Effects of the sequence of isocaloric meals with different protein contents on plasma biochemical indexes in pigs
Chunyan Xie, Xin Wu, Jun Li, Zhiyong Fan, Cimin Long, Hongnan Liu, Patrick P. Even, Francois F. Blachier, Yulong Yin

To cite this version:
Chunyan Xie, Xin Wu, Jun Li, Zhiyong Fan, Cimin Long, et al.. Effects of the sequence of isocaloric meals with different protein contents on plasma biochemical indexes in pigs. PLoS ONE, 2015, 10 (8), 10.1371/journal.pone.0125640 . hal-02641496

HAL Id: hal-02641496
https://hal.inrae.fr/hal-02641496
Submitted on 28 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Abstract

Nutrient composition and pattern of food intake may play a significant role in weight gain. The aim of this study was to document the effects of a daily 3-meal pattern with isocaloric diets containing different dietary protein contents on growth performance and different plasma biochemical indexes including amino acid plasma concentration in castrated male pigs. Then, 21 DLY (Duroc×Landrace×Yorkshire) pigs aged 60 days were assigned randomly into 3 groups: a control group (crude protein, CP 18.1%), a group receiving high then basal and then low CP meals (High-Low group) and a group receiving low then basal and then high CP meal (Low-High group) for 40 days with pigs being feed-restricted. On day 40, after 12 h fasting, blood samples were obtained for analysis. The results showed that the insulin/glucagon ratio was lower in the High-Low group (P < 0.05) when compared with the control group. Compared with the control group, the average daily gain of pigs from the High-Low group increased by 14.10% (P = 0.046). Compared with the control group, serum gamma-glutamyl transferase (GGT) decreased significantly (P < 0.05) in both the High-Low and Low-High groups. Plasma concentrations of branched-chain amino acids (BCAA: valine, isoleucine and leucine) increased in the Low-High group (P < 0.05) when compared with the control group; and plasma methionine and serine decreased in both the two experimental groups (P < 0.05). Compared with the High-Low group, all the BCAA increased significantly (P < 0.05) in the Low-High group. These findings suggest 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. It is proposed that meal pattern with pigs receiving high then basal and then low CP meals daily may help to improve the weight gain of pigs.
Introduction

Pattern of nutrient consumption may reset peripheral circadian clocks and the related metabolic processes [1]. Dietary proteins are believed to participate significantly in the control of blood glucose levels. High-protein (HP) diet not only affects glucose homeostasis, but also may modify the metabolism in peripheral tissues [2]. Plasma amino acid concentrations which largely depend on the food ingested is the net result of protein digestion, amino acid and oligopeptide intestinal absorption, utilization of amino acid in anabolic pathways, release of amino acids from protein etc. [3,4]. 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 (PFAA) concentration measured on the next morning, thus even more than 12 hours after the meal [5].

All aspects of physiology, including sleep-wake cycles, cardiovascular activity, endocrine system, body temperature, gastrointestinal tract motility, and metabolism, are influenced by the circadian clock [6]. Genome-wide circadian expression profiling studies have uncovered potential connections between circadian clocks and many aspects of metabolism, including energy, carbohydrate, amino acid, lipid, and protein metabolism [7]. Diets high in fat or sugar have been shown to alter circadian clock function [8]. Recent studies have linked energy homeostasis to the circadian clock at the behavioral, physiological, and molecular levels [9–11], emphasizing that certain nutrients and the timing of food intake and pattern of meals may play a significant role in weight gain [12].

This represents a paradigm shift in pig feeding, since the optimal dietary nutrient level is more and more considered as a dynamic process. Hence, we made the working hypothesis that a daily 3-meal pattern with different dietary protein contents may affect biochemical circulating parameters as well as body physiology and metabolism. Blood metabolite concentrations may help in interpreting some metabolic changes associated with different dietary modifications. Thus, the present study reports on the effects of feeding a daily 3-meal pattern with different dietary protein contents daily on several parameters including blood insulin, glucagon, and AA concentrations in the growing castrated male pigs.

Materials and Methods

Animals and experimental design

Twenty-one DLY (Duroc×Landrace×Yorkshire) barrows aged 60 days were obtained from Hunan New Wellful Co., Ltd. (Changsha city, 410005, China) [13]. The pigs were randomly distributed in 3 experimental groups according to the average body weight (BW = 20.80±1.74 kg, n = 21): control group, High-Low and Low-High groups, with 7 animals in each experimental groups. The pigs were housed in individual pens[14]. The barrows in the control group were fed with control diet (crude protein (CP), 18.1%) at 08:00, 13:00 and 18:00h. High-Low group of pigs was fed with high-CP diet (CP, 21.0%) at 08:00h, control diet at 13:00h and low-CP diet (CP, 15.3%) at 18:00h, while the Low-High group of pigs was fed with low-CP diet at 08:00h, control diet at 13:00h and high-CP diet at 18:00h. The pigs were feed restricted, and daily food amount was adjusted to 4.0% of BW and was given to pigs in 3 meals each day, each time with about one third of the total amount, and equal feed was given in the morning and evening for each group.

The nutrients were adequate for pigs and met the NRC-recommended requirement within the weight range used in the present study (NRC, 1998). The control diet was balanced on the calculated content of digestible energy (DE) (13.72 MJ/kg), CP (181.1 g/kg), apparent ileal
digestible limiting amino acids (Lys, Met+Cys, Thr, Trp), Ca and P (Table 1). The control diet, high-protein and low-protein diets were composed as indicated in Table 1. All animals had free access to drinking water. The dietary intervention lasted 40 d.

Samples collection

Body weights of individual pigs were measured immediately before feeding at the beginning and end of the trial[15]. On day 40, after 12 h fasting, the pigs were immobilized and then blood samples were collected by venipuncture from the venous sinus into two tubes: heparinized tubes and non-heparinized tubes[16]. The plasma was obtained by centrifugation at 3,000×g for 10 min at 4°C and then stored immediately at –80°C until assayed [13,17].

Feed intake and average daily gain (ADG) were recorded, and feed intake/ADG (F/G) was calculated for all pigs[18]. No animals were sacrificed in this study. This study was performed in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences[19].

Serum biochemical indices

An Automated Biochemistry Analyzer (Synchron CX Pro, Beckman Coulter, Fullerton, CA, USA) was used to determine the concentrations of serum glucose, urea nitrogen, gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, total protein according to the commercial kits and manufacturer’s instructions [20]. All the kits were purchased from Beijing Chemlin Biotech Co., Ltd (Beijing, China).

Table 1. Composition of the pig diets (as-fed basis).

| Ingredients                                      | High-Protein diet | Control diet | Low-Protein diet |
|--------------------------------------------------|-------------------|-------------|-----------------|
| Corn (8% CP; < 13% H2O)                          | 54.6              | 63.5        | 72.4            |
| Soybean expanded (43% CP)                        | 29.00             | 21.27       | 13.54           |
| Enzymatically decomposed soybean meal (CP 48%)   | 8.35              | 7.50        | 6.65            |
| L-lysine98%                                      | 0.00              | 0.20        | 0.40            |
| L-methionine                                     | 0.00              | 0.03        | 0.06            |
| L-threonine                                      | 0.05              | 0.10        | 0.15            |
| Soybean oil                                      | 4.00              | 3.40        | 2.80            |
| Premix¹                                          | 4.00              | 4.00        | 4.00            |

Total 100.00 100.00 100.00

Nutrient levels**(%)

| CP 21.04 18.11 15.29 |
|-----------------------|
| Lysine 1.06 1.02 0.99 |
| Methionine 0.33 0.32 0.31 |
| Methionine+Cystine 0.67 0.62 0.57 |
| Threonine 0.88 0.79 0.70 |
| Calcium 0.58 0.60 0.55 |
| Total Phosphorus 0.59 0.62 0.56 |
| Available Phosphorus 0.41 0.42 0.39 |
| Digestible energy (MJ/kg) 13.68 13.72 13.77 |

¹ Providing the following per kg diet (%): polymineral 25; carnitine 0.5; mixed vitamin 11.5; gourmet power 2.5; antioxidant 5; mildew preventive 12.5; Fe2(SO3)3 5; MgSO3 10; Cr 5; P-Ca 285; limestone 300; chaff 91.5; NaCl 75; feed carrier with zeolite powder. CP is Crude protein.

**nutrient level as calculated value.

doi:10.1371/journal.pone.0125640.t001
Serum hormone analyses
Serum insulin and glucagon were determined by radioimmunoassay according to corresponding reagent kit manufacturer’s instructions (China Institute of Atomic Energy, Beijing, China) [21].

Determination of amino acids in plasma
Plasma (0.5 mL) was deproteinized with 0.5 mL of 1.5 mM HClO₄, followed by addition of 0.25 ml of 2 M K₂CO₃ [22]. The neutralized extract was analyzed for amino acids using high-performance liquid chromatography in Beijing Aminolabs (Beijing, China). This method involved the precolumn derivatization of amino acids with ω-phthaldialdehyde and fluorescence detection [23]. Amino acids in samples were quantified on the basis of known amounts of standards (Sigma Chemicals, St. Louis, MO, USA).

Statistical analysis
Data are presented as the mean ± SEM obtained from triplicate experiment. Differences between mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by Post-hoc Tukey tests for multiple comparisons when interactions were significant using SPSS 13.0 [24]. Differences between experimental groups were considered significant for P less than 0.05.

Results
Growth performance
The effects of pattern of consumption of diets with different protein contents daily on body weigh are shown in Table 2. The results showed that, compared with the control group, the ADG of pigs from the High-Low group increased by 14.1% (P = 0.046).
Compared with the control and Low-High groups, F/G in the high-low group was not significantly different but tended to modestly decrease (0.05 < P < 0.1) by 12.3% and 10.5%, respectively (Table 2).

Serum biochemical indices
Serum biochemical indices are shown in Table 3. Compared with the control group, serum glucose concentration from the High-Low and Low-High groups was not significantly different but tended to modestly increased by 6.2% and 10.02%, respectively. Serum GGT decreased significantly (P < 0.05) in both High-Low and Low-High groups, compared with the control group (Table 3).

Table 2. Effects of the daily 3-meal pattern with different dietary protein contents on growth performance of growing pigs.

| Items             | Control group | High-Low group | Low-High group | P value |
|-------------------|---------------|----------------|----------------|---------|
| Initial BW (kg)   | 20.91±0.56    | 20.87±0.69     | 20.64±0.79     | 0.95    |
| Daily feed intake (g) | 1313.64±8.96 | 1324.32±14.05 | 1309.04±14.11 | 0.69    |
| Daily gain (g)    | 528.13±21.41<sup>b</sup> | 606.79±25.32<sup>a</sup> | 546.07±21.39<sup>ab</sup> | 0.048   |
| Feed/Gain         | 2.46±0.06     | 2.15±0.10      | 2.41±0.09      | 0.074   |

Values are expressed as mean ± SEM, n = 7 pigs in each experimental groups.
<sup>a,b</sup> indicate that means with different letters are significantly different (P<0.05).
Compared with the Low-High group, serum LDH in the High-Low group was lower ($P < 0.05$). Compared with the control group, serum urea concentration was not significantly different in the High-Low group and Low-High group but tended to increase by 20.14% and 16.67% (Table 3).

Serum insulin and glucagon

Serum hormone concentrations are shown in Table 4. No difference was observed for insulin, but serum glucagon was higher in the High-Low and Low-High groups, the difference being significant however only in the High-Low group. Accordingly, the insulin/glucagon ratio was lower in both the High-Low and the Low-High groups than in the control one ($P < 0.05$) (Table 4). No difference was observed in the serum insulin concentrations after a High-Low or Low-High meal intake.

Plasma amino acids

For the essential amino acids, compared with the control group, plasma valine was higher in the Low-High group ($P < 0.05$), plasma leucine was lower in the High-Low group ($P < 0.05$) and plasma methionine was lower by 20.4% ($P < 0.05$) and 19.4% ($P < 0.05$) respectively in the High-Low and Low-High (Fig 1).

For the non-essential amino acids, plasma serine and taurine were lower in the two experimental groups ($P < 0.05$) (Fig 2). Differences were also observed between the two experimental groups; plasma concentrations of BCAA (valine, isoleucine and leucine) were larger and plasma arginine was lower in the Low-High group than in the High-Low group.

Similarly, according to the dietary pattern, diets containing an equivalent amount of nutrients had different effects on several indicators of the metabolism of amino acids (Fig 3). Indeed,
Fig 1. Effects of the daily 3-meal pattern with different dietary protein contents on plasma essential amino acids in pigs. N = 7. Means within a row with different letters differ (P < 0.05). Lys, L-lysine; Met, L-methionine; Thr, L-threonine; Trp, L-tryptophan; Phe, L-phenylalanine; His, L-histidine; Val, L-valine; Ile, L-isoleucine; Leu, L-leucine; Arg, L-arginine.

doi:10.1371/journal.pone.0125640.g001

Fig 2. Effects of the daily 3-meal pattern with different dietary protein contents on plasma non-essential amino acids in pigs. n = 7 pigs. Bars with different letter are significantly different (p < 0.05). Ser, L-serine; Tau, taurine; Asp, L-aspartic acid; Asn, L-asparagine; Tyr, L-tyrosine; Cit, L-citrulline; Glu, L-glutamic acid; Gln, L-glutamine; Orn, L-Ornithine; Cys, L-cystine; Ala, L-alanine; Pro, L-proline; Gly, glycine.

doi:10.1371/journal.pone.0125640.g002
plasma 3-Methyl-L-histidine (MHis) was 23.1% higher ($P < 0.05$) and plasma homocysteine (Hyp) was $x\%$ higher ($P < 0.05$) in the Low-High than in the High-Low group.

**Discussion**

Circadian clock plays a crucial role in the regulation of numerous physiological processes and the beneficial versus deleterious effects of a given diet may be partly related to the sequence of dietary intake in the daytime [12]. Circadian rhythms are generated by one of the most ubiquitous timing systems related to numerous behavioral, physiological, cellular and molecular processes that are controlled by an endogenous clock which depends on environmental factors including light, food and stress [25]. Particularly, circadian control of metabolism has been widely studied. The circadian clock controls food intake and energy homeostasis by regulating the expression and/or activity of enzymes involved in the metabolism of numerous compounds including cholesterol, amino acid, lipid, glycogen, and glucose, as well as the secretion of many hormones involved in metabolism, such as insulin, glucagon, leptin, and ghrelin hormones that exhibit circadian oscillation [26]. In addition, diet-induced thermogenesis is known to show circadian variation, with the highest response in the morning and the lowest in the evening in humans [27]. The body’s nutrient digestion, absorption and utilization may differ according to the different periods of time (morning, noon and night), and according to nutritional needs. Thus, eating habits may contribute to body weight changes. The results obtained in the present study, which are primarily related to optimal pig growth in an agronomic context, may be also relevant in terms of human nutrition. Pigs, like humans, are omnivorous animal and make individual meals [28]. Besides their importance in livestock production, pigs are of particular biomedical interest because of their phylogenetic relation to humans, and sharing
many physiological similarities with humans, offers several specific advantages (when compared to non-human primates). Castrated pigs can avoid unwanted or uncontrolled reproduction, and are easy for management. Interestingly, ADG in response to the High-Low feeding method was higher than that measured in the control group and, since caloric intake was an equivalent food efficiency also tended to be increased. In a recent study, modifications of the daily-phase feeding system did not significantly modify body weight but reduced N excretion by 12% ($P < 0.01$) [29]. Whatever the dietary pattern of the nutrient consumption (including protein consumption) during the day, some homeostasis of the circulating and tissue nutrient concentration has to be maintained. Glucose being the primary fuel source for most cells, it is for instance important for blood glucose levels to remain relatively steady. As a matter of fact, blood glucose levels may be affected by the timing of meals and snacks. Experimental data suggest that breakfast frequency and quality may be related in a causal way to appetite and blood sugar control [30]. High-CP diets have been reported to have positive effects on glucose homoeostasis in rats [31] and humans [32]. In the present study, serum glucose concentration from the High-Low and Low-High groups was not significantly different but tended to modestly increased by 6.2% and 10.02% when compared with the control group.

Transamination in cells and tissues is accomplished by enzymes called transaminases or aminotransferases. This process is an important step in the synthesis of some non-essential amino acids and urea synthesis. The activities of enzymes as well as some endocrine responses are related to these metabolic pathways and are also known to be modified by time-restricted feeding [33]. Elevated levels of serum GGT have been found to predict the risk of development of type 2 diabetes in adults and children [34]. In the present study, daily 3-meal pattern with different dietary protein contents affected fasting plasma GGT significantly, suggesting that the transamination system via urea-cycle is affected in the liver at 12 hours after the ingestion of a variable nutritional levels during the day. Further experiments are needed to confirm this hypothesis. However, our results may indicate that daily 3-meal pattern with different dietary protein contents may affect amino acid requirements and metabolic availability. It has been reported recently that elevated ALT levels, within the normal physiological range, are associated with unfavorable nocturnal glucose profiles in Chinese subjects with normal glucose regulation [35].

It is noteworthy that behaviors and physiological activities in animals display circadian rhythms, allowing the organisms to anticipate and prepare for the diurnal changes in the living environment [36]. Plasma amino acid profiles also exhibit a circadian rhythm [37,38]. Amino acids, normally supplied by dietary protein or by endogenous production, are necessary for anabolic metabolism and signaling purposes including the biosynthesis of polypeptides and proteins, and the synthesis of nucleotides. In the present study, we found that among amino acids, the most significant differences between experimental groups were observed for plasma BCAA concentrations and for some urea-cycle related compounds [5]. Plasma concentrations of the BCAA (leucine, isoleucine, and valine) are more prominently affected than the concentrations of other amino acids by changes in dietary-caloric, protein, fat, and carbohydrate-intake in man [39]. In our study, it is interesting to note that plasma BCAA concentrations significantly increased in pigs receiving the Low-High diet in comparison with what is observed in the High-Low group; and tended to be higher when compared with the control group. Among BCAA, leucine has been shown in animal models to promote nitrogen retention and protein synthesis as well as inhibition of protein degradation [40,41]. This finding is consistent with previous observations indicating that a high protein meal in the evening significantly increased the plasma BCAA concentration on the next morning, even more than 12 hours after the meal [5], demonstrating that varying the protein content in the meal pattern with different dietary protein contents may affect the use of some essential amino acids. These changes...
appear partly related to the changes of transaminating enzyme activities, even if possible causal links between these parameters remain to be demonstrated.

It is well known that proteins have a notable impact on glucose homeostasis mechanisms, predominantly through their effects on insulin, incretins, gluconeogenesis, and gastric emptying [42]. Insulin and glucagon are two important hormones for blood glucose regulation. Protein, but not carbohydrate, lead to increased glucagon secretion following a meal [43,44]. According to classical studies, administration of carbohydrate has a protein-sparing effect in the fasting subject [45,46]. Protein induces an increase in insulin concentrations when ingested in combination with carbohydrate. It is reported that a mixture of wheat protein hydrolysate, free leucine, phenylalanine, and carbohydrate can be applied as a nutritional supplement to strongly elevate insulin concentrations [47]. The insulin/glucagon ratio determines whether glucose uptake or output production [48]. It has been reported that the insulin/glucagon ratio is highest during daylight on a high protein diet and in late night on a control diet [49]. In the present study, it is interesting to note that in the High-Low group, we found no effect on insulin, but higher plasma glucagon than in the control group (this tendency was also observed in the Low-High group). In addition the insulin to glucagon ration was very significantly decreased in both experimental groups. This implies that the varying the protein content in the meal affected plasma glucagon and increased the drive for glucose production in the fasting state. Serum glucagon concentration has been significantly correlated with protein intake [50]. Feeding a high-protein diet resulted within 3 hours in an increase in the concentration of insulin (+100%) and glucagon (+220%) [48].

Previous studies have shown that the glucagon response to feeding with protein depends on the increase in plasma amino acid concentrations [51]. It has been reported that the insulin response is closely related to the increase in plasma amino acids, especially leucine, isoleucine, valine, phenylalanine and arginine, regardless of the rate of gastric emptying; while the glucagon response is linearly related to the increase in plasma amino acids, regardless of the rate of gastric emptying or meal composition. Among the plasma amino acids, tyrosine and methionine have been closely related to the plasma glucagon response, and it has been shown that the glucagon response to feeding with protein depends on the increase in plasma amino acid concentrations [51].

**Conclusion**

The findings reported in the present study strongly suggest that the varying the protein content in the meal affects the insulin/glucagon ratio, and amino acids including BCAA, methionine and serine in pigs. In this study, it was found that the varying meal protein content affects circulating biochemical parameters, which may impact the growth performance of pigs, and then may be useful for pig production. In practice, this study suggests that the High-Low feeding procedure improved growth and feed efficiency.

**Acknowledgments**

This paper was jointly supported by grants from the NSFC (31110103909, 31101730), the National Key Technology Research and Development Program of the Ministry of Science and Technology of China (2012BAD39B00), Chinese Academy of Sciences and Knowledge Innovation Project (CXJQ120113-1), Chinese Academy of Sciences President’s International Fellowship Initiative Grant (No.2015VBA009, 2013T250014), China Postdoctoral Science Foundation funded project (2014M552022). Tianyong Zhang from from Henan Guang’an Biology Technology Co. Ltd contributed materials for the completion of the study, Tian-zheng
Gao, Liao Hui and Peng-fei Gao from Henan Guang'an Biology Technology Co. Ltd. provided assistance during the experiments.

**Author Contributions**

Conceived and designed the experiments: CX XW YY. Performed the experiments: XW JL ZF CL. Analyzed the data: FB HL. Contributed reagents/materials/analysis tools: CX XW. Wrote the paper: CX XW HL FB. Helped write the manuscript: PCE.

**References**

1. Oike H, Oishi K, Kobori M. Nutrients, Clock Genes, and Chrononutrition. Curr Nutr Rep. 2014; 3: 204–212. PMID: 25101217
2. Oishi K, Uchida D, Itoh N. Low-carbohydrate, high-protein diet affects rhythmic expression of gluconeogenic regulatory and circadian clock genes in mouse peripheral tissues. Chronobiol Int. 2012; 29: 799–809. doi: 10.3109/07420528.2012.699127 PMID: 22823864
3. Fernstrom JD, Wurtman RJ, Hammarstrom-Wiklund B, Rand WM, Munro HN, Davidson CS. Diurnal variations in plasma concentrations of tryptophan, tyrosine, and other neutral amino acids: effect of dietary protein intake. Am J Clin Nutr. 1979; 32: 1912–1922. PMID: 570361
4. Nasset ES, Heald FP, Calloway DH, Margen S, Schneeman P. Amino acids in human blood plasma after single meals of meat, oil, sucrose and whiskey. J Nutr. 1979; 109: 621–630. PMID: 571014
5. Nishioka M, Imaizumi A, Ando T, Tochikubo O. The overnight effect of dietary energy balance on post-prandial plasma free amino acid (PFAA) profiles in Japanese adult men. PLoS One. 2013; 8: e62929. doi: 10.1371/journal.pone.0062929 PMID: 23667542
6. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002; 418: 935–941. PMID: 12198538
7. Panda S, Hogenesch JB. It's all in the timing: Many clocks, many outputs. Journal of Biological Rhythms. 2004; 19: 374–387. PMID: 15534318
8. Oosterman JE, Kalsbeek A, la Fleur SE, Belsham DD. Impact of nutrients on circadian rhythmicity. Am J Physiol Regul Integr Comp Physiol. 2014; ajpregu 00322 02014.
9. Froy O. Metabolism and Circadian Rhythms-Implications for Obesity. Endocrine Reviews. 2010; 31: 1–24. doi: 10.1210/er.2009-0014 PMID: 19854863
10. Marcheva B, Ramaey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature. 2010; 466: 627–631. doi: 10.1038/nature09253 PMID: 20562862
11. Turek FW, Joshu C, Khozsaka A, Lin E, Ivanova G, McDearmon E, et al. Obesity and metabolic syndrome in circadian Clock mutant mice. Science. 2005; 308: 1043–1045. PMID: 15845877
12. Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian timing of food intake contributes to weight gain. Obesity (Silver Spring). 2009; 17: 2100–2102.
13. Yin J, Duan JL, Cui ZJ, Cui Z, Ren WK, Li TJ, et al. Hydrogen peroxide-induced oxidative stress activates NF-kB and Nrf2/Keap1 signals and triggers autophagy in piglets. RSC Adv., 2015; 5: 15479–15486
14. Xiao H, Wu MM, Shao F, Tan BE, Li TJ, Ren WK, et al. Metabolic profiles in the response to supplementation with composite antimicrobial peptides in piglets challenged with deoxynivalenol. J Anim Sci. 2015; doi: 10.2527/jas2014-8229
15. Yin J, Ren WK, Duan JL, Wu L, Chen S, Li TJ, et al. Dietary arginine supplementation enhances intestinal expression of SLC7A7 and SLC7A1 and ameliorates growth depression in mycotoxin-challenged pigs. Amino Acids, 2014; 46:883–892. doi: 10.1007/s00726-013-1643-5 PMID: 24368521
16. Yin YL, Huang RL, Li TJ, Ruan Z, Xie MY, Deng ZY, et al. Amino acid metabolism in the portal-drained viscera of young pigs: effects of dietary supplementation with chitosan and pea hull. Amino Acids 2010. 39:1581–1587 doi: 10.1007/s00726-010-0577-4 PMID: 20361217
17. Wu X, Ruan Z, Gao Y, Yin Y, Zhou X, Wang L, et al. Dietary supplementation with L-arginine and N-carbamylglutamate enhances intestinal growth and heat shock protein 70 expression in weanling pigs fed a corn- and soybean meal-based diet. Amino Acids. 2010; 39: 831–839. doi: 10.1007/s00726-010-0538-y PMID: 20213438
18. Wu X, Ruan Z, Gao Y, Wang L, Ruan Z, Liu ZQ, et al. Dietary supplementation with L-arginine or N-carbamyl glutamate enhances intestinal growth and heat shock protein-70 expression in weanling pigs fed
19. Yin YL, Yao K, Liu Z, Gong Z, Ruan D, Deng BE, et al. Supplementing L-leucine to a low-protein diet increases tissue protein synthesis in weanling pigs. Amino Acids. 2010; 39:1477–1486. doi: 10.1007/s00726-010-0612-5 PMID: 20473536

20. Deng J, Wu X, Bin S, Gong Z, Ruan D, Deng BE, et al. Dietary amylose and amylopectin ratio and resistant starch content affects plasma glucose, lactic acid, hormone levels and protein synthesis in splanchic tissues. Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde 2010; 94: 220–226

21. Tang ZR, Yin LY, Nyachoti CM, Huang RL, Li TJ, Yang CB, et al. Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharide on serum parameters and the insulin like growth factor-I mRNA expression in early-weaned piglets. Domestic Animal Endocrinology 2005; 28: 430–441. PMID: 15826777

22. Ren WK, Yin J, Wu MM, Liu G, Yang G, Xion Y, et al. (2014) Serum Amino Acids Profile and the Beneficial Effects of L-Arginine or L-Glutamine Supplementation in Dextran Sulfate Sodium Colitis. PLOS ONE, 2014, 9: e88335 doi:10.1371/journal.pone.0088335 PMID: 24505477

23. Yin YL, Huang R, Zhong HY (1993) Comparison of the ileorectal anastomosis and conventional method for the measurement of ileal digestibility of protein sources and mixed diets in growing pigs. Animal Feed Science and Technology 42: 297–308.

24. Huang RL, Yin YL, Wu GY, Zhang YG, Li TJ, Li LL, et al. (2005) Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. Poultry Science, 84: 1383–1388. PMID: 16206559

25. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002; 418: 935–941. PMID: 12198538

26. Froy O. The relationship between nutrition and circadian rhythms in mammals. Front Neuroendocrinol. 2007; 28: 61–71. PMID: 17451793

27. Romon M, Edme JL, Boulenguez C, Lescroart JL, Frimat P. Circadian variation of diet-induced thermogenesis. J Clin Nutr. 1993; 37: 476–480. PMID: 8460600

28. Groenen MA, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, Rothschild MF, et al. Analyses of pig genomes provide insight into porcine demography and evolution. Nature. 2012; 491: 393–398. doi: 10.1038/nature11622 PMID: 23151582

29. Pomar C, Pomar J, Dubeau F, Joannopoulos E, Dussault JP. The impact of daily multiphase feeding on animal performance, body composition, nitrogen and phosphorus excretions, and feed costs in growing-finishing pigs. Animal. 2014; 8: 704–713. doi:10.1017/S1751731114000408 PMID: 24739349

30. Pereira MA, Erickson E, McKee P, Schrankler K, Raatz SK, Lytle LA, et al. Breakfast frequency and quality may affect glycemia and appetite in adults and children. J Nutr. 2011; 141: 163–168. doi: 10.3945/jn.110.114405 PMID: 21123469

31. Blouet C, Mariotti F, Azzout-Marniche D, Bos C, Mathe V, Tome D, et al. The reduced energy intake of rats fed a high-protein low-carbohydrate diet explains the lower fat deposition, but macronutrient substitution accounts for the improved glycemic control. J Nutr. 2006; 136: 1849–1854. PMID: 16772448

32. Bowen J, Noakes M, Trenerry C, Clifton PM. Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. J Clin Endocrinol Metab. 2006; 91: 1477–1483. PMID: 16772448

33. Lamia KA, Storch KF, Weitz CJ. Physiological significance of a peripheral tissue circadian clock. Proc Natl Acad Sci U S A. 2008; 105: 15172–15177. doi:10.1073/pnas.0806717105 PMID: 18779586

34. Lee SY, Sung E, Chang Y. Elevated serum gamma-glutamyltransferase is a strong marker of insulin resistance in obese children. Int J Endocrinol. 2013; 2013: 578693. doi:10.1155/2013/578693 PMID: 23573088

35. Zhou J, Mo Y, Li H, Ran X, Yang W, Li Q, et al. Alanine aminotransferase is associated with an adverse nocturnal blood glucose profile in individuals with normal glucose regulation. PLoS One. 2013; 8: e56072. doi: 10.1371/journal.pone.0056072 PMID: 23424646

36. Dunlap JC. Molecular bases for circadian clocks. Cell. 1999; 96: 271–290. PMID: 9988221

37. Riggo O, Merli M, Pieche U, Romiti A, Casu A, Coppola A, et al. Circadian rhythmicity of plasma amino acid variations in healthy subjects. Recent Dev Med. 1989; 80: 591–593. PMID: 2623324

38. Tsai PJ, Wu WH, Huang PC. Circadian variations in plasma neutral and basic amino acid concentrations in young men on an ordinary Taiwanese diet. J Formos Med Assoc. 2000; 99: 151–157. PMID: 10770030
39. Adibi SA. Metabolism of branched-chain amino acids in altered nutrition. Metabolism. 1976; 25: 1287–1302. PMID: 790082

40. Yin Y, Yao K, Liu Z, Gong M, Ruan Z, Deng D, et al. Supplementing L-leucine to a low-protein diet increases tissue protein synthesis in weanling pigs. Amino Acids. 2010; 39: 1477–1486. doi: 10.1007/s00726-010-0612-5 PMID: 20473536

41. Suryawan A, Jeyapalan AS, Orellana RA, Wilson FA, Nguyen HV, Davis TA. Leucine stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing mTORC1 activation. Am J Physiol Endocrinol Metab. 2008; 295: E668–875. doi: 10.1152/ajpendo.90314.2008 PMID: 18682538

42. Ranawana V, Kaur B. Role of proteins in insulin secretion and glycemic control. Adv Food Nutr Res. 2013; 70: 1–47. doi: 10.1016/B978-0-12-416555-7.00001-1 PMID: 23722093

43. Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. Am J Clin Nutr. 2003; 77: 91–100. PMID: 12499328

44. Ahmed M, Nuttall FQ, Gannon MC, Lamusga RF. Plasma glucagon and alpha-amino acid nitrogen response to various diets in normal humans. Am J Clin Nutr. 1980; 33: 1917–1924. PMID: 6998275

45. Munro HN. Factors Affecting Requirements for Carbohydrate, Fat and Amino Acids. Nutr Dieta Eur Rev Nutr Diet. 1963; 5: 298–304. PMID: 14112467

46. Yin FG, Zhang ZZ, Huang J, Yin YL (2010) Digestion rate of dietary starch affects systemic circulation of amino acids in weaned pigs. Brit J Nutr 103: 1404–1412 doi: 10.1017/S00071145099993321 PMID: 20102672

47. van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ. Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr. 2000; 72: 96–105. PMID: 10871567

48. Tiedgen M, Seitz HJ. Dietary control of circadian variations in serum insulin, glucagon and hepatic cyclic AMP. J Nutr. 1980; 110: 876–882. PMID: 6246227

49. Peret J, Foustock S, Chanez M, Bois-Joyeux B, Assan R. Plasma glucagon and insulin concentrations and hepatic phosphoenolpyruvate carboxykinase and pyruvate kinase activities during and upon adaptation of rats to a high protein diet. J Nutr. 1981; 111: 1173–1184. PMID: 7019397

50. Tovar AR, Ascencio C, Torres N. Soy protein, casein, and zein regulate histidase gene expression by modulating serum glucagon. Am J Physiol Endocrinol Metab. 2002; 283: E1016–1022. PMID: 12376330

51. Calbet JA, MacLean DA. Plasma glucagon and insulin responses depend on the rate of appearance of amino acids after ingestion of different protein solutions in humans. J Nutr. 2002; 132: 2174–2182. PMID: 12163658