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Effect of Coated Lipase Supplementation on Growth, Digestion and Intestinal Morphology in Weaning Piglets

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Abstract: A total of 72 weaning piglets with an average initial body weight of 8.72±1.01 kg were randomly allotted into one of three groups to investigate the effects of lipase on growth, digestive enzymes activities and intestinal morphology. Pigs were fed on the same basal diet supplemented with 0 and 200 mg kg^{-1} Uncoated Lipase (UC-LIP) or Coated Lipase (CT-LIP). After 28 days feeding trail, 18 pigs from the control, 200 mg kg^{-1} UC-LIP and 200 mg kg^{-1} CT-LIP groups were slaughtered to collect samples for assay. The results showed that supplemental lipase increased ADG ($p<0.05$) and decreased F:G ratio ($p<0.05$) and diarrhea rate ($p<0.05$) of pigs and the coated lipase is more effective than uncoated lipase. With the supplementation of CT-LIP, concentration of TP, FFA and LIP activity in serum was increased ($p<0.05$) and concentration of LDL, SUN, TG was decreased ($p<0.05$). The supplemental lipase increased lipase activity in pancreas ($p<0.05$) and the activities of trypsin, lipase, amylase and FFA concentration in duodenal content ($p<0.05$). Compared to the UT-LIP, the CT-LIP is more effective on the above digestive enzymes ($p<0.05$). The supplemental increased villus height, VH:CD ratio and decreased crypt depth in duodenum and jejunum ($p<0.05$). The results indicated that dietary supplementation of coated-lipase has positive effect on growth, digestion and intestinal morphology in weaning piglets.

Key words: Lipase, pigs, growth, digestion, intestine morphology

INTRODUCTION

Lipase as one kind of glycoprotein has important physiological functions in domestic animals. Exogenous fat will not go through cell membrane and be stored or mobilized without lipase digestion. Monoacylglycerol, Free Fat Acid (FFA) and glycerol resolved from Triglyceride (TG) by lipase not only provide energy but also are the precursor of important lipoid such as phospholipids and sphingolipid (Xiong and Zhang, 2001).

The fat energy is 2.25 times of carbohydrate and can improve the absorption and utilization of soluble vitamin and some hormones and is also one resource of essential unsaturated fatty acid (Leng, 2007; Su, 2006). The lack of lipase will influence the fat metabolism and so that be harmful to livestock health. Due to early weaning stress, the endogenous digestive enzyme secretion of piglet is greatly retarded which will last for 2 weeks (Lindemann *et al*., 1986; Owsley *et al*., 1986; Zhang and Jiang, 1999). It's reported that the dietary supplementation with exogenous enzymes can stimulate the secretion of endogenous digestive enzyme to exert beneficial impacts for pig and poultry (Pluske *et al*., 1997; Wang *et al*., 2007). However, Dierick and Decuypere (2002) found that when compound feed was supplemented with regular lipase, fat digestion was no big difference between with and without lipase supplementation. The great portion of lipase will be destroyed when it goes through the stomach. The protection of active substance in feed is not a fresh issue, its booming puts forward more specific request. Previous studies have indicated that coating can hide the ugly odor and taste, secure ingredient stability, directionally transfer and release active substance and finally improve utilization of active substance (Zhou and Cheng, 2011).

To the best of the knowledge, the coated-lipase has not been investigated in livestock. The study reported here was conducted to evaluate the effects of coated-lipase supplementation on growth performance, digestion and intestinal morphology in weaning piglets.

MATERIALS AND METHODS

Coated Lipase (CT-LIP, 9012 U g^{-1}) and Uncoated Lipase (UC-LIP, 10935 U g^{-1}) were provided by Feed Science Institute of Zhejiang University.

Experimental design and diets: A total of 72 weaning piglets (Landrace x Yorkshire x Duroc) weighing 8.72±1.01 kg were randomly divided into three groups,

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each treatment with three replicates, 8 pigs per replicate. Pigs were fed on the same basal diet supplemented with 0 (the control), 200 mg kg^{-1} UC-LIP or 200 mg kg^{-1} CT-LIP. The basal diet was formulated to meet National Research Council (NRC, 1998) requirements (Table 1). During the feeding period, pigs for experiment were free access to diets and water, 7 days preliminary trial, 28 days for formal feeding trial. Feed consumption, diarrhea and death case was daily recorded. Pigs were weighed to calculate Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed/Gain ratio (F/G). The diarrhea rate was also calculated. Diarrhea rate = Total diarrheal piglets/(Total piglets x Experimental days). The Animal Welfare Committee of Zhejiang University approved the animal care protocol used for this experiment.

Blood profiles: At the end of feeding trial, 18 piglets from control group, 200 mg kg^{-1} UC-LIP group and 200 mg kg^{-1} CT-LIP group (fasting for 12 h and free to water), 2 pigs per replicate and 6 pigs per treatment were selected and slaughtered for samples collection. Blood samples were collected into 15 mL vacuum tube and let alone at 37°C for 10 min, then were centrifuged at 3000 r min^{-1} for 10 min to separate serum. Serum biochemical indexes include Total Protein (TP), Triglyceride (TG), Cholesterol (CHL), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Free Fatty Acid (FFA), Lipase (LIP), Glucose (GLU), Serum Urea Nitrogen (SUN), Ca, P, Alkaline Phosphatase (ALP), Glutamic-Oxalacetic Transaminase (GOT), Glutamic-Pyruvic Transaminase (GPT) were determined with Olympus AU500, full-automatic biochemical analyzer by the Second People's Hospital of Hangzhou.

Table 1: Ingredient inclusion and chemical composition of basal diet as-fed basis

Ingredients (g kg^{-1})	Values
Corn	500.00
Soybean meal	230.00
Extruded soybean	80.00
Fish meal	40.00
Whey powder	80.00
Soybean oil	30.00
Limestone	12.00
Calcium phosphate	18.00
Salt	4.00
Mineral premix[a]	3.00
Vitamin premix[b]	3.00
Chemical composition (analyzed, g kg^{-1})[c]	
Digestible energy (MJ kg^{-1})	14.46
Crude protein	201.50
Calcium	9.20
Phosphorus	7.30
Lysine	12.50
Methionine	6.50

[a]All data were analyzed values except digestible energy which was calculated using swine NRC (1998) values; [b]Contained per kg of diet: Cu, 40 mg from $CuSO_4.5H_2O$; Fe, 180 mg from $FeSO_4.H_2O$; Mn, 30 mg from $MnSO_4.5H_2O$; Se, 0.3 mg from $Na_2SeO_3.5H_2O$; I, 0.3 mg from KI; [c]Contained per kg of diet: vitamin A, 5000 IU; vitamin D_3, 500 IU; vitamin E, 60 IU; vitamin K, 4.4 mg; riboflavin, 6.8 mg; D-pantothenic acid 20.2 mg; niacin, 33 mg; choline chloride 700 mg; vitamin B_{12}, 22 μg; D-biotin, 300 μg; folic acid, 2.5 mg

Pancreatic digestive enzymes: The abdominal cavity of pigs was opened and the entire gastrointestinal tract was removed. The pancreas was carefully dissected free, cleaned of extraneous tissue, weighed and immediately frozen at -80°C. The pancreas was thawed and homogenized using an Ultra Turrax T 25 with a S25KG-18G probe in ice-cold 0.9% NaCl (nine times volumes of pancreatic weight). The homogenate was centrifuged at 3000 r min^{-1} (4°C for 10 min) and aliquots of the supernatant were stored in Eppendorf tube at -80°C. The determination of trypsin, lipase and amylase activity follows the specification of reagent kit provided by Nanjing Jiancheng Biotechnology Institute.

Duodenal content digestive enzymes and FFA: Chyme from the same part of duodenum was collected, then frozen at -70°C for digestive enzyme and FFA analysis. The determination of trypsin, lipase and amylase activity follows the specification of reagent kit provided by Nanjing Jiancheng Biotechnology Institute. The determination of FFA was analyzed with Olympus AU500, full-automatic biochemical analyzer by the Second People's Hospital of Hangzhou.

Small intestinal morphology: The 0.2 cm sections of duodenum, jejunum, ileum was trimmed into square, washed with running saline water, fixed in 10% buffered formalin, embedded in paraffin after dehydration, sectioned at 4 μ and stained with hematoxylin and eosin for observation with Leica universal microscope and LeicaQwin soft for quantitative analysis. Every piece of section was used for villus height and crypt depth determination.

Statistical analysis: All statistical analyses were computed using the general linear models procedure of SAS Software (SAS, 1996). A software program using Duncan's multiple range test to compare treatment means were applied. A $p<0.05$ was considered statistically significant.

RESULTS

Growth performance: As shown in Table 2, the ADG of pigs was increased by 13.65% ($p<0.05$) and 27.30% ($p<0.05$) and the F:G ratio was decreased by 11.26% ($p<0.05$) and 22.94% ($p<0.05$), respectively with the

Table 2: Effect of CT-LIP on growth performance and diarrhoea in weaning piglets[a]

Items	Control	UC-LIP (mg kg^{-1})	CT-LIP (mg kg^{-1})	SEM[c]
Initial weight (kg)	8.63	8.75	8.68	0.05
Final weight (kg)	17.20^{A}	18.49^{B}	19.59^{C}	0.17
ADG (g day^{-1})[b]	306.07^{A}	347.86^{B}	389.64^{C}	6.28
ADFI (g day^{-1})	706.85	713.39	694.94	9.94
F/G	2.31^{A}	2.05^{B}	1.78^{C}	0.05
diarrhea rate (%)	8.17^{A}	6.41^{B}	4.54^{C}	0.34

[a]Values are presented as means; n = 3 per treatment with 8 pigs per pen contributing to a pen mean; [b]Means in a low with different capital letter differ p<0.05; [c]Standard error of the mean

Table 3: Effect of CT-LIP on serum biochemical indices in weaning piglets[a]

Items	Control	UC-LIP (mg kg^{-1})	CT-LIP (mg kg^{-1})	SEM[c]
GLU (mmol L^{-1})[b]	5.32	6.09	6.12	0.20
TP (g L^{-1})	53.68^{A}	56.03^{B}	64.26^{B}	1.79
HDL (mmol L^{-1})	0.70	0.71	0.77	0.03
LDL (mmol L^{-1})	1.93^{A}	1.54^{B}	1.45^{B}	0.08
SUN (mmol L^{-1})	6.23^{A}	5.33^{A}	4.97^{B}	0.25
TG (mmol L^{-1})	0.68^{A}	0.56^{B}	0.43^{C}	0.03
CHL (mmol L^{-1})	2.91	2.78	2.71	0.11
Ca (mmol L^{-1})	2.36	2.44	2.50	0.03
P (mmol L^{-1})	2.74	2.82	2.63	0.08
FFA (mmol L^{-1})	0.29^{A}	0.40^{B}	0.51^{C}	0.03
LIP (U L^{-1})	18.75^{A}	21.25^{B}	24.50^{C}	0.81
ALP (U L^{-1})	235.67	239.33	255.50	7.97
GOT (U L^{-1})	94.33	87.00	95.67	3.92
GPT (U L^{-1})	51.25	54.50	55.00	1.28

[a]Values are presented as means; n = 3 per treatment with 2 pigs per pen contributing to a pen mean (6 pigs per treatment in total); [b]Means in a low with different capital letter differ (p<0.05); [c]Standard error of the mean

supplementation of 200 mg kg^{-1} UT-LIP or CT-LIP. Compared with the UC-LIP group, the ADG of CT-LIP was increased by 12.01% (p<0.05) and F:G ratio was decreased by 13.17% (p<0.05). The diarrhea rate of pigs in UT-LIP or CT-LIP group was decreased by 21.54% (p<0.05) and 44.43% (p<0.05), respectively. The diarrhea in CT-LIP was decreased by 29.17% (p<0.05) compared with the UC-LIP group.

Serum biochemical index: As shown in Table 3, serum TP concentration of CT-LIP group was 19.71% (p<0.05) and 14.69% higher (p<0.05) than the control and UC-LIP group, respectively. Supplementation of lipase decreased TG concentration by 17.65% (p<0.05) and 36.76% (p<0.05), as well as LDL concentration by 24.87% (p<0.05) and 20.21% (p<0.05). The SUN concentration in CT-LIP group was decreased by 20.22% (p<0.05) with the addition of CT-LIP in the diet. The FFA concentration and LIP activity in UT-LIP or CT-LIP group was increased by 37.93% (p<0.05), 75.86% (p<0.05) and 13.33% (p<0.05), 30.67% (p<0.05), respectively. Simultaneously, FFA concentration and LIP activity in CT-LIP group was increased by 27.50% (p<0.05) and 15.29% (p<0.05) compared with the UC-LIP group.

Table 4: Effects of CT-LIP on digestive enzyme activity in pancreas in weaning piglets[a]

Items	Control	UC-LIP (mg kg^{-1})	CT-LIP (mg kg^{-1})	SEM[c]
Trypsin (U mg^{-1} prot)[b]	4070.62	4078.97	4182.60	99.89
Lipase (U g^{-1} prot)	32.07^{A}	45.30^{B}	52.36^{C}	3.00
Amylase (U mg^{-1} prot)	18.31	18.09	22.68	1.44

Table 5: Effects of CT-LIP on digestive enzyme activity and FFA content in duodenal contents in weaning piglets[a]

Items	Control	UC-LIP (mg kg^{-1})	CT-LIP (mg kg^{-1})	SEM[c]
Trypsin (U/mg prot)[b]	1278.08^{A}	1504.43^{B}	1693.26^{C}	54.67
Lipase (U/g prot)	89.63^{A}	129.85^{B}	153.06^{C}	7.57
Amylase (U/mg prot)	3.29^{A}	4.07^{AB}	5.57^{B}	0.41
FFA (mmol L^{-1})	1.26^{A}	1.37^{A}	1.81^{B}	0.09

Table 6: Effects of CT-LIP on disaccharide enzyme activity in jejunal mucosal in weaning piglets[a]

Items	Control	UC-LIP (mg kg^{-1})	CT-LIP (mg kg^{-1})	SEM[c]
Maltase (U/mg prot)[b]	23.54	25.99	26.00	2.83
Sucrase (U/mg prot)	43.96	45.69	49.55	3.62
Lactase (U/mg prot)	11.72^{A}	16.32^{AB}	18.15^{B}	1.21

[a]Values are presented as means; n = 3 per treatment with 2 pigs per pen contributing to a pen mean (6 pigs per treatment in total); [b]Means in a low with different capital letter differ (p<0.05); [c]Standard error of the mean

Endogenous digestive enzymes: Supplemental UT-LIP or CT-LIP increased lipase activity in pancreas by 15.58% (p<0.05) and 63.27% (p<0.05), respectively (Table 4). There is no significant difference in trypsin and lipase activity in pancreas. Compared with the control group, trypsin activity in duodenal content was increased by 17.71% (p<0.05) and 32.48% (p<0.05) with the supplementation of 200 mg kg^{-1} UC-LIP and 200 mg kg^{-1} CT-LIP, respectively as well as lipase activity in duodenal content increased by 44.87% (p<0.05) and 70.77% (p<0.05), respectively (Table 5). The activity of trypsin and lipase in duodenal content of CT-LIP group was 12.55% (p<0.05) and 17.87% (p<0.05) higher than that in UT-LIP group. The amylase activity in duodenal content of CT-LIP group was increased by 69.30% (p<0.05). As shown in Table 6, the FAA level in duodenal content was increased by 43.56% (p<0.05) and 32.12% (p<0.05) with the addition of UT-LIP or CT-LIP.

Intestinal morphology: Compared with the control group, supplemental UC-LIP and CT-LIP increased villus height of duodenum by 7.24% (p<0.05) and 15.25% (p<0.05), respectively as well as VH/CD by 13.66% (p<0.05) and 29.51% (p<0.05), respectively. Crypt depth of duodenum in CT-LIP group was decreased by 10.99% (p<0.05) (Table 7). Villus height of jejunum was increased by 6.52% (p<0.05) and 14.68% (p<0.05) with supplementation of lipase, the crypt depth was decreased by 12.20% (p<0.05) and 16.81% (p<0.05), VH/CD of jejunum was increased by 20.90% (p<0.05) and 37.29% (p<0.05), respectively.

Table 7: Effects of CT-LIP on intestinal morphology in weaning piglets[a]

Items	Control	UT-LIP (mg kg^{-1})	CT-LIP (mg kg^{-1})	SEM[c]
Duodenum				
Villus height (μm)[b]	346.98^{A}	372.11^{B}	399.91^{C}	4.82
Crypt depth (μm)	190.14^{A}	179.33^{AB}	169.25^{B}	2.83
VH/CD	1.83^{A}	2.08^{B}	2.37^{C}	0.05
Jejunum				
Villus height (μm)	322.37^{A}	343.39^{B}	369.70^{C}	4.59
Crypt depth (μm)	183.14^{A}	160.80^{B}	152.36^{B}	3.39
VH/CD	1.77^{A}	2.14^{B}	2.43^{C}	0.06
Ileum				
Villus height (μm)	334.54	336.81	342.89	2.30
Crypt depth (μm)	179.09	176.42	174.96	1.25
VH/CD	1.87	1.91	1.96	0.02

[a]Values are presented as means; n = 3 per treatment with 2 pigs per pen contributing to a pen mean (6 pigs per treatment in total); [b]Means in a low with different capital letter differ ($p<0.05$); [c]Standard error of the mean

Simultaneously, the villus height and VH/CD in CT-LIP group was 7.66% ($p<0.05$) and 13.55% ($p<0.05$) higher than that in UT-LIP group. There was no significant difference of ileum morphology between control and lipase treated group.

DISCUSSION

The moderate addition of exogenous digestive enzymes, such as amylase, proteinase and lipase is beneficial to digestion, intestinal problem reduction and growth. Some researchers have reported that the addition of lipase to dietary feed containing grease can increase ADG and feed efficiency of early weaning piglets (Dierick and Decuypere, 2002; Dierick *et al*., 2003; Decuypere and Dierick, 2003) which is in line with the present result that both CT-LIP and UT-LIP can increase ADG, reduce F/G. Insufficient secretion of endogenous digestive enzymes can induce low-level utilization of nutrients and results in diarrhea (Fu *et al*., 2010) which can be alleviated by addition of exogenous lipase. The reason maybe is that the improved utilization of nutrients, especially fat, reduces intestine stickiness to alleviate diarrhea.

Polyunsaturated Fatty Acid (PUFA) family (n-3 and n-5) restrains the gene transcription of liver fatty acid synthetase and glycolytic enzyme, n-3 fatty acid can restrain TG synthesis and secretion of LDL to maintain the low-level TG (Jump and Clarke, 1999). In the current study, the reduction of TG and LDL by adding lipase may be due to PUFA from soya-bean oil hydrolysis by lipase. The significant increase of lipase activity and FFA concentration indicates that supplemental lipase is beneficial to serum fat metabolism. Meanwhile, the FFA concentration in duodenal content was increased significantly which revealed that the dietary fat hydrolyzed sufficiently by lipase and was absorbed by intestine mucosa when passing digestive tract. Serum SUN is a relatively accurate index for the balance between protein metabolisms *in vivo* and feed amino acid (Zhang and Lu, 2000). The low-level SUN means high-level nitrogen deposition *in vivo* and high protein utilization. The present experiment demonstrated that CT-LIP can reduce serum SUN concentration which may indicate that the increased ADG and feed efficiency is resulted from lipase improve protein accreation by lipase supplementation.

It's reported that the supplementation of exogenous digestive enzymes to piglet with corn-soybean diet enhanced trypsin and amylase activity in pancreas (Shen, 2002). And the addition of lipase to diet with grease increased trypsin activity, reduced pancreatic lipase activity and made no significant difference in pancreatic amylase activity (Su, 2006). The results of the present experiment were partly in agreement with the above research. That is pancreatic lipase activity was increased significantly and the effect of CT-LIP was more positive while there is no significant difference in trypsin and amylase. When piglets was fed diet containing grease supplemented with lipase, increased trypsin activity, reduced lipase activity and increasing trend of amylase activity in duodenal content was observed (Su, 2006). That is in line with the present experiment. The supplementation of CT-LIP increased trypsin activity, lipase activity, amylase activity in duodenal content significantly.

The increase of small intestinal villus height will expand contact area between small intestine and nutrients to enhance its absorptive capacity and better itself immunity (Yamauchi *et al*., 2006; Chen *et al*., 2008). Crypt depth reflects the speed of epithelial cell update (Wei *et al*., 2010). Villus height/Crypt depth comprehensively reflects small intestine capacity of which ratio increasing means better digestibility and ratio reduced following mucosa injury, diarrhea and putrefactive bacteria proliferation. The corn-soybean diet supplemented with exogenous digestive enzymes (amylase and proteinase) can alleviate the villus injury degree (Shen, 2002). When piglets was offered diets containing grease supplemented with lipase, jejunum villus height was increased and crypt depth was reduce, duodenum and ileum exhibited no obvious change (Su, 2006). The present experiment showed that duodenum and jejunum villus height, villus height/crypt depth had been increased, crypt depth reduced while ileum had no obvious change. Wu *et al*. (2004) reported that exogenous digestive enzymes improve intestine morphology by enhance nutrients digestive absorption (Wu *et al*., 2004).

CONCLUSION

The results obtained from this study indicated that coated lipase has the potential to improve growth performance, digestion and intestinal morphology in weaning piglets.

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