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Effects of octacosanol extracted from rice bran on blood hormone levels and gene expressions of glucose transporter protein-4 and adenosine monophosphate protein kinase in weaning piglets


 Lei Long ^{a,b}, Shugeng Wu ^a, Jing Sun ^b, Jing Wang ^a, Haijun Zhang ^a, Guanghai Qi ^{a,*}
^a Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agriculture Sciences, Beijing 100081, China

^b Tianjin NaEr Biotechnology Co., Ltd., Tianjin 300457, China

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ABSTRACT

The object of this study was to explore the regulatory mechanism of octacosanol to the body of animals and the effects of octacosanol on blood hormone levels and gene expressions of glucose transporter protein (GLUT-4) and adenosine monophosphate protein kinase (AMPK) in liver and muscle tissue of weaning piglets. A total of 105 crossbred piglets (Yorkshire × Landrace) × Duroc with an initial BW of 5.70 ± 1.41 kg (21 d of age) were used in a 6-wk trial to evaluate the effects of octacosanol and tiamulin supplementation on contents of triiodothyronine (T_3), thyroxine (T_4), growth hormone (GH), glucagon (GU) and adrenaline (AD) in blood and gene expressions of GLUT-4 and AMPK in liver and muscle. Piglets were randomly distributed into 3 dietary treatments on the basis of BW and sex. Each treatment had 7 replicate pens with 5 piglets per pen. Treatments were as followed: control group, tiamulin group and octacosanol group. The results showed that compared with control group and tiamulin group, octacosanol greatly promoted the secretion of T_3 , GH, GU and AD ($P < 0.01$) and significantly up-regulated the gene expressions of GLUT-4 and AMPK in muscle and liver tissues ($P < 0.05$). There was no significant difference between the control group and tiamulin group in T_3 , T_4 , GH, GU or AD ($P > 0.05$). Results of the present study has confirmed that octacosanol affects energy metabolism of body by regulating secretion of blood hormones and related gene expression in tissue of weaning piglets, which can reduce stress response and has an impact on performance.

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1. Introduction

Octacosanol, which mainly exists in a natural wax product that naturally occurs in fruits, leaves and on the surface of plants (Taylor et al., 2003), is a kind of natural senior fatty alcohol with prominent physiological activity. Because only very few amounts can be ingested from diets, octacosanol must be supplied in extra to gain

health benefits. Most studies that were evaluating the efficacy of octacosanol used either a wheat-germ oil extract (policosanols) or a natural mixture of primary alcohols isolated from sugar cane wax (*Saccharum officinarum* L.), of which octacosanol is the main component (Saint-John and McNauhgtton, 1986; Kato et al., 1995). Recent studies in humans and rodents have demonstrated that octacosanol is involved in the functions of antifatigue (Kim et al., 2003), regulating lipid metabolism (Kato et al., 1995), reducing cholesterol (Hernandez et al., 1992), cytoprotective activity (Carbajal et al., 1996) and promoting the energy metabolism (Oliaro-Bosso et al., 2009) and so on. Now, octacosanol has been widely used in health food, medicine and other fields. With the gradual improvement of the concerns to food safety, the development and application of green feed additives become the research hot spots. Octacosanol with high safety (Castaño et al., 1995) and effective functions can be regarded as a new feed additive, which will have great development and application value. Tiamulin is a

* Corresponding author.

E-mail address: qiguanghai@caas.cn (G. Qi).

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kind of double terpene antibiotics, which can inhibit bacteria and promote the growth of animals by combining with the 50S subunit of bacterial ribosome. However, the safety problems of antibiotics and drug residues have been paid more and more attention in recent years.

There are few studies on octacosanol in the domestic animals. The research in broilers showed that feed supplementation with 25 mg/kg octacosanol increased the body weight by 4% and improved the feed efficiency by 5.9% compared with the control diet (Xu and Shen, 1997). In our previous study in weaning piglets, we found dietary octacosanol supplementation obviously increased the average daily gain (ADG) by 6.45% and significantly improved feed conversion ratio (FCR) by 6.35% compared with the control diet, and had no differences in growth performance compared with the tiamulin diet. Moreover, pigs fed octacosanol had lower diarrhea ratio and higher antioxidant ability (Long et al., 2015). The mechanism may be that octacosanol can regulate the glycogen in body and maintain the balance of energy metabolism (Xiang et al., 2012a). At present, the studies on the energy metabolism of octacosanol in the domestic animal are few reported. In order to probe the active mechanism of octacosanol in animal body and amplify the application in livestock and poultry productions, this study therefore, discussed the variation of indexes on energy metabolism through determining triiodothyronine (T₃), thyroxine (T₄), growth hormone (GH), glucagon (GU) and adrenaline (AD) in blood of weaning piglets, and analyzing the gene expressions of glucose transporter protein (GLUT-4) and adenosine monophosphate protein kinase (AMPK) in the liver and muscle tissue. Meanwhile, by comparing with the effects of tiamulin, the different regulatory mechanism on animal body was illustrated between octacosanol and antibiotics, which provided a theoretical basis for the application of octacosanol in feed field.

2. Materials and methods

2.1. Materials

Octacosanol (purify 53.7%, batch number 131125) was extracted from rice bran.

2.2. Experimental design and management

The animal protocols for the present study were approved by the Animal Care and Use Committee of Feed Research Institute of the Chinese Academy of Agriculture Sciences. A total of 105 cross-bred piglets ([Yorkshire × Landrace] × Duroc) with an initial body weight (BW) of 5.70 ± 1.41 kg (21 d of age) were assigned randomly to 3 dietary treatments based on their sex and BW. Each pen housed 5 pigs, and there were 7 replicate pens per treatment. All pigs were housed in a temperature and humidity controlled room. Each pen was equipped with a 1-sided, stainless-steel self-feeder and a nipple drinker that allowed pigs ad libitum access to feed and water. Dietary treatments were as followed: control group (basal diet, without antibiotics); tiamulin group (basal diet supplemented with tiamulin at 33 mg/kg feed) and octacosanol group (basal diet supplemented with octacosanol at 8 mg/kg feed). All nutrients in diets were formulated to meet or exceed the recommendation of NRC (2012) for weaning pigs and fed in a mash form (Table 1). The experiment lasted 6 weeks.

2.3. Sample collection

On the last day of the experiment, 2 piglets (1 male, 1 female), with BW close to the average weight were selected from each replicate and killed after a 12 h fasting. Blood samples were

Table 1

Composition and nutrient levels of the basal diet (as-fed basis).

Item	Phase 1 (29 to 42 days of age)	Phase 2 (43 to 70 days of age)
Ingredient, %		
Extruded corn	44.49	61.97
Soybean meal	16.20	25.30
Fermented soybean meal	5.00	2.50
Fish meal	3.50	
Soy oil	2.55	1.05
Lactose	8.30	
Whey	10.00	5.00
CaHPO ₄	1.50	1.50
Sugar	3.00	
Plasma powder	3.00	
L-Lys	0.39	0.46
DL-Met	0.30	0.24
L-Thr	0.19	0.20
Choline chloride	0.10	0.10
Permix ¹	0.30	0.30
Limestone	0.98	1.13
NaCl	0.20	0.25
Total	100.00	100.00
Nutrient level², %		
ME, MJ/kg	14.80	14.26
CP	20.00	19.00
Lysine	1.50	1.35
Methionine	0.62	0.53
Methionine + Cysteine	0.97	0.84
Ca	0.95	0.90
Total P	0.75	0.70
Available P	0.55	0.43
EE	5.02	3.98
Crude fiber	1.87	2.45

ME = metabolizable energy; CP = crude protein; EE = ether extract.

¹ The premix provided the following per kg of the diet: vitamin A, 11,025 IU; vitamin D₃, 1,103 IU; vitamin E, 44 IU; vitamin K₃, 4.4 mg; riboflavin, 8.3 mg; nicotinic acid, 50 mg; thiamine, 4 mg; D-pantothenic acid, 29 mg; choline chloride, 166 mg; vitamin B₁₂, 33 µg; Fe (as FeSO₄), 50 mg; Cu (as CuSO₄), 12 mg; Zn (as ZnSO₄), 85 mg; Mn (as MnSO₄), 8 mg; I (as KI), 0.28 mg; Se (as Na₂SeO₃), 0.15 mg.

² All nutrient levels were calculated values except crude protein level.

collected via anterior vena cava puncture. At the time of collection, blood samples were collected into both nonheparinized tubes and vacuum tubes containing 75 µL 10% EDTA-Na₂ and 100 µL trasylol to obtain serum and plasma, respectively. After collection, serum samples were centrifuged (3,000 × g) for 10 min at 4°C and plasma samples were put on ice for 15 min then centrifuged (3,000 × g) for 15 min at 4°C. All supernate fluid was split into 1.5 mL centrifuge tubes and immediately stored at -70°C until required for the analysis of hormone levels.

Liver (rear-left profile) and semitendinosus samples were collected (approximately 10 g of each tissue to remove as much blood as possible) and frozen in liquid nitrogen immediately and stored at -70°C until required for mRNA analysis.

2.4. Hormone measurements

Serum levels of T₃ and T₄, plasma levels of GH, GU and AD were determined by radio immunoassay using assay kits obtained from Beijing North Biotechnology Institute (Beijing, China) and Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China), respectively.

2.5. Determination of gene expressions of GLUT-4 and AMPK in liver and muscle

2.5.1. Total RNA extraction

According to the method of Yang et al. (2012), 100 mg liver and muscle samples were pulverized in liquid nitrogen. Total RNA was

isolated from homogenate using the TRIZOL reagent (Invitrogen, USA) and extracted according to the operation of kit specification. The integrity of RNA was checked by agarose gel electrophoresis. The quality of RNA was determined by ultraviolet spectroscopy (Thermo Fisher Scientific, DE, USA).

2.5.2. cDNA synthesis

First strand cDNA was synthesized with a RevertAid RT-PCR kit (Fermentas) and stored at -20°C immediately.

2.5.3. Primer design and synthesis

The nuclear encoded β -actin gene and GLUT-4, AMPK genes in liver and muscle were used to assess mtDNA copy number (relative to nuclear genome). The primers were designed using Oligo 6.0 (Molecular Biology Insights, Cascade, CO) and are presented in Table 2. The primers were synthesized by Shanghai Jikang Biotechnology CO., Ltd. (Shanghai, China).

2.5.4. Real-time PCR

Real-time quantitative PCR analyses was performed using a CFX96 Real-Time System (Bio-Rad Laboratories, Hercules, CA) using the SYBR Green PCR Core Reagents (Beijing TransGen Biotech, Beijing, China). The reaction mixture (10 μL) contained 1 μL template cDNA, 5 μL SYBR Green I, 3.4 μL DEPC water, 0.2 μL ROX Reference Dye (50 \times), and 0.2 μL each of forward and reverse primers. Thermal conditions for PCR were as follows: pre-denaturation 95°C for 10 s, followed by 45 cycles of 95°C for 10 s, 54.7°C for 30 s, and 75°C for 15 s. Each sample was run in triplicate for analysis. At the end of the PCR cycles, melting curve analysis was performed to validate the specific generation of the expected PCR product. The relative mRNA expression ratio of target genes were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method (Livak and Schmittgen, 2001).

2.6. Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS 17.0 for windows and expressed as mean and pooled SEM. Means were compared using Duncan's multiple range test. Differences were considered statistically significant at $P < 0.05$ and greatly significant at $P < 0.01$ unless otherwise stated.

3. Results

3.1. Effects of octacosanol on hormone levels in blood of weaning piglets

The results of hormone levels in blood of weaning piglets are presented in Table 3. Dietary octacosanol supplementation significantly increased the level of T_3 compared with the control and tiamulin diets ($P < 0.01$). No differences in T_4 level were observed in all groups ($P > 0.05$). Piglets fed 8 mg/kg octacosanol diet had the highest levels of GH, GU and AD, which increased by 12.81, 7.24 and 67.12% compared with piglets fed the control diet ($P < 0.01$) and by

Table 3
Effects of dietary octacosanol on hormone levels in blood of weaning piglets.¹

Item	Control group	Tiamulin group	Octacosanol group
T_3 , ng/mL	0.49 \pm 0.01 ^B	0.48 \pm 0.01 ^B	0.56 \pm 0.01 ^A
T_4 , ng/mL	58.35 \pm 0.79	57.37 \pm 1.47	58.09 \pm 1.58
GH, ng/mL	2.03 \pm 0.03 ^B	1.94 \pm 0.03 ^B	2.29 \pm 0.05 ^A
GU, pg/mL	187.81 \pm 2.66 ^B	185.82 \pm 2.70 ^B	201.40 \pm 1.14 ^A
AD, ng/mL	0.73 \pm 0.01 ^B	0.75 \pm 0.02 ^B	1.22 \pm 0.02 ^A

T_3 = triiodothyronine, T_4 = thyroxine, GH = growth hormone, GU = glucagon, AD = adrenaline.

^{A,B} In the same row, values with the same or no letter superscripts mean no significant difference ($P > 0.05$), while with different capital letter superscripts mean greatly significant difference ($P < 0.01$).

¹ $n = 7$ per treatment with 14 pigs each.

18.04, 7.24 and 62.67% compared with piglets fed tiamulin diet ($P < 0.01$), respectively. There were no differences between tiamulin and control groups in T_3 , T_4 , GH, GU or AD ($P > 0.05$).

3.2. Effects of octacosanol on the gene expressions of GLUT-4 and AMPK in liver and muscle of weaning piglets

3.2.1. The gene expression of GLUT-4

The results of dietary octacosanol supplementation on gene expression of GLUT-4 in liver and muscle of pigs are presented in Fig. 1 and Fig. 2A. Piglets fed octacosanol had higher ($P < 0.05$) gene expression of GLUT-4 in both liver and muscle tissue than piglets fed control or tiamulin diets. No differences were observed in gene expression of GLUT-4 between tiamulin and control groups ($P > 0.05$).

3.2.2. The gene expression of AMPK

Gel electrophoretogram of AMPK gene RT-PCR products are shown in Fig. 3. One light belt of amplified products, which was close to 250 bp, was seen in sample lanes. The results in Fig. 2B indicated that octacosanol supplementation significantly up-regulated the gene expression of AMPK in liver and muscle compared with the control or tiamulin diet ($P < 0.05$). Similarly, no differences were observed in gene expression of AMPK between tiamulin and control groups ($P > 0.05$).

4. Discussion

Thyroid hormones, including T_3 and T_4 , can take part to regulate body metabolism and have two-ways regulation effects to the growth of animals (Li et al., 2012). The increasing levels of thyroid hormones can promote cell differentiation, organs upgrowth and anti-stress property (Duran-Montg e et al., 2009). Growth hormone is a peptide hormone secreted by adenohypophysis cells. Research has confirmed that GH plays an important role in physiological regulation, which can regulate energy metabolism in body, promote protein synthesis and accelerate lipid disintegrating by inhabiting the consuming of glycogen (Luna et al., 2013). Thereby,

Table 2
The design of the gene sequences.

Gene	Sequence accession No.	Primer sequence (5' – 3')	Fragment size, bp
GLUT-4	NM_001128433.1	F: CTGGACCCATCCCGTGGTTCAT R: CAAATGTCCGCCCTCTGGATTACG	234
AMPK	NM_001167633.1	F: AGGTGTACGGAGCTATGCAGCA R: AGCCGTGAGCTGTGAAGAAC	213
β -actin	U07786	F: CTCCTCCGTGGAGAAGAGCTA R: CCTTCTGCAACCTGTCCGCAA	248

GLUT-4 = glucose transporter protein; AMPK = adenosine monophosphate protein kinase.

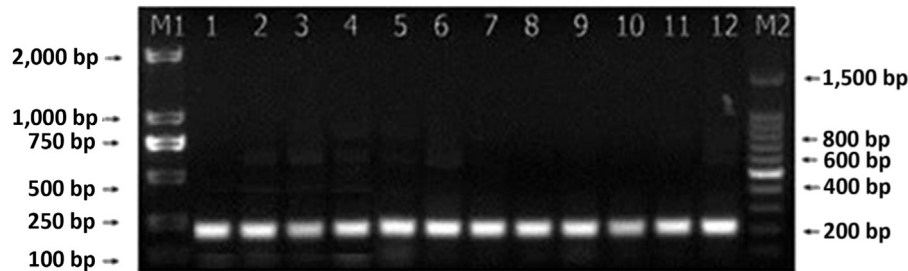


Fig. 1. Agarose gel electrophoretogram of glucose transporter protein (GLUT-4) gene RT-PCR products. M1, DNA marker1 DL2000; 1, 2, Control group muscle; 3, 4, Control group liver; 5, 6, Tiamulin group muscle; 7, 8, Tiamulin group liver; 9, 10, Octacosanol group muscle; 11, 12, Octacosanol group liver; M2, DNA marker2 DL1500.

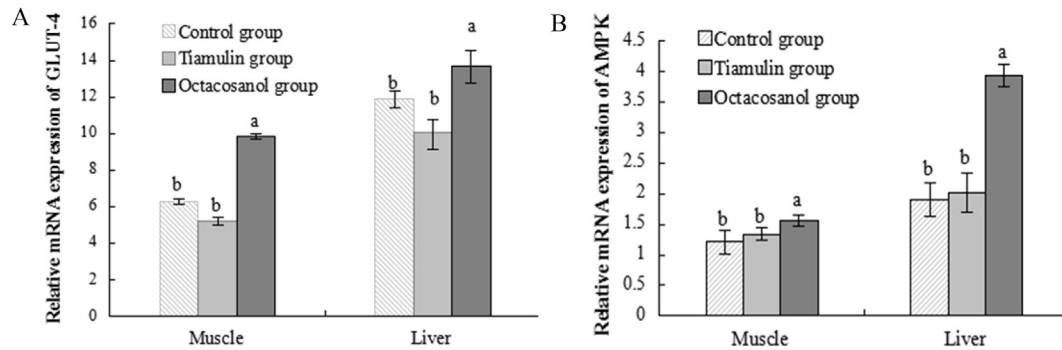


Fig. 2. The relative gene expressions of glucose transporter protein (GLUT-4) and adenosine monophosphate protein kinase (AMPK) in muscle and liver of weaning piglets. Piglets were fed control ($n = 7$), tiamulin ($n = 7$), or octacosanol product ($n = 7$) diets for 6 weeks. Results concerning (A) relative gene expression of GLUT-4, and (B) relative gene expression of AMPK are shown. Data are presents as means \pm SEM; values without a common superscript on bars differ ($P < 0.05$).

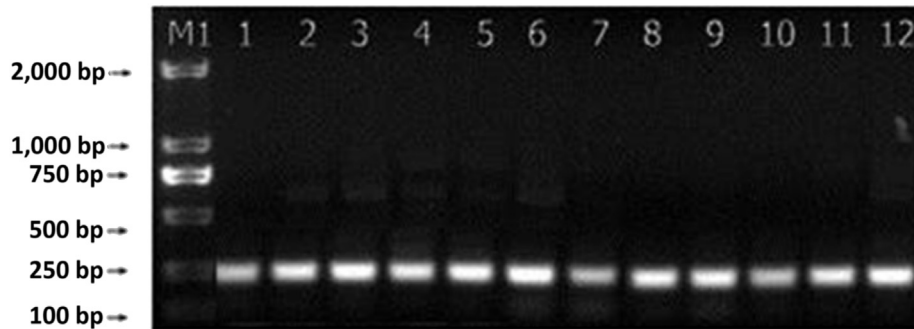


Fig. 3. Agarose gel electrophoretogram of adenosine monophosphate protein kinase (AMPK) gene RT-PCR products. M1, DNA marker1 DL2000; 1, 2, Control group muscle; 3, 4, Control group liver; 5, 6, Tiamulin group muscle; 7, 8, Tiamulin group liver; 9, 10, Octacosanol group muscle; 11, 12, Octacosanol group liver.

energy can be utilized by lipid metabolism instead of glycogen metabolism in the body (Zhou et al., 2015). Ren et al., (2007) also indicated that GH was a main physiological factor to improve the protein deposition of pigs in the grower phase. In this study, dietary octacosanol supplementation increased the levels of T_3 and GH in blood. Similar results were obtained that dietary supplementation of octacosanol promoted GH secretion in blood of rats (Yang, 2012). Accordingly, it can be presumed that octacosanol in body may result in protein deposition and maintain energy balance by promoting the secretion of thyroid hormones and GH, thus reduce utilization of protein for energy by accelerating lipid oxidation in stress situations and obtain the improvement of growth performance of piglets.

Glucagon and AD are both glycogen metabolic hormones. Glucagon is secreted by pancreatic alpha cell in pancreas of the vertebrate. It can activate adenylate cyclase by c-AMP-PK system and excite phosphorylase to promote glycogenolysis and increase

blood sugar consequently (Sapsee et al., 2002). The secretion of GU is regulated by the concentration of blood sugar and increased with the reducing of the concentration of blood sugar and lacking of energy supplementation (Chiou et al., 1990). Adrenaline, which is a physiological accommodation hormone, is secreted by adrenal medulla and released into blood. Adrenaline has prominent functions in promoting catabolism, enhancing glycogenolysis in liver, regulating balance of blood sugar and improving ability of anti-stress (Rosa, 1997; Shilov et al., 2005; Sirota et al., 2008). The secretion of AD in body can induce the adaptive changes of the metabolism of carbohydrates, lipids and proteins, so as to relieve the stress effect. Studies showed octacosanol was effective in modulating lipid metabolism and enhancing glycogen storage or saving glycogen utilization (Kato et al., 1995). Research in running rats indicated octacosanol reduced the utilization of muscle and blood glucose, and promoted the oxidation of fat in muscle tissue (Crowley et al., 1996). The latest study showed

0.75% octacosanol supplementation for 4 weeks significantly increased the contents of glycogen in muscle of rats (Kim et al., 2003). In present study, dietary supplementation of octacosanol promoted the secretion of GU and AD in weaning piglets, which was accordance with the study result that the secretion of GU and AD in rats blood was increased by octacosanol supplementation (Xiang et al., 2012b). Accordingly, from this study, it is illuminated that the regulation of octacosanol on glycogen metabolism in animals is mainly through promoting secretion of GU and AD, which can improve the anti-stress ability and reduce the diarrhea ratio of weaning piglets. The current study also showed that the level of the blood hormone in piglets fed tiamulin diet was not affected, which indicated that the growth of the animal fed tiamulin diet was not promoted by the change of hormone levels, and the mechanism of action between tiamulin and octacosanol was different.

Glucose transport protein can transport glucose for the cellular oxidative respiration and regulate body energy metabolism (Tozzo et al., 1995). The experiment in mice proved that inhibiting the expression of GLUT-4 gene in the muscle could reduce the tolerance ability to glucose, on the contrary, it could significantly improve the blood glucose level of diabetic model mice (Chiappe and Caldiz, 2004). The trial in human demonstrated that insulin could significantly increase the expression of GLUT-4 in patients with type I diabetes mellitus (Schalin-Jäntti et al., 1994), which indicated the level of blood glucose was closely related to the expression of GLUT-4. Belke et al. (2001) also reported through the over-expression of GLUT-4 gene in rat heart, the rates of glucose uptake and glycolysis were significantly improved, and the content of glycogen was increased, which indicated that GLUT-4 could activate the process of glycolysis and promote the synthesis of glycogen. In this experiment, dietary octacosanol supplementation significantly up-regulated the gene expression of GLUT-4 in liver and muscle of weaning piglets compared with tiamulin and control diets. It may be explained that octacosanol can promote glucose uptake, glycogen synthesis and glycolysis, and maintain the energy balance by up-regulating the gene expression of GLUT-4 in the body, which reveals the mechanism of the effect of octacosanol on the growth performance of weaning piglets. No effect was observed in the gene expression of GLUT-4 in tiamulin group, which showed that tiamulin had no regulation function to the glycogen in the animal body.

Adenosine monophosphate protein kinase system is an important signal protein in the regulation of energy change of skeletal muscle. In the activated state, AMPK may induce the glucose transport in tissue cells and supply ATP to meet the consumption of the body (Tsou et al., 2011). Furthermore, AMPK activation increases cardiac muscle glucose uptake through translocation of GLUT-4 via a pathway that is independent of phosphatidylinositol 3-kinases (PI3K) (Russell and Bergeron, 1999). The current study showed that dietary supplementation of octacosanol significantly promoted the gene expression of AMPK in liver and muscle tissue of weaning piglets compared with the control and tiamulin diets. Oliaro-Bosso et al. (2009) research also indicated that octacosanol enhanced phosphorylation of AMPK, promoted the generation of ATP, and inhibited the consumption of ATP during fatty acid and cholesterol synthesis process. Accordingly, it can be known that octacosanol can activate AMPK, rapidly increase the supply of ATP *in vivo*, and thus maintain the balance of energy metabolism and relieve stress. It explains the mechanism of octacosanol to increase the sensitivity and anti-stress ability of the animal in molecular level. And then it may clarify the mechanism of octacosanol on relieving diarrhea degree of weaning piglets. As the same as GLUT-4, tiamulin had no regulation effect to the gene expression of AMPK.

5. Conclusion

In summary, different from the mechanism of tiamulin, octacosanol can promote the secretion of T₃, GH, GU and AD in blood of weaning piglets, improve the gene expressions of GLUT-4 and AMPK in muscle and liver tissues, which control the body's energy balance by two pathways of hormones and gene expression, so as to improve the growth performance, relieve weaning stress, and reduce the ratio of diarrhea. This information may provide theoretical support for the development of octacosanol as a safe and efficient feed additive and the possibility of application in domestic animals, which suggests that octacosanol product may be an alternative to use as antibiotics in diets for weaning piglets.

Conflict of interest statement

We certify that there is no conflict of interests with any financial, professional or personal that might have influenced the performance or presentation of the work described in this manuscript.

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References

- Belke DD, Larsen TS, Gibbs EM, Severson DL. Glucose metabolism in perfused mouse hearts overexpressing human GLUT-4 glucose transporter. *Amer J Physiol Endocrinol Metab* 2001;280:420–7.
- Carbajal D, Molina V, Valdes S, Arruzazabala L, Rodeiro I, Mas R, et al. Possible cytoprotective mechanism in rats of D-002, an anti-ulcerogenic product isolated from beeswax. *J Pharm Pharmacol* 1996;48:858–60.
- Castano G, Más R, Nodarse M, Illnait J, Fernández L, Fernández JC. One-year study of the efficacy and safety of policosanol (5 mg twice daily) in the treatment of type II hypercholesterolemia. *Curr Ther Res* 1995;56:296–304.
- Chiappe CG, Caldiz CI. Insulin resistance and GLUT-4 glucose transporter in adipocytes from hypertensive rats. *Metabol Clin Exp* 2004;53:382–7.
- Chiou GC, Shen ZF, Zheng YQ. Adjustment of blood sugar levels with insulin and glucagon eyedrops in normal and diabetic rabbits. *J Ocul Pharm* 1990;6:233–41.
- Crowley MA, Willis WT, Matt KS, Donovan CM. A reduced lactate mass explains much of the glycogen sparing associated with training. *J Appl Physiol* 1996;81:362–7.
- Duran-Montgé P, Theil PK, Lauridsen C, Esteve-García E. Fat metabolism is regulated by altered gene expression of lipogenic enzymes and regulatory factors in liver and adipose tissue but not in semimembranosus muscle of pigs during the fattening period. *Anim Int J Anim Bio* 2009;3:1580–90.
- Hernandez F, Illait J, Mas R, Castano G, Fernandez L, Gonzalez M, et al. Effects of policosanol on serum lipids and lipoproteins in healthy volunteers. *Curr Ther Res* 1992;51:568–75.
- Kato S, Karino KI, Hasegawa S, Nagasawa J, Nagasaki A, Eguchi M, et al. Octacosanol affects lipid metabolism in rats fed on a high-fat diet. *Br J Nutr* 1995;73:433–41.
- Kim H, Park S, Han DS, Park T. Octacosanol supplementation increases running endurance time and improves biochemical parameters after exhaustion in trained rats. *J Med Food* 2003;6:345–51.
- Li QM, Mair C, Schedle K, Hammerl S, Schodl K, Windisch W. Effect of iodine source and dose on growth and iodine content in tissue and plasma thyroid hormones in fattening pigs. *Eur J Nutr* 2012;51:685–91.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods* 2001;25:402–8.
- Long L, Gao MZ, Peng K, Sun J, Wang SX. Effects of octacosanol extracted from rice bran on production performance and blood parameters in weaning piglets. *J Chin Cereals Oils Assoc* 2015;30:94–100 [in Chinese].
- Luna M, Rodríguez-Méndez AJ, Luna-Acosta JL, Carranza M, Arámburo C. Expression and function of chicken bursal growth hormone (GH). *Gen Compar Endo* 2013;190:182–7.
- National Research Council. Nutrient requirements of swine. 11th rev ed. Washington, DC: National Academic Press; 2012.
- Oliaro-Bosso S, Calcio Gaudio E, Mantegna S, Giraudo E, Meda C, Viola F, et al. Regulation of HMGCoA reductase activity by policosanol and octacosadienol, a new synthetic analogue of octacosanol. *Lipids* 2009;44:907–16.
- Ren JB, Zhao GY, Li YX, Meng QX. Influence of dietary lysine level on whole-body protein turnover, plasma IGF-I, GH and insulin concentration in growing pigs. *Livest Sci* 2007;110:126–32.

- Rosa LF. Effect of adrenaline on lymphocyte metabolism and function. A mechanism involving cAMP and hydrogen peroxide. *Cell Biochem Funct* 1997;15:103–12.
- Russell RR, Bergeron R. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. *Amer J Physi Heart Circulatory Physi* 1999;46:H643.
- Saint-John M, McNaughton L. Octacosanol ingestion and its effects on metabolic responses to submaximal cycle ergometry, reaction time and chest and grip strength. *Int Clin Nutr Rev* 1986;6:81–7.
- Sapse AM, Rothchild R, Jain Duli C, Unson Cecilia G. The role of salt bridge formation in glucagon: an experimental and theoretical study of glucagon analogs and peptide fragments of glucagon. *Mole Med* 2002;8:251–62.
- Schalin-Jääntti C, Yki-Järvinen H, Koranyi L, Bourey R, Lindström J, Nikula-Ijäs P, et al. Effect of insulin on GLUT-4 mRNA and protein concentrations in skeletal muscle of patients with NIDDM and their first-degree relatives. *Diabetologia* 1994;37:401–7.
- Shilov YI, Lanin DV, Orlova EG. Effect of adrenaline on the functions of rat peripheral blood phagocytes under stress and upon hydrocortisone administration. *Dokl Biol Sci* 2005;400:39–41.
- Sirota TV, Yelisyyeva OP, Khunderyakova NV, Makhotina OA. Amaranth seed oil: effect of oral administration on energetic functions of rat liver mitochondria activated with adrenaline. *Biochem Mosc Suppl Ser A Membr Cell Biol* 2008;2:40–7.
- Taylor JC, Rapport L, Lockwood GB. Octacosanol in human health. *Nutrition* 2003;19:192–5.
- Tozzo E, Shepherd PR, Gnudi L, Kahn BB. Transgenic GLUT-4 overexpression in fat enhances glucose metabolism: preferential effect on fatty acid synthesis. *Amer J Physi* 1995;268:956–64.
- Tsou P, Zheng B, Hsu CH, Sasaki AT, Cantley LC. A fluorescent reporter of AMPK activity and cellular energy stress. *Cell Metabo* 2011;13:476–86.
- Xiang Y, Yang H, Wu X, Liu JP. Research and application progress of energy metabolism of body controlled by octacosanol. *J Xinxiang Univ* 2012a;29:44–6 [in Chinese].
- Xiang Y, Yang H, Li LL, Wu X. Rat body GS gene expression regulating by octacosanol. *J Xinxiang Univ Nat Sci Ed* 2012b;29:47–51 [in Chinese].
- Xu RP, Shen H. Application of octacosanol in broilers diet. *Feed Res* 1997;5:26 [in Chinese].
- Yang H. The preparation of octacosanol and the mechanism of the impact on energy metabolism. *Changsha University of Science And Technology*; 2012 [in Chinese].
- Yang H, Li F, Kong X, Yuan X, Lian G, Geng M, et al. Molecular cloning, tissue distribution and ontogenetic expression of Xiang pig chemerin and its involvement in regulating energy metabolism through Akt and ERK1/2 signaling pathways. *Mol Biol Rep* 2012;39:1887–94.
- Zhou YL, Wei XH, Zi ZH, Zou BJ, Xia SS, Lu NS, et al. Potassium diformate influences gene expression of GH/IGF-I axis and glucose homeostasis in weaning piglets. *Livest Sci* 2015;172:85–90.