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lower intake of the N-free diet resulted in more N excretion suggesting that protein catabolism may occur in the body of rabbit to meet maintenance requirements for N when the dietary N intake was very low.

1.

The domestic rabbit (*Oryctolagus cuniculus*) was descended from the European wild rabbits and developed into various breeds of modern domestic rabbits from the 18th century. There are now several hundred varieties throughout the world varying in size, colour, type of hair coat and other characteristics (NRC, 1977). These breeds could be used as different purposes such as for meat, wool and for research. The use of laboratory animals is considered to be necessary, for testing new surgical techniques, the study of new chemical and pharmaceutical substances and the production of vaccines and antibodies (Amalia, 2004). The preparation of antiserum is one of the most important usages of laboratory rabbits (Wei, 1998).

A new strain of laboratory rabbit with consistently inherited black eye trait was produced by inbreeding from a mutant of the Japanese White rabbit. Different to the red eye color of the Japanese White rabbit, this new strain is characterized by black eye trait, so it has been known as white hair and black eye (WHBE). It has been observed that the WHBE rabbit was sensitive to changes in environment and adapted for biomedical research, especially in immunology research. Compared with other breeds of laboratory rabbits, the WHBE rabbit could produce higher antibody titer response to some special antigen (Chen et al., 2005). As far as these special characteristics in WHBE rabbits were concerned, it was assumed that their nutritional requirements may be different from other breeds. Amalia (2004) suggested that laboratory rabbits had quite different nutrient requirements from rabbits that were sold commercially. However, due to the absence of feed standards for laboratory rabbit, researchers have to rely on the nutrient requirements of meat-type rabbits or personal experience in determining the feed allowances, which may compromise the ability of the WHBE rabbit to express maximal potential in immune and other biological aspects. Furthermore, excess dietary nitrogen could not only place strain on the kidney, but also cause environmental pollution (Maerten et al., 1997). It is, therefore, necessary to establish feeding allowances for WHBE rabbits.

Energy and protein (amino acids) are the most critical dietary components for animals. Although the requirements of protein (amino acids) for meat-type rabbits have been established (NRC, 1977), little information is available for laboratory rabbits. Most studies of the nutrient requirement of rabbits were based on feeding trials either in China or abroad (Li and Wei, 2002; NRC, 1977; Maerten, 1992) and there was little examination of nutritional parameters. In factorial analysis, the requirements for nutrients may be divided into various components including maintenance, weight gain, hair growth, *etc.* With respect to protein (amino acid) requirements, that for maintenance is significant and it is important that it is quantified such that total requirements can be established allowing differences between animal species to be determined.

In the current study, a series of nitrogen (N) balance trials were carried out to investigate the requirement of crude protein (CP) for maintenance in WHBE rabbits.

2.

These experiments were approved by the Animal Care and Use Committee of Zhejiang Province and carried out in Laboratory Animal Research Center, Zhejiang Chinese Medicine University, China. The rabbits used in the trials were in good health and met the criteria for microbiology and parasitology (AQSIQ, 2001a,b). The numbers of laboratory-animal-quality certification and experimental facility license were SCXK(ZHE) 2005-0022 and SYXK(ZHE) 2003–0004, respectively.

1
The composition of experimental diets used in the series of nitrogen balance tests.

Items[a]	Crude protein (g/kg)				
	120	140	160	180	200
Digestible energy (MJ/kg DM)	10.4	10.4	10.4	10.4	10.4
Crude protein (g/kg DM)	115	138	159	175	205
Crude fibre (g/kg DM)	123	125	113	115	116
Ca (g/kg DM)	15	15	15	15	15
P (g/kg DM)	8	8	8	8	8
Lysine (g/kg DM)	6.6	7.9	9.0	10.1	11.3
Methionine + cysteine (g/kg DM)	4.9	5.7	6.5	7.3	8.1
Threonine (g/kg DM)	5.0	5.8	6.7	7.5	8.3

[a] The digestible energy, Ca, P and amino acids were calculated from the ingredients of the diets, while the contents of crude protein and crude fibre were determined values (AOAC, 1990).

2.1. Nitrogen balance test

Sixty weanling rabbits (42-day-age, male:female = 1:1) were selected and housed individually in cages in a room with controlled temperature and a regular photoperiod (12 h light and 12 h dark) and given free access to feed and water. Nitrogen balance tests were carried out at the age of 10 and 15 weeks, corresponding to the growing and finishing periods, respectively. In each period, 30 rabbits (male:female = 1:1) were selected and divided into 5 equal groups. Each group with six replicates was randomly allocated to an isoenergetic ration with different content of CP ranging from 120 to 200 g/kg (Table 1). In all experimental diets, the contents of lysine, methionine plus cystine and threonine in CP were similar. All the rabbits for N balance test were confined in individual metabolism cages for collection of faeces and the data were individually recorded.

Each period consisted of 12 days, the first 9 days of which were for adaptation and the subsequent 3 days for collection. During the latter period, faeces and urine were collected respectively at 8:00 a.m. every day. Total faecal output for 3 days was mixed with 20 ml tartaric acid (10%) and dried in an air-force drier at 65–70 °C. After being mixed, ground (40 Mesh) and weighed, a 10% sample of faeces was retained. Urine was acidified with 10% tartaric acid and refrigerated at 4 °C until the end of the collection period. A 10% sample of urine was retained and frozen for subsequent N analysis. Feed, faeces and urine samples were analyzed for N content by Kjeldahl process (methods number 984.13; AOAC, 1990). Feed samples were also analyzed for crude fibre content (method number 962.09). Digestible energy, and Ca and P were calculated from the ingredients of the experimental diets.

2.2. Nitrogen balance test with N-free ration

Twenty rabbits at the age of 16 weeks were used in a nitrogen balance trial with N-free ration. They were randomly divided into five groups (A–E). Before the test, all the rabbits were kept individually in cages and offered a diet with 160 g CP/kg and 10.4 MJ DE/kg for 7 days.

In the subsequent balance period, the rabbits were fed on a N-free diet, based on maize starch with the addition 140 g pure cellulose and 40 g premix (consisting of multi-vitamins and trace minerals) per kilogram. The daily allowance was 55, 45, 35, 25 and 0 g/day for groups (Table 2).

The experimental period lasted for 6 days and excreta collection commenced on the 4-594.commence eTa

2
The experimental design for nitrogen (N) balance with N-free diet (NFD).

Period	Groups				
	A	B	C	D	E
Interim period					
Duration (day)	7	7	7	7	7
Feed	Normal[a]	Normal	Normal	Normal	Normal
Daily intake (g/day)	160	160	160	160	160
Experimental period					
Duration (day)	6	6	6	6	6
Feed	NFD	NFD	NFD	NFD	Fasting
Daily intake (g/day)	55	45	35	25	0
Days to start collection of excreta	4	4	4	4	After 40 h
Duration of sampling (day)	3	3	3	3	40 h

[a] A diet with 160 g CP/kg and 10.4 MJ DE/kg.

3.

3.1. Nitrogen balance test

The results of N balance for rabbits over two periods are shown in Table 3. Net protein utilization (NPU) tended to increase with the increased levels of CP, but no significant difference was found among the five CP contents ($P>0.05$). Age had a significant influence on the NUP ($P<0.001$) that was lower during the growing period than during finishing period ($P<0.01$) possibly due to the fact that the mature rabbits can engage in coprophagy to use the non-protein nitrogenous compounds more efficiently than growing rabbits (Hoover and Heitmann, 1975). Because the contribution of microbial protein to young rabbits is small (Varela-Alvarez, 1991), the growing rabbits should be supplied with good-quality protein. On the other hand, the digestible capacity in the finishing rabbits should be higher than in the grower, resulting in a higher NPU in finishing period.

3
Content of crude protein (CP) in the diet, intake and retention of nitrogen (N), and net protein utilization (NPU) in WHBE rabbit at different ages.

Experimental period (age)	Designed CP (g/kg)	Initial weight (kg)	CP (g/kg)	Nitrogen balance (g/day)		NPU (g/kg)	
				Intake	Retention	NPU	Mean
Growing period (6–12 wks)	120	2.1	115	2.8	1.2	440	445
	140	2.1	137.5	3.0	1.2	405	
	160	2.1	159	3.8	1.7	454	
	180	2.2	175	4.2	1.8	427	
	200	2.2	205	5.2	2.6	499	
Finishing period (13–17 wks)	120	2.8	115	2.9	1.2	424	497
	140	2.7	137.5	3.5	1.8	521	
	160	2.6	159	3.9	2.0	501	
	180	2.7	175	4.3	2.2	501	
	200	2.8	205	5.1	2.7	535	
S.E.M.		0.02		0.04	0.04	8.9	
P	Age	<0.001		0.052	0.004	0.005	
	CP	0.193		<0.001	<0.001	0.061	
	Age × CP	0.226		0.0097	0.255	0.210	

3.2. The requirement of protein for maintenance estimated from N balance test

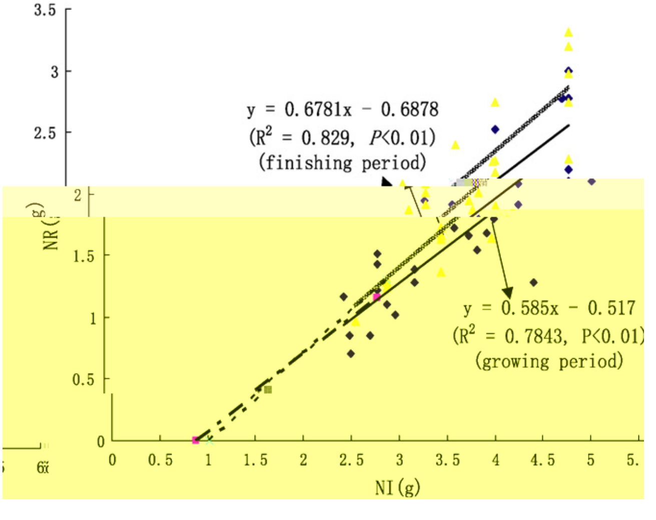
Taking the N retained in the body (NR) as the dependent variable and the N intake (NI) as the independent variable, the data were subject to linear regression. The results are shown in Fig. 1. The requirement of N for maintenance (Nm) may normally be estimated by extrapolating from the linear regression (McDonald et al., 2001) and is equivalent to NI where the relationship is extrapolated to zero N retention. The N requirement would increase with the increasing age and body weight, but no significant difference was found between the two periods when the Nm was express as the value per unit of metabolic body size ($kg^{0.75}$). The result was consistent with Miyasaki et al. (1997) who showed that the CP requirement expressed as gram per unit of metabolic body size remained constant with advancing age in broilers.

It was estimated that the requirement of Nm in WHBE rabbits was, on average, 485 mg/kg $BW^{0.75}$, which is equal to 3.03 g CP/kg $BW^{0.75}$ per day. The value is lower than growing Angora wool-type rabbits (4.87 g CP/kg $BW^{0.75}$, Liu et al., 1991). The body weight of finishing rabbits was about 3.0 kg, so they need 6.91 g CP for maintenance. Given that the daily feed intake of a finishing rabbit was 150 g/day, the CP concentration of feed for maintenance is about 45.6 g/kg, which was lower than that recommended by NRC (1977) for meat-type rabbits (120 g/kg). The lower CP requirement for maintenance in WHBE rabbits than in wool-type and meat-type rabbits may be attributable to a lower basic metabolic rate in WHBE rabbits.

Assuming that the digestibility coefficient of dietary CP is 0.70, the equations in Fig. 1 may be converted to DCPI = 2.14 + 1.23CPR for growing rabbits, and DCPI = 2.11 + 1.03CPR for finishing rabbits, where DCPI and CPR are digestible CP intake and CP retention, as g/kg $BW^{0.75}$ per day. From these two equations, the intercepts (2.14 and 2.11 g/kg $BW^{0.75}$ per day) correspond to the DCP requirement for maintenance, and 0.813 (1/1.23) and 0.969 (1/1.03) are the amounts of dietary digestible CP used for growth that is retained in the bodies of growing and finishing rabbits, respectively. These constants are lower compared with those in the equation: DCPI = 2.88 + 1.78CPR for growing rabbit recommended by Fraga (1998), but resemble those of Spanish Giant rabbits (DCPI = 3.8 + 1.116CPR, de Blas et al., 1985).

3.3. Nitrogen balance test with N-free diet

The results for N balance test with N-free ration are shown in Table 4. When the rabbits are offered N-free diet, the total N excreted (TNE) may be considered as the requirement of Nm. It was found from



. **1.** Linear equation between nitrogen retained in the body (NR) and nitrogen intake (NI) during growing (♦) and finishing (▲) periods.

4

The result for nitrogen (N) balance with the N-free ration and estimated N requirement for maintenance.

Groups	Body weight	Feed intake	N in excreta	N for maintenance	
	kg	g/day	mg/day	mg/kgBW	mg/kgBW$^{0.75}$
A	2.7	47.1	680	251	322
B	2.6	38.2	750	275	349
C	2.7	31.6	839	308	396
D	2.6	19.2	925	353	449
E	2.6	0	1002	383	486
S.E.M.	0.05	0.46	26.2	13.4	15.5
P	0.936	<0.001	0.010	0.046	0.029

the trial that the lower the intake of N-free diet (FI), the greater the TNE. When the data were fitted to the linear regression, the following equations were obtained for the TNE (g/day) and FI (g/day): TNE = 1.022 − 0.007FI (R^2 = 0.637, P<0.01).

In fasting animals, some body protein might be catabolized to be used as a source of glucose via gluconeogenesis. In the animals offered N-free diet, the metabolic fecal N should be low due to low feed intake. Therefore, the TNE was mainly from the endogenous urinary N. The increased intake of N-free diet will help reduce the protein catabolism, resulting in less endogenous urinary N, and hence less TNE.

The rabbits in group E were fasted and the measured Nm was 486 mg/kg BW$^{0.75}$. This was in good agreement with the value obtained from the previous N balance test, confirming the result of N requirement. It implies that the extrapolation from the linear regression of NR on NI was valid, although none of the treatments were close to zero NR in the current study.

In summary, the requirement of Nm for WHBE rabbit is about 485 mg/kg BW$^{0.75}$ per day. Further work is needed to study the requirement of other nutritional ingredients such as amino acids for this new strain of laboratory rabbit.

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