

Effects of Calcium Dibutyryl Cyclic Adenosine Monophosphate on Growth Performance and Serum Biochemical Parameters in Finishing Pigs (Postprint)

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Abstract

This experiment aimed to investigate the effects of calcium dibutyryl cyclic adenosine monophosphate on growth performance and serum biochemical indices of finishing pigs. Eighteen three-way crossbred finishing pigs (Landrace × Yorkshire × Duroc) with similar age and body weight [(83.18±\$1.62) kg] and good health status were selected and randomly divided into 2 groups with 9 replicates per group. The control group was fed the basal diet, while the experimental group was fed the basal diet supplemented with 40 mg/kg calcium dibutyryl cyclic adenosine monophosphate. The experimental period lasted 30 days. The results showed that, compared with the control group: 1) dietary supplementation of calcium dibutyryl cyclic adenosine monophosphate increased the average daily gain of finishing pigs by 30.12 g ($P > 0.05$); 2) dietary supplementation of calcium dibutyryl cyclic adenosine monophosphate significantly increased serum lactate dehydrogenase activity ($P < 0.05$), while significantly decreasing serum urea content ($P < 0.05$); 3) dietary supplementation of calcium dibutyryl cyclic adenosine monophosphate significantly decreased the contents of serine, glycine, and aspartic acid in serum non-essential amino acids ($P < 0.05$), while significantly increasing the contents of threonine, tryptophan, valine, alanine, and isoleucine in essential amino acids ($P < 0.05$). These results indicate that calcium dibutyryl cyclic adenosine monophosphate can improve the growth performance of finishing pigs to a certain extent, which may be related to its promotion of amino acid metabolism.

Full Text

Effects of Dibutyryl Cyclic Adenosine Monophosphate-Calcium on Growth Performance and Serum Biochemical Indices of Finishing Pigs

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Abstract: This study investigated the effects of dibutyryl cyclic adenosine monophosphate-calcium (dbcAMP-Ca) on growth performance and serum biochemical indices of finishing pigs. Eighteen healthy “Landrace × Large White × Duroc” crossbred finishing pigs with similar age and body weight [(83.18±\$1.62) kg] were randomly allocated into two groups with nine replicates per group. The control group was fed a basal diet, while the experimental group received the basal diet supplemented with 40 mg/kg dbcAMP-Ca. The trial lasted for 30 days. The results showed that, compared with the control group: (1) dietary supplementation of dbcAMP-Ca increased average daily gain by 30.12 g ($P>0.05$); (2) dbcAMP-Ca significantly increased serum lactic dehydrogenase activity ($P<0.05$) while significantly decreasing serum urea content ($P<0.05$); and (3) dbcAMP-Ca significantly reduced serum contents of the non-essential amino acids serine, glycine, and aspartic acid ($P<0.05$), while significantly increasing serum contents of the essential amino acids threonine, tryptophan, valine, alanine, and isoleucine ($P<0.05$). These findings indicate that dbcAMP-Ca can improve growth performance in finishing pigs, likely by modulating amino acid metabolism.

Keywords: dibutyryl cyclic adenosine monophosphate-calcium; finishing pigs; free amino acids; protein deposition; amino acid metabolism

Cyclic adenosine monophosphate (cAMP) is a non-specific, small-molecule, highly bioactive substance that serves as a second messenger for various hormones including growth hormone and epinephrine, and is widely distributed in organisms [1]. Formed through the cyclization of ATP catalyzed by adenylate cyclase upon stimulation by external signals (such as certain hormones or other molecular signals), cAMP plays crucial roles in regulating enzyme activity [2], gene expression [3], ion channel activity [4], and cell growth [5], thereby exerting broad regulatory effects on nutritional metabolism. As a promising

animal growth regulator, dibutyryl cyclic adenosine monophosphate-calcium (dbcAMP-Ca) is a cAMP derivative that, upon absorption, releases calcium ions (Ca^{2+}) to accelerate calmodulin binding and elevate cAMP levels while maintaining relative stability. Consequently, dbcAMP-Ca shares the same physiological functions as cAMP, including regulating glucose metabolism through activation of the cAMP-protein kinase system and glycogen phosphorylation [6], and modulating lipid and protein metabolism by enhancing lipase activity, with effects varying by developmental stage—primarily promoting protein metabolism during growth phases and lipid metabolism during finishing phases [7].

Additionally, dbcAMP-Ca functions as a growth regulator with low polarity and lipophilic properties, enabling it to cross cell membranes and exert intracellular effects. This characteristic overcomes limitations of cAMP, allowing direct supplementation via feed. After fulfilling its physiological role, excess dbcAMP-Ca can be degraded by phosphodiesterase without residual accumulation, offering enhanced safety [8-9] and greater chemical stability *in vivo*. However, research on the effects of dbcAMP-Ca on growth performance and amino acid metabolism regulation in finishing pigs remains limited. Therefore, this study primarily investigated the influence of dbcAMP-Ca on growth performance and serum biochemical indices in finishing pigs.

1.1 Experimental Design

Eighteen healthy “Landrace \times Large White \times Duroc” crossbred finishing pigs with similar age and initial body weight [(83.18 \pm 1.62) kg] were randomly divided into two groups, each comprising nine replicates with one pig per replicate. The control group received a basal diet formulated according to NRC (2012) standards, with composition and nutrient levels shown in Table 1. The experimental group was fed the basal diet supplemented with 40 mg/kg dbcAMP-Ca (calculated as pure product based on 98% purity). Pigs were fed twice daily at 08:00 and 14:00, with approximately 3.3 kg/d feed allocation per pig, and provided *ad libitum* access to water. The trial was conducted at the experimental base of Changsha Xingjia Bio-Engineering Co., Ltd., lasting 30 days under strict adherence to farm biosecurity and management protocols.

1.2 Experimental Materials

The dbcAMP-Ca used in this study had a purity of 98% and was provided by Hangzhou Meiya Pharmaceutical Co., Ltd.

1.3 Sample Collection and Analysis

1.3.1 Growth Performance Metrics

Pigs were fasted for 12 hours to record initial and final body weights. Daily feed intake was monitored using an automatic feeding system to calculate average

daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.3.2 Serum Biochemical Indices

At the conclusion of the trial, six pigs were randomly selected from each group and fasted for 12 hours before jugular vein blood collection (10 mL). Blood samples were placed in vacuum tubes, allowed to clot at room temperature for 15 minutes, then centrifuged at 4,000 rpm for 10 minutes. Serum was harvested, aliquoted into EP tubes (5 mL each), and stored at -80°C for subsequent analysis. Serum biochemical parameters were measured using a Mindray BS-190 automatic biochemical analyzer with corresponding reagent kits.

1.3.3 Serum Free Amino Acid Content

A 300 L serum sample was mixed with 8% sulfosalicylic acid at a 1:1 ratio, vortexed, and incubated at 4°C for approximately 8 hours. After centrifugation at 10,000 rpm for 10 minutes, the supernatant was filtered through a 0.45 μm membrane into amino acid sample vials. Concentrations of glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), alanine (Ala), arginine (Arg), tyrosine (Tyr), cysteine (Cys), valine (Val), methionine (Met), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys), and proline (Pro) were determined using an L-8800 automatic amino acid analyzer (Beckman).

1.4 Data Processing and Statistical Analysis

Experimental data were processed using Microsoft Excel 2007 and expressed as mean ± standard error of the mean (SEM). Independent samples t-tests were performed using SAS 6.0 software to compare differences between experimental and control groups, with statistical significance set at $P < 0.05$.

2 Results and Analysis

2.1 Effects of dbcAMP-Ca on Growth Performance of Finishing Pigs

As shown in Table 2, no significant differences were observed in growth performance between the experimental and control groups ($P > 0.05$). The experimental group exhibited a 2.6% increase in daily feed intake and a 30.12 g improvement in daily gain, though the feed-to-gain ratio remained comparable between groups.

2.2 Effects of dbcAMP-Ca on Serum Biochemical Indices of Finishing Pigs

Table 3 demonstrates that dietary dbcAMP-Ca supplementation significantly reduced serum lactic dehydrogenase (LDH) activity by 6.82% ($P < 0.05$) while significantly increasing serum urea content by 49.35% ($P < 0.05$). No significant differences were detected in other serum biochemical parameters between the two groups ($P > 0.05$).

2.3 Effects of dbcAMP-Ca on Serum Free Amino Acid Content of Finishing Pigs

As presented in Table 4, compared with the control group, the experimental group showed significantly decreased serum concentrations of the non-essential amino acids serine (Ser), glycine (Gly), and aspartic acid (Asp) ($P < 0.05$), while significantly increasing levels of the essential amino acids threonine (Thr), valine (Val), isoleucine (Ile), phenylalanine (Phe), and tryptophan (Trp) ($P < 0.05$). No significant differences were observed for other amino acids between groups ($P > 0.05$).

3 Discussion

3.1 Effects of dbcAMP-Ca on Growth Performance of Finishing Pigs

As a cAMP derivative, dbcAMP-Ca exhibits similar physiological functions [10]. Dibutyryl cyclic adenosine monophosphate (dbcAMP) possesses low polarity and lipophilic properties that enable cellular membrane penetration and intracellular activity, overcoming the limitations of cAMP and facilitating direct feed supplementation. Previous research has demonstrated that cAMP and its derivatives can activate protein kinases, enhance metabolic enzyme activity, and promote substrate protein phosphorylation, thereby accelerating histone replication and transcription, enhancing acidic protein transcription, and ultimately stimulating nucleic acid and mRNA synthesis to improve protein synthesis and animal production performance [11-12]. Zhang et al. [13] reported that cAMP injection significantly promoted weight gain and improved feed conversion ratio in broilers. Navegantes et al. [14] found that elevating intracellular cAMP levels in rats significantly reduced proteolytic enzyme activity in skeletal muscle, thereby increasing net protein deposition and lean meat percentage. Tian [15] observed that dietary dbcAMP supplementation significantly improved daily gain and carcass lean percentage while reducing feed-to-gain ratio in growing-finishing pigs. Feng et al. [16] reported significant growth-promoting effects of subcutaneous cAMP injection in 10 kg piglets. Yang et al. [17] demonstrated marked improvements in daily gain following one month of subcutaneous cAMP administration in pigs.

In contrast, the present study found only non-significant improvements in growth performance in finishing pigs, which contradicts previous findings. This discrepancy may be attributed to differences in physiological stages. Gao et al. [7] utilized pigs weighing less than 30 kg that were in the growth phase dominated by protein anabolism, where growth-promoting effects are more pronounced. In contrast, the current study employed pigs weighing approximately 83 kg in the finishing phase, where dbcAMP-Ca primarily promotes lipid metabolism, leading to substantial fat deposition in subcutaneous and abdominal tissues and affecting carcass quality, while exerting minimal effects on protein metabolism. Consequently, growth performance improvements were less pronounced than in previous studies. Consistent with our findings,

Yan et al. [18] reported that dbcAMP supplementation significantly improved daily gain and reduced feed-to-gain ratio during the growth phase, but did not significantly affect growth during the finishing phase, where it primarily promoted lipolysis and reduced body fat deposition.

3.2 Effects of dbcAMP-Ca on Serum Biochemical Indices and Free Amino Acid Content

Alterations in serum biochemical indices reflect changes in metabolic function and cellular permeability [19]. Serum LDH activity and urea content serve as indicators of carbohydrate and protein metabolism efficiency in livestock. LDH is a key enzyme in glycolysis that catalyzes the conversion of pyruvate to lactate [20]. Serum urea content accurately reflects protein metabolism and amino acid balance, with lower urea levels indicating higher protein synthesis rates [21]. The present study found significantly lower serum LDH activity in the experimental group, suggesting that dbcAMP-Ca may inhibit glycolysis during the finishing phase. The significantly higher serum urea content in the experimental group may be functionally related to the primary effect of dbcAMP-Ca on adipose tissue metabolism rather than protein metabolism during finishing. Under adequate nutrient intake, serum albumin may increase as a nutritional reserve while decreasing in metabolic pathways, reducing protein deposition and consequently elevating serum urea content. Hahn et al. [22] similarly reported that lower urea concentrations more effectively promote muscle growth, which aligns with our observation of non-significant growth performance effects.

Serum free amino acids are closely associated with amino acid metabolism and protein deposition, reflecting the nutritional status of animals [23]. The current results indicate that dbcAMP-Ca supplementation promoted amino acid metabolism, as evidenced by significantly reduced serum concentrations of the non-essential amino acids Ser, Gly, and Asp. Aspartic acid primarily participates in the tricarboxylic acid cycle through deamination to oxaloacetate and is closely involved in the urea cycle. Through transamination, Asp generates oxaloacetate and Glu, with Glu participating in interconversion with other amino acids and contributing to urea synthesis [24]. The elevated serum urea content in the experimental group may be associated with Asp-facilitated urea cycle activity. Furthermore, the essential amino acid Thr was significantly increased in the experimental group. Ser, Gly, and Thr are three critical amino acids in nucleotide metabolism that can interconvert through enzymatic reactions: Ser can be converted to Gly via methyltransferase catalysis; threonine dehydrogenase, the primary degradative enzyme for Thr, converts approximately 80% of Thr to Gly; and Gly can be reversibly converted to Ser by SHMT [25]. The observed reduction in Ser and Gly alongside increased Thr suggests partial conversion from Ser and Gly to Thr. As the second or third limiting amino acid in pigs, Thr constitutes an important component of body protein and participates in protein synthesis [26]. Appropriate Thr supplementation can compensate for Gly deficiency and modulate feed intake, with numerous studies demonstrating its

significant influence on amino acid metabolism [27-28]. In addition to Thr, the branched-chain amino acid Ile was also significantly elevated. Branched-chain amino acids function in oxidative energy supply, promote protein synthesis, and inhibit protein degradation [29]. These findings suggest that dbcAMP-Ca may affect amino acid metabolism in finishing pigs, though the specific mechanisms require further investigation.

In conclusion, dbcAMP-Ca can improve growth performance in finishing pigs, potentially by promoting amino acid metabolism, significantly affecting serum amino acids related to nucleotide metabolism (Ser, Gly, Thr), and increasing branched-chain amino acids (Val, Ile) as well as Phe and Trp content.

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