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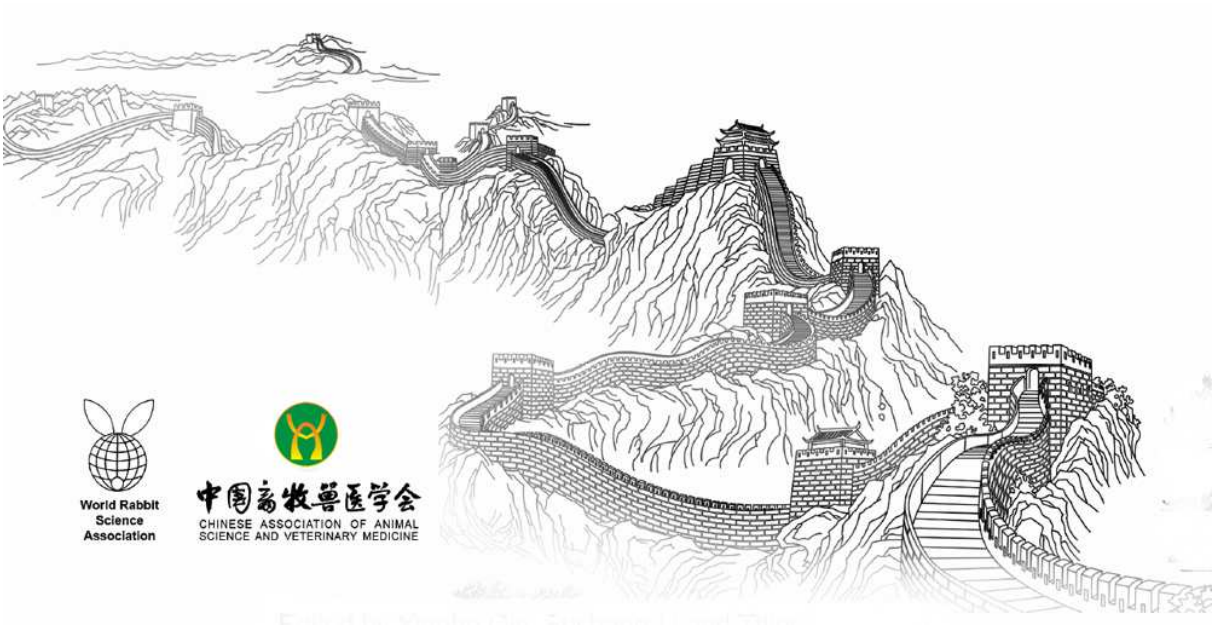

EFFECT OF DIETARY ARGININE ON RABBIT GROWTH AND MRNA EXPRESSION OF GM-CSF IN JEJUNUM.

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ABSTRACT

We investigated the effects of dietary arginine on the rabbit growth and mRNA expression of granulocyte–macrophage colony stimulation factor (GM-CSF) in jejunum. Sixty 49-d growing rabbits (709.5±10.2 g BW) were divided into 3 equal groups, and their diets were supplemented with arginine (0, 0.2, or 0.4% /kg dry matter (DM)) for 42 days. At the end of trial, five rabbits per group were slaughtered to sample jejunum. Adding arginine increased the final weight and average daily gain ($P<0.05$). The mRNA expression of GM-CSF was similar among the 3 groups. Adding arginine improved the growth, but failed to modify the mRNA expression of GM-CSF in jejunum.

Key Words: dietary arginine, intestine immune protein, growing rabbit

INTRODUCTION

Arginine is a semi-essential dibasic amino acid. It is an essential component of the urea cycle and is converted by the arginase into ornithine and urea (Ratner, 1973). Arginine plays multiple metabolic roles including the detoxification of ammonia (being part of the urea cycle), as a precursor for the formation of ornithine, polyamines, and proline, and the production of creatine among many others. Arginine also plays a key role in both innate and adaptive immunity being a substrate for the production of nitric oxide and an essential amino acid for the normal function of T-lymphocytes. Arginine availability is uniquely determined by myeloid cells during immune activation through the induction and/or release of the enzyme arginase 1 (ARG1). Regulation of T-lymphocyte function through arginine depletion has been discovered as a novel function of myeloid cells expressing ARG1, thus giving them the name myeloid-derived suppressor cells (MDSC). GM-SCF is the important proteins to influence the immunity system. It is a cytokine inducing the proliferation and differentiation of hematopoietic progenitor cells to macrophages and/or granulocytes and activates the differentiated cells, is a key determinant of myeloid lineage differentiation and required for the optimal function of tissue-resident mononuclear phagocytes (MNPs), including macrophages and DCs, thereby promoting host protection against environmental pathogens and vaccine responses (Jinushi et al, 2008; Zhan et al, 2012). Arginine and GM-SCF all refers to myeloid cells, so whether arginine regulates the body immunity through GM-SCF? This is a hypothesis.

In this trial, we investigated the effects of dietary arginine on the growth performance and mRNA expression of GM-CSF in small intestine of growing rabbits, and explore the new way on arginine regulating immunity system.

MATERIAL AND METHODS

Animals, Diets, and Feeding Procedures

Sixty 49-d growing rabbits were housed in individual cages (66 cm × 44 cm × 52 cm) and provided ad libitum access to pelleted feed. Ingredients and chemical composition of the basal diet are shown in Table 1. The basal diet was formulated according to the recommended nutritional requirements for rabbits (NRC, 1977; Liu, 1991; Klaus, 1985). Feed was offered twice per day (08:30 h and 16:00 h) in 2

equal portions (the amount of feed increasing per week according to body weight). Animals had free access to tap water throughout the experimental period (49d to 91d).

Table 1 Ingredients and proximate chemical composition of the basal diet

Ingredient	%[1]
Alfalfa meal	40.0
Corn	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
Arginine (% / kg DM)	0.99

[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

All animals were fed the basal diet for 7d, and they equally allotted according to BW and litter into three experimental treatment groups (n= 20/group). Diets were subsequently supplemented with arginine (0, 0.2, or 0.4% /kg DM) using one-way completely random design. Arginine was added to the premix using finely ground maize as a carrier, and the premix was combined with the concentrate. The experiment lasted 42 d.

Sample Collection

At the end of the study, five 91-d-rabbits (similar BW) per group were sacrificed. The samples of the jejunums were collected using surgical scissors, two samples were labeled and immediately frozen (−20ºC), and another two samples were immediately frozen (-196 ºC) using liquid nitrogen until analysis.

Real-time Polymerase Chain Reactions

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™ (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, 58 °C/30 s)×40. The dissociation curve was performed and analyzed using the ABI 7900HT software. The target gene was analyzed and normalized to β-actin. The pairs of primers were listed in the following, respectively: GM-CSF sense 5' GCTGGGCGAAATGGTAGAA 3' antisense 5' TGATAAACTCGGTCTCACAGGAA 3'; β-actin sense 5' TCACCATGGATGATGATATCGC 3' antisense 5'CGTGCTCGATGGGGTACTTCA 3'. 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 (β-actin)]. The relative expression level of target gene and β-actin was calculated as $2^{(-\triangle\triangle CT)}$ (Livak and Schmittgen, 2001).

Statistical Analysis

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

RESULTS AND DISCUSSION

Arginine is one of the most versatile AA, which serves as a precursor for synthesis of urea, nitric oxide (NO) and polyamines and regulates key metabolic pathways that are critical to health, growth, reproduction and homeostasis of the animals (Morris, 2009).

The effects on growing performance

Adding arginine improved linearly the growth and the final weight ($P<0.05$, table 2), and the mortality tended to be lower ($P>0.05$). Other positive results were shown previously, such that infusion of arginine could improve the N metabolism in heifers (Davenport et al, 1990) and milk production in cows (Chew et al, 1984). Parenteral administration of arginine in ewes decreased embryonic loss, increased lamb birth weight, and improved survival rate of fetal lamb to term in prolific ewes (Lassala et al, 2010; 2011). Wu et al (2010) reported that dietary supplementation with 0.6% Arg or 0.08% NCG enhanced intestinal HSP70 gene expression, intestinal growth and integrity, and the availability of dietary nutrients for whole-body weight gain in postweaning pigs fed a CSM-based diet.

Table 2 : The effect of dietary arginine on performance of growing rabbits in the trial

Item	Control	0.2% Arg group	04% Arg group
initial weight	704.1±21.6	712.7±22.6	710.2±19.3
final weight	1376.5±62.5b	1512.8±74.0ab	1597.7±59.9a
average daily gain	16.2±1.20b	18.6±1.59ab	21.1±1.58a
mortality	15.0	10.0	10.0

Means with different letters on the same row differ significantly ($P < 0.05$).

The effects on mRNA expression of GM-CSF

The mRNA expression of GM-CSF appear to be higher in 0.2% and 0.4% Arg group (figure 1) but this was not significant.

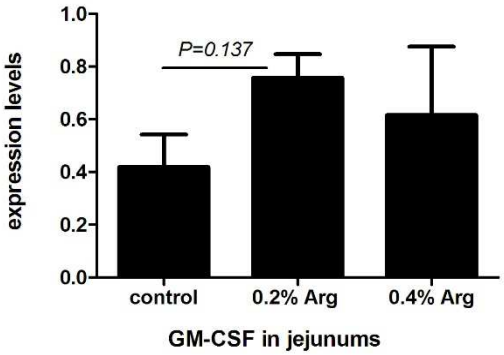

Figure 1. mRNA expression level of GM-CSF in rabbit jejunum, according to the dietary arginine level.

It has been reported that arginine stimulates phagocyte activity and accelerates endotoxic elimination in the gut (Adjei et al, 1995). Arginine also benefits mucosal microcirculation and absorption (He et al, 2009; Liu et al, 2008; Yao et al, 2011). However, the uncovered key role for GM-CSF in the maintenance of intestinal tolerance is consistent with previous studies showing that absence of GM-CSF can also contribute to lupus-like disease, insulitis, and age-related glucose intolerance (Jinushi et al, 2007; Enzler et al, 2007) and further emphasizes the critical role of tissue-resident phagocytes in the maintenance of tissue integrity.

We did not detect a clear effect of arginine on the mRNA expression of GM-CSF in rabbit jejunum. Very interestingly, GM-CSF overexpressing transgenic mice show accumulation of macrophages, blindness and a fatal syndrome of tissue damage (Lang *et al*, 1987). Physiological GM-CSF concentration in the serum range from 20 to 100 pg/ml (Conti and Gessani, 2008) and its levels rise in serum and tissues following stimulation with cytokines, antigens, microbial products or inflammatory agents such as IL-1, TNF-a or lipopolysaccharide (LPS) (Conti and Gessani, 2008). GM-CSF plays a critical role under inflammatory or immunomodulatory conditions by inducing specialized cell types from precursors or by influencing phenotypes of mature cell populations.

CONCLUSION

In this trial, adding arginine in the diet improved the performance, but failed to influence the mRNA expression of GM-CSF in rabbit jejunum. Further measurement with a larger number of samples is necessary, to elicit our initial hypothesis about potential role of arginine on body immune system.

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