

EFFECT OF DIETARY LYSINE ON PRODUCTION PERFORMANCE, INSULIN LIKE GROWTH FACTOR-I (IGF-I) MRNA EXPRESSION IN GROWING RABBITS

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ABSTRACT

An experiment was conducted to study the effects of different amounts of dietary lysine on production performance, serum growth hormone (GH), insulin-like growth factor-I (IGF-I) concentration and IGF-I mRNA expression of growing rabbits. One hundred weaned New Zealand rabbits were allocated to individual cages and randomly divided into five groups. Five diets, containing 5.5 (L1), 6.5 (L2), 7.5 (L3), 8.5 (L4) and 9.5 g/kg (L5) lysine per kg diet, respectively. Average daily gain (ADG) of L3, L4 and L5 were 21.70, 34.37, 23.46 and 13.27, 24.93, 14.79% high than those of L1 and L2 ($P<0.05$), respectively. The feed gain ratio (F/G) of L4 and L5 were 22.73, 15.45 and 17.27, 9.49% low than those of L1 and L2 ($P<0.05$), respectively. Lysine did not affect serum GH concentrations ($P>0.05$). Serum IGF-I concentration tended to have a quadratic increase from L1 to L5 ($P=0.07$). Hepatic and muscular IGF-I mRNA relative abundance tended to increase when dietary lysine level increased ($P=0.053$, $P=0.082$, respectively). Providing a diet mainly consisted of corn, wheat bran and peanut vine, the most appropriate dietary lysine level for weaner to 70-day-old growing meat rabbits was 8.5g/kg diet. IGF-I may be an important factor controlling body growth of growing rabbits.

Key words: New Zealand rabbit, lysine, production performance, IGF-I, mRNA expression

INTRODUCTION

Nutritional status is known to be a key factor that regulates the circulation levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I). Fasting and protein and energy restrictions result in hormone perturbation. However, a single amino acid deficiency was also shown to reduce IGF-I levels in rats (Takenaka et al., 2000) and pigs (Katsumata et al., 2002). Lysine is a major nutrient and limiting amino acid for rabbits. An adequate supply of dietary lysine is essential for protein metabolism and utilization in growing rabbits. Many researchers had studied the effects of appropriate dietary lysine levels on the growth performance of rabbits (Parigi-Bini et al., 1988; Furlan and Martins, 1995). However, there have been few reports on the effect of the dietary lysine level on GH and IGF-I levels in growing rabbits. It is also not clear whether the effect of lysine on protein accretion is regulated by these hormones. The aim of this study was to investigate the influence of the dietary lysine level on growth performance, serum GH and IGF-I levels; and IGF-I expression.

MATERIALS AND METHODS

Animals and diets

One hundred weaned New Zealand rabbits (30 days old) were allocated to five groups with respect to the average body weight (20 rabbits/group). Rabbits were individually housed in self-made metabolism cages (60×40×40 cm), in a closed and ventilated building (temperature ranged from 18 to 28°C maximum, and the relative humidity ranged from 50% to 60%). A cycle of 12 h of light time (6:30 to 18:30) and 12 h of dark time was used throughout this trial.

Five diets that contained 5.5 (L1), 6.5 (L2), 7.5 (L3), 8.5 (L4) and 9.5 g (L5) lysine per kg diet (raw basis)

were formulated (table 1). The diets had equal calculated digestible energy and crude protein, and were pelleted by the use of pressure and the diameter of the pellets was 4 mm.

Table 1: Ingredients and calculated chemical composition of the experimental diets

Ingredients (g/kg diet)	L1	L2	L3	L4	L5	Nutrient (as fed basis)					
						L1	L2	L3	L4	L5	
Corn	150	150	150	150	150	CP, g/kg	162	162	162	162	162
Wheat Bran	338	303	299	295	291	DE (MJ/kg)	10.46	10.46	10.46	10.46	10.46
Peanut vine (<i>Arachis hypogaea liana</i>)	408.39	445.27	451.71	458.14	463.59	CF, g/kg	112	117	117	118	118
Soya bean meal	84	80	76	72	69	Ca, g/kg	5.0	4.6	4.7	4.6	4.7
Salt	5	5	5	5	5	Total P, g/kg	4.6	4.3	4.2	4.2	4.1
Methionine (90%)	1.88	2.21	2.29	2.37	2.44	Lys, g/kg	5.5	6.6	7.6	8.6	9.6
Threonine (90%)	2.73	2.96	3.04	3.12	3.20	Met, g/kg	4.4	4.4	4.5	4.5	4.5
Lysine (90%)		1.56	2.96	4.37	5.77	Met. +Cys, g/kg	6.0	6.0	6.0	6.0	6.0
Premix 1%	10	10	10	10	10	Thr, g/kg	6.0	6.0	6.0	6.0	6.0

CP, crude protein; DE, digest energy; CF, crude fiber; ^a Premix composition (by kg diet): Lys, 1.5g; Met, 1.5g; Cu, 50mg; Fe, 100mg; Zn, 50mg; Mn, 30mg; Mg, 150mg; I, 0.1mg; Se, 0.1mg; vitamin A, 8000 IU; vitamin D, 800 IU; vitamin E, 50g. DE was calculated according to the tables of feed composition and nutritive values in China (the 20th revised edition, 2009).

Experimental procedures

The experiment lasted for 40 days which included a 7-day adjustment period and the rabbits were fed ad libitum. The feed residual were collected daily and weighed.

Individual live weights were measured at the beginning (d 30) and the end (d 70) of the trial and the average daily gain (ADG) was calculated. The average daily feed intake (ADI) was recorded and feed to gain (F/G) ratio were calculated.

Blood sample of all 40 rabbits was carried out by cardiac puncture in 8:00-9:00 of the same day and centrifuged at 3000 rpm/s for 10 min and rabbits were killed by exsanguination from the carotid artery. The isolated serum samples were stored at -20°C for further analysis. Liver and muscle samples were collected, frozen immediately in liquid nitrogen and subsequently stored at -70°C before RNA extraction.

Chemical analysis and statistics

Serum GH and IGF-I were analyzed using commercial RIA kits (GH, 1.5-100 ng/ml, PR, one-step, 37 °C, 2.5 h; IGF-I, 10-500 ng/ml, PR, one-step, 4 °C, 24 h) supplied by Tianjin Jiuding Company and radioactivity was determined in DFM-96 10 tubes RI γ -counter.

Total RNA was extracted from samples by a single-step isolation procedure using Trizol reagent (Invitrogen, USA). Semi-quantitative RT-PCR was performed to determine the IGF-1 gene expression. The forward primer (5'-CCCTCTGCTTGCTCACCTT-3') and the reverse primer (5'-TACATCTCCAGCTCCTCA-3') were designed according to published IGF-1 gene sequence (Genebank accession no. OCU75390). The results were expressed as a ratio of the target to standard signal.

Linear, quadratic and cubic effects of concentration of lysine on all parameters were analyzed by SAS (1985) using the regression procedures. Root mean square error (RMSE) and coefficient of determination (R²) were used to evaluate the goodness of fit for the different effects.

RESULTS AND DISCUSSION

The effect of different amounts of dietary lysine on growth performance

The effect of dietary lysine level on the ADG and F/G was quadratic (Table 2, P=0.013, P=0.034, resp.). This agreed with the previous studies reporting increase of body weight, feed intake and feed conversion ratio with increased dietary lysine content (Parigi-Bini *et al.*, 1988; Furlan and Martins, 1995). The ADI did no significantly differ among different groups.

Table 2: Growth performance of rabbit according to dietary lysine level.

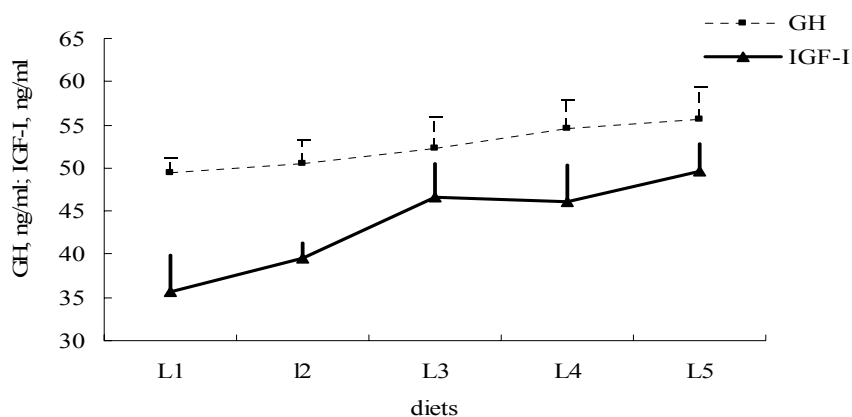
Traits	L1	L2	L3	L4	L5	RMSE	P level *		
							Linear	Quadratic	Cubic
IBW 30 d, g	849	846	834	838	848	84.41	0.82	0.79	0.70
ADG, g/d	25.02 ^b	26.91 ^b	30.48 ^a	33.62 ^a	30.89 ^a	5.15	0.33	0.013	0.091
ADI, g/d	105	104	118	114	115	12.98	0.15	0.090	0.053
F/G	4.40 ^a	4.11 ^a	3.85 ^{ab}	3.40 ^b	3.72 ^b	0.94	0.20	0.034	0.108

P *, P value for linear, quadratic or cubic response. IBW, initial body weight; ADG, average daily gain; ADI, average daily intake; F/G, feed/gain ratio. RMSE, root mean square error. The values with different superscript in the same row differ significantly (P<0.05).

The effect of different amounts of dietary lysine on serum GH and IGF-I concentration

As shown in Figure 1, serum IGF-I tends to increase with dietary lysine concentration (P=0.07). These results are in concordance with the study reported by Ren et al (2007). This might be related to the extent of lysine deficiency and the number of experimental animals.

There were no significant differences in serum GH among different treatments (p=0.15) as in previous reports where increasing dietary lysine level or lysine deficiency did not affect plasma GH (Roy *et al.*, 2000; Ren *et al.*, 2001).

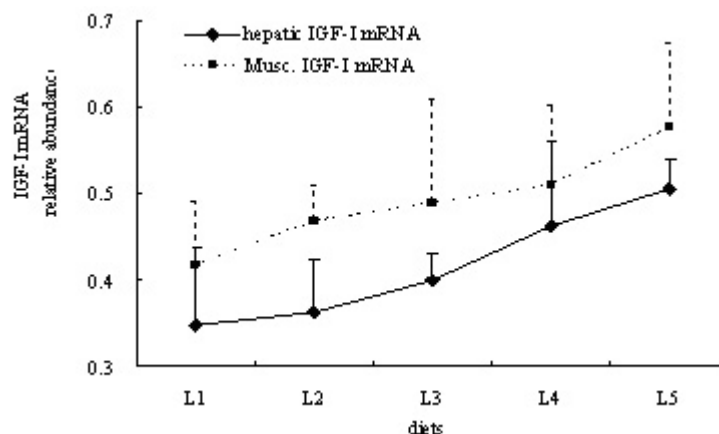


GH, growing hormone; IGF-I, insulin-like factor I; RMSE for GH and IGF-I were 2.61 and 5.78, respectively.

Figure 1: Serum GH and IGF-I concentrations

The effects of different amounts of dietary lysine on IGF-I mRNA expression in liver and muscle

As shown in Figure 2, liver IGF-I mRNA relative abundance shows a quadratic increase (P=0.053) with dietary lysine. There was no significant difference in muscular IGF-I mRNA expression among experimental groups (P=0.08).



RMSE for hepatic and muscular IGF-I mRNA relative abundance were 0.067 and 0.153, respectively

Figure 2: IGF-I mRNA expression in liver and muscle of the growing rabbit.

Pao *et al.* (1993) reported the stimulatory effect of total amino acids on IGF-I mRNA was associated with increased gene transcription. The results of this study demonstrated that the IGF-1 mRNA relative abundance were higher in muscles than in liver of growing rabbits ($P=0.011$), which is consistent to the view that extrahepatic tissues express high levels of IGF-I mRNA (Brameld *et al.*, 1999).

CONCLUSION

The dietary total lysine had quadratic effects on daily gain and feed conversion efficiency of growing rabbits. The lysine tended increase the IGF-I expression and the circle IGF-I concentration. One of the potential mechanism that could explain the improvement of rabbit might be an increased synthesis and secretion of IGF-I.

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