Research note: effects of different anticoccidial regimens on the growth performance, hematological parameters, immune response, and intestinal coccidial lesion scores of yellow-feathered broilers

Ting Lei,[*,1] Dawei Wu,[†] Zheng Song,[*] Yu Ren,[†] Qiang Yu,[*] Changxue Qi,[*] Pan Xiao,[*] and Jun Gong[*]

[*]*Elanco (Shanghai) Animal Health Co., Ltd, Shanghai, China; and* [†]*Hesheng Food Group Co., Ltd, Jiangsu, China*

ABSTRACT Yellow-feathered broiler chickens generally have a longer growth cycle than white broilers and therefore face different coccidiosis challenges. General single vaccine and drug regimens struggle to prevent coccidiosis for yellow broilers. In this study, a single vaccine, and a combination of coccidiostat regimens was employed to explore the preventative and control effects of different pilot regimens on coccidiosis in yellow-feathered broilers. A total of 2,000 one day old Chinese Huang Youma female broilers were allocated into 4 experimental groups, each with 5 replicates. All birds were fed the same starter feed from Days 1 to 25, and all groups were inoculated with a vaccine on Day 4. After Day 26, the groups were then fed as follows: (1) Negative control group: basal diet + vaccine **(NC)**; (2) NC + maduramycin **(NCMD);** (3) NC + narasin **(NCNR)**; and (4) NC + salinomycin **(NCSL).** From Days 26 to 75, the NCNR group had a lower FCR than the other groups. The 75-d BW was higher in the NCNR group than in the NCSL group but was not significantly higher than that in the NC and NCMD groups. The growth performance followed the same trend during the whole experiment (Days 1−75). Compared to the NC group, the NCNR and NCSL groups had higher intestinal mucosa SIgA concentrations at Day 40 and Day 60 ($P < 0.001$); however the NCMD group had lower IgG levels at Day 40 and Day 60 ($P = 0.036$, $P = 0.006$ respectively). The combination groups had significantly reduced AKP levels and urine acid concentrations at Day 60 in comparison to those of the NC group ($P = 0.004$). The NCNR and NCMD groups had less severe intestinal coccidiosis lesion scores than the NC and NCSL groups in older birds. Thus, a single vaccine and/or combinations with different coccidiostats had different effects on broilers. The NCNR group showed comparatively better growth performance, blood biochemical indices, immune response, and coccidiosis lesion scores.

Key words: anticoccidial, narasin, growth performance, immunity, broiler

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INTRODUCTION

Chicken coccidiosis is one of the main factors affecting the performance of broilers and causes economic losses as high as $3 billion per year in the global poultry industry (Kadykalo et al., 2018). Compared with white-feathered broiler chickens, yellow-feathered broilers have a longer coccidia incubation period; the main epidemic strains are *Eimeria necatrix, Eimeria acervulina, Eimeria maxima* and *Eimeria tenella.* At present, the coccidial control measures in yellow-feathered broiler chickens are mainly single coccidial control regimens or vaccines. However, the 2 regimens are not well studied compared to single vaccination or supplemental anticoccidial regimens. For example, when ionophores are used alone, it causes increased mortality and decreased growth performance caused by the challenge of E. *tenella*, and the chemical coccidial drugs are prone to develop drug resistance and limit their effectiveness when used alone (Harfoush et al., 2010).When vaccines alone are used, chickens experience higher risks of enteritis at younger ages, as well as higher risks of necrotic enteritis when they are older due to insufficient prevention and control of coccidiosis, and have lower production performance (Williams, 2002). At present, there are few domestic reports on the efficacy of combining these programs. Related other reports indicate that using vaccines in combination with ionophore coccidial drugs in the feed, rather than vaccination alone, showed the best results for production efficiency and immune function of commercial broilers (Mathis et al., 2014). However, the effects of the combination in yellow-feathered broilers

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[1]Corresponding author: leitingwhat@qq.com

remain unclear. In this experiment, the vaccine alone or in combination with ionophores was used to explore the effects of different anticoccidial combinations on control of coccidiosis and enteritis control and production performance to provide insight in yellow-feathered chickens.

MATERIALS AND METHODS

The present experiment was conducted at the Hesheng Research Center farm, which is located in Jiangsu Province, China. The animal protocols were approved by the Animal Care and Welfare Committee of Hesheng Food Group Co., Ltd. The management and husbandry of the birds strictly followed the Chinese government's regulations on animal welfare. This live animal research on followed the guidelines approved by the institutional animal care and use committee.

Experimental Material

The Monteban (10% narasin) used in the trial was provided by Elanco (Shanghai) Animal health Co., Ltd, Shanghai, China; 1% maduramycin was provided by Zhejiang Huineng Biological Company, Zhejiang province, China; 12% salinomycin premix was provided by Inner Mongolia Baike Biological Company, Mongolia, China.

Birds, Diets, and Management Practices

The study was carried out at the Hesheng Research Center farm (A mimic commercial set up) and the test chicks were provided by Zhuxuan Breeder Farm, Taizhou Branch of Hesheng Group. In the experiment, 2,000 healthy one d old female yellow-feathered broiler (*Huang youma*) chicks were selected and randomly divided into 4 groups, each with 5 replicates of 100 chickens. The first group was the control group without any anticoccidial drugs but only coccidia vaccine; the other groups had a basal diet supplemented with different types of anticoccidial drugs combined with coccidia vaccine. The experimental design and treatment are shown in Table 1. The experiment lasted 75 d, of which days 1 to 25, 26 to 45, 46 to 65, and 66 to 75 had different feed stages. The feed was based on the China Feed Industry Association group standards for laying hens and broilers (Association 2018). On the fourth day, all the chicks were immunized with a coccidial vaccine by mixing it with the feed. The coccidial vaccine was a trivalent vaccine (including *E. tenella, E. maxima* and *E. necatrix* strains; a total of 780,000 ± 150,000/mL oocysts) provided by Tianjin Hlinte Company.

The broilers were raised on floor pens with new litter in a closed shelter with free access to food and water and 24 h of light; the room temperature was kept at 32 to 34°C for the first week and then decreased by 2 to 3°C every week until it reached 26°C. All birds were kept under uniform management conditions in a well-ventilated shed.

Sample and Data Collection

Growth Performance On Days 25 and 75, the whole group was weighed on an empty stomach (after fasting for 12 h). The feed intake during the test period was recorded, and the average daily weight gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**) at 1 to 25 d of age, 26 to 75 d of age, and 1 to 75 d of age were calculated in addition to the mortality rate.

Blood Biochemical Indices On the 40th and 60th d of the experiment, 2 broilers with similar body weights were randomly selected from each replicate and weighed. Blood was drawn from the carotid artery and collected in a clean centrifuge tube. After the serum was precipitated, it was centrifuged at 4°C for 10 min (4,000 r/min), taken into aliquots, and stored in a −20°C freezer until testing. Serum albumin (**ALB**), serum total protein (**TP**), alkaline phosphatase (**AKP**), blood urea nitrogen (**BUN**), and blood uric acid (**UA**) contents were determined by corresponding commercial kits (DiaSys Diagnostic System, Shanghai, China) using an automatic biochemical analyzer (Beckman Synchron CX4 PRO, CA).

Immune Response The 40- and 60-day-old serum IgG, intestinal mucosa IgG and SIgA of broiler chickens were determined by enzyme-linked immunosorbent assays (**ELISA**s). The kits were provided by Nanjing Jiancheng Bioengineering Institute.

Intestinal Coccidiosis Lesion Score On the 20th, 30th, 40th, 50th, and 60th days of the experiment, 2 broilers were randomly selected from each replicate of each treatment group, and necropsy was performed after death by exsanguination after inducing unconsciousness with CO_2. Coccidial lesions were scored using a scale from 0 to 4, referring to the ELANCO Broiler Disease Reference Guide 2019 .

Determination of Leg Yellowness of Broilers On the 75th day of the experiment, 4 hens with similar body weights were randomly selected from each replicate (20 from each group), and the yellowness of the broiler shank was scored with a Robotmation color chart. The

Table 1. Experimental design.

Treatment	Vaccination status	Ionophore amount added at different ages		
		Days 1−25	Days 26−75	Days 66−75
Negative Control (NC)	+	-	-	-
Narasin (NCNR)	+	-	10% narasin (700 g/t)	-
Maduramycin (NCMD)	+	-	1% maduramycin (500 g/t)	-
Salinamycin (NCSL)	+	-	12% salinomycin (500 g/t)	-

same part of the right shin was measured for each chicken to see the status of pigment deposition.

Statistical Analyses

Data were analyzed using one-way analyses of variance and general linear models (JMP15.1, 2020). Detection of significant differences ($P < 0.05$) among multiple means was performed using Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Growth Performance

The bird performance results are shown in Table 2.

During the test period (Days 26−75), the NCNR and NC groups were significantly higher than the NCMD and NCSL groups in ADG and FCR (P = 0.003 and P = 0.002 respectively) but no difference within the 2 group itself. Other indicators had no difference among the groups.

Over the whole period (Days 1−75), the 75-d BW and ADG of the NCSL group were significantly lower than those of the other three groups (P = 0016). The FCR of the NCNR and NC groups were significantly higher than those of the NCMD and NCSL groups (P = 0.007). Other indicators had no difference among the groups.

The performance result is consistent with the results of many previous studies. Yan et al. (2021) found that adding narasin to white-feathered broiler diets improved growth performance and intestinal flora abundance. Whelan et al. (2019) found that narasin reduced mortality and FCR. Waldenstedt et al. (1999) found that the addition of narasin improved the body weight gain and feed conversion ratio of broilers.

Table 2. Effects of different anticoccidial drugs on the growth performance of broilers.

	NC	NCMD	NCNR	NCSL	P Value
1-d BW (g)	38.66	38.3	38.42	38.42	0.780
25-dBW (g)	377.42	396.15	395.14	389.91	0.415
75-d BW (g)	1742.76[ab]	1670.26[ab]	1750.04[a]	1654.68[b]	0.016
1−25 d					
ADG (g/day)	13.55	14.32	14.27	14.06	0.392
ADFI (g/day)	30.58	30.53	30.95	30.51	0.637
Mortality rate (%)	0.40	1.00	1.00	0.40	0.45
FCR	2.26	2.13	2.18	2.17	0.390
26−75 d					
ADG (g/day)	27.86[a]	26.00[b]	27.65[a]	25.81[b]	0.003
ADFI (g/day)	89.96	86.82	88.34	86.55	0.227
Mortality rate (%)	2.40	3.00	1.20	3.00	0.265
FCR	3.23 [bc]	3.34[ab]	3.20[c]	3.35[a]	0.002
1−75 d					
ADG (g/day)	22.72 [ab]	21.76[ab]	22.82[a]	21.55[b]	0.016
ADFI (g/day)	66.99	64.98	66.23	64.74	0.220
Mortality rate (%)	2.80	4.00	2.20	3.40	0.543
FCR	2.95 [ab]	2.99[a]	2.90[b]	3.00[a]	0.007

[a,b]Within a row, numbers with different superscripts significantly differ at $P < 0.05$.

Abbreviations: NC, negative control; NCMD, NC + maduramycin; NCNR, NC + narasin; NCSL, NC + salinomysin; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Immune Response

At 40 d of age, the intestinal mucosa sIgA in the NCNR and NCSL groups was significantly higher than that in the NC and NCMD groups ($P < 0.001$). It was lowest in the NCMD group and significantly lower than that in the other three groups ($P < 0.001$).

At 60 d of age, the intestinal mucosa sIgA in the NCNR group was significantly higher than that in the NC and NCMD groups ($P < 0.001$) but was not different from that in the NCSL group. sIgA is the main antibody of the intestinal mucosa, and the intestinal sIgA levels reflect immune function to a certain extent, the increase in sIgA levels indicates that the immune function of the body is enhanced (Fan et al., 2020). In this study, the mucosal antibody IgA levels were higher, indicating that narasin and salinomycin play a role in improving the mucosal immune function of the intestinal tract.

Blood Biochemical Indices

At 60 d of age. The AKP in the NC group was significantly higher than that in the NCNR and NCSL groups ($P = 0.004$), but there was no difference between the NC and NCMD groups. The BUA in the NC group was significantly higher than that in the other 3 treatment groups ($P = 0.004$). Other indicators, such as TP, ALB, and BUN, did not significantly differ among the groups.

AKP levels are generally considered to be a typical indicator of bone growth (Shuang, 2020). In this study, the AKP levels of 40-day-old broilers were generally higher than those of 60-day-old broilers, but there were no significant differences among the groups, indicating differences between yellow-feathered and white-feathered broiler breeds.

Nitrogenous substances such as amino acids and proteins ingested by poultry are eventually metabolized to uric acid and excreted from the body (Dandie, 2021). In this study, blood uric acid levels in the NC group were significantly higher than those in the other groups in this study, indicating that combining ionophores and a vaccine effectively improved the utilization of protein.

Leg Yellowness

There was no significant difference in the yellowness of the leg among the various treatment groups, but numerically different.

Leg yellowness is an important economic indicator of yellow-feathered chickens and is generally related to the intestinal absorption capacity of these pigments. Allen (1987) found that infection with coccidiosis caused damage to the intestinal mucosa, as the newly replaced mucosal tissue lacked the ability to absorb carotenoids. Therefore, mild coccidial infection affects the absorption of pigments. In this study, the leg yellowness of the treatment groups did not significantly differ, but the numerical value showed that the values of the NCSL and NC groups were slightly lower than those of the NCNR and NCMD groups, indicating that these 2 groups may suffer from more serious mucosal damage. This corresponds to higher severity of *E. necatrix* lesions in older birds in the NC and NCSL groups than those in the other 2 groups, as shown by the intestinal coccidiosis lesion score of necropsies

Intestinal Coccidiosis Lesion Score

The coccidiosis lesion score, which assesses the coccidial challenge degree in the intestine and can distinguish different coccidia types, was first proposed by Johnson and Reid (1970). It has been widely used all over the world. Barrios et al. (2017) found a positive relationship between the microscores and gross lesion scores, confirming that intestinal coccidial lesion scores represent the degree of intestinal damage. In this study, all treatment groups established good immunity to *E. acervuline, E. maxima* and *E. tenella* at approximately 40 d of age; the challenges of mainly poisoning coccidia were higher. The coccidial lesions of the NCNR and NCMD groups were less severe than those of the NC and NCSL groups in older birds, indicating that combination of narasin or maduramycin with the vaccine was better than that of the vaccine alone.

DISCLOSURES

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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