

Effects of Florfenicol and Chromium (III) on Humoral Immune Response in Chicks

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ABSTRACT : One hundred and sixty day-old Hainan chicks were randomly allotted into eight pens to investigate the effect of different dietary concentrations of chromium (Cr) in the form of chromium chloride, and different dosages of florfenicol on humoral immune responses by determining antibody titers to Newcastle disease (ND) vaccines using the hemagglutination inhibition test. The results indicated that ND antibody titers were significantly higher in chicks receiving Cr at low (5 mg/kg feed) and middle (10 mg/kg feed) dose compared with the control ($p < 0.01$). However, ND antibody titers were significantly decreased in chicks receiving Cr at a high dosage of 500 mg/kg feed ($p < 0.01$), though the ND antibody titers of the early days (d 21 and d 28 of age) were higher than that of the control group. It is suggested that excessive Cr intake has detrimental effects on ND antibody production in chicks. No significantly lower response was measured in chicks that received florfenicol at a low dosage of 50 mg/kg feed ($p > 0.05$), but the ND antibody titers were significantly decreased in chicks receiving 200 and 400 mg/kg feed of the drug ($p < 0.01$). The ND antibody titers of group receiving 200 mg/kg feed of florfenicol plus 10 mg/kg Cr were slightly higher than that of the group receiving single florfenicol of 200 mg/kg although, no significant differences were observed between these two treatments. It is suggested that the humoral immune response impaired by florfenicol (200 mg/kg feed) could not be significantly reversed by Cr (10 mg/kg feed). (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 3 : 366-370)

Key Words : Chromium, Florfenicol, Hainan Chicks, Humoral Immune Response, Newcastle Disease Virus Antibody Titers

INTRODUCTION

Florfenicol is D-d-threo-3-fluoro-2-dichloroacetamide-1-(4-methylsulfonyl-phenyl)-1-propanol, a chemosynthetic, structural analog of chloramphenicol with a broad spectrum and similar mechanism of action. Chloramphenicol has been banned for use in food-producing animals worldwide because of its potential toxicity to human beings and strong immunosuppression of animals at the therapeutic dosage. Compared to chloramphenicol, florfenicol does not have the p-nitro group that has been associated with irreversible aplastic anemia in man (Neu and Fu, 1980; Yunis, 1981; Skolimowski et al., 1983), and has shown greater *in vitro* activity against pathogenic bacteria than either of its structural analogs, thiamphenicol or chloramphenicol. Florfenicol is also active against some bacteria that are resistant to chloramphenicol (Syriopoulou et al., 1981), and has a longer elimination half-life in animals (Varma et al., 1986; EL-Banna, 1998; Hu et al., 2002). Therefore, florfenicol has basically become a substitute of chloramphenicol and fills the clinical therapeutic niche

previously occupied by chloramphenicol. However, there is increasing proof that chloramphenicol and its analogs have adverse effects on the immune function. The immunosuppression of thiamphenicol is about six times as much as that of chloramphenicol (Neu et al., 1980; Yunis, 1981; Page et al., 1990). Florfenicol also significantly suppressed the phagocytosis of ³²P-labelled staphylococcus by bovine blood neutrophils (Bretzlaff et al., 1987), reduced the proliferation of fish T and B cells and depressed phagocytic ability of fish polymorphonuclear and mononuclear cells (Sieroslawka et al., 1998). But investigation of the effects of florfenicol on humoral immune response in poultry has not yet been reported.

Cr (III) seems to be an essential trace element because of its activity as an insulin potentiator. Anderson (1987) suggested that Cr (III) is an integral part of glucose tolerance factor (GTF) and may regulate carbohydrate storage into muscle or adipose tissue through improved insulin sensitivity. A considerable body of research has shown that Cr (III) over a wide range of dosages (2-400 mg/kg feed) increased growth, improved carcass quality, egg production performance and egg quality (Page et al., 1992; Kegley et al., 1996; Mooney et al., 1997; Luo et al., 2002). Cr (III) will likely be used widely for animals in the future as a micronutrient supplement. Asmatullah et al. (1999) described the effect of hexavalent chromium on egg laying capacity, hatchability of eggs and thickness of egg shell. Generally speaking, the effects of immunoregulator on the immune response depend on the dose administered.

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Table 1. Composition of chick basal diets

Ingredients	Percentage (%)
Com	62.75
Soybean meal	28.75
Limestone	7.36
Dicalcium phosphate	0.50
Salt(Sodium chloride)	0.30
Vitamins and trace minerals mixture*	0.23
DL-Methionine	0.11
Total	100

* The vitamin mix supplied (per kilogram of diet): vitamin A, IU; vitamin D₃, 1.200 IU; vitamin E, 20 IU; vitamin K, 1.0 mg; vitamin B₁, 6.0 mg; vitamin B₂, 6.0 mg; vitamin B₆, 12.0 mg; d-biotin, 0.04 mg; choline chloride, 800.0 mg. The trace minerals mix supplied (in milligrams per kilogram): Fe (FeSO₄·H₂O), 50; Cu (CuSO₄·H₂O), 10; Zn (Zn SO₄·7H₂O), 50; Mn (Mn SO₄·H₂O), 80.

The concentration of Cr (III) in the basal diet was 0.47 mg/kg.

Excessive intake of most macro- and micronutrients has adverse effects on immune functions, and there is increasing proof that excess intakes of some nutrients could inhibit the immune response and increase the risk of infection in animals and humans (Kumari and Chandra, 1993; Friedman et al., 1998). Though Cr (III) could enhance cell-mediated immune responses and serum alkaline phosphates (AKP) of chicks, the effects of Cr (III) on the humoral immune responses remain to be proved.

The present study describes the effects of different dosages of florfenicol and Cr (III) on the humoral immune response in chicks by determining the changes of Newcastle disease (ND) antibody titers.

MATERIALS AND METHODS

Drug and chromium source

Florfenicol was supplied by Yikan Medical Chemistry Factory, Huang Yan, Zhejiang, China, and the content was 99.5%. Chromium chloride (CrCl₃·6H₂O) was manufactured by Shanghai Chemical Agents Company, Chinese Medicine Group (Lot NO F2000523) and used as the Cr (III) source.

Chicks and management

One hundred and sixty day-old Hainan chicks were purchased from a local hatchery and were randomly allotted into eight groups, 20 chicks each. Each group was kept in a separate pen with a layer of saw dust on the floor which was replaced on alternate days. The room temperature was maintained at 30°C during the first week by using electrically heated battery brooders and then was kept constant at 25°C±2°C. The chicks were given commercial chick basal diets (Table 1).

Administration of florfenicol and Cr (III)

Of the eight groups (n=20), group 1 was fed the basal diet (control), and group 2, 3, 4 fed the basal diets supplemented with 50, 200 and 400 mg/kg florfenicol for 7 days started from d 3 of age, and group 5, 6, 7 fed with 5,

10 and 500 mg/kg Cr (III) in the form of chromium chloride (CrCl₃·6H₂O) during the entire period of the experiment, respectively, and the last group fed the basal diet supplemented with 200 mg/kg florfenicol for 7 days from d 3 of age plus 10 mg/kg Cr (III) during the entire period of the experiment. Water was provided *ad libitum*. The dosage design of florfenicol was based on the facts that the drug has been approved by many countries including EU for incorporation into animal rations at concentrations of 40-60 g/ton feed for preventing diseases and promoting animals growth, and that the drug is added to feed usually at a dosage of 200-400 g/ton feed for 3 d in treating the acute serious infectious diseases.

Vaccinations

Vaccinations were performed according to standard programs practiced in the local chick farm. Chicks were vaccinated against ND virus by intramuscular inoculation on d 14 of age (attenuated live virus), followed by a booster intramuscular injection administered on d 42 of age (heated-killed ND virus). The chicks were eliminated after blood samples were drawn from the heart at d 21 of age. Blood samples were collected from the pterygoid vein at d 28, 35, 42, 49, 56, 63 and 70 of age.

Determination of antibody titers

ND antibody titers in serum were determined by hemagglutination inhibition test. In brief, 25 µl of PBS (0.01 mol/L, pH 7.2), were put into bores of a "V" type reaction board, and 25 µl serum sample was added into the first bore and mixed with 25 µl of the PBS, then 25 µl of the mixture drawn from the first bore was added to the second bore and mixed with 25 µl of the PBS, and so on, till to the last bore. Then, 25 µl of ND standard antigen were put into above bores. At the same time, the antigen, masculine serum and diluent control were set. The reaction board was shaken on the minim oscillator, the mixtures were placed in the incubator for about half an hour at 37°C, then, 25 µl 1% red cell suspension of chick was added to every bore, the mixtures were reincubated as above. The ND antibody titer levels were transformed by the value of log₂ of the highest dilution times of the detected serum sample, which could inhibit the hemagglutination reaction.

Statistical analysis

ND antibody titers of the treatment groups were compared with those of the control using student's "t" test. The level of significance tested was either p<0.01 or p<0.05.

RESULTS

Effects of florfenicol on ND antibody titers in chicks

Figure 1 shows the effects of florfenicol on the humoral response in chicks. ND antibody titers were not

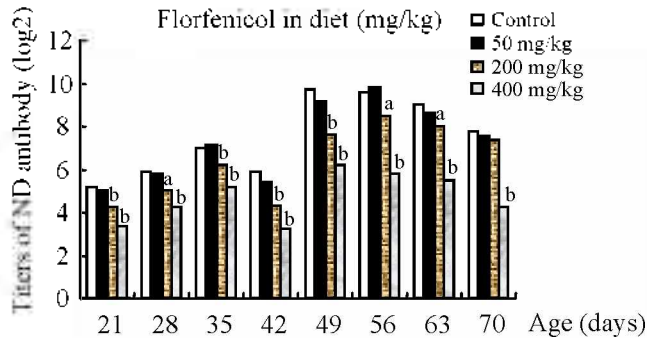
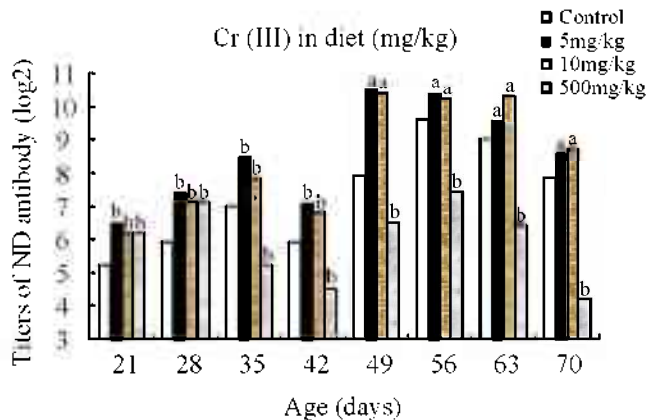


Figure 1. Effects of different dosages of florfenicol added in diet on production of anti-Newcastle disease antibody in chicks. All data are presented as mean±SEM for n=10 birds per group. Significant differences between the control group and treatment groups are indicated by letter a ($p<0.05$) or b ($p<0.01$).



Figures 2. Effects of different dosages of Cr (III) added in diet on production of anti-Newcastle disease antibody in chicks. All data are presented as mean±SEM for n=10 birds per group. Significant differences between control group and treatment groups are indicated by letter a ($p<0.05$) or b ($p<0.01$).

significantly decreased in chicks that were fed the basal diet supplemented with a low dosage of 50 mg/kg florfenicol for 7 days from 3 d of age. ND antibody titers were significantly lower in chicks that were fed basal diet added with a dosage of 200 mg/kg florfenicol during 21 to 49 d of age ($p<0.01$), at 56 d of age ($p<0.05$), and no significant difference was measured at 63 d and 70 d of age ($p>0.05$). ND antibody titers in chicks receiving 400 mg/kg florfenicol were significantly lower than those of the control at all ages (21, 28, 35, 42, 49, 56, 63 and 70 d).

Effects of Cr (III) on ND antibody titers in chicks

The effects of Cr (III) added to the basal diet on the antibody titers to ND virus in chicks were progressive with the age and inoculation (Figure 2). After boosting, the titers of all groups increased. Compared with control group ND antibody titers were significantly higher in chicks fed the basal diets supplemented with 5 and 10 mg/kg Cr (III)

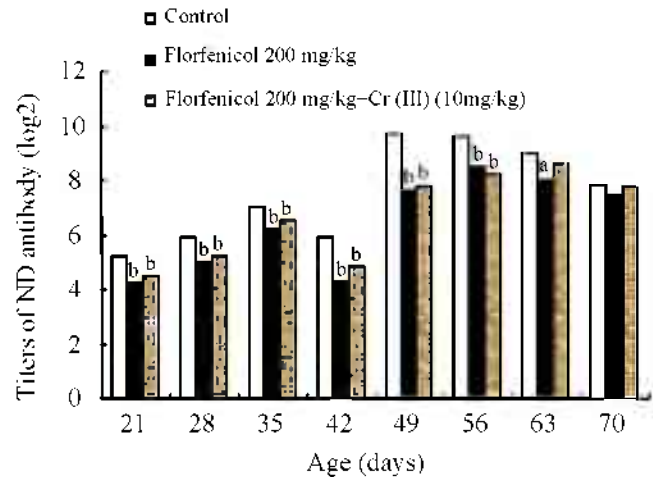


Figure 3. Effects of combination of florfenicol and Cr (III) on ND antibody titers in chicks. All data are presented as mean±SEM for n=10 birds per group. Significant differences between the control group and treatment groups are indicated by letter a ($p<0.05$) or b ($p<0.01$).

during 21 to 63 d of age ($p<0.01$), and ND antibody titers were still higher than those of control at 70 d of age ($p<0.05$). However, no significant differences were observed between the treatments of 5 and 10 mg/kg during the whole experimental period. However, ND antibody titers were significantly decreased in chicks receiving Cr (III) feed added at a high concentration of 500 mg/kg ($p<0.01$), when compared to the response of control group, during the period from 35 to 70 d of age, though the titers of the early days (21 to 28 d of age) were higher in a short period ($p<0.01$). After the second booster, the anti-ND virus immune response of all groups decayed with time when the titers reached peak levels; however, the response of the group receiving 500 mg/kg feed Cr (III) appeared to decay at a faster rate.

Effects of combination of florfenicol and Cr (III) on ND antibody titers in chicks

The effects of combination of florfenicol and Cr (III) on the production of ND antibodies in chicks were shown in Figure 3. Thus, up to the first booster injection (14 d of age) and the second booster injection (42 d of age), ND antibody titers were significantly decreased in chicks fed the basal diets added florfenicol with a dosage of 200 mg/kg or florfenicol (200 mg/kg) plus Cr (III) (10 mg/kg) when compared to those of control group during 21 to 56 d of age ($p<0.01$). No significant difference was measured at 70 d of age ($p>0.05$). No significant differences were observed between the florfenicol and florfenicol plus Cr (III) treatments during the whole experimental period ($p>0.05$), though the ND antibody titers were a little higher in chicks receiving 10 mg/kg Cr (III) plus 200 mg/kg florfenicol than those of chicks treated with a same dosage of florfenicol.

The results indicated that the immunosuppression caused by florfenicol at a dosage of 200 mg/kg couldn't be reversed and the antibody titers couldn't be adjusted to the control levels by Cr (III) (10 mg/kg).

DISCUSSION

Florfenicol was found to have adverse effects on the humoral immune response in healthy chicks in this study, and the effects were dependant on the dosages of the drug administered. ND antibody titers were significantly decreased in chicks receiving the basal diets added florfenicol with 200 mg/kg and 400 mg/kg, whereas no significantly lower response was observed with a low dosage of 50 mg/kg. The mechanism of immunosuppression induced by florfenicol may be the same as that of chloramphenicol and thiamphenicol. Page et al. (1990) reported that chloramphenicol and its analog had adverse effects on cell-mediated immunity. High dosage of florfenicol can inhibit the synthesis of protein and then impair the production of specific antibody in animals. Lundena et al. (1999) also reported that florfenicol at a dosage of 20 mg/kg BW didn't have any significant effects on antibody production in the rainbow trout immunized with a commercial oil-based divalent (furunculosis/vibriosis) vaccine. Considering the effects of florfenicol on the immune response, it is safe that chicks be fed the diet supplemented with a dosage of 50 mg florfenicol/kg feed for preventing or treating infectious diseases.

In chicks examined, optimal effects of Cr (III) for ND antibody production were observed with a limited dose range. The highest antibody production occurred in chicks that received 5 or 10 mg/kg Cr (III) feed. The effects of Cr (III) on ND antibody production became apparent after boosting: highest antibody titers and longest duration of the immune response were obtained by a concentration of 5 mg Cr (III) /kg feed added in the basal diet after the first boosting, and by a concentration of 10 mg Cr (III)/kg feed after the second boosting, but no significant differences were observed between the two dose treatments ($p>0.05$).

This study has shown that excessive intakes of Cr (III) could impair ND antibody production in chicks. ND antibody titers were significantly decreased in chicks receiving the basal diet supplemented with a high dosage of 500 mg Cr (III)/kg, compared with the control levels, though the ND antibody titers of the early ages (21 and 28 d of ages) were higher.

It is suggested that the reasonable or safe dose of Cr (III) as a feed supplement should be about 5 or 10 mg/kg feed, and excessive intakes of the element are not beneficial to the immune system in chicks.

The humoral immune response impairment induced by florfenicol could not be reversed by Cr (III) even at a proper

dosage. Florfenicol, an analog of chloramphenicols, may impair antibody production by inhibiting mitochondrial protein synthesis (Yunis et al., 1973), whereas Cr (III) at a proper dosage may enhance antibody production by reducing the concentration of corticoid in the serum (Mowat, 1993; Burton, 1995; Heugten and Spears, 1997).

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