Ecotoxicology and Environmental Safety 109 (2014) 70–76

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Toxicological effects of dietary nickel chloride on intestinal microbiota

Bangyuan Wu [a], Hengmin Cui [a,b,*], Xi Peng [a,b], Kangcheng Pan [a,b], Jing Fang [a,b], Zhicai Zuo [a,b], Junliang Deng [a,b], Xun Wang [c], Jianying Huang [a]

[a] Key Laboratory of Animal Diseases and Environmental Hazards of Sichuan Province, Sichuan, Ya'an, China
[b] College of Veterinary Medicine, Sichuan Agricultural University, Ya'an 625014, Sichuan, China
[c] College of Animal Science and Technology, Sichuan Agricultural University, Ya'an 625014, Sichuan, China

ARTICLE INFO

Article history:
Received 3 July 2014
Received in revised form
1 August 2014
Accepted 2 August 2014
Available online 28 August 2014

Keywords:
Nickel chloride
Bacterial counts
Microorganism diversity
Intestine
Broiler

ABSTRACT

This study was designed to evaluate the toxicological effect of dietary nickel chloride ($NiCl_2$) on the counts of intestinal bacteria and diversity of microorganisms in broilers. Plate counting and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) assays were used. A total of 240 one-day-old avian broilers chicks were divided into four equal groups and kept on corn-soybean basal diet along with supplementation of 0, 300, 600 and 900 mg/kg $NiCl_2$ for 42 days. Samples were taken at 21 and 42 days of age during the experiment. The bacterial count results showed that dietary $NiCl_2$ in the range of 300 to 900 mg/kg decreased the counts of *Bifidobacterium spp.* and *Lactobacillus*, increased *Escherichia coli (E. coli)* and *Enterococcus spp.* in the ileum and cecum. PCR-DGGE analysis showed that bacterial band numbers, profile similarity, and the Shannon index of the ileum and cecum were all decreased in the 300, 600, and 900 mg/kg groups at 21 and 42 days of age. In conclusion, dietary $NiCl_2$ affected the amount and diversity of intestinal microbiota in the ileum and cecum of broilers. This finding implies that $NiCl_2$ has toxicological effect on the intestinal ecosystem and, possibly functions.

1. Introduction

Nickel is one of the essential elements present in abundance in the earth's crust, at an average concentration of about 75 μg/g. Although nickel has oxidation states of −1, 0, +1, +2, +3, and +4, it exists principally in the stable, divalent state (Ni^{2+}) (Poonkothai and Vijayavathi, 2012). In biological systems, nickel in its ionic state forms stable complex components with various ligands and binds to organic material (Scott-Fordsmand, 1997). For example, Khan and Moheman (2006) have reported that nickel interacts with iron present in hemoglobin and helps in oxygen transport, stimulates metabolism, and is a key metal in several plants and animal enzyme systems.

Nickel is considered an essential element in animals, microorganisms and plants and is a constituent of enzymes and proteins (Poonkothai and Vijayavathi, 2012; Hausinger, 1987; Henriksson and DaSilva, 1978). Some microorganisms (like the *Cyanobacterium Oscillatoria sp.* or *Pseudomonas flava*) have an absolute metabolic requirement for nickel (Van Baalen and Donnell, 1978; Tabillion et al., 1980), and nickel is an indispensable element for the chemolithotrophic growth of several microbial species (Tabillion et al., 1980; Bartha and Ordal, 1965; Tabillion and Kaltwasser, 1977). Nickel also is the core metal in the tetrapyrrole ring of methanogenic bacteria, which is essential for the growth of these microbes (Baker et al., 2000; Welch, 1995).

Although nickel is omnipresent and vital for the function of many organisms, its concentrations in some areas of the world, from both anthropogenic release and natural sources, may be toxic to living organisms. The toxicity of nickel or nickel compounds is of interest partly because of its wide distribution throughout the environment (Sunderman et al., 1988). Humans are exposed to nickel via food, water, and air produced from sources such as mining, extraction, refining, electroplating, food processing, and waste disposal (Nielsen et al., 1984). Several known toxic effects of nickel in higher animals and humans are allergies, carcinogenesis, and cardiovascular and renal disorders (Chowdhury et al., 2008). In humans, nickel can cause damage to the liver, kidney, spleen, and brain; vesicular eczema; and lung and nasal cancer on acute exposure (International Programme on Chemical Safety (IPCS), 1992). Nickel also is a potent teratogen in animals; inhalation and exposure of nickel carbonyl compounds to rats and hamsters caused fetal death, diminished weight gain, and eye malformations (Sevin, 1980). The growth of metals mixture (Zn+Ni) exposed fish species was significantly lesser than that of control fish (un-stressed) (Naz and Javed, 2013). A laboratory experiment

* Corresponding author at: College of Veterinary Medicine, Sichuan Agricultural University, Ya'an 625014, Sichuan, China. Fax: +86 0835 2882340.
E-mail addresses: wubangyuan2008@163.com (B. Wu), cuihengmin2008@sina.com, cui580420@sicau.edu.cn (H. Cui).

http://dx.doi.org/10.1016/j.ecoenv.2014.08.002
0147-6513/

also suggested that four fish species (*Catla catla*, *Ctenopharyngodon idella*, *Cirrhina mrigala* and *Labeo rohita*) showed highest sensitivity to nickel, and dose dependent metal accumulation in fish was also observed (Kousar and Javed, 2014).

Despite the numerous references cited above, studies on the effects of nickel or nickel compounds on the intestinal microbiota in animals and man have not been reported. Our previous studies have proved that dietary nickel chloride ($NiCl_2$) can cause intestinal oxidative damage, inhibit intestinal development, and decrease cytokine mRNA expression levels or increase the cellular apoptosis of cecal tonsil and spleen in broilers (Wu et al., 2013a, 2013b, 2013c, 2014a, 2014b; Huang et al., 2013).

The present study was designed to evaluate the effects of dietary $NiCl_2$ on intestinal bacterial counts and microbial diversity in broilers, as measured by plate counting and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) assays, and to show the toxicological effect of $NiCl_2$ on the intestinal ecosystem and functions.

2. Materials and methods

2.1. Broilers and diets

Two hundred and forty one-day-old healthy avian broilers were randomly assigned to one of four groups, with 60 broilers in each group and five broilers for endpoint analysis (21 and 42 days of age). Broilers were housed in cages with electrically heated units and provided with water and the experimental diets, described below, ad libitum for 42 days.

A corn-soybean basal diet formulated by The National Research Council (NRC) (1994) was the control diet. $NiCl_2 \cdot 6H_2O$ was mixed into the corn-soybean basal diet to produce experimental diets with 300, 600, or 900 mg/kg of $NiCl_2$, as described by Wu et al. (2014b).

2.2. Sample treatment

At 21 and 42 days of age, five broilers in each group were humanely killed, and the intestinal tracts were immediately removed and were kept on ice. Equal-length segments of the same place of the ileum and cecum were excised, and then the digesta were removed from the lumen of the ileum and cecum into sterile tubes for bacterial counting within 1 h. Meanwhile, equal amounts of ileal or caecal digesta from the five broilers within each group were combined for preparation of bacterial samples that were subsequently used for DNA extraction. And samples for DNA extraction were frozen in liquid nitrogen and stored at −70 °C.

2.3. Determination of bacterial counts by plate counting

Bacterial counting was performed as described (San Martín et al., 2007). For bacterial counting, digesta samples were serially diluted from 10^{-1} to 10^{-7} with sterile 0.9 percent sodium chloride (NaCl) solution. Dilutions were subsequently plated on appropriate selective agar for enumeration of target bacterial groups. Plates with 30–300 colonies were replica-plated onto de Man–Rogosa–Sharpe agar (for *Lactobacillus*), *Bifidobacteria* selective medium agar (for *Bifidobacterium spp.*) and MacConkey agar (for *E coli*). After the samples were seeded on the surface of the plates, the plates were incubated 37 °C aerobically for 24 h or anaerobically for 36 h for colony counting. The number of bacterial colonies was calculated, and the results were expressed as $\log^{10}$ colony-forming units per gram (CFU/g) of fresh digesta.

2.4. Microorganism diversity analysis by PCR-DGGE

Bacterial samples prepared from five chickens were subjected to five freeze-thaw cycles, alternating between liquid nitrogen and 65 °C for 5 min in the presence of β-mercaptoethanol (5 μl/ml), followed by bead-beating as described by Satokari et al. (2001)to lyse cells. DNA was extracted from cell lysates using the method of phenol-chloroform extraction and ethanol precipitation as described by Zoetendal et al. (2001) with some modifications (Kraatz et al., 2006). These DNA preparations were used as template DNA in the PCR. Primers 339-GC forward: 5′-CGC CCG GGGCGC GCC CCG GGC GGG GCG GGG GCA CGGGGG GAC TCC TAC GGG AGG CAG CAG T-3′ and 539 reverse: 5′-GTA TTA CCG CGGCTG CTG GCA C-3′ were used to amplify the V3 regions of bacterial 16S rDNA.

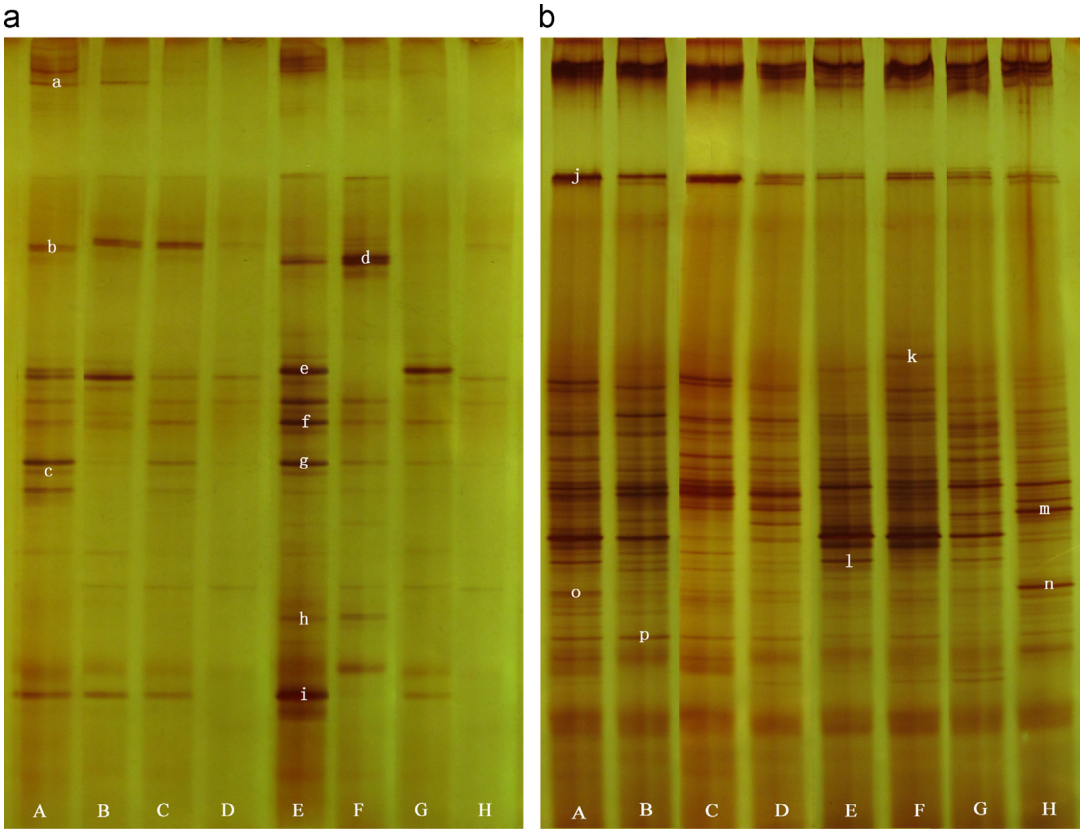

Fig. 1. PCR-DGGE DNA profiles of the 16 S rDNA of bacteria in ileum (a) and cecum (b) at 21 and 42 days of age. A: Control group, B: 300 mg/kg group, C: 600 mg/kg group, D: 900 mg/kg group at 21 days of age; E: Control group, F: 300 mg/kg group, G: 600 mg/kg group, H: 900 mg/kg group at 42 days of age. The lowercase represent the different PCR-DGGE DNA profiles.