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Evaluation of synbiotic and cinnamon (*Cinnamomum verum*) as antibiotic growth promoter substitutions on growth performance, intestinal microbial populations and blood parameters in *Japanese* quail

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Abstract This study was conducted to evaluate the effects of dietary supplementation with cinnamon powder, cinnamon oil, and synbiotic as growth promoter agents on growth performance, blood parameters, and intestinal microbial populations in *Japanese* quails. A total of 420 one-day-old *Japanese* quails were randomly assigned to 7 treatments with 4 replicates. The dietary treatments consisted of the basal diet as control, 200 mg virginiamycin/kg, 100 and 200 mg cinnamon oil, 1 and 2 g cinnamon powder/kg, and 500 mg synbiotic/kg added to the basal diet. Birds were given feed and water ad libitum. Body weight gain and feed intake of quails were determined at day 1, 21, and 35, and feed conversion ratio was calculated. At day 35, two birds per replicate were slaughtered for determination of bacteria colony count. Compared to control and 1 g cinnamon powder/kg diet, supplementing 200 mg cinnamon oil/kg increased body weight gain of quails at day 35 ($P<0.05$). Feeding 200 mg cinnamon oil/kg and virginiamycin improved feed conversion ratio compared to control group at day 35 ($P<0.05$). Count of lactobacillus bacteria increased in birds fed 200 mg cinnamon oil/kg diet in comparison with the birds fed control, virginiamycin and 2 g cinnamon powder/kg diets. Dietary supplementation of 200 mg cinnamon oil/kg diet and virginiamycin decreased the number of coliforms in the ileum. In conclusion, 200 mg cinnamon oil/kg diet can be applied as an alternative to antibiotic for *Japanese* quails diets to improve growth performance, and it can also increase the number of lactobacillus bacteria and decrease coliforms.

Keywords: cinnamon powder, cinnamon oil, *Japanese* quail, synbiotic

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Introduction

Antibiotic growth promoters (AGP) were used in the poultry diet for three purposes: improving their growth performance, treating sick animals, and preventing the outbreak of disease among animals susceptible to infections for more than half a century (Kamphues and Hebeler, 1999). The AGP have been banned in the European Union since 2006 due to the appearance of residues in animal products and bacterial resistance (Brenes and Roura, 2010). Therefore, it is important to find alternative growth promotors (Griggs and Jacob, 2005). There is a large variety of products that can be used as a replacement for AGP with synbiotics and plant extracts (essential oils) as the candidates (Bedford, 2000; Ghasemi et al., 2014). The antimicrobial effects of essential oils have been demonstrated, but the reports about their effect on growth performance in poultry are variable needing more investigations (Burt, 2004; Brenes and Roura, 2010).

The main pharmacological effects of cinnamon are attributed to its cinnamaldehyde content followed by eugenol, which are the main bioactive components of cinnamon (Gurdip et al., 2007; Kaskatepe et al., 2016). It has long been considered that cinnamon has several beneficial effects on either human or animals due to its antimicrobial, antifungal, and antioxidant properties (Lee et al., 2001, 2007; Cocchiara et al., 2005; Goni et al., 2009; Ojaghian et al., 2014). Although cinnamon dietary supplementation improved growth performance of broiler chickens in some instances (Lee et al., 2004; Garcia et al., 2007; Al-Kassie, 2010), some studies did not find a beneficial effect (Hernandez et al., 2004; Koochaksaraie et al., 2011).

Synbiotic is a combination of both probiotics and prebiotics which can improve the survival of live microbial dietary supplements in the gastrointestinal tract of the host animal and its performance. There is very limit-

ed information available on *Japanese* quails performance fed essential oils or their components. This study was conducted to investigate the potential of applying cinnamon (oil and powder) and a synbiotic as antibiotics substitutions on growth performance, intestinal microbial populations and blood parameters in *Japanese* quails.

Materials and methods

Birds and diets

The experiment was conducted in accordance with the animal welfare guidelines at the Veterinary Control and Research Institute of Kerman, Iran. A total of 420 one-day-old male *Japanese* quails (*Coturnix japonica*) were randomly allotted to 7 treatments with 4 replicates. The experiment lasted 5 weeks, consisting of a starter phase from day 0 to 21 and a finisher phase from day 22 to 35. The diets were formulated according to the requirements recommended by Shim and Pran Vohra, (1984), NRC (1994) and Altine et al. (2016). The diets fed in any period had the same composition. The composition of the basal diets is shown in Table 1. Dietary treatments were: 1) basal control diet without any added compounds (CON); 2) Control + 100 mg cinnamon oil/kg diet (CIO1); 3) Control + 200 mg cinnamon oil/kg diet (CIO2); 4) Control + 1 g cinnamon powder/kg diet (CIP1); 5) Control + 2 g cinnamon powder/kg diet (CIP2); 6) Control + 200 mg virginiamycin (antibiotic)/kg diet (VIM); and 7) Control + 500 mg synbiotic (Biomin GmbH, Herzogenburg, Austria)/kg diet (SYN), this synbiotic contained *Enterococcus faecium as* the probiotic strain and fructo-oligosaccharides as the prebiotic.

Fresh cinnamon inner barks were purchased in autumn, sun-shade dried and then was ground to obtain cinnamon powder. Cinnamon barks (50 mesh particle size) were hydrodistilled using a Clevenger's apparatus for preparation of the essential oils (Clevenger, 1928), the plant materials were boiled in water. The chemical compositions of the cinnamon oil was determined by gas chromatography–mass spectrometry (Gurdip et al., 2007), with cinnamaldehyde being the major oil constituent (72%). The amount of the supplement was chosen to provide approximately 72 and 144 ppm cinnamaldehyde for CIO1/CIP1 and CIO2/CIP2, respectively.

The birds were reared on floor pens (15 birds/m^2) under similar conditions. The lighting program consisted of 24 h/d fluorescent lighting (20 lux light intensity). The initial temperature of 37°C was gradually reduced according to the age of the birds until reaching 25°C at the end of the experiment. Feed and water were provided ad libitum. The quails were not vaccinated.

Performance and biochemical determination

Birds and feeds were weighed at day 1, 21, and 35 on a pen basis. The body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined. The pens were checked for mortality twice a day and the birds that died during the experiment were weighed and sent to the pathology laboratory for necropsy. At day 35, two birds per replicate (8 birds per treatment) were randomly selected and slaughtered. Duodenum, ileum, and cecum were dissected out and their length and weight were determined and expressed as a percentage of live body weight (BW).

Serum biochemical parameters

At the end of the experiment, one bird per pen was selected randomly and blood samples were collected in

Table 1. Ingredient composition and calculated values (g/kg) of the basal diets (as fed basis)

Item	1-21 day	22-35 day
Ingredient (%)		
Corn	53.00	58.90
Soybean meal	36.60	32.20
Corn oil	6.05	5.00
Dicalcium phosphate	1.60	1.60
Calcium Carbonate	1.70	1.30
DL-methionine	0.15	0.10
Vitamin premix [1]	0.25	0.25
Mineral premix [2]	0.25	0.25
Salt	0.40	0.40
Calculated nutrient values		
Metabolizable energy (kcal/kg)	3100	3100
Crude protein (%)	22.70	20.60
Calcium (%)	1.00	0.91
Available phosphorus (%)	0.71	0.66
Sodium (%)	0.15	0.15
Total Met + Cys	0.75	0.70
Total Lysine (%)	1.18	1.00
Total Threonine (%)	1.15	1.02
Total Methionine (%)	0.48	0.45

[1]Provided per kilogram of diet: 15,000 IU of vitamin A (retinol); 3,750 IU of vitamin D3 (Cholecalciferol); 37.5 mg of vitamin E (tocopheryl acetate); 2.55 mg of vitamin K3; 3 mg of thiamin; 7.5 mg of riboflavin; 4.5 mg of vitamin B6 (pyridoxine); 24 µg of vitamin B12 (cyanocobalamin); 51 mg of niacin; 1.5 mg of folic acid; 0.2 mg of biotin; 13.5 mg of pantothenic acid; 250 mg of choline chloride; 100 mg of antioxidant.

[2]Provided per kilogram of diet: 37.5 mg of Zn (ZnO, 80.35% Zn); 37.5 mg of Mn ($MnSO_4.H_2O$, 32.49% Mn); 37.5 mg of Fe ($FeSO_4 \cdot 7H2O$, 20.09% Fe); 3.75 mg of Cu ($CuSO_4 \cdot 5H2O$); 0.83 mg of I (KI, 58% I); 62.5 mg of Sulfur; 0.23 mg of Se ($NaSeO_3$, 45.56% Se).

non- heparinized tubes by puncturing the brachial vein. The blood sample was centrifuged (SIGMA 4-15 Lab Centrifuge, Germany) at 2000×g for 15 min to obtain serum. Individual serum samples were analyzed for blood glucose level, cholesterol (CHL), high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol and triglyceride (TG) using standard commercial kits (Sigma Chemical Co., St. Louis, MO 63178-9916) (Nazifi, 2005).

Enumeration of the intestinal microflora

Upon slaughter on day 35 of age, contents of the ileum were sampled for enumeration of total bacteria, Lactobacillus and Coliform. Intestinal microflora, including total bacteria, Lactobacillus and Coliform, were determined. Intestinal digesta samples (1 g) were diluted with sterile 0.9% NaCl to 10 folds and then, a specific agar was used to culture bacteria as follows: Nutrient-agar medium was used to count total anaerobic bacteria (incubation for 24 h at 37 °C); MRS-agar medium for Lactobacillus (72 h incubation at 37 °C) and MacConkey-agar medium for Coliform (24 h incubation at 37 °C). The counts were reported as log10 CFU/g of digesta (Leusink et al., 2014).

Statistical analysis

The data were statistically analyzed using the GLM procedure (SAS, 2013) as a completely randomized design with the pen being defined as the experimental unit. Differences among the means were determined by the Tukey's test at *(P*<0.05).

Results and discussion

Growth performance

Mortality rate was low (0.5%) and the deaths were not associated with any specific treatment. The means for BWG, FI, and FCR at days 21 and 35 are presented in Table 2. In general, FI was not affected by the experimental treatments. There were differences (P<0.05) in the BWG of *Japanese* quails fed the dietary treatments (Table 2). Birds receiving VIM supplementation had significantly higher BWG during 1 to 35 days of age compared to CIP1 or CON diet. Although the BWG of quails fed CIO2 and VIM diet was similar during 1 to 35 days (P<0.05).

Supplementing CIO2 and VIM improved FCR compared to CON group during 1 to 35 days (P<0.05). The results showed that additives used in the current study did not have any harmful effects on growth performance parameters, furthermore, some of them had the same growth-promoting effect compared to AGP.

Al-Kassie (2010) also showed that supplementation of 200 mg/ml oil extract derived from thyme or cinnamon in broiler diets significantly improved the weight gain and feed conversion ratio during a growing period of 6 weeks. In addition, Toghyani *et al.* (2011) reported that BW of broiler chickens at 28 and 42 was higher in the groups fed the diet containing 2 g cinnamon powder (containing 60 ppm cinnamaldehyde) compared to those fed the CON diet. Moreover, Lee *et al.* (2004) showed that adding cinnamon to the broiler diet improved their growth performance. However, Hernandez *et al.* (2004) reported that the broilers fed a diet supplemented with 34 ppm cinnamaldehyde in starter diet and 60 ppm cinnamaldehyde in grower diet had the same growth performance parameters as those received the un-supplemented diet. Koochaksaraie *et al.* (2011) also reported that dietary cinnamon powder (250 to 2000 mg/kg diet) had no growth-promoting effect on broilers.

In this experiment, birds fed with VIM and CIO2 recorded the greatest BWG among all experimental gro-

Table 2. Effects of experimental diets on growth performance in quail chickens[1]

Treatment	BWG (g/b/d)		FI (g/b/d)		FCR (g/g)	
	(1-21 day)	(1-35 day)	(1-21 day)	(1-35 day)	(1-21 day)	(1-35 day)
CON	4.96	5.34 ^{b}	10.97	15.32	2.21	2.87 ^{a}
CIO1	5.07	5.49 ^{ab}	11.10	15.46	2.19	2.81 ^{abc}
CIO2	5.07	5.69 ^{ab}	10.78	15.30	2.12	2.68 ^{c}
CIP1	4.97	5.34 ^{b}	10.98	15.01	2.20	2.81 ^{abc}
CIP2	4.82	5.43 ^{ab}	10.70	15.34	2.20	2.82 ^{ab}
VIM	5.08	5.75 ^{a}	11.04	15.65	2.17	2.72 ^{bc}
SYN	4.86	5.55 ^{ab}	10.63	15.60	2.18	2.81 ^{abc}
SEM [2]	0.044	0.041	0.316	0.106	0.014	0.015
P value	0.716	0.017	0.920	0.841	0.757	0.002

[a-c]Means in the same row with common superscript (s) do not differ (P>0.05).

[1]Abbreviation: basal control diet (CON); basal control diet with 100 mg cinnamon oil/kg (CIO1); basal control diet with 200 mg cinnamon oil/kg (CIO2); basal control diet with 1 g cinnamon powder/kg (CIP1); basal control diet with 2 g cinnamon powder/kg (CIP2); basal control diet with 200 mg virginiamycin/kg (VIM); basal control diet with 500 mg synbiotic/kg (SYN).

[2]Standard errors of mean. Data are means of 4 replicate pens of 15 birds each.

ups. It is known that antibiotics affect intestinal microflora, thus, decreasing energy loss and intestinal thickness, therefore, BW would be improved (Miles et al., 2006; Mehdipour et al., 2014). Cinnamaldehyde may improve growth performance parameters via improvement in endogenous digestive enzyme secretion' also it has beneficial effects on the protection of the intestinal villi through intercellular antioxidant activity which may positively affect the absorption of digested nutrients (Jamroz et al., 2005). Also cinnamon has antimicrobial activity against bacteria found in the intestine, limiting the growth and colonization of numerous pathogenic and nonpathogenic species in the gut (Dorman and Deans, 2000; Cabuk et al., 2006). The active substances of mentioned aromatic plants alter the cell membrane structure of enteropathogenic strains, causing ion leakage out of the cells, and death of pathogens (Windisch et al., 2008). The aldehyde existing in cinnamon improves the growth of beneficial intestinal microflora like lactobacilli (Castillo et al., 2006).

The favorable effect of growth promoting substances in the present study, such as VIM and CIO2, on growth performance may be related to an efficient use of nutrients, which in turn results in an improved FCR. The improvement of BWG and FCR are due to the active materials found in cinnamon that improve the efficiency of feed, thus, increase growth (Garcia et al., 2007). In this experiment, quails fed CIP2 and CIO2 received 144 ppm cinnamaldehyde, but these diets had different effects on growth performance, perhaps it was due to the differences in the form of providing cinnamon because CIP2 was in powder form of cinnamon bark but CIO2 was hydrodistilled to obtain oil form. Therefore, it seems CIO2 was more effective than CIP2.

Table 3 shows the effect of dietary treatments on gastrointestinal tract segments length and weight. Duodenal length was shorter in the group which was fed CIO2 than CIP1 and CON groups. Decreasing duodenal length can be due to decreasing bacterial harmful effects (Visek, 1978). Generally, increasing the number of beneficial bacteria and decreasing the harmful one can improve intestine morphology by causing intestinal epithelium and microvilli increment (Kalavathy et al., 2003). As it was shown in the current study that the number of harmful bacteria like coliforms was decreased in CIO2 group. Therefore, the

decreased duodenal length in CIO2 group might be due the CIO2 antibacterial effects (Botsoglou et al., 2003; Goni et al., 2009), and decreased pathogenic activity.

Serum biochemical parameters

There were no significant differences in the blood parameters (P>0.05) between CON and other groups (data not shown).

Enumeration of intestinal microflora

The compositions of intestinal microflora of at day 35 of age are shown in Table 4. The counts of total anaerobic bacteria were not influenced by the treatments. However, the count of the coliform bacteria was lower in birds fed CIO2 and VIM diet compared to CON, CIO1, CIP1, CIP2, and SYN groups. The counts of lactobacilli were higher in CIO2 group compared to VIM, CIP1, and CON groups.

As antibiotics, plant extracts could control and limit the growth and colonization of numerous pathogenic and

Table 3. Effect of experimental diets on the relative length (cm) and weight (% of BW) of intestine (duodenum, ileum, and cecum) in Japanese quails on day 35[1]

Treatment	Weights			Length(cm)		
	Duodenal	Ceca	Small intestinal	Duodenal	Ceca	Small intestinal
CON	0.68	0.51^{ab}	1.72	6.26^{a}	8.79	24.03
CIO1	0.71	0.47^{ab}	1.66	5.94^{ab}	8.46	24.87
CIO2	0.79	0.59^{a}	1.84	5.29^{b}	8.16	23.28
CIP1	0.72	0.50^{ab}	1.62	6.22^{a}	7.80	24.65
CIP2	0.68	0.46^{b}	1.80	5.89^{ab}	8.28	25.86
VIM	0.72	0.45^{b}	1.70	5.82^{ab}	7.06	24.54
SYN	0.72	0.52^{ab}	1.90	5.84^{ab}	8.82	25.44
SEM [2]	0.021	0.013	0.036	0.072	0.244	0.339
P value	0.882	0.041	0.367	0.005	0.505	0.501

[a,b]Means in the same row with common superscript (s) do not differ (P>0.05).

[1]Abbreviation: basal control diet (CON); basal control diet with 100 mg cinnamon oil/kg (CIO1); basal control diet with 200 mg cinnamon oil/kg (CIO2); basal control diet with 1 g cinnamon powder/kg (CIP1); basal control diet with 2 g cinnamon powder/kg (CIP2); basal control diet with 200 mg virginiamycin/kg (VIM); basal control diet with 500 mg synbiotic/kg (SYN).

[2]Standard errors of mean.

Data are means of 4 replicate pens of 2 birds each (n = 8).

Table 4. Effect of experimental diets on microflora composition (log10 CFU/g) at 35 day of quail chickens[1]

Treatment	log10 CFU/g		
	Total anaerobes	Lactobacillus	Coliforms
CON	9.32	8.10 [b]	5.14 [a]
CIO1	9.22	8.42 [ab]	5.12 [a]
CIO2	8.97	9.10 [a]	4.34 [b]
CIP1	9.28	8.09 [b]	5.13 [a]
CIP2	9.22	8.41 [ab]	5.01 [ab]
VIM	9.01	7.86 [b]	4.33 [b]
SYN	9.28	8.28 [ab]	5.11 [a]
SEM [2]	0.049	0.101	0.083
P value	0.356	0.018	0.0006

[a,b]Means in the same row with common superscript (s) do not differ (P>0.05).
[1]Abbreviation: basal control diet (CON); basal control diet with 100 mg cinnamon oil/kg (CIO1); basal control diet with 200 mg cinnamon oil/kg (CIO2); basal control diet with 1 g cinnamon powder/kg (CIP1); basal control diet with 2 g cinnamon powder/kg (CIP2); basal control diet with 200 mg virginiamycin/kg (VIM); basal control diet with 500 mg synbiotic/kg (SYN).
[2]Standard errors of mean. Data are means of 4 replicate pens of 1 birds each (n = 4).

nonpathogenic species of bacteria in the gut (Dorman and Deans, 2000). Although Gram-positive bacteria such as lactobacillus were highly sensitive to AGPs, the growth of Gram-negative bacteria like Escherichia coli could be inhibited by essential oils (Bento et al., 2013). The antibacterial properties of plant extracts could be attributed mainly to their phenolic components and their mechanisms of action on the microbial cell (Burt, 2004; Penalver et al., 2005). Si *et al.* (2006) reported that it is possible to select plant extract compounds with a strong antimicrobial action against gut pathogens while not harming the beneficial bacteria such as bifidobacteria and lactobacilli. The intestinal lactobacilli and bifidobacteria compete with potential pathogens for nutrients and binding sites, thereby reducing the population of intestinal pathogens (Rolfe, 2000).

The main phenolic compound of bark cinnamon is cinnamaldehyde which has antibacterial effects. Cinnamaldehyde is reported to cause cell membrane disintegration and release the bacterial cell contents, penetrate the bacterial cell membrane to impair the enzyme system, reduce the intracellular pH, and cause depletion of adenosine triphosphate. Cinnamaldehyde disrupts the membrane integrity, which affects pH homeostasis and equilibrium of inorganic ions (Helander et al., 1998; Oussalah et al., 2006). In the current study, birds fed CIO2 had lower count of coliforms and higher lactobacilli compared to VIM, CIP1, and CON groups. Therefore, CIO2 could improve the intestinal microbial population by increasing number of lactobacillus bacteria and decreasing coliforms.

Conclusion

Cinnamon antimicrobial compounds may improve digestion, gut microflora and absorption. Particularly, birds fed CIO2 showed increasing beneficial (lactobacillus bacteria) and decreasing harmful ones (coliforms) bacteria leading to the same increase in BWG and improving FCR as an antibiotic group. Overall, these responses showed that cinnamon oil (CIO2) in feed, may display comparative growth promoting effects to those of traditional antibiotics; therefore, this product might be a potential alternative to the AGP for improving BWG, FCR, and intestinal microbial populations in quails.

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بررسی اثر سینبیوتیک و دارچین در مقایسه با آنتی بیوتیک محرک رشد بر عملکرد رشد، جمعیت میکروبی روده و پارامترهای خون در بلدرچین های گوشتی

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چکیده در این آزمایش، تاثیر استفاده از پودر و اسانس دارچین و سینبیوتیک بعنوان محرک رشد بر پارامترهای عملکرد، پارامترهای خونی و میکروفلورای روده در جوجه بلدرچین های گوشتی بررسی شد. این آزمایش با مجموع ۴۲۰ جوجه یکروزه بلدرچین در قالب طرح کاملا تصادفی با ۷ تیمار و ۴ تکرار ر به اجرا در آمد. تیمار های آزمایشی شامل ۱) جیره شاهد (فاقد هرگونه افزودنی)، ۲) جیره شاهد + ۱۰۰ میلی گرم در کیلوگرم اسانس دارچین، ۳) جیره شاهد + ۲۰۰ میلی گرم در کیلوگرم اسانس دارچین، ۴) جیره شاهد + ۱ گرم پودر دارچین، ۵)جیره شاهد + ۲ گرم پودر دارچین، ۶) جیره شاهد + ۲۰۰ میلی گرم در کیلوگرم ویرجینیامایسین، ۷) جیره شاهد + ۵۰۰ میلی گرم در کیلوگرم سینبیوتیک. آب و خوراک بصورت آزاد در اختیار پرندگان قرار گرفت. وزن بدن و مصرف خوراک در روزهای ۱، ۲۱ و ۳۵ اندازه گیری و ضریب تبدیل محاسبه گردید. در ۳۵ روزگی، دو پرنده بصورت تصادفی از هر تکرار برای اندازه گیری میکروفلور روده ای کشتار شد. در سن ۳۵ روزگی، جوجه های تغذیه شده با ویرجینیامایسین و اسانس دارچین (۲۰۰ میلی گرم در کیلوگرم) افزایش وزن بیشتری در مقایسه با گروه شاهد و پودر دارچین (۱ گرم در کیلوگرم) برخوردار بودند($P<۰/۰۵$). همچنین در سن ۳۵ روزگی، در جوجه های تغذیه شده با ویرجینیامایسین و اسانس دارچین (۲۰۰ میلی گرم در کیلوگرم) بهبود ضریب تبدیل مشاهده شد($P<۰/۰۵$). بیشترین شمارش کلونی باکتری های لاکتوباسیل در تیمار اسانس دارچین (۲۰۰ میلی گرم در کیلوگرم) مشاهده گردید، که نسبت به تیمار شاهد، ویرجینیامایسین و پودر دارچین (۲ گرم در کیلوگرم) اختلاف معنی داری را نشان داد ($P<۰/۰۵$). کمترین شمارش باکتری کلی فرم در تیمارهای آنتی بیوتیک و اسانس دارچین (۲۰۰ میلی گرم در کیلوگرم) مشاهد شد($P<۰/۰۵$). هیچکدام از گروه ها تاثیر معنی داری بر پارامترهای خونی نداشت. یافته های این پژوهش نشان داد، افزودن ۲۰۰ میلی گرم در کیلوگرم اسانس دارچین می تواند بعنوان یک جایگزین مناسب برای آنتی بیوتیک محرک رشد در جیره بلدرچین های گوشتی برای بهبود عملکرد رشد استفاده شود، همچنین می تواند باعث افزایش لاکتوباسیلوس ها و کاهش کلی فرم ها در فلور میکروبی روده شود.