Oral administration of dibutyryl adenosine cyclophosphate improved growth performance in weaning piglets by enhancing lipid fatty acids metabolism

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A B S T R A C T

Dibutyryl adenosine cyclophosphate (dbcAMP-Ca), an analog of cyclic adenosine monophosphate (cAMP), plays greater roles in regulating physiological activities and energy metabolism than cAMP. The aim of this study was to investigate the effect of oral administration of dbcAMP-Ca on growth performance and fatty acids metabolism in weaning piglets. A total of 14 early weaning piglets (7 ± 1 d of age, 3.31 ± 0.09 kg, Landrace × Large White × Duroc) were randomly divided into 2 groups: control group and dbcAMP-Ca group, and the piglets received 7 mL of 0.9% NaCl or 1.5 mg dbcAMP-Ca dissolved in 7 mL of 0.9% NaCl per day for 10 d, respectively. The results showed that the average daily gain (ADG) increased by 109.17% (P < 0.05) in the dbcAMP-Ca group compared with the control group. Besides, dbcAMP-Ca significantly decreased blood high density lipoprotein cholesterol (HDL-C) concentration (P < 0.05) and significantly increased blood low density lipoprotein cholesterol (LDL-C) concentration (P < 0.05) compared with the control group. Further, liver C18:2n6t content significantly increased in dbcAMP-Ca group (P < 0.05) compared with the control group. With the increase of C18:2n6t content, the mRNA expression levels of peroxisome proliferator-activated receptor α (PPARα) and hormone sensitive glycerol three lipase (HSL), of which genes are related to lipid metabolism, were also significantly increased (P < 0.05 or P < 0.01). All of the results indicated that dbcAMP-Ca improved the ADG, which was probably done by regulating fatty acids metabolism in the liver of weaning piglets.

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1. Introduction

Neonatal piglets face heavy challenge to adapt to the shift between intrauterine and extraterine environments because of weak gut absorptive capacity, low immunity and adaptability, etc (Tanghe et al., 2014; Wang et al., 2017). Normally, weaning usually occurred in an early period at around 21 d of age. However, under the integrated production, weaning time of piglets gets earlier and earlier. Weaning in piglets may lead to worse situation and result in weaning stress in piglets, thus may affect their health and welfare with a decline in feed intake and be vulnerable to disease (Duan et al., 2015).
Milk lipids are the main sources of energy for sucking piglets. An earlier study has found that respiratory entropy of newborn piglets was reduced after birth which indicated that piglets used large amounts of fatty acids to provide energy (Hales, 1997). A former study also showed that lipids in the milk provided nearly 50% of the energy for suckling piglets (Hobbins, 1997). However, it has been reported that the activity of pancreatic lipase increased with age but weaning made it sharply decline (Aumaitre and Corring, 1978; Cera et al., 1990). Therefore, fatty acid, 1 of the 3 major nutrients, plays significant roles in growth, metabolism and physiological functions in newborn mammals because of their considerable energy needs and defective dietary capacity (Gruppuso et al., 1994; Hardy and Kleinman, 1994; Goodyer et al., 2001). Herein, the decomposition and utilization of fatty acids are of great significance to newborn piglets.

Interestingly, cyclic adenosine monophosphate (cAMP), which has been shown to mediate the hormonal regulation of lipid metabolism (Butcher et al., 1968; Gagelin et al., 1999), is vital in regulating and utilizing fatty acids (Luiken et al., 2002; Madsen et al., 2008). Moreover, dibutyryl adenosine cyclophosphate calcium (dbcAMP-Ca, Fig. 1), an analog of cAMP, can regulate the lipid metabolism remarkably in growing and finishing pigs (Gao et al., 2010). dBcAMP affects the metabolism of fatty acids and the growth performance in weaning piglets. Therefore, the present study was intended to seek the effect of oral administration of dbcAMP-Ca on growth performance and lipid fatty acids metabolism in weaning piglets.

2. Materials and methods

2.1. Animals and treatments

The animal experiment was approved by the Protocol Management and Review Committee of the institute of Subtropical Agriculture, Chinese Academy of Science. Pigs were fed by artificial breast feeder and had ad libitum access to water and the basal milk.

2.2. Samples collection

Before slaughter, 5 mL blood samples were collected from the jugular vein, and plasma samples were obtained by centrifugation at $3,000 \times g$ for 10 min at 4 °C, followed by being immediately stored at −80 °C for later lipid profiles analysis (Wu et al., 2016). Liver samples were taken from each animal, followed by being flash frozen in liquid nitrogen and stored at −80 °C prior to RNA isolation and at −20 °C for fatty acid analysis, respectively.

2.3. Fatty acids analysis in liver of piglets

The extraction of fatty acids from 500 mg of the liver tissue and the methylation were performed. The concentration of individual fatty acids was quantified according the peak area and expressed as a percentage of total fatty acids by gas chromatography (GC-2010, Shimadzu Corp, Japan) as previously described (Tan et al., 2009; Raj et al., 2010).

2.4. RNA extraction and cDNA synthesis

About 100 mg of the liver tissue was pulverized in liquid nitrogen. Total RNA was isolated from the homogenate using TRIzol reagent (Invitrogen, CA, USA). The concentration of total RNA was quantified spectrophotometrically (Nanodrop ND-1000; Thermo Fisher Scientific, DE, USA) at 260 nm, and the ratio of 260 nm to 280 nm was used to assess RNA quality, then cDNA synthesis was carried out using a PrimeScript RT reagent Kit With gDNA Eraser (TaKaRa, Dalian, China). Primers (Table 2) were designed by Primer 5.0 based on GenBank (http://www.ncbi.nlm.nih.gov/pubmed/), and Oligo Synthesis was conducted by Sangon Biotech (Shanghai, China). β-actin was chosen as a reference gene.

| Ingredients (%) and nutrient levels (%) of the basal milk (air-dry basis). |
|--------------------|-----------------|-----------------|
| Ingredients          | Content          | Nutrient levels | Content          |
| Skimmed milk powder  | 85.0             | DE, Mj/kg       | 14.65            |
| Dried whey           | 5.0              | CP              | 20.50            |
| Glucose              | 2.5              | Ca              | 0.70             |
| Plasma proteins      | 3.5              | Total P         | 0.60             |
| Premix*              | 4.0              | Lys             | 1.45             |
| Total                | 100.0            | Met             | 0.48             |
|                     |                  | Try             | 0.29             |

* The premix provided the following for per kg of the basal milk: vitamin A1 500 IU, vitamin D3 200 IU, vitamin E 85 IU, D-pantethenic acid 35 mg, vitamin B6 12 mg, folic acid 1.5 mg, nicotinic acid 35 mg, vitamin B12 2.5 mg, biotin 0.2 mg, vitamin B12 0.05 mg, copper (as copper sulfate) 15 mg, ferrum (as ferrous sulfate) 100 mg, manganese (as manganese sulfate) 20 mg, iodate (as calcium iodate) 1.0 mg, selenium (as sodium selenite) 0.35 mg, cobalt (as cobalt sulfate) 0.2 mg, and chromium (as chromium picolinate) 0.2 mg.
monounsaturated fatty acid (MUFA) C18:1n9t and C18:1n9c decreased by 12.50% and 5.14% \((P < 0.05)\) in dbcAMP-Ca group, respectively. Meanwhile, the liver content of C18:3n3 decreased by 30.00% in dbcAMP-Ca group \((P < 0.05)\) compared with the control group.

### 3.4. mRNA expression levels of lipid metabolism related genes

To further confirm the role of lipid metabolism in the liver, the mRNA expression levels of the lipid metabolism related genes, fatty acid synthases (FAS), hormone sensitive glycerol three lipase (HSL), acetyl-CoA carboxylase \(\alpha\) (ACC\(\alpha\)), carnitine palmitoyl transferase 1\(\alpha\) (CPT-1\(\alpha\)), carnitine palmitoyl transferase 1\(\beta\) (CPT-1\(\beta\)), peroxisome proliferator-activated receptor \(\alpha\) (PPAR\(\alpha\)), were detected by qRT-PCR. As shown in Fig. 3, compared with the control group, dbcAMP-Ca significantly increased the mRNA expression level of PPAR\(\alpha\) \((P < 0.05)\) and extremely significantly increased that of HSL \((P < 0.01)\) in the liver of piglets. However, there were no differences between the control group and dbcAMP-Ca group for the mRNA expression levels of FAS, ACC\(\alpha\), CPT-1\(\alpha\) and CPT-1\(\beta\).

### 4. Discussion

Dibutyryl adenosine cyclophosphate (dbcAMP-Ca), as an analog of cAMP, exerts effects via stimulating cAMP signaling pathway (Arnold et al., 2010), such as cell proliferation and differentiation, hormones release and regulation (Boghaert et al., 1991; Chrenek et al., 2010, 2013). And former studies have also found that

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**Table 2**

| Genes   | Primers Sequence (5' to 3') | Size, bp |
|---------|------------------------------|----------|
| \(\beta\)-actin | Forward TGCGGACATCAAGGAGAAC | 216      |
| FAS     | Forward GTCCTGCTAAGCTCAACTC | 206      |
| PPAR\(\alpha\) | Forward GCCATCATTTGTCGCGGAGAC | 139      |
| CPT-1\(\alpha\) | Forward CACTAAAAACTGCTCTTCTAG | 118      |
| CPT-1\(\beta\) | Forward CGCAAGTCGTCAGGATACAAA | 100      |
| ACC\(\alpha\) | Forward TGGGCTTGGGAAACAGAAGAC | 211      |
| HSL     | Forward GAGGCGGAGCTATGGGCC | 130      |

**Table 3**

| Item      | Groups\(^1\) | P-value |
|-----------|--------------|---------|
| TC        | Control      | dbcAMP-Ca |         |
| TG        | 2.47±0.15    | 2.38±0.12 | 0.65    |
| HDLC      | 0.36±0.03    | 0.32±0.04 | 0.56    |
| LDL-C     | 1.21±0.07\(^*\) | 1.03±0.04 | 0.04    |
| T-bil     | 0.80±0.07    | 0.93±0.07\(^*\) | 0.03 |
| HDLC      | 20.70±2.06   | 21.39±4.76 | 0.80 |
| D-bil     | 2.54±0.50    | 1.97±0.88 | 0.51    |
| TBA       | 24.17±6.57   | 14.07±2.69 | 0.18 |

\(^{1}\) Control group: a basal diet; dbcAMP-Ca group: the basal diet supplemented with 1.5 mg/d of dbcAMP-Ca. \(^\ast\) Indicates a significant difference \((P < 0.05)\) between the control group and dbcAMP-Ca group.
growth hormone could be better stimulated through Ca$^{2+}$ and cAMP-dependent interactive mechanism thus enhance the production performance of animals (Sartini et al., 1996; Pahan et al., 1998). In this study, the supplement of dbcAMP-Ca significantly increased the ADG and promoted the growth of early weaning piglets, which might be caused by the interactive effect of Ca$^{2+}$ and dbcAMP.

Lipid, as a kind of necessary substance for animals, plays a vital role in maintaining cell structure and function (Smith et al., 2003; Liu et al., 2017). Blood lipid concentrations were regarded as the status of dynamic lipid absorption and nutritional in animals and humans (Li et al., 2016). Notably, increasing levels of blood constituents are associated with the increasing of dietary nutrients (Brungardt, 1963; Sink et al., 1973). In this regard, the current result of the elevated blood concentrations of LDL and HDL was influenced by dbcAMP-Ca treatment when compared with the control group. In our results, LDL concentration increased while HDL decreased, which seemingly shows lipid metabolic disturbance and it might confer the risk for cardiovascular disease according to the former studies (Gupta and Rajagopal, 2007; Shin, 2009). However, for the fast growing piglets, high concentrations of LDL and low HDL may represent a high level of nutrition, which is in line with the significant change in the ADG. Furthermore, fatty acids composition in the liver also reflects the changes in fatty acid metabolism. The contents of polyunsaturated fatty acid (PUFA) and MUFA decreased whereas the content of saturated fatty acid (SFA) increased in dbcAMP-Ca group when compared with the control group, and this could be explained by the high LDL and low HDL in blood. According to the former studies, the proportion of PUFA is affected by many factors, such as synthesis rate, and mutual conversion (Enser et al., 2000). Besides, PUFA can be oxidized to supply energy to the organism (Tebby et al., 1994; Clarke, 2000). Our study indicated that the supplement of dbcAMP-Ca could promote the oxidation of PUFA and might provide energy for piglets to meet the requirements of growth and development. For the further researches on lipid metabolism in the liver, we detected the mRNA expression levels of lipid metabolism related genes. Hormone sensitive glycerol three lipase is a rate-limited enzyme in triglyceride decomposition which cleaves fatty acids from triglycerides and diglycerides (Watt, 2013). Peroxisome proliferator-activated receptor α (PPARα) has been regarded as the major regulators of lipid metabolism (Ajuwon et al., 2003). Triglyceride (TG) is synthesized via FAS catalyzing acetyl coenzyme A and malonate coenzyme A. Moreover, it is the major form required for body fat deposition (Semenkovich, 1997; Yan et al., 2002). In this experiment, dbcAMP-Ca increased the mRNA expression levels of PPARα and HSL in the liver, which indicated that the addition of dbcAMP-Ca mainly promoted lipolysis and inhibited lipid deposition in the liver, thereby promoted the usage of milk lipids, and thus provided more energy for sucking piglets (Luiken et al., 2002; Madsen et al., 2008; Jia et al., 2018).

Taken together, oral administration of dbcAMP-Ca can significantly increase the weight gain of piglets and affect blood HDL and LDL concentrations, decrease the content of PUFA and enhance metabolism of fatty acids in the liver, which may be through the decomposition of lipids by PPARα and HSL in the liver to provide more energy to ensure the healthy growth of piglets.

5. Conclusions

Conclusively, the present study suggests that dbcAMP-Ca has a significant effect on the growth performance mainly by its regulation effects on lipid metabolism. In the future, more well-designed researches will be needed to investigate the effects of dbcAMP-Ca on early weaning piglets.

Conflicts of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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**Table 4**

Long chain fatty acid content (%) in liver (n = 7).

| Item          | Groups $^1$ | P-value |
|---------------|------------|---------|
|               | Control    | dbcAMP-Ca |
| C18:0         | 0.02 ± 0.003 | 0.02 ± 0.003 | 0.69 |
| C18:1n9t      | 0.08 ± 0.03 | 0.08 ± 0.03$^*$ | 0.03 |
| C18:1n9c      | 0.75 ± 0.27 | 0.75 ± 0.27 | 0.09 |
| C16:0         | 0.07 ± 0.01 | 0.07 ± 0.01 | 0.34 |
| C16:1         | 15.73 ± 0.56 | 15.73 ± 0.56 | 0.59 |
| C17:0         | 0.41 ± 0.03 | 0.41 ± 0.03 | 0.75 |
| C18:0         | 21.24 ± 0.61 | 22.21 ± 1.08 | 0.47 |
| C20:0         | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.61 |
| C16:1         | 0.91 ± 0.21 | 0.93 ± 0.37 | 0.94 |
| C17:1         | 0.04 ± 0.01 | 0.03 ± 0.00 | 0.23 |
| C18:0n6t      | 0.08 ± 0.02 | 0.07 ± 0.04 | 0.79 |
| C18:0n6c      | 17.73 ± 0.98 | 16.77 ± 0.75 | 0.45 |
| C20:0n6t      | 0.01 ± 0.003 | 0.013 ± 0.008$^*$ | 0.04 |
| C20:0n6c      | 15.95 ± 0.37 | 16.07 ± 0.67 | 0.88 |
| C18:3n6       | 0.16 ± 0.02 | 0.17 ± 0.02 | 0.76 |
| C18:3n3       | 0.17 ± 0.02 | 0.12 ± 0.05 | 0.14 |
| C20:0         | 0.31 ± 0.03 | 0.28 ± 0.03 | 0.47 |
| C20:3n6       | 2.45 ± 0.20 | 2.53 ± 0.32 | 0.83 |
| C22:0n6       | 17.67 ± 0.37 | 17.36 ± 0.82 | 0.76 |
| SFA           | 6.70 ± 0.76 | 6.49 ± 0.23 | 0.82 |
| MUFA          | 39.12 ± 0.6 | 40.00 ± 0.70 | 0.28 |
| MUFA          | 17.82 ± 0.98 | 16.85 ± 0.76 | 0.44 |
| PUFA          | 80.52 ± 0.86 | 77.48 ± 1.11 | 0.08 |

$^*$ Indicates a significant difference ($P < 0.05$) between the control group and dbcAMP-Ca group.

$^1$ Control group: a basal diet; dbcAMP-Ca group: the basal diet supplemented with 1.5 mg/d of dbcAMP-Ca.
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