

# Effects of pantothenic acid on growth performance and antioxidant status of growing male white Pekin ducks

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**ABSTRACT** An experiment was conducted to investigate the effects of dietary pantothenic acid levels on growth performance, carcass traits, pantothenic acid status, and antioxidant status of male white Pekin ducks from 15 to 42 D of age and to evaluate the requirement of this vitamin for growing ducks. Different levels pantothenic acid (0, 2, 4, 6, 8, and 10 mg/kg) were supplemented to a corn-soy isolate protein basal diet to produce 6 dietary treatments with different analyzed total pantothenic acid levels (4.52, 6.44, 8.37, 9.88, 12.32, and 14.61 mg/kg). A total of 240 15-day-old male white Pekin ducks were allotted to 6 dietary treatments with 8 replicate pens of 5 birds per pen. At 42 D of age, growth performance, carcass traits, tissue pantothenic acid concentrations, and antioxidant status of white Pekin ducks were examined. Significant effects of dietary pantothenic acid on BW, average daily weight gain (ADG), plasma, and liver pantothenic acid concentrations were

observed ( $P < 0.05$ ) but not carcass traits. The growing ducks fed the basal diet without pantothenic acid supplementation had the lowest BW, ADG, plasma, and liver pantothenic acid content among all ducks ( $P < 0.05$ ). In addition, the ducks fed the basal diet without pantothenic acid supplementation showed the lowest antioxidant capacity indicated by greatest plasma malondialdehyde content and lowest liver total antioxidant capacity ( $P < 0.05$ ). And, these criteria responded linearly as dietary pantothenic acid levels increased ( $P < 0.05$ ). These results indicated that dietary pantothenic acid supplementation improved growth performance and antioxidant status of the growing ducks. In accordance with the broken-line model, the pantothenic acid requirements (based on dietary total pantothenic acid) of male white Pekin ducks from 15 to 42 D of age for BW, ADG, and plasma and liver pantothenic acid contents were 10.18, 10.27, 12.06, and 10.79 mg/kg, respectively.

**Key words:** duck, pantothenic acid, requirement, growth performance, antioxidant status

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## INTRODUCTION

Pantothenic acid, an essential water-soluble vitamin, functions as a component of coenzyme A and acyl-carrier protein, which is involved in various metabolic reactions including lipids, proteins, and carbohydrates (Smith and Song, 1996; Miller and Rucker, 2012). The symptoms of pantothenic acid deficiency, such as high mortality, growth retardation, dermatosis, and rough feathers, have been observed in chicks, turkeys, and geese (Jukes, 1939;

Bauernfeind et al., 1942; Lepkovsky et al., 1945; Kratzer and Williams, 1948; Hegsted and Riggs, 1949; Wang et al., 2016). Similar to other avian species, pantothenic acid is also needed by ducks, and the deficiency of this vitamin could lead to growth depression, an excess of secretion from the eyes, high mortality, and poor feather in starter Pekin ducks (Hegsted and Perry, 1948). Besides, it is shown that pantothenic acid could protect the cell membrane against damage caused by lipid peroxidation (Slyshenkov et al., 1995, 2001; Walczak-Jedrzejowska et al., 2013). Previous studies have demonstrated that supplementation of pantothenic acid in the diet could increase antioxidant capability in geese (Wang et al., 2016) and fish (Qian et al., 2015). However, it is still unclear whether dietary pantothenic acid affects antioxidant status of growing Pekin ducks, which requires further investigation.

Recently, we estimated the pantothenic acid requirements of modern starter Pekin ducks, which confirmed

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that pantothenic acid recommendation of [NRC \(1994\)](#) during the starter period (11 mg/kg) is still sufficient for modern Pekin ducks strains (unpublished data). Although the pantothenic acid recommendation of growing Pekin ducks provided by the [NRC \(1994\)](#) refers to the requirement for starter ducks from 1 to 2 wk of age (11 mg/kg) ([Hegsted and Perry, 1948](#)), no documentation was provided to support this value, and the pantothenic acid requirements of growing ducks are virtually lacking until now. It has been demonstrated that the vitamin requirements varies with different ages in poultry ([Heuser et al., 1938](#); [Tang et al., 2015](#)). Thus, it is unknown whether the pantothenic acid requirement of starter ducks also applies to birds in growing period. Therefore, the objectives of the present study were to investigate the effects of dietary pantothenic acid levels on growth performance, carcass traits, pantothenic acid status, and antioxidant status of growing white Pekin ducks. The optimum dietary pantothenic acid requirement for these ducks was also evaluated.

## MATERIALS AND METHODS

All experimental procedures of the present study were approved by the Animal Care and Use Committee of the Institute of Animal Sciences of Chinese Academy of Agricultural Sciences and performed as per the

guidelines for animal experiments established by the National Institute of Animal Health.

## Animals and Housing

Dose-response experiment with 6 dietary pantothenic acid levels was conducted with growing male white Pekin ducks from 15 to 42 D of age. A total of 300 1-day-old male white Pekin ducks obtained from Pekin duck breeding center in the Chinese Academy of Agricultural Sciences were randomly allotted to 20 raised plastic-floor pens with 15 birds per pen, and then, they were raised until 14 D of age. At 14 D of age, after fasting for 12 h, all the ducks were weighed individually, and 240 ducks selected from these ducks were divided into 6 dietary treatments, each containing 8 replicate pens with 5 birds per pen. Each pen had similar bird weight. All ducks were raised on plastic-wire floors in an environmentally controlled house. The temperature was kept at 30°C from 1 to 3 D of age and then reduced gradually to approximately 25°C until 14 D of age and was kept at approximately 16°C to 24°C thereafter. Feed and water were given ad libitum, and 24-h constant lighting was provided.

## Diets

All the ducks were raised with a common corn-soybean meal starter diet ([Table 1](#)), and this diet was

**Table 1.** Composition of common starter feed from hatch to 14 D of age and pantothenic acid-deficient basal diet from 15 to 42 D of age (% as-fed).

Item	Common starter feed (from hatch to 14 D)	Basal feed (from 15 to 42 D)
Ingredient, %		
Corn	62.95	82.94
Soybean	33.3	-
Soy isolate protein	-	12.9
Limestone	0.8	1.0
Dicalcium phosphate	1.5	1.6
Vitamin and trace mineral premix	1.0 <sup>a</sup>	1.0 <sup>b</sup>
Sodium chloride	0.3	0.3
DL-Methionine	0.15	0.22
L-Tryptophan	-	0.04
Calculated composition, %		
Metabolizable energy <sup>c</sup> , kcal/kg	2,919	3,189
Crude protein	20.02	18.07
Calcium	0.93	0.93
Nonphytate phosphorus	0.36	0.43
Lysine	1.11	0.91
Methionine	0.45	0.45
Methionine + cysteine	0.79	0.70
Threonine	0.83	0.68
Tryptophan	0.22	0.20
Arginine	1.38	1.19
Pantothenic acid <sup>d</sup> , mg/kg	18.90	4.52

<sup>a</sup>Supplied per kilogram of total diet: Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O), 10 mg; Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O), 60 mg; Zn (ZnO), 60 mg; Mn (MnSO<sub>4</sub>·H<sub>2</sub>O), 80 mg; Se (NaSeO<sub>3</sub>), 0.3 mg; I (KI), 0.2 mg; choline chloride, 1,000 mg; vitamin A (retinyl acetate), 10,000 IU; vitamin D<sub>3</sub> (Cholecalciferol), 3,000 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 20 IU; vitamin K<sub>3</sub> (menadione sodium bisulfate), 2 mg; thiamin (thiamin mononitrate), 2 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 4 mg; cobalamin, 0.02 mg; d-calcium-pantothenate, 11 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.2 mg.

<sup>b</sup>Supplied per kilogram of total diet: Cu (CuSO<sub>4</sub>·5H<sub>2</sub>O), 10 mg; Fe (FeSO<sub>4</sub>·7H<sub>2</sub>O), 60 mg; Zn (ZnO), 60 mg; Mn (MnSO<sub>4</sub>·H<sub>2</sub>O), 80 mg; Se (NaSeO<sub>3</sub>), 0.3 mg; I (KI), 0.2 mg; choline chloride, 1,000 mg; vitamin A (retinyl acetate), 10,000 IU; vitamin D<sub>3</sub> (Cholecalciferol), 3,000 IU; vitamin E (DL- $\alpha$ -tocopheryl acetate), 20 IU; vitamin K<sub>3</sub> (menadione sodium bisulfate), 2 mg; thiamin (thiamin mononitrate), 2 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 4 mg; cobalamin, 0.02 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.2 mg.

<sup>c</sup>The value is calculated as per the AME of ducks ([Ministry of Agriculture of China, 2012](#)).

<sup>d</sup>The values were analyzed by HPLC coupled with triple quadrupole mass spectrometry.

formulated to meet the nutrient recommendation for starter ducks provided by the [Ministry of Agriculture of China \(2012\)](#). The starter diet included 11 mg/kg of supplemental pantothenic acid. The basal diet during the growing period was formulated to be pantothenic acid deficient ([Table 1](#)), and all nutrients except pantothenic acid met the recommendations for growing ducks provided by the [Ministry of Agriculture of China \(2012\)](#). To produce 6 experimental diets, the basal diet was prepared as mash and then supplemented with 0, 2, 4, 6, 8, and 10 mg crystalline calcium pantothenate/kg diet, respectively. All diets were cold-pelleted at room temperature. The crystalline d-calcium pantothenate (purity, 99%) was obtained from Hangzhou Xinfu Technology Co. Ltd. (Hangzhou, Zhejiang, China). The pantothenic acid content of all experimental diets was analyzed by HPLC coupled with triple quadrupole mass spectrometry. The analyzed values of 6 experimental diets were 4.52, 6.44, 8.37, 9.88, 12.32, and 14.61 mg pantothenic acid/kg, respectively.

### Sample Preparation and Data Collection

At 42 D of age, after fasting for 12 h, the ducks and residual diet from each pen were weighed to determine average daily weight gain (ADG), average daily feed intake, and feed conversion ratio. Two ducks were selected randomly from each pen and bled via a wing vein. The blood samples were collected into heparin sodium-containing tubes and centrifuged at  $1,520 \times g$  for 15 min to obtain plasma and then stored at  $-20^{\circ}\text{C}$  until further analysis. Afterward, these selected ducks were sacrificed by  $\text{CO}_2$  inhalation and immersed into hot water at  $60^{\circ}\text{C}$  for 2 min. Then, they were scalded, picked, and eviscerated manually. The breast meat (including both the pectoralis major and pectoralis minor), leg meat (including the thighs and the drum sticks), and abdominal fat were removed from carcasses and weighed as described by [Xie et al. \(2006\)](#). The percentage yield for the breast meat, leg meat, and abdominal fat was expressed as relative weight to fasted live BW at processing. The liver from these selected ducks was sampled immediately and then stored at  $-20^{\circ}\text{C}$  until further analysis.

### Measurements

Pantothenic acid concentrations in feed, plasma, and the liver were determined by HPLC coupled with triple quadrupole mass spectrometry (Agilent 6470) as per the methods described previously by [Lu et al. \(2008\)](#). Agilent 1290 HPLC system consisting of ZORBAX Eclipse Plus C18 column ( $3.0 \times 150$  mm i.d.,  $1.8 \mu\text{m}$ ) was applied for pantothenic acid separation. The column oven was maintained at  $35^{\circ}\text{C}$ , and the flow rate of the mobile phase was 0.2 mL/min. The binary mobile phase used consisted of acetonitrile and water containing 0.1% formic acid. Before HPLC coupled with triple quadrupole mass spectrometry analysis, feed samples were prepared as per the methods described by [Woollard et al. \(2000\)](#). Frozen liver samples ( $\sim 0.2$  g) were thawed, minced, and then added to 10 volumes of 50 mmol/L  $\text{KH}_2\text{PO}_4\text{-K}_2\text{HPO}_4$  buffer (pH 7.0) and homogenized. The mixture was centrifuged at 12,000 rpm for 15 min at  $4^{\circ}\text{C}$ . Then, 1 mL of the supernatant was retained, and 1 mL 3% trichloroacetic acid was added to each sample, vortexed, and centrifuged at 12,000 rpm for 15 min at  $4^{\circ}\text{C}$ . The supernatant was filtered through the  $0.22\text{-}\mu\text{m}$  filter and injected directly into the chromatographic system. Plasma samples were prepared as per the method described by [Petteys and Frank \(2011\)](#). The peak was identified and quantified by using the pure authentic standards purchased from Sigma-Aldrich (St. Louis, MO).

The malondialdehyde content and total antioxidant capacity in the plasma and liver tissue were measured by colorimetric methods using commercial kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, Jiangsu, China). These parameters were expressed as units per milliliter for plasma and units per milligram of protein for liver tissue.

### Statistical Analyses

The 1-way ANOVA were performed using SAS software ([SAS Institute, 2011](#)), with pen used as the experimental unit for analysis. The linear and quadratic polynomial contrasts were performed to determine the effect of dietary pantothenic acid levels in ducks. The

**Table 2.** Effect of dietary pantothenic acid levels on growth performance of male white Pekin ducks.<sup>1</sup>

Dietary pantothenic acid (mg/kg)	Final BW (g)	ADG (g/d/bird)	ADFI (g/d/bird)	FCR (g/g)
4.52	2,931	88.8	167	1.89
6.44	2,944	89.3	169	1.89
8.37	2,966	90.0	174	1.93
9.88	3,026	92.2	177	1.92
12.32	3,031	92.3	167	1.83
14.61	3,013	91.8	172	1.87
SEM	12.1	0.43	1.24	0.01
<i>P</i> -value				
Pantothenic acid	0.039	0.041	0.126	0.058
Pantothenic acid linear	0.009	0.008	0.942	0.061
Pantothenic acid quadratic	0.163	0.186	0.068	0.083

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.

<sup>1</sup>Results are means with  $n = 8$  per treatment.

**Table 3.** Effect of dietary pantothenic acid levels on carcass traits of male white Pekin ducks at 42 D of age.<sup>1</sup>

Dietary pantothenic acid (mg/kg)	Relative weight (% of live BW) <sup>2</sup>			
	Carcass	Breast meat	Leg meat	Abdominal fat
4.52	87.7	12.1	9.80	0.80
6.44	87.5	12.6	9.80	0.79
8.37	87.4	12.2	9.63	0.80
9.88	87.4	11.7	9.39	0.82
12.32	86.8	12.8	9.91	0.78
14.61	87.1	12.1	9.53	0.88
SEM	0.23	0.13	0.085	0.021
<i>P</i> -value				
Pantothenic acid	0.880	0.217	0.513	0.822
Pantothenic acid linear	0.259	0.848	0.554	0.396
Pantothenic acid quadratic	0.834	0.854	0.646	0.434

<sup>1</sup>Results are the means of 8 replicates of 2 ducks each.

<sup>2</sup>The percentage yield is calculated using the following equation: Yield = (carcass, breast meat, leg meat, or abdominal fat weight) × 100%/live BW.

variability in the data was expressed as the SEM, and a probability level of  $P < 0.05$  was considered to be statistically significant. The broken-line regression analysis (Robbins et al., 2006) was used to estimate the pantothenic acid requirements for ducks. The broken-line model was provided as follows:

$$y = l + u(r - x)$$

where  $y$  = growth performance (BW or ADG) and plasma or liver pantothenic acid content,  $x$  = dietary total pantothenic acid level (mg/kg),  $r$  = pantothenic acid requirement,  $l$  = the response at  $x = r$ ,  $u$  = the slope of the curve. In this model,  $y = l$  when  $x > r$ .

## RESULTS AND DISCUSSION

### Growth Performance and Carcass Traits

A series of studies have shown that dietary pantothenic acid deficiency could cause growth retardation in chicks, turkey poults, geese, and ducks, which can be alleviated by pantothenic acid supplementation (Jukes, 1939; Bauernfeind et al., 1942; Lepkovsky et al., 1945; Hegsted and Perry, 1948; Kratzer and Williams, 1948; Hegsted and Riggs, 1949; Wang et al., 2016). In the present study, growth depression was also the typical symptom for pantothenic acid-deficient growing ducks. Supplementation of pantothenic acid in the diet affected BW and ADG significantly ( $P < 0.05$ , Table 2) but not average daily feed intake and feed conversion ratio ( $P > 0.05$ , Table 2). Among all ducks, the birds fed the basal diet with no supplementation of pantothenic acid

showed the lowest BW and ADG ( $P < 0.05$ , Table 2). These criteria increased linearly as dietary pantothenic acid increased and reached a plateau when dietary pantothenic acid was higher than 9.88 mg/kg ( $P < 0.05$ , Table 2). These data indicated that the basal diet was pantothenic acid deficient, and the deficiency was alleviated by supplementation of this vitamin. The results in the present study are in line with those of previous studies aforementioned (Bauernfeind et al., 1942; Lepkovsky et al., 1945; Hegsted and Perry, 1948; Kratzer and Williams, 1948; Beer et al., 1963; Gries and Scott, 1972). On the other hand, dietary pantothenic acid levels did not affect carcass traits of male Pekin ducks at 42 D of age, such as the percentages of carcass, breast meat, leg meat, and abdominal fat ( $P > 0.05$ , Table 3).

The broken-line regression has been widely used to estimate the pantothenic acid requirements for ducks (Hegsted and Perry, 1948), chicks (Hegsted and Riggs, 1949), shrimp (Shiau and Hsu, 1999), and fish (Wen et al., 2009; Lin et al., 2012; Qian et al., 2015). Furthermore, BW and ADG of ducks showed a linear response to increasing dietary pantothenic acid levels (Table 2). Therefore, this analytical method was also used to predict the pantothenic acid requirements of growing ducks in the present study. As per this regression, the pantothenic acid requirements of growing male white Pekin ducks from 15 to 42 D of age for BW and ADG were 10.18 and 10.27 mg pantothenic acid/kg diet, respectively (Table 4). At present, the pantothenic acid requirement of growing white Pekin ducks recommended by the NRC (1994) was 11 mg/kg, which refers to the value of starter ducks (Hegsted and Perry, 1948). To our best knowledge, this is the first time to estimate the pantothenic acid requirement of the growing ducks. And,

**Table 4.** Pantothenic acid requirements of male white Pekin ducks from 15 to 42 D of age based on broken-line regression analysis.

Response criterion	Regression	Requirement (mg/kg)	95% confidence interval (mg/kg)	<i>P</i> -value	R <sup>2</sup>
BW	$Y = 3014.6 - 16.71 \times (10.18 - x)$	10.18	6.57 to 13.83	0.009	0.999
ADG	$Y = 91.80 - 0.59 \times (10.27 - x)$	10.27	6.52 to 14.01	0.009	0.999
Plasma pantothenic acid	$Y = 1.41 - 0.11 \times (12.06 - x)$	12.06	9.92 to 14.20	<0.001	0.893
Liver pantothenic acid	$Y = 28.85 - 1.01 \times (10.79 - x)$	10.79	7.54 to 14.04	<0.001	0.978

Abbreviation: ADG, average daily gain.



**Table 5.** Effect of dietary pantothenic acid levels on plasma and liver pantothenic acid concentrations of male white Pekin ducks.<sup>1</sup>

Dietary pantothenic acid (mg/kg)	Plasma pantothenic acid (μmol/L)	Liver pantothenic acid (μg/g)
4.52	0.61	22.1
6.44	0.82	25.0
8.37	0.95	26.5
9.88	1.21	27.7
12.32	1.34	29.6
14.61	1.49	28.1
SEM	0.055	0.61
<i>P</i> -value		
Pantothenic acid	<0.001	0.014
Pantothenic acid linear	<0.001	<0.001
Pantothenic acid quadratic	0.332	0.079

<sup>1</sup>Results are means with n = 8 per treatment.

the values estimated in this study were close to the recommendation of the [NRC \(1994\)](#), indicating the pantothenic acid recommendation of the [NRC \(1994\)](#) is sufficient for the modern white Pekin ducks strains.

### Pantothenic Acid Status

Tissue pantothenic acid may be a useful biomarker for pantothenic acid status. To date, no information was available how tissue pantothenic acid content responds to the supplementation of this vitamin in poultry. Previous studies have shown that liver pantothenic acid was markedly decreased in pantothenic acid deficient shrimp and fish ([Shiau and Hsu, 1999](#); [Lin et al., 2012](#); [Qian et al., 2015](#)). In agreement with these previous studies, the birds fed the basal diet with no pantothenic acid supplementation showed the lowest plasma and liver pantothenic acid concentrations among all ducks at 42 D of age in the present study ( $P < 0.05$ , [Table 5](#)), which also showed the poorest growth ([Table 2](#)). However, these bad statuses could be reversed by increasing dietary pantothenic acid levels. The plasma and liver pantothenic acid concentrations increased linearly as dietary pantothenic acid increased ( $P < 0.05$ , [Table 5](#)), which was also accompanied with the simultaneous improvement of growth performance ([Table 2](#)). Similar to previous studies in shrimp ([Shiau and Hsu, 1999](#)) and fish ([Lin et al., 2012](#); [Qian et al., 2015](#)), tissue pantothenic acid concentrations increased linearly with

increasing pantothenic acid levels in the diet. Therefore, tissue pantothenic acid concentrations were used to estimate pantothenic acid requirement of growing Pekin ducks. As per the broken-line analysis, the pantothenic acid requirements of growing male white Pekin ducks from 15 to 42 D of age for plasma and liver pantothenic acid were 12.06 and 10.79 mg pantothenic acid/kg diet, respectively ([Table 4](#)). And the pantothenic acid requirements estimated for tissue saturation were greater than those for growth performance in our study ([Table 4](#)), which is consistent with the results observed in fish previously ([Lin et al., 2012](#); [Qian et al., 2015](#)). One explanation is that tissue pantothenic acid concentration responds more rapidly to dietary vitamin intake than weight gain ([Lin et al., 2012](#)).

### Antioxidant Status

It has been reported that pantothenic acid deficiency can cause oxidative damage in geese ([Wang et al., 2016](#)) and fish ([Qian et al., 2015](#)). Similar, among all ducks, we found that the birds fed the basal diet with no supplementation of pantothenic acid had the greatest plasma malondialdehyde content and lowest liver total antioxidant capacity activity in the present study ( $P < 0.05$ , [Table 6](#)). These results indicated that pantothenic acid deficiency could cause oxidative stress in growing Pekin ducks, which is in line with those of previous studies ([Qian et al., 2015](#); [Wang et al., 2016](#)).

**Table 6.** Effect of dietary pantothenic acid levels on plasma and liver antioxidant status of male white Pekin ducks.<sup>1</sup>

Dietary pantothenic acid (mg/kg)	MDA		T-AOC	
	Plasma (nmol/mL)	Liver (nmol/mg prot)	Plasma (U/mL)	Liver (U/mg prot)
4.52	5.73	0.53	2.20	1.01
6.44	4.56	0.51	2.57	1.00
8.37	4.40	0.50	2.36	0.98
9.88	4.53	0.40	2.33	0.99
12.32	4.41	0.41	2.51	1.22
14.61	4.00	0.44	2.64	1.37
SEM	0.13	0.017	0.23	0.040
<i>P</i> -value				
Pantothenic acid	0.006	0.094	0.197	0.009
Pantothenic acid linear	<0.001	0.017	0.072	<0.001
Pantothenic acid quadratic	0.065	0.271	0.794	0.050

Abbreviations: MDA, malondialdehyde; T-AOC, total antioxidant capacity.

<sup>1</sup>Results are means with n = 8 per treatment.

Pantothenic acid could protect cells against oxidative stress by increasing the levels of glutathione and promoting cellular repair mechanisms by potentiating synthesis of membrane phospholipids (Slyshenkov et al., 1995, 2001; Walczak-Jedrzejowska et al., 2013).

In conclusion, among all the growing Pekin ducks, the birds fed the basal diet without pantothenic acid supplementation had the lowest growth performance and pantothenic acid concentrations in plasma and the liver of growing male white Pekin ducks, as well as lowest anti-oxidant capacity in both plasma and the liver. All these negative effects could be avoided by increasing dietary concentration of this vitamin. As per the broken-line regression analysis, the pantothenic acid requirements of the male white Pekin ducks from 15 to 42 D of age for BW, ADG, pantothenic acid contents in plasma and the liver were 10.18, 10.27, 12.06, and 10.79 mg/kg, respectively.

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