potassium iodate 0.8, zinc sulfate 5.24, thiamine 2, riboflavin 3, pyridoxine HCl 3, nicotinic acid 30, calcium pantothenate 20, folic acid 1, biotin 0.8, cyanobalamin 0.025, retinol acetate 0.069, cholecalciferol 0.0093, DL-tocopherol 50, phylloquinone 0.15, ascorbic acid 700.

Dissection protocol

At 15 d of age, fasting piglets, deprived of food for at least 8 h, were anesthetized by retro-ocular injection with a 50:50 mixture of ketamine:propofol (1 mL/kg). The abdominal wall was opened and the entire gastrointestinal tract was removed. Using scissors, the mesentery was cut, and five intestinal tissue samples, each 1 cm in length, were removed at a point 10%, 25%, 50%, 75%, and 90% of the length of the small intestine and kept in Bouin fixative until analysis. In addition, mucosa samples from the jejunum and ileum were removed by scraping the entire luminal surface with a glass