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Original Research Article

# Effects of a two-meal daily feeding pattern with varied crude protein levels on growth performance and antioxidant indexes in pigs

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ABSTRACT

The present study aimed to evaluate the effects of daily feeding pattern on growth performance, blood biochemistry, and antioxidant indexes in pigs. One hundred and eighty female Duroc × Landrace × Yorkshire (DLY) pigs with similar body weight (11.00 ± 0.12 kg) were randomly assigned to 3 groups: the control group (fed 17.01% CP diet, twice daily); high-low group (H-L group, fed 18.33% CP diet in the morning, followed by 15.70% CP diet in the afternoon); and low-high group (L-H group, fed 15.70% CP diet in the morning, followed by 18.33% CP diet in the afternoon) ($n = 6$). Comparable amounts of their respective diets were given at 05:30 and 15:00 throughout the experimental periods to make all the treatments consumed the same type of food and the same amount of calories on a daily basis. On day 30, one pig was randomly selected per litter for blood samples. Compared with the control group, ADG in the H-L and L-H groups increased by 8.11% and 16.23%, but not significant ($P > 0.05$); and blood urea nitrogen (BUN) in the H-L and L-H groups decreased by 26.76% and 41.04% ($P < 0.05$), respectively. The H-L group feeding pattern could significantly improve levels of serum superoxide dismutase (SOD), when compared with the control group. These findings suggest that the two-meal daily feeding pattern with varied levels of CP affects serum levels of BUN and SOD. These changes could effectively silightly improve growth performance and antioxidant capacity in pigs without incurring increased feeding costs.



## 1. Introduction

Studies have shown that circadian rhythms are widely observed in plants, animals, fungi, and cyanobacteria and are regulated by endogenous molecular oscillators referred to as circadian clocks (Schibler, 2005; Panda et al., 2002). In mammals, important daily activities, such as sleep/wake cycles, metabolic homeostasis, cardiovascular activity, the endocrine system, regulation of body temperature, gastrointestinal tract motility and metabolism, are governed by the endogenous circadian clock (Green et al., 2008; Hastings et al., 2003; Reppert and Weaver, 2002, Dunlap, 1999). Physiological processes have intrinsic biological rhythm and exhibit intrinsic circadian phenomena (Feng and Lazar, 2012). In pigs, nutrient digestion, metabolism, and other aspects of physiological activity have shown typical circadian changes. Their digestive capacity and basal metabolic rate tends to be lower at the afternoon than during the daytime (Wu and Yin, 2015). In mammals, nutrient intake in the morning plays an important role in circadian regulation and metabolism (Holt et al., 1999). Digestive function and the basal metabolic rate decline during the afternoon and diet-induced thermogenesis is maximal in the morning and minimal at the afternoon (Romon et al., 1993). Studies have shown that the timing of carbohydrate and fat intake on

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a given day can significantly affect glucose tolerance and the insulin index, thereby increasing body weight (Bray et al., 2010). Antioxidant enzymes have a circadian rhythm, which has been thought to be an important part of the physiological response to oxidative stress in living things (Krishnan et al., 2008).

Difference in dietary protein contents affects circadian rhythm of nutrient metabolism. In a recent report, it has been reported that a high protein meal given in the evening (40% of energy as protein) significantly increases the plasma free amino acids concentration measured on the next morning, which is more than 12 h after the meal (Nishioka et al., 2013). Compared with the normal group, a high protein meal fed in the morning and a low protein meal fed in the evening significantly increases the average daily gain (ADG) of growing pigs (Xie et al., 2014). In the past, ration formulation according to the dietary requirements of specific developmental phases has formed the basis of pig feeding practices. In the present study, we focused on the effects of a two-meal daily feeding pattern with varied levels of dietary protein on several parameters in pigs, including growth performance, blood biochemistry, and antioxidant indexes.

## 2. Materials and methods

### 2.1. Diet composition

The nutrient levels of the experimental diets met the NRC (2012) recommendations for pigs within the weight range used in present study and the Feeding Standard of Swine (NY/T 65-2004). The control diet was based on a digestible energy (DE) of 14.00 MJ/kg and CP content of 17.01%. Diet composition and nutrient levels are presented in Table 1.

### 2.2. Animals and experimental design

Female Duroc × Landrace × Yorkshire (DLY) pigs ($n = 18$) with similar body weight (11.00 ± 0.12 kg) were obtained from Henan Guang'an Biology Technology Co., Ltd. (Zhengzhou, China) and randomly assigned to three groups. The control group was fed a control CP diet, twice daily; the high-low (H-L) group was fed a high CP diet and a low CP diet (in that order) daily; and the low-high (L-H) group was fed a low CP diet and a high CP diet (in that order) daily ($n = 6$). The experiment lasted 30 d. Comparable amounts of their respective diets were given at 05:30 and 15:00 throughout the experimental periods to make all the treatments consumed the same type of food and the same amount of calories on a daily basis. On day 30, one pig was chose from each litter and blood samples were obtained for serum.

Pigs in the control group were fed the control diet (CP, 17.01%; DE, 14.00 MJ/kg) at 05:30 and 15:00. Pigs in the H-L group were fed the high-CP diet (CP, 18.33%; DE, 14.17 MJ/kg) at 05:30 and the low-CP diet (CP, 15.70%; DE, 13.83 MJ/kg) at 15:00, whereas pigs in the L-H group were fed the low-CP diet at 05:30 and the high-CP diet at 15:00. To ensure that all pigs consumed the same type of food and the same amount of calories daily, pigs in the H-L and L-H groups were fed comparable amounts of their respective diets at 05:30 and 15:00 throughout the experimental period, according to feed intake in the morning of pigs.

Feed intake was recorded during the investigation, and values for average daily gain (ADG) and feed intake/ADG (F/G) were calculated at the end of the study. No animals were sacrificed in the present study.

**Table 1**
Diet composition and nutrient levels.

| Item | Basal diet | High CP level diet | Low CP level diet |
|---|---|---|---|
| Ingredients, % (air-dry basis) | | | |
| Corn | 68.34 | 64.84 | 71.83 |
| Soybean meal (CP 46%) | 17.73 | 20.73 | 14.74 |
| Wheat bran | 4 | 3.3 | 4.7 |
| Wheat middling | 1.32 | 1.32 | 1.32 |
| Fish meal | 3 | 3.5 | 2.5 |
| Lys (98%) | 0.42 | 0.42 | 0.42 |
| Met (99%) | 0.1 | 0.1 | 0.1 |
| Thr (98.5%) | 0.09 | 0.09 | 0.09 |
| Soybean oil | 1.00 | 1.70 | 0.3 |
| Premix[1] | 4.00 | 4.00 | 4.00 |
| Total | 100.00 | 100.00 | 100.00 |
| Nutrient levels, % (DM basis)[2] | | | |
| CP | 17.01 | 18.33 | 15.70 |
| Lys | 1.17 | 1.26 | 1.08 |
| Met | 0.41 | 0.43 | 0.39 |
| Met + Cys | 0.67 | 0.62 | 0.57 |
| Thr | 0.88 | 0.79 | 0.70 |
| Ca | 0.69 | 0.70 | 0.66 |
| TP | 0.64 | 0.65 | 0.62 |
| AP | 0.37 | 0.39 | 0.35 |
| EE | 4.14 | 4.76 | 3.53 |
| DE, MJ/kg | 14.00 | 14.17 | 13.83 |

[1] The premix provided the following per kg of diets: Fe 100 mg, Zn 25 mg, Cu 20 mg, Mg 0.01 mg, I 0.20 mg, Mn 10.2 mg, Se 0.1 mg, VA 1,500 IU, $VD_3$ 110 IU, $VB_1$ 1 mg, $VB_2$ 15 mg, $VB_{12}$ 0.03 mg, VE 18 IU, citric acid 12 mg, carnitine 0.5 mg, antioxidant 5 mg, mildew preventive 12.5 mg, chromium picolinate 5 mg, $Ca(H_2PO_4)_2$ 285 mg, limestone 300 mg. Feed carrier was zeolite powder.

[2] The nutrient levels were calculated values.

### 2.3. Sample collection

Body weights of individual pigs were measured immediately before feeding at the beginning and end of the trial. On day 30, following 12 h of fasting, 6 piglets that were identified as being closest in BW to the average within each pen were randomly selected from each group. Blood was sampled by venipuncture of the venous sinus and collected in non-heparinized tubes. Serum was obtained by centrifugation at 3000 × $g$ for 15 min at 4°C, and stored immediately thereafter at −20°C until further analysis.

### 2.4. Determination of biochemical parameters

The levels of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), glucose (GLU), lactate dehydrogenase (LDH), total protein (TP), serum ammonia (AMM), blood urea nitrogen (BUN), immunoglobulin G (IgG), calcium (Ca), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and holestenone ( CHO ) were determined using commercial kits (Sino-German Beijing Leadman Biotech Ltd., Beijing, China) and a biochemical analyzer (Beckman CX4, Beckman Coulter Inc., Brea, CA, USA).

### 2.5. Determination of serum antioxidant indexes

The serum levels of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA), and total antioxidant capacity (T-AOC) were determined according to the manufacturer's instructions of the respective commercial kits. All kits were purchased from Nanjing Jiancheng Biotech Co., Ltd (Nanjing, China).

### 2.6. Statistical analysis

Statistical analyses were carried out using one-way ANOVA within the SPSS Statistics 13 software (SPSS Institute, Inc.). All results were expressed as means ± SEM. $P < 0.05$ was considered statistically significant. Differences between individual means were determined by the Duncan's new multiple range test.

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