Journal of Animal and Veterinary Advances 12 (2): 215-220, 2013
ISSN: 1680-5593

Effects of Dietary Vitamin D_3 on *MHC-II-β* Gene Expression in Immune Tissues of *Monopterus albus*

[1]De-Bing Li, [1]Chao Li, [1]Shan-Shan Shao, [1]Gong-Wei Zhang,
[2]Yong Tang, [1]Ding-Gang Zhou and [1]Xue-Wei Li
[1]College of Animal Science and Technology, Sichuan Agricultural University, 625014 Ya'an, China
[2]Leshan Vocational and Technical College, 614000 Leshan, China

Abstract: To investigate the effects of dietary Vitamin D_3 (VD_3) on *MHC-II-β* gene expression in *Monopterus albus* (*M. albus*). A total of 540 healthy *M. albus* (weigh, 21.7±2.1 g) were randomly assigned to six groups (3 replicates per group, 30 *M. albus* per replicate) and fed with dietary VD_3 at various concentrations (0 (control), 250, 500, 1,000, 2,000 and 4,000 IU kg^{-1}). After 20, 40 and 60 days of feeding, researchers randomly selected six *M. albus* from each group and collected tissues (hepatopancreas, spleen, head kidney and hindgut) for detection of *MHC-II-β* gene expression using real-time quantitative PCR. MHC-II-β was expressed in four tissues with expression in the head kidney significantly higher ($p<0.01$) than that in the spleen, hindgut and hepatopancreas. At 20 days, the highest MHC-II-β expression was detected in the 4,000 IU kg^{-1} group in the head kidney and in the 2,000 IU kg^{-1} group in the spleen, both of which were significantly higher than that of the other groups ($p<0.05$). At 40 days, the 1000 IU kg^{-1} group showed the highest MHC-II-β expression in the head kidney, hindgut and hepatopancreas. At 60 days, the 500 IU kg^{-1} group showed the highest MHC-II-β expression in the head kidney and spleen and was significant higher than that of the control ($p<0.05$) while the 4,000 IU kg^{-1} group showed significantly lower MHC-II-β expression compared to the control ($p<0.05$). Relative to the MHC-II-β expression in the head kidney, the results demonstrate that short-term (20 days) and high dose dietary provision of VD_3 (4,000 IU kg^{-1}) significantly increased MHC-II-β expression in *M. albus* immune organs. However, the highest MHC-II-β expression was observed with long-term (60 days) dietary provision of VD_3 at a dose of 500 IU kg^{-1}.

Key words: *Monopterus albus*, vitamin D_3, *MHC-II-β* gene, dose, head

INTRODUCTION

In recent years, *Monopterus albus* (*M. albus*) has become one of the most important species in Chinese aquaculture and is favored by worldwide customers due to its nutritional and medical properties. As a result of this aquaculture, various diseases of *M. albus* have arisen which have become a major factor in limiting large-scale farming of *M. albus*. Recently, the use of nutritional strategies to regulate the immune system and to enhance resistance to pathogenic microorganisms has become one of the most effective approaches to the prevention and control of animal diseases.

Vitamin D_3 (VD_3) which is an essential nutrient for animals is hydroxylated and to become active 1, 25-$(OH)_2$-D_3 that specifically binds to intracellular Vitamin D Receptors (VDRs). Furthermore, VD_3 also participates in a wild range of biological processes through its classical regulation of calcium and phosphorus (Zhang *et al*., 2009). To date, increasing numbers of studies have confirmed that VDRs are distributed in most immune cells including monocytes, macrophages and activated T and B lymphocytes (Chen, 2011). This indicates that these immune cells are also the targets of VD_3 which may participate in the regulation of immune functions. The *MHC-II-β* gene is critically associated with vertebrate resistance, predisposition, immunity and growth performance in response to many diseases. It is also an important candidate gene in evaluating general resistance to disease in animals. The encoded MHC-II-β molecules are mainly expressed in immune cells, the expression level of which is regulated not only by various transcription factors but also by physical factors (temperature) and pathogens (virus, bacteria, etc.) (Zhou, 2007).

Head kidney and spleen are primary peripheral immune organs in fish where immune cells differentiate, mature, settle and proliferate and where the immune

Corresponding Author: Ding-Gang Zhou, College of Animal Science and Technology, Sichuan Agricultural University, 625014 Ya'an, China

response is elicited. However, little is known about the effects of VD_3 on the immune function and growth performance of *M. albus* or the capacity of VD_3 to regulate the expression of the *MHC-II-β* gene in *M. albus*. The study used fluorescent RT-PCR to evaluate the effects of dietary VD_3 on MHC-II-β expression levels in peripheral immune organs of *M. albus*, to elucidate the relationship between VD_3 and *MHC-II-β* gene expression and to provide the theoretical basis for investigating the immunoregulatory functions and mechanisms of VD_3.

MATERIALS AND METHODS

Experimental diets: The basal diet, prepared according to the feed formula reported by Cheng *et al*. (2009), contained fishmeal and soybean meal as protein sources, fish oil as a fat source and inorganic salts and vitamin (excluding VD_3) as supplements. The composition and nutrition levels of the basal diet are shown in Table 1. The six experimental diets were then prepared by the addition of VD_3 (Bayer Animal Health Co., Ltd. Sichuan, China) to the basal diet at various concentrations (0 (control), 250, 500, 1000, 2000 and 4000 IU kg^{-1}). All ingredients were sieved (80-mesh), mixed well, extruded into 2 mm long feed, dried naturally and preserved for future use.

Experimental animals and feed management: A total of 540 healthy wild *M. albus* (weight, 21.7±2.1g) were obtained from Caoba Town, Yucheng District, Ya'an, Sichuan Province. *M. albus* were randomly assigned to the six experimental groups designated 0 (control), 250, 500, 1000, 2000 and 4000 IU kg^{-1} group with three replicates per group and 30 *M. albus* per replicate. Aquarium (100×60×60 cm) and aerated tap water (height of the water surface was 15-20 cm^2) were used to still-culture *M. albus* in the Fish Physiology Laboratory of Sichuan Agriculture University for 15 days. *M. albus* experiments began after a period of adjustment to the aquarium environment and the experimental diet. The water conditions were as follows: temperature, 20±3°C; pH, 6.8-7.5, dissolved oxygen concentration, >5 mg L^{-1}. *M. albus* were fed with an amount equivalent to 2% of the body weight at 18:00 each day and the feeding amount was adjusted according to their intake conditions to ensure food were consumed within 30 min. Aquarium water (approximately 2/3 of the total volume) was changed at 09:00 each day to allow the removal of contaminants. The experiment lasted for 60 days.

Table 1: Composition and nutrient levels of basal diets (air dried weight %)

Ingredients	Content	Nutrient levels	Content
Corn	5.27	Crude protein	42.00
α-starch	15.50	Calcium	2.00
Wheat meal	4.00	Phosphorus	1.53
Soybean meal	26.00	Lys	3.07
Fish meal	46.00	Met + Cys	1.42
Fish oil	2.00	-	-
Monocalcium phosphate	0.20	-	-
Vitamin premix	0.03	-	-
Choline	0.50	-	-
Mineral premix	0.50	-	-
Total	100.00	-	-

Contents of the following in per kilogram diet: VA 6000 IU, VE 50 mg, VK 5 mg, VB_1 15 mg, VB_2 15 mg, VB_3 25 mg, VB_5 30 mg, VB_6 10 mg, VB_7 0.2 mg, VB_{11} 3 mg, VB_{12} 0.03 mg

Sample collection: At 20, 40 and 60 days after the start of the experiment, six *M. albus* were randomly selected from each group for tissue (hepatopancreas, spleen, head kidney and hindgut) collection. Tissues were washed with ice-cold PBS to remove blood and intestinal content, snap-frozen in liquid nitrogen and preserved at -80°C for future use.

Total RNA extraction and cDNA synthesis: Total RNA were isolated using RNAiso Plus (TaKaRa, Dalian, China) according to the manufacturer's instructions. Extracted total RNA was detected using the nucleic acid analyzer NanoVue (GE, USA) and the OD_{260}/OD_{280} values were between 1.82-1.89. The 28S and 18S mRNA showed clear and bright strips with clean edges after 1% agarose gel electrophoresis and the brightness of the 28S band was approximately twice that of the 18S band which met the requirements for RT-PCR. The RNA concentration of each sample was then adjusted to approximately 480 ng μL^{-1} and cDNA was synthesized using PrimeScript RT reagent kit according to the instructions (TaKaRa, Dalian, China)

Real-time PCR: Real-time PCR amplification was performed using the CFX96 PCR System (Bio-Rad, USA) with the synthesized cDNA as templates. According to the *M. albus MHC-II-β* gene sequence (Genbank No. EU880835), the primer was designed for *MHC-II-β* gene as: 5'-CAATCACCGTCTGCCATCTC-3' and 5'-CCCTCTGCTGGTCAACATCC-3'. The HPRT (Genbank No. EU880835) expression was analyzed as an internal control for reaction efficiency, the primers were 5'-ACTCAAGTGGCGACAATC-3' and 5'-TGGCTCTATCTAAGACAATCAAT-3'. The reaction system comprised: 10 μL SYBR Premix Ex Taq II (2x) (TaKaRa, Dalian, China), 0.8 μL upstream and downstream primers (10 μM), 1.6 μL cDNA template and 6.8 μL ddH_2O. The reaction conditions were: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 10 sec, annealing at 57°C for 30 sec, 40 cycles. Dissociation curve temperatures were set between 65-95°C, heating rate was set at 0.5°C/sec and the plate was read once per second. Each sample contained two replicates and ddH_2O was used as a blank control.

Purified and sequenced PCR products were serially diluted (10 fold) to $1:10^5$ to construct the standard curve. Gene amplification efficiency was set above 90% (amplification efficiency: HPRT, 97.1%; MHC-II-β, 90.6%). The samples were quantified using the Ct value according to the linear calculation formula obtained from the standard curve.

Statistical analysis: Gene expression data are presented as mean±SD. The SPSS 17.0 Software was used for Analysis of Variance (ANOVA). LSD test was used for multiple comparisons. Significance was defined at $p<0.05$ and extreme significance was defined at $p<0.01$.

RESULTS

MHC-II-β expression levels in different tissues: The MHC-II-β expression level in the hepatopancreas of the control group at 20 days was arbitrarily set at 1 as the basal control for all experimental groups.

MHC-II-β expression levels of the control group at different time points are listed in Table 2. MHC-II-β was expressed in all four tissues at each time point with the highest expression in the head kidney, followed by spleen, hindgut and hepatopancreas. The differences in expression levels between each time point were extremely significant ($p<0.01$).

Effects of dietary VD_3 on MHC-II-β expression in the head kidney: The MHC-II-β expression levels in the head kidney of the experimental groups were differentially elevated by VD_3 at 20 days in a dose-dependent manner, with the highest expression level of MHC-II-β at 4,000 IU kg^{-1} (Table 3). The 2,000 IU kg^{-1} group (2.3 fold vs. Control) and the 4,000 IU kg^{-1} group (3.9 fold vs. control) showed significant increases in MHC-II-β expression compared to the control ($p<0.05$) while there were no significant differences in the other experimental goups compared to the control ($p>0.05$). At 40 days HC-II-β expression levels were significantly elevated in all the experimental groups compared to the control ($p<0.05$). The 1,000 IU kg^{-1} group had the highest MHC-II-β, expression (5.9 fold vs. control). At 60 day, MHC-II-β expression levels in the 250, 500, 1,000 and 2,000 IU kg^{-1} groups were significantly upregulated ($p<0.05$) with the highest expression levels in the 500 IU kg^{-1} group while the MHC-II-β expression in 4,000 IU kg^{-1} group was significantly downregulated ($p<0.05$), compared to the expression level at 20 days.

Analysis of the effects of VD_3 on MHC-II-β expression levels in the head kidney revealed that in the 250 and 500 IU kg^{-1} groups, expression levels significantly increased ($p<0.05$) with the feeding period, reaching the highest levels at 60 days. In the 1,000 and 2,000 IU kg^{-1} groups, MHC-II-β expression levels at 40 and 60 days were significantly higher than that at 20 days ($p<0.05$) with the highest expression at 40 days. However, MHC-II-β expression levels in the 4,000 IU kg^{-1} group were significantly downregulated ($p<0.05$) after 20 days and reached the lowest at level at 60 days. From these results researchers infer that long-term feeding of high dose VD_3 suppresses the expression of MHC-II-β in *M. albus* head kidney.

Effects of dietary VD_3 on MHC-II-β expression levels in the spleen: Spleen MHC-II-β expression levels were significantly upregulated at 20 and 40 days ($p<0.05$) in all experimental groups compared to the control (Table 4). At 20 days, the 2,000 IU kg^{-1} group had the highest MHC-II-β expression level (2.8 fold vs. control). At 40 days, both the 2,000 and 4,000 IU kg^{-1} groups had significantly elevated MHC-II-β expression levels (2.7 fold and 2.9 fold vs. control, respectively) and the elevations were significantly higher ($p<0.05$) than those in the other experimental groups. At 60 days, spleen MHC-II-β expression levels were significantly upregulated ($p<0.05$)

Table 2: MHC-II-β expression levels in different tissues (n = 6)

Tissue (days)	Hepatopancreas	Hindgut	Spleen	Head kidney
20	1.00 ± 0.11^{d}	2.38 ± 0.41^{c}	9.69 ± 0.56^{b}	62.11 ± 5.920^{a}
40	1.32 ± 0.13^{d}	2.56 ± 0.48^{c}	11.04 ± 0.35^{b}	71.30 ± 8.970^{a}
60	1.46 ± 0.16^{d}	2.77 ± 0.39^{c}	13.72 ± 1.41^{b}	94.18 ± 10.43^{a}

Different superscript lowercase letters indicate extremely significant differences ($p<0.01$) between data within the same column

Table 3: Effects of dietary VD_3 on MHC-II-β expression levels in the head kidney (n = 6)

Dietary VD_3 level (IU kg^{-1})	20 days	40 days	60 days
0	62.11 ± 5.92^{Bc}	71.30 ± 8.970^{Bd}	94.18 ± 10.43^{Ad}
250	76.39 ± 2.48^{Cc}	147.63 ± 6.830^{Bc}	181.35 ± 14.91^{Ac}
500	78.77 ± 14.28^{Cc}	217.61 ± 17.39^{Bab}	252.15 ± 19.25^{Aa}
1000	89.43 ± 12.42^{Cc}	416.74 ± 35.45^{Aa}	223.59 ± 7.45^{Bab}
2000	142.85 ± 12.42^{Cb}	317.37 ± 19.87^{Ab}	184.46 ± 18.63^{Bbc}
4000	242.22 ± 6.210^{Aa}	222.34 ± 26.71^{Ac}	73.46 ± 4.350^{Bd}

Different superscript lowercase letters indicate significant differences ($p<0.05$) between data within the same column; different superscript uppercase letters indicate significant differences ($p<0.05$) between data within the same row

Table 4: Effects of dietary VD_3 on MHC-II-β expression in the spleen (n = 6)

Dietary VD_3 level (IU kg^{-1})	20 days	40 days	60 days
0	9.69 ± 0.68^{Bc}	11.04 ± 0.35^{Bd}	13.72 ± 1.41^{Ac}
250	20.05 ± 0.58^{Bb}	23.34 ± 0.12^{ABc}	26.44 ± 0.77^{Ab}
500	19.57 ± 0.58^{Cb}	26.83 ± 0.58^{Bbc}	33.51 ± 0.48^{Aa}
1000	18.11 ± 1.16^{Bb}	26.63 ± 1.16^{Abc}	16.27 ± 0.39^{Cc}
2000	27.31 ± 0.87^{Aa}	29.44 ± 0.48^{Aab}	14.24 ± 0.58^{Ccd}
4000	15.42 ± 0.87^{Bbc}	32.16 ± 0.77^{Aa}	8.46 ± 0.68^{Cd}

Different superscript lowercase letters indicate significant differences ($p<0.05$) between data within the same column; different superscript uppercase letters indicate significant differences ($p<0.05$) between data within the same row

in the 250 IU kg^{-1} group (1.9 fold vs. control) and 500 IU kg^{-1} group (2.4 fold vs. control) while the 1,000 and 2,000 IU kg^{-1} groups showed no significant differences compared to the control. In contrast, the 4,000 IU kg^{-1} group showed a significant decrease ($p<0.05$) in MHC-II-β expression level with expression levels reaching only 60% of the control levels.

Analysis of the effects of dietary VD_3 on MHC-II-β expression levels in the spleen revealed significantly increased expression levels with the feeding period in the 250 and 500 IU kg^{-1} groups ($p<0.05$), reaching the highest levels at 60 days. The differences were significant compared to that observed at 20 days. In the 1,000 and 2,000 IU kg^{-1} groups, MHC-II-β expression levels first increased and then decreased over the feeding period with the highest MHC-II-β expression at 40 days while the expression levels at 60 days were significantly lower ($p<0.05$) than that at 20 days.

Effects of dietary VD_3 on MHC-II-β expression levels in the hindgut: The hindgut MHC-II-β expression levels in each experimental group were significantly upregulated ($p<0.05$) at 20, 40 and 60 days compared to that in the control (Table 5). The 1,000 IU kg^{-1} group had the most obvious elevations of 6.9, 12.8 and 14.9 fold of the control at 20, 40 and 60 days, respectively. These elevations were significantly higher ($p<0.05$) than those in the other experimental groups.

Analysis of the effects of VD_3 on MHC-II-β expression levels in the hindgut revealed that MHC-II-β expression levels in the 1,000 and 2,000 IU kg^{-1} groups significantly increased ($p<0.05$) over the feeding period and MHC-II-β expression at 60 days was significantly higher ($p<0.05$) than that at 20 and 40 days.

Effects of dietary VD_3 on MHC-II-β expression levels in the hepatopancreas: Expression levels of MHC-II-β in the hepatopancreas were significantly upregulated ($p<0.05$) in all experimental groups (excluding the 4,000 IU kg^{-1} group) compared to the control (Table 6). The 1,000 IU kg^{-1} group exhibited the most obvious elevation (5.6 fold vs. control) and was significantly higher ($p<0.05$) than that in the other experimental groups. At 40 days, the 1,000 IU kg^{-1} group showed the highest MHC-II-β expression level that was significantly higher ($p<0.05$) than that in the other experimental groups while the MHC-II-β expression level in the 4,000 IU kg^{-1} group was significantly lower ($p<0.05$) than that in the control. At 60 days, the MHC-II-β expression level in the 4,000 IU kg^{-1} group was significantly lower ($p<0.05$) than that in the control, although there were no significant differences between other experimental groups and the control.

Table 5: Effects of dietary VD_3 on MHC-II-β expression in the hindgut (n = 6)

Dietary VD_3 level (IU kg^{-1})	20 days	40 days	60 days
0	2.38 ± 0.41^{Bd}	2.56 ± 0.48^{Ae}	2.77 ± 0.39^{Ae}
250	7.25 ± 0.16^{Ac}	4.23 ± 0.16^{Bd}	7.53 ± 0.16^{Ad}
500	10.54 ± 0.16^{Ab}	7.98 ± 1.15^{Bc}	8.64 ± 0.52^{Bd}
1000	16.44 ± 1.25^{Ca}	30.32 ± 1.31^{Ba}	35.39 ± 1.51^{Aa}
2000	5.95 ± 0.78^{Cc}	12.16 ± 0.94^{Bb}	28.85 ± 4.54^{Ab}
4000	6.47 ± 0.37^{Bc}	3.34 ± 0.26^{Cd}	12.88 ± 2.35^{Ac}

Table 6: Effects of dietary VD_3 on *MHC-II-β* gene expression in the hepatopancreas (n = 6)

Dietary VD_3 level (IU kg^{-1})	20 days	40 days	60 days
0	1.00 ± 0.11^{Bd}	1.32 ± 0.13^{Ab}	1.46 ± 0.16^{Aa}
250	2.41 ± 0.09^{Abc}	1.69 ± 0.10^{Bb}	1.62 ± 0.07^{Ba}
500	3.12 ± 0.20^{Ab}	1.88 ± 0.07^{Bb}	1.37 ± 0.13^{Cab}
1000	5.61 ± 0.21^{Aa}	2.32 ± 0.05^{Ba}	1.59 ± 0.12^{Ca}
2000	2.34 ± 0.16^{Abc}	1.66 ± 0.03^{Bb}	1.19 ± 0.05^{Cab}
4000	1.52 ± 0.04^{Acd}	0.64 ± 0.04^{Bc}	0.45 ± 0.02^{Bb}

Different superscript lowercase letters indicate significant differences ($p<0.05$) between data within the same column; different superscript uppercase letters indicate significant differences ($p<0.05$) between data within the same row

Analysis of the effects of VD_3 on MHC-II-β expression levels in the hepatopancreas revealed that MHC-II-β expression levels were significantly downregulated over the feeding period with the highest expression at 20 days followed by progressively reduced expression at 40 and 60 days.

DISCUSSION

Fish are vertebrates that possess both specific and non-specific immunity. The development, maintenance and normal functioning of the fish immune system are dependent on the expression and regulation of diverse immune factors. MHC-II molecules are expressed by a specific population of immune cells (dendritic cells, macrophages and mature B lymphocytes) which mainly function in the recognition of exogenous antigens thus regulating animal immune responses. The essential role and significant functions of MHC-II molecules in immune responses is a significant focus of research in the field of immunogenetics and anti-disease dietetics. Since the 1990s, studies have increasingly been conducted on *MHC-II* genes in a variety of fish including zebrafish, flounder, rainbow trout, Atlantic salmon, sea bass, grass carp and carp (Juul-Madsen *et al*., 1992; Hordvik *et al*., 1993; Bingulac-Popovic *et al*., 1997; Rodrigues *et al*., 1998; Michel *et al*., 2009). These studies have shown that fish *MHC-II* gene expression exhibits obvious tissue-specificity with highest expression levels in the thymus, gill, spleen, head kidney and small intestine, low expression in the hepatopancreas and minimal or absent expression in muscle (Rodrigues *et al*., 1995). In the study, researchers first investigated the distribution of MHC-II-β in four different tissues of *M. albus*. The results indicated

that the *MHC-II-β* gene is expressed in *M. albus* hepatopancreas, hindgut, spleen and head kidney and that the expression abundance increases sequentially with the lowest expression in the hepatopancreas and highest expression in the head kidney.

Currently, the non-classical functions of VD_3, especially in the regulation of immune responses, cell proliferation and differentiation as well as in glucose metabolism are receiving increasing attention. However, the studies are limited to laboratory animals and humans with few reports describing the roles and mechanism of VD_3 in the immune systems of livestock or aquiculture animals. Studies by Zhang *et al.* (2011), Li and Zhou (2009) and Lei (2009) indicated that appropriate VD_3 levels could induce the expression of the antimicrobial peptides, β-defensin and cathelicidin-1 in chickens and could enhance chicken immunity and growth performance. The research of Li *et al.* (2001) also confirmed that appropriate dietary VD_3 improved swine immune functions and growth performance while high levels of VD_3 were shown to be toxic and suppress cellular immune functions in piglets. The results of the study suggest that both short and long-term feeding of dietary VD_3 significantly regulate MHC-II-β expression in *M. albus* peripheral immune tissues. This study has confirmed the feasibility of using dietary VD_3 to regulate *M. albus* immune functions and provides new insights into the mechanisms underlying the immunoregulatory effects of VD_3.

The study also indicates that dietary VD_3 exerts similar regulatory functions in different immune organs of *M. albus*. In short-term feeding (20 days), high levels of VD_3 (2,000 and 4,000 IU kg^{-1}) significantly up-regulated MHC-II-β expression in the head kidney and spleen. As the feeding period was extended, low levels VD_3 (250 and 500 IU kg^{-1}) exhibited a dose-dependent increase in MHC-II-β expression in the head kidney and spleen. However, long-term feeding of high levels of VD_3 (4,000 IU kg^{-1}) inhibited the expression of MHC-II-β. Table 5 and 6 show that as the feeding period was prolonged, hindgut MHC-II-β expression in the 1,000 and 2,000 IU kg^{-1} group was continuously and significantly increased ($p<0.05$) while in the hepatopancreas, MHC-II-β expression was down-regulated in these two groups. This indicates that dietary VD_3 differentially regulates MHC-II-β expression in organs such as the hindgut and hepatopancreas. The distinct functions of VD_3 could be attributed to organ cell construction or to *VDR* gene polymorphism (Wang *et al.*, 2011) and this also reflects the pleiotropic functions of VD_3 regulation.

CONCLUSION

Taking the MHC-II-β expression in the head kidney as the major reference, researchers conclude that short-term (20 days) feeding of dietary VD_3 (4,000 IU kg^{-1}) significantly increased MHC-II-β expression in *M. albus* immune organs. With long-term (60 days) feeding, the highest MHC-II-β expression was detected in *M. albus* fed with 500 IU kg^{-1} VD_3.

ACKNOWLEDGEMENT

This project was sponsored by the Natural Science Fund of Education Department, Sichuan Province (0914ZA146).

REFERENCES

Bingulac-Popovic, J., F. Figueroa, A. Sato, W.S. Talbot and S.L. Johnson, 1997. Mapping of MHC class I and class II regions to different linkage groups in the zebrafish, Danio rerio. Immunogenetics, 46: 129-134.

Chen, Z.C., 2011. Re-understanding of vitamin D. Herald Med., 30: 555-559.

Cheng, Y.B., Y.H. Cao, L.Z. Xia, Y. Ren, D. Wu, Q.M. Cai and T.T.S. Pan, 2009. Different sources and levels of dietary protein on *M. albus* growth performance and meat quality. Feed Indust., 30: 21-24 (In Chinese).

Hordvik, I., U. Grimholt, V.M. Fosse, O. Lie and C. Endresen, 1993. Cloning and sequence analysis of cDNAs encoding the MHC class IIβchain in Atlantic salmon (Salmo salar). Immunogenetics, 37: 437-441.

Juul-Madsen, H.R., J. Glumann, H.O. Madsn and M. Simonsen, 1992. MHC class II beta-chain expression in the rainbow trout. Scand. J. Immunol., 35: 687-694.

Lei, S.P., 2009. Effects of dietary vitamin D_3 on immune function and growth performance of sichuan mountain silky chicken. Dissertation of Master's degree. Ya'an, Sichuan Agricultural University, Pages: 17-25.

Li, D.F., H.L. Liu, P.B. Xi, Y. Chen and Y.H. Li, 2001. Effects of dietary vitamin D3 on growth performance and immune functions of weaned piglets. J. China Agric. Univ., 5: 87-94 (In Chinese).

Li, S.M. and D.G. Zhou, 2009. Regulatory effects of dietary vitamin D3 on β-defensin expression in silky chicken. Chin. J. Anim. Nutri., 21: 573-579 (In Chinese).

Michel, C., L. Bernatchez and J. Behrmann-Godel, 2009. Diversity and evolution of MHC II beta genes in a non-model percid species-the Eurasian perch (*Perca fluviatilis* L.). Mol. Immunol., 46: 3399-3410.

Rodrigues, P.N.S., T.T. Henmsen, A. Van Maanen, A.J. Taverne-Thiele, J.H.M.W. Rombout, B. Dixon and R.J.M. Stet, 1998. Expression of MHC Cyca class I and class II molecules in the early life history of the common carp (*Cyprinus carpio* L.). Dev. Comp. Immunol., 22: 493-506.

Rodrigues, P.N.S., T.T. Hermsen, J.H.W.M. Rombout, E. Egberts and R.J.M. Stet, 1995. Detection of MHC class II transcripts in lymphoid tissues of the common carp (*Crprinus carpio* L.). Dev. Comp. Immunol., 19: 483-496.

Wang, J., K. Tang, Q. Hou, X. Cheng, Y. Liu and C. Liu, 2011. Theoretical studies on the structural rearrangement of ligand binding pocket in human vitamin Dreceptor. Int. J. Quantum Chem., 111: 3928-3937.

Zhang, G.W., D.B. Li, S.J. Lai, S.Y. Chen, R.P. Lei and D.G. Zhou, 2011. Effects of dietary Vitamin D3 supplementation on AvBD-1 and chCATH-1 genes expression in chicken. Japan Poult. Sci., 48: 254-258.

Zhang, G.W., D.G. Zhou, R.P. Lei and D.B. Li, 2009. Effects of 1,25-(OH)2-D3 on antimicrobial peptide expression and regulation. Chinese J. Anim. Sci., 45: 59-63 (In Chinese).

Zhou, G.Y., 2007. Principle of Immunology. Shanghai Science and Technology Press, Shanghai (In Chinese).