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Effect of applying selenium fertilizer to improve soil and increase selenium level in food for prevention and treatment of Kaschin-Beck disease

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Abstract— For prevention and treatment of Kaschin-Beck disease by applying Se fertilizer to soil to increase Se up to normal level for the wheat grains is introduced in this paper. After use this measure the intake of Se by the residents in the exemplary area increased from 10.4 μg to 33.6 μg per day in average. After supplementing Se to human body one year, there is no new patient found

* Li Jiyun and Chen Daizhong were in charge of this program.

among 300 healthy children in the exemplary area but 4 patients found among 264 healthy children in the control area. It is shown that by applying Se fertilizer to KBD area is a effective way for preventing and cure this disease.

Keywords: selenium; prevention; treatment; Kaschin-Beck disease.

1 Introduction

It has been proved by much research in the last decade that the Kaschin-Beck disease (KBD) is prevalent among residents in low selenium environment areas (Li, 1982; Chen 1985; Hou, 1984) and the residents in the disease area are at low Se nutrient metabolic level. Under the circumstances good results have been obtained by applying Se to human beings for preventing and curing the disease.

The measures which have been used in supplement of Se to human body are as follows: taking sodium selenite pill or sodium selenite added to salts, spraying Se solution on crop leaves and so on (Li, 1979; Liang 1982; Wang 1983; Yang, 1982). The more popular one is the use of table salt with Se. All of these measures have some good effect in the experiments, but have their own shortage and limitation in their application to larger areas. Therefore, it will be helpful to the KBD control and treatment to improve the low Se environment if a method can be found which is simple, reliable and safe for human body and suitable for application.

Watkinson *et al.* (Watkinson, 1966) succeeded in controlling the white muscle disease in livestock by applying sodium selenite to pasture land. The government of New Zealand adopted a law of Se application in pasture in 1982 and the white muscle disease was under control shortly (Watkinson, 1966). During that period the New Zealand's scientists found that the Se content in wheat grain in vast areas was very low and the Se uptake amount of the residents in south island was only 30 μg per day. So the wheat contained higher Se was imported from Australia for supplement of Se to the human body. Se complex fertilizer was applied to farmland in a nativewide scale in Finland in 1983, this measure made Se intake level of people to increase from 50 μg$-$100μg a day, so as to improve their health situation (Viaranta, 1983). In addition, Kissel-Nielsen (Danmark) and Cary (U. K.) *et al.* reported on applying Se into soil to supplement the Se nutrition of human and animal bodies (Gissel, 1973; Cary, 1973). The authors carried out an experiment on Se fertilizer in the KBD area, Yongshou County, Shaanxi Province from 1983$-$1986 and the results indicated that applying sodium selenite 15 g per mu (1 ha$=$15mu) to the main soil (red soil and bicolour soil) in this region made the Se content of wheat grain to increase to the same level as that in the disease-free areas and added Se in soil can

last for three years.

On the basis of the above research result, since 1986 we have carried out experiment on a large scale for the prevention and cure of KBD by applying Se fertilizer to improve soil.

2 Experimental methods and basic conditions in the experimental area

The experiment area was located in Bin County, Shaanxi Province, where is in semi-humid and semi-arid forest steppe region in the temperate zone with Heilu soil (light mollisol soil), the annual mean precipitation is about 564 mm and the annual cumulation of temperatures $\geqslant$ 10 ℃ is approximately 3658 ℃ . The natural landscape of the area belongs to the hilly region of loess plateau. It is a medium serious KBD area.

Experiment was conducted in a Se fertilizer experiment area (South Yuan— Yuan is a table land with abruptly descending edges) and a control area (North Yuan) with a boundary line along Jinghe River to urban district of Bin County so as to prevent the disturbance of human factors to experiment results. The two experiment areas are 20 km apart from each other. Both areas are on the broken loess plateau with a narrow and uneven surface caused by soil erosion. The soil distributed in this area is Clay Heilu soil and Shan soil (loess like Entisol) developed loess parent material. The crop growing in the area is mainly winter wheat (70% of the crop land), with a small ratio of other crops harvesting in fall such as maize, sorghum, beans and millets. The economy in this area is mainly of self-supporting type. Due to the limitation of living conditions, diet of the local residents is composed mainly of cereals with less vegetable and other food-stuffs. Their nutrition level is very low.

The demonstration area convered seven neighboring villages in Didian district with 3700 people. The control area was sited to cover the five villages in Tandian and Xiaozhang districts. The physical conditions, living habits and board levels in both areas were very similar to each other. These similarities and the comparabilities in degrees and features of the disease were considered carefully in order to get reliable experimental data. Comparison in the disease situation between both areas is shown in Table 1.

The granulated complex fertilizer was made by mixing urea, superphosphate and sodium selenite in a given composition, consisting of N 10%, P 10% and Se 0.05%. The complex fertilizer as a top dressing was applied to soil between the ridges of winter wheat in an area of about 500 hectares at the time of seedling turning green in February 1987 at a rate of 150－200 kg per hectare (as sodium selenite of 150－200g/ha).

Effect of treating with Se among 3－15 years old children were observed. About 500 children were examined separately in each of both areas. X-ray was used to examine the hands of the subjects at regular intervals before and after the Se fertilizer was applied.

Table 1 Comparison of X-ray diagnoses in pathological changes between demonstration and control areas

Villages in demonstration area	X-ray detected, %	Met. part[1] detected, %	Epi.end[2] detected, %	Activity index	Severity index
Xinzhuang	22.7	18.2	9.1	26.5	36.1
Hanjia	36.7	31.7	13.9	51.7	43.6
Dache	38.7	28.0	20.0	56.6	52.5
Nantouao	48.8	44.2	25.6	67.9	60.7
Zhangjia	41.2	30.9	21.7	50.9	51.2
Wangjia	44.3	39.7	22.9	53.2	66.7
Liujia	42.9	33.9	14.3	55.0	51.2
Average	40.2	33.4	19.2	52.7	53.7
Villages in control area	X-ray detected, %	Met. part[1] detected, %	Epi.end[2] detected, %	Activity index	Severity index
Dongwu	48.6	26.4	40.3	29.0	65.8
Liucun	47.7	45.4	20.8	62.3	71.9
Liuchou	47.1	36.1	38.7	48.6	71.8
Dizhuang	65.4	50.0	40.4	76.9	80.1
Zhangjia	36.3	26.3	25.3	37.8	50.3
Average	47.4	35.3	32.9	49.0	67.9

1. Metaphyses part changes; 2. Control-epiphyseal end changes.

3 Experimental results

3.1 Increase in Se content in soil, grain and staple foods by applying Se fertilizer

Addition of Se to soil is to increase not only the content of total Se, but also the amount of available Se in soil. In fact, the total Se content in demonstration area soil was found to be only 89.6 ppb before Se fertilizer was applied. About four months later, the total Se content reached 148.3 ppb although some of it had been uptaken by wheat roots during the growing period from spring to summer.

The content of water soluble Se was 1.66－2.42 ppb before Se application with a mean of 2.05 ppb. After the harvest time, the soluble Se content in soil was still as high as 3.42 ppb. In other words, it was 66% higher than that if soil before fertilizing with Se (Table 2).

Table 2 Comparison of Se content in soils between the demonstration and control areas before and after Se fertilizer application (Unit: ppb)

Demonstration area					Control area		
Before Se fertilizer application			After Se fertilizer application				
Villages	Total Se content	Water-soluble Se content	Total Se content	Water-soluble Se content	Villages	Total Se content	Water-soluble Se content
Liujia	81.6±8.0	1.66±0.14	138.93±26.9	2.25±0.20	Dizhuang	86.5±6.8	1.71±0.25
Wangjia	86.1±11.8	1.87±0.61	153.0±28.1	2.90±0.37	Liuchou	86.3±10.8	1.74±0.43
Zhangjia	101.2±11.1	2.61±0.83	171.0±47.9	2.89±0.18	Leicun	79.0±3.9	1.91±0.21
Dache	88.8±6.7	1.92±0.46	155.0±29.4	3.39±1.42	Dongwu	90.5±6.3	2.07±0.37
Hanjia	94.7±13.4	2.42±0.83	131.8±15.3	4.88±2.52			
Nantouao	87.7±13.6	1.82±0.30	136.9±53.3	4.20±3.35			
Average	89.6	2.05	148.3	3.42	Average	87.0	1.92

Table 3 Comparison of Se content in wheat grains between the demonstration and control areas before and after Se fertilizer application (Unit: ppb)

Demonstration area				Control area			
	Before Se fertilizer	After Se fertilize application					
Villages	Application			Villages	Application		
	1986	1987	1988		1986	1987	1988
Liujia	5.3±1.2 (9)	71±49 (33)	84±16 (8)	Dizhuang	6.7±1.9 (8)	5.6±0.7 (5)	6.2±0.2 (4)
Wangjia	4.6±2.3 (9)	90±43 (108)	34±16 (9)	Lichou	7.6±3.6 (6)	7.0±0.4 (5)	6.9±0.8 (6)
Zhangjia	6.6±2.7 (10)	77±48 (21)	24±11 (8)	Leicun	8.6±2.0 (10)	7.6±0.5 (5)	6.6±0.8 (6)
Dache	5.7±2.3 (9)	82±34 (50)	36±23 (8)	Dongwu	5.6±1.5 (9)	5.9±2.3 (15)	6.6±0.6 (6)
Xinzhuang	9.4±2.7 (7)	100±42 (24)	52±21 (7)	Zhangjia	7.3±2.4 (10)	7.2±1.9 (12)	7.5±0.4 (6)
Hanjia	5.5±2.1 (9)	64±36 (23)	42±25 (9)				
Nantouao	9.2±3.1 (10)	70±45 (71)	68±48 (9)				
Average	6.6	79	48.6	Average	7.1	6.7	6.8

The Se content in wheat grain was increased rapidly from 4.6－9.4 ppb with a mean of 6.6 ppb before Se fertilizer application to a value of 64－100 ppb with a mean of 79 ppb after the first harvest, hanging an increase by about 12 times (July 1987). After the second harvest (July 1988), the Se content in wheat grains was still as high as 24－84 ppb with a mean of 48.6ppb, remaining 7 times higher than the background level in wheat, which reaches or surpasses the Se content in wheat grains harvested from a disease-free area (Table 3).

3.2 Increase in the content of many kinds of amino acid in wheat grain by applying Se fertilizer

It is known that crops absorb Se mainly through concentrating it in their seed protein in the form of amino acids in general. So the wheat grain was separately collected from 20 households living in each of the demonstration and control areas and was analyzed for amino acid content. The results showed that the contents of all kinds of amino acid, except Methionine, in the wheat grain are higher from the demonstration area than from the control area, and particularly the content of Cystine containing Se and S increases by up to 61.8% that is very important for improving Se nutrition of human beings. The Lysine content in wheat grains from control arca was 0.19%, but that from the demonstration area was 0.26% and the relative percentage higher than former is 25.9%. It may play a good role in improving the children's health in the disease area.

Table 4 Comparison of content of various amino acids in the wheat grains between demonstration and control areas[1]

Amino acids	Content in wheat grain, mg/g		Increase by applying Se to soil in demonstration area, %	*P*
	Demonstration area	Control area		
Cystine	2.05±0.67	1.36±0.62	50.7	<0.01
Methionine	0.91±0.42	0.84±0.45	8.3	>0.50
Lysine	2.92±0.56	2.32±0.73	25.9	<0.50
Phenylanine	4.49±1.07	2.32±0.71	93.5	<0.01
Leucine	6.71±1.51	4.73±1.88	41.9	<0.01
Isoleucine	3.11±0.93	2.03±1.06	53.2	<0.01
Valine	4.62±2.17	2.67±0.89	73.0	<0.01
Threonine	2.74±0.55	2.09±0.83	31.1	<0.01

1. Average of 20 samples taken from households living in each of the demonstration and control areas

3.3 Increase in Se amount in staple food for residents by applying Se fertilizer

As mentioned above, the local residents take a very simple diet which consists of wheat grain as staple food and less subsidiary food and vegetable. In addition, drinking water in this area has a very low Se content (0.02ppb－0.1ppb), so that the level of Se uptake by residents is largely determined by the Se content in wheat grain.

In order to find out whether the intake the amounts of Se by the residents from staple food increased or not after applying Se fertilizer, samples were collected from the meals of residents in some households in the demonstration, control and disease free areas (suburb of Xi'an City). All of the samples were treated by tissue homogenizer and then 50 g of each sample were taken out, put into a small pot, covered by a polythene lid, dried in vacuum till constant weight, and then digested in HNO_3/H_2O_2 under high-pressure microwave. Finally, each sample was determined for content of Se by the method of catalytic polarographic analysis.

Before applying Se fertilizer, the Se contents in staple food collected from the resident households living in the demonstration and control areas were 13 ppb and 8.7 ppb, respectively, without significant differences found. In the same time, the Se content in staple food collected from the disease-free area was 23ppb ($p<0.001$). Having estimated that each resident took 817g (DW) food per day, the Se intake of each resident in the demonstration and control area was 10.4μg and 7.0 μg, respectively, but that was 18 μg in the disease-free area, Xi'an. In December of the same year it was just the time when the residents consumed the food made from the grains that had been applied with Se fertilizer. Four months later, the Se intake level of residents in the demonstration area was increased up to 33.6 μg with the incidence of 42 ppb Se in their food. But in control area, the Se content in the food and Se intake level of residents were 6.3 ppb and 5.0μg, respectively. This means the both of the Se content of food and Se intake level of residents in the demonstration area were 7 times higher than those in control area, and double those in the disease-free area.

3.4 Increase in both the metabolic level of selenium in human body and the effect of prevention and treatment of KBD

In order to determine the nutrient metabolic level in human body, human hair measurement method base on population epidemiology was used. This method is simple, stable and reliable, and there is a good linear correlation between the Se contents in hair and blood.

Li Jiyun *et al.* indicated that the Se content in children's hair was below 110 ppb found in KBD disease areas. The background values in 12 villages in both the

demonstration and control areas are between 54.1－97.9 ppb. The average hair Se contents of children living in the demonstration and control areas were 82.3 ppb and 64.6 ppb, respectively. At three months after consuming seleniferous wheat from the demonstration area applied with Se fertilizer, it reached up to 162－274 ppb, and the average was 215.1 ppb, two times greater than the background value. Nine months after consuming the seleniferous wheat, the Se content increased to 262－330 ppb, reaching the mean value of hair Se contents in disease-free areas, while the Se content of hair in the control areas was still 70.5 ppb (Table 5). This fact showed that Se applied to soil could enter human body through the grain and increase the Se nutrient metabolic level in the body.

Table 5 Comparison of Se content in resident's food among demonstration, control and disease-free areas

Villages	Demonstration area			
	Before Se food supply May, 1987		After Se food supply May, 1988	
	Se content, ppb	Daily uptake, μg/d	Se content, ppb	Daily uptake, μg/d
Liujia	16	12.8	51	40.8
Dache	13	10.44	39	31.2
Nantouao	11	8.8	37	29.6
Mean	13	10.44	42	33.6
Villages	Control area			
	May, 1987		May, 1988	
	Se conytent, ppb	Daily uptake, μg/d	Se content, ppb	Daily uptake, μg/d
Dizhuang	8.4	6.4	6.4	5.1
Liuchou	9.0	7.2	6.1[n]	4.9
Mean	8.7	7.0	6.3	5.0
Villages	Non-disease area			
	May, 1987		May, 1988	
	Se content, ppb	Daily uptake, μg/d	Se co tent, ppb	Daily uptake, μg/d
Yangjiagou (Xi'an)	23	18.4	21	16.8

We had the children from both the demonstration and control areas examined twice with X-ray in May, 1987 and 1988, nine months after the children consuming Se wheat, the results, as compared with control area, indicated that significant prevention and treatment effectiveness have been achieved (Table 6).

Table 6 Comparison of Se content in children's hair between the demonstra

Unit: ppb

Demonstration area						Control area		
	Before consuming		After consuming		After consuming			
Villages	Se food, May, 1987		Se food, Nov., 1987		Se food, May, 1988	Villages	May, 1987	May, 1988
Liujia	83.0±48.0	(15)	211±133	(10)	330±111 (17)	Dizhuang	60.8±11.4 (11)	65.5±10.9 (20)
Wangjia	74.6±29.9	(10)	247±125	(18)	285±104 (82)	Liuchou	69.1±17.9 (15)	70.6±9.5 (20)
Zhangjia	78.2±24.1	(10)	274±118	(12)	201±141 (69)	Leicun	70.2±21.5 (14)	67.0±8.3 (15)
Dache	77.0±26.6	(10)	214±101	(12)	299±147 (64)	Dongwu	54.1±22.9 (15)	73.9±12.7 (15)
Xinzhuang	97.9±29.5	(10)	225±99	(16)	325±129 (67)	Zhangjia	68.9±20.2 (16)	75.8±12.9 (15)
Hanjia	79.2±26.2	(10)	173±103	(12)	269±88 (66)			
Nantouao	86.0±59.8	(16)	162±89	(10)	262±88 (62)			
Average	82.3		215		294	Average	64.6	70.5

The numbers of children examined with X-ray in 1987 and 1988 were approximately equal (about 500), who were 3－13 years old. In comparison of the results in 1988 with those in 1987, other indexes were lower remarkably in the demonstration area than in the control area, except a small change in control-epiphyseal end changes.

The ratio of subjects with positive results in X-ray examination was reduced by 7.19% in the demonstration area (3.04% in the control area), the ratio of subjects with changes in metaphyses part found in the demonstration area was reduced by 12.99% (only 3.77% in the control area), especially the active index, which is a comprehensive index indicating the seriousness and affecting factors of the disease factors of the disease, in 1988 was reduced by 22.21% as compared with that in 1987, while the active index in the control area was reduced only by 2.08%. It can be seen that consumption of seleniferous wheat plays an important role in the repair of the child's metaphyses part changed, and the patient's conditions have taken remarkably favorable turn.

Individual observation was made between X-ray examinations in 1987 and 1988

for the same purpose (Talbe 7).

Table 7 Results from colonial observation with X-ray examination before and after Se fertilizer application

Group	Year	No. of children observed	X-ray changes, No.	%	Metaphyses changes(++), No.	%	Metaphyses changes(++), No.	%	Epiphyseal end changes, No.	%	Active index	Severity index
Control area	1987	580	275	47.4	205	35.34	28	4.82	191	32.93	49.00	67.91
	1988	577	256	44.4	182	31.54	28	4.85	185	32.06	46.92	62.34
Demonstration area	1987	590	237	40.2	197	33.39	38	6.44	113	19.15	52.68	53.67
	1988	570	188	33.0	147	25.79	6	1.05	99	17.35	29.87	46.28

Table 8 Results from individual observation with X-ray examination before and after Se application

Group	Patient group											
	No.of cases	Recovery, No.	%	Remarkable improvement, No.	%	Improved, No.	%	Deteriorated, No.	%	No-change, No.	%
Demonstration area	173	20	11.56	22	13.29	52	30.06	0	0	77	44.51
Control area	184	0	0	1	0.54	14	7.61	25	13.59	144	78.26

Group	Health group			Number of children observed
	No.of cases	New discoveried, No.	%	
Demonstration area	300	0	0	473
Control area	264	4	1.52	448

As shown in Table 8, the number of patients (173) in the demonstration area was approximately equal to that in the control area (184), no case of illness was recovered in the control area, while in the demonstration area, 94 cases of illness were recovered, with the pathological changes in X-ray examination disappeared thoroughly in 1990, and the ratio of recovery was 54.8%. The ratio of improvement in the changes in the demonstration area reached 78.8%, i. e., much greater than the ratio

of self improvement in the control area (in contrast, the ratio of deterioration confirmed by X-ray in the control area is as high as 13.6%, while no case being deteriorated in the demonstration area). This result demonstrated that the increase in Se uptake of resident by applying Se fertilizer to soil has a good curing effect on early child KBD and metaphyses part changes.

Moreover, in 1988, we found 4 patients who were among 264 healthy children in control area in 1987, the rate of new incidence was 1.52%, while no new incidence being among 300 children who were healthy in demonstration area in 1987. This result preliminarily showed that applying Se fertilizer to soil has preventive effect on developing new incidence of KBD.

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