Effects of Olaquindox and Cyadox on Immunity of Piglets Orally Inoculated with *Escherichia coli*

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ABSTRACT : A 2×3 factorial arrangement of treatments was used to determine the effects of olaquindox and cyadox on immune response of Landrace×Large-White geld piglets that had been orally given 10^{10} CFU of *Escherichia coli* (*E. coli*, O_{139}:K_{88}). Factors included (1) *E. coli* inoculation or control, and (2) no antimicrobials, 100 mg/kg olaquindox and 100 mg/kg cyadox in the basal diet respectively. *E. coli* inoculums were orally administered 7 days after the diets were supplemented with olaquindox and cyadox. The effects of the two antimicrobials were assessed in terms of: (1) average daily gain (ADG), (2) systemic immune response (the number of white blood cells and lymphocytes, leukocyte bactericidal capacity, lymphocyte proliferation response to PHA, immunoglobulin concentrations, and total serous hemolytic complement activity), and (3) intestinal mucosal immunity including the number of intraepithelial lymphocytes (IELs) and immunoglobulin A secreting cells (ASCs) in the intestinal lamina propria. *E. coli* inoculation reduced ADG ($p<0.05$) during the period of d 0 to d 14 after the challenge while the antimicrobial supplementations improved ADG ($p<0.01$) during the experiment. ADG in cyadox-supplemented pigs was higher ($p<0.05$) than that in olaquindox-supplemented pigs. The antimicrobials decreased IEL and ASC counts in the jejunum and ileum ($p<0.01$) while *E. coli* inoculation caused them to increase ($p<0.01$). Jejunal ASCs in the cyadox-supplemented pigs were lower ($p<0.05$) than those in the olaquindox-supplemented. *E. coli* elicited increase ($p<0.05$) in white blood cell counts, leukocyte bactericidal capacity, lymphocyte proliferation rate, serous IgA concentrations, and serous hemolytic complement activity. The antimicrobials decreased the measured systemic immune parameters, but not significantly ($p>0.05$). The data suggest that olaquindox and cyadox suppress *E. coli*-induced immune activation, especially intestinal mucosal immune activation, which may be involved in the observed growth promotion. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1320-1325)***

Key Words : Quinoxalines, Antimicrobials, Immunity, Growth, Pigs

INTRODUCTION

Antimicrobials have been used extensively in the livestock industry as growth promoters for more than fifty years. Mechanisms by which they enhance growth are still speculative. The growth promotion of antimicrobials is generally believed to be due to the intestinal microflora modifications, including the suppression of intestinal pathogenic microorganisms (such as *E. coli*) (Visek, 1978; Samiento et al., 1988b; Copet, 1999). Since the gut mucosal immune system contains half the lymphocytes in the body (Mestecky and Meghee, 1987) and is directly exposed to intestinal microorganisms, the alterations of the intestinal microflora may affect immunity. Some studies suggested that immune system activations affected animal growth rate (Sauber et al., 1999; Jones et al., 2001; Roberts et al., 2004). It is not clear whether antimicrobials promote growth by their effects on immunity.

Among antimicrobials, the quinoxalines olaquindox and cyadox, possess the traits of obvious growth promotion (Broz and Sevcik, 1979; Uovsova, 1983). However, there are few data about effects of the two antimicrobials on immunity of piglets. The objective of the present study was to investigate the effects of olaquindox and cyadox on immunity of piglets following the *E. coli* experimental inoculation in order to probe into immune modulations of antimicrobial growth promoters.

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MATERIALS AND METHODS

Antimicrobial growth promoters

Cyadox: synthesized by the Institute of Veterinary Pharmaceutics, Huazhong Agricultural University, Wuhan, China. Olaquindox: provided by Hubei Zhongmu Anda Ltd. Hubei, China. It was indicated with the broth microdilution test that the minimal inhibitory concentrations of cyadox and olaquindox against *E. coli* to be inoculated were 4 and 2 μg/ml respectively.

Pigs

Five-week-old Landrace×Large-White geld piglets were selected from litters of sows whose offspring had shown K_{88} susceptibility by the *in vitro* villous-adhesion assay (Sarmiento et al., 1988a). It was investigated that the piglets and their parents both had no enteric diseases and were not inoculated by any *E. coli* vaccines. The piglets were housed five per pen in isolation units, where feed and water were

Table 1. The complement hemolytic assay procedure

Items	Tube number									Spontaneous lysis	100% lysis
	1	2	3	4	5	6	7	8	9		
Surum sample (ml)	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	-	-
Barbital buffer (ml)	1.40	1.35	1.30	1.25	1.20	1.15	1.10	1.05	1.00	1.50	-
Sensitized erythrocyte (ml)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50
Distilled water (ml)	-	-	-	-	-	-	-	-	-	-	2.00

freely available. Environmental temperature of the rooms was controlled at about 20°C.

Experimental design

One hundred and fifty piglets (average initial weight: 10.3±1.6 kg; five week old) were randomly allotted to one of six treatments. A 2×3 factorial arrangement of treatments was employed with the following factors: 1) inoculation or control; *E. coli* was used as the challenge agent and 2) no any antimicrobial, 100 mg/kg olaquindox and 100 mg/kg cyadox in the basal diet (according to NRC, 1998) respectively. The treatments included three non-inoculated groups (control, olaquindox and cyadox) and three *E. coli*-inoculated groups (E control, E+olaquindox and E+cyadox). *E. coli* inoculums were orally administered 7 days after the diets were supplemented with the antimicrobials. The method which was described by Sarmiento et al. (1988a) was used for *E. coli* inoculation. Each pig to be inoculated was given 10^{10} CFU/ml of *E. coli* (serotype O_{139}:K_{88}; China Institute of Veterinary Drug Control, Beijing, China) via an orogastric tube. Each of the non-inoculated pigs was given equal volume of alkaline broth as a placebo. The inoculated and non-inoculated pigs were housed in completely separated rooms.

One randomly selected pig from each pen (five pigs per group) at d 7, 14 and 21 after the challenge respectively was anesthetized and sacrificed for intestinal samples immediately after blood samples were taken from anterior neva cava. Body weight was determined at weekly time points.

Measurements of systemic immune parameters

White blood cells and lymphocytes : 2 ml of heparinized blood from each sample was measured using CELL-DYN3700 Automated Hematology Analyzer.

Leukocyte bactericidal capacity : The leukocyte bactericidal capacity was examined by the method of Liping (2000). 2 ml of heparinized blood was blended with equal volume of 3% glutin saline solution, and incubated at 37°C for 30 min. The blood plasma layer rich in leukocytes was removed and suspended in RPMI-1640 medium (imported from Gibco-BRL, Gaitheersbrg, MD, USA by Tian Yuan Biotech Co. Ltd, Wuhan, China) to a concentration of 5×10^6 cells/ml. The leukocyte solution, *Staphylococcus aureaus* suspension (5×10^7 CFU/ml; China Institute of Veterinary Drug Control, Beijing, China) and normal swine serum (volume ratio 5:1:4) were added into one tube sequentially and incubated at 37°C for 2 h. Another tube was prepared as the same method except incubation. The two tubes were centrifuged at 800×g for 10 min. The number of *Staphylococcus aureaus* in the supernatant fluid in each tube was determined using a agar plate culture method. The result (a killing index) was calculated as follows: $K_2 = \log N_0 - \log N_2$ (N_0: The mean number of *Staphylococcus aureaus* in the supernatant without 2 h incubation; N_2: the mean number of *Staphylococcus aureaus* in the supernatant with 2 h incubation).

Lymphocyte proliferation rate : *In vitro* lymphocyte proliferation response to Phytohemagglutinin (PHA) was monitored by a method of Blecha (1983). Approximately 5 ml of heparinized blood was mixed with RPMI-1640 complete culture medium (containing 25 mM HEPES, 14 μM 2-mercaptoethamol, 10% fetal calf serum, 100 units of penicillin and 100 units of streptomycin/ml). Blood mononuclear cells were isolated by gradient centrifugation and placed in quadruplicate to a 96-well plate at a concentration of 2×10^6 cells/ml. Phytohemagglutinin-M was used as a mitogen at a concentration of 100 μg/ml and complete culture medium without PHA was used as a negative control. Cells were incubated at 37°C in a 5% CO_2 atmosphere for 48 h. The cultures were then incorporated with H-thynidine, incubated for an additional 18 h, and then collected for liquid scintillation counting using an automated cell harvester. Stimulation index (SI) was calculated.

Total serous hemolytic complement activity : The total serous hemolytic complement activity (CH_{50}) was done by the modified Mayer's method (Liping and Xueqing, 2000). Briefly, antibody-sensitized sheep erythrocyte solution was prepared by mixture of 2% sheep erythrocyte solution with equal volume of hemolysin solution (2 U, China Institute of Veterinary Drug Control, Beijing, China). Serum samples were diluted 1:10 with 7.4 pH barbital buffer. The diluted serum and the antibody-sensitized erythrocyte solution were added into a series of tubes (Table 1). A 50% hemolytic solution standard was prepared by mixture of 100% hemolytic solution with equal amount of the buffer. The tubes were bathed at 37°C for 30 min. After centrifugation, the OD_{542} value of the supernatant fluid in each tube was measured. Hemolytic titer of a sample (U/ml) = 10/x (x: added serum volume (ml) in a tube with the OD value approximating to that of the 50% hemolytic solution).

Table 2. Effect of olaquindox and cyadox on average daily gain (g)[a]

Time	-*E. coli*			+*E. coli*			SEM	P-value		
	Control	Olaquindox	Cyadox	Control	Olaquindox	Cyadox		*E. coli*	Quinoxalines	Interaction
Wk 1[b]	346	361	403	304	344	375	7.7	0.020	0.001	0.699
Wk 2	441	500	539	382	479	503	11.5	0.019	0.0001	0.652
Wk 3	486	524	589	437	490	527	14.8	0.103	0.035	0.948
Overall[b]	424	462	508	375	438	468	8.8	0.001	0.0001	0.590

[a] The results are presented for 3 weekly periods and overall after the *E. coli* inoculation.

[b] The mean of average daily gain in the cyadox-supplemented was different from that in the olaquindox-supplemented ($p<0.05$).

Immunoglobulin concentrations : Rabbit anti-swine IgA serum and swine IgA standard were kindly donated by professor Yangqian, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China. IgM was isolated from porcine colostrum with the method of Reyero (1977) and identified by specific anti-swine IgM serum (Serotec Ltd, Kidlington, UK) with polyacrylamide gel electropheresis. Rabbit anti-swine IgG and IgM sera were prepared by swine IgG (Beijing Medical University, Beijing, China) and IgM immunizing rabbits respectively.

Concentrations of IgG, IgM and IgA in samples were determined using a radial immunodiffusion method in three repetitions per sample. Ring diameters were measured using an optical micrometer and values calculated against standard curves which were made according to measurements of known concentrations of immunoglobulin standards.

Measurements of intestinal mucosal immunity

Tissue section preparation : A 1- to 2-cm-long cross-section, which was taken from mid-jejunum and mid-ileum respectively, was lightly put into a phosphate-buffered paraformaldehyde (40 g/L) solution along with a small piece of rough paper on its chorion side to prevent curliness. After 24 h, 1-mm thick cross-sectional pieces were cut from each sample and embedded in a paraffin block. Tissues were sectioned at 4 μm, mounted on poly-lysine dealt slides, de-paraffinized and re-hydrated sequentially.

Intraepithelial lymphocytes : Three slides from each sample were used for intraepithelial lymphocyte (IEL) counts. The sections were stained with hematoxylin and eosin (H&E). At 400×magnifications, ten well-developed villi were selected from ten different fields in each section for IEL counts. The IEL numbers were determined per 100 enterocytes. The mean value of each sample was given.

IgA secreting cells : Another three slides were used for IgA secreting cell (ASC) enumeration with a Strept-Avidin-Biotin Complex (SABC) technique. Endogenous peroxidase activity was extinguished by incubation with 3% H_2O_2-PBS (pH 7.3, 0.01 M) for 10 min. The sections were digested with complex enzymes for 5 min at room temperature. After washed with PBS, the sections were blocked by goat serum (1:50 dilution) at 37°C for 20 min in a humid chamber. The serum being shaken off, the sections were acted with a 1:100 dilution of rabbit anti-swine-IgA antibody (kindly presented by professor Yangqian, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China) at 37°C for 1 h. The sections were washed and incubated with biotinylated goat anti-rabbit-IgG antibody (SABC kit provided by Wuhan Boster Biological Technology Ltd., Wuhan, China) at 37°C for 20 min. After incubation, peroxidase-conjugated streptavidin label was added. Diaminobenzidine was used as substrate. Following development of desired staining, sections were washed and counterstained with Mayer's hematoxylin. Negative controls for all immunohistochemical procedures consisted of exclusion of primary antibodies from tissue sections. ASC counts were conducted with a light microscope connected to a video-based and computer-linked system (HPIAS-1000) that was programmed to perform cell-count analyses. Since ASCs were mainly distributed in jejunal and ileal lamina propria between glandular cavities, ASCs and interstitial cells (mainly lymphocytes) were counted respectively in ten fields of the ASC well-distributed lamina propria in each section examined under 400×magnifications. The mean ASC percentage in interstitial cells each sample was given.

Statistical analysis

Data were analyzed using least squares analysis of variance according to the General Linear Models procedure of SAS (1999). Pen means were used to analyze ADG data. Differences between treatment means were evaluated by a *t*-test following a significant *F*-test.

RESULTS

Growth performance

There were no *E. coli*×quinoxaline interactions ($p>0.05$) on average daily gain during the entire experiment (Table 2). *E. coli* inoculation reduced ADG during the period of d 0 and d 14 ($p<0.05$) after the challenge. ADG was improved ($p<0.01$ during the period of d 0 to d 14; $p<0.05$ during d 15 to d 21) when the diets were supplemented with the antimicrobials. ADG in cyadox-supplemented pigs was higher ($p<0.05$) than that in olaquindox-supplemented pigs during the first week and the whole experiment. ADG in cyadox group was improved by 19.9%, which was higher

Table 3. Effect of olaquindox and cyadox on systemic immune parameters[ab]

Item		*E. coli* inoculation				Quinoxalines				
		-	+	SEM	P	Control	Olaquindox	Cyadox	SEM	P
White blood cells, (10^9/L)	d 7	17.6	22.7	2.54	0.025	22.6	19.0	18.8	1.21	0.292
	d 14	17.7	20.0	1.23	0.183	21.1	18.7	16.8	1.19	0.154
	d 21	17.1	19.2	1.05	0.169	19.4	17.1	17.9	0.69	0.427
Lymphocytes (10^9/L)	d 7	9.5	11.9	1.43	0.078	10.1	10.9	11.1	0.21	0.813
	d 14	10.6	12.9	1.16	0.081	12.6	11.5	11.2	0.44	0.623
	d 21	10.8	13.0	1.12	0.055	13.9	11.0	10.9	1.10	0.057
Leukocyte bactericidal capacity	d 7	0.83	1.04	0.10	0.023	1.02	0.92	0.87	0.04	0.380
	d 14	0.78	0.90	0.06	0.094	0.89	0.83	0.81	0.03	0.606
	d 21	0.74	0.74	0.01	0.977	0.75	0.73	0.73	0.01	0.929
Lymphocyte proliferation rate (%)	d 7	158	159	0.767	0.735	152	162	161	3.22	0.148
	d 14	164	171	3.77	0.111	167	169	168	2.56	0.958
	d 21	167	176	4.40	0.026	176	170	169	2.15	0.280
IgG (g/ml)	d 7	15.0	14.7	0.14	0.866	16.1	14.1	14.3	1.95	0.554
	d 14	18.7	19.4	0.37	0.608	20.5	18.7	18.0	0.72	0.377
	d 21	20.2	22.1	0.96	0.255	23.8	20.5	19.3	1.33	0.095
IgM (mg/ml)	d 7	2.45	2.33	0.06	0.849	2.60	2.27	2.31	0.11	0.889
	d 14	2.53	2.81	0.14	0.637	3.02	2.60	2.41	0.18	0.690
	d 21	2.44	2.57	0.07	0.841	2.72	2.58	2.23	0.15	0.816
IgA (mg/ml)	d 7	0.89	0.92	0.01	0.843	0.93	0.89	0.89	0.01	0.963
	d 14	0.93	1.21	0.14	0.050	1.30	1.00	0.91	0.12	0.077
	d 21	0.87	1.03	0.08	0.261	1.13	0.92	0.80	0.10	0.188
CH_{50} (U/ml)	d 7	28.7	37.3	4.26	0.005	33.9	34.5	30.6	1.22	0.459
	d 14	32.8	38.8	2.99	0.130	34.8	35.4	37.2	0.72	0.869
	d 21	32.5	38.7	3.11	0.060	34.7	35.0	37.0	0.72	0.813

[a] The results are presented for 3 weekly periods and overall after the *E. coli* inoculation.

[b] There were no *E. coli*×quinoxaline interactions (p>0.05).

than that in olaquindox group (9.0%). ADG was improved by 10.5% in E+cyadox group and by 3.3% in E+olaquindox group while ADG in E control group was decreased by 11.6%.

Systemic immune response

There were no *E. coli*×quinoxaline interactive effects (p>0.05) on systemic immune parameters (Table 3). *E. coli* inoculation led to an increase (p<0.05) in white blood cell counts and leukocyte bactericidal capacity at d 7. Lymphocyte proliferation response to PHA at d 21. IgA concentrations at d 14, and total serous hemolytic complement activity at d 7 after the challenge. The antimicrobials did not affect the measured systemic immune parameters (p>0.05). No difference was observed (p>0.05) in the immune parameters between the olaquindox-supplemented and the cyadox-supplemented during the whole trial.

Intestinal intraepithelial lymphocytes

There existed *E. coli*×antimicrobial interaction on IELs in the jejunum and ileum (p = 0.077) (Table 4). Due to the induction of *E. coli* inoculation, Jejunal IELs increased (p = 0.017) at d 7, maximized (p<0.0001) at d 14 and remained higher level (p = 0.010) at d 21, while ileal IELs inclined (p = 0.025) at d 7 and declined to the level of the control (p = 0.344) at d 21 after the inoculation. The antimicrobials reduced IELs in the jejunum and ileum during the experiment (p<0.05 at d 7 and d 21; p<0.01 at d 14). However, the jejunal and ileal IELs in the olaquindox-supplemmented were not different (p>0.05) from those in the cyadox-supplemented during the experiment.

IgA secreting cells

An *E. coli*×antimicrobial interaction on ASCs was observed in the jejunum (p = 0.006) and in the ileum (p = 0.092) at d 14 after the inoculation (Table 4). *E. coli* inoculation elicited jejunal ASCs to increase (p<0.001 at d 14 and p<0.05 at d 21), and ileal ASCs to increase (p<0.05 at d 7 and d 21; p<0.01 at d 14). The supplementations decreased ASCs in the jejunum (p<0.05 at d 7; p<0.01 at d 14 and d 21) and in ileum (p<0.01 at d 7; p<0.05 at d 14 and d 21). Jejunal ASCs in the cyadox-supplemented were lower (p<0.05) than those in the olaquindox-supplemented at d 14.

DISCUSSION

Effects of quinoxalines on systemic immunity are controversial. Giurgea (1976) reported that carbadox (a quinoxaline), significantly decreased gamma globulin concentrations as well as while blood cell counts when it

Table 4. Effect of olaquindox and cyadox on intraepithelial lymphocytes and IgA secreting cells[a]

Item		*E. coli*			Quinoxalines				p value		
		-	+	SEM	Control	Olaquindox	Cyadox	SEM	*E. coli*	Quinoxaline	Interaction
Intraepithelial lymphocytes (IELs), IEL/100 enterocytes											
Jejunum	d 7	19.4	23.4	2.00	24.0	20.1	20.1	1.30	0.017	0.081	0.447
	d 14	20.6	29.4	4.40	29.1	24.4	21.5	2.21	0.0001	0.002	0.076
	d 21	21.9	26.6	2.33	27.9	23.1	21.8	1.85	0.010	0.016	0.350
Ileum	d 7	16.3	19.9	1.87	20.9	16.9	16.4	1.49	0.025	0.043	0.482
	d 14	18.2	21.3	1.53	22.8	18.7	17.7	1.56	0.018	0.005	0.077
	d 21	18.9	20.3	0.73	23.1	18.4	17.3	1.78	0.344	0.011	0.812
IgA secreting cells (ASCs), %											
Jejunum	d 7	3.17	3.55	0.18	3.86	3.25	2.97	0.26	0.171	0.033	0.765
	d 14[b]	3.57	5.89	1.16	5.88	4.63	3.67	0.64	0.0001	0.0001	0.006
	d 21	3.68	4.30	0.31	4.80	3.73	3.43	0.41	0.041	0.002	0.246
Ileum	d 7	2.01	2.52	0.26	2.78	2.22	1.79	0.28	0.042	0.009	0.873
	d 14	2.33	4.85	1.24	4.58	3.24	2.95	0.50	0.0001	0.013	0.092
	d 21	2.65	3.61	0.48	3.77	3.04	2.57	0.35	0.016	0.044	0.596

[a] The results are presented for 3 weekly periods and overall after the *E. coli* inoculation.

[b] The mean of IgA secreting cells in the cyadox-supplemented was different from that in the olaquindox-supplemented ($p<0.05$).

was admixed to feed ration for chicken. However, the results of Yen (1987) showed that no differences were observed ($p>0.05$) in the number of white blood cells and lymphocytes, and lymphocyte blostogenesis response to PHA between the control and the carbadox-supplemented. In the present study, addition of olaquindox and cyadox to diets did not affect ($p>0.05$) swine systemic immune parameters measured during the period of d 0 to d 21 after the inoculation, although *E. coli* inoculation elicited elevation ($p<0.05$) in white blood cell counts, leukocyte bactericidal capacity, lymphocyte proliferation response to PHA, IgA concentrations and serous hemolytic complement activity.

IELs represent a very large and probably the most unusual population of lymphocytes in the body. Their numbers are influenced by the number and properties of intestinal bacteria. In the current study, IELs in control group were much lower than those given by Chu et al (38.9±3.5 IELs/100 enterocytes). This difference might be due to different microenvironments in which the animals were bred. ASCs produce secretory antibodies to participate in the control of intestinal pathogenic microorganisms. The study of Ahren (1998) indicated that ASC levels are relatively sensitive indicators in reflecting intestinal immune responses. In this study, the antimicrobials decreased IELs and ASCs in the jejunum and ileum ($p<0.01$). Since olaquindox and cyadox exhibited *in vitro* antimicrobial activities in our experiment and Shengxian's (Shengxian et al., 2000), this reduction was probably due to reducing the stimulation of the inoculated *E. coli* to the intestinal mucosal immune system.

The mode of *E. coli* or its endotoxin (lipopolysaccharide, LPS) challenge is commonly used for studying effects of dietary regimes, stressors and medicines on immunity of weaned pigs (Sarmiento and Moon, 1988b; Heugten et al., 1997; Jones, 2001). Although the minimal inhibitory concentrations (MIC) of olaquindox and cyadox were close, their bacteriostatic effects *in vivo* are not fully understood. Spierenburg (1988) estimated that the protective concentrations of 100 mg/kg olaquindox and 100 mg/kg cyadox in diets were found till oral part of jejunum and mid of jejunum respectively according to their concentration profiles along the gastrointestinal tract after in-feed medication and their MIC values. This probably gives an explanation for the result in our study that ASCs in the cyadox-supplemented were lower than those in the olaquindox-supplemented.

For this study, cyadox improved ADG by 19.9% (higher than olaquindox did), which was similar to that reported By Broz (1979). The difference in ADG between the cyadox-supplemented and the olaquindox-supplemented might be associated with the amplitude of suppressing the immune activations in the experimental pigs. Studies verified that immune system activations resulted in metabolic shifts, including the increasing utilization of glucose by peripheral tissues and the enhancing gluconeogenesis from lactate and glucogenic amino acids, by involving cytokines such as INF-α, TNF, IL-1, IL-2 (Grunfeld and Feingold, 1992; Heugten et al., 1994; Tingshi and Yuming, 2004). These cytokines act on animal growth by the nutrient redistribution away from the growth process and toward support of immune system function.

IMPLICATIONS

Based on the large economic impact of piglets' postweaning diarrhea caused by enterotoxigenic *E. coli* and the increasing fear for the potential development of antibiotic resistance, it is worthy to investigate growth-promoting mechanisms of quinoxalines (antimicrobial promoters) so as to seek for alternatives to improve growth

and efficiency of livestock production. This experiment indicated that olaquindox and cyadox might improve the growth performance by reducing the stimulation of the *E. coli* to the intestinal immune system and suppressing *E. coli*-induced immune activation.

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