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Excess of dietary benzoic acid supplementation leads to growth retardation, hematological abnormality and organ injury of piglets

Yan Shu, Bing Yu, Jun He, Jie Yu, Ping Zheng, Zhengcai Yuan, Daiwen Chen *, Xiangbing Mao *

Key Laboratory of Animal Disease-Resistance Nutrition, Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China

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ABSTRACT

Benzoic acid has been widely used in feed industry as an organic acidifier and preservative. However, it is unknown whether excessive benzoic acid in diets would have a potential risk on pigs. This study was conducted to investigate the safety of benzoic acid that was used in diets of piglets. A total of 120 weaned pigs [(Yorkshire × Landrace) × Duroc] with initial average BW of 8.16 ± 0.09 kg (28 ± 1 d of age) were randomly allotted to four groups receiving diets supplementing 0%, 0.5%, 2.5% and 5.0% benzoic acid for 56 d. Supplementing 0.5% benzoic acid in the diet had no negative effects on the growth of piglets, and increased antioxidant enzyme (CAT and GSH-Px) activities in the liver ($P < 0.05$). Dietary 2.5% benzoic acid supplementation decreased ADFI and ADG of pigs from 1 to 28 d ($P < 0.05$), reduced the white blood cell and globulin on d 56 ($P < 0.05$), and resulted in spleen injury on d 28 and d 56. In addition, besides impairing growth performance of pigs during the whole experiment ($P < 0.05$), supplementing 5.0% benzoic acid in the diet increased the relative liver weight on d 56 ($P < 0.05$), enhanced the serum alanine aminotransferase and aspartate aminotransferase of pigs on d 28 ($P < 0.05$), and led to liver injury. Moreover, dietary 5.0% benzoic acid supplementation also decreased the red cell pressure product, red blood cell volume and increased red cell distribution width-SD/CV on d 28 ($P < 0.05$), and resulted in the serious spleen damage. When compared with pigs fed by the diet supplemented 0.5% benzoic acid, pigs fed the diets containing 2.5% or 5.0% benzoic acid would have higher benzoic acid residues in the liver and kidneys on d 28 and d 56 ($P < 0.05$). These results suggested that dietary 0.5% benzoic acid supplementation had the beneficial effects on piglets, but supplementing excessive (2.5% and 5.0%) benzoic acid in the diets could lead to growth retardation, hematological abnormality and the injury of some organs (liver and spleen).

1. Introduction

As a weak organic acid, benzoic acid has been widely used in feed and food additives. In 2003, the European Union authorized that it could be used in the feed of growing pigs at the dose of 0.5–1.0%. Many studies have proposed that supplementing proper benzoic acid in diets may improve growth performance of weaned piglets via increasing nutrients digestibility and intestinal health (Kluge et al., 2006; Guggenbuhl et al., 2007; Torrallardona et al., 2007a; Halas et al., 2010; Diao et al., 2015, 2016). However, Amaechi and Anueyiagu (2012) have found that while dietary 0.6% and 1.2% benzoic acid supplementation increase the weight gain of 120 d-age broiler, supplementing 1.8% and 2.4% benzoic acid in the diets lead to poor growth and the increasing mortality. Previous studies also have shown that excessive benzoic acid intake may impair the health of humans and mice, such as metabolic acidosis, hyperpnea and allergic reaction (Nair, 2001; Hong et al., 2009).

Following oral administration, benzoic acid can be rapidly absorbed in the gastrointestinal tract and metabolized in the liver (Cong et al., 2001). Benzoic acid and its analogue, including sodium benzoate, will be conjugated with glycine to form benzoyl-glycine that may be excreted by the urinary system (Snapper et al., 1946; Gregus et al., 1993). Furthermore, benzoic acid is distributed in the following penetration scale: forehead > abdomen > thigh > chest > arm > back, through the blood circulation (Rougier et al., 1986).

The research about the impact of excessive benzoic acid intake in humans and animals is relatively limited. Some studies have indicated that the acute toxicity symptoms resulted by benzoic acid mainly include tear, diarrhea, muscle weakness, tremor, nervousness, anxiety and hypoactivity, and the chronic toxicity

* Corresponding authors.
E-mail addresses: dwchen@sicau.edu.cn (D. Chen), acatmxb2003@163.com (X. Mao).

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reactions embody in weight loss, anorexia and pathological changes (Wiley and Bigelow, 1908; Kreis et al., 1967; Bedford and Clarke, 1972). The World Health Organization has summarized that the oral median lethal dose (LD50) of benzoic acid in rats are 2100–4070 mg/kg, the LD50 of benzoic acid in mice are 1940–2263 mg/kg, and the LD50 of benzoic acid in cats are 420–890 mg/kg (WHO, 2000). Consequently, excessive benzoic acid intake might cause a potential poisoning risk to humans and animals. However, it is unknown about the impacts of supplementing excessive benzoic acid in diets on growth performance and health of piglets. Therefore, this study was conducted to investigate the effects of dietary excessive benzoic acid supplementation on growth performance, blood components and organ pathobiology of piglets.

2. Materials and methods

The experimental protocol used in the following experiment was reviewed and approved by the Animal Experimental Committee of Sichuan Agricultural University. The experiment was conducted at the Animal Experiment Center of Sichuan Agricultural University.

2.1. Animals and housing

A total of 120 healthy crossbred [(Yorkshire × Landrace) × Duroc] weaned pigs (60 males and 60 females) with an initial average body weight (BW) of 8.16 ± 0.09 kg were housed in rooms with temperature maintained at 25 ± 1 °C, relative humidity controlled at 60–70%, and the natural lighting. The diets were supplied 4 times daily at 08:00, 12:00, 16:00 and 20:00 h, and water could be ad libitum accessed for pigs.

2.2. Diets and experimental design

Two control diets were formulated to meet or exceed the National Research Council- recommended nutrient requirements (NRC, 1998). The ingredients and nutrient composition of control diets were listed in Table 1. Benzoic acid added to diets was purchased from DSM (China) Limited (Shanghai, China), which of purity was 99.5%. The benzoic acid supplementing diets were the control diets supplemented with 0, 0.5%, 2.5% and 5.0% benzoic acid via substituting the same amount of rice mill by-product in control diets.

All pigs were allocated with completely randomized design according to their initial body weight (BW) and sex dietary in a 56-day experiment. Days 1–28: 4 treatments, 6 replicates (pens) per treatment, 5 pigs per pen; days 29–56: 4 treatments, 6 replicates (pens) per treatment, 4 pigs per pen. Pigs in different treatments were fed with the diets supplementing 0%, 0.5%, 2.5% and 5.0% benzoic acid, respectively.

2.3. Data and sample collection

The feed intake per pen measured daily and the weight of each pig measured in the morning of days 1, 29 and 57 were used to calculate average daily feed intake (ADFI), average daily weight gain (ADG) and feed/gain ratio (F: G). One pig, which of BW close to the average BW per pen, was selected to obtain blood samples via the jugular veins after 12 h fasting on day 1, 29 and 57. The 2 mL of blood was collected into EDTA anticoagulative tubes to examine blood routine indices. Another 10 mL of blood was collected into vacutainer tubes and centrifuged at 3000 rpm for 15 min to obtain serum for the measurement of blood biochemical indices. On day 29 and 57, the selected pigs were slaughtered by an intracardiac injection of Na pentobarbital (50 mg/kg body weight) and jugular exsanguinations. The liver, spleen and kidneys were removed. Tissue samples (about 5 cm^3 of a regular shape) were excised at the same site, and immediately immersed into 4% neutral buffered paraformaldehyde for histopathological examination. Liver tissues (approximately 2 g) were collected, and stored at −80 °C for analyzing antioxidant indices. About 10 g of liver and kidneys samples were collected, and stored at −20 °C for measuring benzoic acid residue.

Table 1
Ingredients and nutrient composition of the control diets during days 1–28 and days 29–56 (air dry basis).

Items	Days 1–28	Days 29–56
Ingredients, %		
Corn, 8% CP	52.10	63.68
Fish meal, 64.5% CP	5.40	0.00
Whey powder, 3% CP	6.00	0.00
Dehulled soybean meal, 47.9% CP	11.00	0.00
Soybean protein concentrate	5.00	0.00
Extruded soybean	10.00	26.60
Rice mill by-product	5.00	5.00
Soybean oil	3.00	2.50
Chloride choline, 50%	0.15	0.15
Salt	0.30	0.30
Calcium carbonate	0.60	0.72
Dicalcium phosphate	0.70	0.72
L-Lysine · Hcl, 78%	0.20	0.00
Vitamin premix[a]	0.03	0.03
Mineral premix[b]	0.50	0.30
Sweeteners	0.02	0.02
Nutrient composition[c]		
Digestible energy, MJ/kg	14.29	13.86
Crude protein, %	20.02	17.03
Calcium, %	0.78	0.60
Total phosphorus,%	0.67	0.50
Available phosphorus, %	0.46	0.28
Lysine, %	1.33	0.86
Methionine, %	0.41	0.27
Methionine and cysteine, %	0.77	0.57
Threonine, %	0.72	0.65
Tryptophan, %	0.54	0.20

[a] Provided the following per kilogram of two control diets: vitamin A 9000 IU, 8000 IU; vitamin D_3 3000 IU, 4000 IU; vitamin E 20 IU, 20 IU; vitamin K_3 3.0 mg, 1.0 mg; vitamin B_1 1.5 mg, 1.5 mg; vitamin B_2 4.0 mg, 5.0 mg; vitamin B_6 3.0 mg, 2.0 mg; vitamin B_{12} 20 μg, 30 μg; nicotinic acid 30 mg, 30 mg; D-pantothenic acid 15 mg, 15 mg; folic acid 0.75 mg, 0.5 mg; biotin 0.1 mg, 0.1 mg. Respectively.

[b] Provided the following per kilogram of two control diets: Fe 75 mg, 100 mg as iron sulfate; Cu 150 mg, 30 mg as copper sulfate; Zn 75 mg, 100 mg as zinc sulfate; Mn 60 mg, 60 mg as manganous sulfate; I 0.35 mg, 0.35 mg as potassium iodate; Se 0.35 mg, 0.35 mg as sodium selenite. Respectively.

[c] Calculated nutrient levels.

2.4. Sample analysis

2.4.1. Blood routine and biochemical indices

Blood routine indices, including white blood cell count (WBC), lymphocyte count (LY), red blood cell count (RBC), red cell pressure product (HCT), red blood cell volume (MCV), red cell distribution width-SD/CV (RDW-SD/CV) and mean hemoglobin (MCH), were measured by blood cell analyzer (Sysmex XT-1800, Japan). Blood biochemical indices, including total protein (TP), albumin (AL), globulin (GLOB), blood urea nitrogen (BUN), creatinine (CR), alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (AKP), were measured by automatic biochemistry analyzer (Hitachi 7020, Japan).

2.4.2. Antioxidant indices

Antioxidant indices in the serum and liver, including glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD),