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EFFECTS OF CRUDE PROTEIN LEVELS ON GROWTH PERFORMANCE, NITROGEN BALANCE, DIGESTIBILITY AND CRUDE PROTEIN REQUIREMENT OF GROWING FUXING PIGS

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ABSTRACT

The purpose of this study was to determine the protein requirements of growing Fuxing pig, and to provide basic parameters for determining the feeding standards of growing Fuxing pig. In this experiment, a single factor randomized block design was used, and forty healthy weaned pigs with a body weight of（20.45±1.65kg）were randomly selected and divided into 4 groups, with 10 pigs in each group. Each group was fed diets with CP levels（CP12、CP14、CP16、CP18）during the 67 days feeding trail, the 7 days preliminary experimental period and the 60 days experimental period. Three healthy pigs were randomly selected from each group. Before the last 7 days of the experiment, all urine and feces were collected to determine the nutritional digestibility and metabolic. The results showed that the addition of CP16 to the diet could reduce the F/G. The CP16 group decreased by 8%, 6.9%, and 3.7% compared with that of the CP12, CP14, and CP18 groups, respectively. There was no difference between the CP16 and CP18 groups （P>0.05）. The digestibility of EE in the CP16 and CP18 groups was significantly higher than that in the CP12 group (P≤0.05). There was no significant difference between the CP14, CP16, and CP18 groups (P>0.05). With the increase of the crude protein level, the digestibility of EE in the CP, DCP (digestible crude protein) CP16and CP18 groups was significantly higher than that of the CP12 group, CP14 group (P≤0.01), CP16 group. In the case of the CP12 group and the CP14 group, NI (nitrogen intake), UN (urine nitrogen), RN (retention nitrogen), N%, were all significantly higher. To sum, Fuxing pig DCP intake was used as the dependent variable (Y) and ADG as the independent variable (X) for linear regression analysis. The digestible CP requirement the regression equation with Fuxing pig's growing period was as follows: DCP=1.93 $W^{0.75}$+0.34△W (DCP: g/d；$W^{0.75}$: kg；△W: g/d).

Keywords: Fuxing Pig, Protein Requirement, Growth Performance, Nitrogen Balance, Regression Model

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INTRODUCTION

Proteins in livestock and poultry have structure and function. Li *et al*., (2018) proposed that protein restriction is associated with certain diseases. Therefore, the protein intake of livestock is of utmost importance since insufficient protein intake can negatively impact growth performance and physiological status. Assessing optimal protein intake for growing pigs has been crucial to pig nutrition research in recent years (Wecke and Liebert, 2009). Aquilani *et al*., (2019) found the optimal protein requirement for growing Cinta Sense pigs.

Regression can predict or control the value of another specific variable by the value of one or several variables and give the accuracy of this prediction or control. Using regression analysis, Soto *et al*., (2019) predicted the influence of dietary detergent fibre on pig carcass yield. The regression equation was carcass yield, %=0.03492±0.02633×WP(d)-0.05092±0.02862×NDF1(%)-0.06897±0.02931×NDF2(%)-.00289±0.00216×(NDF2 [%]×WP [d]) ＋ 76.0769±1.33730. The regression equation is evidently an effective mathematical model. Remus *et al*.,(2020) also proposed establishing egression to predict individual blood pressure changes over time to determine the protein content of a person's daily weight gain.

Fuxing pigs are excellent local breeding pigs in Dejaing city, Guizhou Province, China. This pig breed has the advantages of a high tolerance for tough feeding, strong resistance to stress, good carcass quality, a high intramuscular fat content, and delicious meat. There are currently no feeding standards for pigs of the Dejiang Fuxing breed. According to NRC (2012), the optimal crude protein intake for growing-finishing pigs weighing 20–50 kg is CP18. In addition, protein needs were dependent on breed, environment, feeding, and management. Appropriate dietary protein can improve livestock production performance, increase feed conversion rate, and reduce environmental pollution from livestock and poultry production. In light of the regression equation, this experiment was conducted to predict the CP requirements of growing Dejiang Fuxing pigs.

MATERIALS AND METHODS

Experimental animals, diets and design: Forty healthy weaned pigs, with parity similar, body weight (20.45±1.65 kg) were selected randomly and divided into 4 groups, each group of 6 heads. Each group was fed dietary of CP levels (CP12、CP14、CP16、CP18) respectively in 67 days feeding trail, 7 days preliminary experimental period and 60 days experimental period. All urine and feces were selected to determine the nutrition digestibility and metabolic rates before the last 7 days experiment. The experimental diet composition and nutrition level are shown in Table 1.

Table 1. Different crude protein diet formulas and nutrient levels（air-dry basis）

Items（%）	*Dietary crude protein levels*			
	CP12 Group	*CP14 Group*	*CP16 Group*	*CP18 Group*
Corn	66.59	65.00	70.63	65.90
Wheat bran	16.71	12.71	1.53	0.33
Soybean meal	9.73	16.13	23.84	29.79
Soybean oil	2.78	2.11	0.00	0.00
L-Lysine	0.18	0.06	0.00	0.00
DL-Methionine	0.02	0.00	0.00	0.00
Premix[1]	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00
Nutrient levels[2]				
DE/（MJ/kg）[2]	13. 30	13.30	13.30	13.30
CP[3]	12.08	14.13	16.28	17.94
CF[3]	3.05	2.99	2.48	2.60
Calcium	0.74	0.76	0.77	0.79
NP[3]	0.26	0.26	0.24	0.25
Lysine	0.80	0.80	0.88	1.01
Methionine + Cystine	0.41	0.45	0.53	0.59
Threonine	0.45	0.55	0.66	0.75
Tryptophan	0.14	0.17	0.21	0.24

[1] The premix provided the following for per kilogram of the basal diet：VA 250 000 IU, VD_3 175 000 IU, VE 250 IU, VK_3 133 mg, VB_1 22 mg, VB_2 88 mg, VB_6 55 mg, VB_{12} 0.32 mg, biotin 5.5 mg, pantothenic acid 165 mg, nicotinic acid 275 mg, folic acid 8.8 mg, Lys 150 g, copper 3360 mg, iron 3200 mg, zinc 2600 mg, manganese 750 mg, iodine 16.5 mg, selenium 8 mg, calcium 130 g, TP 22 g, salt 102 g.
[2]CP and CF were actual measured values, While the rest were calculated values.
[3] DE=digestion energy CP=crude protein CF=crude fiber NP=available phosphorus

Sample collection and chemical analyses: Fecal were taken at 1/10 of the total weight, and 10mL 10% H_2SO_4 solution was added for every 100g of fecal for nitrogen fixation. The fecal were stored in the refrigerator at -20℃ for further testing. Before urine collection, 100mL 10%H_2SO_4 solution was added into the 400mL urine collection container, and collected and filtered with double-layer gauze. The volume was accurately measured and recorded, and then 10% urine was taken out and placed in bottles, stored at -20℃ refrigerator for future measurement.

The method of analysis of feces, urine and feed in this experiment is as follows: dry matter (AOAC, 2012, reference: 934.01), crude protein (AOAC, 2012, reference: 976.05), ether extract (AOAC, 2012, reference: 950.46), Ash (AOAC, 2012, reference:942.05), NDF (Goering 1970), Nitrogen (AOAC, 2012, reference: 984.13).

Calculations and statistical analyses: The following parameters in all tanks were calculated using the following equations:

ADG (Average daily gain, g/d) = Total gain/60d

ADFI (Average daily feed intake, g/d) = Total feed intake/60d
F/G (feed/gain) = ADFI/ADG
Apparent digestibility of a nutrient in feed, %=[(an amount of a nutrient in the feed－the amount of a nutrient in the feces)/an amount of a nutrient in the feed]×100
Nitrogen absorption = Nitrogen intake － Nitrogen excretion in feces
Nitrogen absorption, % = (Nitrogen intake－Nitrogen excretion in feces)/ Nitrogen intake
Nitrogen retention = Nitrogen intake－Nitrogen excretion in feces－Nitrogen excretion in urine
Nitrogen retention (NPU,%) = (Nitrogen intake － Nitrogen excretion in feces － Nitrogen excretion in urine)/ Nitrogen intake
Nitrogen epigenetic titer = Nitrogen retention/(Nitrogen intake－Nitrogen excretion in feces)

After preliminary sorting of the experimental data using Excel 2003, SPSS19.0 software was used for single-factor ANOVA difference analysis, Duncan's multiple comparison, P ≤ 0.05 means significant difference, P≤ 0.01 means extremely significant difference. Use y= a + bx linear model to set up the regression equation.

RESULTS

Effects of Different CP Levels on The Growth Performance of Growing Fuxing Pig: The effects of different crude protein levels on the performance of Dejiang Fuxing pigs are shown in Table 2. The results show that different crude protein levels have an effect on the body weight of Dejiang Fuxing pigs. The CP14, CP16, and CP18 groups are significantly higher than the CP12 group (P≤0.01), the CP16 crude protein group's weight gain is the highest. There is no significant effect on feed intake (P>0.05). The average daily gain of CP16 group is significantly higher than that of the CP12 group (P≤0.05). The feed weight ratio of the CP16 group is significantly lower than that of the CP12 group and CP14 group (P≤0.05), by 8% and 6.9%, respectively.

Effects of Different Crude Protein Levels on Nutrient Apparent Digestibility of Growing Fuxing Pig: The effects of different crude protein levels on the apparent digestibility of Dejiang Fuxing pig nutrients are shown in Table 3. Different protein levels have no significant effect on the DM and NDF digestibility (P>0.05). The apparent digestibility of CP, CF and Ash in CP16 and CP18 groups is significantly higher than that in the CP12 and CP14 groups (P≤0.01), and CP14 group is significantly higher than CP12 group (P≤0.05). However, the difference between the CP16 and CP18 groups is not obvious (P>0.05). The apparent digestibility of EE in the CP16 and CP18 groups is significantly higher than that in the CP12 group (P≤0.05). There is no significant difference between the CP14, CP18 groups and the CP12 grpup (P>0.05).

Effects of Different Crude Protein Levels on Nitrogen Balance of Growing Fuxing Pig: The effects of different CP levels on the nitrogen balance of Dejiang Fuxing pigs are shown in Table 4. With the increase of protein level, the CP intake and DCP intake of the CP14 group are significantly higher than those of the CP12, CP16and CP18 groups (P≤0.01), the differences among the other groups are not significant (P>0.05). The nitrogen intake in the CP16 and CP18 groups is significantly higher than that in the CP12 and CP14 groups (P≤0.01), and the CP14 group is higher than the CP12 group (P≤0.05). There is no significant difference between other groups (P>0.05). The FN in the CP16 and CP18 groups is significantly higher than that of the CP12 group and the UN is significantly increased (P≤0.05). The RN CP12 group is significantly lower than the other three groups (P≤0.01). There is no significant difference among the other groups (P>0.05). The nitrogen digestibility of the CP16 group is higher than that of the CP12, CP14 groups (P≤0.01). There is no significant difference between CP16 group and CP18 group (P>0.05). The NPU rate and the NBV in the CP12 group are significantly higher than those in the CP14, CP16, and CP18 groups (P≤0.05). The NPU rate and the NBV in the CP14 group is significantly higher than those in the CP16 and CP18 groups (P≤0.05). Thereis no significant difference between the other two groups (P>0.05). There is no significant difference in the GE digestibility among the groups (P>0.05). The CP14 group's the GE metabolism rate and DE metabolism rate are significantly higher than those in the CP12, CP16 and CP18 groups (P≤0.05). There is no significant difference in other groups (P>0.05).

Table 2 Effects of different crude protein levels on the growth performance of growing Fuxing pig

Items[1]	*Dietary crude Protein levels*				*SEM[2]*	*P-value*
	CP12 Group	*CP14 Group*	*CP16 Group*	*CP18 Group*		
IBW/kg	20.35	20.58	20.25	20.28	0.26	0.533
FBW/kg	46.30[a]	48.57[ab]	51.62[b]	49.07[ab]	0.60	0.013
ADFI/(g/d)	1751.97	1853.39	1872.70	1824.00	20.84	0.184
ADG/(g/d)	438.60[a]	469.17[ab]	510.88[b]	473.97[ab]	10.07	0.084
F/G	4.02	4.01	3.68	3.91	0.07	0.235

In the same row, values with the same or no letter superscripts mean no significant difference(P>0.05), while with different small letter superscripts, mean significant difference(P≤0.05). The following table with.

[1]IBW=initial body weight FBW=final body weight ADFI=average daily feed intake ADG=average daily gain F/G=ADFI/ADG

[2]SEM=standard error of the mean.

Table 3 Effects of different crude protein levels on nutrient apparent digestibility of growing Fuxing pig

Items[1]	*Dietary crude Protein levels*				*SEM*	*P-value*
	CP12 Group	*CP14 Group*	*CP16 Group*	*CP18 Group*		
DM/%	82.23	82.44	82.61	83.05	0.37	0.915
CP/%	76.16[c]	79.17[b]	82.53[a]	82.12[a]	0.78	≤0.001
EE/%	70.32[a]	73.16[ab]	76.08[b]	74.86[b]	0.82	0.08
CF/%	50.18[c]	54.79[b]	60.03[a]	61.36[a]	1.40	≤0.001
Ash/%	40.32[c]	43.06[b]	45.81[a]	46.20[a]	0.76	≤0.001
NDF//%	74.18	73.57	74.71	72.93	0.28	0.63

[1]DM=dry matter CP=crude protein EE=ether extract CF=crude fiber Ash=crude ash

Table 4 Effects of different crude protein levels on nitrogen balance of growing Fuxing pig

Items[1]	*Dietary crude Protein levels*				*SEM*	*P-value*
	CP12 Group	*CP14 Group*	*CP16 Group*	*CP18 Group*		
Intake CP/(g/d)	210.24[c]	259.47[b]	299.79[a]	328.30[a]	10.69	≤0.001
DCP/(g/d)	160.12[c]	205.42[b]	247.41[a]	269.60[a]	9.73	≤0.001
NI/(g/d)	33.64[c]	41.52[b]	47.97[a]	52.53[a]	1.70	≤0.001
FN/(g/d)	8.02[c]	8.65[b]	8.38[b]	9.39[a]	0.25	≤0.001
UN/(g/d)	5.01[d]	9.05[c]	13.84[b]	16.03[a]	0.66	≤0.001
RN/(g/d)）	20.61[c]	23.82[ab]	25.75[a]	27.11[a]	1.04	≤0.001
N/%	76.16[c]	79.17[b]	82.53[a]	82.12[a]	≤0.01	≤0.001
NPU/%	61.27[a]	57.36[b]	53.68[c]	51.61[c]	0.01	≤0.001
NBV/%	80.445[a]	72.47[b]	64.97[c]	62.84[c]	0.01	0.014

[1]DCP=digestible crude protein NI=nitrogen intake FN=fecal nitrogen UN=urine nitrogen
[1]RN=Nitrogen retention NPU,%=nitrogen retention (NPU,%) NBV=Nitrogen epigenetic titer

Analysis of Protein Requirement: There is a linear regression relationship between the DCP intake and ADG during the growth period of Fuxing pigs. The model y=a+bx required for CP is determined according to the factor analysis method, and the regression equation was established. The equation obtained by analyzing the experimental results is as follows:

Y =25.24+0.3428X R^2 = 0.8933, P≤0.05.

Note: Y is the mean DCP intake (g/d) of Fuxing pigs, X is the mean ADG of Fuxing pigs, and R^2 is the correlation coefficient. When X ADG is zero, the Y value at this time is the maintenance requirement of digestible protein (25.24g/d). The metabolic body weight is calculated at $W^{0.75}$, and the growth stage of the revitalized pig protein Vitter requires 1.93g/d of metabolic body weight per kilogram.

DISCUSSION

Effects of Different CP Levels on The Growth Performance of Growing Fuxing Pig: CP is an important nutrition (Bertolo and Ma ,2016). The content and type of CP in the diet will affect the growth performance of pigs (Rafiee *et al*., 2019). Pigs will have different performances when they eat different levels of CP, which are mainly manifested in pig's feed intake, daily gain, and F/G. When CP is deficient in the diet, the production performance of pigs will be reduced, resulting in slow growth and weight loss (Nyachoti *et al*., 2006). When there is too much CP content in the diet, pigs will regulate the nitrogen metabolism through their own nitrogen balance mechanism, usually without long-term adverse effects. However, if the CP content exceeds the maximum acceptable level for pigs, the performance of pigs will be significantly reduced (Chen *et al*., 1999; Kerr *et al*., 2003). Zhao *et al*.,(2020) studied the CP requirements of Yimeng black pigs during the growth and fattening period and found that the growth performance of the pigs was affected by the protein level. The optimal protein level was 90% of the recommended amount. Such high or low crude protein level was not conducive to the pig's growth, which was basically consistent with previous studies. Htoo *et al*.,(2007) reduced dietary protein levels by balancing the AA (amino acids) levels in pig's diets, but had no effect on pig's growth performance. In summary, the performance of pigs is strongly related to dietary CP levels, AA supplement levels and AA supplement methods.

Effects of Different Crude Protein Levels on Nutrient Apparent Digestibility of Growing Fuxing Pig: The CP content in the diet is an important factor affecting the apparent digestibility of various nutrients in pigs. As one of the energy materials required for animal nutrition, protein participates in various life activities of the body. Meyer J H and Garrett *et al*.,(1967) showed that the CP level of diet can significantly affect the digestibility of various nutrients such as organic matter, DM, EE and CP at the same energy level. Qui *et al*.,(2018) also found that dietary protein levels had a significant effect on nutrient digestibility. If pigs consume too much protein(more than 18.25% of the diet), it will increase the burden on the gastrointestinal tract, reduce the activity of digestive enzymes secreted by the gastrointestinal tract (Lee *et al*., 2022), increase the number of intestinal peristalsis, and ultimately shorten the retention time of nutrients in the gastrointestinal tract. When the protein level is too low to meet the minimum requirements of the animal, it will affect the growth and development of the animal, gradually weaken the digestive function of the animal, reduce the desire to eat food, and then reduce its CP. The fiber digestibility was also increased gradually, which is very similar to the results of this study. In this study, the protein digestibility of 20~50kg Fuxing pigs first increase and then decrease with the increase of dietary protein levels, which is consistent with previous studies.

In addition, the results of this study show that when the crude protein level of 20-50kg Fuxing pigs is CP12, the apparent digestibility of various nutrients is reduced compared with the other three groups. The reason may be due to insufficient dietary protein content. Pigs cannot meet their minimum requirements after eating. The lack of protein synthesis in pigs hinders protein turnover and metabolic efficiency. There are interactions between various nutrients, so the digestion of other nutrients is obvious. The rate will also be limited to some extent.

Effects of Different Crude Protein Levels on Nitrogen Balance of Growing Fuxing Pig: Protein intake and deposition are affected by the protein level. As the protein level increases, the protein intake and deposition will also increase accordingly; but too high or too low will adversely affect it (Millet *et al*., 2018). Gatel *et al*.,(2010) and Wu *et al*.,(2018) reported that reducing dietary protein levels would significantly reduce the excretion of fecal nitrogen and urine nitrogen. Ball *et al*.,(2013) reported that the apparent nitrogen digestibility gradually increased, and the nitrogen excretion decreased slowly with increasing protein levels. When the protein level is not sufficient for the animal body, the efficiency of amino acid synthesis will be reduced. Therefore, the deposition of nitrogen will be significantly reduced (Lenis *et al*., 1999; Lei *et al*., 2019). There is a certain correlation between the quality of pig nitrogen metabolism balance and the biological value of nitrogen, and its value generally fluctuates between 50% and 80%. Yamazaki *et al*.,(2019) showed that appropriate down-regulation of dietary protein levels can significantly increase the biological value of nitrogen. Wen *et al*.,(2019) found that the biological value of nitrogen increased with decreasing dietary protein concentration, with values was ranging from 40% to 55%. In this study, the dietary protein level was significantly higher than that of 20-50kg Dejiang Fuxing pigs. The law of nitrogen metabolism is basically consistent with the previous research results.

Analysis of Protein Requirement: The regression model parameters were obtained by designing the protein single factor experiment. The one-variable linear regression equation of the average daily intake of digestible crude protein and the average daily weight gain was fitted to establish a mathematical model of protein requirement and body weight, which can determine the animal requirement of protein maintenance. The results of this experiment showed that the digestible protein maintenance requirement of Fuxing pigs in the growth stage was 25.24g/d or 1.93g/$W^{0.75}$·d.

According to the principle of factorial method, the total digestible crude protein of Fuxing pigs in the growth stage needs a mathematical model are shown in Figure1:

$$DCP=1.93\ W^{0.75}+0.34\triangle W$$

(R^2 value is 0.8933, $P\leq0.05$, so it is feasible.)

DCP: g/d; $W^{0.75}$: kg; $\triangle W$: g/d;

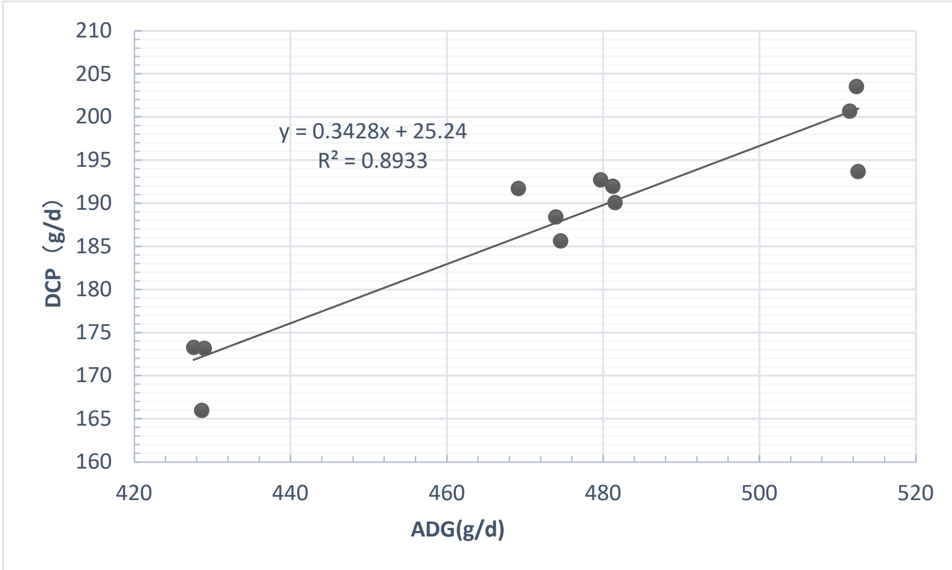

Figure1 Regression analysis of protein requirement of growing Fuxing pig

Conclusion: Based on the above results, the suitable protein level and the maintenance requirement of digestible protein were CP16% and 25.24g/d or 1.93g/$W^{0.75}$ d in Dejiang Fuxing pigs at the growth stage.

Factorial model is: DCP=1.93$W^{0.75}$+0.34△W

DCP: g/d; $W^{0.75}$: kg; △W: g/d.

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