EFFECTS OF DIETARY VITAMIN B6 ON SKELETAL MUSCLE PROTEIN METABOLISM OF GROWING RABBITS

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ABSTRACT

This research aimed to evaluate the effects of dietary vitamin B6 on skeletal muscle protein metabolism of growing rabbits. Two hundred health rabbits with similar body weight were randomly assigned to one of five dietary groups, with 40 animals per group. The dietary groups consisted of different vitamin B6 supplementation levels: 0(control), 5, 10, 20 and 40 mg/kg. The feeding trial lasted 60 d. The results showed that dietary vitamin B6 had significant effects on fore legs and hind legs muscle ratio ($P<0.05$), and on serum total amino acid (T-AA), blood urea(UR) and insulin-like growth factor-1(IGF1)content ($P<0.05$). Additionally, vitamin B6 had significant effects on muscle insulin-like growth factor 1(IGF1), myogenic determination factor(MYOD) and myogenin(MYOG), myocyte regulation factor 5 (MYF5), myostation(MSTN) and WW contains E3 proteasome ubiquitin ligase 1 (WWP1) mRNA expression($P<0.05$). The results of western blot also showed that IGF1 (MW = 15 KD), WWP1 (MW = 36 KD) and MYOG (MW = 38 KD) significantly expression with the dietary vitamin B6($P<0.05$). This study indicated that addition of vitamin B6 could obviously modify protein metabolism of growing rabbits, and the appropriate vitamin B6 supplementation level was 20 mg/kg for growing rabbits.
Keywords: rabbits; vitamin B6; slaughter performance; serum constituents; protein metabolism.

INTRODUCTION

Nutritional factors are the major determinants of animal growth, and direct nutrient effects on the expression of growth-regulatory genes, and in particular those of the growth hormone insulin-like growth factor (GH-IGF) axis have been postulated (Brameld, 1997). Vitamin B6 is part of the vitamin B complex group, Pyridoxal 5'-phosphate (PLP) is the co-factor for >120 enzyme-catalyzed reactions. These include reactions in pathways for the synthesis and catabolism of amino acids and amines that act as neurotransmitters in the central nervous system (Clayton, 2006). Lack of vitamin B6 will cause animal anorexia, growth retardation, neurological disorders, anemia and other symptoms, especially can lead to disorder of protein metabolism. Research also shows that, add vitamin B6 in prawn feed, the protein of diet can effective deposition in vivo, when adding a higher content of pyridoxine in the Atlantic salmon feed, the body can produce more protein (Albektsen et al., 1993; Giri et al., 1997).

The objective of the present study was to investigate and discuss the effects of vitamin B6 on slaughter performance, serum biochemical, serum hormone and skeletal muscle protein metabolism of growing rabbits. In addition, the appropriate vitamin B6 supplemental level was also determined for growing rabbits.

MATERIALS AND METHODS

Animals and experimental design

In this study, 200 healthy growing rabbits of similar body weight were randomly assigned to one of the five diets, with 40 animals per dietary group. The 60-d feeding trial included a seven day adjustment period and a 53-d experimental period.

Chemical analysis of experimental diets

The experimental diet (Table 1) used in this study was formulated to meet the recommended nutrient requirements of growing rabbits (NRC, 1977). Five different vitamin B6-supplemented diets were prepared: 0 (control), 5, 10, 20, and 40 mg/kg. The measured levels of vitamin B6 in the diets were 4.51, 9.66, 14.64, 24.32, and 44.81 mg/kg, respectively. The vitamin B6 form was pyridoxine

hydrochloride (98%, Jiangxi Tyson Pharmaceutical Co., Ltd., China).

Table 1: Composition and nutrient levels of the experimental diet (as-fed basis, %)

Ingredients	Percentage	Nutrient levels [2)]	Content
Corn	15.00	Digestible energy(MJ/kg)	10.28
Soybean meal	10.00	Crude protein	16.20
Wheat bran	12.00	Crude fiber	17.47
Big wheat (skin)	10.00	Crude ash	11.75
Peanut vine	30.00	Ether extract	2.79
Sunflower meal	8.00	Lysine(Lys)	0.60
Rice bran	10.00	Methionine(Met)	0.27
Premix [1)]	5.00	Calcium	0.97
Total	100.00	Phosphorus	0.43

[1)] Premix provided the following per kg of diets, vitamin A: 10000 IU; vitamin D_3: 2000 IU; vitamin E: 50 mg; vitamin K_3: 2.5 mg; vitamin B_1: 5 mg; vitamin B_2: 10 mg; nicotinic acid: 20 mg; pantothenic acid: 50 mg; folic acid: 2.5 mg; vitamin B_{12}: 1 mg; choline chloride: 400 mg; Fe: 100 mg; Zn: 50 mg; Cu: 40 mg; Mn: 30 mg; I: 0.5 mg; Se: 0.05 mg; $CaHPO_4$: 15000 mg; NaCl: 5000 mg; Lys:1500 mg; Met: 1500 mg; 10% bacitracin zinc: 300 mg; the rest is miscellaneous meal carrier complement。
[2)] Measured values.

Statistical analyses

The data were analyzed by ANOVA and Duncan's test using the GLM Procedure of SAS 9.1.3 statistical software. The data were expressed as mean and root mean square error (R-MSE). $P < 0.05$ and $P < 0.01$ were considered to be significant and extremely significant, respectively.

RESULTS AND DISCUSSION

Effect of vitamin B6 on slaughter performance

The effects of dietary vitamin B6 on slaughter traits were displayed in Table 2. Dietary vitamin B6 affected the fore legs muscle ratio(P=0.0010) and hind legs muscle ratio(P=0.0295).

Table 2: Effects of dietary vitamin B6 on slaughter performance of growing rabbits

Items	Dietary vitamin B6 level (mg/kg)					R-MSE	*P*-value
	0	5	10	20	40		
Slaughter ratio（%）	85.95	85.67	85.99	85.65	86.22	1.0274	0.7875
Eviscerated ratio（%）	54.51	54.06	56.07	56.98	56.17	1.9430	0.0913
Loin muscle ratio（g/kg）	111.83	120.99	130.18	126.93	116.15	14.0083	0.0771
Fore legs muscle ratio（g/kg）	101.67^{c}	114.51^{b}	122.12^{a}	121.60^{a}	115.49^{b}	9.5989	0.0010
Hind legs muscle ratio（g/kg）	207.34^{b}	220.69^{ab}	238.31^{a}	233.65^{a}	238.42^{a}	21.8307	0.0295

R-MSE, root mean square error.
a, b: Treatments with different letters are different at $P < 0.05$.

Effect of vitamin B6 on serum biochemical

The serum biochemical analysis results are shown in Table 3, dietary vitamin B6 had significant influences on the content of serum T-AA and UR (P=0.0481 and P=0.0358, respectively), and T-AA highest or UR lowest was observed in 20 mg/kg group.

Table 3: Effects of dietary vitamin B6 on serum biochemical of growing rabbits

Items	Dietary vitamin B6 level (mg/kg)					R-MSE	*P*-value
	0	5	10	20	40		
GLU /(mmol/L)	6.89	5.59	7.36	7.16	7.84	1.6428	0.6232
T-AA /(mmol/L)	11.73^{b}	12.26^{ab}	13.99^{ab}	17.17^{a}	14.49^{a}	8.3946	0.0481
UR /(mmol/L)	4.48^{a}	4.33^{ab}	3.93^{b}	3.79^{b}	3.86^{b}	0.6352	0.0358
AKP /(U/L)	136.06	148.00	139.13	119.31	109.75	46.6081	0.4798
Ca^{2+} /(mmol/L)	2.95	2.92	3.06	2.93	3.05	0.2198	0.5212
IP /(mmol/L)	2.20	2.13	2.24	1.95	2.30	0.4626	0.6026

GLU, glucose; T-AA, total amino acids; UR, urea; AKP, alkaline phosphatase; Ca^{2+}, Calcium ion; IP, inorganic phosphorus; R-MSE, root mean square error.
a, b: Treatments with different letters are different at $P < 0.05$.

Effect of vitamin B6 on serum hormones

There were no overall differences in serum GH and INS (P=0.4311 and P=0.0685, respectively). Vitamin B6 had a significant influence on serum IGF1 concentration (P=0.0423, Table 4), and increase with vitamin B6 level.

Table 4: Effects of dietary vitamin B6 on serum hormones of growing rabbits

Items	Dietary vitamin B6 level (mg/kg)					R-MSE	*P*-value
	0	5	10	20	40		
GH / (ng/ml)	49.12	49.85	50.34	51.76	51.32	6.9547	0.4311
INS / (uIU/ml)	4.68	5.10	5.94	7.18	9.10	4.2650	0.0685
IGF1 / (ng/ml)	214.56^{c}	274.75^{b}	276.53^{b}	274.93^{b}	292.12^{a}	23.9249	0.0423

GH, growth hormone; INS, insulin; IGF1, insulin-like growth factor 1; R-MSE, root mean square error.
a, b: Treatments with different letters are different at $P < 0.05$.

Effect of vitamin B6 on related mRNA expression

As shown in Figure 1, dietary vitamin B6 had significant influence on IGF1, MYOD, MYOG, MYF5, MSTN and WWP1 ($P < 0.05$), and no significant effects were observed on GF1R mRNA expression ($P > 0.05$).

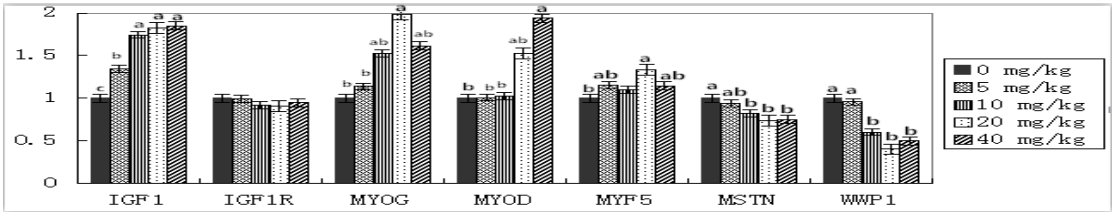

Figure 1: Effects of dietary vitamin B6 on mRNA expression levels of growing rabbits

Effect of vitamin B6 on related protein expression

In order to verify the accuracy of the fluorescence quantitative PCR results, IGF1, MYOG, MSTN and WWP1 were selected for western blotting, to further validate the difference in the protein profile of muscle tissue from different treat rabbits. The results (Figure 2) showed that IGF1 (MW = 15 KD), MYOG (MW = 38 KD)and WWP1 (MW = 36 KD) had significant affected by vitamin B6 in diets (P< 0.05), vitamin B6 had no significant effects on MSTN(MW=42KD).

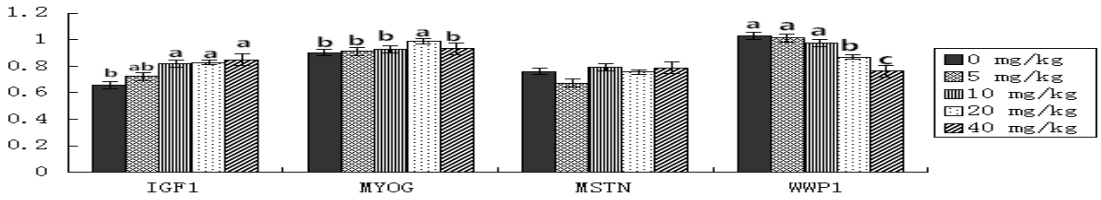

Figure 2： The relative expression of proteins in skeletal muscle of growing rabbits

CONCLUSIONS

In the current study, one of the potential mechanisms for vitamin B6 to improve slaughter performance in growing rabbits was through increasing the synthesis and secretion of IGF1. For a ration mainly composed of corn, wheat bran and peanut straw, the most appropriate dietary vitamin B6 content for 3 to 5-month-old growing rabbits was 20 mg/kg.

ACKNOWLEDGEMENTS

We acknowledge support from the earmarked fund for Modern Agro-industry Technology Research System(CARS-44-B-1)and the Special Fund for Agro-scientific Research in the Public Interest (2000903006).

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