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LYSINE SUPPLEMENTATION IMPROVES NUTRIENTS DIGESTION, GROWTH PERFORMANCE AND LIVER FUNCTION OF FEMALE BLUE FOXES (*Alopex lagopus*) IN GROWING PHASE

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ABSTRACT

The present study investigated the effects of lysine supplementation in low-protein diets on nutrients digestibility, growth performance and healthy status of growing female blue foxes (*Alopex lagopus*). Control animals were fed a diet containing normal protein level (32% of dry matter, DM). Animals in the treatment groups were challenged with low-protein diets supplemented with 0.00%, 0.20%, 0.40%, 0.60%, 0.80% and 1.00% lysine, corresponding to 0.87%, 1.07%, 1.27%, 1.47%, 1.67% and 1.87% total lysine of DM, respectively. Results showed that 1.47% lysine supplementation exerted beneficial effects ($P < 0.05$) on blue foxes, as evidenced by improved digestibility of crude protein (CP) and several amino acids (aspartic acid, glycine, methionine, isoleucine and tyrosine). Similar effect was observed in N retention ($P < 0.05$) and the growth performance ($P < 0.05$). Additionally, the 0.87% lysine group exhibited a significant decrease in blood albumin (ALB) as opposed to the other groups ($P < 0.01$). Collectively, these findings indicate that quality of low-protein diet can be improved by supplemented lysine without influencing health of female blue foxes at the growing phase. The optimum lysine for maximum performance and low N emission of the growing female blue foxes in approximately 1.47% of DM.

Key words: lysine, low protein diets, female blue foxes, growing phase, growth performance

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INTRODUCTION

The blue fox (*Alopex lagopus*) is one of the widely farmed fur-bearing animals in the world. As a primary carnivorous mammal, blue foxes require high amount of dietary animal protein. Traditional ways of using high quality protein sources remain the first-line option for most fox ranches. The plant-based protein, widely employed in fur-bearing animal feeds, are less digestible as compared to animal proteins (Qingkui Jiang, Li, Zhang, Zhang, Gao, Xing, *et al*., 2018; WeiLi, Yeye, HanLu, Wei, DanLi, BaiYang, *et al*., 2009) that results in higher dietary CP concentration in order to meet their protein requirement. However, excessive dietary protein leads to various detrimental consequences like high farming cost and nitrogen excretion (Aquilani, Sirtori, Franci, Acciaioli, Bozzi, Benvenuti, *et al*., 2019; Q. Jiang, Zhang, Li, Zhang, Gao, Xing, *et al*., 2020). The plant-based protein feedstuffs also limit the availability of amino acids to the digestive process of animals, which might affect animal growth and health.

Individual amino acids present in the feedstuff protein are what the animals truly require. Supplemented amino acids to low protein diet greatly benefit animal farming industry by saving protein ingredients, decreasing nitrogen excretion, production cost and the risk of health disorders without impairing growth performance (Corley, Esch, Bahr, & Easter, 1983; van Harn, Dijkslag, & van Krimpen, 2019; Wang, Zhou, Wang, Cai, Zeng, & Qiao, 2018). The available blue fox nutrient requirements do not include recommendations on amino acids (Council, 1982). Information on the physiological demands of amino acids of the fox, especially during a particular physiological stage, still remains very limited.

In growing animals, additional body tissue is being synthesized, which require either high dietary protein concentration or more balanced amino acids composition. The wide use of plant source protein in modern fox farming industry do not allow to reduce dietary CP levels, which would limit lysine supply to foxes (Liu, Wu, Bryant, & Roland Sr, 2005). Therefore, physiological functions of lysine need to be explored in

order to formulate feed with balanced amino acids. Keeping this concept in view, the present study was designed to investigate if lysine supplementation to low-protein diets could affect growth performance, nitrogen balance, nutrient digestibility, and serum parameters of female blue foxes in their growing period.

MATERIALS AND METHODS

Animals, diets and management: The investigation was performed at the Fur Animal Breeding Base of Institute of Special Animal and Plant Science, Chinese Academy of Agricultural Sciences (44.02° N, 126.15° E) in the northeast of China, with the experimental protocol being evaluated and approved by the Animal Care Committee of the Institute. All procedures followed the Laws, Regulations, and Guidelines for Animal Research in China (Ogden, Pang William, Agui, & Lee, 2016) and were abided by the Welfare of Animals Kept for Fur Production of European Commission. A total of 105 eight-week-old female blue foxes were housed individually in roofed, open-sided standard sheds holding two rows of cages. The cages, measured 100 x 70 x 75 cm, are made of strong wire mesh and raised 80cm above the ground. Animals had access to the respective diets twice a day *ad libitum* at 0800 and 1600 (Beijing time) with drinking water taken freely.

Animal weights were recorded at the start of experiment and distributed randomly into seven dietary groups of 15 animals each with increasing lysine levels (0.87%, 1.07%, 1.27%, 1.47%, 1.67%, 1.87%) or without lysine supplementation as control diet containing normal protein level of 32% (Table 1). The lysine-supplemented groups were offered 2% less CP than that of control group.

Increasing level of dietary carbohydrates were used to compensate the decreasing dietary protein levels (in the experiment from 300.0 to 320.2 g/kg DM). Therefore, all the diets were isocaloric (14.64 MJ/kg to 14.48 MJ/kg) with ether extract (EE) content (g/kg DM) within the range from 119.7 to 121.0 g/kg DM. The ingredients and chemical composition of four diets was listed in Table 1, and amino acid content in Table 2.

Blood sampling and measurement: Blood samples were collected to determine serum biochemical indicators at the end of the experiment. Plasma and serum were immediately separated by centrifugation at 2,500 × g at 4 ℃ for 5 min. Serum was frozen at -80℃ for biochemical indicators determination. Blood urea nitrogen (BUN), total protein (TP), glutamate pyruvate transaminase (GPT) and blood glucose (GLU) were determined by automatic biochemistry analyzer (Hitachi 7020, Hitachi High Technologies, Inc., Ibaraki, Japan). Test kits were purchased from Nanjing Jiancheng Biochemical Corporation (Nanjing, China).

Table 1: Composition and nutrient levels of the experimental diets (air-dry basis, %).

Items	Basal diet (0.87% lysine)	Control diet
Ingredients		
Extruded corn	46.00	42.10
Soybean meal	10.00	10.00
Bone and meat meal	10.00	12.00
Corn germ meal	10.00	10.00
Fish meal	13.80	16.00
Soybean oil	8.70	8.40
Salt	0.30	0.30
Premix†	1.00	1.00
Lys	0.00	0.00
Met	0.20	0.20
Total	100.00	100.00
Nutrient levels‡		
CP (%)	30.00	32.02
EE (%)	11.97	12.10
Lys (%)	0.87	1.32
Met (%)	0.77	0.86
ME (MJ/kg)	14.64	14.48
% of ME		
CP	23.88	25.48
EE	35.66	35.09
CC	40.46	39.43

† The premix contained per kg: Vitamin A 1000,000 IU; Vitamin D_3 200,000 IU; Vitamin E 6000 IU; Vitamin B_1 600 mg; Vitamin B_2 800 mg; Vitamin B_6 300 mg; Vitamin B_{12} 10 mg; Vitamin K_3 100 mg; Vitamin C 40 000 mg; Nicotinic acid 4 000 mg; Pantothenic acid 1 200 mg; Alkaloid 20 mg; Folic acid 80 mg; Choline 30 000 mg; Fe 8 200 mg; Cu 800 mg; Mn 1 200 mg; Zn 5 200 mg; I 50 mg; Se 20 mg; Co 50 mg.

‡ Results in % of dry matter (DM), except density of metabolizable energy (ME), in MJ/kg DM. Energy distribution as % of ME. Values of crude carbohydrate (CC) was calculated, and the rest were measured.

Chemical analysis: Starting from d 45, seven animals from each treatment were randomly selected and assigned to individual metabolism cages for 3 days of digestive and nitrogen balance trial. Customized trays and bottles were placed under each cage to collect urine and feces, respectively. 10-mL 10% H2SO4 per 100 mL urine was used to combine the nitrogen with addition of five drops of methylbenzene to prevent nitrogen loss. Processed urine samples were stored at −20 °C until being analyzed. Fecal and diet samples were dried in an air-drying oven at 65 °C before being ground for further analysis. DM and N content in wet samples of diets were analyzed (International, Horwitz, & Association of Official Analytical, 2005). Analysis of CP, EE and crude carbohydrate (CC) in air-dried samples of diets and feces were carried out: DM, CP (Kjeldahl-N × 6.25), EE and CC were determined after acid hydrolysis according to

standard procedures (International, Horwitz, & Association of Official Analytical, 2005). Concentration of amino acids in diets and feces were analyzed by amino acid analyzer (L-8900, HITACHI, Japan), as described by Lissbrant, Hammarsten, Lissbrant, Ferrara, Rudolfsson, and Bergh (2004). Calculation of metabolizable energy (ME) content and the proportional composition of ME were based on the digestibility coefficients achieved and the following values of ME: protein 18.8 MJ/kg, EE 39.8 MJ/kg and carbohydrate 17.6 MJ/kg (Clauss, Kleffner, & Kienzle, 2010) .

Table 2: Chemically analyzed amino acids contents of experimental diets (% DM).

Items	Basal diet (0.87% lysine)	Control diet
Aspartic acid	1.43	1.41
Threonine	0.75	0.77
Serine	0.87	0.90
Glutamic acid	2.72	2.81
Glycine	0.94	0.97
Alanine	1.15	1.18
Valine	0.82	0.85
Methionine	0.77	0.86
Isoleucine	0.79	0.81
Leucine	1.96	2.15
Tyrosine	0.65	0.70
Phenylalanine	0.96	1.02
Lysine	0.87	1.32
Histidine	0.45	0.46
Arginine	1.10	1.06
Proline	0.77	0.77

Growth performance evaluation: Animals were inspected twice a day. Individual live weights of each blue fox were recorded at regular intervals using an electronic scale, on d 0 (beginning of the experiment), d 15, d 30 and d 45.

Statistical analysis: The data were analyzed using one-way ANOVA procedure according to the completely randomized design (CRD) design using GraphPad Prism (version 6.01, GraphPad Software, Inc., San Diego, California, USA). Results were presented as mean ± SD. $P < 0.05$ stands for statistical significance and $P < 0.01$ for highly statistical significance.

RESULTS

Nutrients digestibility: Low dietary protein with lysine supplementation did not affect DM intake and apparent EE digestibility of blue foxes, as compared to the control group with normal diet (Table 3). However, apparent DM and CP digestibility were significantly increased in groups with 1.47% or higher dietary lysine ($P < 0.05$), as compared to the ones with low lysine supplementation. This suggests that insufficient lysine in low protein diets could reduce apparent digestibility of DM and CP. In contrast, digestibility of isoleucine and tyrosine were significantly elevated in groups fed with 1.47% or less lysine, as opposed to the 1.87% lysine group (Table 4).

Nitrogen balance: N intake in the low-protein-diet groups were lower than that in the control group, with 1.47% and 1.87% groups consuming the most N in the supplementation groups (Table 5). Fecal nitrogen was significantly low at 1.27 and 1.47% lysine content ($P < 0.05$) than that of the 0.87% group. N in urine showed non- significant difference. Diet with 1.47% lysine markedly increased N retention of blue foxes, comparing with the 0.87%, 1.07% and 1.67% groups ($P < 0.05$).

Growth performance: Animals fed with 1.47% and 1.67% dietary lysine had markedly improved final body weight in contrast to the 0.87% lysine group ($P < 0.05$) (Table 6). Comparable body weight gain from d 16 to d 30 was observed in the 1.47% lysine and the control group, which are significantly higher than that of the 0.87% lysine group ($P < 0.05$).

Serum biomarkers: Low-protein diets with supplementary lysine did not affect total protein (TP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in serum (Table 7). Blood albumin (ALB) increased linearly with dietary lysine concentration and peaked at 1.47% lysine group, which is a significant increase than that of 0.87 and 1.07% lysine groups ($P < 0.05$). Similar trend was found in the level of alkaline phosphatase (ALP) ($P < 0.05$).

Table 3: Effect of lysine supplementation on feed intake and nutrients digestibility of blue foxes.

Items	Lysine						Control	RMSE	P-value
	0.87%	1.07%	1.27%	1.47%	1.67%	1.87%			
DM intake, g/d	240.92	238.29	240.86	241.85	242.14	241.33	242.14	3.84	0.6590
DM output, g/d	100.81^{a}	94.88^{ab}	83.17^{c}	86.58^{bc}	88.30^{bc}	91.82^{abc}	91.11^{abc}	9.50	0.0232
DM digestibility, %	58.15^{a}	60.29^{ab}	65.46^{c}	64.20^{bc}	65.53^{bc}	61.93^{abc}	62.38^{bc}	3.87	0.0109
CP digestibility, %	57.31^{a}	58.04^{a}	62.57^{ab}	66.01^{b}	62.49^{ab}	61.75^{ab}	62.59^{ab}	4.93	0.0198
EE digestibility, %	88.90	86.87	86.96	87.80	87.08	85.63	85.65	2.36	0.2030

DM = dry matter; CP = crude protein; EE= ether extract; RMSE = root mean square error.
[a,b and c]Within a row, means without a common superscript letter differ significantly ($P < 0.05$).

Table 4: Effect of lysine supplementation on amino acids digestion of blue foxes.

Items	Lysine						Control	RMSE	P- value
	0.87%	1.07%	1.27%	1.47%	1.67%	1.87%			
Aspartic acid	54.76	54.47	54.44	58.52	51.90	47.46	50.11	7.19	0.1604
Threonine	67.67	67.67	69.84	68.97	67.56	62.63	66.61	4.29	0.0920
Serine	56.56	56.84	60.89	58.94	55.36	51.48	55.67	6.27	0.2350
Glutamic acid	55.39	55.99	53.27	59.92	56.64	50.30	52.17	6.64	0.1950
Glycine	41.90	41.60	48.23	46.75	42.12	36.56	38.13	8.06	0.1339
Alanine	50.42	52.68	52.90	56.22	50.48	48.80	49.75	5.58	0.3020
Methionine	88.13	88.01	88.84	87.38	86.39	89.78	90.27	2.88	0.2350
Isoleucine	55.57^{a}	55.62^{a}	54.94^{a}	58.58^{a}	53.84^{ab}	44.96^{b}	52.73^{ab}	7.49	0.0430
Leucine	46.49	47.48	45.29	51.91	46.97	39.24	40.62	8.59	0.1478
Tyrosine	94.37^{a}	94.68^{a}	94.34^{a}	95.33^{a}	93.90^{ab}	91.37^{bc}	91.29^{c}	1.99	< 0.0001
Phenylalanine	47.72	53.67	54.60	55.79	51.40	44.04	46.95	8.59	0.1372
Lysine	68.57	67.70	67.44	69.66	68.00	62.85	67.35	4.19	0.1302
Histidine	87.48	87.64	88.69	88.39	87.98	85.60	86.95	2.01	0.1357
Arginine	58.65	55.99	58.48	60.84	58.84	57.96	57.66	5.84	0.9116
Proline	71.39	71.31	72.31	76.48	68.58	68.53	67.61	7.01	0.3506
Valine	63.00	63.42	64.30	66.25	62.66	58.78	60.92	4.86	0.1825

RMSE = root mean square error.
[a,b and c]Within a row, means without a common superscript letter differ significantly ($P < 0.05$).

Table 5: Effect of lysine supplementation on nitrogen metabolism of blue foxes.

Items	Lysine						Control	RMSE	P-value
	0.87%	1.07%	1.27%	1.47%	1.67%	1.87%			
N intake,g/d	11.36^{a}	10.94^{b}	11.10^{b}	11.93^{c}	11.50^{a}	12.08^{c}	12.58^{d}	0.57	< 0.0001
Fecal nitrogen,g/d	4.85^{a}	4.60^{ab}	4.15^{b}	4.06^{b}	4.31^{ab}	4.62^{ab}	4.71^{ab}	0.56	0.0482
Urine nitrogen,g/d	3.39	2.59	2.77	2.14	3.23	3.28	2.53	1.26	0.5379
N retention,g/d	3.12^{a}	3.75^{ab}	4.18^{abc}	5.74^{c}	3.96^{ab}	4.18^{abc}	5.34^{bc}	1.53	0.0217

N = nitrogen. RMSE = root mean square error.
[a,b and c]Within a row, means without a common superscript letter differ significantly ($P < 0.05$).

Table 6: Growth performance of blue foxes from day (d) 1 to 45.

Item	Lysine						Control	RMSE	P-value
	0.87%	1.07%	1.27%	1.47%	1.67%	1.87%			
Body weight (kg)									
D0	2.07	2.04	2.03	2.04	2.07	1.99	2.04	0.27	0.9080
D15	2.91	2.92	2.87	2.97	3.04	2.90	2.94	0.29	0.5680
D30	3.74	3.93	3.89	4.05	4.04	3.91	4.05	0.39	0.8480
D45	4.29^{a}	4.52^{ab}	4.57^{ab}	4.71^{b}	4.81^{b}	4.59^{ab}	4.67^{ab}	0.41	0.0127
Daily weight gain (g)									
D0-d15,	58.82	58.82	55.52	61.98	61.26	57.60	60.30	11.08	0.2970
D16-d30	55.43^{a}	67.49^{ab}	68.22^{ab}	71.84^{b}	63.13^{ab}	64.02^{ab}	73.71^{b}	15.02	0.0277
D31-d45	36.47	39.24	45.00	44.53	48.80	43.48	41.09	17.73	0.3651

RMSE = root mean square error.
[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 7: Effect of lysine supplementation on serum biomarkers of blue foxes.

Items	Lysine						Control	RMSE	P-value
	0.87%	1.07%	1.27%	1.47%	1.67%	1.87%			
TP, g/L	58.58	60.71	57.90	62.79	61.31	59.71	57.79	5.51	0.6223
ALB, g/L	32.16^{a}	32.10^{a}	33.12^{ab}	34.40^{b}	33.93^{b}	33.87^{b}	33.84^{ab}	1.54	0.0105
ALP, U/L	291.9^{a}	300.4^{ab}	319.3^{abc}	346.3^{c}	313.3^{abc}	306.5^{abd}	312.17^{bcd}	27.37	0.0368
GOT, U/L	53.22	41.07	46.32	51.01	52.35	45.01	46.40	36.79	0.3532
GPT, U/L	152.90	160.35	153.81	171.23	181.38	169.42	186.56	10.39	0.6250

TP = total protein, ALP = alkaline phosphatasein, GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; ALB = Blood albumin; RMSE = root mean square error.
[a,b,c and d]Within a row, means without a common superscript letter differ ($P < 0.05$).

DISCUSSION

It has been well established that amino acids supplementation to low-protein diets promote diets' metabolic value and growth performance of poultry, pig, dog and mink (Aletor, Hamid, Niess, & Pfeffer, 2000; Blaza, Burger, Holme, & Kendall, 1982; Easter & Baker, 1980; Guo, Wu, Zhang, Liu, Cui, Yang, *et al*., 2015; Kerr, McKeith, & Easter, 1995; Zhang, Li, Xing, Ren, Yang, & Yang, 2012). Lysine is characterized as the first limiting amino acid in many feedstuffs for animals. However, it's significance in the development of blue foxes has not been fully appreciated.

Lysine is involved in numerous physiological processes including muscle protein accretion and whole-body growth (Liao, Wang, & Regmi, 2015). Although previous study reported that excess lysine would not affect the growth of pigs (Yen, Klindt, Kerr, & Buonomo, 2005), it has been shown that a reasonable lysine level is critical to facilitate nutrients' digestion and utilization (Zeng, Yan, Wang, Zhang, Zhu, Shu, *et al*., 2013). In the present study, digestibility of DM and CP increased with levels of lysine supplementation and peaked at 1.47% lysine treatment, which is a significant increase as compared to the group without lysine addition (0.87%). This is consistent with studies in pigs reporting restricted dietary lysine decreased DM and CP digestibility (Yang, Jin, Yoon, Choi, Shinde, Piao, *et al*., 2008). Our findings suggest that low protein diet (30% of DM) containing 1.47% lysine may promote the digestibility of nutrients in female blue foxes of growing phase. Indeed, low or high dietary lysine supplementation could result in detrimental effect on nutrients digestibility, as reported by Jin, Oh, Piao, Jang, Choi, Heo, *et al*. (2010) and Zeng, *et al*. (2013) in pigs. However, the potential adverse effect of high lysine in low protein diets for female blue foxes needs to be further investigated.

Mammalians are not able to store excess amino acids. Under normal conditions, catabolism of amino acids takes place to separate nitrogen from the carbon skeleton. The nitrogen can be either incorporated for biosynthesis of protein or excreted as the form of urea and NH3 in the urine and causes environmental pollution. The nitrogen emission issue is more severe in fur farm as fur animals consume diets with high protein content. Therefore, digestibility of amino acids is critical for reducing nitrogen pollution. In the present investigation, isoleucine, and tyrosine were mostly influenced by lysine supplementation among the amino acids studied, achieving highest digestibility at the 1.47% lysine group. These results indicate that with 1.47% lysine addition, low protein diet (30% of DM) could contribute to diminishing nitrogen emission by promoting digestibility of certain amino acids.

Principally, N retention is determined by the first limiting amino acid. Serving as the first limiting amino acids in pigs, lysine supplementation increases total retention and reduces fat deposition (Noblet, Henry, & Dubois, 1987). Once the requirement is met, further supplementation of the first limiting amino acids would not affect N retention. In contrast, study in pigs indicated that supplementing the second limiting amino acid (threonine) to diets containing the first limiting amino acid (lysine) improved daily gain, feed efficiency as well as N retention (Eckert & Allee, 1974). Addition of less limiting amino acids optimized feed conversion of broilers (Kidd, Kerr, Allard, Rao, & Halley, 2000). In blue foxes, the role of less limiting amino acids on N retention has not been fully understood yet. In the current study, total N intake was related to DM intake. However, significant decrease of fecal nitrogen was observed in groups with 1.27% and 1.47% lysine. As a result, N retention tended to respond positively to the digestibility of nutrients, suggesting that lysine influenced N retention of growing female blue foxes in a dose-dependent manner. Moreover, unlike the first limiting amino acid, the dose-response relationship between lysine and N retention may not be linear. Lysine deficiency or excess could produce adverse effect on N retention and growth of blue foxes. Dahlman, Valaja, Niemelä, and Jalava (2002) found lysine supplementation in low protein diets reduced early growth and skin length of male blue foxes, possibly due to the relatively low protein level 22.5% and 15% of ME from protein, or the imbalance in dietary amino acid content. This is in agreement with our findings on the final body weight and daily weight gain from d 15 to d 30 and d 30 to d 45 (Table 6), during which blue foxes experienced rapid growth and development and deposit majority of their muscle tissue. Due to the nature of protein, N retention is especially critical for muscle deposition and live weight increase. Therefore, based on our investigation, 30% protein level supplemented with 1.47% - 1.67% lysine is recommended for optimal growth of female blue foxes at growing period.

The liver performs four main tasks related to protein metabolism: formation of blood proteins, amino acid interconversion, amino acids deamination, and urea synthesis (Eghtesad, Poustchi, & Malekzadeh, 2013). Hepatic functions might be impaired by nutritional status, especially by dietary protein and amino acids content (Wu, 2009). These damages could be evaluated by several biochemical markers in the blood such as TP, ALB, ALP, GOT and GPT. One of the hallmarks of liver disease is the reduced circulating proteins synthesized by the liver, including ALB (Peck-Radosavljevic, 2000). In this study, none or low lysine addition (0.87% and 1.07% dietary lysine) tended to decrease ALB level as opposed to the group with higher lysine level (1.47% and 1.67%). The lower ALB level is likely caused by the lysine-limited protein deficiency, which results in dysregulation of protein metabolism in liver (Charlton, 1996). Indeed,

ALP, a standard biochemical marker for hepatic function, was found significantly lower in the none or low lysine groups, indicating potential protein malfunction (Ray, Singh, Jena, Behera, & Ray, 2017; Simko, 1991). Taken together, these results suggest that inadequate dietary lysine could lead to functional impairment of liver.

Conclusion: Supplementation of lysine with appropriate level (1.47%) could protect growing female blue foxes from low digestibility of DM, CP and certain amino acids caused by low dietary protein (30% of DM). N retention, growth performance and live weight were considerably elevated in the 1.47% lysine group. Meanwhile, the 30% of DM protein with 1.47% lysine level is characterized by less N excretion and lower production cost without undermining welfare of female blue foxes. In summary, low protein diet (30% of DM) with 1.47% lysine level could maintain normal feed utility, growth performance and health status of female blue foxes at growing phase as compared with the regular protein diet (30% of DM) group and contribute to reduction of farming cost and nitrogen emission to environment.

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REFERENCES

Aletor, V.A., I.I. Hamid, E. Niess, and E. Pfeffer. (2000). Low‐protein amino acid‐supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole‐body composition and efficiencies of nutrient utilisation. J. Sci. Food Agric., 80(5), 547-554.

Aquilani, C., F. Sirtori, O. Franci, A. Acciaioli, R. Bozzi, D. Benvenuti, M. Čandek-Potokar, and C. Pugliese. (2019). Effects of Different Protein Levels on the Nitrogen Balance, Performance and Slaughtering Traits of Cinta Senese Growing Pigs. Animals, 9(12), 1021.

Blaza, S.E., I.H. Burger, D.W. Holme, and P.T. Kendall. (1982). Sulfur-containing amino acid requirements of growing dogs. The J. Nutrition, 112(11), 2033-2042.

Charlton, M.R. (1996). Protein metabolism and liver disease. Baillieres Clin Endocrinol Metab, 10(4), 617-635.

Clauss, M., H. Kleffner, and E. Kienzle. (2010). Carnivorous mammals: nutrient digestibility and energy evaluation. Zoo. Biol, 29(6), 687-704.

Corley, J.R., M.W. Esch, J.M. Bahr, and R.A. Easter. (1983). Amino acid supplementation of low-protein diets for swine: effects of gestation treatment on reproductive performance of gilts and sows. J. Anim. Sci., 56(1), 108-117.

Council, N.R. (1982). Nutrient Requirements of Mink and Foxes,: Second Revised Edition, 1982. Washington, DC: The National Academies Press.

Dahlman, T., J. Valaja, P. Niemelä, and T. Jalava. (2002). Influence of protein level and supplementary L-methionine and lysine on growth performance and fur quality of blue fox (Alopex lagopus). Acta. Agric. Scand. A. Anim. Sci, 52(4), 174-182.

Easter, R.A., and D.H. Baker. (1980). Lysine and protein levels in corn-soybean meal diets for growing-finishing swine. J. Anim. Sci., 50(3), 467-471.

Eckert, T.E., and G.L. Allee. (1974). Limiting amino acids in milo for the growing pig. J. Anim. Sci., 39(4), 694-698.

Eghtesad, S., H. Poustchi, and R. Malekzadeh. (2013). Malnutrition in Liver Cirrhosis:The Influence of Protein and Sodium. Middle. East. J. Dig. Dis, 5(2), 65-75.

Guo, J., X. Wu, T. Zhang, Z. Liu, H. Cui, F. Yang, and X. Gao. (2015). Effects of dietary methionine supplementation on growth performance of cubs, nutrient digestibility, nitrogen metabolism and serum biochemical indicators of female blue foxes (Alopex lagopus). Anim. Nutr, 1(4), 378-382.

International, A., W. Horwitz, and C. Association of Official Analytical. (2005). Official methods of analysis of AOAC International. Section 4, 24-43.

Jiang, Q., G. Li, T. Zhang, H. Zhang, X. Gao, X. Xing, and F. Yang. (2018). Application of formulated diets and their effects on nutrient digestibility and reproductive performance of female mink (Neovison vison) during gestation. J. Appl. Anim. Res., 46(1), 125-129.

Jiang, Q., T. Zhang, G. Li, H. Zhang, X. Gao, X. Xing, F. Yang, and X. Wu. (2020). DIETARY PROTEIN LEVELS AFFECT THE NURSING PERFORMANCE AND SERUM BIOCHEMICAL PROFILE OF LACTATING MINK (Neovison vison). J. Anim. Plant. Sci, 30(4), 836-844.

Jin, Y., H. Oh, L. Piao, S. Jang, Y. Choi, P. Heo, Y. Jang, and Y. Kim. (2010). Effect of dietary lysine restriction and energy density on performance, nutrient digestibility and meat quality in

finishing pigs. Asian-Australas. J. Anim. Sci., 23(9), 1213-1220.

Kerr, B., F. McKeith, and R. Easter. (1995). Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. J. Anim. Sci., 73(2), 433-440.

Kidd, M., B. Kerr, J. Allard, S. Rao, and J. Halley. (2000). Limiting amino acid responses in commercial broilers. J. Appl. Poult. Res, 9(2), 223-233.

Liao, S.F., T. Wang, and N. Regmi. (2015). Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. SpringerPlus, 4, 147.

Lissbrant, I.F., P. Hammarsten, E. Lissbrant, N. Ferrara, S.H.g.m. Rudolfsson, and A. Bergh. (2004). Neutralizing VEGF bioactivity with a soluble chimeric VEGF-receptor protein flt(1-3)IgG inhibits testosterone-stimulated prostate growth in castrated mice. Prostate, 58(1), 57-65.

Liu, Z., G. Wu, M. Bryant, and D. Roland Sr. (2005). Influence of added synthetic lysine in low-protein diets with the methionine plus cysteine to lysine ratio maintained at 0.75. J. Appl. Poult. Res, 14(1), 174-182.

Noblet, J., Y. Henry, and S. Dubois. (1987). Effect of Protein and Lysine Levels in the Diet on Body Gain Composition and Energy Utilization in Growing Pigs1. J. Anim. Sci., 65(3), 717-726.

Ogden, B.E., W. Pang William, T. Agui, and B.H. Lee. (2016). Laboratory Animal Laws, Regulations, Guidelines and Standards in China Mainland, Japan, and Korea. ILAR. J, 57(3), 301-311.

Peck-Radosavljevic, M. (2000). Thrombocytopenia in liver disease. Can J Gastroenterol, 14 Suppl D, 60d-66d.

Ray, C.S., B. Singh, I. Jena, S. Behera, and S. Ray. (2017). Low Alkaline Phosphatase (ALP) in adult population an indicator of Zinc (Zn) and Magnesium (Mg) deficiency. Curr. Res. Nutr. Food. Sci, 5(3), 347-352.

Simko, V. (1991). Alkaline phosphatases in biology and medicine. Dig Dis, 9(4), 189-209.

van Harn, J., M.A. Dijkslag, and M.M. van Krimpen. (2019). Effect of low protein diets supplemented with free amino acids on growth performance, slaughter yield, litter quality, and footpad lesions of male broilers. Poult. Sci, 98(10), 4868-4877.

Wang, Y., J. Zhou, G. Wang, S. Cai, X. Zeng, and S. Qiao. (2018). Advances in low-protein diets for swine. J. Anim. Sci. Biotechnol, 9, 60-60.

WeiLi, S., G. Yeye, L. HanLu, Z. Wei, L. DanLi, L. BaiYang, and L. GuangYu. (2009). Comparative research on apparent digestibility of dry matter and crude protein of different protein source diets in blue fox. Chin. J. Anim. Nutr, 21(6), 953-959.

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. Amino Acids, 37(1), 1-17.

Yang, Y., Z. Jin, S. Yoon, J. Choi, P. Shinde, X. Piao, B. Kim, S. Ohh, and B. Chae. (2008). Lysine restriction during grower phase on growth performance, blood metabolites, carcass traits and pork quality in grower finisher pigs. Acta. Agric. Scand. A. Anim. Sci, 58(1), 14-22.

Yen, J.T., J. Klindt, B.J. Kerr, and F.C. Buonomo. (2005). Lysine requirement of finishing pigs administered porcine somatotropin by sustained-release implant. J. Anim. Sci., 83(12), 2789-2797.

Zeng, P.L., H.C. Yan, X.Q. Wang, C.M. Zhang, C. Zhu, G. Shu, and Q.Y. Jiang. (2013). Effects of Dietary Lysine Levels on Apparent Nutrient Digestibility and Serum Amino Acid Absorption Mode in Growing Pigs. Asian-Australas. J. Anim. Sci., 26(7), 1003-1011.

Zhang, H.-h., G.-y. Li, X.-m. Xing, E.-j. Ren, Y. Yang, and F.-h. Yang. (2012). Effect of low-protein diet with supplementing different levels of DL-methionine on production performance of minks in growing-furring period. J. For. Res, 23(1), 151-155.