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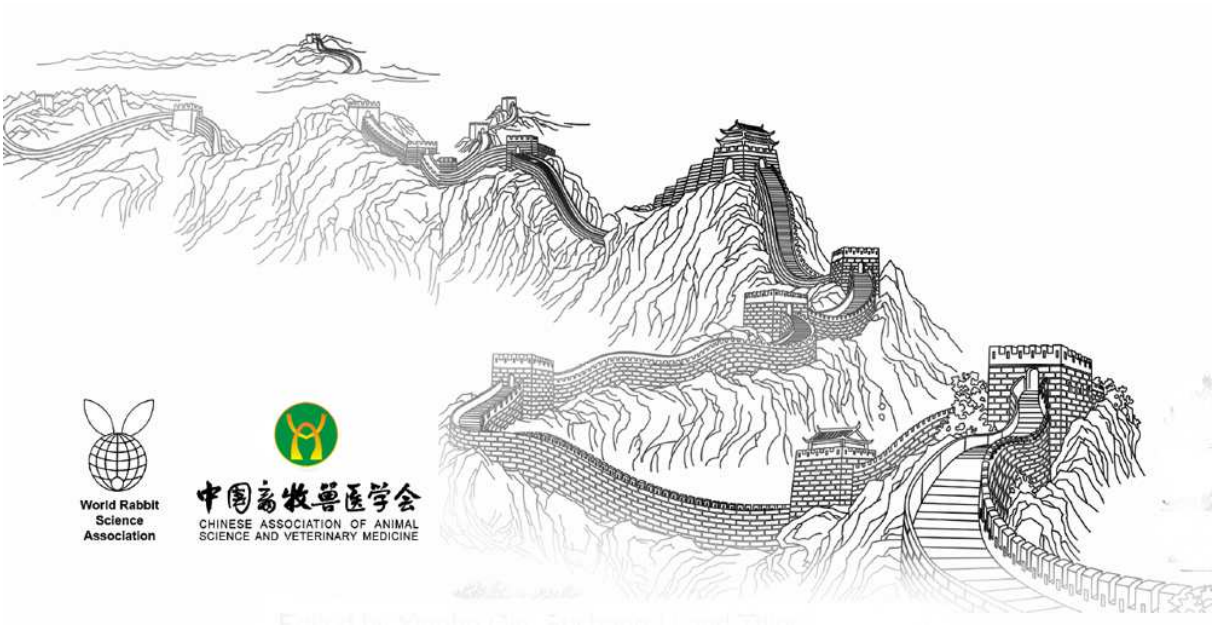

GROWTH AND EXPRESSION OF CaBP-D28K IN SMALL INTESTINE OF THE RABBIT, ACCORDING TO WAVE LENGTHS OF LIGHT.

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ABSTRACT

We investigated the effects of different wavelengths of light on the rabbit growth and mRNA expression of calbindin-D28K (CaBP-D28K) in small intestine. Eighty New Zealand rabbits (1486 ±2 g BW, 40 males and 40 females) were divided into 4 groups: R group (red light/ LD:14:10/60lx), G group (green light/LD:14:10/60lx), D (dark/0 lx) group and control group (daily lighting period). After 6 weeks, at the end of trial, five rabbits per group were slaughtered to sample small intestine. The growth rate in D group was 25% lower compared to R group (P<0.05). mRNA expression of CaBP-D28K was 20% lower in D group compared to R group (P<0.05).

Key Words: light, small intestine, growth performance ,CaBP-D28K, rabbits

INTRODUCTION

In addition to the role in vision, light strongly affect mammals health and well-being (Hughes S. *et al.*, 2015). Changing the duration of the daily lighting period through the seasons affects the behaviour, physiological parameters, and some other productive performance of rabbits. The natural photoperiod and the lighting schedules in rabbit houses may thus have significant roles in productive performance and other traits. Questions regarding the effects of colour and intensity of light still remain. Therefore we aimed to improve rabbit light requirements in production.

MATERIALS AND METHODS

Animals and experimental design

Eighty New Zealand rabbits (1486±2 g BW, 40 males and 40 females) were housed in individual cages. Ingredients and chemical composition of the basal diet are shown in table 1.

Table 1 Ingredients and proximate chemical composition of the basal diet

Ingredient	%[1]
Alfalfa meal	40.0
Corn (maize)	22.0
Wheat bran	20.5
Soybean meal	10.0
Dicalcium phosphate	2.0
Salt	0.5
Premix[2]	5.0
Chemical composition[3]	
Digestible energy (MJ/kg)[4]	11.45
Crude protein, %	16.82
Crude Fibre, %	14.25
Calcium, %	1.15
Phosphorus, %	1.14

[1]As fed basis; [2]Contained per kg of premix: 5320 mg $FeSO_4.H_2O$, 1080 mg $CuSO_4.5H_2O$, 560 mg $MnSO_4.H_2O$, 3652 mg $ZnSO_4.H_2O$, 1000 mg $CoCl_2.6H_2O$, 180,000 IU of vitamin A, 18,000 IU of vitamin D, 900,000 IU of vitamin E.; [3]measured values, except DE.[4]DE was calculated according to tables of ingredients

Feed was offered, ad libitum, twice per day in two equal portions. Rabbits had free access to tap water throughout the experimental period. All rabbits were fed the basal diet for 7 days, and then, they were allocated randomly into four different rooms in separate cages (one rabbit per cage): R group (red light/ LD:14:10/60lx), G group (green light/LD:14:10/60lx), D (dark/0 lx) group and control group (daily lighting period). Rabbits exposed to red light or green light, received 14 h of light exposure (14 h light, 10h dark). Rabbits of D group received 0h of light while rabbits of control group received daily lighting of light exposure. The intensity of light was 60 ± 0.65 lux as measured using a TES-1336A light meter (TES Electrical Electronic Crop., Taipei, China).The experiment lasted for six weeks.

Sampling
At the end of the study, five rabbits (similar BW) per group were sacrificed. The samples of the small intestine were collected using surgical scissors, two samples were labelled and immediately frozen (−20ºC), and another two samples were immediately frozen (-196 ºC) using liquid nitrogen until analysis.

Real-time quantitative polymerase chain reaction (PCR)
Total RNA from tissues was isolated with Trizol agent (Invitrogen Life Technologies, USA). RNA concentration was determined by a spectrophotometer at A260 and the purity designated valid if the ratio of A260/A280 ranged from 1.8 to 2.0. Using the RT system (Promega, Madison, WI), cDNA was synthesized from 3 μg RNA at 42 °C for 60 min followed by 72°C for 15 min. Real-time PCR was performed with SYBR Premix Ex Taq^{TM} (Perfect Real Time) (TaKaRa Bio Inc, Dalian) using the ABI 7900HT real-time thermocycler (Applied Biosystems, Forster, CA) with the following program : 95 °C/10 min, (95 °C/15 s, 49 °C/45 s)×40. The dissociation curve was performed and analyzed using the ABI 7900HT software. The target gene was analyzed and normalized to GAPDH. The pairs of primers were listed in the following, respectively: CaBP-D28K sense 5' TCCAACATACAAGAAGAGCA 3' antisense 5' GCACAAAGAATAAGAGCAAGA 3'; GAPDH sense 5' GCAAGTTCCACGGCACAG 3' antisense 5' GGTTCACGCCCATCACAA3'. The CT value represents the number of cycles required for the fluorescence signal to reach the threshold for each reaction [△CT=CT (target gene)−CT (GAPDH)]. The relative expression level of target gene and β-actin was calculated as $2^{(-\triangle\triangle CT)}$ (Livak and Schmittgen, 2001).

Statistical analyses
Data was analyzed as a completely randomized block using one way ANVOA procedure in SPSS 17.0 program. All cages of rabbits served as the experimental unit for data. Differences among means were tested using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The effects on growth performance
No mortality occurred during the study, except one dead in D and in C group. The growth rate of R group was higher than in D group (+25%, $P < 0.05$). It was investigated the effect of blue light on the performance of rabbit does(Gerencsér et al., 2011b), compared to white light: kits in blue light weighed 3611 g and those in white light (3498 g). According to the literature the minimum light intensity for rabbit does is 30–40 lx (Lebas *et al.*, 1997) or 50 lx (EFSA, European Food Safety Authority Journal, 2005). This level is necessary for visual contact with each other, to investigate their surroundings and to show active behaviour.

There is little experimentally based data about the effect of light intensity on production and behaviour of rabbit does. Rabbits are active during night and therefore their colour vision is limited. They can detect the wavelengths between blue and green (Nuboer, 1986). We suggest that in the future it would be worthwhile to examine the effect of light colour on production of rabbit does.

Table 2: the effect of different light on performance of growing rabbits in the trial

	R group	G group	D group	Control
initial weight	1486±27	1486±24	1487±27	1485±27
final weight	2342±76a	2284±81ab	2090±90b	2299±77ab
average daily gain	19.9±1.7a	18.7±1.5a	14.3±1.6b	19.3±1.9a
mortality	0	0	1	1

Means with different letters on the same row differ at level P of 5%.

The effects on mRNA expression of CaBP-D28K

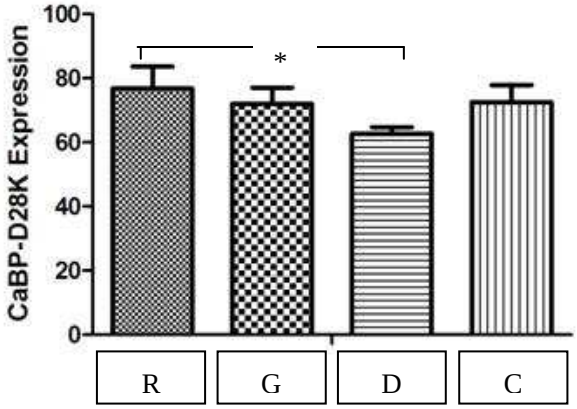

Fig. 1 – Results from the real-time quantitative PCR analysis showing normalized (against GAPDH) Ct-values for CaBP-D28K. Data are expressed as mean ± SEM, and * $p \leq 0.05$.

We find that normalized (against GAPDH) Ct-values for CaBP-D28K RNA expression in R group was higher than in D group ($p<0.05$). The vitamin D3 dependent calcium binding protein, calbindin-D 28K(CaBP-D28K) is believed to be involved in the ATP-dependent transcellular calcium transport in the epithelia of the intestine, and would play an important role in transepithelial calcium translocation which can reflect the absorption of calcium in intestinal (Hwang *et al.*, 2012; Jung *et al.*, 2011).

CONCLUSIONS

Different wavelengths of light in livestock shed may have effects on physiological parameters, behaviour and production of rabbits. We find that darkness impair the growth compared to red light. Further knowledge should be obtained to provide clear recommendations for commercial producers. A new research direction could be the testing of LED lamps.

ACKNOWLEDGEMENTS

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