Biol Trace Elem Res (2016) 172:320–325
DOI 10.1007/s12011-015-0606-y

Critical Thresholds of Antioxidant and Immune Function Parameters for Se deficiency Prediction in Dairy Cows

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Received: 7 October 2015 / Accepted: 21 December 2015 / Published online: 8 January 2016

Abstract The aim of this study was to determine the plasma selenium (Se) levels of lactating cows and to evaluate its association with antioxidant ability and immune function. In a descriptive study, 20 healthy Holstein cows with normal Se level (C) and 30 Holstein cows with subclinical Se deficiency (T) were randomly selected between 14 and 21 days postpartum from a dairy farm, according to a cutoff point of 70 mg/L Se in plasma. Analysis of biochemical parameters of antioxidant and immune function were performed on all the cows, and the risk prediction thresholds for subclinical Se deficiency were determined by area under receiver operating characteristic curve. Cows in the T group had significantly lower plasma Se concentrations compared with cows in the C group (52.16 ± 8.81 vs. 80.37 ± 8.46 μg/L, $P = 0.02$). There was a marked decrease in plasma glutathione peroxidase (GSH-Px) activity in the T group that correlated positively with the plasma Se level ($R = 0.65$, $P = 0.00$), and a significant increase of plasma methane dicarboxylic aldehyde (MDA), total nitric oxide synthase, and lipid peroxidation that correlated negatively with plasma Se levels ($R = -0.47$, $P = 0.01$; $R = -0.33$, $P = 0.04$; $R = -0.40$, $P = 0.03$). Furthermore, there were significantly lower plasma tumor necrosis factor-α and immunoglobulin G levels in the T group that correlated positively with plasma Se levels ($R = 0.41$, $P = 0.01$ and $R = 0.45$, $P = 0.01$), and a markedly lower plasma interleukin-6 level that correlated negatively with plasma Se levels ($R = -0.38$, $P = 0.02$). In addition, if plasma GSH-Px activity was less than 42.37 U/ml, the risk of Se deficiency was significantly increased in lactating cows. These results suggest that low plasma Se levels may reduce the antioxidant ability and immune function, and the risk of low plasma Se level may be predicted effectively by plasma GSH-Px activity in lactating cows.

Keywords Dairy cows · Se deficiency · Antioxidant ability · Immune function · Risk prediction · Threshold

Introduction

Early in the 20th century, selenium (Se) was acknowledged as a necessary trace element in animals [1]. The main causes of oxidative stress are the excessive accumulation of free radicals in an animal's body, which may cause impaired organ function [1–4]. The free radicals can be scavenged by antioxidant compounds [2, 3]. Antioxidant activity that comprises two systems for scavenging free radicals, an enzymatic system and a non-enzymatic system, is a response to a poor environment and disease [1–3]. The enzymatic system, which mainly includes superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase, can remove a large amount of oxygen free radicals from the animal's body. The non-enzymatic system refers to vitamin C, vitamin E, Se, etc., and this may scavenge excess free radicals through combination with them [3, 4]. Cooperation between the two types of antioxidant may be able to maintain the balance of free radicals in the animal's body. The total antioxidant capacity (T-AOC) is the total capacity of the two kinds of antioxidant system. When the T-AOC levels decrease, the organism is suffering from external stimuli or

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lipid peroxidation damage and is unable to keep the antioxidant system active, and lipid peroxides and free radicals abound. Therefore, T-AOC may be used to evaluate the activity of antioxidant enzymes and the non-enzyme system in the body [5, 6]. In addition, Se may enhance an animal's resistance to disease by improving the function of the immune system and may regulate the body's immune function through cellular immunity, humoral immunity, and nonspecific immunity [4, 5]. High plasma Se concentration will not only increase the immunoglobulin G concentration and the ability of lymphocytes to secrete IL-2, thus reducing the incidence of mastitis, but will also improve the antimicrobial activity of neutrophils in blood and milk [7–9].

At present, Se deficiency in dairy cows is receiving much attention. The plasma Se concentration in normal dairy cows ranges from 70 to 100 μg/L [10]. When Se content in plasma is less than 70 ug/L, a dairy cow will show Se deficiency [11–13], which has many deleterious effects on cows. By detection of Se, oxidative stress, and immune function in plasma from lactating cows, this experiment was designed to clarify the relationship of plasma Se with oxidative stress and immune function in lactating cows with Se deficiency, explore early warning indicators and the threshold for the risk of Se deficiency, and provide technical support for preventing Se deficiency in lactating cows.

Materials and Methods

Animals

This experiment was carried out in an intensive dairy herd containing more than 4700 Holstein cows that were maintained in free-stall housing using a complete dairy management software (Valley Agricultural Software, Tulare, CA, USA) system in Heilongjiang, China. From May to June 2014, 50 multiparous cows were selected randomly to provide 20 control cows and 30 cows with Se deficiency, which had similar age, parity, body condition score and milk yield. The cows were considered to have Se deficiency (T, 52.16 ± 8.81 μg/L) if they had plasma Se concentration <70 μg/L; otherwise, they were healthy controls (C, 80.37 ± 8.46 μg/L) [10, 11]. All the cows were fed a total mixed ration (TMR) during the early lactation, which consisted of 21.40 % corn, 6.44 % bean pulp, 3.20 % concentrated feed, 47.70 % maize silage, 20.50 % hay, 0.35 kg fat, 0.15 % calcium hydrophosphate, 0.33 % sodium bicarbonate, and 0.28 % salt. Its nutritional levels were 75.60 % dry matter (DM), 11.65 % crude protein (CP), 20.32 MJ/kg DM net energy for lactation (NEL), 4.10 % crude fat, 21.42 % crude fiber, 0.65 % Ca, and 0.44 % P, with dry matter intake (DMI) of 20 kg. The animals were held with permission of the Local Dairy Cattle Association at Mishan city, Heilongjiang province, China, and were treated in conformance with commonly practiced ethical standards.

Sample Collection

Each blood sample was taken from the left jugular vein into a 1.5 ml tube containing 10 % heparin between 14 and 21 days postpartum, before the morning feed. All blood samples were immediately centrifuged at 1400×*g* for 3–5 min at room temperature. The plasma samples were aliquoted into 1 ml Eppendorf tubes and stored at −80 °C until analysis. All haemolyzed samples were discarded. This experiment was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA. All animal experiments were conducted according to the practices and standards approved by the Animal Welfare and Research Ethics Committee at Heilongjiang Bayi Agricultural University, China.

Laboratory Analysis

The concentration of Se in plasma was measured by means of an atomic absorption spectrophotometer (ICE 3500, Thermo Fisher Scientific India Pvt. Ltd.). The levels of SOD, methane dicarboxylic aldehyde (MDA), nitric oxide (NO), (CAT), GSH-Px, total nitric oxide synthase (T-NOS), and lipid peroxidation (LPO) in plasma were detected by kits from Beijing Strong Biotechnology Company, Beijing, China. The concentrations of interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and immunoglobulin G (IgG) in plasma were analyzed by the ELISA method using kits from Shanghai Desai Biotechnology Company, Shanghai, China.

Statistical Analysis

Student's *t* test was used to analyze any significant differences in plasma concentrations of SOD, MDA, NO, CAT, GSH-Px, T-NOS, LPO, IL-1β, IL-2, IL-6, TNF-α, IgG, and Se between the T group and the C group. The analyses were performed using SPSS software (Version 19.0, IBM, New York, USA). All data are presented as mean and standard deviation (SD) in Table 1, and $P < 0.05$ or 0.01 was considered to represent statistical significance.

Given that plasma Se concentration has been accepted as a gold standard for the diagnosis of Se deficiency and is also considered an optimal herd monitoring biomarker [12, 14], Spearman's correlation coefficients were utilized to reveal the correlations between Se and the other parameters in Tables 2 and 3.

Table 1 Clinical information on two groups of lactating dairy cows

Parameters	T	C
No.	30	20
Se, μg/L	52.16 ± 8.81[A]	80.37 ± 8.46[B]
Age	3.91 ± 1.29	4.02 ± 1.34
Parity	2.22 ± 0.73	2.36 ± 1.02
BCS	2.94 ± 0.58	2.75 ± 0.55
MY, kg/day	26.45 ± 9.72	27.70 ± 9.30

In the same row, the different capital letters represent significant differences ($P < 0.01$); no letters indicate no significant difference between the results ($P > 0.05$)

T cows with low plasma Se level, *C* cows with normal plasma Se level, *BCS* body condition score, *MY* milk yield per day

Subsequently, two logistic regression models were established to obtain risk factors for antioxidant ability and immune function associated with Se deficiency. Model 1 included SOD, GSH-PX, MDA, T-NOS, CAT, NO, and LPO to determine the relationships between antioxidant ability and Se deficiency. Other risk factors for immune function included in model 2 were IL-1β, IL-2, TNF-α, IL-6, and IgG to investigate the associations between immune function and Se deficiency.

Finally, the cutoff points of the risk factors were determined using area under receiver operating characteristic (ROC) curve (AUC) analysis [15], in which Se was included as a continuous outcome parameter. Furthermore, curves of sensitivity versus specificity were plotted using AUC analysis for IL-6, MDA, GSH- Px, TNF-α, IL-2, etc., to include all possible threshold values of the parameters assessed. Youden's index was computed to determine the optimal thresholds of the selected parameters [16]. The AUC was used to assess the diagnostic precision of the eligible parameters [17].

Results

Antioxidant Ability of Lactating Cows

The clinical information on all the experimental cows is listed in Table 1. Cows in the T group had significantly low plasma Se levels compared with in the C group, but the other clinical parameters were not significantly different.

The biochemical parameters are listed in Table 2. The results showed a significantly lower GSH-Px activity and a markedly higher MDA, T-NOS, and LPO in plasma samples of the T group compared with the C group.

In the 40 cows, there was a significant positive correlation between the plasma concentrations of Se and GSH-Px ($R = 0.65$, $P = 0.00$), and also a prominent negative correlation between the plasma concentrations of Se and MDA ($R = -0.47$, $P = 0.01$), T-NOS ($R = -0.33$, $P = 0.04$), or LPO ($R = -0.40$, $P = 0.03$). However, there was no significant correlation between the plasma concentrations of Se and SOD, T-AOC, CAT, or NO ($P \geq 0.05$).

Immune Function of Lactating Cows

The biochemical parameters are listed in Table 3. There were significantly lower levels of IL-1β, TNF-α, and IgG in plasma samples from the T group compared with the C group.

In the 40 cows tested, there was a significant correlation between the plasma concentrations of Se and TNF-α ($R = 0.41$, $P = 0.01$), IgG ($R = 0.45$, $P = 0.01$), or IL-6 ($R = -0.38$, $P = 0.02$). However, there was no significant correlation between the plasma concentrations of Se and IL-1β or IL-2 ($P \geq 0.05$).

Risk Prediction

During regression analysis in the first logistic model, plasma SOD, T-AOC, CAT, or NO ($P > 0.05$) were removed and

Table 2 Antioxidant ability and its correlation with plasma Se of the dairy cows tested

Parameters	T	C	Mean	Correlation
No.	30	20	40	40
Se, μg/L	52.16 ± 8.81A	80.37 ± 8.46B	70.27 ± 8.60	
SOD, U/ml	66.46 ± 5.75	69.59 ± 8.57	67.99 ± 7.14	$R = 0.30$, $P = 0.45$
GSH-PX, U/ml	50.19 ± 6.46a	60.03 ± 12.33b	53.70 ± 10.35	$R = 0.65$, $P = 0.00$
MDA, nmol/ml	2.48 ± 0.97a	1.73 ± 4.36b	2.21 ± 0.91	$R = -0.47$, $P = 0.01$
T-NOS, U/ml	49.10 ± 2.59a	46.81 ± 3.29b	48.27 ± 3.05	$R = -0.33$, $P = 0.04$
CAT, U/ml	1.08 ± 0.44	0.99 ± 0.24	1.05 ± 0.38	$R = -0.19$, $P = 0.50$
NO, μmol/L	5.74 ± 3.55	5.49 ± 4.36	5.54 ± 1.36	$R = -0.13$, $P = 0.55$
LPO, μmol/ml	1.72 ± 0.45a	1.33 ± 0.30b	1.58 ± 0.44	$R = -0.40$, $P = 0.03$

In the same row, the different lowercase letters represent significant differences ($P < 0.05$); no letters indicate no significant difference between the results ($P > 0.05$). "–" represents negative correlation

Table 3 Immunological function and its correlation with plasma Se in the experimental dairy cows

Parameters	T	C	Mean	Correlation
No.	30	20	40	40
IL-1β, ng/L	57 ± 6a	69 ± 15b	64 ± 13	$R = 0.15$, $P = 0.34$
IL-2, ng/L	115 ± 16	132 ± 35	125 ± 28	$R = 0.13$, $P = 0.40$
TNF-α, ng/L	547 ± 29a	645 ± 115b	600 ± 98	$R = 0.41$, $P = 0.01$
IL-6, ng/L	430 ± 140	447 ± 139	444 ± 137	$R = -0.38$, $P = 0.02$
IgG, mg/ml	3.78 ± 0.70a	4.95 ± 1.28b	4.48 ± 1.19	$R = 0.45$, $P = 0.01$

In the same row, the different lowercase letters represent significant differences ($P < 0.05$); no letters indicate no significant difference between the results ($P > 0.05$). "–" represents negative correlation

GSH-Px, MDA, T-NOS, and LPO ($P < 0.05$) were retained, suggesting that a decrease in GSH-Px and an increase in MDA can predict the risk of oxidative stress due to Se deficiency. In the second logistic model, plasma TNF-α, IL-6, and IL-2 ($P < 0.05$) were retained, and IL-1β and IgG ($P > 0.05$) were eliminated, suggesting that a decrease in plasma TNF-α, IL-6, and IL-2 can predict the risk of immune dysfunction due to Se deficiency.

From the ROC curve analysis, the prediction thresholds, sensitivity, specificity, SE, and AUC for MDA, GSH-Px, TNF-α, IL-6, and IL-2 are presented in Table 4. The optimal prediction thresholds were determined by the Youden's index to be more than 4.41 mmol/ml for MDA, with 100 % sensitivity and 88 % specificity, less than 42.37 U/ml for GSH-Px, with 100 % sensitivity and 93.8 % specificity, less than 523.17 ng/L for TNF-α, with 91.7 % sensitivity and 100 % specificity, less than 699.39 ng/L for IL-6, with 100% sensitivity and 93.7 % specificity, and less than 90.53 ng/L for IL-2, with 87.5 % sensitivity and 100 % specificity.

The areas under the ROC curves for MDA, GSH-Px, TNF-α, IL-6, and IL-2 are shown in Table 4. As GSH-Px had a positive correlation with Se, the area above the ROC curve was selected. The AUC was greater than 0.70 for GSH-Px. Therefore, if plasma GSH-Px activity is less than 42.37 U/ml in a lactating cow, it may be used to predict the risk of oxidative stress due to Se deficiency. However, MDA, TNF-α, IL-6, and IL-2 may be of value in the diagnosis of immune dysfunction due to Se deficiency.

Discussion

This study investigated selenium concentration in the plasma of lactating cows from an intensive dairy farm. The plasma selenium concentration of normal dairy cows should be above 70 μg/L [10–12], but the average level of selenium in plasma was below this value. This illustrates that the prevalence of Se deficiency was high, and this was associated with insufficient supply of Se in the diet on the farm.

Selenium has an important antioxidant function in the cells, since it is a major component of GSH-Px, which can remove excess free radicals, and also is a regulator of lipid antioxidation [1–3]. The main purpose of SOD is to scavenge free radicals and to protect dehydrogenase from superoxygen free radical damage [5, 13]. MDA is an important index of oxidative stress to investigate the degree of membrane lipid peroxidation [6, 14]. CAT is one of the key enzymes in the biological defense system in certain tissues to removes hydrogen peroxide for preventing cell damage [5, 6]. T-AOC is a comprehensive index of antioxidant function to reflect the ability to compensate for external stress and the metabolic status of free radicals [6.7, 16]. The chronic Se deficiency may cause organ damage due to a higher NOS activity producing excess

Table 4 The cutoff point, sensitivity, specificity, and area under the receiver operating characteristic curve (AUC)

Parameters	Cutoff point	Sensitivity (%)	Specificity (%)	AUC analysis
MDA, nmol/ml	4.41	100	93.7	0.88, $P = 0.01$
GSH-Px, U/ml	42.37	100	93.8	0.80, $P = 0.003$
TNF-α, ng/L	523.17	91.7	100	0.68, $P = 0.114$
IL-6, ng/L	699.39	100	93.7	0.66, $P = 0.150$
IL-2, ng/L	90.53	87.5	100	0.52, $P = 0.889$

NO, and H_2O_2 from in the organ metabolism may be broken down by GSH-Px and CAT [10, 15, 18].

The present researches have already showed that Se was added to the diet of dairy cows, the activity of GSH-Px and SOD in serum was increased, the MDA content decreased, levels of free radicals drop, and thus cell damage was reduced [5, 6, 15]. It suggests that Se may scavenge active oxygen, reduce the production of peroxide, and protect the cell membrane from damage inflicted by oxides. In this experiment, the activities of SOD, GSH-Px and NOS in plasma were lower in the Se-deficient group, and the MDA level was higher, but the T-AOC showed no obvious change. This suggests that Se deficiency can cause oxidative stress to compromise the animal health.

Some reports have already determined that Se is an important cofactor of the antioxidant enzyme glutathione peroxidase and plays the important role in redox catalysis [1, 5, 6]. It is tempting to speculate that Se may promote the production of interferon, increase the activity of IFN-gamma in vitro, and strengthen the cytotoxia effects of natural killer (NK) cell, thus protect the target cell membranes [7–9]. Moreover, Se can prevent lipid peroxidation damage induced by tertiary butyl peroxide hydrogen to remove the toxic effect of peroxide on animals [5–7]. In this experiment, Pearson correlation analysis showed that there was a positive correlation between Se and GSH-Px, and with SOD, and a negative correlation between Se and T-AOC, MDA, T-NOS, LPO, NO, and CAT in lactating cows, including those with Se deficiency. From these results, we found that Se deficiency occurred in lactating cows, it is tempting to speculate that Se may be weaken the ability of GSH-Px to scavenge free radicals; thus, the large amounts of free radicals and lipid peroxide were produced to induce oxidative tissue damage. Therefore, Se deficiency may induce a state of oxidative stress in lactating cows.

In addition, Se deficiency is able to disrupt the immune system which includes cellular immunity, humoral immunity, and nonspecific immunity. Cellular immunity mediated by T cells is a specific defense reaction [4–6]. The cellular immune response not only targets a specific antigen, but it also enhances the humoral immunity [16]. IL-2 is produced by activated T cells and combines with the IL-2 receptor on the surface of the T lymphocyte to stimulate T cell proliferation further [7, 8]. IgG is produced mainly by plasma cells in the spleen and lymph nodes and has immune activity with antiviral and antibacterial effects [9, 13]. This experiment showed that plasma levels of IL-2, TNF alpha, and IgG were lower in the Se deficient group, which suggests that Se deficiency had weakened the animals' immune function. Se can stimulate lymphocytes to secret IL-1 and IL-2 and thus improve the synthesis of IgG [8, 9, 13]. In addition, IL-1B is a cell factor and polypeptide adjustment factor generated by mononuclear macrophages, which has an immunoregulatory function. IL-6 is a lymphatic factor produced by activated T cells and fibroblasts, which can stimulate B cell precursor cells to produce antibodies and enhance the function of NK cells [5, 6, 16]. In this experiment, Spearman correlation analysis showed a positive correlation between Se and TNF-α, IgG, IL-β, and IL-2, and a negative correlation between Se and IL-6 in lactating cows, including those with Se deficiency. Thus, Se deficiency is able to reduce immune function in lactating cows.

Generally, a guide for classifying the accuracy (AUC value) of a diagnostic test is the traditional academic point system: 0.90–1 = excellent (A), 0.80–0.90 = good (B), 0.70–0.80 = fair (C), 0.60–0.70 = poor (D), 0.50–0.60 = fail (F) [17]. In Table 4, a diagnostic test (AUC value) of GSH-Px, MDA, TNF-α, IL-β, and IL-2 to Se deficiency was above 0.50, and only AUC of GSH-Px was good and significant ($P < 0.05$). These results may be related to the degree of Se deficiency.

Selenium is a necessary trace element in animals, and its deficiency will be very harmful to cattle health; therefore, it should be quite important to develop a strategy to prevent Se deficiency on dairy farms. In this study, the plasma GSH-Px of lactating cows was less than 42.37 U/ml, which will have increased the risk of Se deficiency. The level of MDA in plasma should not be more than 4.41 nmol/ml; these can be used as auxiliary diagnostic indicators.

Acknowledgments The authors would like to thank 8511 dairy farm for the experimental animals and other supports, also thank International Science Editing in Ireland for editing the manuscript, and then appreciate the financial support from the National Science and Technology Support Program (2012BAD12B05-2, 2012BAD12B 03–2, and 2013BAD21B01), the National Science Foundation Committee (31672625), China Spark Program (2012GA670001) and NCET (1252-NCET-003) for the research, and/or publication of this article.

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