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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.

3. Results

3.1. Growth performance

The effects of a two-meal daily feeding pattern with varied levels of crude protein on body weight are presented in Table 2. The results showed that compared with the control group, ADG in the H-L and L-H groups increased by 8.11% and 16.23%, but not significant ($P > 0.05$).

3.2. Serum biochemistry

In comparison to the control group, the BUN concentration decreased by 26.76% ($P < 0.05$) in the H-L group, and in the L-H group by 41.04% ($P < 0.05$). Differences observed in serum ALP, AST, GLU, LDH, TP, AMM, IgG, CA, TG, LDL, and HDL were not significant among the 3 groups ($P > 0.05$) (see Table 3).

3.3. Antioxidant indexes

The serum SOD concentration in the H-L group was increased by 14.25% ($P < 0.05$) over that in the control group. However, the T-AOC, CAT and MDA levels in the H-L and L-H groups were not significantly affected by the various feeding patterns ($P > 0.05$) (see Table 4).

4. Discussion

The sleep/wake cycle and fast/feed cycle are the most obvious manifestations of the circadian clocks in mammals. Circadian clocks can affect homeostasis via a series of physiological and behavioral processes. The digestion, absorption, and utilization of nutrients are all regulated by a certain periodicity. This might be the reason for the differences observed in the growth performance of animals fed varied diets on a daily basis (Panda et al., 2002; Reppert and Weaver, 2002).

Food intake resets circadian clocks in peripheral tissues. It is reported that a high protein meal in the evening significantly increased the plasma branched-chain amino acid (BCAA) concentration on the next morning, even more than 12 h after the meal, demonstrating that varying the protein content in the meal pattern with different dietary protein contents may affect the use of some essential amino acids (Nishioka et al., 2013). Our previous study also found that the sequence and quantity of alimentary protein intake affect the insulin/glucagon ratio, as well as amino acid concentrations including BCAA, methionine and serine (Xie et al., 2015). Strong associations exist among the important indexes, ADG, ADFI, and F/G, all of which reflect growth and development in pigs. A high protein meal fed in the morning and a low protein meal fed in the evening can significantly increase the ADG of growing-finishing pigs, a finding that is consistent with those of the present study (Xie et al., 2014).

Serum biochemical parameters are important indicators of physiological and metabolic functions and aid in the evaluation of the general health of the animal. Serum BUN is synthesized via the Krebs–Henseleit cycle and is the final product of protein and amino

Table 3
Effect of daily two-meal pattern with different crude protein levels on serum biochemical indexes of pigs ($n = 6$).[1]

Item	Control group[2]	H-L group[2]	L-H group[2]	*P*-value
ALP, U/L	212.40 ± 17.37	245.25 ± 13.55	204.60 ± 31.18	0.323
AST, U/L	69.80 ± 8.40	53.25 ± 3.99	57.00 ± 4.78	0.426
GLU, mmol/L	3.79 ± 0.22	3.98 ± 0.35	4.63 ± 0.38	0.536
LDH, U/L	600.33 ± 40.54	626.60 ± 37.08	616.00 ± 59.06	0.429
TP, g/L	54.12 ± 1.10	54.78 ± 1.34	56.72 ± 1.76	0.689
Ca, mmol/L	2.24 ± 0.03	2.12 ± 0.05	2.20 ± 0.06	0.745
CHO, mmol/L	2.80 ± 0.28	2.58 ± 0.09	2.59 ± 0.11	0.574
TG, mmol/L	0.61 ± 0.06	0.65 ± 0.03	0.58 ± 0.07	0.689
LDL, mmol/L	1.20 ± 0.13	1.01 ± 0.06	1.00 ± 0.04	0.342
HDL, mmol/L	1.81 ± 0.20	1.86 ± 0.08	1.85 ± 0.09	0.849
IgG, g/L	61.24 ± 7.31	68.06 ± 10.04	55.57 ± 11.73	0.211
AMM, μmol/L	85.33 ± 6.18	71.94 ± 5.34	70.58 ± 8.91	0.209
BUN, mmol/L	4.41 ± 0.37[a]	3.23 ± 0.29[b]	2.60 ± 0.38[b]	0.0001

ALP = alkaline phosphatase; AST = aspartate aminotransferase; GLU = glucose; LDH = lactate dehydrogenase; TP = total protein; Ca = calcium; CHO = cholestenone; TG = triglyceride; LDL = low density lipoprotein; HDL = high density lipoprotein; IgG = immunoglobulin G; AMM = ammonia; BUN = blood urea nitrogen.

[1] Values without letter superscripts within the same row were not significantly different ($P > 0.05$). Different lowercase letter superscripts denoted significant differences ($P < 0.05$).

[2] Pigs in the control group were fed with 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.

Table 4
Effects of daily two-meal pattern with different crude protein levels on blood antioxidation indexes of pigs ($n = 6$).[1]

Item	Control group[2]	H-L group[2]	L-H group[2]	*P*-value
SOD, U/mL	15.72 ± 0.40[b]	17.96 ± 0.31[a]	17.02 ± 1.16[ab]	0.021
T-AOC, U/mL	0.20 ± 0.02	0.22 ± 0.02	0.24 ± 0.05	0.669
CAT, U/mL	27.24 ± 11.72	21.37 ± 4.23	32.30 ± 3.10	0.223
MDA, nmol/mL	0.51 ± 0.04	0.44 ± 0.02	0.46 ± 0.04	0.406

SOD = superoxide dismutase; T-AOC = thetotal antioxidant capacity; CAT = catalase ; MDA = malondialdehyde.

[1] Values without letter superscripts within the same row were not significantly different ($P > 0.05$). Different lowercase letter superscripts denoted significant differences ($P < 0.05$).

[2] Pigs in the control group were fed with 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.

Table 2
Effect of two-meal daily feeding pattern with different levels of crude protein on growth performance in pigs ($n = 6$).[1]

Item	Control group[2]	H-L group[2]	L-H group[2]	*P*-value
Initial of average weight, kg	10.96 ± 0.33	11.12 ± 0.21	10.91 ± 0.42	0.971
End of average weight, kg	22.36 ± 0.71	22.87 ± 0.51	23.39 ± 1.01	0.793
ADFI, kg/d	0.65 ± 0.02	0.68 ± 0.02	0.70 ± 0.03	0.853
ADG, kg/d	0.37 ± 0.02	0.40 ± 0.02	0.43 ± 0.02	0.626
F/G	1.77 ± 0.06	1.70 ± 0.06	1.62 ± 0.02	0.314

ADFI = average feed intake; ADG = average daily gain; F/G = feed intake/ADG.

[1] Values without letter superscripts within the same row were not significantly different ($P > 0.05$). Different lowercase letter superscripts denoted significant differences ($P < 0.05$).

[2] Pigs in the control group were fed with 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.

acid metabolism in animals (Sun et al., 2007). The relatively low levels of BUN observed in the present study suggest that the H-L and L-H feeding patterns contributed to improvement of the rate of protein synthesis. The BUN level in pigs of other experimental groups was significantly lower than that in the control group. This reduction may have relation with the ADG of pigs later in the fattening period. In our previous study, we found that dietary amylose and amylopectin ratio and resistant starch content affects plasma glucose, lactic acid, hormone levels and protein synthesis in splanchnic tissues (Deng et al., 2010). In the present study, H-L or L-H feeding patterns also had the different ratios of starch with protein for different meal time, which may improve the use of dietary protein.

Reactive oxygen species (ROS) were reported to be directly involved in causing oxidative damage in cellular macromolecules such as lipids, proteins, and nucleic acids in tissues leading to oxidative stress. Excessive amounts of these harmful free radicals can be removed by antioxidants and antioxidant enzymes that are capable of protecting tissues from oxidative damage (Xu et al., 2008; Yang et al., 2013). Superoxide dismutase can effectively remove the super-oxide anion, protect cells from damage, and strengthen the antioxidant capacity of pigs. Thus, SOD activity can indirectly reflect the capacity to remove oxygen free radicals. In the present study, the H-L feeding pattern significantly increased levels of serum SOD and improved the antioxidant capacity of pigs. Free radicals are involved in the damage to protein synthesis. In the present study, H-L or L-H feeding patterns may decrease oxidative damage. However, possible causal links between these parameters needs further investigations.

5. Conclusion

In summary, the results of the present study demonstrated that dynamic nutrition can significantly affect levels of serum BUN and SOD, and to some extent, can also affect growth performance. These findings may be attributed to the effects of the circadian clock on homeostasis via a series of physiological and behavioral processes.

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