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SYMPOSIA AND ORAL PRESENTATIONS

Animal Health: Probiotics, Performance and Antioxidants

1073 Thiamine status of feedlot cattle fed high concentrate diet. T. Karapinar*, M. Dabak, and O. Kizil, *University of Firat, Faculty of Veterinary Medicine*, *Elazig, Turkey*.

Thiamine deficiency causes a decrease in transketolase activity and an increase in thiamine pyrophosphate (TPP) effect on the erythrocytes. The objective of this experiment was to determine if ruminal acidosis alters erythrocytic transketolase enzyme activity as an indicator of thiamine level in feedlot cattle. A total of 65 feedlot cattle (1–2 years old) were fed either high concentrate diet (HCD, n = 50) or low concentrate diet (LCD, n = 15). The HCD group was fed a mixture of 75% cracked barley, 8% bran, 7% cotton seed meal and 10% straw for at least the last 3 mo, whereas the LCD group was fed a mixture of 30% cracked barley, 10% bran, 10% sugar beet pulp, 10% cotton seed meal and 40% straw. Rumen fluid samples of all cattle were obtained by rumenocentesis to determine ruminal pH. After ruminal samples were obtained, blood samples were collected from a jugular vein for erythrocytic transketolase enzyme activity using the Clausen's colorimetric method. Mean pH values of ruminal fluid samples in the HCD and LCD groups were 5.3 and 6.1, respectively. The mean TPP effect % in the HCD group (47.16 ± 3.17) was significantly higher than in the LCD group (19.53) \pm 2.51) (P < 0.01). Ruminants with functional rumen are considered to have no specific dietary thiamine requirement due to extensive thiamine synthesis by rumen microbes. Thiamine deficiency in both chronic ruminal acidosis and acute ruminal lactic acidosis may occur because of inadequate synthesis of thiamine, bacterial production of thiaminase in the acidotic ruminal fluid. Furthermore, a decrease in ruminal pH may result in the release of bacterial thiaminases. The present study demonstrated that thiamine deficiency could occur in ruminants fed under intensive fattening regimens.

Key Words: polioencephalomalacia, transketolase, vitamin B1

1074 The effect of five herbal extracts on performance, carcass characteristics and immune system in broilers. M. Alempour¹, S. Rahimi*¹, M. A. Karimi Torshizi¹, and A. Rahimi², ¹Tarbiat Modares University, Tehran, Iran, ²Islamic Azad University, Tehran, Iran.

The purpose of this study was to evaluate the effect of 5 herbal extracts and Virginiamycin antibiotic on growth performance, carcass characteristics and immune system in broilers. A total of 720 1-d-old broiler chicks (ROSS 308) were assigned to 9 treatments with 4 replicates of 20 birds per pen as follows: the basal diet, and basal diet supplemented with 15 ppm Virginiamycin, 1% aqueous extract of garden thyme (*Thymus vulgaris*), garlic (*Allium sativum*), thyme (*Thymus kotschyanus*), sage (*Salvia officinalis*), peppermint (*Mentha piperita*), a blend of garden thyme and peppermint and blend of thyme and sage. Performance and feed conversion were calculated at 14, 28 and 42 d of age. At end of the experiment 2 birds from each replicate (8 birds per treatment) were randomly selected to evaluate the carcass characteristics, relative weight of lymphoid organs and fat pad. Greatest and least body weight were belonged to virginiamycin and sage, (P < 0.05) respectively. Lowest and highest feed conversion ratio were related to virginiamycin and

control groups (P < 0.05) respectively. There was no difference in carcass characteristics, fat pad and digestive organs weight. Relative weights of lymphoid organs (spleen and bursa of Fabricius) as 2 immune indices were unaffected by the treatments. According to results of this experiment, the herbal extracts did not show significant difference in performance and lymphoid organs weight in broilers.

Key Words: herbal extract, performance, immune system

1075 Comparison the effect of five herbal extracts and virginiamycin on serum lipids and immune system in broilers. M. Alempour¹, S. Rahimi¹, M. A. Karimi Torshizi¹, and A. Rahimi*², ¹Tarbiat Modares University, Tehran, Tehran, Iran, ²Islamic Azad University, Tehran, Tehran, Iran.

The objective of this study was to investigate the effect of 5 herbal extracts and virginiamycin on blood metabolites in broilers. A total of 720 1-d-old (Ross 308) male broiler chicks were assigned to 9 treatments with 4 replicates of 20 birds per pen as follows: the basal diet (control) and basal diet supplemented with 15 ppm virginiamycin, 1% aqueous extract of garden thyme (*Thymus vulgaris*), garlic (*Allivum sativum*), common thyme (Thymus kotschvanus), common sage (Salvia officinalis), peppermint (Mentha piperita), a blend of garden thyme and peppermint and blend of common thyme and common sage with the same dose in drinking water as a completely randomized design.. At the end of the experiment 2 birds from each replicate were randomly selected to measure total cholesterol (TC), LDL cholesterol, triglycerides (TG) and HDL cholesterol levels. Garlic (Allivum sativum) significantly reduced the levels of TC, LDL, TG (97.89, 21.49 and 37.45 mg/dl respectively) and significantly increased the level of HDL (68.91 mg/dl) (P < 0.05). According to results of this experiment the herbal extracts could have beneficial effect on blood lipids in comparison with the control and antibiotic fed birds. Garlic was the most effective treatment in this trial.

Key Words: broiler, blood factors, herbal extract

1076 Characterization of a yeast autolysate in vitro and effect on piglet performance in vivo. A. Ganner*¹, S. Masching², M. Pelz¹, and G. Schatzmayr¹, ¹Biomin Research Center, Tulln, Austria, ²Biomin Holding GmbH, Herzogenburg, Austria.

Dietary yeast derivatives have been proposed to improve piglet health by preventing infectious diseases, by modulating the immune system and by controlling pathogenic bacteria such as *E.coli* and *Salmonella*. Aim of the study was an in vitro characterization of a yeast autolysate and its effect on piglet performance. As an in vitro model the murine macrophage cell line J774A.1 was used. Cells were incubated for 48 hours with LPS of *E. coli* 0127:B8 and the autolysate. TNF-alpha, IL-12, TGF-beta and IL-10 were determined in the supernatant of the cultures with ELISA. Additionally the autolysate was examined for its capacity to bind *E. coli* F4 with a quantitative microplate-based assay by measuring the optical density as growth parameter of adhering bacteria. Subsequently, a feeding trial was conducted to evaluate the efficacy of the autolysate on performance

of weaning piglets in a 56 days study. 40 piglets were divided into 2 groups with 2 replicates: control group A, group B (0.1%). Statistical analyses were performed with t-test, SPSS 18. TGF-beta was enhanced up to 100% in comparison to the control (LPS of E. coli); no induction of IL-10. IL-12 was inhibited 60 to 80%; no effect on TNF-alpha could be observed. In the microbiological assay E. coli F4 adhered with 10⁶ CFU/mg to the autolysate. In the course of the feeding trial a positive influence could be observed by the autolysate. Weight on day 56 (43.13 kg) and daily weight gain (dwg 604g) were improved in comparison to the control (40.92 kg weight day 56, P = 0.048; 505g dwg, P = 0.025). Feed consumption was increased ($P \ge 0.05$), FCR (kg/kg) of the trial group was 1.74, of the control 1.71 ($P \ge 0.05$). Pathogen binding and modulation of the immune system, as shown in vitro, might have been reasons for the increase in performance of piglets in the feeding trial. In vitro and in vivo results indicate that this particular yeast autolysate is a health and performance improving agent.

Key Words: yeast autolysate, cytokines, piglet performance

1077 Effect of several feed additives on growth performance and microbial load in Escherichia coli challenged broilers. A. R. Valipouri¹, S. Rahimi*¹, T. Zahraei Salehi², and A. Rahimi³, ¹Tarbiat Modares University, Tehran, Iran, ²University of Tehran, Tehran, Iran, ³Islamic Azad University, Tehran, Iran.

The objective of this study was to compare the effects of different feed additives on performance and microbial load in E. coli challenged broiler chickens. A total of 528 d-old Ross 308 male broilers were used to study the effect of antibiotic, probiotic, prebiotic and organic acid on performance, cecal coliform load, immune system and internal organs weight. The birds were placed into 6 groups with 4 replicates and 22 birds per pen. Six dietary treatments include: 1) negative control as basal diet without any antibiotic growth promoter and coccidiostat (Ctl-); 2) Diet 1+ 0.9 g/kg of feed Primalac (Prim); 3) Diet 1+0.1 g/kg of feed Bactocell (Bact); 4) Diet 1+15ppm virginiamycin (VM); 5) Diet 1+2 g/kg of feed Fermacto (Ferm); and 6) Diet 1+2 g/kg of feed Formycin (OA). At d 7 all birds were orally gavaged with a 0.5 mL of 10⁷ CFU of mixed culture of pathogenic E. coli (O2K12 and O78K80) verified for presence of genes including stx1, stx2, eaeA and hlyA. Eight birds from each group were killed for detection of challenged bacteria in liver, spleen and cecum content at d 14, 28 and 42. Overall weight gain (P < 0.01) and feed conversion (P < 0.05) were significantly improved in VM (2460.00 and 1.64) and Prim (2337.25 and 1.70) groups compared with control group (2280.50 and 1.82). The challenged serotypes were recovered only from Ctl- (46%) and VM (25%) fed groups. Feed supplementation with probiotic, prebiotic or organic acid, significantly decreased coliform population at all intervals in comparison with Ctland antibiotic treatments (P < 0.05). Feed additives supplementation had no influence on the internal organs weight at all intervals.

Key Words: alternative, antibiotic, Escherichia coli

1078 Improvement of microbial flora of broilers digestive system by medicinal plants supplementation. A. Niknam, S. Rahimi*, J. Azimi, K. Seifi, M. Hoseinzade, and M. Moradi Nejad, *Tarbiat Modares University, Tehran, Iran.*

The objective of this study was to compare the effects of dietary administration of medicinal plants, with antibiotics and probiotics on microbial flora of digestive tract in broilers. A total of 210, day old male broiler chicks (Arbor Acres Plus) were randomly allocated into 7 treatments. The treatments were as follow: control; dry peppermint (*Mentha piperita*); thyme (*Thymus vulgaris*); basil (*Ocimum basilicum*) leaves; or garlic (*Allium sativum*) bulb (15kg/ton feed); Virginiamycin (150g/ton

feed); and Primalac (1kg/ton feed). At 42 d of experiment 9 birds from each treatment were randomly selected and sacrificed. Samples were collected from crop and ileocecal section of intestine and diluted in phosphate buffer saline (PBS). Then, samples were plated onto Mann Rogosa Sharpe (MRS), MacConkey and Plate-count agar to enumerate lactobacilli, coliforms and total aerobic bacteria, respectively. Crop contents of garlic and peppermint treatments contained the highest and lowest number of lactobacillus bacteria (5.88 vs. 3.68 Log10 cfu/g), respectively (P < 0.05). There was no significant difference in number of coliforms in crop. The maximum and minimum number of total aerobic bacteria observed for control and basil treatments (5.69 vs. 3.93 Log10 cfu/g), respectively (P < 0.05). Supplementation of diet with garlic increased number of lactobacillus bacteria in ileum (8.76 Log10 cfu/g) compared with other treatments (P < 0.05). Primalac increased both coliforms and total aerobic bacteria in ileum higher than other treatments (P < 0.05), while lowest number of these bacteria belongs to basil and control treatments (9.34 and 8.96 vs. 8.24 and 8.08 Log10 cfu/g), respectively. Microbial changes in crop and ileum of chicken demonstrate that administration of medicinal plants can increase number of lactobacillus and improve bacterial balance in gut.

Key Words: medicinal plants, digestive system, broilers

1079 Peripartal intravaginal probiotics lowered uterine infections and improved reproductive performance of Holstein dairy cows. B. N. Ametaj*1, Q. Zebeli¹, S. Iqbal¹, M. Gänzle¹, Y. Wang¹, D. J. Ambrose², and S. M. Dunn¹, ¹University of Alberta, Edmonton, Alberta, Canada, ²Alberta Agriculture and Rural Development, Edmonton, Alberta, Canada.

Uterine infections affect 1 in 2 dairy cows after parturition lowering their reproductive performance and increasing culling rates. The objective was to investigate the prophylactic effect of probiotic bacteria on postpartum metritis and the overall reproductive performance of dairy cows. Eighty pregnant multiparous and primiparous dairy cows 2 wk before the expected day of calving were assigned to one of 2 groups receiving: 1) 1 mL of carrier only (reconstituted skim milk), or 2) 1 mL of probiotic bacteria in reconstituted skim milk at 10 10 to 10 12 cfu/ treatment. Intravaginal infusions were performed once during wk -2, -1, +1, +2, +3, and +4 relative to parturition with probiotic bacteria isolated from vaginal tracts of healthy cows, Lactobacillus sakei FUA 3089, Pediococcus acidilactici FUA 3140, and P. acidilactici FUA 3138. All cows were observed for reproductive performance and reproductive disease until next pregnancy. Probiotic treatment lowered the incidence of uterine infections in both multiparous (17 vs 48%; P < 0.01) and primiparous (16 vs 58%; P < 0.03) cows. The interval from calving to conception tended to be shorter (93 vs 145 d; P < 0.10) in treated cows, and pregnancy rate at first insemination in multiparous cows tended to be higher (P < 0.10). Both multiparous and primiparous cows receiving probiotics had lower incidence of purulent (P < 0.008 and P < 0.02) and foul-smelling (P < 0.06 and P < 0.05) discharges on +3 wk. Moreover, multiparous cows had lower rate of uterine horn fluctuations (P < 0.05), smaller cervix size on wk +3 (P < 0.001) and +5 (P < 0.06), and lower uterine horn asymmetry on wk +3 (P < 0.01) and +5 (P < 0.01). There was a tendency for probiotic treatment to reduce early calving (P <0.11), the number of medications per cow (P < 0.09), and the number of cows in the clean-up program (P < 0.06). Further research is warranted to understand the beneficial effects of intravaginal probiotics on reproductive performance of dairy cows.

Key Words: dairy cow, probiotics, uterine infections

1080 Changes in ruminal-rectal temperature relationship associated with consumption of endophyte infected tall fescue. B. Scharf*, J. S. Johnson, H. L. Vellios, R. L. Weaber, and D. E. Spiers, *University of Missouri*, *Columbia*.

Little is known about changes in ruminal temperature after consumption of endopyte infected tall fescue. Twenty-four Angus steers (318 \pm 8 Kg BW) were housed in the Brody Environmental Center (University of Missouri), and randomly assigned to a diet with either endophyte infected tall fescue seed (E+; 40µg ergovaline/kg/d) or endophyte free seed (E-; 0µg ergovaline/kg/d). Animals were housed for 7d at an ambient temperature (T_a) of 21°C (TN) before heat stress (HS), which consisted of daily cyclic T_a (26°C night: 36°C day) for 7d. A telemetric, temperature transmitter (SmartStock, Pawnee, OK) was placed into the rumen (T_{rum}) of each animal before the study. Rectal temperature (T_{re}) and respiration rate (RR) were measured 6 times daily. At TN, no differences were found in RR, $T_{\rm re}$, or $T_{\rm rum}$ between E+ and E- animals (P= 0.11). T_{rum} - T_{re} difference was also not significant across treatments, with T_{rum} maintained ~1.1°C higher than T_{re} . Both groups increased RR during HS (P < 0.001), with E+ steers maintaining the higher rate (89.8 vs 76.7 \pm 3.2bpm; P < 0.01). Similarly, T_{re} increased for both groups during HS (P < 0.01), with E+ animals showing the greatest increase $(0.9 \text{ vs } 0.6 \pm 0.1 ^{\circ}\text{C}; P < 0.01)$. T_{rum} also increased with HS. However, T_{re} showed a much greater rise than T_{rum} (1.0 vs 0.2 ± 0.2 °C). T_{rum} - T_{re} difference decreased from TN to HS (1.1 vs 0.6 ± 0.1 °C), with large differences between treatments. E+ steers showed a large increase in T_{re} but only a minimal change in T_{rum} during HS for a small T_{rum} - T_{re} difference (0.12 \pm 0.1°C). E- animals showed a smaller increase in T_{re} and a similar increase in T_{rum} resulting in a significantly higher T_{rum} - T_{re} value (0.67 \pm 0.1°C) than for E+ animals (P < 0.05). Consumption of endophyte infected tall fescue seed caused a considerable increase in rectal temperature. Unexpectedly, ruminal temperature showed only a minimal increase which is likely due to a decline in feed intake and an associated reduction in heat production.

Key Words: cattle, heat stress, transmitters

1081 Effect of dietary antioxidants and prepartum cooling on oxidative status and neutrophil function of periparturient Holstein cows during summer in Florida. D. Wang*, J. H. Shin, M. Garcia, J. E. P. Santos, and C. R. Staples, *University of Florida, Gainesville.*

The objective of this study was to evaluate the effect of supplementation with 0 or 250 mg of Agrado Plus per kg of dietary DM (Novus International; St. Louis, MO) on oxidative status and neutrophil function of periparturient Holstein cows managed under cooled (C; shade, fans, and sprinklers) or noncooled (NC; shade alone) conditions prepartum. Primiparous (n = 22) and multiparous (n = 13) pregnant Holstein cows were assigned randomly to one of 4 treatments arranged in a 2 × 2 factorial design at 35 d before calculated calving date. Upon calving, all cows were housed in a cooled free-stall barn and remained on assigned diets. Blood was collected at -15, 1, 8, 15, and 29 DIM for oxidative markers. Phagocytosis and oxidative burst of neutrophils were measured in whole blood collected at -15, 0, 7, and 14 DIM. Rectal temperature of cooled cows was lower prepartum (39.2 vs. 39.7C). Mean erythrocyte glutathione peroxidase activity (GPx) corrected for packed cell volume were increased for NC vs. C multiparous cows fed the control diet (8,854 vs. 12,247 nmol/min/mL) but feeding antioxidants reversed this pattern (10,720 vs. 8697 nmol/min/mL); GPx in primiparous cows was not affected by treatments (diet by cooling by parity interaction, P < 0.05). Mean plasma concentrations of thiobarbituric acid reactive substances were reduced in NC vs. C cows fed the control diet (1.73 vs. 2.33 nmol/mL) but were unchanged by cooling cows fed antioxidants (1.83 vs. 1.78 nmol/mL). Mean concentrations of superoxide dismutase activity in erythrocytes were not affected by treatments. Cooled cows had fewer lymphocytes (3455 vs. 5411/uL). Mean phagocytosis and mean florescence intensity by neutrophils was increased by cooling multiparous cows (77.5 vs. 72.0% and 37.3 vs. 33.8, respectively) but was decreased by cooling primiparous cows (74.3 vs. 81.0% and 55.4 vs. 68.7, respectively). Cooling prepartum and feeding antioxidants influenced oxidative status and neutrophil function.

Key Words: antioxidant, immunity

1082 Isolation, characterization and antioxidant activity of an exopolysaccharide produced by *Enterobacter cloacae* **Z0206.** M. L. Jin*¹, Y. M. Wang¹, Z. Q. Lu¹, M. Huang¹, C. L. Xu², and Y. Z. Wang¹, ¹Zhejiang University, Hangzhou, China, ²Northwestern Polytechnical University, Xi'An, China.

A water-soluble extracellular polysaccharide (EPS-1) was isolated from the submerged culture broth of Enterobacter cloacae Z0206 through fermentation, ethanol precipitation, anion-exchange and gel-permeation chromatography. Its structural characteristics were investigated by chemical analysis, high performance liquid chromatography (HPLC) and Fourier transform infrared (FTIR) spectrophotometer. Bioactivity tests were carried out to investigate the antioxidant activity of EPS-1.40 ICR (Institute of Cancer Research) male mice $(18 \pm 2 \text{ g})$ were randomly divided into 4 groups of 10 each. Three immunosuppressed groups were administered with EPS-1 (0, 200 and 400 mg/kg body weight (BW)) by gavage once daily, and cyclophosphamide (CP) was given intraperitoneally at 50 mg/kg BW on the 12th day. Control mice received the same volume of 0.9% normal saline. The experiment lasted for 14 d. Activities of antioxidant enzymes such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD) in liver were studied. The results indicated that the average molecular weight of EPS-1 was about 23982 Da. It was hypothesized that EPS-1 belongs to the α -type heteropolysaccharide with pyran group, consisting of glucose, mannose and galactose with a molar ratio of 6.860: 1.180: 0.455. CP, as expected, showed suppressive effect on antioxidant status. Compared with CPtreated animals, activities of GSH-Px, CAT and SOD were increased by 22.38% (P < 0.05), 16.89% (P < 0.05) and 5.67% (P > 0.05) respectively, and recovered to the normal levels in animals treated with EPS-1 (400 mg/kg BW). It is suggested that EPS-1 produced by Enterobacter cloacae Z0206 could provide protection against CP-induced oxidative damage in mice, and it may act as a potent antioxidant agent.

Key Words: polysaccharide, *Enterobacter cloacae*, antioxidant

1083 Chinese medical plants and extracts moderating effects on antioxidant status of small intestinal mucous and IEC-6 cells under heat stress. K. J. Guo^{1,3}, X. Z. Song², G. L. Cheng^{1,3}, W. L. Luan¹, F. H. Liu*^{1,3}, and J. Q. Xu⁴, ¹Department of Animal Science and Technology, Beijing University of Agriculture, Beijing, China, ²College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, 330045, P.R. China, ³Beijing Key Laboratory of TCVM, CAU-BUA TCVM Teaching & Research Team, Beijing, China, ⁴TCVM Laboratory, CAU-BUA TCVM Teaching & Research Team, College of Veterinary Medicine, China Agricultural University, Beijing, China.

The aims of the study were to determine: 1. the effects of supplemental Chinese medicine additives on antioxidant status of intestinal mucous in piglets under heat stress; 2. the effects of active components of above studied medicine: *Herba Agastachis* essential oil and *Cortex Phellodendri* alkaloid on antioxidant status of IEC-6 cells after high-temperature treatment. In exp. 1, 16 35-d-old weaned Chinese experimental mini

piglets $(6.50 \pm 1.05 \text{kg})$ were randomly divided into 4 groups: Normal temperature control group, NTC; High temperature stress group, HTS; High temperature + 0.5% Chinese medicine additives group 1, CMA1 and High temperature + 0.5% Chinese medicine additives group 2, CMA2. NTC pigs were housed under the condition of 23°C and 60% humidity for 10days, while the other 3 groups were housed under the same conditions with NTC but treated with 40°C from 10:00 to 15:00 each day for 10 consecutive days. At the end of experiment, duodenum, jejunum, ileum samples of piglets were collected and SOD, GSH-PX activity, MDA contents of intestinal mucous were determined by kit. In exp. 2, IEC-6 cells were cultured under 37°C for 41 h as control and those under 37°C for 38 h and 41°C for last 3h as experimental group (EG). 200μg/mL, 100μg/mL, 50μg/mL essential oil or alkaloid were added into EG and the contents of SOD, GSH-PX activity, MDA were analyzed. The results showed: (1) Chinese medicine additives could significantly increase SOD (P < 0.05), GSH-PX (P < 0.05) activity and decrease MDA content (P < 0.05) in heat stressed piglets. (2) 200µg/ mL essential oil and 100 µg/mL alkaloid significantly increased the levels of SOD (P < 0.05) and GSH-Px (P < 0.05) and decreased the content of MDA (P < 0.05) in IEC-6 cells treated by high temperature. In conclusion, Chinese medicine additives can play important role on improving the antioxidant status of intestinal mucous in piglets. Cortex Phellodendri alkaloid and Herba Agastachis essential oil are the main effective components.

Key Words: Chinese medical plants and extracts, heat stress, antioxidant function

1084 Immune responses and gene expression in red swamp crayfish (*Procambarus clarkii*), induced by selenium-enriched exopolysac-charide (Se-ECZ-EPS) from *Enterobacter cloacae* Z0206. X. X. Wang*, Z. Q. Lu, Y. F. Zhang, L. N. Zhu, Y. Ren, and Y. Z. Wang, *Feed Science Institute of Zhejiang University*, *Hangzhou city*, *Zhejiang province*, *China*.

Selenium-enriched exopolysaccharide (Se-ECZ-EPS), produced by *Enterobacter cloacae* Z0206, is an important polysaccharide but

little information is available about the potential crustacean immune response to this compound. To investigate the modulation of immunity in crustaceans, we examined phenoloxidase activity (PO), respiratory bursts ability (O2⁻¹) and superoxide dismutase (SOD) levels, as well as expression profiles of several immune-related gene in red swamp crayfish (Procambarus clarkii) that were individually injected with Se-ECZ-EPS at 1 μg g⁻¹. The protection of crayfish against white spot syndrome virus (WSSV) by Se-ECZ-EPS was also investigated. During the experiment period, the water temperature was maintained at $22 \pm 1^{\circ}$. Duncan's multiple comparison tests were used to compare significant differences among treatments. At 24, 48 and 72h post-injection, PO activities of crayfish in treatment groups were 0.26±0.03, 0.40±0.02 and 0.38 ± 0.03 respectively, and significantly (P < 0.05) higher than those of control groups (0.16±0.03, 0.15±0.01 and 0.11±0.01). Respiratory burst levels were 0.74 ± 0.05 , 1.21 ± 0.28 and 1.53 ± 0.28 and SOD activities were 34.00±2.52, 43.60±3.41 and 42.00±5.76, respectively at 24, 48 and 72h post-injection, and were significantly (P < 0.05) higher than those of their respective control groups; 0.61 ± 0.04 , 0.61 ± 0.03 and 0.51 ± 0.04 for respiratory burst, and 24.23±2.68, 24.91±2.46 and 24.97±2.98 for SOD activities. Among the examined genes, at 24h and 48h after Se-ECZ-EPS treatment, the mRNA expression of serine proteinase (9.94±1.86 and 2.97±0.69, respectively), HSP70 (9.38±1.91 and 12.96±1.02, respectively) and Mn-SOD (2.72±0.22 and 4.95±0.84, respectively) were significantly up-regulated (P < 0.05). No significant expression changes of proPO were observed from 24h to 72h. The survival rate of crayfish that received Se-ECZ-EPS was significantly higher than that of control group after 14 days (59.7% and 0%, respectively). It can be concluded that Se-ECZ-EPS is an efficient immunostimulant and can improve immunity of crayfish.

Key Words: immune responses, exopolysaccharide, *Procambarus clarkii*