Polygonatum sibiricum polysaccharide affects growth performance and immune response in cyclophosphamide-induced immunosuppressed chickens

Dan Xu

Sichuan Agricultural University

Jin Zhao

sichuan agricultural university

Chonglin Ran

sichuan agricultural university

Lu Xu

sichuan agricultural university

Shaojun Ren

sichuan agricultural university

Yuanlin Huang

sichuan agricultural university

Ziting Tang

sichuan agricultural university

Juchun Lin

sichuan agricultural university

Hualin Fu

sichuan agricultural university

Wei Zhang

sichuan agricultural university

Changliang He

sichuan agricultural university

Xiaoling Zhao

sichuan agricultural university

Gang Shu (✉ dyysg2005@sicau.edu.cn)

Sichuan Agricultural University

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Abstract

Background: This study aims to identify the effects of dietary *Polygonatum sibiricum* polysaccharide (PP) on growth performance and blood parameters in immunosuppressed chickens induced by cyclophosphamide (CY). **Results:** The results showed that PP enhanced the growth performance and immune response in chickens treated with cyclophosphamide characterized by increasing relative weight of thymus, total antioxidant capacity (T-AOC), superoxidase dismutase (SOD), albumin (ALB), immunoglobulin M (IgM), and antibody titers of Newcastle disease (ND) and avian influenza (H9), and decreasing feed conversion ratio (FCR) and malondialdehyde (MDA) level. **Conclusions:** PP enhanced chickens' growth performance via improving antioxidant ability and alleviating CY-induced immunological stress.

Background

Infectious diseases and mycotoxins induce immunosuppression, a disorder of immune system in broilers, which reduces immune response to vaccines, increases morbidity and mortality of flocks [1, 2] and causes higher feed conversions and lower average daily weight gains [3, 4].

Cyclophosphamide (CY) is an alkylating agent widely used to build immunosuppression and stress animal models via intramuscular [5, 6] or intraperitoneal injection. It inhibited both humoral and cellular immunity, causing B cell damage irreversibly [5] and depressed both antibody formation and immunoglobulin synthesis [7]. Strategies to minimize immunosuppressed diseases are mainly via vaccination and stress control [2]. However, inactivated vaccine cannot produce sufficient antibody, attenuated vaccine induces reactivation and infection [8]. Therefore, new methods to control stress are in great demand.

Polygonatum sibiricum polysaccharide (PP) is a recently isolated water soluble component of rhizome polygonatum, which has medicinal efficiency in enhancing immune function [9], vital essence replenishment, dryness removal, and promotion of fluid secretion. In laboratory animals, PP has the potential as a treatment of diabetes and ocular complications, an alternative to estrogen to treat osteoporosis, a neuroprotective agent, a prevention for osteoporosis, and a remedy for inflammatory disorders [10-13]. Liu had shown that the polysaccharide from *Polygonatum sibiricum* played an important role in the protection of the Cy-treated immunosuppressed mice and could be used as a potential immunostimulant agent [14]. However, its protective effect against immunosuppression in chickens is poorly understood. Therefore, the objective of the present study was to elucidate the effects of PP on growth performance, immune and biochemical traits of immunosuppressed chickens induced by CY.

Results

Growth Performance

Growth performance indices were affected by CY and PP during the overall period. From d 21 to d 28, PP+CY group had lower DFI and FCR compared with CY group ($p < 0.05$). CY group had lower DFI and DWG from d 1 to d 35 compared with the Control ($p < 0.05$) (Table 1).

Serum Antioxidant Indices

PP group had the highest T-AOC and SOD and the lowest MDA among all groups at all time points. CY group had the lowest T-AOC and SOD, and the highest MDA at all time points, compared with other groups. PP+CY had lower MDA compared with CY group on d 14 and 21 ($p < 0.05$) (Table 2).

Blood Biochemical Indices

PP group had higher TP ($p < 0.05$) than CY and PP+CY groups on d 14. CY group had lower ALB than other groups on d 14 ($p < 0.05$). PP group had higher ALB compared with other groups on d 28 ($p < 0.05$) (Table 2).

Immune Organ Relative Weights

For spleen, PP groups were higher than CY and PP+CY groups on d 14 and 28 ($p < 0.05$). For relative weights of thymus, the Control and PP groups were higher than CY and PP+CY on d 14 ($p < 0.05$). PP+CY group was higher than CY group on d 21 ($p < 0.05$). For bursa, the Control and PP groups were higher than CY and PP+CY groups on d 14, 21, and 28 ($p < 0.05$) (Table 3).

Effect of PP on Immunoglobulin

IgG levels had no difference among four groups. For IgM, the Control was the lowest on d 14 ($p < 0.05$). PP group was higher than CY and PP+CY group on d 28 ($p < 0.05$) (Table 4).

Effect of PP on Antibody Assay

For ND, CY group was the lowest on d 14 ($p < 0.05$). PP+CY group was higher than CY at all time points ($p < 0.05$). The Control was higher than CY at all time points ($p < 0.05$). For H9, CY was lower than PP and PP+CY on d 14 ($p < 0.05$). CY group was the lowest on d 21 ($p < 0.05$). PP was the highest and CY group was the lowest compared to other treatments on d 28 ($p < 0.05$) (Table 5).

Discussion

In the present study, we built the model of immunosuppression chickens induced by CY in order to study PP's function in growth promoting and immune booster in broilers. The results indicated that the growth performance, relative weights of bursa, Spleen and thymus, antibody titers to ND and H9 of CY group decreased ($p < 0.05$) compared to the Control, which were consistent with the immunosuppressed model in other studies with bursa atrophy that showed depression of ND and HI antibody production [15].

Studies have indicated that the deficiency of immune system resulted in poor growth performance, low DFI and DWG and high FCR [16]. Consistent with this finding, CY lowered DWG and DFI from d 1 to d 35 compared to Control group in our study ($p<0.05$). However, PP group had higher DWG and DFI from d 1 to d 35 and lower FCR from d 7 to d 21 compared with PP+CY group ($p<0.05$), which indicated that PP displayed positive effect on chicken growth and promoted the feed conversion efficiency of birds. In consistent with other studies, *Astragalus* polysaccharide, and 2 g/kg polysaccharide extracts of *Lentinus edodes* inclusion increased broilers' BWG and decreased FCR [17, 18].

T-AOC is an integrate and sensitive antioxidant index that identifies oxidative status in vivo [19]. MDA is the product of lipid peroxidation and tissue damage manifestation [20]. CY caused overproduction of reactive oxygen species (ROS) [21], which is an inclusion of superoxide anion (O_2^-), hydroxyl radical ($\cdot OH$), and hydrogen peroxide (H_2O_2). ROS cause damage of nuclear acids, proteins and lipids [22]. SOD catalyzes O_2^- to O_2 , produces H_2O_2 , and maintains body within a low superoxide concentration. According to the reduction in T-AOC on d 14 and d 21, and SOD on d 14 in PP+CY group compared with PP group, administration of CY in chickens induced oxidative stress in the present study. PP+CY group decreased MDA on d 14 and 21 ($p<0.05$) compared to CY group, indicating PP restored oxidative effect of CY, which is in consistent with former studies. Polysaccharides extracted from *Platycodon grandiflorum* [23], *Rhododendron aganniphum* [24], *Pleurotus eryngii* [25], and *Glycine soja* [26] have great antioxidant capacity in elevating SOD and decreasing MDA concentration in pheochromocytoma cells and showing good radical scavenging activity for 1,1-diphenyl-2-picrylhydrazyl radicals in vitro.

Serum biochemical parameters reflect nutritional status in broilers [27]. Serum total protein contains albumin and globulin, reflecting protein anabolism, immune status [28], and hepatic protein metabolic status [29]. Elevation of ALB reflects increased antioxidant, immunomodulatory, and detoxification functions [30]. The TP concentration on d 14 in PP group was higher compared with PP+CY group ($p<0.05$) and PP group had the highest ALB, compared with other groups on d 28 ($p<0.05$), which was in consistent with increased antioxidant status and improved growth performance in PP groups in this study. The mechanism of PP on protein synthesis or catabolism to improve protein anabolism needs to be further investigated.

Immune responses of broilers were evaluated by assessing immune organ relative weights, concentrations of IgG and IgM, and antibody titers of ND and H9 in the current study. CY intramuscular administration decreased relative weights of thymus on d 14 and 21, bursa on d 14, 21, and 28 ($p<0.05$) compared to the Control, which are in line with previous studies [31]. However, PP+CY increased relative weights of thymus on d 21 compared to CY group, PP group had higher relative weights of spleen on d 14 and 28 and bursa during the whole period compared with PP+CY group, indicating that PP could resist the immunosuppression on development of immune organs induced by CY. Previous studies proved the positive effect of polysaccharides' immune on spleen [17, 32] and relative weights of thymus and bursa [1].

IgG is the principle antibody in immune response for anti-infection, and IgM arises in initial stage of anti-infection immune response [2]. The levels of IgG and IgM are indicators of antiviral response capacity [33]. In the current study, PP group had higher IgM on d 28 than PP+CY group and on d 21 than Control group ($p < 0.05$), which indicated that PP enhances anti-infection and anti-virus ability of chickens. Similar results have been reported in previous studies. Polysaccharides extracted from *Astragalus membranaceu*, *Astragalus*, sulfated epimedium, *Tremella fuciformis*, and *Lentinus edodes* increased production of IgG and IgM in chickens [34].

Antibody titers manifest humoral immune response mediated by B lymphocyte. The results showed PP+CY had higher antibody titer of ND and H9 compared with CY group during the whole period ($p < 0.05$). This indicates that PP resisted the decreased antibody formation and immunoglobulin synthesis induced by CY, which confirms immunomodulatory effects of polysaccharides. Polysaccharide from Epimedium polysaccharide-propolis flavone, *Astragalus*, sulfated epimedium, and achyranthan had higher anti-ND titers ($p < 0.05$) than the control in chickens [1, 2].

Conclusions

PP at a dose of 800 mg/kg in diets enhanced immune response in a local meat-type chicken induced by CY and improved its growth performance and antioxidant levels. PP may be a potential feed additive to mount a successful defense against immunodeficiency diseases in chickens and other animals as well.

Methods

Reagents and Viruses

Polygonatum sibiricum polysaccharide (content>99%) was purchased from Zhejiang Shengshi Biotechnology Co. Ltd (Zhejiang, P. R. China).

Cyclophosphamide with the purity of 83.84% was purchased from Jiangsu Hengrui Medicine Co. Ltd (Jiangsu, P. R. China). The chicken-specific ELISA assay kits for total antioxidant capacity (T-AOC), malondialdehyde (MDA), superoxidase dismutase (SOD), total protein (TP), albumin (ALB), Immunoglobulin G (IgG), and Immunoglobulin M (IgM) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, P. R. China). Avian influenza (H9 subtype) and Newcastle disease (ND) virus were purchased from Wens Dahuanong Biotechnology Co. Ltd (Guangdong, P. R. China).

Animals Husbandry and Immunosuppressed Model Design

The objective of the present study was to elucidate the effects of PP on growth performance, immune and biochemical traits of immunosuppressed chickens induced by CY.

Four hundred and eighty one-day-old Erlang mountainous chickens (line SD02) were provided by Poultry Breeding Farm of Sichuan Agricultural University and had free access to water and basal diets which met

the NRC requirements [35]. And the formulation and nutrient levels were according to the National Research Council and shown in Table 6. Chickens were raised in cages in a ventilated house maintained at 50% relative humidity. The photoperiod was 24 h for the first 14 days and 20 h for the subsequent days. Temperature was 34°C initially and decreased by 2°C per week to 24°C, which was maintained till the end of the experiment.

Polygonatum sibiricum polysaccharide at 0 or 800 mg/kg of the diet and CY at 0 or 80 mg/kg were used in a 2 × 2 factorial designed with 4 randomized complete blocks consisting of initial BW of 37.83±3.45g chickens. They were allocated into 4 groups: 1) non CY-induced control (CON; birds fed basal diets and treated with sterile saline); 2) CY-induced control (CY; birds fed basal diets and treated with 80 mg/kg CY); 3) non CY-induced group + PP (PP; birds fed basal diets supplemented with 800 mg/kg PP and treated with sterile saline); 4) CY-induced group + PP (PP + CY; birds fed basal diets supplemented with 800 mg/kg PP and treated 80 mg/kg CY). There were four replicates (30 birds per replicate) randomly assigned into each of 4 diet groups. CY and sterile saline were delivered through intramuscular injection on d 7, 8, and 9, respectively with a dose of 80 mg/kg of body weight according to the previous report. Our previous study showed basal diet supplemented with 800 mg/kg PP had better effect than 200 mg/kg, 400 mg/kg, and 1200 mg/kg on increasing daily weight gain (DWG), daily feed intake (DFI), relative weights of bursa, spleen, thymus and SOD, TP, ALB, T-AOC, and decreasing feed conversion ratio (FCR) and MDA.

Growth Performance

Feed intake was monitored for each cage every week after a 12 h fast. Average DWG and DFI were weighed on d 1, 7, 14, 21, 28, and 35. FCR was calculated as DWG/DFI. These growth performance parameters were adjusted by mortality. On d 14, 21, and 28, five chickens close to mean body weight were euthanized by injecting pentobarbital sodium intravenously at a dose of 100 mg/kg without any fear and agony, as pentobarbital sodium can inhabit the ascending reticular activating system of brainstem, leading to the death of animals. And tissues were collected and stored at -80°C for further analysis.

Biochemical Parameter Assay

On d 14, 21, and 28, 1mL of blood was obtained from the brachial vein and centrifuged at 2,000 x g for 5min. Supernatant liquid of blood serum was collected and stored at -20°C for further analysis. The contents of T-AOC, SOD, MDA, TP, and ALB were measured using ELISA kits according to manufacturer's instructions.

Immune Organ Relative Weight

Spleen, thymus, and bursa were collected and weighed on d 14, 21, and 28. Immune organ relative weights were expressed as the ratio of organ weight to body weight (mg/g).

Serum Immunoglobulin and Antibody Assays

Blood samples of 2.0 mL were extracted from the main brachial vein of chicken and clotted at 37 °C for 2 h. To collect serum, the blood samples were centrifuged at 2,000 x g for 3 min. IgG and IgM were measured using ELISA kits according to manufacturer's instructions. The serum antibody titers of subtype avian influenza viruses H9 (H9) and Newcastle disease virus (ND) were assessed by hemagglutination assay (HA) and hemagglutination inhibition (HI) assay. The concentrations of titer were recorded as log₂ value. The reciprocal of the highest dilution was the complete agglutination.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) via SPSS 19.0. software. Duncan's multiple range test was used to determine the differences among groups. Significance was assigned at $p < 0.05$.

Abbreviations

PP: *Polygonatum sibiricum* polysaccharide, CY : cyclophosphamide, T-AOC: total antioxidant capacity, SOD: superoxidase dismutase, ALB: albumin, IgM: immunoglobulin M, IgG: Immunoglobulin G, ND: antibody titers of Newcastle disease, H9: avian influenza, FCR: decreasing feed conversion ratio, MDA: malondialdehyde, TP: total protein, CON: control, DWG : daily weight gain, DFI : daily feed intake , HI: hemagglutination inhibition, HA: hemagglutination

Declarations

Ethics approval and consent to participate

All procedures for raising and slaughtering chickens were performed according to the Guide for the Care and Use of Laboratory Animals of the Chinese Association for Laboratory Animal Sciences and approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University with an issued number DYY-S20130538.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SG, ZJ and ZXI conceived and designed the experiments. XD, XL, RSj and ZW performed the experiments. SG, XD, RCI and HCI finished the Draft of the manuscript. HYI, TZt, LJC and FHI analyzed the data. All authors had read and approved the manuscript.

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Tables

Table 1 Effect of *Polygonatum sibiricum* polysaccharide on growth performance in chickens (Mean $\pm$ SE)

Item	Age (d)	0 g of PP		800 mg/kg PP		SEM	<i>p</i> -value
		Saline	CY	Saline	CY		
DFI	1-7	6.40	6.34	6.37	6.32	0.035	0.427
	7-14	17.61 ^a	15.70 ^{bc}	16.52 ^b	15.45 ^c	0.250	0.000
	14-21	29.42 ^a	27.17 ^b	28.86 ^a	27.04 ^b	0.438	0.002
	21-28	43.71 ^a	42.36 ^a	42.04 ^a	40.25 ^b	0.466	0.011
	28-35	49.85 ^a	46.55 ^a	49.21 ^a	42.75 ^b	1.054	0.002
	1-35	29.19 ^a	27.69 ^{bc}	28.31 ^{ab}	26.36 ^c	0.357	0.008
DWG	1-7	2.48	2.44	2.67	2.54	0.083	0.299
	7-14	9.47 ^a	6.67 ^b	9.14 ^a	6.87 ^b	0.203	0.000
	14-21	17.40 ^a	13.81 ^b	17.02 ^a	13.49 ^b	0.332	0.000
	21-28	20.35	20.18	21.36	20.83	0.271	0.061
	28-35	23.53 ^a	20.51 ^b	22.64 ^a	19.58 ^b	0.630	0.002
	1-35	14.71 ^a	13.05 ^b	14.62 ^a	12.65 ^b	0.187	0.000
FGR	1-7	2.59	2.62	2.40	2.49	0.086	0.303
	7-14	1.87 ^b	2.36 ^a	1.83 ^b	2.26 ^a	0.064	0.000
	14-21	1.69 ^b	1.98 ^a	1.72 ^b	2.02 ^a	0.055	0.001
	21-28	2.12 ^a	2.10 ^a	1.97 ^b	1.94 ^b	0.030	0.030
	28-35	2.12	2.29	2.18	2.19	0.072	0.483
	1-35	1.99	2.12	1.94	2.09	0.038	0.057

DFI: daily feed intake (g); DWG: daily weight gain (g); FCR: feed conversion ratio; SEM: pooled standard error of the means (n=4).

a-c Means in a row without the same superscripts differ significantly ($p < 0.05$)

Table 2 Effect of dietary PP on serum biochemical indices of chickens (Mean $\pm$ SE)

Item	0 g PP		800 mg/kg PP		SEM	<i>p</i> -value
	Saline	CY	Saline	CY		
T-AOC (U/mL)						
d 14	9.81 ^b	6.71 ^c	11.41 ^a	7.53 ^c	0.448	0.000
d 21	8.36 ^b	6.10 ^c	11.32 ^a	7.32 ^{bc}	0.593	0.000
d 28	9.83 ^{ab}	7.51 ^c	11.67 ^a	9.47 ^{bc}	0.648	0.004
SOD (U/mL)						
d 14	173.24 ^a	113.02 ^b	190.34 ^a	124.01 ^b	10.95	0.001
d 21	145.93	123.45	167.77	130.91	11.62	0.103
d 28	307.07	291.13	315.39	302.14	8.38	0.262
MDA (mmol/mL)						
d 14	1.65 ^b	2.25 ^a	1.43 ^b	1.69 ^b	0.134	0.013
d 21	0.79 ^c	2.19 ^a	0.60 ^c	1.49 ^b	0.111	0.000
d 28	1.19	2.06	1.06	1.56	0.099	0.001
TP (g/L)						
d 14	37.81 ^{ab}	33.57 ^b	42.33 ^a	34.82 ^b	1.426	0.028
d 21	30.27	35.58	35.26	33.30	1.364	0.253
d 28	31.22	31.96	31.80	29.21	2.101	0.878
ALB (g/L)						
d 14	16.00 ^a	13.12 ^b	15.76 ^a	15.89 ^a	0.623	0.024
d 21	16.64	17.38	15.57	16.47	1.147	0.766
d 28	12.34 ^b	14.51 ^b	17.96 ^a	11.85 ^b	1.007	0.002

T-AOC: total antioxidant capacity (U/mL); MDA: malondialdehyde (mmol/L); SOD: superoxidase dismutase (U/mL); TP: total protein (g/L); ALB: albumin (g/L); SEM: pooled standard error of the means (n=4).

^{a-c} Means in a row without the same superscripts differ significantly ($p < 0.05$).

Table 3 Effect of PP on relative weights of spleen, thymus, and bursa of chickens (Mean $\pm$ SE)

Relative weight (% of body weight)	0 g PP		800 mg/kg PP		SEM	<i>p</i> -value
	Saline	CY	Saline	CY		
Spleen						
d 14	1.49 ^{ab}	1.02 ^b	1.91 ^a	1.02 ^b	0.153	0.001
d 21	1.30	1.33	1.40	1.24	0.090	0.659
d 28	1.84 ^{ab}	1.48 ^b	2.20 ^a	1.52 ^b	0.132	0.003
Thymus						
d 14	4.38 ^a	2.03 ^b	4.56 ^a	2.04 ^b	0.365	0.000
d 21	5.76 ^a	4.21 ^b	5.94 ^a	6.40 ^a	0.416	0.003
d 28	6.59	6.37	7.08	6.69	0.374	0.601
Bursa						
d 14	2.39 ^a	1.10 ^b	2.70 ^a	1.28 ^b	0.209	0.000
d 21	4.14 ^a	1.09 ^b	3.92 ^a	1.32 ^b	0.202	0.000
d 28	4.94 ^a	1.28 ^b	5.02 ^a	1.68 ^b	0.224	0.000

SEM: pooled standard error of the means (n=4).

ab Means in a row without the same superscripts differ significantly ($p < 0.05$).

Table 4 Effect of PP on serum globulin of chickens (Mean $\pm$ SE)

Item	0 g PP		800 mg/kg PP		SEM	<i>p</i> -value
	Saline	CY	Saline	CY		
IgG (g/L)						
d 14	3.22	3.66	3.34	3.66	0.211	0.401
d 21	2.31	2.40	2.93	2.45	0.152	0.088
d 28	2.21	1.86	2.72	2.12	0.167	0.073
IgM (g/L)						
d 14	1.42 ^b	1.97 ^a	2.08 ^a	1.94 ^a	0.154	0.039
d 21	1.51	1.83	1.60	1.65	0.106	0.248
d 28	1.00 ^{ab}	1.76 ^b	2.37 ^a	1.82 ^b	0.243	0.040

IgG: immunoglobulin G (g/L); IgM: immunoglobulin M (g/L); SEM: pooled standard error of the means (n = 4).

^{ab} Means in a row without the same superscripts differ significantly ($p < 0.05$).

Table 5 Effect of PP on serum antibody titer[#] of chickens (Mean $\pm$ SE)

Item	0 g PP		800 mg/kg PP		SEM	<i>p</i> -value
	Saline	CY	Saline	CY		
ND						
d 14	3.8 ^b	3.6 ^c	4.8 ^b	5.0 ^a	0.332	0.018
d 21	3.0 ^b	2.8 ^b	4.0 ^{ab}	4.2 ^a	0.284	0.009
d 28	6.2 ^a	2.5 ^c	7.2 ^a	4.6 ^b	0.319	0.000
H9						
d 14	4.0 ^{ab}	3.6 ^b	4.8 ^a	4.8 ^a	0.245	0.006
d 21	3.4 ^a	2.0 ^b	3.8 ^a	3.2 ^a	0.332	0.009
d 28	5.4 ^{ab}	3.0 ^c	6.0 ^a	4.8 ^b	0.329	0.000

H9: avian influenza H9 subtype; ND: Newcastle disease; SEM: pooled standard error of the means (n = 4).

^{a-c} Means in a row without the same superscripts differ significantly ($p < 0.05$).

[#] The antibody titers were shown as log₂ value of the reciprocal of the highest dilution.

Table 6 Composition and nutrient levels of the basal diet (air-dry basis) (g/kg)

Ingredients	1-21 d	22-35 d	Nutrient levels ^b	1-21 d	22-35 d
Corn	565.0	621.4	ME (MJ/kg)	12.13	12.63
Soybean meal	365.7	306.8	CP	205.0	182.0
Admixture oil	28.0	32.9	Lys	11.50	10.10
Limestone	13.1	14.1	Met + Cys	8.10	7.30
CaHPO ₄	12.6	10.9	Ca	9.00	8.50
NaCl	12.6	10.9	TP	5.70	5.30
Premix ^a	3.00	3.00			
50% choline (50%)	10	10			
Lys	0.20	0.40			
Met	2.4	2.2			

^a The premix provided the following per kg of the diet: Cu 8 mg; Fe 90 mg; Zn 50 mg; Mn 80 mg; I 0.30 mg; Se 0.15 mg; vitamin A(Retinol) 10000 IU; vitamin D₃(Cholecalciferol) 2,100 IU; vitamin E(tocopherol) 14.97 IU; vitamin K₃(menaphthone) 0.6 mg; vitamin B₁(thiamine) 2.0 mg; vitamin B₂(Riboflavin) 4.0 mg; vitamin B₁₂(cyanocobalamin) 0.01 mg; nicotinic acid 30.0 mg; folic acid 0.6 mg; biotin 0.15 mg; D-pantothenic acid 11 mg; phytase 700 U.

^b Nutrient levels are calculated value.

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