

Effects of Dietary Manganese on Cu, Fe, Zn, Ca, Se, IL-1 β , and IL-2 Changes of Immune Organs in Cocks

Xiaofei Liu · Zhipeng Li · Chunran Han · Ziwei Zhang ·
Shiwen Xu

Received: 15 December 2011 / Accepted: 23 February 2012 / Published online: 9 March 2012
© Springer Science+Business Media, LLC 2012

Abstract Manganese (Mn) is an essential trace element required for normal development and bodily function. However, little is known about the effect of excessive amounts of Mn in immune organs of poultry. The aim of this study was to investigate the effect of dietary Mn on the content of trace elements, such as copper (Cu), iron (Fe), zinc (Zn), calcium (Ca), and selenium (Se), and the mRNA level of IL-1 β and IL-2 in immune organs (spleen, thymus, and bursa of Fabricius) and the content of IL-1 β and IL-2 in serum of poultry. Fifty-day-old male Hyline cocks were fed either a commercial diet or a Mn-supplemented diet containing 600, 900, and 1,800 mg/kg. The immune organs were collected at 30, 60, and 90 days, respectively, and the content of trace elements and the mRNA level of IL-1 β and IL-2 were examined; the serum were collected and the IL-1 β and IL-2 contents detected. The results

showed that Mn content in immune organs increased and Fe, Zn, and Ca contents decreased; however, Cu and Se contents showed no difference. IL-1 β and IL-2 mRNA levels and IL-1 β and IL-2 contents decreased. The present study demonstrates that excess exposure to Mn results in metal accumulations in immune organs. Manganism can disturb the balance of trace elements in immune organs and induce immune suppression in the molecular level; therefore, the immune function of cocks are also suppressed after manganism.

Keywords Manganese · Cocks · Immune organ · Trace elements · IL-1 β · IL-2

Introduction

As an essential nutrient, manganese (Mn) is necessary for normal homeostatic processes controlling reproduction, formation of connective tissue and bone, carbohydrate, lipid metabolism, and brain function [1].

Increased Mn has been reported in association with maneb-adulterated food, potassium permanganate, which is used to purify drinking water [2], and, in particular, methylcyclopentadienyl manganese tricarbonyl, an automobile fuel octane enhancer [3]. However, exposures to high levels of Mn are associated with severe damage to the liver [4], lung [5], and the reproductive [6], nervous [7], and immune systems [8, 9]. Yin et al. [10] reported that Mn exposure triggered apoptosis and cell death via the ERK signaling pathway and reduced electron transport chain activity [11, 12], leading to decreased ATP. Mn exposure can influence the subcellular distribution and expression of molecules associated with Fe transport in the choroid plexus [13].

In recent years, researches on manganism are increased, but most of the research activity mainly focused on human

Xiaofei Liu and Zhipeng Li contributed equally to this work.

All other authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the Journal.

X. Liu · C. Han

Key Laboratory for Food Science and Engineering,
Harbin University of Commerce,
Harbin 150076, People's Republic of China

X. Liu

e-mail: liuxiaofei72@163.com

C. Han

e-mail: hanchr@hrbcu.edu.cn

X. Liu · Z. Li · Z. Zhang · S. Xu (✉)

College of Veterinary Medicine, Northeast Agricultural University,
Harbin 150030, People's Republic of China
e-mail: shiwenxu@neau.edu.cn

Z. Li

e-mail: shiwenxu@neau.edu.cn

Z. Zhang

e-mail: zhangziwei@sina.com

and rats; researches on the toxicity of Mn focus on nervous and genital systems, while reports on the immune system were seldom, even of poultry. In light of the potential for the increased exposure of the general population to Mn, it is imperative that we gain a better understanding of the fundamental causes of Mn neurotoxicity. Therefore, we designed experiments to evaluate the effects of manganese on the immune system of cocks. First, Mn is an important cofactor for a variety of enzymes, and Mn impacts on energetic metabolism [14]. Due to a variety of enzymes linking to some metal elements in the immune system, metal elements impact on energetic metabolism [15]. To investigate whether manganese affects immune system function through metal elements, the contents of Mn, Cu, Fe, Zn, Ca, and Se in immune organs (spleen, thymus, and bursa of Fabricius) were detected. Second, IL-1 β and IL-2 are important cytokines in the immune system. However, little is known about the effect of manganese on IL-1 β and IL-2 in the immune system. Thus, the mRNA level of IL-1 β and IL-2 in the serum and the content of IL-1 β and IL-2 in immune organs were determined along with the increase of Mn-supplemented diets and days on cocks. In the present study, our work was completed with the aim of better understanding the mechanisms of Mn-induced immunotoxicity in poultry using male Hyline cocks as a model.

Materials and Method

Poultry and Experimental Design

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. Four hundred 50-day-old male Hyline cocks were divided randomly into four groups. The control group was fed a basal diet, the low-dosed group (L group) fed with the basic diet supplemented with 600 mg/kg MnCl₂, the middle-dosed group (M group) fed with the basic diet supplemented with 900 mg/kg MnCl₂, and the high-dosed group (H group) was fed the basal diet supplemented with 1,800 mg/kg MnCl₂ according to one fifteenth, one tenth, and one fifth of the median lethal dose (LD₅₀) for cocks. Poultry were maintained in the Laboratory Animal Center, College of Veterinary Medicine, Northeast Agricultural University, China. Food and water were provided ad libitum. On the 30th, 60th, and 90th days of the experiment, respectively, 30 cocks in each group were selected randomly. The blood was collected via cardiac puncture and the serum obtained by centrifugation at 1,000 \times g for 10 min at 4 $^{\circ}$ C for the next experiment. Following euthanasia with sodium pentobarbital, the immune tissues of the cocks were quickly removed, blotted, and then rinsed with ice-cold, sterile deionized water, frozen immediately in liquid nitrogen, and stored at -80 $^{\circ}$ C until required.

Determination of the Concentrations of Mn, Cu, Fe, Zn, Ca, and Se in Immune Tissues

The contents of Mn, Cu, Fe, Zn, and Ca in immune organs were detected using flame atomic absorption spectrometry (FAAS) [16–18]. The device working parameters (air, acetylene, optics, and electronics) were adjusted for maximum absorption for each element. Acetylene was of 99.99% purity. All analyses were made in triplicate and the mean values reported. Under the optimum established parameters, standard calibration curves for metals were constructed by plotting absorbency against concentration. The optimal operating conditions of Mn, Cu, Fe, Zn, and Ca were shown in Table 1.

The samples of frozen immune tissues were cut into small pieces with a stainless steel knife and were baked at 110 $^{\circ}$ C for 12 h in a baking oven; then, 0.5 g samples were transferred into beakers. For digestion, 25 mL of concentrated HNO₃/HCl (4:1) was added to each beaker and warmed on a low-temperature electric hot plate to solution transparency. The samples were adjusted to 10 mL using a volumetric flask by 0.5% HNO₃, except for Ca determination. The samples for Ca content were metered volume by LaNO₃ solution; the final concentration of La³⁺ was 2.0 g/L. All samples were measured by FAAS. The contents of Mn, Cu, Fe, Zn, and Ca were calculated from a standard curve, respectively.

The Se content in immune tissues was estimated by the method described by Hasunuma et al. [19, 20]. The assay is based on the principle that the Se contained in samples is converted to selenous acid in response to acid digestion. The Se content was calculated by reference to a standard curve.

Determination of IL-1 β and IL-2 Concentrations in Serum

The blood was collected via cardiac puncture, and then the serum was obtained by centrifugation at 1,000 \times g for 10 min at 4 $^{\circ}$ C. The contents of IL-1 β and IL-2 were detected using IL-1 β -¹²⁵I and IL-2-¹²⁵I RIA Kit (Beijing North Institute of Biological Technology, PR China), respectively, by radioimmunoassay and following the protocol of the manufacturer. Radioactivity was determined using an automatic gamma counter [21].

Determination of IL-1 β and IL-2 mRNA Levels by qPCR

Total RNA was extracted from the tissue samples using the RNAfast200 Kit (Fastagen, China). The concentration of the extracted total RNA was quantified with a spectrophotometer. RNA integrity was evaluated by observing the 18S and 28S ribosomal bands after electrophoresis on a 1% agarose gel in the presence of ethidium bromide. cDNA was synthesized from 5 μ L of RNA using the Reverse Transcriptase M-MLV system (TaKaRa, China). Synthesized cDNA was diluted five times with sterile water and stored at -80 $^{\circ}$ C before use.

Table 1 Optimal operating conditions of Mn, Cu, Fe, Zn, and Ca

Elements	Wavelength, λ (nm)	Light current (mA)	Slit (nm)	Acetylene discharge (L/min)	Air discharge (L/min)
Mn	278.24	2	0.2	1.5	6.5
Cu	323.46	2	0.3	1.5	6.5
Fe	248.30	3	0.2	2.0	8.0
Zn	213.90	3	0.3	1.2	6.8
Ca	422.70	3	0.2	2.0	8.0

Specific primers for IL-1 β , IL-2, and β -actin were designed by Primer Premier Software 5.0 (PREMIER Bio-soft International, USA; Table 2). The specificity of the primers was firstly confirmed by general PCRs [22, 23]. Quantitative RT-PCR (qPCR) was used to detect the expression of IL-1 β and IL-2 and the β -actin gene in immune tissues. Reaction mixtures were performed in the ABI PRISM 7500 real-time PCR system (Applied Biosystems, USA). Reactions consisted of the following: 10 μ L of 2 \times SYBR Green I PCR Master Mix (TaKaRa), 2 μ L of either diluted cDNA, 0.4 μ L of each primer (10 μ M), 0.4 μ L of 50 \times ROX reference Dye II, and 6.8 μ L of PCR grade water. PCR was performed under the following conditions: 1 cycle at 95 $^{\circ}$ C for 30 s, 40 cycles at 95 $^{\circ}$ C for 15 s, and at 56 $^{\circ}$ C for 30 s. The melting curve analysis showed only one peak for each PCR product. Electrophoresis was performed with the PCR products to verify primer specificity and product purity. The mRNA relative abundance was calculated according to the method of Pfaffl [24], accounting for gene-specific efficiencies, and was normalized to the mean expression of β -actin.

Statistical Analysis

Statistical analysis of all data was performed using SPSS procedures (SPSS Inc., Chicago, IL, USA). A significant value ($p < 0.05$) was obtained by one-way ANOVA. Differences between means were assessed using Tukey's honestly significant difference test for post hoc multiple comparisons. Data are expressed as the mean \pm SD.

Results

Effects of Concentrations of Cu, Fe, Zn, Ca, and Se in Immune Tissues

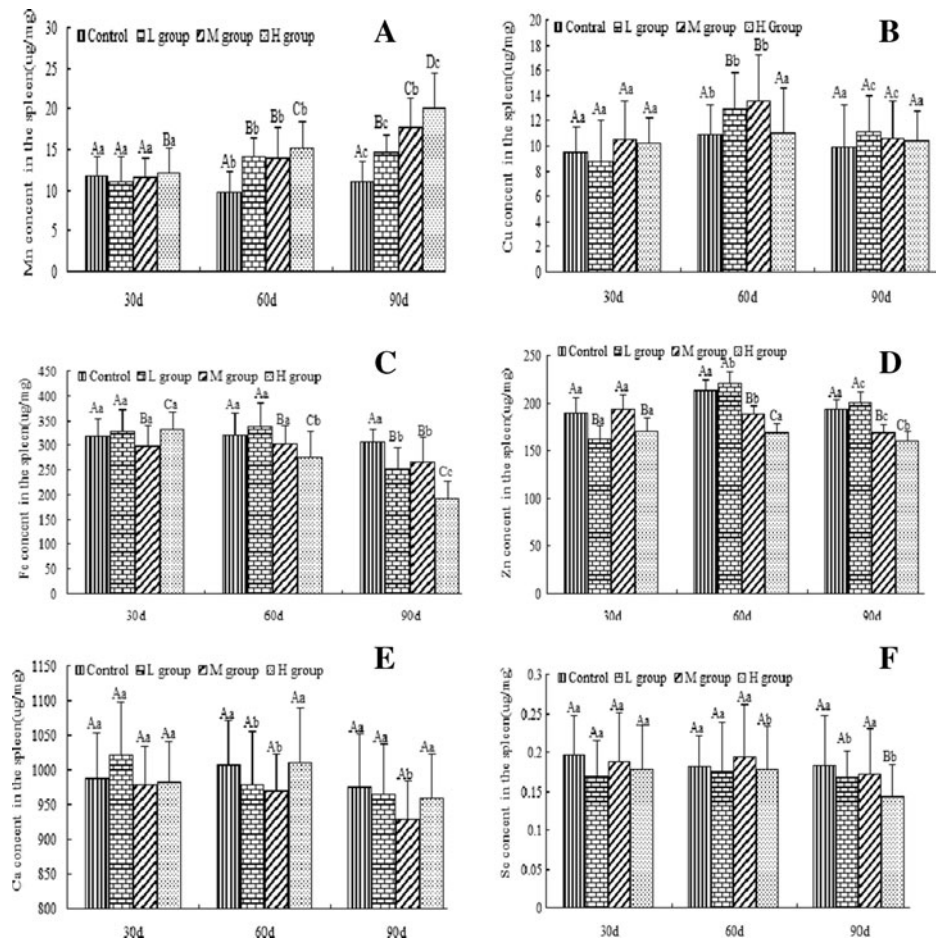
The effects of the different concentrations of dietary Mn on Cu, Fe, Zn, Ca, and Se contents in the spleen are shown in Fig. 1. Mn content in the spleen appeared in a dose- and time-dependent fashion. On the 30th day, the Mn level showed no statistically significant differences. On the 60th day, the Mn level in the H group increased significantly compared with the control ($p < 0.01$). On the 90th day, the Mn level in the M group increased more notably than the L group ($p < 0.05$; Fig. 1a). Besides the 60th day where the Cu level in the L and M groups elevated significantly more than the control ($p < 0.05$), there were no significant differences in the different groups on other days (Fig. 1b). On the 60th day, the Fe level in the H group showed significant differences compared with the control ($p < 0.05$); on the 90th day, the Fe level in the H group decreased extremely than the control ($p < 0.01$; Fig. 1c). On the 60th and 90th days, the Zn level in the M and H groups showed significant differences compared with the control and L groups, respectively ($p < 0.05$; Fig. 1d). The Ca level presented no obvious regularity in different groups, besides the Ca level in the H group which showed a time-dependent fashion (Fig. 1e). The Se level had no obvious regularity in different groups and in different times (Fig. 1f).

The effects of the different concentrations of dietary Mn on Cu, Fe, Zn, Ca, and Se contents in the thymus are shown in Fig. 2. The Mn content in the thymus had a dose- and time-dependent fashion. On the 30th day, there was no significant

Table 2 Primers used for quantitative real-time PCR

Target gene (cocks)	GeneBank accession no	Prime	Sequence (5'-3')	PCR fragment length (bp)
IL-1 β	NM204524	Forward	5'-GGTCAACATCGCCACCTACA-3'	86
		Reverse	5'-CATACGAGATGCAAACCAGCAA-3'	
IL-2	NM204153	Forward	5'-TGGAGCATCTCTATCAG-3'	167
		Reverse	5'-TAACCACTTCTCCCAGGTAACAC-3'	
β -actin	L08165	Forward	5'-CCCGCAAATGCTCTAAACC-3'	93
		Reverse	5'-CCAATCTGTCTTGTATTATGC-3'	

Fig. 1 Contents of Mn, Cu, Fe, Zn, Ca, and Se in the spleen after manganese (in micrograms per milligram). **a** Mn content. **b** Cu content. **c** Fe content. **d** Zn content. **e** Ca content. **f** Se content. Bars represent the mean \pm standard deviation ($n=30$ /group). Bars with different capital letters are statistically significantly different in the same group. Bars with different small letters are statistically significant at the same concentrations of dietary Mn



increase of the Mn level compared with the control. On the 60th day, the Mn level in the H group increased higher than the control ($p<0.05$). On the 90th day, the Mn level in the M and H groups increased significantly more than the L group ($p<0.01$; Fig. 2a). Besides the 90th day where the Cu levels in the M group elevated significantly more than the control ($p<0.05$; Fig. 2b), on the 30th and 60th days, there were no statistically significant differences; on the 90th day, the Fe level in the M and H groups had a tendency to decrease along with increasing days (Fig. 2c). On the 60th day, the Zn level in the H group decreased significantly more than the control ($p<0.05$); on the 90th day, the Zn level in the H group showed extreme changes compared with the other groups ($p<0.01$; Fig. 2d). The Ca level presented no obvious regularity in different groups, but the Ca level showed a time-dependent fashion in the control and H groups (Fig. 2e). The Se level had no obvious regularity in different groups and in different times (Fig. 2f).

The effects of the different concentrations of dietary Mn on Cu, Fe, Zn, Ca, and Se contents in the bursa of Fabricius are shown in Fig. 3. The Mn content in the bursa of Fabricius had a dose- and time-dependent fashion. On the 30th and 60th days, the Mn level in the H group were higher than the control ($p<0.05$). On the 90th day, the Mn level in the H group increased significantly more than the L group ($p<0.01$; Fig. 3a). On the

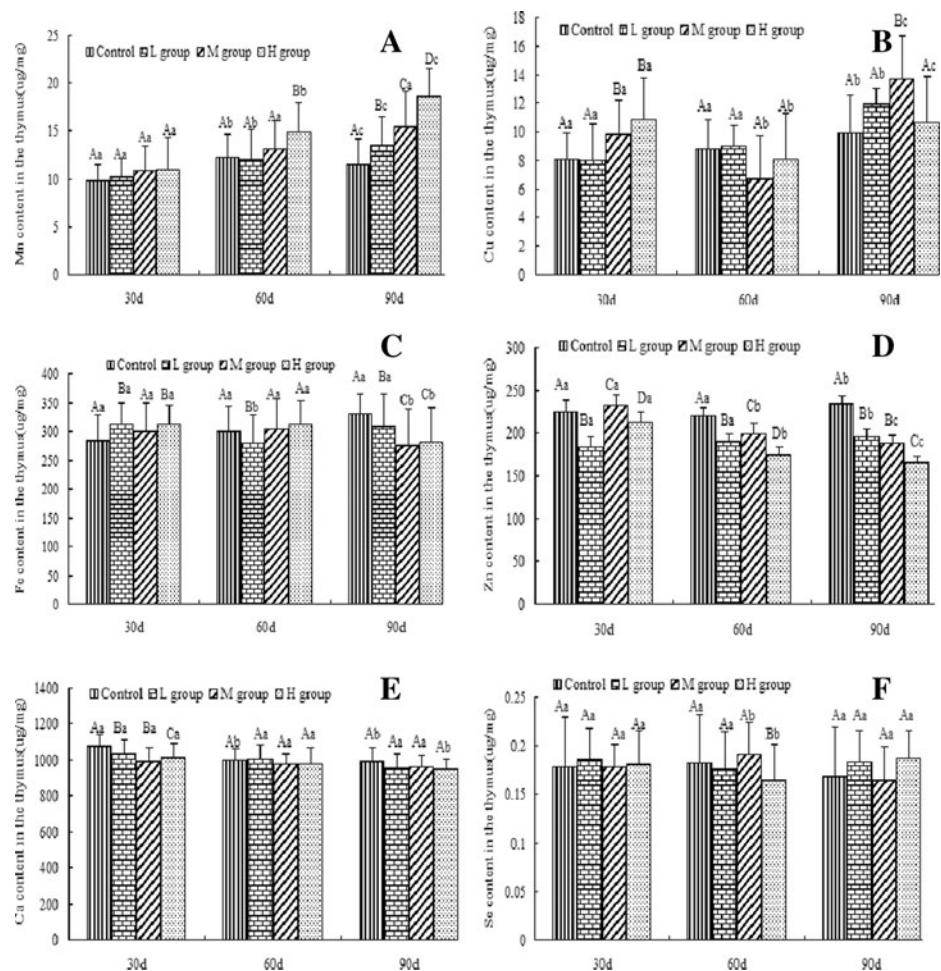
30th, 60th, and 90th days, the Cu level in the M group elevated significantly more than the control, respectively ($p<0.05$; Fig. 3b). The Fe level in different groups had no obvious regularity along with the increase of days (Fig. 3c). The Zn level in the M and H groups had a tendency to decrease along with the increase of days. On the 60th day, the Zn level in the H group showed extreme differences more than the control ($p<0.01$; Fig. 3d). The Ca level in different groups had no obvious regularity, but it showed a time-dependent fashion (Fig. 3e). The Se level had no obvious regularity in different groups and in different times (Fig. 3f).

The contents of Fe, Cu, Zn, Ca, and Se were changed by excess Mn in immune organs of cocks. It was illustrated that the balance of trace elements was disturbed and that the function of absorption in trace elements was disbalanced.

Effects of IL-1 β and IL-2 Concentrations in Serum

As shown in Fig. 4, the IL-1 β and IL-2 levels in the serum were changed significantly on the 30th, 60th, and 90th days along with the increase of Mn-supplemented diets. On the 30th day, the IL-1 β content increased firstly, then decreased. On the 60th day, the IL-1 β content decreased slightly; in the M group, the IL-1 β content decreased slightly compared with

Fig. 2 Contents of Mn, Cu, Fe, Zn, Ca, and Se in the thymus after manganese (in micrograms per milligram). **a** Mn content. **b** Cu content. **c** Fe content. **d** Zn content. **e** Ca content. **f** Se content. Bars represent the mean±standard deviation ($n=30$ /group). Bars with different capital letters are statistically significantly different in the same group. Bars with different small letters are statistically significant at the same concentrations of dietary Mn



the control ($p<0.05$); the IL-1 β content in the H group decreased significantly ($p<0.01$). On the 90th day, the IL-1 β content decreased significantly; in different groups, the IL-1 β content showed statistically significant differences ($p<0.01$; Fig. 4a).

On the 30th day, the IL-2 content increased firstly, then decreased along with the increase of Mn-supplemented diets. The IL-2 content in the L group increased significantly more than the control ($p<0.05$). In the M and H groups, the IL-2 content decreased remarkably more than the control ($p<0.05$). On the 60th day, the IL-2 content reduced compared with the control ($p<0.05$). In the H group, it decreased significantly more than the control ($p<0.01$). On the 90th day, in all groups, the IL-2 content showed statistically significant differences ($p<0.01$; Fig. 4b).

Effects of IL-1 β and IL-2 on the mRNA Level of Immune Organs

The distribution of the IL-1 β and IL-2 mRNA expression measured by qPCR is shown in Figs. 5 and 6. In this experiment, the control group was used as rating (1) on different days. The mRNA expression of IL-1 β in immune organs in

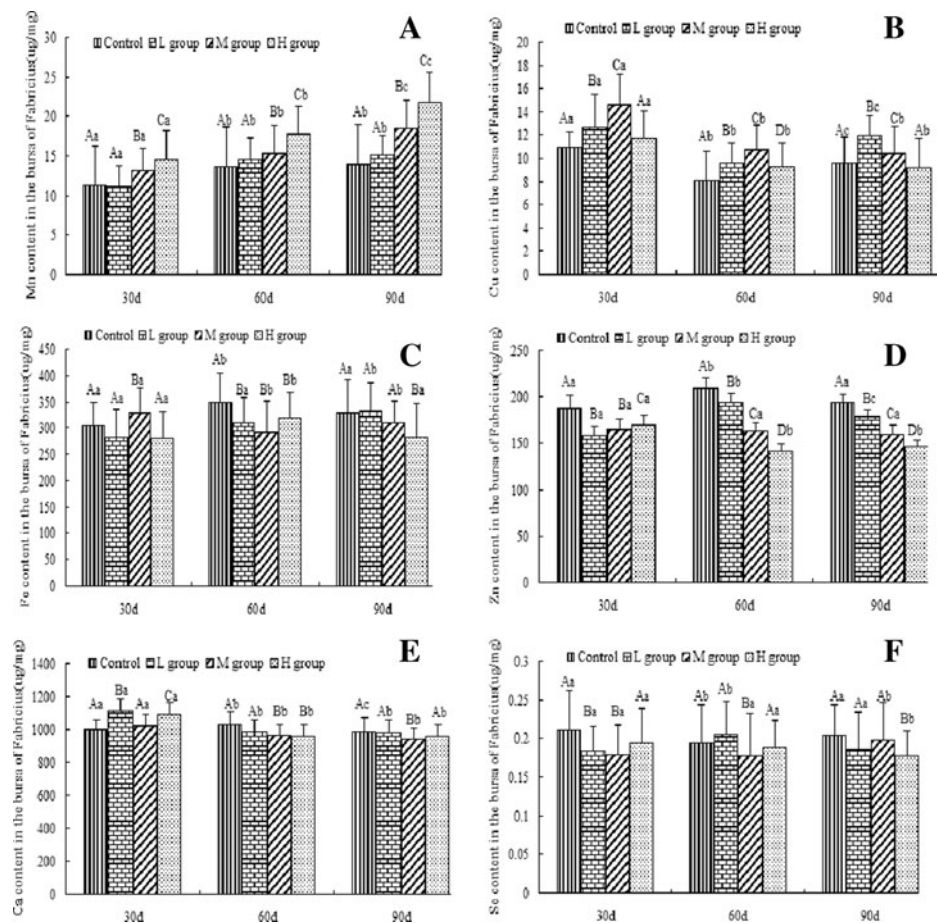
different days and in different dosed groups decreased along with the increase of Mn-supplemented diets; the dosage effect of Mn was shown obviously, and the time-dependent fashion of Mn was shown (Fig. 5a–c).

On the 30th day, the IL-2 mRNA level in the spleen and thymus increased firstly, then decreased (Fig. 6a, b); however, in the bursa of Fabricius, it decreased firstly, then increased slightly (Fig. 6c). On the 60th and 90th days, the IL-2 mRNA level fell off gradually following increased Mn-supplemented diets in immune organs; the time-dependent fashion of Mn was shown. The results showed that the IL-2 mRNA expression in the spleen and thymus was higher than normal in the low-dosed manganese and in the short-term group, but the expression decreased significantly in the high-dosed and in the long-term group.

Discussion

Mn is an essential element for humans, animals, and plants, which is required for growth, development, and maintenance of health. In animals, eating too little manganese can interfere with normal growth, bone formation, and reproduction [25].

Fig. 3 Contents of Mn, Cu, Fe, Zn, Ca, and Se in the bursa of Fabricius after manganese (in micrograms per milligram). **a** Mn content. **b** Cu content. **c** Fe content. **d** Zn content. **e** Ca content. **f** Se content. Bars represent the mean \pm standard deviation ($n=30$ /group). Bars with different capital letters are statistically significantly different in the same group. Bars with different small letters are statistically significant at the same concentrations of dietary Mn



The conditions were reversible to some extent by supplying the affected animals with Mn. Mn exposure suppressed fundamental immune mechanisms of Norway lobsters [26]. Both excess and deficiency of Mn supply led to impaired growth. In previous studies, it was reported that the presence of Mn was detected in various organs of rats [27–30], bovine [6], hen [31], and humans [32]. Among them, the predominant research was on nervous tissues. However, research of Mn in avian immune organs remains unknown, until now. In this study, a manganese model was established and the results revealed that Mn content in immune organs increased gradually along with the increase of Mn-supplemented diets and days.

The concentration of nutrients required to maintain health and the productivity of chicken is challenged due to the reduction in feed intake under high Mn exposure [33]. Trace element status as better nutrition can affect the immune function not only in a direct way but also indirectly by modulating the plasma levels of hormones that regulate the development and function of host defense cells. In the present study, trace elements such as Cu, Fe, Zn, Ca, and Se were detected after increased Mn-supplemented diets.

Cu is an essential element for all known living organisms [34]. Cu content has a positive relationship with Mn and Mn advanced in activity. For example, high Mn exposure inhibited

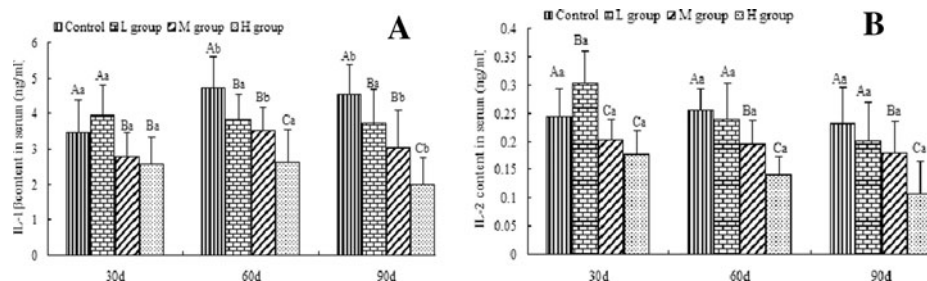
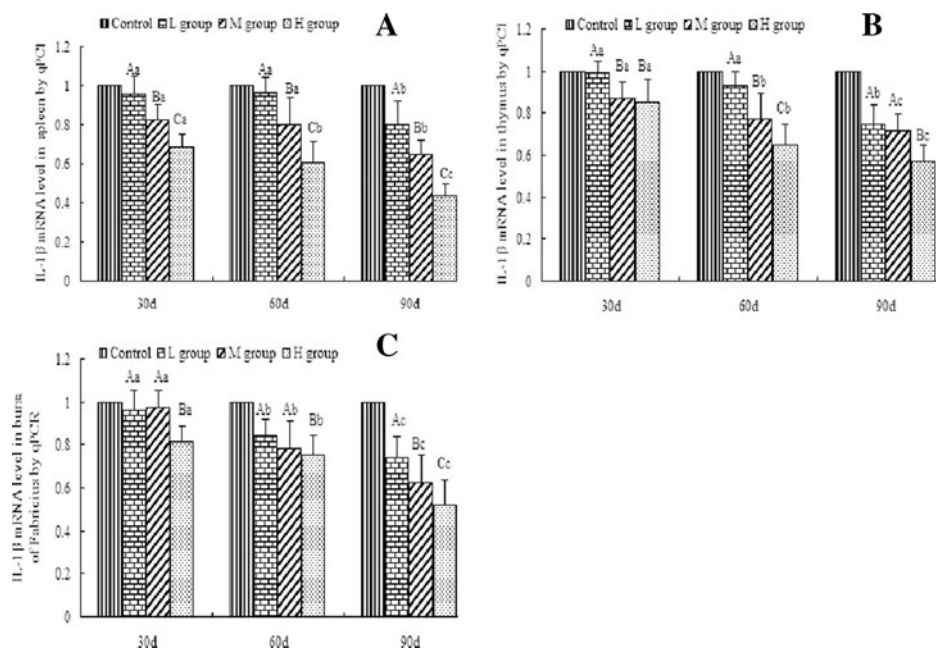


Fig. 4 Contents of IL-1 β and IL-2 in serum (in nanograms per milliliter). **a** IL-1 β content. **b** IL-2 content. Bars represent the mean \pm standard deviation ($n=30$ /group). Bars with different capital letters are statistically

significantly different in the same group. Bars with different small letters are statistically significant at the same concentrations of dietary Mn

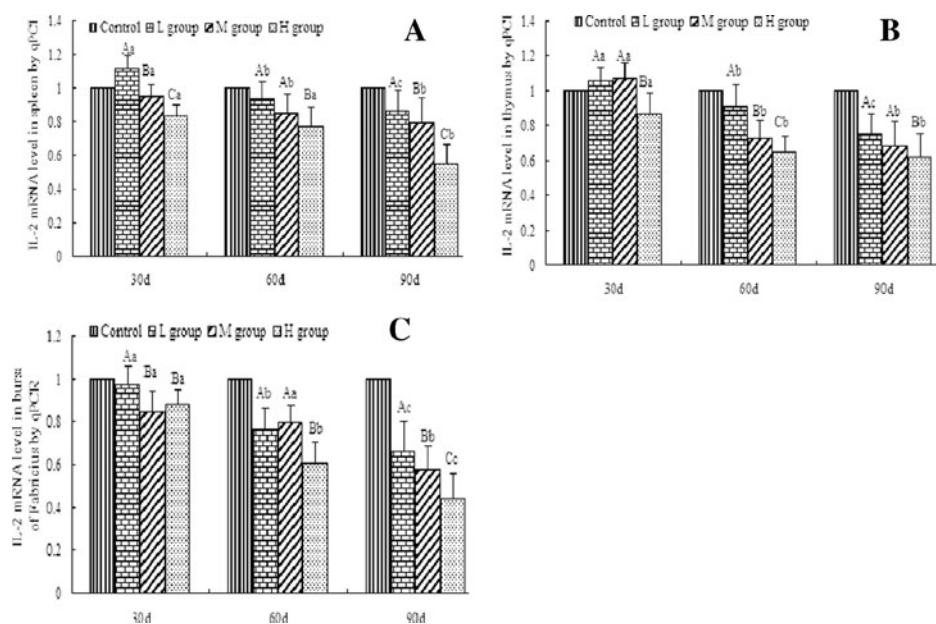
Fig. 5 Determination of the IL-1 β mRNA level by qPCR. **a** Spleen. **b** Thymus. **c** Bursa of Fabricius. Bars represent the mean \pm standard deviation ($n=30$ /group). Bars with different capital letters are statistically significantly different in the same group. Bars with different small letters are statistically significant at the same concentrations of dietary Mn



Cu intake and influenced immune globulin content. In their study, Khandelwal et al. [35] demonstrated that Cu could help reduce Mn accumulation to weaken Mn toxicity. The Cu level showed no significant difference among four groups after mother rats were administrated Mn in the experiment of Zhang et al. [36]. Our study showed that Cu content in the spleen, thymus, and bursa of Fabricius increased at first and then decreased with Mn addition. There was no significant effect of manganese on the Cu content in immune organs. The result was consistent with this report. Manganism regulation in the organism is very close to that of Fe [37, 38]. Chronic Mn exposure alters iron homeostasis possibly by expediting the unidirectional influx of iron from the systemic circulation to

cerebral compartment. The action appears likely to be mediated by manganese-facilitated iron transport through the blood-brain barrier [39]. Mn exposure facilitates Fe deficiency. In an experiment conducted in developing rats treated with a high-Mn diet through breast milk, decreased Fe in plasma was detected [40]. In this study, the Fe content decreased along with the increase of Mn-supplemented diets in immune organs. It was obvious that iron metabolism was disturbed. This evidence strongly supports the hypothesis of Mn and Fe competition for transport mechanisms. A diet deficient in Zn leads to atrophy of the thymus [41], a reduction in spleen weight of rats [42], and the loss of T helper cell function [43]. Zn has a unique role in thymus-dependent “T”-cell-mediated immune response.

Fig. 6 Determination of the IL-2 mRNA level by qPCR. **a** Spleen. **b** Thymus. **c** Bursa of Fabricius. Bars represent the mean \pm standard deviation ($n=30$ /group). Bars with different capital letters are statistically significantly different in the same group. Bars with different small letters are statistically significant at the same concentrations of dietary Mn



Zhang et al. [36] reported that the Zn levels in newborn rats' livers were lower than the control group after mother rats were exposed to Mn. In this study, the Zn content decreased along with Mn accumulating in immune organs. The result was consistent with this report. Our study showed that the Ca content had no significant differences in low-dosed groups, but it decreased gradually along with the increase of using days. Mn can, however, act as a toxicant to organisms when the concentrations are elevated and start affecting neuromuscular transmission by interacting with mitochondrial Ca²⁺ and disturbing the ion balance in muscle membranes [44]. When Mn is inside the cell, it preferentially accumulates into the mitochondria, mainly as Mn²⁺ via the Ca²⁺ uniporter [45]. Se can improve the immune status and anti-inflammatory action [46]. Se compounds have been reported to regulate the function of neutrophils, NK cells, B lymphocytes, and T cells [47] and affect the incorporation of Se into immune-important organs, such as the spleen and lymph nodes [48]. In this study, the Se level had no obvious regularity in different groups and in different times.

Chronic exposure to high levels of Mn in diets leads to its accumulation in immune organs, resulting in the balance of trace elements such as Cu, Fe, Zn, Ca, and Se being disturbed in immune organs, as shown. It is uncertain whether Mn accumulated in immune organs to increase Fe, Zn, and Ca contents decreased; however, Cu and Se contents showed no difference. Therefore, the reason needs further study.

Cytokines play a crucial role in regulating the immune response in that they help determine whether a response to an immunogenic stimulus leads to a potent effector response or the induction of immune tolerance [49]. IL-1 β and IL-2 fulfill their role mainly in the inflammatory and in the immune response. Inflammatory mediators engaged in blood and dialyzer membrane interactions include the activation of monocytes, leading to the increased release of IL-1 β , which in turn leads to the release of IL-2 by monocytes [50, 51]. Oweson et al. [52] demonstrated that an accumulation of Mn in different species had effects on the immune system. Hernroth et al. [26] revealed that a surplus of Mn affected several immunological processes of *Nephrops norvegicus* because Mn suppressed the number of hemocytes by inducing apoptosis. In our experiment, emphasis was placed on IL-1 β and IL-2 solely because of the importance of their function in the immune system, not excluding the importance of the other interleukins. IL-1 β and IL-2 mRNA levels in immune organs decreased along with the increase of Mn-supplemented diets, which presented obvious regularity in a dose- and time-dependent fashion. The same regularity was shown in serum of IL-1 β and IL-2 contents. The IL-1 β and IL-2 mRNA levels and content increased in the low-dose and short-term groups. The results revealed that the immune system of cocks was stimulated by Mn of low dosage; the function of the immune system enhanced. However, the IL-1 β and IL-2 mRNA levels and content decreased along in

the high-dosed and long-term groups. The function of immune organs may be destroyed; therefore, the secretion ability of IL-1 β and IL-2 was limited. It was proved that high Mn exposure induced immune suppression in the molecular level. In addition, Cu deficiency resulted in a decreased secretion of IL-2. This is perhaps related to the decrease of IL-2 because IL-2 is required for T lymphocyte proliferation and is the principal cytokine responsible for the progression of T lymphocytes from the G1 phase to the S phase of the cell cycle [53].

In conclusion, the present study demonstrates that excessive Mn accumulated largely in immune organs exposed to manganese and disturbed the unbalance of the microelement metabolisms and induced immune suppression in the molecular level; therefore, the immune system of cocks is injured and the immune functions are also suppressed. The mechanism of this effect in the developmental toxicity of Mn remains to be further studied.

Acknowledgments This study was supported by the National Natural Science Foundation of China (no. 30871902).

References

1. Bourre JM (2006) Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. *J Nutr Health Aging* 10(5):377–385
2. Xu XR, Li HB, Wang WH et al (2005) Decolorization of dyes and textile waste water by potassium permanganate. *Chemosphere* 59(6):893–898
3. Park RM, Bowler RM, Roels HA (2009) Exposure–response relationship and risk assessment for cognitive deficits in early welding-induced manganese. *J Occup Environ Med* 51(10):1125–1136
4. Klos KJ, Ahlskog JE, Kumar N, Cambern S et al (2006) Brain metal concentrations in chronic liver failure patients with pallidal T1 MRI hyperintensity. *Neurology* 67(11):1984–1989
5. Iregren A (1999) Manganese neurotoxicity in industrial exposures: proof of effects, critical exposure level, and sensitive tests. *Neurotoxicology* 20(2–3):315–323
6. Bansal AK, Bilaspuri GS (2008) Effect of manganese on bovine sperm motility, viability, and lipid peroxidation in vitro. *Anim Reprod* 5:90–96
7. Zhang DH, Kanthasamy A, Anantharam V (2011) Effects of manganese on tyrosine hydroxylase (TH) activity and TH-phosphorylation in a dopaminergic neural cell line. *Toxicol Appl Pharmacol* 254(2):65–71
8. Misselitz B, Muhler A, Weinmann HJ (1995) A toxicological risk for using manganese complex? A literature survey of existing data through several medical specialties. *Investig Radiol* 30(10):611–620
9. Avila DS, Gubert P, Fachineto R et al (2008) Involvement of striatal lipid peroxidation and inhibition of calcium influx into brain slices in neurobehavioral alterations in a rat model of short-term oral exposure to manganese. *Neurotoxicology* 29(6):1062–1068
10. Yin Z, Aschner JL, Santos AP, Aschner M (2008) Mitochondrial-dependent manganese neurotoxicity in rat primary astrocyte cultures. *Brain Res* 1203:1–11
11. Malecki EA (2001) Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. *Brain Res Bull* 55(2):225–228

12. Wang X, Miller DS, Zheng W (2008) Intracellular localization and subsequent redistribution of metal transporters in a rat choroid plexus model following exposure to manganese or iron. *Toxicol Appl Pharmacol* 230(2):167–174
13. Milatovic D, Zaja-Milatovic S, Gupta RC et al (2009) Oxidative damage and neurodegeneration in manganese-induced neurotoxicity. *Toxicol Appl Pharmacol* 240(2):219–225
14. Rivera-Mancia S, Rios C, Montes S (2011) Manganese accumulation in the CNS and associated pathologies. *Biomaterials* 24:811–825. doi:10.1007/s10534-011-9454-1
15. Zwingmann C, Leibfritz D, Hazell AS (2004) Brain energy metabolism in a sub-acute rat model of manganese neurotoxicity: an ex vivo nuclear magnetic resonance study using [^{1-13}C] glucose. *Neurotoxicology* 25(4):573–587
16. Sogut O, Percin F (2011) Trace elements in the kidney tissue of bluefin tuna (*Thunnus thynnus* L. 1758) in Turkish seas. *Afr J Biotechnol* 10(7):1252–1259
17. Harmanescu M, Popovici D, Gergen I (2008) Heavy metals contents for different honey samples from Berini Timis county. Proceeding of the International Conference Bioatlas, Transilvania University of Brasov, Romania
18. AL-Gahri MA, Almussali MS (2008) Microelement contents of locally produced and imported wheat grains in Yemen. ISSN: E-J Chem 5(4):838–843
19. Hasunuma R, Ogawa T, Kawanishi Y (1982) Fluorometric determination of selenium in nanogram amounts in biological materials using 2,3-diaminonaphthalene. *Anal Biochem* 126(2):242–245
20. Yu D, Li JL, Xu SW et al (2011) Effects of dietary selenium on selenoprotein W gene expression in the chicken immune organs. *Biol Trace Elem Res* 144:678–687. doi:10.1007/s12011-011-9062-5
21. Li JL, Gao R, Xu SW et al (2010) Testicular toxicity induced by dietary cadmium in cocks and ameliorative effect by selenium. *Biomaterials* 23:695–705
22. Sun B, Wang R, Xu SW (2011) Dietary selenium affects selenoprotein W gene expression in the liver of chicken. *Biol Trace Elem Res* 143:1516–1523. doi:10.1007/s12011-011-8995-z
23. Li JL, Li HX, Li S, Jiang ZH, Xu SW, Tang ZX (2011) Selenoprotein W gene expression in the gastrointestinal tract of chicken is affected by dietary selenium. *Biomaterials* 24(2):291–299
24. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29(9):e45
25. Idowu OMO, Ajuwon RO, Oso AO et al (2011) Effect of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens. *Int J Poult Sci* 10(3):225–230
26. Hemroth B, Baden SP, Holm K et al (2004) Manganese induced immune suppression of the lobster, *Nephrops norvegicus*. *Aquat Toxicol* 70(3):223–231
27. Milatovic D, Yin ZB, Gupta RC et al (2007) Manganese induces oxidative impairment in cultured rat astrocytes. *Toxicol Sci* 98(1):198–205
28. Zhang P, Hatter A, Liu B (2007) Manganese chloride stimulates rat microglia to release hydrogen peroxide. *Toxicol Lett* 173(2):88–100
29. Zhao F, Cai TJ, Liu MC, Zheng G et al (2009) Manganese induces dopaminergic neurodegeneration via microglial activation in a rat model of manganese. *Toxicol Sci* 107(1):156–164
30. Milatovic D, Gupta RC, Yu YC et al (2011) Protective effects of antioxidants and anti-inflammatory agents against manganese-induced oxidative damage and neuronal injury. *Toxicol Appl Pharmacol* 256(3):219–226
31. Swiatkiewicz S, Koreleski J (2008) The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality. *Vet Med* 53(10):555–563
32. Guilarte TR (2010) Manganese and Parkinson's disease: a critical review and new findings. *Environ Heal Perspect* 118:1071–1080
33. Bauman DE, Currie WB (1980) Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J Dairy Sci* 63(9):1514–1529
34. Bokoye CO, Ibeto CN, Ihedioha JN (2011) Assessment of heavy metals in chicken feeds sold in south eastern, Nigeria. *Adv Appl Sci Res* 2(3):63–68
35. Khandelwal S, Ashguin M, Tandon SK (1984) Influence of essential elements on manganese intoxication. *Bull Environ Contam Toxicol* 32(1):10–19
36. Zhang BY, Chen S, Ye FL (2002) Effect of manganese on heat stress protein synthesis of new-born rats. *World J Gastroenterol* 8(1):114–118
37. Fitsanakis VA, Piccola G, Aschner JL, Aschner M (2006) Characteristics of manganese (Mn) transport in rat brain endothelial (RBE4) cells, an in vitro model of the blood–brain barrier. *Neurotoxicology* 27(1):60–70
38. Fitsanakis VA, Thompson KN, Deery SE, Milatovic D et al (2009) A chronic iron-deficient/high-manganese diet in rodents results in increased brain oxidative stress and behavioral deficits in the Morris water maze. *Neurotox Res* 15(2):167–178
39. Zheng W, Zhao QQ, Slavkovich V et al (1999) Alteration of iron homeostasis following chronic exposure to manganese in rats. *Brain Res* 833(1):125–132
40. Garrick MD, Singleton ST, Vargas F, Kuo HC et al (2006) DMT1: which metals does it transport? *Biol Res* 39(1):79–85
41. Prasad AS, Oberleas D (1971) Changes in activities of zinc dependent enzymes in zinc dependent tissues of rats. *J Appl Physiol* 31(6):842–846
42. Mengheri E, Bises G, Gaetani S (1988) Differentiated cell-mediated immune response in zinc deficiency and in protein malnutrition. *Nutr Res* 8(7):801–812
43. Fraker PJ, Haas SM, Leucke RW (1977) Effect of zinc deficiency on the immune response of the young adult A/J mouse. *J Nutr* 107(10):1889–1895
44. Gavin CE, Gunter K, Gunter TE (1999) Manganese and calcium transport in mitochondria: implications for manganese toxicity. *Neurotoxicology* 20(2–3):445–453
45. Gunter TE, Miller LM, Gavin CE, Eliseev R et al (2004) Determination of the oxidation states of manganese in brain, liver, and heart mitochondria. *J Neurochem* 88(2):266–280
46. Leng L, Bobzek R, Kuriková S et al. (2003) Comparative metabolic and immune responses of chickens fed diets containing inorganic selenium and Sel-Plex™ organic selenium. In: Nutritional biotechnology in the feed and food industry. Proceedings of Alltech's 19th Annual Symposium, United Kingdom, pp 131–145
47. Kiremidjian-Schumacher L, Rol M, Wishe H et al (1992) Regulation of cellular immune responses by selenium. *Biol Trace Elem Res* 33(1–3):23–35
48. Hawkes WC, Kelley DS, Taylor PC (2001) The effects of dietary selenium on the immune system in healthy men. *Boil Trace Elem Res* 81(3):189–213
49. Ganesh BB, Bhattacharya P, Gopisetty A et al (2011) IL-1 β promotes TGF- β 1 and IL-2 dependent foxp3 expression in regulatory T cells. *PLoS One* 6(7):e21949. doi:10.1371/journal.pone.0021949
50. Ward RA (1994) Phagocytic cell function as an index of biocompatibility. *Nephrol Dial Transplant* 9(suppl2):46–56
51. Rysz J, Banach M, Cialkowska-Rysz A et al (2006) Blood serum levels of IL-2, IL-6, IL-8, TNF- α and IL-1 β in patients on maintenance hemodialysis. *Cell Mol Immunol* 3(2):151–154
52. Oweson C, Baden SP, Hemroth BE (2006) Manganese induced apoptosis in haematopoietic cells of *Nephrops norvegicus*. *Aquat Toxicol* 77(3):322–328
53. Bonham M, Connor O, Hannigan JM et al (2002) The immune system as a physiological indicator of marginal copper status? *Br J Nutr* 87(5):393–403