The Effect of Complementary Access to Milk Replacer to Piglets on the Activity of Brush Border Enzymes in the Piglet Small Intestine

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ABSTRACT : The activity of brush border enzymes (sucrase, lactase and maltase) in the piglet small intestine was evaluated as well as piglet performance during the weaning period in the present study. There were two treatment groups: Piglets of six litters were fed dry feed plus milk replacer (Group M) and of six litters fed dry pelleted feed (Group C). One piglet from each litter was sacrificed on day 3 before weaning, and day 3, 10 and 17 postweaning, respectively. Providing milk replacer caused an increased piglet live weight at weaning ($p<0.001$) and until termination of the experiment ($p<0.001$). A slightly higher ($p<0.16$) level of protein was measured in the jejunum of group M piglets as compared with group C piglets. Before weaning the activity of lactase was high in the jejunum of group C piglets. The activity of lactase in the jejunum was lowered in the jejunum of group C piglets and in distal jejunum of group M piglets during the postweaning period as compared with pre-weaning period ($p<0.05$). Lowered activity of lactase in the distal jejunum of piglets was found at day 10 and 17 postweaning, respectively. No treatment differences were found in the activity of lactase in the piglet jejunum. No treatment differences were seen in the activity of maltase and sucrase in the piglet jejunum also. However, weaning caused a higher activity of sucrase in the distal jejunum of group M piglets as compared with pre-weaning period. In conclusion, providing milk replacer to piglets caused an improved growth performance. Feeding milk replacer did not influence the activity of lactase, maltase and sucrase in the jejunum of piglets. Weaning resulted in a markedly lowered activity of lactase, while no dramatic changes in the activity of maltase took place during the period around weaning. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1617-1622)***

Key Words : Lactase, Maltase, Sucrase, Performance, Piglets

INTRODUCTION

The mucosal epithelium in the neonatal pig small intestine is regarded as anatomically and functionally immature, and there are dramatic changes in function at weaning (Hampson, 1986a; Pluske et al., 1996a). At weaning the piglets experience a number of stresses such as being separated from the sow, being moved and being offered an alien feed type and form. Numerous studies have shown that weaning can cause morphological changes in the piglet small intestine, and these changes including villous atrophy, increased crypt depth, reduced disaccharidase concentration and reduced absorption (Kenworthy, 1976; Hampson, 1986a, b; Hampson and Smith, 1986; Miller et al., 1986; Dunsford et al., 1989; Kelly et al., 1991a, b; Pluske et al., 1996a, b) are generally associated with the poor performance observed as they are thought to result in a temporary decreased digestive and absorptive capacity of the small intestine. Major changes in villous architecture and reductions in specific enzyme activity are occurring in the piglet small intestine in the first one or two weeks following the immediate post-weaning period (Armstrong and Clawson, 1980). It is always interesting of attempting to alleviate these changes through various methods including dietary strategy. The introduction of creep feeding before weaning appears to have some ameliorative effects (Makinde et al., 1997). Creep feeding during the suckling period and enhancing the immune response of piglets by nutritional means might be beneficial to the pig health (Nabuurs and Hoogendoorn, 1993; Lien et al., 2005). More morphological changes in the jejunum than in the other intestinal segments were also reported by Koga and Kimura (1980) and Komai and Kimaru (1979). Previous study of piglets performed by Cera et al. (1988) indicated that the hydrolysis of feed constituents and absorption of nutrients should take place primarily in the mid-portion of the small intestine.

The present study was designed to test the effect of complementary access to milk replacer to piglets on the activity of brush border enzymes in the proximal and distal jejunum as well as piglet performance around the weaning period.

MATERIALS AND METHODS

This experiment was carried out at the animal experimental facility of the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences. This experiment was performed in accordance with the recommendations of *the Guide for the Care and*

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Table 1. Chemical composition (g/kg DM) of the pelleted feed and the milk replacer

	Pelleted feed	Milk replacer
Dry matter (%)	90.6	95.6
Crude protein (N×6.25)	153.3	209.2
HCl-fat	36.7	365.7
Ash	55.2	72.9
Gross energy (MJ/kg DM)	16.2	20.2
Lysine	10.1	ND[3]
Threonine	5.8	ND
Methionine	3.9	ND
Cystine+methionine	6.5	ND
Vitamin/mineral*	[1]	[2]

[1] Supplied (/kg feed): vitamin A: 5,000 IU; vitamin D_3 500 IU; vitamin E 80 mg; calcium 5.95 g; phosphorus 5.61 g; sodium 2.65 g; 0.35 mg Se as Na_2SeO_3; 25 mg Cu as $CuSO_4.5H_2O$.

[2] Supplied (/kg milk replacer): vitamin A 25,000 IU; vitamin D_3 3,000 IU; vitamin E 100 mg; vitamin B_1 8 mg; vitamin B_2 16 mg; vitamin B_6 5 mg; vitamin B_{12} 0.03 mg; vitamin C 100 mg; biotin 0.20 mg; niacin 48 mg; D-pantothenic acid 28 mg; Co 800 mg; folic acid 0.8 mg; calcium 5.95 g; phosphorus 5.61 g; sodium 2.65 g; Mg 1.1 g; 0.50 mg Se as Na_2SeO_3; 5.05 mg Cu as $CuSO_4.5H_2O$.

[3] Not determined.

Use of Laboratory Animals established by the Ethical Committee for Animal Experiments in Uppsala, Sweden.

There were two treatment groups: Piglets of six litters were fed dry pelleted feed plus milk replacer (Group M) and of six litters fed pelleted dry feed (Group C).

Animals, housing and feeding

Around 30 days before farrowing, twelve gestating sows (Swedish Landrace×Yorkshire) taken from the minimal disease pig farm at Västeras, were used to provide piglets. Sows were fed diet containing 15.3% crude protein (CP) and 15.8 MJ/kg DM gross energy (GE) twice daily (0800 and 1500). From gestation day 114 the sows' feed allowance was kept at 1.5-2.5 kg/d. After farrowing, feed allowance was increased by 400 g daily until reaching a maximum of 7.5-9.5 kg/d based on the number of piglets.

After farrowing, the piglets' teeth were clipped at the first day of age. Then each piglet was injected with 200 mg of iron dextran (URSOFERRAN®, i.m.) in a volume of 1 ml, containing Fe^{3-} 200 mg, NaCl 15 mg, phenol 5 mg at day 4 and 14 of age, respectively. Male piglets were castrated at day 4 of age. Piglets were not allowed access to the sow's diet. The sows and piglets were housed in an environmentally smooth-walled pen on conventional concrete floor with a room temperature of 18-20°C and with light from 0700 to 0900. On the floor straw was used as bedding and cleaned twice daily to maintain hygiene.

Piglets were given a standard dry pelleted feed (Table 1) manufactured by Skånska Lantmännen in Sweden *ad libtum* from day 22 of age until termination of the experiment. The C group piglets did not receive any milk replacer. Beyond the standard dry pelleted feed, piglets in group M were also offered free access to milk replacer.

The milk replacer (Table 1) manufactured by Skånska Lantmännen in Sweden was mixed with 40°C water, with a ratio of 130 grams of milk powder per liter water in the morning (0830) and afternoon (1500) everyday. The milk was provided to the piglets in self-drinkers (one self-drinker per litter). The amount of remaining milk in the drinkers was recorded, and the drinkers were thoroughly cleaned daily in the morning and afternoon everyday. New milk was provided in drinkers.

Postmortem procedure

All piglets were weaned at 34 d of age. Milk replacer was fed to group M piglets from day 22 until day 21 post-weaning (d 21 pw). One animal from each litter was randomly selected and killed at d 30 (4 days pre-weaning) and at d 37 (d 3 pw), d 44 (d 10 pw), d 51 of age (d 17 pw), respectively.

Piglets were removed from the sow at d 30 about 2 h prior to sacrification. Piglets were starved about 2 h, anaesthetized with a mixture (1.2 ml/10 kg BW, i.m.) of Zoletil® 100 (Virbac Lacboratories, Carros, France) and Stresnil (40 mg azaperon/ml; JANSSEN-CILAG PHARMA, Vienna, Austria) with a ratio of 1:1 (v/v), and then restrained on a surgical table in a dorsal position and killed by an injection with overdose of sodium pentobarbitone (100 mg/ml, i.p.; 2.5 ml/kg BW). A midline laparotomy was performed to expose the gastrointestinal tract. The cardia of stomach and the rectum were ligated using plastic ties, later the entire gastrointestinal tract was removed immediately and dissected from the mesentery.

Tissue samples of small intestine (14-cm in length) were taken at distances of 25% (proximal jejunum) and 75% (distal jejunum) from the pylorus to the ileocaecal valve. Tissue samples were dissected free of its mesentery, rinsed gently in fresh chilled physiological saline solution, and then cut into 5-cm cross-sections.

Brush border enzymes and protein concentration

The 5-cm cross-section tissue samples were immediately frozen in liquid nitrogen and stored at -80°C prior to preparation of mucosa for enzyme and protein measurements.

Tissue samples for enzyme analysis were prepared according to Dahlqvist (1964). In brief, tissue samples stored at -80°C were thawed and flushed with isotonic saline at 4°C. The mucosa was scraped off with a blunt spatula, weighed and homogenized in ice-cold Milli-Q water, using the ratio 1:25 (w/w), and then centrifuged (2,000 g, 30 min, 4°C). The supernatant was diluted with Milli-Q water with the ratio of 1:5 (w/w) for determination of lactase (β-galactosidase; EC 3.2.1.23) and sucrase

Table 2. Effect of complementary access to milk replacer to piglets on piglet live weight and daily live weight gain

	Treatment[1]		SEM	Significance[2]
	C	M		
Piglet live weight (kg)				
Birth	1.6	1.6	0.05	NS
22-d-old	7.0	6.9	0.18	NS
Weaning (34-d-old)	11.3	12.6	0.28	***
37-d-old	11.8	13.3	0.29	***
44-d-old	14.1	16.4	0.38	***
51-d-old	17.3	20.3	0.53	***
End (58-d-old)	22.9	26.6	0.71	***
Average daily gain (g)				
22-d-old - weaning	477	621	15	***
Weaning - 37-d-old	192	222	21	NS
37-d-old - 44-d-old	324	435	14	***
44-d-old - 51-d-old	453	543	24	*
51-d-old - 58-d-old	577	631	20	0.077
22-d-old - 58-d-old	446	544	16	***
Weaning - 58-d-old	437	517	16	**
Average daily milk intake (liter/piglet, day)[3]				
22-d-old - weaning	-	0.94		
Weaning - 37-d-old	-	1.78		
37-d-old - 44-d-old	-	2.37		
Weaning - 44-d-old	-	2.19		
44-d-old - 51-d-old	-	2.83		
51-d-old - 55-d-old	-	2.99		
22-d-old - 55-d-old	-	2.14		
Weaning - 55-d-old	-	2.62		
Average daily dry feed intake (g/piglet, day)				
22-d-old - weaning	64	14		
Weaning - 37-d-old	151	28		
37-d-old - 44-d-old	538	148		
Weaning - 44-d-old	422	112		
44-d-old - 51-d-old	730	303		
51-d-old - 58-d-old	1,179	832		
22-d-old - 58-d-old	604	330		
Weaning - 58-d-old	796	443		

The results are least square means and based on individual values.
[1] C, the control group (Group C); M, the milk supplemented group (Group M).
[2] NS: Not significant, $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.
[3] The milk was made using milk replacer mixed with water in a ratio of 130 g per liter water (45°C).

(sucrose-α-glucosidase; EC 3.2.1.48) activities. For measurement of maltase (β-glucosidase; EC 3.2.1.20), the supernatant was diluted 1:50 (w/w) with Milli-Q water. Lactase activity was estimated by a 30 min incubation with 90 mM lactose in 100 mM sodium citrate buffer (pH 6.0) containing 0.1 mM P-chloromercuribenzoate to inhibit lysosomal β-galactosidase at 37°C (Koldovsky et al., 1969). Sucrase and maltase were estimated by incubating with 90 mM sucrose or maltose for 30-60 min in 90 mM NaCl, 4 mM disodium succinate buffer (pH 6.0). Homogenates were pre-heated at 55°C for 45 min before estimating maltase in order to destroy the ability of sucrase-isomaltase to hydrolyse maltase (Dahlqvist, 1960). The reaction was terminated by submerging the tubes in boiling water. The free glucose liberated by the action of mucosal enzymes was then measured using a glucose-oxidase kit (Sigma Aldrich, St. Louis, MO, USA). The activities of lactase, maltase and sucrase were expressed as unit (U) which is defined as the amount of enzyme that hydrolyses 10 μmol of substrate per minute.

The protein content of the supernatant was assayed using the method described by Bradford (1976) using bovine serum albumin (BSA) as standard.

Statistical analysis

The enzyme activities were expressed as units (μmol substrate hydrolysed per minute at 37°C) per gram mucosa. All statistical tests were performed on a computerized statistical software package. Analysis of variance was performed using the GLM procedure (SAS, 1990). Significance was declared at $p<0.05$. Results are presented as least square means with their standard error of means (SEM).

RESULTS

Piglet performance

There were no significant differences in live weight at birth and 22 days of age, in live weight gain from weaning to 3 days postweaning between treatment groups (Table 2). However, providing milk replacer resulted in an increased piglet live weight at weaning ($p<0.001$), and until termination of the experiment ($p<0.001$).

The concentration of protein in the epithelium of the jejunum

At d 30 and d 3 pw, there were no significant differences found in the concentration of protein of jejunal epithelium between treatment groups. A higher level of protein in the proximal jejunal epithelium of group M piglets was observed at d 30 ($p<0.10$) and d 3 pw ($p<0.05$) as compared with group C piglets.

Independent of treatment group, before weaning the concentration of protein in the jejunal epithelium was high. After weaning it tended to decrease. However, the concentration of protein in the distal jejunum was slightly higher at d 17 pw than at d 10 pw.

Brush border enzymes in the epithelium of the jejunum

There were no significant differences found in the activity of lactase in the epithelium of the proximal and the distal jejunum between the control and the milk groups from d 30 to d 17 pw (Table 3). However, the highest activity of lactase was observed at d 30 in the epithelium of both the proximal (group C) and the distal (groups C and

Table 3. Effect of *ad libtum* access to milk replacer to piglets on the concentration (mg/g tissue) of protein, the activity (U/g tissue) of lactase, maltase and sucrase in the mucosa of the proximal and distal jejunum at age of days[1] (weaning (d 34))

Parameter	Treatment[3]	Proximal jejunum (at day)				Distal jejunum (at day)			
		d 30	d 37	d 44	d 51	d 30	d 37	d 44	d 51
Protein									
	C	64.6^{A}	55.7^{AB}	46.0^{AB}	41.8^{B}	62.2^{A}	54.9^{AB}	44.6^{B}	49.3^{AB}
	M	60.2	55.1	63.3	63.1	60.3^{A}	60.0^{AB}	46.7^{B}	57.7^{AB}
	SEM	10.22	4.75	6.69	5.34	6.53	5.54	3.82	4.49
	Significance[2]	NS	NS	NS	*	NS	NS	NS	NS
Lactase									
	C	13.6^{A}	8.2^{B}	5.9^{B}	4.9^{B}	4.9^{A}	1.3^{B}	0.1^{B}	0.1^{B}
	M	11.8	6.8	5.8	4.6	4.9^{A}	2.2^{B}	0.4^{B}	0.1^{B}
	SEM	2.52	0.89	1.08	0.99	1.21	0.53	0.14	0.05
	Significance	NS	NS	NS	NS	NS	NS	NS	NS
Maltase									
	C	21.2	21.1	21.2	25.5	19.0^{AB}	20.9^{AB}	10.2^{A}	25.9^{B}
	M	20.7	20.2	20.8	22.8	14.9^{A}	18.2^{A}	14.7^{A}	32.0^{B}
	SEM	3.46	3.83	3.03	2.97	2.65	4.19	2.80	6.28
	Significance	NS	NS	NS	NS	NS	NS	NS	NS
Sucrase									
	C	8.7^{A}	4.4^{B}	3.2^{B}	4.3^{B}	5.4^{AB}	6.8^{A}	3.6^{B}	5.5^{AB}
	M	7.7	4.4	3.9	4.5	4.2^{B}	6.0^{AB}	5.9^{AB}	7.3^{A}
	SEM	2.17	1.08	0.54	0.56	0.62	1.02	0.77	1.06
	Significance	NS	NS	NS	NS	NS	NS	NS	NS

[1] Values represent least square means of six observations.
[2] NS: Not significant, $p>0.05$; * $p<0.05$.
[3] C, the control group (Group C); M, the milk supplemented group (Group M).
[A, B] Means values within the same site in the same row with different superscript letters were significantly different ($p<0.05$).

M) jejunum followed by a gradual decrease with age increasing after weaning.

Furthermore, no treatment differences occurred in the activity of maltase in the epithelium of the jejunum (Table 3). In the jejunum, the activity of maltase reached a minimum at d 44, while the highest activity was measured at d 17 pw independent of treatment group.

The activity of sucrase in the epithelium of the jejunum was unaffected by treatment group throughout the experimental period except for a higher activity ($p<0.10$) of sucrase at d 10 pw in jejunum of group M piglets as compared with that of group C piglets (Table 3). The highest level of sucrase in the proximal jejunum was seen before weaning, whereas the activity of sucrase in the distal jejunum reached maximum levels at d 3 pw and d 17 pw in group C piglets and group M piglets, respectively. At d 3 pw, the activity of sucrase in the proximal jejunum decreased in piglets of group C. This suggests that changes in the activity of enzymes to age and weaning at various sites along the gastrointestinal tract might be different.

DISCUSSION

The present data have clearly indicated that feeding of supplemental milk replacer around the weaning period dramatically improved piglet growth performance. Similarly, Dunshea et al. (1999) reported that piglets supplemented with milk replacer had a higher live weight gain during the three-week period following weaning (20 to 41 d of age). However, a study of Royeaerd et al. (1989) has shown that only a marginal increase in piglet live weight at weaning was achieved from feeding milk replacer and a tendency to have higher live weight gain for supplemented piglets was observed during the 28-56 d of age period.

Numerous studies concerning the hydrolytic activities of the intestinal mucosa towards sucrose and lactose have been conducted. The hydrolytic activity towards sucrose in the small intestine mucosa is due to one of the α-glucosidase as referred to sucrase (Manners and Stevens, 1972). The hydrolytic activity towards lactose in the small intestine mucosa is due to two types of enzyme (lactase and hetero β-galactosidase; Asp et al., 1969). One with β-galactosidase and β-glucosidase activity is attached to the brush border and is a true digestive enzyme with a pH optimum of 5.5-6.0 (Manners and Stevens, 1972).

Berg et al. (1973) observed that the morphology of the mucosa and activity of the various mucosal enzymes varied from one segment to another along the small intestine of pigs. In the present study, lactase activity in the proximal jejunum was higher than in the distal jejunum throughout the study. The corresponding values were on average 12.7 versus 4.9, 7.5 versus 1.7, 5.8 versus 0.3, 4.8 versus 0.06 U/g, at d 30, d 3 pw, d 10 pw and d 17 pw, respectively. This suggests that the hydrolysis action of lactose can be mainly performed in the proximal region of small intestine. Similarly, earlier findings performed in humans (Asp et al.,

1969) demonstrated much lowered activities of disaccharidases in the ileum compared with that in the jejunum. High activity of lactase was seen in the jejunal mucosa before weaning, which is of importance for the hydrolysis of dietary lactose during the sucking period. Lactose is consisted of the monosaccharides glucose and galactose linked by a β-1,4-glycosidic bond and is the most abundant carbohydrate in milk. The intestinal hydrolysis of lactose occurs through the action of the enzyme lactase, which is located on the brush border of the intestinal mucous (Raiten et al., 1998). Kliegman and Sparks (1985) reported that after hydrolysis, glucose and galactose were easily absorbed with more than 90% of the absorbed glucose and galactose arriving at the portal vein. Several investigators have demonstrated that the highest activities of lactase in the small intestine of piglets aged 14-22 days are observed in the region from 5-30% along the small intestine and the lowest activities in the segment from 70-75% along the small intestine, irrespective of whether activities are expressed as per gram protein, per gram mucosa or per unit length of the small intestine (Kidder and Manners, 1980; Hampson and Kidder, 1986; Kelly et al., 1991b).

In the present study, a much lower lactase activity in the distal jejunum was observed after weaning, suggesting much more limited hydrolysis of lactose occurred in the distal region of small intestine. This should be closely related to the phenomena of poor performance, the lowered digestive capacity and low feed intake during the weaning period.

Weaning has marked effects on the development of the carbohydrases and on intestinal morphology in pigs (Hampson and Smith, 1986; Dunsford et al., 1989; Kelly et al., 1991; Pluske et al., 1996a, b). In the present study, lactase activity in the pig proximal and distal jejunal mucosa was high before weaning (d 30) and then decreased gradually with the age of piglets up to d 17 pw. This agrees well with numerous findings showing that in suckling piglets the activity of lactase undergoes a marked reduction from two to five weeks of age followed by a period when it remains relatively unchanged or continues to diminish gradually up to eight weeks of age (Miller et al., 1986; Chapple et al., 1989; Kelly et al., 1991b; Sangild et al., 1991). Kidder and Manners (1980) also observed that the activity of lactase in the small intestine of piglet was high at birth and began to fall during two months of age. A reduction (68-83%) in the total activity of brush border enzymes took place in the period following weaning (Miller et al., 1986). Similarly, we found that the reduction of lactase activity in the proximal and distal jejunum reached 41% and 65%, respectively, at d 3 pw. However, much higher variability in the activity of lactase with age in the small intestine of pig was seen as compared with other carbohydrases (Kidder and Manners, 1980).

In the present study, the activity of maltase in the jejunal mucosa is relatively stable as compared with the enzyme lactase during the weaning period and no significant differences between the proximal and distal jejunum were observed throughout the experiment except for observing a lowered activity of maltase in the distal jejunum at d 10 pw. In contrast, a higher activity of maltase in the region of 10-15% along the small intestine than in the terminal ileum of piglets aged 5-8 weeks were found by Kidder and Manners (1980). This should be explained by the fact that from 22 d of age all piglets were given dry pelleted feed in the current study and consequently weaning caused little effect on gut environment of piglets.

It is well known that drastic and complementary changes in the activities of lactase and sucrase in the mucosa of the small intestine take place during the early postnatal development of piglets. However, during the weaning period we found that, unlike the enzyme lactase, there were no differences observed in the activity of sucrase among the various regions along the small intestine except for measuring a slightly high activity of sucrase before weaning. This suggests that sucrase activity around the weaning period is relatively stable.

There were no significant differences in the activity of sucrase between treatment groups tested throughout the experiment, which implies that administration of milk replacer fails to cause a significant increase in sucrase activity in the small intestine. In contrast to our present findings, Gay et al. (1976) reported that weaning caused declined activity of intestinal lactase and sucrase activity and to a lesser extent maltase activity.

In conclusion, feeding milk replacer around the weaning period resulted in an increased live weight gain of piglets. However, providing milk replacer did not affect the activity of brush border enzymes in the piglet small intestine. Weaning caused a markedly lowered activity of lactase, while no dramatic changes in the activities of maltase and sucrase took place during the weaning period. Further studies are required to investigate the mechanisms by which growth performance of piglets given milk replacer can be improved.

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