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ASSESSMENT OF MIXED MINERALS BY OBSERVING INTESTINAL EPITHELIAL CELL ALTERATIONS IN PIGLETS

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ABSTRACT

The experiment was conducted to assess the effect of dietary Mixed Minerals (MM) on intestinal epithelial cell morphology, villus height and area and growth performance in piglets. Thirty two-month-old hybrid piglets (15 kg BW) (Large White × Landrace × Duroc), consisting of 15 castrated males and 15 females, were allocated into three experimental groups with five replicates of one castrated male and one female per replicate. The basal diet was supplemented with MM at 0 (control), 0.05 and 0.1% for 30 days. Compared with dome-shaped epithelial cells on the intestinal villus apical surface, further protuberated dome-shaped cells were observed in the 0.05% MM group and cell clusters comprised of dome-shaped cells appeared in the 0.1% MM group. However, the villus height and villus area as well as growth performance were not affected, except that the feed intake and average daily feed intake of the 0.1% MM group increased compared with those of the 0.05% MM group ($p<0.05$); as well, body weight gain of the 0.1% MM group was 4% greater than the control. These results suggest that MM can stimulate functions of epithelial cells with increasing levels of MM, but that they have no power to improve body weight gain resulting from increased villus activity and that MM have no function to affect growth performance but might affect other biochemical functions, such as immunity processes in the body.

Keywords: Growth Performance, Intestinal Epithelial Cells, Minerals, Piglets, Scanning Electron Microscope, Villus Area, Villus Height

1. INTRODUCTION

Market weight is an important economic factor in pig production. In the USA, pigs were marketed at between 120 and 130 kg live body weight, while European countries marketed pigs between 105 and 125 kg (NASS, 2003). Pig market weight has increased by 12% during the last two decades in the USA (NASS, 2003), because pig production costs decrease with increasing market weight. This continuous increase of market weight seems to be attributable to several factors, such as genetic improvements, improved nutritional management and development of new feed additives. In particular, to accelerate market weight, crossbreeding methods resulting from gene improvements have been widely practiced. Such crossbreeding of purebred pig species may change the fundamental morphology and function of the gastrointestinal tract in. Consequently, it is necessary to use crossbred piglets when researchers develop a new supplement aimed at improving intestinal function.

During the neonatal and weaning period, most of the digestive system grows and develops more rapidly than the body and the intestinal maturation process includes dramatic changes in histology, brush-border enzyme activities, as well as ion and nutrient transport capacity (Marion *et al*., 2005). Particularly for pigs, the weaning process is a major critical and difficult period of pig rearing, because they are stressed by a change in dietary nutrition and by being removed from their mother and placed in a new environment. Such stress causes marked

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structural and functional alterations in the gastrointestinal tract. Consequently, to improve growth performance, this morphological hypotrophy of the intestine due to weaning must be repressed by adding supplements to basal diets.

It has long been recognized that the availability of minerals in animal feed sources can be low. However, in weaned nursery pigs fed pharmacological concentrations of Zinc (Zn) as Zinc Oxide (ZnO), growth performance increased (Hahn and Baker, 1993; Hill *et al*., 1996; Smith *et al*., 1997). Such increased growth was thought to be induced by enhanced nutrient absorption resulting from an altered intestinal morphology due to high Zn supplementation (Carlson *et al*., 1999). These reports suggest that increasingly high mineral levels may affect intestinal function. The previous low availability of minerals in animal feed sources is thought to be due to the presence of phytate (Larsen, 1993). Recently, Mixed Minerals (MM) in the form of hydroxides were offered for plant to increase soil bacteria (Core®; Kyowa Chemical Industry Co., Ltd., Kagawa Japan).

It is difficult to assess the nutritional value of micronutrients such as minerals. As absorptive epithelial cells distributed on the villus apical surface are known to be easily altered by fed feed ingredients (Maneewan and Yamauchi, 2003; Tarachai and Yamauchi, 2000), morphological alterations of these epithelial cells can be used as a primary index in assessing the nutritional merit of these ingredients. The present aim was to evaluate the nutritional merit of dietary MM by observing epithelial cell alterations and villus morphology, as well as growth performance.

2. MATERIALS AND METHODS

2.1. Animals and Housing

All experimental procedures on animal housing were described in detail in the previous paper (Maneewan *et al*., 2012), with the exception that a total of 30 two-month-old hybrid piglets (15 kg BW) (Large White × Landrace × Duroc), consisting of 15 castrated males and 15 females, were allocated into three experimental groups with five replicates of one castrated male and one female per replicate. The basal diet (**Table 1**) was supplemented with MM (**Table 2**) at 0 (control), 0.05 and 0.1%. The feeding experiment was carried out during 30 days (until the piglets were three months old) and growth performance and histological changes observed.

2.2. Light Microscopy

Villus height, excluding the intestinal crypt, was measured in two villi in each section. Villi that included the lamina propria were chosen and the length from the villus tip to the bottom was measured at 10×10 magnification. Sixteen values of villus height were obtained from eight sections per pig and the average of these values was expressed as the mean villus height for each pig.

To measure villus area, the width of the villus was measured at the base and apex. Two villi that included lamina propria were selected at 10×10 magnification for each section per pig. Sixteen samples were counted from eight sections per piglet. Villus area was calculated from villus height, basal width and apical width. A total of 16 calculations of the villus area were made for each pig and the average of these values was expressed as the mean villus area for each pig.

Table 1. Feed and nutrient compositions of starter basal diet in pigs (%)

Ingredients	% of starter diet
Corn	60.60
Rice bran	5.00
Soybean meal (44% protein)	24.20
Fish meal (60% protein)	3.00
Palm oil	3.70
Bone meal	1.00
Dicalcium phosphate (P-18)	1.80
Salt	0.35
Premix†	0.35
Total	100.00
Chemical composition	
Crude protein (%)	18.00
Calcium (%)	0.89
Phosphorus (%)	0.67
Lysine (%)	0.96
Methionine + cystein (%)	0.63
Tryptophan (%)	0.22
Threonine (%)	0.70
Metabolizable energy (kcal/kg)	3250.00

Starter diet is for nursery pigs: 15-30 kg
Premix supplied per kg diet: Vitamin A, 3333 IU; vitamin D, 667 IU; vitamin E, 0.33 mg; vitamin K, 0.66 mg; vitamin B_2, 1.67 mg; vitamin B_{12}, 0.003 mg; calcium pantothenate, 6.67 mg; cobalt, 3.47 mg; copper, 27.60 mg; iodine, 0.77 mg; manganese, 18.47 mg; zinc, 50.00 mg; and Fe, 60.00 mg

Table 2. Supplemented levels of each mineral in mixed mineral (MM) to basal diet for piglet (mg/kg)

Groups	Ca	Mg	Zn	Fe	Mn	Cu
Control	0	0	0.0	0.0	0	0
0.05% (MM)	150	75	12.5	7.5	2	1
0.10% (MM)	300	150	25.0	15.0	4	2

3. RESULTS

3.1. Growth Performance

Compared with the control, feed intake, body weight gain, average daily feed intake, average daily gain and feed efficiency were not different in the 0.05 and 0.1% MM groups; the 0.05% group had lower values, but the 0.1% group showed higher values in each item of growth performance than the control (**Table 3**). The 0.1% MM group had higher values in feed intake and average daily feed intake than those of the 0.05% group ($p<0.05$).

3.2. Light Microscopy

Villus height and villus area of the duodenum, jejunum and ileum were not different among groups (**Table 4**).

3.3. Scanning Electron Microscopy

On the villus apical surface of the duodenum (**Fig. 1**), the control (A) had dome-shaped cells protuberating into the intestinal lumen (white arrow) and deeper cells at the sites of recently exfoliated cells (black arrow). Furthermore, in the 0.05% dietary MM group (B), protuberated dome-shaped cells (white arrows) were closely distributed. In the 0.1% dietary MM group (C), cell clusters (stars) comprised of dome-shaped cells, in addition to single dome-shaped cells (white arrow), appeared.

On the villus apical surface of the jejunum (**Fig. 2**), the control (A) was distributed with flat cells, resulting in a smooth surface. In the 0.05% dietary MM group (B), dome-shaped cells (white arrows) and deeper cells at the sites of recently exfoliated cells (black arrows) appeared. In the 0.1% dietary MM group (C), cell clusters (stars) appeared, in addition to dome-shaped cells (white arrow).

On the villus apical surface of the ileum (**Fig. 3**), the control (A) was distributed with faintly protuberated dome-shaped cell (white arrows). In the 0.05% dietary MM group (B), the deeper cells at the sites of recently exfoliated cells (black arrow) were found among dome-shaped cells (white arrows). In the 0.1% dietary MM group (C), the deeper cells at the sites of recently exfoliated cells (black arrow) were observed on the continued cell clusters (stars).

Table 3. Growth performance of piglets fed 0 (control), 0.05 and 0.1% dietary mixed minerals (MM) during 2 to 3 - month - old (mean ± sem) (n = 5)

Items	Control	0.05% MM	0.1% MM	Grand mean	*P* -value
Feed intake	43.09±0.31	41.78±0.94	44.78±0.59	43.22±0.48	0.099
BW gain	22.00±0.76	21.45±0.60	23.10±0.24	22.18±0.35	0.077
ADFi	1.44±0.01	1.39±0.03	1.49±0.02	1.44±0.02	0.098
ADG	733±25	715±20	770±8	739±12	0.077
F: G	1.97±0.05	1.95±0.04	1.94±0.03	1.95±0.02	0.678

ADFi = Average Daily Feed intake; ADG = Average Daily Gain; F: G = Feed per BW Gain (Feed efficiency)

Table 4. Villus height and villus area of piglets fed 0 (control), 0.05 and 0.1% dietary mixed minerals (MM) during 2 to 3-month- old (n = 4)

	Mineral (%)			
Items	0	0.05	0.10	P Value
Villus height (mm)				
Duodenum	0.34	0.27	0.28	0.2468
Jejunum	0.21	0.20	0.23	0.4316
Ileum	0.20	0.18	0.15	0.3967
Villus area $(mm)^2$				
Duodenum	0.042	0.023	0.027	0.163
Jejunum	0.017	0.016	0.017	0.832
Ileum	0.020	0.015	0.011	0.155

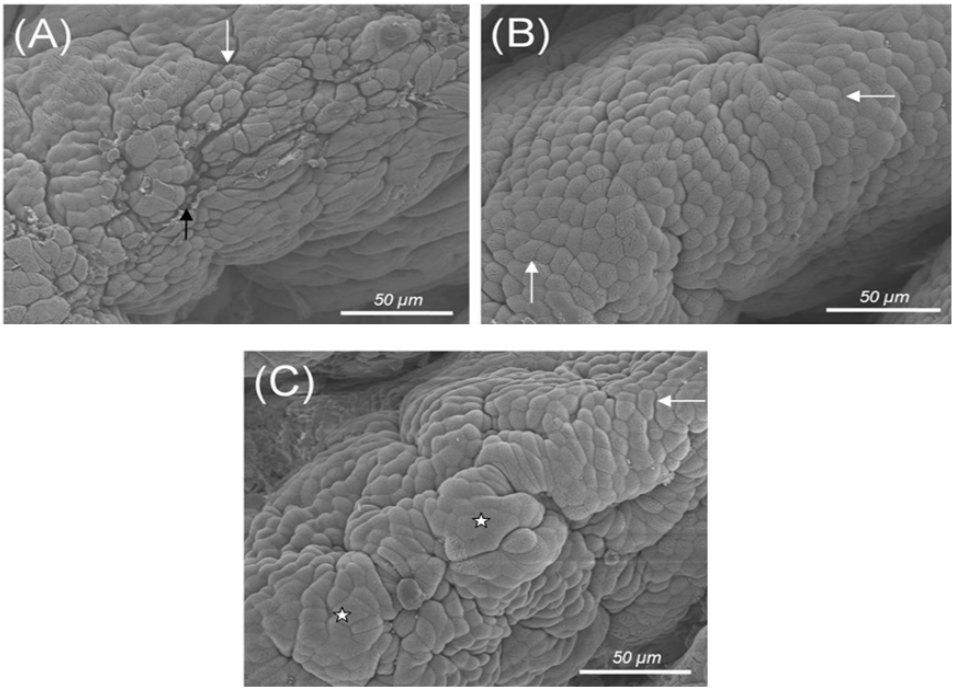

Fig. 1. Duodenal villus apical surface in piglets fed 0 (A), 0.05 (B) and 0.1 (C) % dietary mix mineral diets. White arrow, dome-shaped cells; Black arrow, deeper cells at the sites of recently exfoliated cells; Stars, cell clusters. Scale bar = 50 µm (×700)

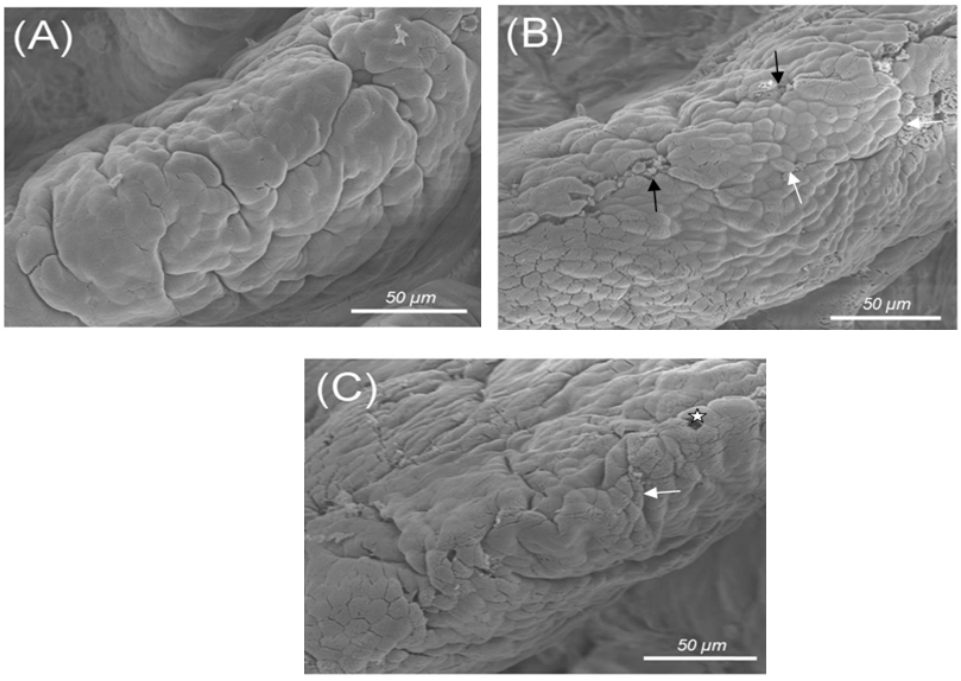

Fig. 2. Jejunal villus apical surface in piglets fed 0 (A), 0.05 (B) and 0.1 (C) % dietary mix mineral diets. White arrow, dome-shaped cells; Black arrow, deeper cells at the sites of recently exfoliated cells; Stars, cell clusters. Scale bar = 50 µm (×700)

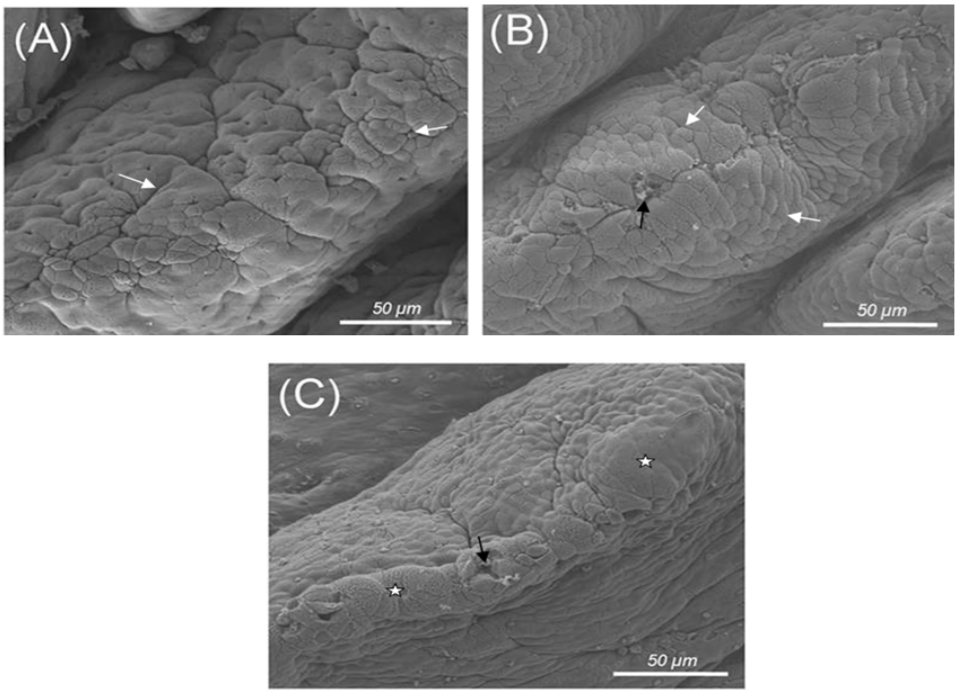

Fig. 3. Ileal villus apical surface in piglets fed 0 (A), 0.05 (B) and 0.1 (C)% dietary mix mineral diets. White arrow, dome-shaped cells; Black arrow, deeper cells at the sites of recently exfoliated cells; Stars, cell clusters. Scale bar = 50 µm (×700)

4. DISCUSSION

The intestine is the site of digestion and absorption of ingested feed and its morphology was related to the diets fed to the animals (Langhout *et al*., 1999; Yasar and Forbes, 1999). Epithelial cells multiply by mitosis in the intestinal crypt and migrate along the villus surface upward to the villus tip within a few days (Imondi and Bird, 1966), where they are extruded into the intestinal lumen within 48 h after birth (Potten, 1998). Therefore, as the exfoliative zone around the center of the villus apical surface is the final stage of cell life, many kinds of morphological alterations of epithelial cells occurred around the central part on the apical surface according to intestinal function (Ibuki *et al*., 2013; Ruttanavut *et al*., 2012). In fact, protuberated cells, deeper cells at the sites of recently exfoliated cells and cell clusters were demonstrated as indicators that the function of these cells was activated (Khonyoung *et al*., 2012; Maneewan *et al*., 2012). These kinds of cellular morphologies were also found in the present villus apical surface. The control duodenal villi had protuberated cells and deeper cells at the sites of recently exfoliated cells. Further protuberated dome-shaped cells were observed in the 0.05% dietary MM group and cell clusters comprised of dome-shaped cells appeared in the 0.1% dietary MM group. The flat cells of the control jejunum developed into dome-shaped cells in the 0.05% dietary MM group and into cell clusters in the 0.1% dietary MM group. In the ileum, in addition to dome-shaped cell in the control, deeper cells at the sites of recently exfoliated cells in the 0.05% dietary MM group and continuous cell clusters in the 0.1% dietary MM group were observed. Such morphological alterations suggest that the epithelial cells were hypertrophied with increasing levels of dietary MM. However, these ultrastructural alterations did not induce activated morphological changes of villus height and villus area nor did they induce improved growth performance. These results might correspond with the fact that, generally, light microscopic parameters, such as villus morphology, were altered in dramatic treatments such as fasting and refeeding (Tarachai and Yamauchi, 2000). In fact, after weaning, reduction of the villus height to 75% of the pre-weaning value was found within 24 h of weaning (Hampson, 1986), but crypt depths increased (Pluske *et al*., 1997). These alterations of the light microscopic parameters induced a decreased activity of the brush-border enzymes lactase and sucrose (Hampson and Kidder, 1986), resulting in delay of growth performance. Therefore, in this study, it may be

reasonable to consider that no change of villus morphology resulting from feeding dietary MM did not induce body weight gain either. However, the values for body weight gain and average daily gain were greater in the 0.1% dietary MM group than those of the control, although they were not significantly greater. When the 22.0 kg body weight gain value of the control is expressed as index of 100, the 23 kg body weight of the 0.1% dietary MM group is 104.5 (a 4% higher value than the control). As it is well known that minerals are essential for the maintenance of good health, with effects on the immune system, as well as on energy, metabolism and antioxidant protection but that they are needed in very small amounts, the present hypertrophied epithelial cells resulting from feeding MM might improve other biochemical processes such as those of the immune system in the body. Further study is being carried out in this direction.

Current swine practices result in weaning at the younger ages and lighter body weight (Mahan *et al*., 1996). The early-weaned pigs have a less mature gastrointestinal tract (De Passille *et al*., 1989) and secrete lower quantities of digestive enzymes (Graham *et al*., 1981; Sloat *et al*., 1985) compared with pigs weaned at older or heavier weights. Even in such early-weaned pigs having physiological immaturity, the initial diets were formulated by adding relatively high levels of dried whey containing high mineral levels, particularly Na, K and Cl (NRC, 1988; Mahan *et al*., 1996). Because the osmotic electrolyte balance in the gut of young weanling pigs may be delicate (Hamilton and Roe, 1977), gastrointestinal upsets due to excessive dietary cations and (or) anions have been considered to be harmful to the weaned pig (Mahan *et al*., 1996). Consequently, post-weaning scours have been attributed to the high ash content (i.e., 8.0%) contributed from whey (Mahan *et al*., 1996). These reports suggest that increasingly high mineral levels may affect intestinal function. In fact, the present study demonstrated that epithelial cells were hypertrophied with increasing MM levels compared with those in the control and that feed intake increased significantly and body weight was higher in the 0.1% dietary MM group than in the 0.05% dietary MM group. It is not clear at present why the 0.05% dietary MM group showed inferior values than the control, but it is possibly related to the fact that the low (0.05%) level of the present supplemented minerals is uncongenial to the minerals in the basal diet and that the higher 0.1% level of the present supplemented minerals negates this incompatibility. Because the early stage diets were formulated by adding high mineral levels even in early-weaned pigs having physiological immaturity (Mahan *et al*., 1996). In addition, the previous low availability of minerals in animal feed sources is thought to be due to the presence of phytate (Larsen, 1993). Excessive amounts of phytate in the diet induced negative effect on mineral balance, because it forms insoluble complexes with minerals and reduced the bioavailability of these minerals (Forbes *et al*., 1984). Phytase synthesis was enhanced by the gastrointestinal microflora (Moore and Veum, 1983). As the present MM was offered for plants to increase soil bacteria in the hydroxide form, the 0.1% high level MM might induce good mineral balance.

5. CONCLUSION

In conclusion, MM can stimulate the functions of the epithelial cells with increasing levels of MM but have no power to improve body weight gain due to villus activity and no function to affect growth performance. However, they might have other biochemical functions, such as a positive effect on immunity processes in the body.

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