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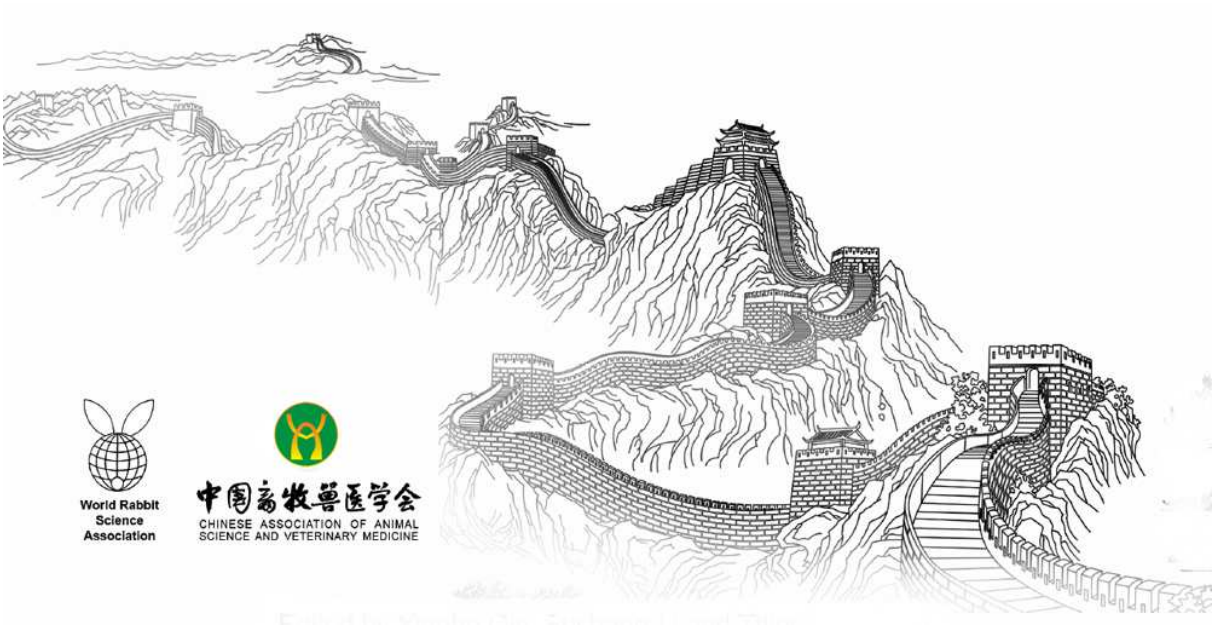

EFFECTS OF DIETARY VITAMIN B6 ON THE NON-SPECIFIC IMMUNE RESPONSE OF GROWING RABBITS

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ABSTRACT

This study evaluated the effects of dietary vitamin B6 on immune organ indexes, serum immune active compounds, and intestinal mucosal immunity of growing rabbits. Two hundred healthy Rex Rabbits 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 and the feeding trial lasted 60 d. The results showed that dietary vitamin B6 had increased thymus and spleen indexes (P<0.05) and serum IgA levels (P<0.05), but not serum IgG or IgE levels (P>0.05). Additionally, vitamin B6 had improved serum interleukin-6 and interferon-γ levels (P<0.05) and on secretory IgA levels in the duodenum and ileum (P<0.05), but not in the jejunum or colon (P>0.05). Splenic *IL-6* and *IFN-γ* mRNA expression levels increased with vitamin B6 (P<0.01). Additionally, vitamin B6 had significant effects on *pIgR* expression in the ileum (P<0.05) and increased microfold cell count in the appendix (P<0.05). The findings revealed that vitamin B6 affects the non-specific immune response of growing rabbits and the recommended vitamin B6 supplemental level is 10–20 mg/kg for growing rabbits.

Keywords: vitamin B6; rabbit; immune organs; serum immune active compounds; intestinal mucosal immunity.

INTRODUCTION

Rex Rabbit is a breed generally recognized by its velvety fur and is raised for fur and meat production, is a major component of farm economies in developing countries. Rabbits are generally afflicted by digestive disorders, infectious diseases of the digestive system currently account for 70% of all rabbit diseases (Carabaño et al., 2008). Adequate nutrition can minimize the risk of digestive disorders (Gidenne et al., 2010). Pyridoxal 5'-phosphate (PLP), the coenzyme form of vitamin B6, participates in amino acid, glucose, and lipid metabolism and regulates the immune system. In healthy individuals, vitamin B6 deficiency decreases the levels of IL-2, T-, and B-lymphocytes (Meydani et al., 1991). Trakatelli (1992) reported that mice and rats fed vitamin B6-deficient diets had reduced lymphocyte maturation, cell proliferation, and cellular activity. Additionally, lack of vitamin B6 decreases serum IgE, IgG1, and IgG2α levels (Inubushi, 2000). The objective of this study was to investigate the effects of vitamin B6 on the immune organs, serum immune active compounds, and intestinal mucosal immunity of growing Rex rabbits. In addition, the appropriate vitamin B6 supplemental level was determined for growing Rex rabbits.

MATERIALS AND METHODS

Chemical analysis of experimental diets

Five different vitamin B6-supplemented diets were prepared: 0 (control), 5, 10, 20, and 40 mg/kg. The vitamin B6 form was pyridoxine hydrochloride (98%, Jiangxi Tyson Pharmaceutical Co., Ltd., China).

Table 1: Composition and nutrient levels of the experimental diet (%)

Ingredients	Percentage	Nutrient levels [2)]	Content, as fed basis
Corn	15.0	Digestible energy(MJ/kg)	10.28
Soybean meal	10.0	Crude protein	16.20
Wheat bran	12.0	Crude fiber	17.47
Barley grain	10.0	Crude ash	11.75
Peanut vine	30.0	Ether extract	2.79
Sunflower meal	8.0	Lysine(Lys)	0.60
Rice bran	10.0	Methionine(Met)	0.27
Premix [1)]	5.0	Calcium	0.97
Total	100.0	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; the rest is miscellaneous meal carrier complement. [2)] Digestible energy was calculated according to the tables of feed composition and nutritive values in China (The 20th revised edition, 2009), while the others were measured values.

Animals and experimental design

200 weaned 30 days old healthy growing Rex rabbits of similar body weight (684±40g) were randomly assigned to one of the five diets in this study. The 60-d feeding trial included a seven day adjustment period and a 53-d experimental period. At the end of the feeding trial, 40 rabbits were bled and the serum was stored at -20°C. The digestive tract, including duodenum, jejunum, ileum, and colon (upper 1 cm segment), was removed, suspended in 5 ml PBS, and washed for 5 min by vortexing. The mucus was collected by centrifugation at 5,000 g for 30 min at 4°C, and the resulting supernatant was stored at -20°C. Splenic and intestinal samples were collected, frozen in liquid nitrogen, and stored at -80°C.

Measurements and analyses

Immune organ indexes: Twelve hours prior to slaughter, rabbits were fasted and weighed. Following slaughter, the thymus, spleen, and liver were carefully removed and weighed. The immune organ indexes (g/kg)= Immune organ weight(g)/Slaughter weight(kg).

Immune active compounds: Serum IgG, IgA, IgE, IL-2, IL-6, and IFN-γ levels were measured by ELISA kit (Shang Hai Lengton Bioscience Co., China) and sIgA in the intestinal mucus was measured by ELISA as described by Sheela *et al.* (2003).

mRNA expression: Total RNA was extracted from tissue samples by a single-step isolation procedure using Trizol (Invitrogen, USA). Semi-quantitative RT-PCR was performed to determine *IL-6*, *IFN-γ*, and *pIgR* mRNA expression levels. *GAPDH* was used as the reference gene.

Intestinal M cell: The intestinal appendix immunohistochemical analysis was performed using the PV-9002 Plink-2 plus Polymer HRPD Detection System for mouse primary antibody (ZSGB-BIO, Beijing, China).

Statistical analyses

The data were analyzed by ANOVA and Duncan's test using the GLM Procedure of SAS 9.1.3 statistical software and mRNA expression were normalized to the internal control *GAPDH* and the relative expression levels were calculated using the $2^{-\triangle\triangle Ct}$ method. 10 vimentin-positive sections were selected from the same group to evaluate the immunohistochemistry results. A total of 30 fields of view (three per section) were analyzed using Image J software (National Institutes of Health, USA). M cell staining intensity was expressed as OD units according to the following calculation, OD= lg(g0/g) where g0 is the mean gray level of the background and g is the gray level of each positive image (Stanley, 2009). OD units are positively correlated with M cell count.

RESULTS AND DISCUSSION

Effect of vitamin B6 on immune organ indexes

Dietary vitamin B6 affected the thymus ($P = 0.0362$) and spleen ($P = 0.0478$) indexes, especially in the 20 and 10 mg/kg groups, respectively (Table 2).

Table 2: Effects of vitamin B6 on immune organ index of growing rabbits (g/kg)

Organ index	Dietary vitamin B6 level (mg/kg)					R-MSE	*P*-value
	0	5	10	20	40		
Thymus	2.3^{b}	2.3^{b}	2.4^{b}	2.6^{a}	2.3^{b}	0.487	0.0362
Spleen	0.3^{c}	0.4^{ab}	0.5^{a}	0.3^{b}	0.3^{b}	0.517	0.0478
Liver	26.1	24.3	23.5	25.9	24.3	3.498	0.5102

Notes: Different lowercase letters in a row represent significant differences at $P < 0.05$. The same as below.

Effect of vitamin B6 on serum immune active compounds and sIgA levels

Dietary vitamin B6 improved IgA levels ($P = 0.0240$; Table 3), especially in the 10 mg/kg group. Vitamin B6 increased serum IL-2 ($P = 0.0353$) and IFN-γ ($P = 0.0142$) levels. Vitamin B6 had significant effects on the sIgA levels in the duodenum ($P = 0.0130$) and ileum ($P = 0.0453$); sIgA levels decreased in the 20 mg/kg group(Table 4).

Table 3: Effects of vitamin B6 on serum immune active compounds of growing rabbits (μg/ml)

Items	Dietary vitamin B6 level (mg/kg)					R-MSE	*P*-value
	0	5	10	20	40		
IgG	19.64	19.83	20.50	23.91	21.75	3.893	0.1848
IgA	132.74^{c}	135.25^{c}	162.78^{a}	148.54^{b}	140.04^{b}	43.329	0.0240
IgE	13.17	14.62	14.82	15.49	15.04	3.650	0.5622
IL-2	28.69	32.44	32.92	34.31	33.10	5.584	0.0501
IL-6	250.28^{b}	251.73^{b}	267.59^{ab}	288.56^{a}	298.61^{a}	49.129	0.0353
IFN-γ	210.12^{b}	211.38^{ab}	221.11^{a}	222.83^{a}	232.16^{a}	43.282	0.0142

Table 4: Effects of vitamin B6 on sIgA levels of growing rabbits (μg/ml)

Items	Dietary vitamin B6 level (mg/kg)					R-MSE	*P*-value
	0	5	10	20	40		
Duodenum sIgA	24.73^{b}	26.73^{b}	27.79^{b}	30.48^{a}	28.71$^{b.}$	1.317	0.0130
Jejunum sIgA	28.00	28.62	29.05	29.14	28.68	1.177	0.0984
Ileum sIgA	30.37^{c}	34.25^{b}	34.81^{b}	36.03^{a}	35.49^{a}	0.775	0.0453
Colon sIgA	28.46	28.72	28.93	30.75	29.12	1.615	0.0738

Effect of vitamin B6 on mRNA expression and on M cell count

Dietary vitamin B6 increased splenic *IL-6* and *IFN-γ* mRNA expression levels ($P < 0.01$, figure 1). Additionally, vitamin B6 had significant effects on intestinal *pIgR* mRNA expression levels($P < 0.05$). Vitamin B6 levels had significant effects on OD units ($P<0.05$; Figure2). With the increasing vitamin B6 from 0 to 5 mg/kg, M cell count in the appendix increased.

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

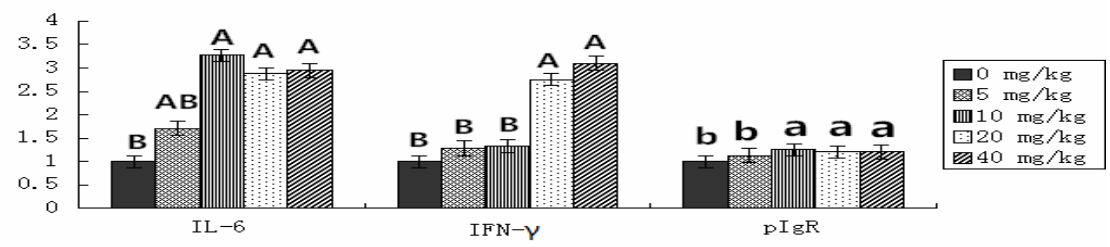
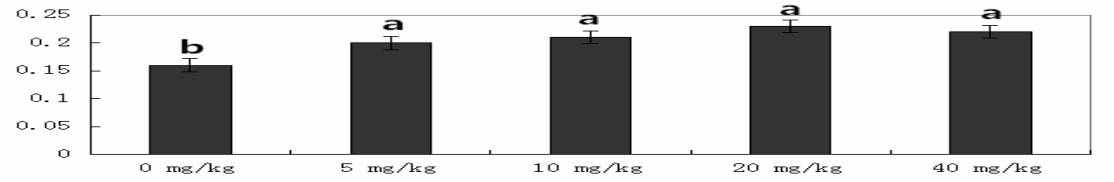


Figure 2： Effects of vitamin B6 on M cell count in growing rabbits



CONCLUSIONS

The results revealed that the increase dietary vitamin B6 from 0 to 40 mg/kg, positively affected the non-specific immune response of growing rabbits. Using a diet mainly consisting of corn, wheat bran, and peanut vine, the appropriate vitamin B6 supplemental level for growing Rex rabbits would be 10–20 mg/kg.

ACKNOWLEDGEMENTS

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Effects of dietary vitamin B6 on the non-specific immune response of growing Rex rabbits

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1.The Message

Weaned rabbits are generally afflicted by digestive disorders (70 % of all rabbit diseases), which contribute to reduced growth performance and health. This study evaluated the effects of vitamin B6 on the non-specific immune response of early weaned Rex rabbits.

2.Introduction

Pyridoxal 5'-phosphate (PLP), the coenzyme form of vitamin B6, participates in amino acid, glucose, and lipid metabolism and regulates the immune system. Adequate nutrition can minimize the risk of digestive disorders (Gidenne et al., 2010). Thus the aim of this study is to evaluate the effects of dietary vitamin B6 on growth performance, immune organ indexes, serum immune active compounds, and intestinal mucosal immunity of growing Rex rabbits.

3.Methods

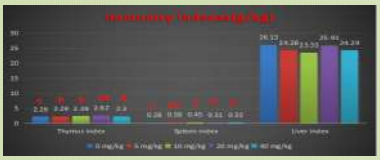
Five different vitamin B6-supplemented diets were prepared: 0 (control), 5, 10, 20, and 40 mg/kg. The vitamin B6 form was pyridoxine hydrochloride (98%, Jiangxi Tyson Pharmaceutical Co., Ltd., China). 200 weaned 30 days old healthy growing Rex rabbits of similar body weight (684±40g) were randomly assigned to one of the five diets in this study. The 60-d feeding trial included a seven day adjustment period and a 53-d experimental period. Individual weight was measured at the beginning and end of the trial and the ADG was calculated. At the end of the feeding trial, 40 rabbits were bled and serum IgG, IgA, IgE, IL-2, IL-6, and IFN-γ levels were measured by ELISA kit (Shang Hai Lengton Bioscience Co., China). 12 hours prior to slaughter, rabbits were fasted and weighed. Following slaughter, the thymus, spleen, and liver were carefully removed and weighed.The digestive tract, including duodenum, jejunum, ileum, and colon (upper 1 cm segment), was removed, suspended in 5 ml PBS, and washed for 5 min by vortexing. The mucus was collected by centrifugation at 5,000 g for 30 min at 4°C, and the resulting supernatant was stored at -20°C, and sIgA in the supernatant was measured by ELISA as described by Sheela et al. (2003). Splenic and intestinal samples were collected for mRNA expression. The intestinal appendix immunohistochemical analysis was performed using the PV-9002 Plink-2 plus Polymer HRPD Detection System for mouse primary antibody (ZSGB-BIO, Beijing, China).

4.Resuts

4.1 Effects of dietary vitamin B6 on growth performance

Items	Dietary vitamin B6 level (mg/kg, as-fed basis)					R-MSE [a]	P-value
	0	5	10	20	40		
Initial body weight (g)	685.5	682.7	685.4	684.3	682.4	99.66	0.867
Final body weight (g)	1305.6[c]	1440.2[b]	1528.4[ab]	1569.4[a]	1509.2[ab]	150.34	0.012
Average daily gain (g/d)	11.7[c]	14.3[bc]	15.9[ab]	16.7[a]	15.6[ab]	2.47	0.034
Average daily feed intake (g/d)	62.5[c]	64.4[bc]	67.3[a]	69.5[a]	65.0[ab]	3.85	0.048
Feed/Gain	5.35	4.41	4.18	4.15	4.78	0.70	0.090
Diarrhea ratio [1] (%)	12.45	9.21	7.32	5.23	5.25	—	—
Mortality [2] (%)	10.25	7.50	5.00	2.50	2.50	—	—

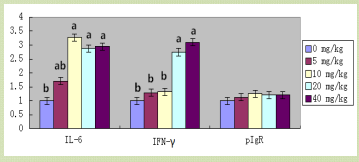
4.2 Effects of dietary vitamin B6 on immune organs development



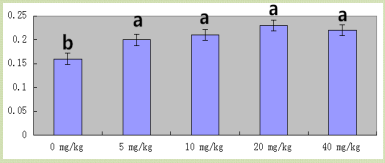
4.3 Effects of vitamin B6 on serum immune active compounds

4.4 Effects of vitamin B6 on intestinal mucus sIgA levels

4.5 Effects of vitamin B6 on related gene mRNA expression



4.6 Effects of dietary vitamin B6 on OD values in Immunohistochemistry resuts



5.Conclusions

Vitamin B6 clearly plays an important role in stimulating the non-specific immune responses of growing Rex rabbits under the experimental conditions.

Using a ration mainly consisting of corn, wheat bran, and peanut vine, the appropriate vitamin B6 supplemental level for weaned (28 -day) to 3-month old growing Rex rabbits is 10–20 mg/kg (the basic diet vitamin B6 content was 4.51 mg/kg).

6.Acknowledgements

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