Physiological Significance of Free Amino Acids in Porcine Milk

(ブタ乳汁中遊離アミノ酸の生理的意義)

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Preface

The pig is omnivorous animal, belongs to the Suidal in the order of Artiodactyla, and has been bred and improved for meat production. Pigs are the most productive among domestic animals, and develop fast and early with high efficiency of feed consumption. In another aspect, pigs has been recognized as one of the most useful animal models for human medical and nutritional research, because pigs have anatomical structure and physiological function fairly similar to those of human.

The body size of common breeds of the pig for meat production is too big to be utilized as laboratory experimental animals, and people has been developed relatively smaller sized pig breeds called miniature pigs with body weight of matured individuals ranging 30-70 Kg. Recently a new aspect of research has been rapidly developed for assessing their possible utility for the donor of the organs for xeno-transplantation to human. More commonly, miniature pigs have widely used as a model of human in physiology, pharmacology and nutritional researches. For example, newborn piglets have been used as an animal model of human infant in pediatrics to study their nutritional necessary of the formula (the formulated artificial milk).

The pig is a very particular omnivorous species with a large body size and a large number of litter. During an implantation process in the pig, the uterine space will be divided among the more advanced embryos, leaving the slow ones with too little space to survive. Fetuses in the midsection of the uterus between the tip and the body sometimes have space available that is marginal, that is, sufficient to survive, but less than enough to normally. Runts arise from these conditions. Unless runts are given special treatment at birth, they rarely survive. Thus, competition for survival starts from the stages of the embryos and the fetuses in the pig.

The competition continues after birth. The pig possesses characteristic nursing behavior; the piglets born normally distribute themselves along the udder due to a tendency for each to suckle consistently only one or two specific teats. This spatial arrangement is called the teat order. Newborn piglets receive no aid from the sow but are usually ambulatory within minutes after birth. Upon standing, piglets move about for seeking teats until they contact a teat, and begin to suckling. The central point of the activity of newborn piglets seems to find a better udder of a dam.

In sows, the mammary gland produce colostrum prior to (1 to several hours) parturition, and secrete continuously in the early 2 or 3 days of lactation, then the secretion is disconnected and the milk is ejected within 10~30 seconds at intervals of 30~90 minutes. At this later stage, suckling stimulus to teats seems to effect on the milk production (Gill and Thomson, 1955). Not only changes in plasma concentrations of hormones, such as prolactin, somatostatin, insulin, glucagon and vasoactive intestinal polypeptide (Kendall et al., 1983; Algers et al., 1991), suckling stimulus to a particular teat must also induce a change in the local environment of each teat through paracrine pathways. Piglets in a litter may vary widely in body weight at birth, and during suckling, piglets stimulate the udder by massaging the teats before and after let-down (Fraser, 1980; Algers and Jensen, 1985). Due to difference in the intensity of suckling behavior on the preferred teat and its positions on the udder among piglets in a litter, the milk production and composition may be varied among the teats of a given sow. Thus, a bigger piglet with the more intense suckling behavior will have a chance to suckle the "better teat".

One of the focal points of pig breeding for obtaining better meat producing animals would have been how to moderate the competition among embryos, fetuses and newborn piglets, as mentioned above. By moderating these competition, one can expect to have a litter being born and growing uniformly. But in modern well-bred pig strains, there are still problems of stillbirth, runts and piglet's diarrhea. In order to accelerate further the efficiency of pig breeding, we have to cope with these problems, and find the genetical background of these phenotypes that results in the economical loss in pig production.

Before starting the research of this thesis, the author had a hypothesis that the piglet's diarrhea should be one of the outcome of the "better teat competition" among piglets. The occurrence of diarrhea in weak and meager piglets would be a good way to remove them from the descendants. If this is the case, composition of the milk should differ among teats. If the teat which more healthy piglets prefer secrete more nourishing milk, the strategy of selection of offspring might work. The question is what nourishing milk is. It is known that colostrum contains high activities of protease inhibitors which limit the breakdown of intake proteins and these inhibitors minimize the potential of colostrum proteins to be digested and becomes a source of free amino acids for neonates. Thus, free amino acids in colostrum have been suggested to be an important source of free amino acids for suckling animals. Furthermore, amino acids as one of the most important nutrients for the neonates distributed in the milk in three form; protein-bound, free peptide and free amino acids. The free amino acids can be absorbed directed by the intestine enterocyte, so that the free amino acids in colostrum and milk will be specially significant for the newborns whose digestive system are undeveloped.

From the background described above, the author tried to analyze free amino acids concentrations in colostrum and milk from individual teats of sows in Chapter I. Because it was found that glutamine concentration differed among the milk samples collected from different teats, glutamine was analyzed in Chapter II, if its concentration can be a cue for teat preference by piglets. In Chapter III, the effect of taurine on new born piglets was examined from various aspects, because taurine was found to be the most abundant free amino acids in colostrum and became most abundant free amino acids in milk as a lactational period progressed. Finally in Chapter IV, effects of glutamine in milk was investigated in relation with the occurrence of diarrhea under glutamine deficient condition.

Chapter I

Free Amino Acids Concentrations in Colostrum and Milk from Individual Teats of Sows and their Influence on Piglets Growth

Abstract

Concentrations of free amino acids in the colostrum and milk that were obtained from individual teats of sow dams were quantified. Taurine was the most abundant free amino acids in the colostrum and maintained at an high level (1-2 mmol/L of defatted milk) throughout a lactational period. Other free amino acids such as glutamine were in trace amounts in colostrum. The concentration of glutamine increased to the greatest extent among all other free amino acids examined during a 25 days of lactational period, and became the most abundant free amino acids in milk (2 mmol/L of defatted milk). Free glycine and glutamic acid also increased to a large extent (0.7-1.2 mmol/L of defatted milk).

Immediately after farrowing, no significant differences in the concentrations of each free amino acid were observed among colostrum samples obtained from different teats. However, the concentrations of free amino acids in milk from different teats became significantly different as lactational period progressed. As for the milk samples obtained from individual teats, there were significantly positive correlations between the total free amino acids, and free glutamine, glutamic acid and glycine concentrations. Piglets were identified every day which teat they suckled. There were also significantly positive correlations between the body weight of each piglet, and milk yield of each teat and the total free amino acids concentration in milk obtained from each teat. Cumulatively, a larger piglet suckled a higher milk yield teat from which higher concentrations of total free amino acids, glutamine,

glutamic acid and glycine were supplied. In light of present findings, the free amino acids in sow dam's milk can be a possible participant to determine the teat preference by piglets.

Introduction

Neonates do not have well developed digestive and regulatory systems to adapt their food and environment, and yet they are stressfully challenged by a new environment at birth. Because newborn mammals do not normally consume solid foods, their mother's milk is the only source of exogenous amino acids for the synthesis of proteins, neurotransmitters, hormones, polyamines, purine and pyrimidine nucleotides, creatine, carnitine, porphyrins and other biologically important molecules. Some amino acids, such as glycine, histidine and taurine, are effective scavengers of free radicals and therefore may help prevent or alleviate potential injury. Thus, milk plays a vital role in the survival and growth of mammalian neonates.

It has been known that colostrum contains high activities of protease inhibitors, which limit the breakdown of intake proteins (Laskowski et al., 1957). Although the presence of protease inhibitors in colostrum has important immunological implications, these inhibitors minimize the potential of colostrum proteins to be digested and becomes a source of free amino acids for neonates. Thus, free amino acids in colostrum have been suggested to be an important source of free amino acids for suckling animals. Furthermore, milk is an important source of taurine, a β-amino acids that is virtually absent in the foods of plant origin. A number of studies have reported the concentrations of free amino acids in the milk from a number of species, including baboon, beagle, cat, chimpanzee, cow, gerbil, guinea pig, horse, sheep and humans (Atkinson et al., 1980; Vina et al., 1987). Taurine has been shown to be the most abundant free amino acid in the milk from beagle, cat, gerbil, mouse and rhesus monkey (Rassin et al., 1978). The abundance of free taurine in the milk is consistent with its important role in the postnatal growth and development of such species as the cat (Sturman,1993).

The pig has been widely used as an excellent animal model for human nutrition research (Burrin et al., 1992). Thus, to substantiate data on the amino acid nutrition of sow's milk, several reports were focus on the concentrations of free and protein-bound amino acids in the colostrum and milk (Beacom and Bowland, 1951; Cuperlovic, 1967; Bengtsson, 1972). Recently Wu et al. (1994) reported that taurine and histidine were the most abundant amino acids in sow's colostrum, whereas glutamine concentrations increased to the greatest extent among all free amino acids during lactation. However these studies neglected the teat-order behavior that specifically observed in piglets society. Piglets may vary widely in body weight within a litter at birth, and they normally distribute themselves along the udder due to a tendency for each to consistently suckle only one or two specific teats. This spatial arrangement being called the teat order. During suckling, piglets stimulate the udder by massaging the teats before and after let-down (Fraser, 1980; Algers and Jensen, 1985), this suckling behavior effects on the milk production (Gill and Thomson, 1956) by changing plasma concentrations of some hormones, such as prolactin, somatostatin, insulin, glucagon and vasoactive intestinal polypeptide (Kendall et al., 1983; Algers et al., 1991). Due to difference in the intensity of suckling behavior on the preferred teat and its positions on the udder among piglets in a litter, the milk production and composition may be varied among the teats of a given sow.

This study was designed to quantify the concentrations of free amino acids in colostrum and milk samples from sows that were separately collected from individual teats at different stages during lactation. By obtaining these informations, significance of free amino acids concentrations for determination of the teat order of piglets was discussed.

Materials and Methods

Chemicals

All chemicals used for analysis of amino acids in this study were purchased from Wako Pure Chemical Industries, Ltd.

Sow dams and collection of colostrum and milk

Six sow dams in the Experimental Station for Bio-animal Science of the University of Tokyo were used in this study. They were F1 of Large White sows and Landrace boars. The range of parity was from 2 to 6. Dams were nursing 10.6 piglets on an average at weaning (ranging from 8 to 13 piglets per litter). Dams were free access to water and fed 3 times a day (0600, 1300 and 1700 hr) with soybean-corn meal-based diet that met NRC recommended requirements. On the day of farrowing (day 1 of lactation), colostrum was collected manually from each teat. The first colostrum samples were obtained 0-1 hr after farrowing before the teats had not been suckled by newborn piglets. Teats were identified by numbering as R (right side) or L (left side) 1, 2 and so on. At later collections on days 3, 5, 10 and 15 of lactation, colostrum or milk samples were obtained after an injection of oxytocin (20 IU units, Atonin-O, Teikoku Zoki Co, Tokyo) via the ear vein to cause milk letdown. Before the oxytocin injection, piglets were isolated from their dams for an hour after the dam finished the diet given on 1300 hr. Two-5 ml colostrum or milk were taken from each nipple and stored at –80℃ until analyses of free amino acids.

Piglets

Piglets were marked by punching the ear number, and weighed at birth, days 3, 5, 10, and 15 of lactation. Observations had been done to certify the teat-order of the piglets in the lactational period.

Analysis of amino acids by HPLC

The milk samples were centrifuged at 10,000 rpm for 10 min at 4℃ to remove fat, mononuclear and other suspended cells and debris. One ml of defatted milk was deproteinized for 2 min with 0.37 mol/L TCA (trichloroacetic acid) , and centrifuged at 10,000 rpm for 5 min at 4℃. The supernatant was filtered through 0.1 μm Ultrafree-MC (Millipore Co.) and were used for amino acid analysis.

All amino acids were determined with an amino acid analyzer (Hitachi High Speed Amino Acid Analyzer, Model L-8500) by using "The 110 min Program for Biological Liquid Analysis" which was supplied by the manufacturer.

Statistical analysis

Data of free amino acid concentrations in the milk were analyzed by t test and the linear multiple regression method. $P<0.05$ was taken as statistical significance.

Results

Free amino acids in sow dam's colostrum and milk

The concentrations of free amino acids in dam's colostrum and milk are shown in Table 1. Taurine was the most abundant free amino acid in colostrum on the beginning of the lactation. Glutamine was almost undetectable. On day 3 of lactation, the concentrations of the most free amino acids increased ($p<0.05$), except for the following amino acids: ornithine, citrulline, and asparagine. On day 10 of lactation, glutamine and glutamic acid increased significantly and they became the most abundant free amino acids in the milk. The increasing trend lasted to the end of this study, the values over 4 mmol/L in some of the teats on day 25 of lactation. Taurine maintained in high level throughout lactational period. It is noteworthy that the concentrations of glycine increased to the second abundant amino acids.

Difference in total free amino acid concentrations among the teats

There were no significant differences in concentrations of free amino acids in the colostrum from different teats during early hours of the delivery. It is noteworthy that the concentrations of free amino acids of milk from the different teats diverged each other later than day 3 of lactation. Table 1-2 shows a representative distribution of total free amino acids among different teats between days 1 and 16 of lactation, and Fig. 1-1 shows changes in the total free amino acids concentrations at each teat in dam-232. On day 3 of lactation in this case, the teats of L2, R2, R6 and R7 became lower in the total free amino acids than other teats ($p < 0.05$), and the teats L1, L3 and L6 became to supply the highest ($p< 0.05$) concentrations of total free amino acids. On day 5 of lactation, R4 and R6 were the lowest in total free amino acids ($p<0.05$) and R3, R1, L1 and L3 became the richest ones. On day 10 of lactation, R6 and R5 were lower in total free amino acids than in the other teats ($p<0.05$), and R6 significantly higher than R5 ($p<0.05$). The teat R4 was atrophied at this moment and milk samples could not be collected hereafter. On day 16 of lactation, R5 was lowest in total free amino acids as compared with the other teats and followed by R1 and L3.

Relationship of total free amino acids in milk and the teat nursing behavior by piglets

Fig. 1-2 shows the changes in the rank of piglet's body weight (an order of body weight from heavier to lighter ones) from the day of birth (day 1 of suckling) to day 15 of 12 piglets delivered from dam-232. Except one piglet (E) who stayed at the first rank, the rest of piglets changed their ranking throughout the experimental period.

Pig E mentioned above seemed to prefer the lower position of the udder at first, but most of time during the first 3 days, alternated his suckling teats between L3 and R3. He spent short times for tasting R4 and then, settled at R3 by keeping his 1st rank for the rest of the period. Relatively higher concentrations of total free amino acids and glutamine, glutamic acid and glycine were continuously detected in the milk samples collected from R3.

The pig I was, the second heaviest birth weight, occupied R 4, 5 and then R6, and finally settled at R4 on day 3. At that moment, the order of the free amino acid concentrations was R4>R5>R6. Perhaps because of interference by pig E, pig I changed his main teat from R4 to R5 on day 5 when the free amino acids concentrations were R5>R4>R6. Between days 11 and 15, it was found that R6 became the fixed teat for pig I, and R4 had atrophied completely. Meanwhile, the body weights rank of pig I dropped from the second to the sixth by day 15, probably due to insufficient milk yield from R4. A similar process was observed in pig D who changed his primary teat from L6 to L5 associating with a drop of the rank from the 3rd (on day 1) to the 9th (on day 14). On the other hand, pig H suckled only R2, raised her rank from the 8th to the 3rd. The total free amino acids of R2 were continuously increasing to relatively higher levels.

Relation between body weight and total free amino acids in milk from each teat

The correlation was taken on different days after parturition, between body weight of the piglets and concentration of the total free amino acids in milk from the teat they suckled. There were some positive correlations. For dam-232 as an example, the correlation coefficients were 0.052~0.579 throughout an experimental period, only on the correlation on day 5 (0.579) was significant ($p < 0.05$). In dam-312, the correlation was significant on day 10. However, during the whole lactational period, the correlation coefficients between the body weight and the total free amino acids or glutamine in milk from the teat they suckled are 0.754 (Fig.1-2) and 0.839 (Fig.1-3), and they are highly significant ($p<0.001$).

Discussion

This study characterizes the distributions of free amino acids in sow dam's colostrum and milk from individual teats during 25 days of lactation. Amino acids as one of the most important nutrients for the neonates distributed in the milk in three forms; protein-bound, free peptide and free amino acids. The free amino acids can be absorbed directed by the intestine enterocyte, so that the free amino acids in colostrum and milk will be specially significant for the newborns whose digestive system are undeveloped. Several studies (indicated that taurine is the most abundant free amino acid in sow's colostrum. We also found the high concentration of taurine (1 mmol/L) in the colostrum before and soon after farrowing. Glutamine was too low to be measurable in the colostrum that was collected before the delivery. Wu and Knabe (1994) reported that histidine followed by taurine was the richest amino acid in the sow dam's colostrum. According to our result, histidine was less than 100th of taurine concentration. It is difficult to reconcile this discrepancy, but more recently, Toshiyuki and Noboru (1995) reported the similar results as mine that histidine was much lower in the sow's colostrum. Taurine is widely distributed in brain, heart, retina, liver and muscle, and it serves in such wide respects of physiological processes as its distribution. Taurine maintained in high level throughout the lactation period in sow's milk, and its actions for the newborn piglets were not so clear that more investigations are needed in future.

Before farrowing, concentrations of free amino acids in the colostrum were not significantly different among the teats where samples were taken. Before the start of suckling the colostrum expected to be ejected from each teat seemed to be uniform in nutrients for the piglets' first suckling, implying that a sow dam gives her offspring with the equal chance to exist at first.

The concentrations of free amino acids such as glutamine, glycine and glutamic acid increased significantly on day 3. From the data reported by Toshiyuki and Noboru (1995), the changes of free amino acids in sow's colostrum occurred early in the 6 hr after parturition. According to Wu and Knabe (1994), the total amount of free amino acids progressively increased with increasing lactation days, whereas the total amounts of protein-bounding amino acids decreased during the first 8 days of lactation and remained constantly throughout the following 3 weeks. Total free α-amino acids were 4.6% of total protein bound amino acids in sow's milk. Also note that free glutamine plus glutamate in the milk accounted for as much as 12% of protein-bound glutamine plus glutamate. For the free amino acids need not digestive process before absorbed by intestine, sows supply the amino acids in free state to her offspring will be more effective than in protein bound way. Some of free amino acids such as glutamine are an important source of energy and metabolic substrate for the enterocyte of intestine (Alice and Rongping, 1998; Laura and Kretchmer, 1988; Wu and Knabe et al., 1995).

After teat suckling by newborn piglets started, a uniform composition of free amino acids in the colostrum among teats became considerably variable. This empirical variation suggests that the activity of mammary gland epithelial cells could be influenced by not only endocrine mechanisms but also paracrine ones by different intensity and/or quality of stimulation to each different teat by each different piglet. Particularly in pigs, external appearance of the udder changes dramatically as lactation progresses. As shown in Reference Figure 1-1, 2 days after farrowing, and all udders looks uniform (A), but on 14 days of lactation the udder of which teat has not been suckled by piglets severely atrophied (B). If a dam nurses only one (Reference Figure 1-2) or two (Reference Figure 1-3) offspring, only utilized udder(s) has grown prominently.

It is well known in many species that suckling-induced teat stimulation influences plasma concentrations of hormones, such as prolactin, oxytocin, somatostatin, insulin, glucagon and vasoactive intestinal polypeptide in sows (Kendall et al., 1983; Algers et al., 1991). Among them, prolactin involves in stimulation of milk production and oxytocin is involved in milk ejection. If the endocrine mechanism carried by various hormones is the only regulator for the mammary gland function and morphology, however, the conspicuous morphological difference of the sow's udder mentioned above should not be induced. Particularly in pigs, some paracrine mechanisms as yet to be defined must control the geographical difference in mammary gland cells function and proliferation. Distinct difference in the concentration of free amino acids among samples collected from different teats will also be regulates by paracrine mechanisms including mechanisms regulating blood flow to a particular part of the udder.

Many investigators have been attracted by the mysterious teat order phenomena that was observed only in the piglets society. It was known that different gland sections of the udder may secrete very different quantities of milk (Donald, 1937a). Lewis (1978) indicated that correlations between pig weight gain, and milk yield and composition were all positive and significant. These former studied clearly indicated that the teat order, if any, promote that the heavier piglet gets the more milk and can function for as a selecting bigger and probably better offspring for the next generation. One of the essential problems that have no satisfactory explanations is "how the piglets can find whether is the high yield teats?" This study showed the solid relationship between the body weight and the total free amino acids, glutamine, glutamic acids and glycine concentration of milk. Further, these parameters for each teat correlated with the milk yield from the respective teat. It is known that glutamine, glutamic acid and glycine can stimulate the taste organ of rat (Grill et al., 1987). If the piglet also could detect these free amino acids by tasting, that will be possible for the free glutamine and other free amino acids such as glycine, glutamic acid could be an indicator of milk yields of the teats.

When the amino acid concentrations in the milk from each teat were compared on different lactational days, glutamine, glutamic acid and glycine concentrations on day 3 correlated with the respective 3 amino acids and the total free amino acids concentrations on day 5 but neither on days 10 or 16. On the other hand, glutamine, glutamic acid and glycine concentrations on day 10 correlated with the respective 3 amino acids and the total free amino acids concentrations on day 16. Correlation coefficients between glutamine and other parameters were generally higher. Thus, glutamine can be a more faithful indicator than glutamic acid and glycine that can predict the milk yield of a particular teat. The stability of glutamine concentration in milk from a particular teat seemed to increase as lactational period progressed. Meanwhile, changes in teat preference by piglets and body weight rank decrease later than day 10 of lactation.

The author would like to propose a working hypothesis; teat preference by piglets and body weight rank changes frequently during the first 10 days of lactational period, because milk yield and thus glutamine concentration are fairly variable among teats, but the final teat order is decided when mammary gland activity to supply milk to each teat becomes stabilized later than day 10 of lactation. Competition for occupying a particular teat will occur among piglets where glutamine concentration in milk is higher than the others. At this stage, sow dam gives her offspring with the uneven opportunity to survive.

In conclusion, free amino acids in sow's milk are modified by the nursing activity of the piglets. Piglets are individually different in birth weight, birth time, character, preferred teats position and relationships with the littermates, so that their nursing activity are different each other, and leading to the mammary gland in different development state. The concentrations of some free amino acids like glutamine, glutamic acid and glycine are closely related with the development of mammary gland, and it serves as not only the important nutrients but also as a signal of the development state of mammary gland that may be recognized by piglets. In this way, the piglets can develop their sensory systems and physical power to exist and growth in the competition with their littermates and adapt to a new environment.

Table 1-1. Free amino acids and nitrogen compounds in sow's colostrum and milk

	Day of Lactation					
	1	3	5	10	15	25
Amino acid			*μ mol/L defatted milk*			
Nutritionally nonessential amino acids						
Ala	15 ± 4	204 ± 82	397 ± 141	443 ± 137	491 ± 155	530 ± 205
$AspNH_2$	0 ± 0	5 ± 13	29 ± 21	62 ± 24	36 ± 35	
Asp	1 ± 4	103 ± 54	187 ± 74	469 ± 125	242 ± 107	108.9 ± 42.6
Cys	0 ± 0	16 ± 6	25 ± 6	32 ± 5	34 ± 11	35.7 ± 16.5
$GluNH_2$	0 ± 0	561 ± 259	1044 ± 396	1205 ± 299	1733 ± 530	1980 ± 945.6
Glu	12 ± 6	362 ± 158	588 ± 191	792 ± 141	608 ± 193	789 ± 412.5
Gly	19 ± 4	299 ± 164	448 ± 205	518 ± 202	822 ± 313	2195 ± 1057
Ser	15 ± 9	72 ± 30	123 ± 41	105 ± 28	159 ± 49	345 ± 142.8
Tyr	0 ± 0	18 ± 9	31 ± 12	38 ± 10	42 ± 13	83.7 ± 17.7
Nutritionally essential amino acids						
Arg	3 ± 9	34 ± 12	43 ± 12	40 ± 9	44 ± 10	126.9 ± 21
His	6 ± 6	30 ± 11	47 ± 12	47 ± 11	63 ± 19	116.7 ± 35.7
Ile	0 ± 0	4 ± 4	7 ± 3	9 ± 3	10 ± 3	23.4 ± 8.4
Leu	0 ± 0	18 ± 7	24 ± 7	24 ± 6	26 ± 7	65.4 ± 15.6
Met	0 ± 0	2 ± 4	6 ± 3	7 ± 2	9 ± 3	14.4 ± 3
Lys	5 ± 8	23 ± 10	26 ± 9	12 ± 9	18 ± 10	86.4 ± 28.8
Phe	0 ± 0	7 ± 5	10 ± 6	15 ± 5	18 ± 5	45.6 ± 7.8
Pro	1 ± 2	74 ± 69	56 ± 26	59 ± 14	69 ± 20	126.9 ± 21
Thr	0 ± 0	56 ± 22	77 ± 27	99 ± 28	79 ± 27	107.1 ± 45.3
Val	0 ± 0	16 ± 8	24 ± 9	30 ± 9	33 ± 12	72.3 ± 24.9
Other amino acids and nitrogen compounds						
P-Ser	59 ± 5	39 ± 6	39 ± 4	39 ± 3	35 ± 4	60 ± 12
Tau	1063 ± 100	1858 ± 377	1991 ± 402	1616 ± 198	1306 ± 360	1360 ± 377.7
PEA	612 ± 49	834 ± 133	408 ± 351	463 ± 78	317 ± 307	742 ± 266.4
Cit	9 ± 6	15 ± 3	21 ± 4	23 ± 5	33 ± 7	41.1 ± 17.4
α-ABA	0 ± 0	0 ± 0	9 ± 7	11 ± 4	23 ± 9	41.1 ± 22.2
Cys	0 ± 0	16 ± 6	25 ± 6	32 ± 5	34 ± 11	35.7 ± 16.5
Cysthi	1 ± 3	99 ± 42	108 ± 40	77 ± 23	35 ± 15	
β-Ala	2 ± 5	7 ± 6	14 ± 6	19 ± 6	24 ± 8	27.9 ± 25.2
$EOHNH_2$	49 ± 2	27 ± 8	20 ± 11	17 ± 9	28 ± 26	80.1 ± 86.7
Orn	21 ± 21	35 ± 18	60 ± 23	36 ± 10	52 ± 17	60 ± 16.8
Urea	1682 ± 137	2866 ± 192	4441 ± 464	5523 ± 482	4621 ± 717	5827 ± 598
NH_3	381 ± 39	260 ± 27	310 ± 112	255 ± 96	428 ± 134	1265 ± 240

Values are means ± SD n=14

PEA: Phospho ethanol amine

α-ABA: α-amino-n-butyric-acid

Cysthi: Cystathionine

Table 1-2. Changes in total free amino acid concentrations in milk samples collected from different teats on different days of lactation*

	Day of lactation				
	1d	3d	5d	10d	16d
Teat number	μmol/L defatted milk				
L1	1937.4	5178.4	6503.6	6614.6	7139.4
L2	1907.7	3411.2	5814.2	6191.8	6440.4
L3	1937.4	4993.4	6279.8	4948.8	5856.0
L4	2034.9	4310.6	6216.6	6290.6	6030.0
L5	1891.5	4029.4	6179.2	5750.6	6582.6
L6	2067.3	4786.6	5294.0	6073.6	6566.0
L7	1867.2	4673.6	5522.6	6136.8	6599.4
R1	2041.2	4598.4	6938.4	6091.8	5728.0
R2	1798.5	2905.6	5278.4	6768.8	7192.8
R3	1830.9	4455.4	6915.0	5310.6	6255.2
R4	1598.1	4774.4	3871.6	※	※
R5	1831.8	4691.7	5078.2	3120.2	870.2
R6	1942.2	1607.6	1630.0	5768.6	6475.6
R7	1806.6	2082.6	5670.2	6745.0	7204.4

※ The production of milk were too little to be sampled

* Data of the sow No.232

Tab. 1-3. Correlation among the concentrations of free amino acids in the sow's milk collected on the different days of a lactational period

	Total free amino acids				Glutamate				Glutamine				Glycine			
	Days of lactation				Days of lactation				Days of lactation				Days of lactation			
	3	5	10	16	3	5	10	16	3	5	10	16	3	5	10	16
Total FAA 3d	1	0.699*	-0.318	-0.292	0.95***	0.667 *	0.141	0.015	0.935***	0.354	-0.111	-0.268	0.926***	0.614*	-0.64	-0.362
Total FAA 5d		1	0.09	0.06	0.563 *	0.911***	0.369	0.369	0.578 *	0.85 ***	0.165	0.148	0.463	0.772**	-0.181	-0.132
Total FAA 10d			1	0.913***	-0.435	0.061	0.772**	0.792***	-0.378	0.26	0.927***	0.905***	-0.441	-0.075	0.845***	0.77**
Total FAA 16d				1	-0.349	-0.02	0.746**	0.833***	-0.322	0.13	0.901***	0.982***	-0.369	-0.083	0.728**	0.839***
Glu 3d					1	0.521	-0.001	-0.111	0.888***	0.236	-0.203	-0.317	0.887***	0.585*	-0.71	-0.359
Glu 5d						1	0.453	0.365	0.574*	0.8***	0.124	0.025	0.514	0.565*	-0.128	-0.231
Glu 10d							1	0.839***	-0.014	0.391	0.876***	0.73**	-0.005	0.081	0.491	0.505
Glu 16d								1	-0.067	0.331	0.851***	0.8***	-0.126	0.095	0.51	0.426
$GluNH_2$ 3d									1	0.236	-0.251	-0.313	0.973***	0.646*	-0.642*	-0.346
$GluNH_2$ 5d										1	0.235	0.253	0.137	0.522	0.166	0.044
$GluNH_2$ 10d											1	0.883***	-0.276	-0.01	0.672*	0.705**
$GluNH_2$ 16d												1	-0.38	0.005	0.709	0.85***
Gly 3d													1	0.522	-0.664*	-0.365
Gly 5d														1	-0.361	-0.151
Gly 10d															1	0.738**
Gly 16d																1

1. * $P<0.05$; ** $P<0.01$; *** $p<0.0001$.
2. n=12

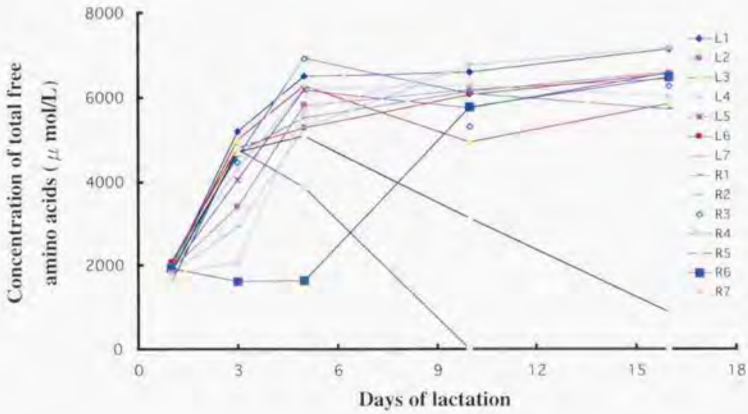


Fig. 1-1.

Changes in total free amino acids in milk samples collected from different teats on different days of lactation. The data presented in Tab. 1-2 are illustrated.

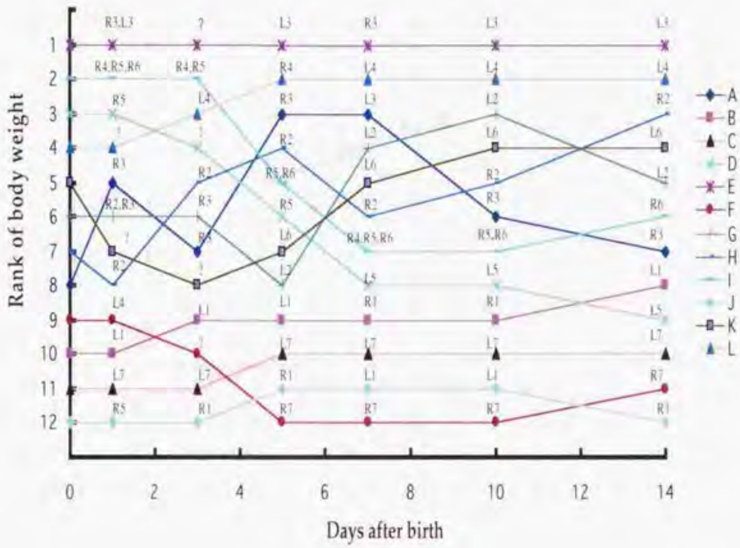


Fig.1-2.

Change in the body weight ranking among a litter of piglets during 14 days of lactation. Symbols in parentheses indicate the teat position on the final day of the experiments. The total free amino acids concentration in the milk from each teat is presented in Tab. 1-2 and Fig. 1-2.

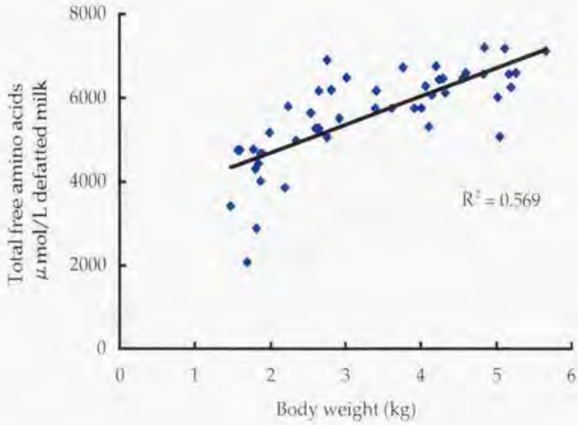


Fig.1-3.

Correlation between body weight and total free amino acids concentrations in defatted milk sampled from different teats of sow No.232 between days 3-16 of lactation, and there is a significant correlation ($p<0.05$).

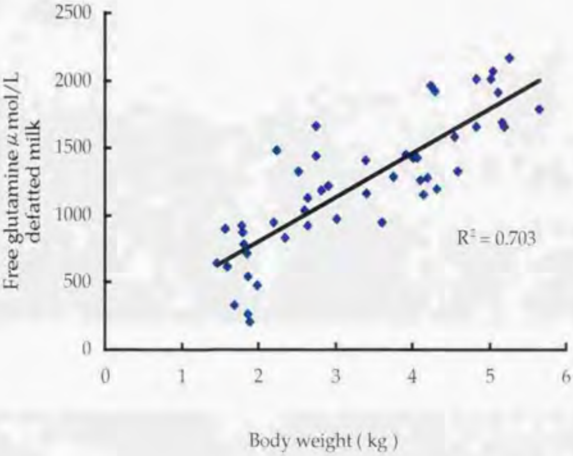
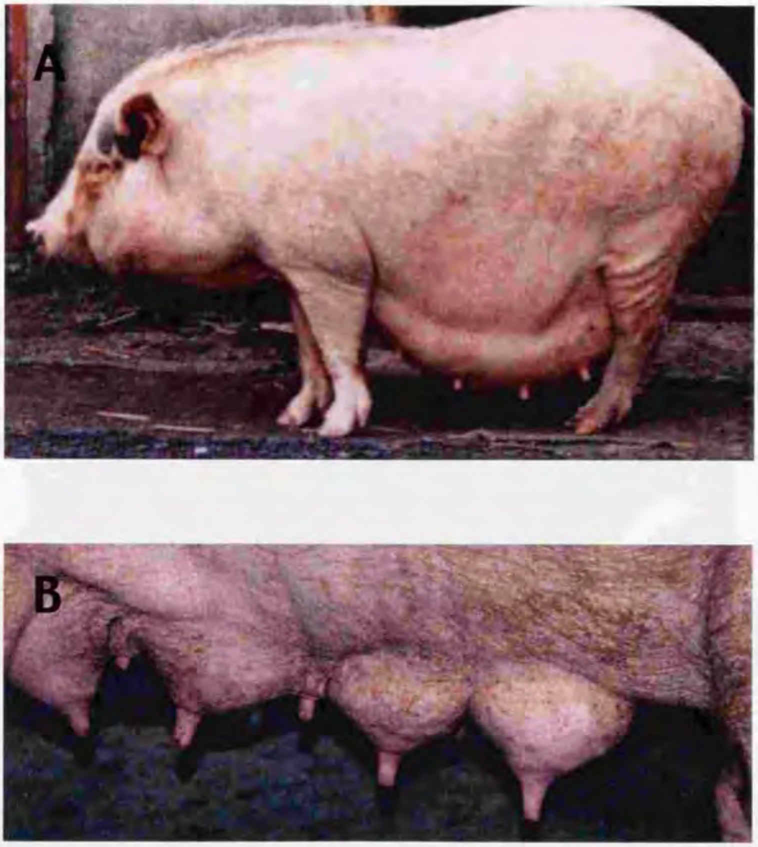


Fig. 1-4.

Correlation between piglets body weight and concentration of free glutamine in defatted milk sampled from different teats of sow No.232 between days 3-16 of lactation, and there is a significant correlation ($p<0.05$).



Reference figure 1.

A: A miniature sow 2 days after farrowing. The mammary develops uniformly.

B: Udders of a sow(F1 dam of Landrace × Large White) on day 14 of lactation. Two teats on the right side (R3, R5) which have not been suckled by any piglets are severely atrophied.

Reference figure 2.
A miniature dam which is nursing one piglet on day 15 of lactation. Only one utilized udder (L3) has grown prominently.

Reference figure 3.
A miniature dam which nursing two piglets on day 15 of lactation. Only the utilized udders (L1 and R6) have grown prominently.

Chapter II

The Effect of Free Glutamine Concentration in Milk on the Teat Preference by Piglets

Abstract

The effect of glutamine concentration in milk on the teat preference behavior by piglets was studied by nursing piglets by means of an auto nursing device (autosow). The autosow which the author developed has 4 teats to feed artificial milk all day long once an hour for 10 min with an antecedent sound signal for starting each milk feeding. Each 4 newborn miniature piglets of a litter were allocated to a particular nipple individually for 2 weeks, where they received the same cow milk supplemented with 0.5% taurine (the training period). Then, they were put together to be able to access any one of the 4 nipples (the selection period). The 2 nipple supplied cow milk supplemented with 0.25% and 0.5% glutamine and the rest of the 2 nipples supplied cow milk without glutamine supplementation.

The preference to each nipple by each piglets (number of accesses and lengths of staying, in connection with the time of milk releasing) was recorded throughout the 1st day of the selection period. The heaviest piglet visited different teats most frequently and finally selected the one of the teat supplying the highest glutamine containing milk. The result indicates that piglets can distinguish the difference in the concentration of glutamine and preferred the higher glutamine concentration. Thus, free glutamine can be at least one of the signals in milk that is preferred by a piglet.

Introduction

An early work by Robinson (1969) suggested that growth rate, growth potential and body composition of growing swine was influenced by nutritional levels during a certain period early in the life and that social behavior determined food intake during this period. Suckling piglets normally distribute themselves along the udder, and intend to suckle consistently only one or two specific teats. This spatial arrangement is called as the teat order. Due to difference in yield (Barber et al., 1955; Gill and Thomson, 1955) and possibly composition (Smith, 1952) of milk from the different teats, the nutritional status of individual piglets may vary widely within litters when the piglets are dependent primarily on their mother's milk for nourishment.

Newborn piglets receive little assistance from the sow after birth and thus, must find food and warmth depending on their own efforts. Piglets show a strong attraction to auditory, visual, olfactory and tactile stimuli immediately after birth (Rohde and Gonyou 1987, 1991; Julie et al., 1990; Toshio et al. 1998). Sensory systems play an important role in maternal-neonatal and neonatal-neonatal behavior in pigs. These sensory system help piglets to find and recognize the teats that they prefer. During the early hours after birth, most of the piglets fight against each other for snatching or protect the teat of their preference, until the establishment of teat order. Canine teeth grown at birth will help them to fight each other (Reference Fig.2-1). Viewing from the point of growth and survival of piglets, the meaning of the teat order will be a nutritional arrangement of the milk production and quality of the teats. It is assumed that the piglets could detect the differences of the milk production or quality among the teats. The question will be how they could do it.

There were great changes in the concentrations of free and protein bound amino acids in sow's colostrum and milk during lactation periods (Wu et al., 1995; Toshiyuki and Noboru, 1995). Our studies in the previous chapter showed significant difference in concentrations of glutamine and other amino acids in the milk samples collected from different teats. The teat with the higher milk yield was shown to produce the more free amino acids. The concentration of free glutamine in sows' milk increased to the greatest extent among all the free amino acids examined. It is known that some of the amino acids such as glutamic acid, glutamine or glycine can be tasted by rat (Gill et al. 1987). The author's question is whether piglets are also sensitive to these free amino acids, and can distinguish the different concentrations as a cue to find a teat to which relatively better developed mammary gland supplies milk. In this chapter, the author examined the effect of glutamine supplementation to cow milk on the behavior of teat preference of piglets reared on an artificial nursing device.

Materials and Methods

Auto feeding device

For feeding the piglets in the way similar to sow's , the author developed an automatic feeding device. Essentially, the device consisted of: (1) a cooling box for keeping the milk fresh; (2) a four-channel roller pump driven by the control unit to pump the milk from the cooling box to the teats; (3) a warm bath for heating milk to 30℃ when the milk is ejected from the teats; (4) the teats made of soft silicon rubber with the size fitting to the piglets mouth; (5) a control unit for programming the time sequence and the volume of milk to be fed; (6) individual chambers with a heating mat on the floor; (7) a cassette tape player, which is switched on and off at the start and the end of each feeding, respectively, playing back a particular music to give the sound signal to the piglets. Fig.2-1 shows the whole view of the automatic feeding device.

Animals and feeding condition

Four male crossbred miniature piglets (Small-ear pig of Taiwan×G ë ttingen miniature schwein×Pittmanmore miniature pig) in the same litter were used. Their growth curves throughout the experimental period were obtained by measuring the body weight once a day.

Piglets were not allowed to access the teat soon after birth and thus, deprived of the colostrum. Then they were bottle-fed with the replacer of cow milk supplemented with 0.5% taurine for

10 to 20 hr at 1-2 hr intervals with the sound signal mentioned above. After the bottle-feeding, they were transferred to the auto feeding device with individual chambers and trained for 2 weeks to suckle from the teat (teat-1 at the left-end to 4 at the right end) equipped with the device at intervals of 30 cm (the training period, Fig. 2-1). The piglets were fed with the replacer for 2.5 min at every hour. Feeding volume increased gradually from 15 to 30 ml/hr.

The piglets were, then on day 15 of birth, placed together in a new cage allowing them to access any of the 4 teats (the selection period, Fig. 2-2). The teats and the bottles were exchanged to new ones. The piglets were designated as A, B, C and D according to the order of body weight (heavier to lighter) by marking the letter on the back. The 2 teat (teat-1 and 2) supplied cow milk supplemented with 0.25% and 0.5% glutamine and the rest of the 2 nipples (teat-3 and 4) supplied cow milk no supplemented with glutamine. All teats were supplemented with 0.5% in the milk. Thirty ml of artificial milk was supplied similarly as during the training period.

Teat selection behavior

The piglet's behavior was recorded by a video camera for 20 hr on the 1st day of the selection period until the teat order was established. Any accesses to each teat by each piglet (a short or long period) were plotted as a function of time. The accesses to or occupations of each teat by each piglet during the feeding period (once every hour for 2.5 min) were picked out from the record above and illustrated.

Results

Body weight gain by the artificial feeding

It was easy to train the piglets to suckle the artificial teat by the help of human hand soon after birth. The growth curve (Fig. 2-3) shows that the body weight at birth were fairly uniform in pigs A, B and C, but pig D was particularly light. Diarrhea was often observed in all the piglets at the start of artificial feeding with cow milk supplemented with 0.25% taurine for 3 days. During this period, body weight increased slowly or sometime decreased and the difference in severity of diarrhea seemed to enhance the difference in boy weight gain. Pigs A, B and C recovered from the digestive trouble after day 4 of artificial feeding, but pig D was continuously suffered from the digestive problem for 2 weeks. By day 14 of artificial feeding, just before the selection period, the body weight of the piglets distributed into a wide range from 628 g to 1385 g.

Teat preference behavior

When the piglets were put together in one cage and allowed to access to any teats, they tended to move from a teat to a teat. At the first moment, teat-1, 2, 3 and 4 were visited by A, B, D and C pigs, respectively (Fig. 2-4). When a particular teat was occupied by a piglet, this kind of seeking behavior by the other littermates induced the positioning of the body so as to make the teat inaccessible to challengers. Aggressive behavior, in the form of groaning, biting or pushing with the nose or shoulder, was often shown by challengers toward the piglet suckling the teats. If the initial defensive maneuver is not successful, the defending piglet returned the same fighting techniques to the challenger.

As is shown in Figs. 2-6 and 2-7, number of contacts with the teat was highest in pig A who was the heaviest among the 4 piglets; he contacted with the 4 teats 280 times. This might be due to his superiority to the other littermates that made him to access any teats freely. After pig A visited the whole teats for about 10 hr, he finally selected teat 1 as his home position for suckling (Fig. 2-5), although he never stop sampling other teats (Fig. 2-4). The second heaviest pig B's final home position for suckling was teat-2, but he also suckled teat 1 when pig A was absent (Fig. 2-5). Pig C (the third heaviest one) stayed on teat 4 soon after the selection period started. Pig D (the lightest one) visited to the teats-1 and 2 at the beginning (Figs. 2-4) and often had chances to take milk from teat-1 or 2 during the first 3 hr, before pig A settled on his home position, teat-1 (Fig. 2-5). But after teats 1 and 2 were occupied by pig A and B, pig D stayed on teat 3.

Discussion

When piglets are fed artificially, it is easy to cause the digestive problem such as diarrhea is often arisen. In the case of piglets with lower birth weight, deprivation of the colostrum sometimes causes fatal consequences. The sow dam produces and secretes the colostrum continuously in a slow rate for 1-2 days after parturition and feeds her litter at hourly intervals, more than 20 times per day. In this way, the piglets can get a small but sufficient amount of the colostrum within a limited time. It is very important for the neonates whose digestive system undeveloped to lessen stresses caused by food intake. It seems particularly difficult to simulate artificially the quality of milk and the way of feeding by the dam during an early stage of lactation. In this study, the piglets were trained to suckle the artificial teat by aid of human hand, and cow milk supplemented with 0.25% taurine was used for the replacer. Though digestive problems occurred, but most of them recovered when they started to be fed with an aid of the auto feeding device, which could lessen the stress caused by irregular feeding intervals.

Donald (1937 a, b) recognized the nutritional significance of the teat order with respect to the milk yield of individual teats. Subsequent studies by Barber et al. (1955), and Gill and Thomson (1955), have substantiated the early report that milk intake and early growth are closely and positively correlated and that milk yield varies greatly among teats on a given sow. The aforementioned researchers reported that anterior teats tend to produce more milk than posterior teats, and therefore piglets that suckle from the anterior teats tend to growth faster.

Thomas et al. (1977) summarized the behavior exhibited by domestic piglets during the establishment of teat specificity into three phases. The initial, or teat seeking phase, is one in which the piglets search vigorously over the surface of objects encountered in their environment; they do not exhibit agonistic behavior during this phase and this phase is terminated by oral contact with a teat. The second phase that of teat sampling is characterized by activity suckling one teat after another, agonistic behavior is common and is initiated at udder during competition for possessing of a teat. The final phase is that of "teat maintenance." This period is one of relatively calm and involves simply an alteration between sleeping and sucking from the specific teat or a pair of teats. In many years, people puzzled with the mystery of the teat-order phenomenon in the piglet's society. There were many researches into the way in which piglets locate their teats. The importance of visual, auditory, olfactory and tactile in teat seeking behavior has been confirmed by some sagaciously designed experiments (Rohde and Gonyou, 1991; Jeppesen 1982 a, b; Morrow-Tesch and McGlone. 1990 a, b; Lewis and Hurinik, 1986). However, some of the nature questions have no perfect answers, such as, how the piglets fined the high yield teats.

Thomas et al. (1977) supposed that piglets could actually distinguished between low and high yielding teats and therefore attempts to claim the anterior teats. But he had not found conclusive evidence to support his hypothesis. In the studies in

Chapter I, the author found that the percent of some free amino acids in the milk from functional teats were increased linearly as lactation progressed, and that the concentrations among the teats were different in a given sow. The fact that the more developed mammary gland secretes the higher concentration of some amino acids lead to consideration whether the free amino acids in milk can be tasted by piglets as a cue for access to a higher yield teats.

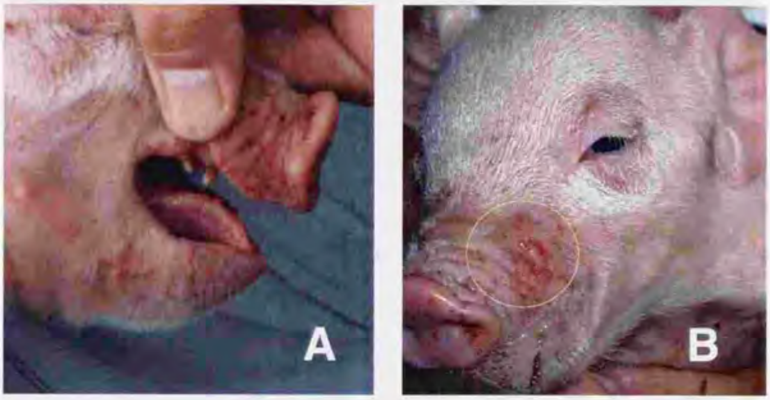
Jeppesen (1981) introduced an artificial sow to study the influence of milk yields on teat preference. He found that piglets quickly took up fixed positions on the single row of teats on the model udder. This provided an ideal opportunity to investigate how piglets recognize their teats and attempt to change the teat. The factors such like odor, light and composition of milk that may influence the piglet's behaviors could be controlled easily, and the activity of piglets could be monitored precisely.

This experiment was designed to simulate the establishment of teat order using miniature piglets at 14 days of age, which were reared on the auto feeding device. Because the piglets had been reared in the individual cage with the similar teats that were used in the group cage, the first phase of teat seeking (Thomas and Graves. 1976) was extremely shorten and the piglets could find and start to suckle the teat immediately. In the following phase of teat sampling, the biggest piglet (pig A) contacted and tried most of the four teats, and finally fixed its home position on the teat which was rich in free glutamine. This result implies that the piglets could distinguish the difference of the concentration of glutamine by tasting and preferred the relatively high glutamine diet. Though the two teats (teat-1 located at the left end and teat-2 located next to teat-1) supplied the same concentration of free glutamine, pig A selected teat- 1. In this concern, selection of the teat by pig C and D, the lightest two, was noteworthy; though the two teats (teat-4 located at the right end and teat-3 located next to teat-4) supplied the replace without free glutamine supplementation, pig C, heavier than pig D selected teat- 4. Thus, it can be premised that if the glutamine concentration is the same, the teat located at the end is better position than the teat located between teats.

Glutamine is an abundant free amino acid in plasma of animals (Hamilton 1945; Wu and Knabe, 1994) and in sow's milk. Glutamine serves as an important fuel for enterocytes (Windmueller and Spaeth, 1980) and as an essential precursor for the synthesis of protein, purine and some other bio molecules. Recent research has shown that glutamine is required for the maintenance of gut-associated lymphatic tissue and intestinal integrity. On the other hand, sow's milk contained other abundant free amino acids such as glutamate, glycine and taurine. The predominance of free glutamine as well as other amino acids which secreted in the higher concentrations in the more developed teats may be candidates for the milk composition signaling the milk yield. If this were the case, it would be perhaps the result of evolutionary selection that free amino acids abundantly yielded by the sow can not only serve as important trophic factors for piglet's survival but also serve as the indicator of the mammary gland development.

In conclusion, the auto feeding device is ideal for artificial feeding of the newborn miniature piglets and for studying the effects of the compositions of milk on the piglet's behavior of teat ordering. Piglets can distinguish the difference in the concentration of glutamine and preferred the higher glutamine concentration. Thus, free glutamine can be at least one of the signals in milk that is preferred by a piglet. This result support the hypothesis supposed by Thomas et al. (1977) that piglets can actually distinguish between low and high yielding teats.

Repeated and additional studies will be necessary to clarify the effects of glutamine; glutamate glycine and some other compositions of milk may be as a signal(s) of high yield teats.



Reference figure 4.

A: Newborn piglets have two sharp tooth (canine and 1st premolar teeth) on both side of the upper and lower jaws. The teeth are used as a weapon to fight against the littermate for the teat preference conflict.

B: A wound on the face of piglet injured by its littermate while fighting with his littermates. The picture was taken 4 hr after the end of parturition.

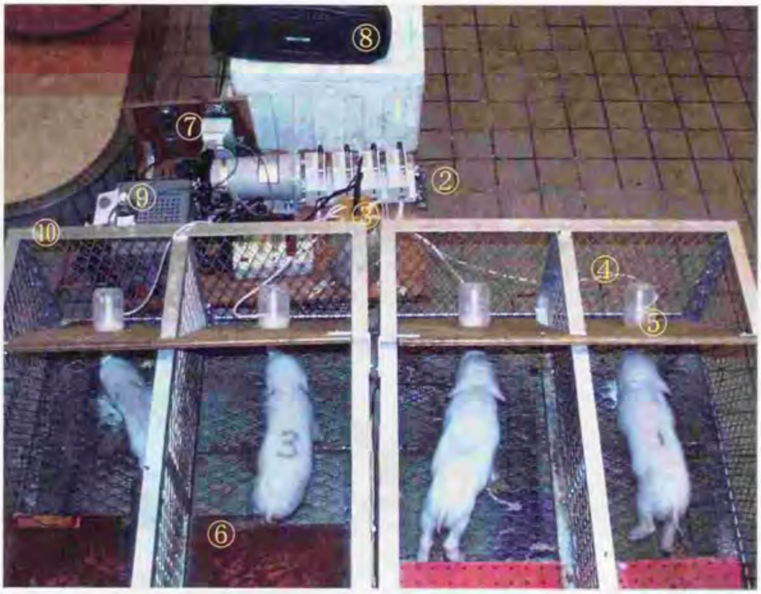


Figure 2-1.

The automatic feeding device for miniature piglets (individually rearing).

①Cool box for keep fresh milk. ②Roller pump (4 channels).
③Hot water bath for heating the passing milk.
④Silicon tubing. ⑤Artificial teat made of silicon rubber.
⑥Warming mat.
⑦Program timer for control the pump and the play of a endless tape recorder. ⑧Tape player. ⑨Power adapter. ⑩Steel cage.

Fig.2-2.
The artificial feeding device for miniature piglets (group rearing). Piglets select any of the teats. Accesses to each teat and suckling from each teat by each 4 piglets were recorded by a video camera on day 14 of age.

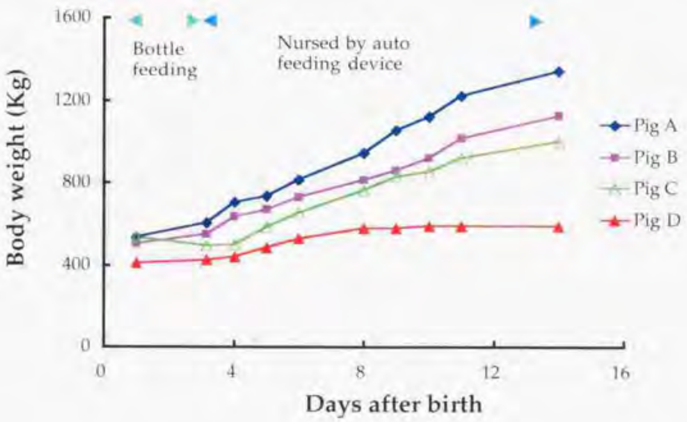


Fig. 2-3.
Growth curve of the miniature piglets which have been nursed artificially immediately after birth by either the bottle for the first or then the auto nursing device.

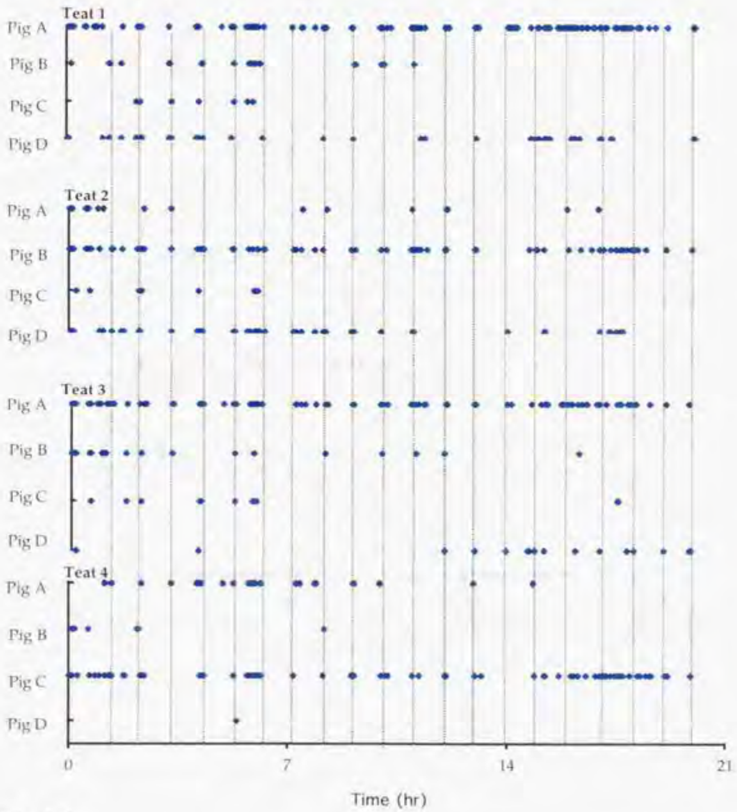


Fig. 2-4.

Accsses to each of 4 teats(1, 2, 3 or 4) by each 4 (A, B, C or D) piglets. Any of the accesses are poltted as a fraction of time based on the record by a video-recorders. The artficial milk was supplied for 2.5 min once every hour (immediately after gree vertical lines). The piglets are designated A to D according to their body weight (heavier to lighter). The teat are arranged from 1 to 4. Teats 1 and 2 supplied cow milk supplemented with glutamine(0.5%) and taurine(0.5%) and teat 3and 4 supply cow milk supplemented with only taurine(0.5%).

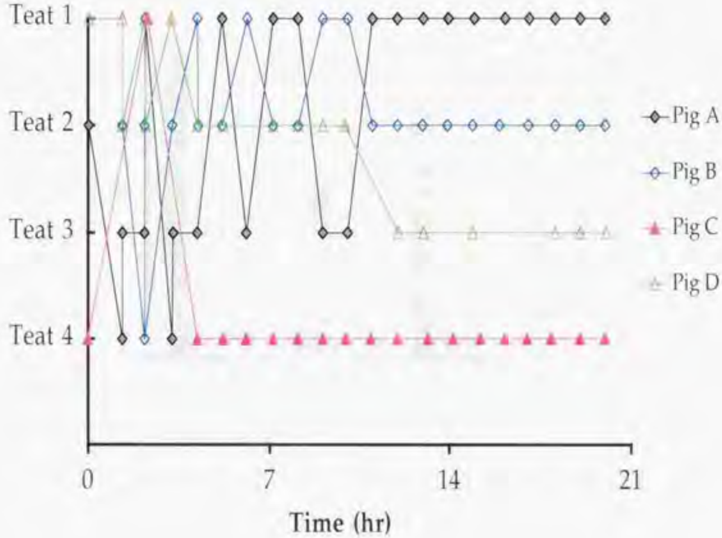


Fig.2-5.

Suckling position of each piglet. This figure is made based on the data in Fig. 2-4, by depicting the position of the piglet only during the period when the milk is supplied. See the legend for Fig. 2-4 for further detail.

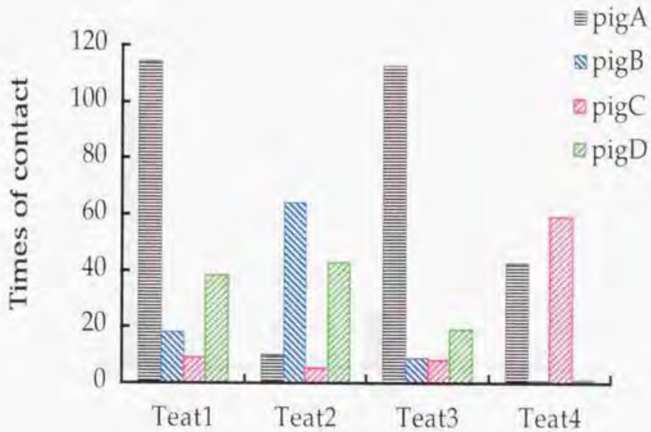


Fig. 2-6.
Frequency of accesses to each teat by each piglets. This figure is made based on the data in Fig. 2-4. Note that the heaviest pig A could most freely visited different teats. See the legend for Fig.2-4 for further detail.

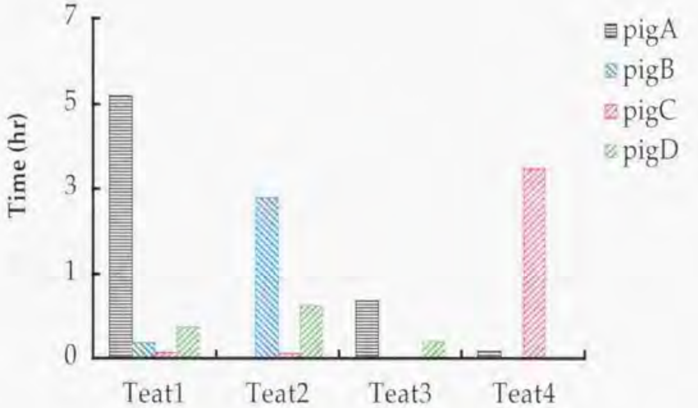


Fig. 2-7.

The occupancy (the length of time) of each teat by piglet during the 20 hr observation. Note that pig D (the lightest one) tried to suckle teat 1 and 2 where glutamine supplemented milk was supplied. But these teats were mainly occupied by pigs A and B, heavier ones. See the legend for Fig. 2-4 for further detail.

Chapter Ⅲ

Effects of Taurine in Milk on New Born Piglets

Abstract

As taurine is found to be the most abundant free amino acid in sow's colostrum, significance of this amino acid on the physiology of newborn piglets was investigated. The newborn miniature piglets were deprived of their dam's colostrum and nursed on a replacer based on fresh cow milk. The piglets from different litters were divided in two groups, one group suckled the replacer supplemented with 0.5% taurine, and the other group suckled the replacer without taurine supplementation (mean taurine concentration of the replacer is approximately 200 μ mol/L or 0.0025%). The first meconium was excreted significantly earlier ($p < 0.05$) in the taurine supplemented group than in the no supplemented group. Moreover, frequency of defecation at the day of birth and the following day was significantly higher ($p<0.05$) in the former group. Intestinal obstruction like the milk curd syndrome that easily happens in formula feeding infants occurred in the group without taurine supplementation. The piglets without taurine supplementation were later found to be affected with retardation of hair growth. Possible mechanisms for these phenotypes were discussed in relation with presence of a large amount of taurine in colostrum of the pig.

Introduction

2-Aminoethane sulfonic acid, or taurine, is a phylogenetically ancient amino acid with a distinct distribution in the biosphere. It is presented in high concentration in algae (Ericson and Carlson 1954; Reynoso and Gamboa 1982) and the animal kingdom. In many animal species including mammals, it is one of the most abundant lower-molecular-weight organic constituents. A 70-kg human contains up to 70 g of taurine. Taurine can be synthesized in some species such as adult rats, but in other species like cats can not synthesis sufficient taurine to meet the need of the body. (Jacobsen and Smith, 1968; Worden and Stipanuk. M. H., 1985). Taurine has been shown to be an essential nutrient for cats, and probably also for primates, especially during an early developmental period. A dietary deficiency of taurine in cats results in malfunctioning of a number of physiological systems; the visual system in which visual acuity, electroretinogram, and visual evoked responses occur along with degeneration of the retina and the tapetum lucidum; the reproductive system in which spontaneous fetal resorption, abortions and stillbirth occur frequently, and surviving offspring have often abnormal ontogeny (Imaki et al., 1986; Sturman et al., 1985; Sturman, 1988; Sturman and Messing, 1991). All of these changes are accompanied by a decreased concentration of taurine throughout the body.

As was presented later in this chapter in Table 3-1, the concentration of free taurine is rich in colostrum in all species of domestic animals examined. Thus, a common characteristic seems that taurine is rich in the colostrum, but it is noteworthy that only in the sow's milk, high concentrations of taurine are maintained even when lactational period progresses. Stephen and Chavez (1991) reported a significant increase in enzymatic activity for taurine synthesis in the brain tissue of piglets from the 3rd to 4th week, suggesting that ingestion of taurine may be a prerequisite for brain development before full accomplishment of enzymatic capacity for synthesizing taurine by themselves. Thus, taurine may be specifically needed by piglets for their normal growth and development in their early life.

The objectives of the present study were to examine the effect of a low taurine or taurine-supplemented milk replacer on the digestive functions of newborn miniature piglets.

Materials and Methods

Colostrum and milk samples were collected from Shiba Goats, Japanese Black Cows, a Thoroughbred Horse and Holstein Cows for determining concentrations of taurine. Colostrum samples were collected on the day of parturition. All of them delivered normal offspring in the Experimental Station for Bio-animal Science of the University of Tokyo.

Newborn miniature piglets were used. Their origins are described in Chapter II. Eighteen piglets born from 8 different dams were separated from their mothers soon after birth before they suckled the colostrum, and were raised in a warm environment (24~29℃). As piglets in natural condition usually suckle their dam's teats for less than 30 min at about 2 hr intervals at their early age, they were bottle-fed at 2 hr intervals on the day of birth and the following day, allowing to access a bottle for 2~3 min once every 2 hr. The bottle contained excess amount of the replacer. The replacer was fresh milk from cows at a middle of lactational period that contained 0.0025% taurine. Ten piglets were nursed with the replacer supplemented with 0.5% (40 mmol/L) taurine, and the rest of 8 piglets were nursed with the replacer without taurine supplementation.

The time between birth and the first excretion of meconium was measured for each group and the frequency of defecation within a day was also estimated from on days 1 and 2 of suckling. The amounts of milk intake at each suckling from days 1 to 5 of suckling (day 1 is the day of birth) were determined by the weight gain of piglets during the nursing period. The milk intake record was omitted when piglets urinated or defecated during the nursing period. The numbers of animals used for obtaining this result are presented in Fig. 3-2.

Other 8 newborn piglets from 4 different dams were divided in two groups (4 animals for each group) and bottle-fed for 2 days after birth and then, in the following days nursed by the auto-nursing device described in Chapter II with the replacer with or without taurine supplement (5%).

Body weight was measured once a day in normal piglets (n=9) nursed by their dams, as well as the piglets of the experimental groups.

Urine samples were collected from a piglet of each group on day 10 of suckling for measuring free amino acids, ammonia and urea by HPLC as described in Chapter II. The samples were deproteinized by 6% TCA (trichloroacetic acid) solution and diluted 40 times by the same solution for determining the free amino acids in the blood samples.

Hair growth of piglets of the 2 groups was compared macroscopically from birth to day 14 of suckling, and hair samples microscopically collected on day 14 of suckling.

Results

As shown in Table 3-1, the concentration of free taurine was rich in the colostrum in all species of domestic animals examined. It is noteworthy that only in the sow's milk, high concentrations of taurine were maintained even when lactational period progressed.

On day 10 of suckling, the piglets nursed with the replacer supplemented with 0.5% taurine (the taurine group) excreted higher concentration of taurine and urea into the urine. However, in the piglets nursed with the replacer without taurine supplementation (the non-taurine group), the concentration of free taurine was too low to be detected in the samples diluted 40-fold (Fig. 3-1).

On days 1 and 2 of suckling (bottle-fed period), the two groups consumed almost same amounts of the replacer. From day 3 or later, the taurine group became to take more the replacer than the non-taurine group (Fig. 3-2). The weight gain of the former group exceeded the latter, but weight gain of both groups were lower than that of piglets naturally nursed by their dams (Fig. 3-3).

The average length of time for the excretion of the first meconium was significantly faster ($p<0.05$) in the taurine group than the non-taurine group (Table 3-2). The frequency of defecation per day on days 1 and 2 of suckling is presented in Fig. 3-4. The frequency was always higher in the taurine group than the non-taurine group. The differences were significant ($p<0.05$) on days 1 and 2 (Fig. 3-4). The piglets in the non-taurine group (fed with only fresh cow milk) appeared to have a trouble in defecation due to the stickiness of feces excreted in a form of like milk curd (Fig. 3-5).

The growth rates were slower in the non-taurine group than in the taurine group. Both of the artificially raised piglets were slower in body growth than the dam-nursed piglets during a 7-days nursing period (Fig. 3-3).

Retardation of hair growth was frequently observed in piglets of the non-taurine group. Hair samples collected at day 14 of suckling showed that some parts of the hair became thin and bent, and rough on the surface (Fig. 3-6).

Discussion

The taurine concentration in sow's colostrum and milk was estimated as approximately 1 mmol/L or 0.0125%. (See Table 3-1). Thus, nursing piglets with cow milk supplemented with 0.5% taurine (the taurine group) will result in highly excessive ingestion of taurine by piglets. But much larger amounts of taurine itself and urea were excreted into the urine in a piglet of the taurine group on day 10 of suckling, suggesting that majority of ingested taurine was absorbed and excreted either directly or after catabolism. The maximal ingestion of taurine was estimated as 2 g, and an excreted amount was estimated as approximately 1.5 g per day, if the increased excretion of taurine and urea into urine was solely resulted from the taurine absorbed. Thus, a major part of taurine ingested should be absorbed and excreted through urination.

It was reported in human, that urinary taurine was significantly lower in infants fed with a lower taurine milk replacer, when compared to infants fed with human milk (Jarvenpaa et al., 1983c; Rassin et al., 1983). Similar phenomena were observed in monkey at 3 months of age (Sturman, 1988). This decrease is restored with supplementation of taurine (Rassin et al., 1981). Therefore, the kidney may work to maintain taurine pools in the way of eliminating the overload taurine and reabsorbing taurine in the deficiency of exogenous taurine.

Piglets nursed with cow milk without taurine supplementation (the non-taurine group) had a poorer appetite than the piglets of the taurine group. The poor appetite may be coupled with prolonged passage time of meconium and feces, and the excrete became sticky. This symptom seemed similar to so called milk curd syndrome in human medical practice that is an intestinal obstruction in neonates as yet little known. Infants with milk curd syndrome have a normal discharge of meconium, and the intestinal obstruction appears usually between the 2nd and 16th day after birth. In most of the cases, the milk curd was found in the distal part of the ileum. The etiology of the milk curd obstruction syndrome remains still unclear. It was supposed that the composition of artificial milk formula in connection with transitory absorption deficiency of amino acids, and the possibility of a temporarily insufficient excretion of bile acids might affect the milk digestion (Pochon and Stauffer, 1978).

Bile acids are detergents because they contain both lipophilic and hydrophilic regions, the latter being hydroxy, sulfate, sulfonate or carboxylate, they serve to solubilize or emulsify fats to make them more accessible for digestion. Bile salts are derivatives of cholesterol. In all vertebrates except for mammals, taurine is the sole amino acid conjugated with cholesterol derivatives to form bile salts. Taurine conjugates are quantitatively the major metabolites of taurine formed in vertebrates, although mammals use glycine in addition to taurine (Haslewood 1978). Under normal conditions, the most common bile salts are either taurocholate or glycocholate, the latter being found only in placental animals. Taurocholate is the more efficient bile salts, as the acidity of the sulfonate function means it remains ionized even under the highly acid conditions that episodically occur in the upper intestine (Hofmann and Small 1967). Carnivores tend to be exclusive taurine conjugators of choleic acids. Herbivores and omnivores tend to be both taurine and glycine conjugators.

Pretreatment for 3 days with taurine 0.5% in drinking water led to a 70% increase of the secretory maximum for chenodeoxycholic acid. Analysis of the bile acids after supplemental taurine demonstrated a large increment of tauroconjugates (Belli et al 1988). There was a linear correlation between fat absorption and duodenal bile acids in the formula fed infants (Jarvevnpaa, 1983 a, b, c). Taurine supplementation improved fat absorption, especially of saturated fatty acids, in preterm infants (Galeano et al., 1987). Jackson et al. (1993) reported that there were differences in bile acid metabolism between breast- and formula-fed infant baboons. Infants fed with human milk passed a great number and softer stool than those who received cows' milk formula (Weaver and Lucas 1993). Cows' milk contained less free amino acids especially lower taurine than human milk, and higher concentration of saturated fatty acids which is lower solubility in water. These results suggest that taurine deficiency and high saturated fatty in cows' milk may cause incomplete digestion of fatty acids in cows' milk feeding piglets or cause the milk curd syndrome in human infants.

This study suggests that feeding on a low taurine formula to the colostrum-deprived piglets may cause the malgrowth of hair. Taurine is synthesized from the sulfur-containing amino acids methionine and cysteine, and thus they are essential components of the diet (Huxtable, 1986). Cysteine residues provide covalent cross-links between the adjacent polypeptides of hair, so that the structure of hair can be affected by the content of cysteine (Albert, 1982). The change of the hair shape in the non-taurine group in this study may be caused by insufficient cysteine which were used to produce taurine to cope with the body needs.

On the other hand, Watanabe et al. (1995) reported that the injected radiolabeled taurine was present in a free form within the skin tissue over the epidermis and external root sheaths near the opening of the hair follicles. The external root sheath just below the insertion of the sebaceous glands and the peripheral part of the sebaceous glands showed a high taurine distribution. Whether taurine can directly affect the formation of hair is still unknown, but this will be another possibility for the malformation of hairs in the non-taurine group piglets in this study.

In conclusion, taurine which the richest free amino acid in colostrum suggested to play important role in the early development and proper function of digestive system of newborn piglets. Taurine deficiency in formula may be the pathogeny of milk curd syndrome. The colostrum deprived miniature piglets may be used as a model of low birth weight infants to study the effect of dietary nutrition.

Tab. 3-1. Concentration of free taurine in defatted milk of various domestic animals

Species	Taurine in defatted milk, μmol/L		
	Less than 3days after birth	On 7 days after birth	More than 30 days after birth
Goat	846 ±303(6)	348 ±11(6)	264 ±83(6)
Japan Black Cow	576 ±153(2)	281 ±23(2)	73 ±25(2)
Horse	241(1)		
Cow	988 ±267(6)	436 ±50(6)	209 ±38(6)
Sow	995 ±487(8)	1186 ±108(8)	1202 ±267(8)

Datas adapted from unpublished data that determined by the author.

Number in parentheses are number of dams used.

Tab. 3-2. The average length of time for the excretion of the first meconium in artificially nursing miniature piglets

Treatment	Average±SE
Taurine group	8.07±1.43* (n=7)
Non taurine group	17.07±3.84 (n=7)

*Significantly shorter than none taurine group ($p<0.05$)

Animals were deprived with colostrum after birth and fed with cow milk with (Taurine group) taurine supplemented in cincentration of 0.5% or without supplementation (non taurine group) in the milk.

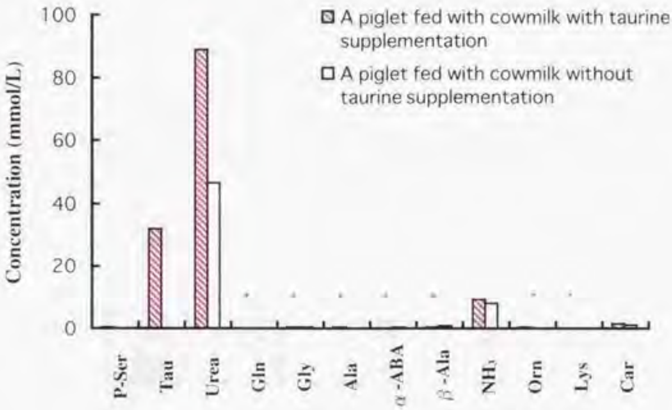


Fig. 3-1.

Concentrations of free amino acids, urea and ammonia in urine samples of artificially nursing miniature piglets on day 10 of suckling. They were fed with cow milk after birth with or without taurine supplementation.

* : too low to be detectable.

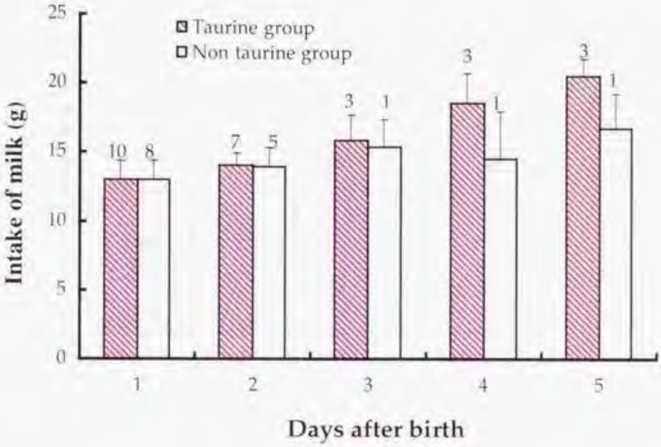


Fig. 3-2.

Changes in milk intake at each suckling period in artificially nursing miniature piglets. Taurine group was fed cow milk supplemented with taurine (0.5%). Non taurine group was fed with cow milk without the supplement. Estimations were made 5 times a day from individual (s) survived. Number above each column is number of piglets arbitrary chosen for the estimation.

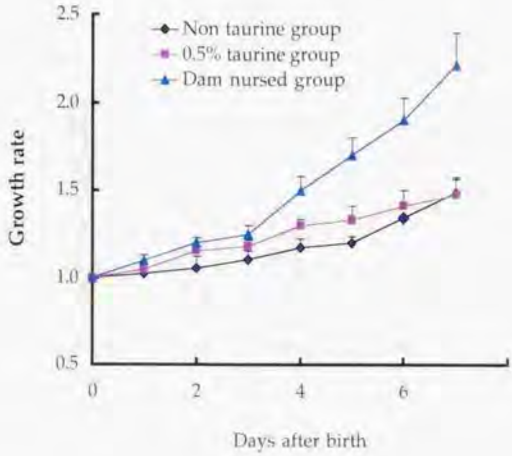


Fig. 3-3.
The growth rate curves of piglets nursed artificially (Non taurine and 0.5% taurine groups) or naturally Dam nursed group). The none taurine group piglets (n=4) were fed with fresh cow milk, the 0.5% taurine group were nursed with fresh cow milk supplemented 0.5% taurine. (Growth rate = body weight/birth weight)

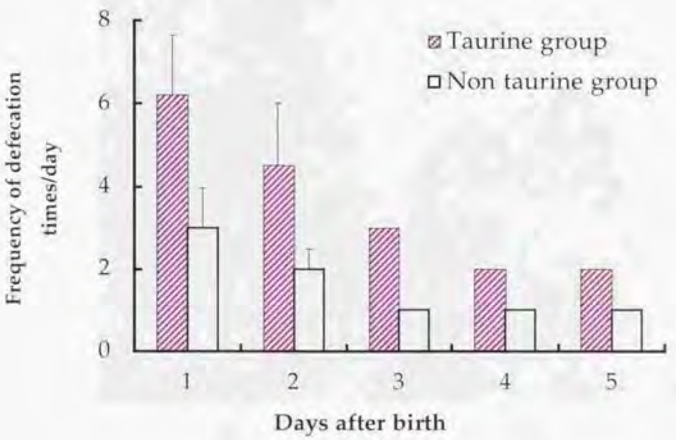


Fig.3-4.

Change in the frequency of defecation per day in artificially nursing piglets fed with cow milk supplemented with 0.5% taurine (Taurine group) or not (Non-taurine group). Values are mean±SE (n=5 for each group for 1 and 2 days after birth, and n=1 each for 4-5 days after birth). Significant difference (P<0.05) on 1 and 2 days after birth.

Fig. 3-5.

Effects of taurine supplementation on the feces of piglets artificial nursing miniature. Feces of none taurine supplemented piglets (A) were harder than those of taurine supplemented piglet (B), and contained undigested milk cores.

Fig. 3-6.
Effects of taurine supplementation on the growth of hair of artificially nursing miniature piglets. Hair samples were collected from the shoulder area on the 14 days after birth. The hair of none taurine supplemented piglets (A) is thin and has bents, but the hair of taurine supplemented piglet (B) grows straight.

Chapter IV

Effects of Free Glutamine in Milk on New Born Piglets

Abstract

During lactational period, glutamine is noticeably increased to the highest concentration of free amino acids in sows' milk. Significance of this amino acid on the early development of intestinal structure of newborn piglets was investigated. The newborn miniature piglets were deprived of their dam's colostrum and nursed on fresh cow milk replacer by an auto feeding device. The piglets from the same litter were divided into two treatments, one group suckled the replacer supplemented with 0.5% taurine only (group A); and other group suckled the replacer supplemented with 0.5% taurine and 0.5% glutamine (group B). The piglets were scored for diarrhea every day and the sections of the small intestine from piglets from each group was histologically examined 14 days after birth. The piglets that were fed with the replacer without glutamine supplement were frequently suffered from diarrhea. The villas in small intestine were higher in the height in group A than in group B. The intraepithelial lymphatic tissue were more developed in group A than in group B. It suggesting that glutamine may be functioning from early life for the normal development of intestinal structure.

Introduction

The studies in the previous chapters have shown that glutamine is one of the most abundant free amino acids in the sows' milk in accordance with several reports (Wu and Knabe, 1994; Toshiyuki and Noboru, 1995). Glutamine is also in the highest concentration in plasma. Such a predominance of glutamine is consistent with its versatile role as an essential precursor for the synthesis of molecules such as proteins, purine and pyrimidine nucleotides, NAD+, and amino sugars, and as a major fuel for rapidly dividing cells (Krebs, 1980).

The small intestine of the postabsorptive adults rat extracts 25-33% of arterial glutamine in a single pass, which accounts for 30% of whole-body glutamine utilization (Windmuller, 1982). Intraluminally delivered glutamate and glutamine are metabolized extensively by the small intestine mucous; 66 and 98% of luminal glutamine and glutamate is catabolized in a single pass by rat jejunum, respectively (Windmuller and Spaeth, 1975; 1976). Similarly, 96 and 95 % of enterally delivered glutamate is extracted in the first pass by the human splanchnic bed (Battezzati et al., 1995) and by porcine portal drained viscera (mainly intestine) (Reeds et al., 1996). It is also reported that most glutamine and almost all glutamate in the diet do not enter the portal circulation and are not available to extra intestinal tissues (Battezzati et al., 1995; Stoll et al., 1998). Indeed, the small intestine is a major organ of glutamine utilization in mammals in the postabsorptive state (Windmuller and Spaeth, 1980; Wu and Knabe, 1994) and converts glutamine into citrulline for endogenous synthesis of arginine (Wu and Knabe, 1995). The latter is an essential amino acid for young and weanling mammals (Visek, 1984) including pigs (Mertz et al., 1952).

Recent research has shown that the supplement of glutamine to the traditional glutamine-free total parenteral nutrition solution prevents intestinal atrophy in humans and rats under catabolic conditions (Buchman et al.,1995; Li et al., 1994; Schroder et al., 1995; Souba et al., 1990; Tamada et al., 1992). In addition, intestinal intraepithelial lymphocyte utilizes glutamine as an important energy source and requires glutamine for proliferation (Dugan et al., 1994; Wu et al., 1996).

In sows' milk an increase in free amino acids (including glutamine) takes place in the early period of lactation. It may be presumed that glutamine will be needed by piglets in the early period of life. The objective of the study in this chapter was to determine the effects of dietary supplementation of glutamine on the growth and development of small intestine in colostrum deprived newborn miniature piglets.

Materials and Methods

Animals and feeding

Four newborn miniature piglets from four sows were nursed artificially. They were deprived of colostrum by separating from their dams soon after birth, and fed by a bottle (training for the artificial teats for 1~2 days after birth) and then fed by the auto feeding device described in Chapter II. The formula was fresh cows' milk with supplementation of 0.5% taurine in the group A, and 0.5% of taurine and 0.5% of glutamine in the group B. The milk were fed for 2.5 min at an hourly interval for 12 days without warming up before feeding and thus, the temperature of milk was 10℃ (stress-feeding). The volume of diet was increased from 12 ml/hr at the third day to 25 ml/hr at the 14th day when this study finished.

The appearance of feces and frequency of defecation were observed and scored daily based on viscosity of feces (0, no diarrhea; 1, stiff flowing feces; 2, easy flowing feces; 3, watery diarrhea).

Intestinal sampling and histology

The piglets were killed on 14 days after birth. Pigs were anesthetized by intramuscular injections of atropine and ketamine at doses of 0.05 and 4.76 mg/kg body wt. After the intestine was removed, the intestine was immediately placed in physiological saline solution and the mesenteric web was removed, allowing the intestine to be lain straight. Samples were placed on a smooth surface in six parts of equal length. This arrangement allowed collection of tissue at seven equidistant points along the length of the small intestine from the duodenum (segment 1) and proximal jejunum (segment 2) to distal jejunum (segment 6) and distal ileum (segment 7) (Cranwell and Moughan 1989). Thus regardless of animal size, tissues were collected from the same relative site along the small intestine. Intestinal content was removed from tissue samples with mild saline washes. Tissue samples (~3 cm) from each segment were weighed and immersed in Bound's Solution. The fixed samples were processed by the standard paraffin method. Sections ($5\mu M$ thickness) were cut and stained with hematoxylin and eosin. Measurements of villous height and crypt depth were made on twenty to fifty well-orientated villa using an ocular micrometer under the microscope of 10×10 condition.

Results

Animal observations

Body weight of the piglets in the two group increased slowly at the first 2 days. During this stage, piglets were trained to fit the teats and the cows' milk based diet. Diarrhea occurred in some of the pigs at this stage. When the piglets began to suckle the milk from the auto feeding device, they appeared to live a regular living style of eating-sleeping-eating. In the piglets in the group B with glutamine supplementation, diarrhea was alleviated and the body weight increased linearly. But in the piglets of the group A without glutamine supplementation, severe diarrhea continued to occur (Figs.4-1, 4-2).

Intestinal morphometry

The piglets that nursed with the formula with 0.5% glutamine supplementation had a well-developed intestinal structure. In the duodenum, many well-developed glandulae duodenales could be fined in the glutamine supplemented group B, but less in the group A (Fig.4-3). In the piglets of group A, the villi of jejunum were in good shaped where many crumples were observed on the out side surface of the villa (Fig. 4-4).

One of the most noticeable differences was that the gut intraepithelial lymphatic tissue in the ileum were much better developed in the group B than in the group A. The lymphatic noduli occupied big areas with clear boundaries among the noduli (Fig 4-5).

The heights of villi and crypt depth in the intestine of different parts were shown in the Tab. 4-1. The average villous height in the small intestine was significantly higher in group B than in group A.

Discussion

The miniature pig offers several advantages for determining the effect of dietary composition on intestinal morphology in human clinical nutrition. First, pig has a gastrointestinal tract structure similar to that of human and has been widely used as an animal model for studying human intestinal growth and parental nutrition (Burrin et al., 1990; Reeds et al., 1993). The miniature pig is small in body size, so that is easy for handling in laboratory condition, and the intestine exhibits a relatively short postnatal period of maturation (Klein and McKenzie, 1983; Moughhan and Rowan, 1989), and its structure is easily affected by weaning or dietary nutrition (Dunsford et al., 1989; Hampson, 1986; Henworthy, 1976).

Glutamine is an abundant free amino acid in the plasma of animals (Hamilton, 1945; Wu et al., 1994) and in sows' milk (Wu and Knabe, 1994). Glutamine has been demonstrated to be a major energy source for the adult rat small intestine (Windmueller and Spaeth, 1980), and it role as a fuel and a precursor for amino acid synthesis in the gut of neonatal pig (Wu et al., 1995). Glutamine is hydrolyzed to glutamate and ammonia at acidic pH (Krebs, 1980). However, Wu (1996) reported that dietary glutamine was not subject to measurable acid hydrolysis in the stomach and upper parts of duodenum. It means that dietary supplementation of free glutamine was indeed available for the small intestine for metabolic utilization. Several reports confirmed that the level of dietary nutritional effect on the growth and integration of the small intestine after weaning (Pluske et al., 1996; Burrin et al., 1991).

According to the previous reports mentioned above and described in Introduction of this chapter, it can rationally expected that a gradual increase in free glutamine concentration in sow's milk does have some favorable effects on the function and development of the intestine. Indeed, the villi of the intestine of piglets in the group B supplemented with glutamine were tall and well shaped with a large surface area due to the presence of many crumples. These phenotypes may be resulted from supplementation of glutamine in the cow milk.

The intestine, traditionally viewed as an organ of digestion and absorption of nutrients, maintains an indispensable immunological function. The intestinal mucous has a unique interface with microbial and chemical environment in which it must protect the internal milieu from potentially hostile pathogens. To that end, the intestinal immune system has developed into a complex organ known as the gut-associated lymphatic tissue. As compare with better known immune organ, such as the reticuloendothelial system, liver and spleen, the gut is the largest immune organs in the body (John, 1990). In this study, lymphoid tissue of the glutamine supplemented piglets developed so well indicates that glutamine of the milk may be used by the immune tissue in the early period of life. In contrast, the piglets fed with low concentration of glutamine took longer time to recover from diarrhea. This symptom should be coupled with the atrophic changes in the intestinal structure.

Diarrhea in piglets is one of the major problem in pig production. It will not be realistic that the milk secreted from every teat simultaneously decreases to an extremely low glutamine concentration. A more realistic situation will be as follows; the number of the teats that secretes milk with sufficient glutamine concentration depends on sow's general nutritional condition. If the dam is in a malnutritional condition, a lager number of offspring forced to suckle the teat supplying insufficient concentration of glutamine. Thus, more piglets will be suffered from diarrhea and have higher risk for survival. However, even in this kind of situation, pig has a good scheme to make heavier or stronger piglets be able to survive selectively by means of introducing uncompromising competition among a litter to seek for the teat with better milk yield and higher glutamine concentration in milk. This "teat order" paradigm will allow the pig, which is a very particular omnivorous species with a large body size and a large number of litter, still to exist on the earth.

Further studies must be needed particularly to analyze the involvement of other amino acids such as glutamate and glycine as a cue for teat selection. Future study on the change in sows' milk composition in different nutritional condition of the dam will be helpful for understanding the mechanism of occurrence of diarrhea in suckling piglets. It is author's unsolved question whether the supplementation of glutamine to the dam can reduce the mortality of piglets.

Tab. 4-1. Villous height and crypt depth of piglets in group A and B

	Position	Group A	Group B	Significance
Villos height(μm)		mean±ES	mean±ES	
	Duodenum	658±13.5	767±25.6	**
	Jejunum	578.2±17.6	813.6±12.2	***
	Ileum	388.1±14.6	534.4±12.7	***
Crypt depth(μm)				
	Duodenum	132.0±13	212.0±14.7	**
	Jejunum	99.1±1.8	130.0±10.4	*

*: p<0.05

**: p<0.01

***: p<0.001

Group A: supplemented taurine(0.5%)

Group B: supplemented taurine(0.5%) and glutamine(0.5%)

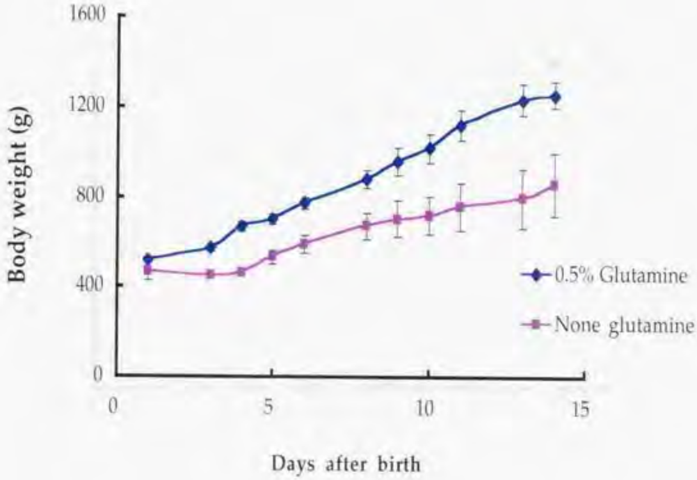


Fig.4-1.

Growth curve of artificially nursing miniature piglets. A received cow milk supplemented only with 0.5% taurine. Group B received cow milk supplemented with glutamine and 0.5% taurine, and group. Values are the mean ± S.E.. of 5 piglets for each group.

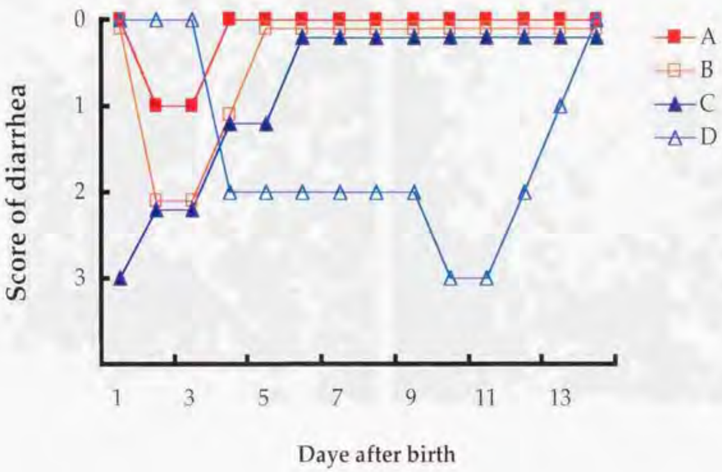


Fig.4-2.

Daily scores of degree of diarrhea in artificially nursing miniature piglets. They were fed with cow milk with a taurine supplementation after birth with (A, B) or without (C, D) an additional glutamine supplementation (0.5%). Diarrhea score: 0; no diarrhea, 1; stiff flowing, 2; easy flowing feces, 3; severe, watery diarrhea.

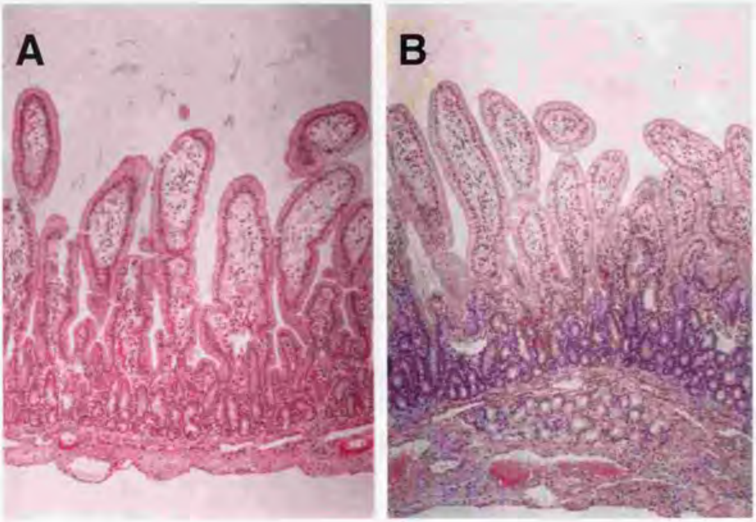


Fig. 4-3.

The section of the duodenum of artificially nursing miniature piglets 14 days after birth. They were fed with cow milk with a taurine supplementation after birth without (A) or with (B) an additional supplementation of glutamine (0.5%).

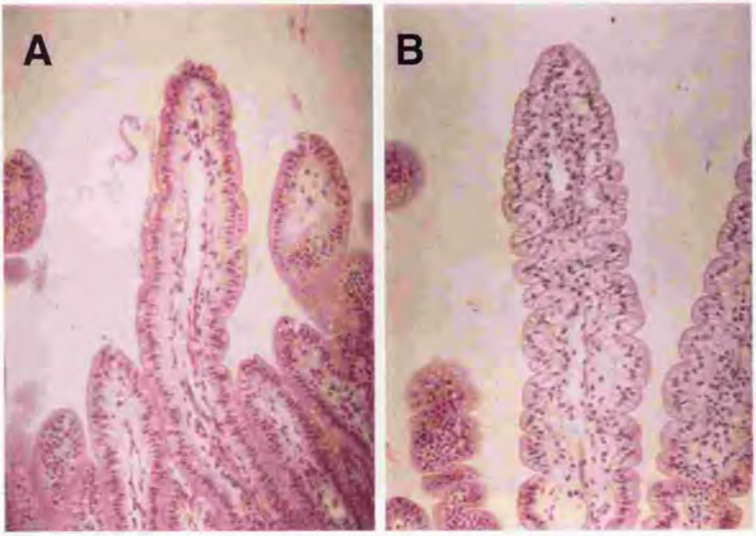


Fig. 4-4.

The sections of the villi in the jejunum of artificially nursing miniature piglets 14 days after birth. They were fed with cow milk with a taurine supplementation after birth without (A) or with (B) an additional supplementation of glutamine (0.5%).

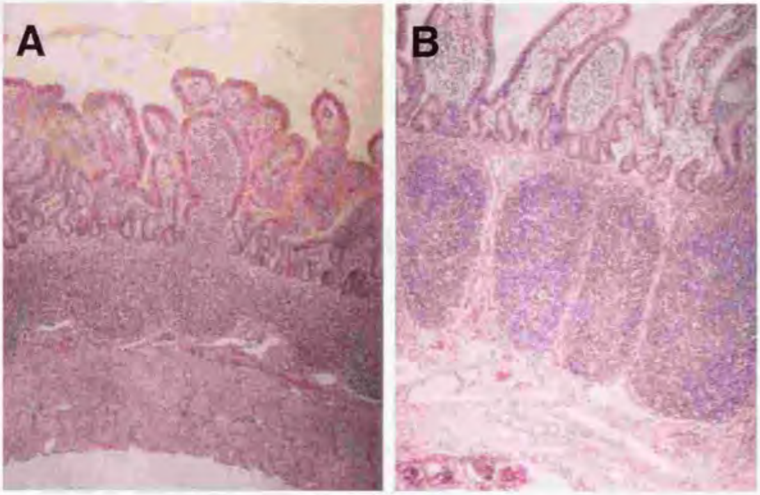


Fig. 4-5

The section of the ileum of artificially nursing miniature piglets 14 days after birth. They were fed with cow milk with a taurine supplementation after birth without (A) or with (B) an additional supplementation of glutamine (0.5%).

General discussion

The pig is most productive among domestic animals, and grows fast with a high efficiency of food consumption. Frequent occurrence of diarrhea in piglets is one of the serious economical problems in pig industry, the prevention of this disease has long been a target of veterinary science. The pig has very particular nursing behavior, which motivated the author to correlate this nursing behavior with the occurrence of diarrhea in piglets. In deal with this problem, the author tried to introduce a new aspect, i.e. free amino acids in sow's milk. Furthermore, because the pig has many anatomical and physiological similarities with human, it has been recognized that the pig is one of ideal animal models for human medical and nutritional research. The study in pigs to analyze the relation between the occurrence of diarrhea and free amino acids in the milk will present suggestive information for the nutrition of human neonates receiving formulated milk.

Neonates do not have well-developed digestive and regulatory systems to adapt their food and environment, and yet they are stressfully challenged by a new environment at birth. Because newborn mammals do not normally consume solid foods, their mother's milk is the only source of exogenous amino acids for the synthesis of proteins, neurotransmitters, hormones, polyamines, purine and pyrimidine nucleotides, creatine, carnitine, porphyrins and other biologically important molecules. Some amino acids, such as glycine, histidine and taurine, are effective scavengers of free radicals and therefore may help to prevent or alleviate potential injury. Thus, milk plays a vital role in the survival and growth of mammalian neonates.

The concentration of free amino acids in milk is different between species and changes during the lactation period. It is known that the colostrum contains high activities of protease inhibitors, which limit the breakdown of intake protein. Although the presence of protease inhibitors in colostrum has important immunological implications, these inhibitors minimize the potential of colostrum proteins to be digested and becomes a source of free amino acids for neonates. Thus, free amino acids in colostrum have been suggested to be an important source of free amino acids for suckling animals. Furthermore, milk is an important source of taurine, a β-amino acid that is virtually absent in the foods of plant origin.

The pig as a multiple pregnancy species has its special characteristics in reproductive process and nursing behavior. In sows, the mammary gland produces the colostrum prior to (1 to several hours) the parturition, and secretes it continuously during the early 2 or 3 days of lactation, and then the secretion ceases. After that, the normal milk is ejected for 10-20 seconds at one episode and the episode recurrs at 30-90 minutes intervals. It has been well known that the piglets born in different body weight and their postnatal growth are significantly different among the littermates. The teat order behavior is the phenomena that observed only in pig. The big piglets seem to have a benefit from the fixed position of the teat.

This study was designed to identify the distribution of free amino acids in each teat during the lactation and the relationship between the growth of piglets and the concentration of free amino acids in the sows' milk.

In chapter I the author dealt with the concentrations of free amino acids in the colostrum and milk which were obtained from individual teats of sow dams during the lactation period. Observations were done to certify the teat-order of the piglets in the lactation period. Taurine was the most abundant free amino acids in the colostrum and maintained at a high level (1-2 mml/L of defatted milk) throughout a lactational period. Other free amino acids such as glutamine were in trace amounts in colostrum. The concentration of glutamine increased to the greatest extent among all other free amino acids examined during a 25 days of lactational period, and became the most abundant free amino acids in milk (2 mmol/L of defatted milk). Free glycine and glutamic acid also increased to a large extent (0.7-1.2 mmol/L of defatted milk).

Immediately after farrowing, no significant differences in the concentrations of each free amino acid were observed among colostrum samples obtained from different teats. However, the concentrations of free amino acids in milk from different teats became significant different as lactational period progressed. As for the milk samples obtained from individual teats, there were significantly positive correlations between the total free amino acids, and free glutamine, glutamic acid and glycine concentrations. There were also significantly positive correlations between the body weight of each piglet, and milk yield of each teat and the total free amino acids concentration in milk obtained from each teat. Cumulatively, a larger piglet suckled a higher milk yield teat from which higher concentrations of total free amino acids, glutamine, glutamic acid and glycine were supplied.

In chapter II, the effect of glutamine supplementation on the teat nursing behavior in piglets artificially fed was analyzed. The author hypothesized that the piglets can distinguish the difference of the concentration of free amino acid by taste sensory. In this study miniature pigs were used. One of the reasons for choosing miniature pigs rather than commercial pigs is that the nursing behavior of the pig is expected to be expressed more prototypically in miniature pigs. In this study, an auto feeding device (autosow) was assembled and used for feeding the newborn miniature piglets. The newborn miniature piglets were reared on the autosow individually with the cow's milk for two weeks. Then, they were transferred into a new environment where 4 of the piglets could access to any of the 4 teats. Two teats ejected the milk supplemented with 0.25% and 0.5% glutamine, respectively and the other two teats ejected the milk without supplementation. The sequence of behavioral events leading to the formation of teat nursing order was described. At the first 1~3 hours, the piglets showed the similar style of teat nursing behavior like which classified as three stages of "teat seeking", "teat sampling" and "teat defense". Finally, the heaviest piglet selected the teat ejecting 0.5% glutamine and the second heaviest selected one ejecting 0.25% glutamine. The lightest two selected the teats ejecting the milk without glutamine supplementation. This result implies that piglets can distinguish different concentrations of glutamine and prefers to higher concentration. To the best of the author's knowledge, this is the first experiment that testifies the role of taste sensory in the teat nursing behavior. It is suggested that the predominant free glutamine and other free amino acids in sows' milk may be tasted by piglets as an indicator of developed mammary gland.

Chapter III and IV were designed to investigate the effects of supplementation of taurine and glutamine on the physiology of newborn miniature piglets. The newborn piglets were deprived of their dam's colostrum and nursed with fresh cow milk. In the first treatment, the piglets from different litters were divided in two groups, one group suckled the replacer supplemented with 0.5% taurine, and the other group suckled the replacer without taurine supplementation (mean taurine concentration of the replacer is approximately 200 μmol/L or 0.0025%). The first meconium was excreted significantly earlier ($p < 0.05$) in the taurine supplemented group than in the no supplemented group. Moreover, frequency of defecation at the day of birth and the following day was significantly higher ($p<0.05$) in the former group. Intestinal obstruction like the milk curd syndrome that happens in formula feeding infants occurred in the group without taurine supplementation. These results suggest that taurine deficiency and high saturated fatty acids in cows' milk may cause incomplete digestion of fatty acids in cows' milk feeding piglets or cause the milk curd-like syndrome in human infants. Another observation is that the piglets without taurine supplementation were later found to be affected with retardation of hair growth. Whether taurine can directly affect the formation of hair is unknown.

In the second treatment the piglets from the same litter were divided into two treatments, one group suckled the replacer added with 0.5% glutamine and 0.5% taurine; and other group suckled the replacer supplemented 0.5% taurine only. The piglets were scored for diarrhea every day and various parts of the small intestine from each pig was histologically examined 14 days after birth. The piglets receiving the replacer without glutamine supplementation were easy to be affected with diarrhea. The villi of the small intestine were higher in height in the glutamine supplemented group. The submucosal lymphatic tissue was well-developed in the glutamine supplemented group. These results indicate that glutamine may be functioning on the intestine development, such as epithelial proliferation and submucosal lymphatic tissue development from early life.

It was shown in this study that sow's udder secrete the milk rich in free amino acids which are different in the concentration among the teats and the stage of lactation period. Free amino acids such as glutamine, glycine and glutamate may play important roles not only as nutrients but also as a signal for determining teat nursing behavior. There are two clues suggesting that some of free amino acids operate as an indicator of the development of the mammary gland. The first, these amino acids seems to be effective to stimulate the taste organ of piglets. The second, concentrations of these amino acids change according to the developmental stages, and the more the mammary gland develops, the more free amino acids are secreted into the milk. In this study, the piglets appeared to prefer to the glutamine-supplemented milk. If piglets could distinguish the difference in glutamine concentration among the teats, they will be rewarded by nutritionally and energetically rich milk for growth and development.

In conclusion, the free amino acids in porcine milk may play important roles in several aspects of breeding of the piglets by effecting on the development of digestive system, immune system and the teat nursing behavior. Further studies on the function of the free amino acids in porcine milk will be helpful to understand the mysterious phenomena of teat order and to develop a practical measure to prevent the occurrence of the piglet diarrhea.

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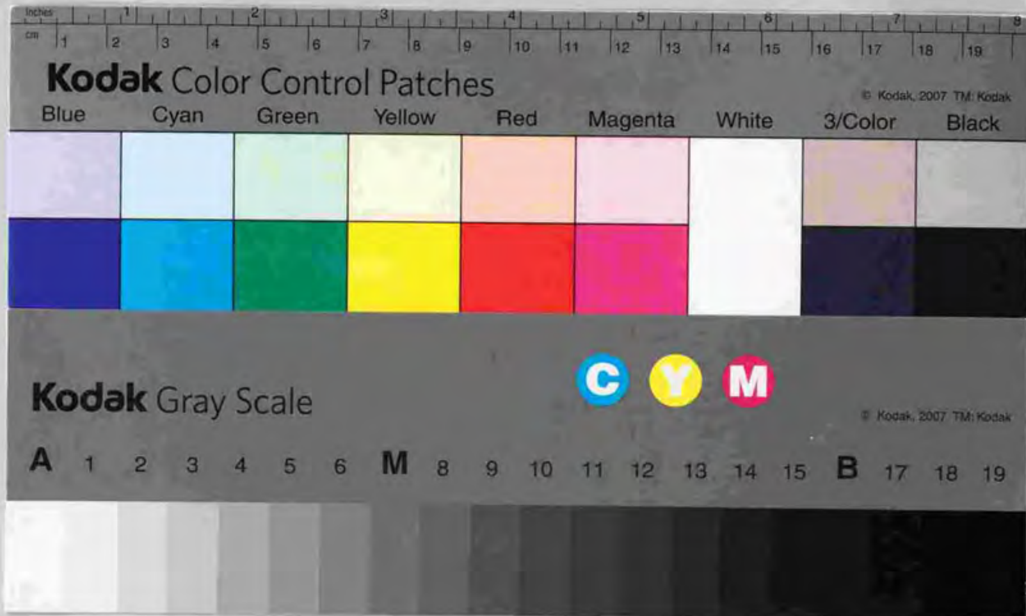
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