Comparison of performance and feed digestibility of the non-antibiotic feed supplement (Novacid) and an antibiotic growth promoter in broiler chickens

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ABSTRACT Antibiotic growth promoters have been widely used in poultry to improve overall performance. The emergence of antibiotic resistance has resulted in sanctions imposed on the use of antibiotics in poultry diets, and alternatives such as herbal extracts are being considered to improve growth performance. The aim of this study was to compare the performance and feed digestibility of the feed supplement Novacid, which contains organic acids, glucomannan, and phytochemicals, with that of the antibiotic growth promoter bacitracin methylene disalicylate (BMD) in commercial broiler chickens. Six hundred 1-d-old Ross × Ross 308 male broiler chicks were randomly and equally assigned to six treatment groups with five replicates each (20 chicks per replicate). The chicks were fed a corn–soybean meal basal diet, and divided into two groups: unchallenged and challenged with *E. coli* (400 mg/kg *Escherichia coli* inoculation). Each of these groups was divided into three study groups: untreated, treated with 0.05% Novacid, and treated with 400 mg/kg BMD. At day 42, inclusion of Novacid or BMD significantly ($P < 0.05$) improved the performance in the unchallenged groups relative to the control group. However, in *E. coli*-challenged groups, Novacid and BMD did not improve performance. Ileal digestibility of crude fat, crude protein, and gross energy were reduced in the Novacid group ($P < 0.05$). BMD and Novacid were equally effective in controlling ileal nutrient digestibility and feed coliform count ($P < 0.05$). Novacid reduced cecal *E. coli* and *Salmonella* count compared to BMD and control. Thus, a phytochemical feed supplement with organic acids and glucomannan could be an effective substitute for antibiotic growth promoters in broiler diets, but cannot replace antibiotics to counter potent infectious agents such as *E. coli*.

Key words: broiler, *Escherichia coli*, feed additive, antibiotic growth promoter, poultry performance

2019 Poultry Science 98:904–911
http://dx.doi.org/10.3382/ps/pey437

INTRODUCTION

The poultry industry is one of the fastest growing agricultural sectors. Infections and disease prevention in poultry is therefore of great public health and economic significance. *Escherichia coli* (*E. coli*) is a gram-negative bacterium that causes colibacillosis in all poultry species. *E. coli* is typically present in animal feces, especially in poultry with associated diseases. Although some *E. coli* strains are not virulent, others cause bloody diarrhea, severe anemia, kidney failure, and urinary tract infections, leading to severe mortality (Manafi et al., 2017). *E. coli* infections can be transferred to broiler meat and subsequently to humans. Pathogenic avian *E. coli* is often resistant to frequently used antibacterial agents due to their abuse (Vandemaele et al., 2002). In addition, the nontherapeutic usage of commercial antibiotics could cause tolerance or resistance in humans as well as animals. Therefore, a worldwide ban has been imposed on antibiotic usage in order to decrease antibiotic resistant bacterial traits in poultry (Smith et al., 2000).

Antibiotic growth promoters (**AGP**) have been used to improve overall poultry performance since the 1950s (Manafi, 2015). However, since sanctions have been imposed on the use of AGP in poultry diet, herbal extracts have received attention as a potential alternative to AGP to improve growth performance. The beneficial effects of herbal extracts are due to their composition of active phytochemicals and secondary compounds such as organic acids, essential oils (**EO**), terpenoids, phenolic compounds, penoids, and aldehydes. These compounds can influence the gut through various mechanisms (Kamel, 2000) including stimulation of secretion of digestive enzymes, bile, and mucus, improvement of nutrient digestibility (Mellor, 2000;

Received October 19, 2017.
Accepted September 1, 2018.
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Platel and Srinivasan, 2004), reduction of oxidative stress (Luna et al., 1994), and antimicrobial effects, thereby enhancing overall performance (Manafi, 2015).

Novacid is a commercial feed additive containing a combination of organic acids (propionic acid, fumaric acid, and lactic acid), glucomannan oligosaccharides, and a proprietary mix of spice extracts. Organic acids have bactericidal effects against *Salmonella* in poultry feeds (Ricke, 2003). Feed with individual or combinations of organic acids could reduce bacterial colonization in the broiler intestine (Hinton and Linton, 1988; Chaveerach et al., 2002), particularly of acid-intolerant bacteria such as *E. coli*, *Salmonella,* and *Campylobacter* (Dibner and Buttin, 2002). Glucomannans are a subclass of mannans (Schröder et al., 2009) mainly comprising mannose and glucose sugars with desirable nutritional and health characteristics, such as dietary fiber (Chen et al., 2008). In addition, glucomannan is a prebiotic ingredient, which can stimulate the growth of *Lactobacillus* and *Bifidobacterium*, suppress pathogenic bacterial growth, and increase the production of short-chain fatty acids (Chen et al., 2005; Elamir et al., 2008; Connolly et al., 2010). Mannan oligosaccharides directly affect broilers gut health by improving the integrity of the intestinal mucosa, attaching to *E. coli* and *Salmonella,* and preventing these pathogens from infiltrating the intestinal wall (Manafi et al., 2016). Organic acids like formic acid could enhance the digestibility of proteins and amino acids by increasing gastric proteolysis and improving digestive processes. In addition, plant extracts such as thymol, carvacrol, cinnamaldehyde, and limonene are used as substitutes for AGP (Applegate et al., 2010). A wide range of EO derived from spice extracts have antimicrobial, antioxidant, and antiseptic properties (Jang et al., 2007). The addition of plant extracts to the diet of broilers has shown varying effects on growth performance (Garcia et al., 2007; Jang et al., 2007; Brenes and Roura, 2010; Manafi et al., 2016).

The aim of this study was to determine the effects of Novacid, in comparison with those of the AGP bacitracin methylene disalicylate (**BMD**), on normal performance, nutrient digestibility, and organ weight of broiler chickens with or without *E. coli* challenge.

MATERIALS AND METHODS

Experimental Protocol and Sample Collection

Six hundred 1-d-old Ross × Ross 308 male broiler chickens were obtained from a local hatchery, weighed, and randomly allocated into six treatment groups with five replicates, each containing 20 birds. The broiler chicks were fed a basal diet consisting of corn and soybean meal (control) and divided into two groups: (1) unchallenged control and (2) control challenged with *E. coli* (400 mg/kg *Escherichia coli* inoculation). Each group was divided into the following study groups: (1) untreated control; birds treated with 0.05% Novacid, and birds treated with 400 mg/kg BMD. On day 10, chicks in the *E. coli* infection groups were intramuscularly (**IM**) inoculated with 0.5 mL of nutrient broth culture containing 10^8 colony-forming units (**CFU**) of *E. coli* per mL (Manafi et. al., 2017) in the right breast.

The Novacid feed supplement (Zeus Biotech Private Limited, Mysore, India) and BMD were procured from a local market in powder form and added to experimental diets by creating a premix to ensure proper distribution throughout the diet. The feeding regimen (Table 1) consisted of starter (1 to 14 d), grower (15 to 28 d), and finisher (29 to 42 d) diets. The diets were provided as mash and prepared with the same batch of ingredients for the starter, grower, and finisher periods, formulated to meet the nutrient requirements specified by the Ross 308 Aviagen rearing guidelines (Aviagen, 2014). All birds had ad libitum access to feed and water. Replicate groups of chicks were housed in independent pens. The rearing conditions and vaccination schedule were based on those described by Manafi et al. (2017); birds were housed in a conventional sided deep litter house at the University Experimental Poultry Farm. All chicks were exposed to 23 h light with 30 lux intensity for the first 7 d, and 10 lux intensity for the rest of the study. Temperature was initially set at 32°C on d 1 and decreased linearly by 0.5°C per day to 21°C by using a thermostatically controlled heating system.

Table 1. Composition of basal and experimental diets (g/kg unless otherwise stated).

	Starter (1 to 14 d)	Grower (15 to 28 d)	Finisher (29 to 42 d)
Ingredients			
Corn (8% CP)	545.5	540	567
Soybean meal (43% CP)	401	390	360
Soybean oil	11	33.2	39
Calcium carbonate	10.6	8.9	8.7
Dicalcium phosphate[a]	19.1	17.3	15.7
DL-methionine	3	2.1	1.6
L-lysine	1.3	-	-
Vitamin premix[b]	2.5	2.5	2.5
Mineral premix[c]	2.5	2.5	2.5
Salt	3.5	3.5	3
Nutrient composition			
Metabolizable energy (MJ/kg)	11.76	12.47	12.76
CP (%)	21.5	21.0	20.0
Ca (%)	0.97	0.86	0.81
Available phosphorous (%)	0.46	0.43	0.40
Methionine + cysteine (%)	1.0	0.9	0.82
Lysine (%)	1.32	1.19	1.11

[a]Dicalcium phosphate contained: 16% phosphorous and 23% calcium.
[b]Vitamin premix per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3(Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin K3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; pantothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg.
[c]Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4.5H_2O$), 10 mg; I (KI, 58% I), 1 mg; Se ($NaSeO_3$, 45.56% Se), 0.2 mg.

Vaccination Schedule

Newcastle disease (**ND**) vaccination was carried out on the first day (by spray in the hatchery), and repeated on day 12 as B_1 (CEVA) added to drinking water, with a booster on day 20 as clone-30 (HIPRAVIAR) added to drinking water. Vaccination against infectious bronchitis virus was carried out twice as follows: first spray at commencement of the experiment, and booster in drinking water on day 10, both as H-120 (CEVA). Vaccination against infectious bursal disease was carried out twice: first on day 15 and second on day 24, both as Gambo-l (CEVA) in the drinking water. The booster, B_1 neurotropic vaccine strain virus (ND 6/10; CEVA) was provided in drinking water at 3 wk-of-age after a hemagglutination inhibition titer test of sera to determine levels of antibody to ND in the blood.

Performance

All birds were weighed individually after arrival and subsequently on days 7, 14, 21, 28, 35, and 42 by using a digital electronic top pan balance with 0.1 g accuracy. Feed consumption was recorded on a pen basis by measuring leftover feed at the same time as the live weight recording. The feed conversion ratio (**FCR**) was calculated as feed to gain ratio. Dead birds were subjected to thorough post-mortem examination to identify the cause of death. The survival percentage of the birds was recorded weekly.

Measurement of Visceral Organs

At the end of the trial, 10 birds from each treatment group were randomly selected and killed by cutting the jugular vein. Each bird was weighed individually, the abdominal cavity opened, and the internal organs removed. The thymus, spleen, and the bursa of Fabricius were removed and weighed on a mono pan balance (0.1 mg accuracy), and the liver, kidney, pancreas, and gizzard were weighed on a digital top pan balance (0.1 g accuracy). The weights were adjusted to 1 kg live weight. The study was approved by the ethics committee of the Malayer University.

Nutrient Digestibility

Ileal contents were removed aseptically from 10 birds from each treatment group (two birds from each replicate) and freeze-dried. For chemical analysis of the ileal digesta samples, dry matter (**DM**) was determined by the method described by AOAC (1984), and gross energy (**GE**) was measured by the AOAC method (2000) by using a bomb calorimeter (Smith et al., 2000). Crude fat content of the excreta was determined by the acid hydrolysis method (AOAC, 1975). Freeze-dried excreta were analyzed for crude protein (**CP**) according to the AOAC method (1984) modified by Naumann and Bassler (1993).

Gastrointestinal Tract and Feed pH

The crop, proventriculus, gizzard, duodenum, jejunum, and ileum were separated from 10 birds in each treatment group (two birds from each replicate). These were cut longitudinally and the digesta contents were removed aseptically into 90 mL sterilized physiological saline (1:10 dilution) in plastic tubes. A digital portable pH meter (HACH IQ150 pH/mV/Temperature System, Loveland, CO) was placed directly into the digesta, and the pH was measured in triplicate. The same pH meter was used to measure the compound feed pH (Xu et al., 2015).

Determination of Cecal and Food Bacterial Count

On day 42, two birds from each cage were slaughtered and cecal contents were extracted and pooled. Microbial populations were determined by serial dilution (10^{-1} to 10^{-9}) of cecal samples in anaerobic diluents before inoculation onto Petri dishes containing sterile agar as described by Gunal et al. (2007). *E. coli* were grown on eosin methylene blue agar, *Salmonella* on *Salmonella Shigella* agar (SS agar; Merck, Germany), and coliforms on MacConkey agar (Darmstadt, Germany). *E. coli* was incubated aerobically at 37°C. Plates were counted between 24 and 48 h after inoculation. CFUs were defined as distinct colonies measuring at least 1 mm in diameter. Nine sterile test tubes with lids containing 9 mL of phosphate buffer solution as diluent were aseptically prepared and approximately 1 g of the cecal contents taken by sterile swab and homogenized for 3 min were added and mixed before transfer to the microbiology laboratory (Gunal et al., 2007). Then, 1 mL from each 10 mL of buffer plus cecal sample was removed, transferred to a tube, and mixed thoroughly. The procedure was continued to complete all the dilutions. One milliliter from each test tube was transferred to selective media agar plates, which were incubated at 37°C for 24 h. Finally, the intestinal bacterial colony populations formed on each plate were counted manually.

Survivability Rate

The survivability rate was calculated based on the mortality data, which were recorded daily throughout the trial, and percentage survivability rates were calculated accordingly.

Statistical Analysis

Data were subjected to one-way ANOVA using the GLM procedure on the SAS 9.2 software (SAS, 2007). These data were analyzed using a completely

Table 2. Effect of dietary treatments on broiler performance.

	Dietary treatments							
	Unchallenged with *E. coli*			Challenged with *E. coli*				
Day	Control	BMD (400 mg/kg)	NOVACID (0.05%)	Control	BMD (400 mg/kg)	NOVACID (0.05%)	*P*-value	SEM
Average body weight (g)								
1	46.2	46.2	46.8	45.8	46.0	46.0	0.73	16.0328
7	202.2^{a}	205.8^{a}	205.2^{a}	180.0^{c}	191.2^{b}	185.0^{b,c}	0.02	64.7514
14	435.2^{a,b}	453.6^{a}	450.0^{a}	422.0^{b}	438.0^{a,b}	443.0^{a}	0.04	149.8153
21	794.8^{b,c}	842.0^{a}	814.6^{b}	744.0^{d}	783.0^{c}	774.4^{c}	0.02	283.5267
28	1325.2^{b}	1375.0^{a}	1365.6^{a}	1264.0^{c}	1320.0^{b}	1315.0^{b}	0.04	434.2138
35	1798.4^{a,b}	1849.8^{a,b}	1821.6^{a,b}	1558.8^{d}	1797.6^{b}	1720.8^{c}	0.01	563.5529
42	2216.6^{b}	2461.8^{a}	2476.8^{a}	2078.4^{c}	2155.6^{b,c}	2112.4^{c}	0.02	810.2538
Feed consumption (g)								
0–42	3892.4^{a}	3769.8^{b}	3763.0^{b}	3956.6^{a}	3916.0^{a}	3904.2^{a}	0.01	1333.5719
Feed conversion ratio (FCR)								
0–42	1.756^{c}	1.532^{d}	1.520^{d}	1.904^{a}	1.814^{b,c}	1.846^{a,b}	0.001	0.6117

[a-d]Means with different superscript letters in a row are significantly different. SEM: standard error of means.
BMD: bacitracin methylene disalicylate.
Challenged *E. coli*: intramuscularly (IM) inoculated into right breast with 0.5 ml of nutrient broth culture containing 10^8 colony-forming units (CFU) of *E. coli* per mL.
NOVACID: combination of organic acids, spice extracts, and glucomannan.

randomized design to examine the overall effect of the treatments. Pen means were used as the experimental units for all variables evaluated. For multiple comparisons of means, Tukey's post-hoc test was used. Differences were considered significant at $P < 0.05$.

RESULTS

Performance

The average body weight (**ABW**), feed consumption, and FCR are shown in Table 2. The ABW of the broiler chickens at the beginning of the study did not differ between treatment groups, indicating uniformity between replicates. At day 42, in the unchallenged groups, ABW was significantly higher in the Novacid and BMD treatment groups ($P < 0.05$) than in the control group. Further, the Novacid-fed groups showed slightly higher ABW compared to the BMD-fed birds. No significant differences were observed between the *E. coli*-challenged groups. A similar trend was observed in terms of feed consumption and FCR; however, feed conversion in broilers challenged with *E. coli* was significantly more efficient in groups receiving Novacid and BMD treatment ($P < 0.05$), compared to the corresponding control group.

Nutrient Digestibility

The digestibility of DM remained unaffected between all treatments. Among the unchallenged *E. coli* groups, crude fat was significantly increased in the BMD-treated group, CP was significantly increased in the Novacid treated group, and GE was significantly decreased in the Novacid-treated group ($P < 0.05$), compared to the corresponding control groups. In the *E. coli*-challenged groups, the digestibility of crude fat and CP were significantly increased and GE significantly decreased in the BMD and Novacid treated groups ($P <$ 0.05), compared to the controls (Table 3).

Gastrointestinal Tract and Feed pH

No significant differences were found in the pH of the feed or gastrointestinal tract among all dietary treatment groups on day 42 (Table 4).

Cecal and Feed Bacterial Count

In the unchallenged groups, addition of Novacid and BMD could significantly decrease ($P < 0.05$) coliform, *Salmonella*, and *E. coli* counts in the cecum, compared with the corresponding control groups. The same trend in cecal bacterial count was observed in the *E. coli*-challenged groups (Table 5). The addition of Novacid and BMD decreased the population of feed coliforms and *Salmonella* significantly ($P < 0.05$) in the unchallenged groups. Novacid treatment reduced the feed *E. coli* count in the unchallenged groups compared to the corresponding control and BMD treatment groups. In the *E. coli*-challenged groups, addition of Novacid and BMD into the diets could significantly ($P < 0.05$) reduce the coliform, *Salmonella* and *E. coli* counts in feed, compared to the respective control treatments (Table 6).

Survival Rate

The dietary treatments showed no significant effects on the percentage survival rate of broilers in the *E. coli*-challenged and unchallenged groups on day 42 (Table 7).

Table 3. Effect of dietary treatment on ileal nutrient digestibility in broilers at 42 d.

	Dietary treatments							
	Unchallenged with *E. coli*			Challenged with *E. coli*				
Apparent digestibility (%)	Control	BMD (400 mg/kg)	NOVACID (0.05%)	Control	BMD (400 mg/kg)	NOVACID (0.05%)	*P*-value	SEM
DM	77.17	79.31	78.04	76.59	77.76	77.67	0.13	27.3427
Crude fat	83.05^{b}	85.83^{a}	84.26^{a,b}	78.47^{d}	81.22^{c}	80.49^{c}	<.0001	28.3168
CP	71.84^{b}	73.12^{a,b}	74.83^{a}	65.74^{d}	70.84^{b,c}	69.24^{c}	<.0001	24.4653
GE	81.88^{b}	85.42^{a}	78.40^{c,d}	75.61^{d}	72.03^{e}	70.67^{e}	<.0001	27.2317

$^{a\text{-}e}$Means with different superscript letters in a row are significantly different. SEM: standard error of means.
BMD: bacitracin methylene disalicylate.
Challenged *E. coli*: intramuscularly (IM) inoculated into right breast with 0.5 ml of nutrient broth culture containing 10^8 colony-forming units (CFU) of *E. coli* per mL.
NOVACID: combination of organic acids, spice extracts, and glucomannan.

Table 4. Effect of dietary treatment on gastrointestinal tract and feed pH at 42 d.

	Dietary treatments							
	Unchallenged with *E. coli*			Challenged with *E. coli*				
pH	Control	BMD (400 mg/kg)	NOVACID (0.05%)	Control	BMD (400 mg/kg)	NOVACID (0.05%)	*P*-value	SEM
Crop	5.16	4.83	4.83	5.16	4.66	4.83	0.75	1.7342
Proventriculus	2.83	3.33	2.16	3.66	3.50	3.33	0.1906	1.0924
Gizzard	3.16	2.16	3.16	2.50	2.50	2.33	0.801	0.9528
Duodenum	6.33	6.16	6.16	6.66	6.16	6.33	0.90	2.1834
Jejunum	6.00	6.16	6.66	6.66	6.50	6.83	0.90	2.2167
Ileum	7.50	7.33	6.66	7.50	7.50	7.33	0.511	2.3641
Feed	6.66	6.16	6.33	6.66	6.66	6.33	0.841	2.2357

SEM: standard error of means.
BMD: bacitracin methylene disalicylate.
Challenged *E. coli*: intramuscularly (IM) inoculated into right breast with 0.5 ml of nutrient broth culture containing 10^8 colony-forming units (CFU) of *E. coli* per mL.
NOVACID: combination of organic acids, spice extracts, and glucomannan.

Table 5. Effect of dietary treatment on cecal bacterial count ($\log_{10}$) of broilers at 42 d.

	Dietary treatments							
	Unchallenged with *E. coli*			Challenged with *E. coli*				
Bacterial count	Control	BMD (400 mg/kg)	NOVACID (0.05%)	Control	BMD (400 mg/kg)	NOVACID (0.05%)	*P*-value	SEM
Coliform	1.90^{b}	1.08^{d}	1.20^{c,d}	2.96^{a}	1.57^{b,c}	1.77^{b}	<.0001	0.7413
Salmonella	1.88^{a,b}	1.02^{c}	1.56^{b}	2.08^{a}	1.60^{b}	1.87^{a,b}	<.0001	0.5438
E. Coli	2.07^{b,c}	1.03^{d,e}	1.48$^{c\text{-}e}$	2.84^{a}	1.66$^{b\text{-}d}$	2.23^{a,b}	<.0001	0.6192

$^{a\text{-}e}$Means with different superscript letters in a row are significantly different. SEM: standard error of means.
BMD: bacitracin methylene disalicylate.
Challenged *E. coli*: intramuscularly (IM) inoculated into right breast with 0.5 ml of nutrient broth culture containing 10^8 colony-forming units (CFU) of *E. coli* per mL.
NOVACID: combination of organic acids, spice extracts, and glucomannan.

Table 6. Effect of dietary treatment on food bacterial count ($\log_{10}$) at 42 d.

	Dietary treatments							
	Unchallenged with *E. coli*			Challenged with *E. coli*				
Bacterial count	Control	BMD (400 mg/kg)	NOVACID (0.05%)	Control	BMD (400 mg/kg)	NOVACID (0.05%)	*P*-value	SEM
Coliform	1.50^{b}	0.99^{c}	1.01^{c}	2.59^{a}	1.13^{c}	1.54^{b}	<.0001	0.5908
Salmonella	1.57^{b}	1.00^{d}	1.34^{b,c}	2.02^{a}	1.54^{b}	1.16^{c,d}	<.0001	0.6862
E. coli	2.07^{a}	2.02^{a}	1.14^{b}	2.00^{a}	1.30^{b}	1.14^{b}	<.0001	0.6615

$^{a\text{-}d}$Means with different superscript letters in a row are significantly different. SEM: standard error of means.
BMD: bacitracin methylene disalicylate.
Challenged *E. coli*: intramuscularly (IM) inoculated into right breast with 0.5 ml of nutrient broth culture containing 10^8 colony-forming units (CFU) of *E. coli* per mL.
NOVACID: combination of organic acids, spice extracts, and glucomannan.

Table 7. Effect of dietary treatments on percent survival of broilers at 42 d.

	Dietary treatments							
	Unchallenged with *E. coli*			Challenged with *E. coli*				
Day	Control	BMD (400 mg/kg)	NOVACID (0.05%)	Control	BMD (400 mg/kg)	NOVACID (0.05%)	*P*-value	SEM
7	99	100	100	100	99	99	0.81	34.8252
14	100	100	100	99	100	100	0.17	36.4297
21	99	100	98	98	98	100	0.63	34.4294
28	100	99	99	98	98	99	0.59	35.7398
35	99	99	100	99	98	98	0.84	34.6927
42	98	99	99	98	99	99	0.63	33.9829

SEM: standard error of means.
BMD: bacitracin methylene disalicylate.
Challenged *E. coli*: intramuscularly (IM) inoculated into right breast with 0.5 ml of nutrient broth culture containing 10^8 colony-forming units (CFU) of *E. coli* per mL.
NOVACID: combination of organic acids, spice extracts, and glucomannan.

Table 8. Effect of dietary treatments on organs relative weight of broilers at 42 d.

	Dietary treatments							
	Unchallenged with *E. coli*			Challenged with *E. coli*				
	Control	BMD (400 mg/kg)	NOVACID (0.05%)	Control	BMD (400 mg/kg)	NOVACID NOVACID (0.05%)	*P*-value	SEM
Breast	$25.00^{a,b}$	27.66^{a}	$26.16^{a,b}$	22.33^{b}	$24.66^{a,b}$	$23.66^{a,b}$	0.012	8.4875
Liver	5.16	5.83	5.66	5.16	5.00	5.16	0.90	1.8927
Kidney	7.16	8.16	8.33	6.66	7.33	7.16	0.10	2.6391
Pancreas	3.83	4.33	4.00	3.50	3.66	3.83	0.93	1.3265
Bursa of Fabricius	1.66	1.50	1.33	1.83	1.50	1.66	0.71	0.5215
Heart	3.83	4.16	4.00	3.66	3.66	3.83	0.99	1.3376

$^{a\text{-}d}$Means with different superscript letters in a row are significantly different. SEM: standard error of means.
BMD: bacitracin methylene disalicylate.
Challenged *E. coli*: intramuscularly (IM) inoculated into right breast with 0.5 ml of nutrient broth culture containing 10^8 colony-forming units (CFU) of *E. coli* per mL.
NOVACID: combination of organic acids, spice extracts and glucomannan.

Relative Weights of Organs

The weight of breast was maximum in the unchallenged group that received BMD treatment, and *E. coli* infection reduced the breast weight significantly in the control group. The addition of Novacid and BMD could partially reverse the adverse effects of *E. coli* infection (Table 8). Inclusion of BMD or Novacid in the diet did not significantly alter the relative organ weights of the liver, kidney, pancreas, bursa of Fabricius, and heart in the *E. coli*-infected or uninfected groups.

DISCUSSION

Antibiotic-mediated growth enhancement was first observed by Moore et al. (1946). Following the discovery by Jukes (1972) that Aureomycin stimulated significant growth in chickens, cattle, and pigs, the use of antibiotic growth promotors in the agriculture industry became widespread. AGP have contributed to large financial profits for producers and low cost for consumers. However, the use of AGP has been linked to the increase in the incidence of antimicrobial-resistant bacterial infections, resulting in widespread sanctions imposed on their use. The discovery and development of effective alternatives to AGP is therefore of great economic significance. In this study, we compared the efficacy of the commercially available phytochemical growth supplement Novacid™ to that of the AGP BMD.

The ABW on day 42 was significantly increased by Novacid and BMD supplementation in the unchallenged groups. Further, the Novacid-fed unchallenged groups showed slightly higher ABW compared to the BMD-fed groups. These findings are in agreement with those of Guo et al. (2000), Jamroz and Kamel (2002), Pereira et al. (2015), and Manafi et al. (2017) who reported that diet supplementation with plant-derived products had a significant beneficial effect on broiler body weight gain. A significant decrease in food consumption and FCR was observed in the Novacid and BMD-fed unchallenged groups, which is similar to the findings of Cross et al (2002) who reported a significant decrease in feed intake upon oregano supplementation of broiler feed. There have been several contradictory reports wherein supplementation with various phytochemicals had no effect on weight or feed intake (Mitsch et al., 2004; Frankic et al., 2009; Puvača et al., 2013; Suganya et al., 2016). Our results were likely different because of the composition of Novacid, which contains organic acids, spice extracts, and glucamannan oligosaccharides, each of which promote immunity and digestive functions. The FCR in broilers challenged with *E. coli* was significantly more efficient in groups receiving Novacid and BMD treatment ($P < 0.05$) compared to the corresponding control group. However, the results in the unchallenged and challenged groups were not comparable, indicating that neither feed supplement could completely ameliorate the effects of the *E. coli* challenge.

Novacid showed a significant effect on food digestibility in broilers. This is likely due to the effects of the organic acids, which promote digestibility of feed components, and spice extracts, which enhance gastric secretions (Hernández et al., 2004). Further, the addition of Novacid or BMD could significantly decrease cecum coliform, *Salmonella*, and *E. coli* counts and feed coliforms and *Salmonella* in the unchallenged groups. This is in agreement with the findings of Jamroz and Kamel (2002), who reported that herbal dietary additives resulted in lower *E. coli and C. perfringens* cecal counts. Novacid treatment reduced the feed *E. coli* count in the unchallenged groups compared to the corresponding control and BMD treatment groups. These effects are likely due to the individual components of Novacid. Organic acids exert antibacterial actions by decreasing the pH of drinking water and reducing the feed buffering (Van Immerseel et al., 2006; Valenzuela-Grijalva et al., 2017). Organic acids can enhance their antimicrobial effect by converting from undissociated to dissociated form; in the undissociated form, acids can diffuse through the cell membrane into the microbial cell (Van Immerseel et al., 2006), where they dissociate and suppress enzymes and nutrient transport systems. Fermentable carbohydrates such as glucomannan affect gut immunity by increasing resistance to microbial colonization and enhancing immune activity (Fernandez et al., 2002; Hedemann et al., 2009; Ito et al., 2009). The antimicrobial properties of spice extracts have also been extensively documented (Liu et al., 2017). In the *E. coli*-challenged groups, addition of Novacid and BMD into the diets could significantly reduce the number of coliform, *Salmonella* and *E. coli* counts in feed.

We did not observe significant effects of feed supplementation on GI-tract pH, indicating that neither feed supplement disrupted the pH balance in the GI tract. There was no significant effect of feed supplementation on survival, indicating that the *E. coli* challenge did not cause death in the birds, despite affecting digestion and body weight. This was similar to the observations of Pereira et al (2015). No significant differences in relative organ weight were observed between the groups; however, supplementation with Novacid or BMD partially reversed the adverse effects of *E. coli* challenge on breast weight. The *E. coli* challenge likely did not significantly affect the various organs, but affected digestion, and therefore body and muscle mass.

Thus, our results demonstrated that the non-antibiotic feed additive Novacid has comparable effects to those of BMD in broilers in terms of performance and digestibility, and feed and cecal bacterial content. Although neither supplement could completely restore performance to the levels observed in the unchallenged groups after *E. coli* challenge, both treatments could partially restore performance (close to the level of the unchallenged control). Moreover, Novacid proved more efficacious than BMD in reducing feed bacterial count. Thus, even in case of *E. coli* challenge, Novacid could prevent significant detrimental effects. Further studies on the physiological effects of Novacid at the cellular level, and on individual components of Novacid and their effect on broiler performance could result in improvement in the efficacy of feed supplements. In conclusion, our present results indicate that the phytochemical feed additives Novacid may be used as an effective environment-friendly alternative to an AGP in healthy broiler chickens. Future research to evaluate the effectiveness of such dietary herbal growth promoters and improve their efficacy in case of bacterial challenge would be of great value.

ACKNOWLEDGMENTS

The authors thank the personnel of the Nutrition and Microbiology Laboratory, Department of Animal Science, Malayer University, for diet formulation, animal care, and assistance with intestinal content sampling.

REFERENCES

Applegate, T. J., V. Klose, T. Steiner, A. Ganner, and G. Schatzmayr. 2010. Probiotics and phytogenics for poultry: Myth or reality? J. Appl. Poult. Res. 19:194–210.

AOAC International. 1975. Official Methods of Analysis of the Association of Official Analytical Chemists. 12th ed. Association of Official Analytical Chemists, Washington, DC.

AOAC International. 1984. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th ed. Association of Official Analytical Chemists, Washington, DC.

AOAC International. 2000. Method 990.03: Periodic acid Schiff staining. In Official Methods of Analysis of AOAC International. 17th ed. Association of Official Analytical Chemists International, Gaithersburg, MD.

Aviagen. 2014. Ross 308: Broiler Nutrition Specification. Aviagen Inc.; Huntsville, AL.

Brenes, A., and E. Roura. 2010. Essential oils in poultry nutrition: Main effects and modes of action. Anim. Feed Sci. Technol. 158:1–14.

Chaveerach, P., D. A. Keuzenkamp, H. A. Urlings, L. J. Lipman, and F. Vanknapen. 2002. In vitro study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. Poult. Sci. 81:621–628.

Chen, H. L., H. C. Cheng, W. T. Wu, Y. J. Liu, and S. Y. Liu. 2008. Supplementation of konjac glucomannan into a low-fiber Chinese diet promoted bowel movement and improved colonic ecology in constipated adults: a placebo-controlled, diet-controlled trial. J. Am. Coll. Nutr. 27:102–108.

Chen, H. L., Y. H. Fan, M. E. Chen, and Y. Chan. 2005. Unhydrolyzed and hydrolyzed konjac glucomannans modulated cecal and fecal microflora in Balb/c mice. Nutrition. 21:1059–1064.

Connolly, M. L., J. A. Lovegrove, and K. M. Tuohy. 2010. Konjac glucomannan hydrolysate beneficially modulates bacterial composition and activity within the faecal microbiota. J. Funct. Foods 2:219–224.

Cross, D. E., T. Acamovic, S. G. Deans, and R. M. McDevitt. 2002. The effects of dietary inclusion of herbs and their volatile oils on the performance of growing chickens. Br. Poultry Sci. 43:33–35.

Dibner, J. J., and P. Buttin. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11:453–463.

Elamir, A. A., R. F. Tester, F. H. Al-Ghazzewi, H. Kaal, A. A. Ghalbon, N. A. Elmegrahai, and J. Piggott. 2008. Effects of konjac glucomannan hydrolysates on the gut microflora of mice. Nutr. Food Sci. 38:422–429.

Fernandez, F., M. Hinton, and B. Van Gils. 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella* Enteritidis colonization. Avian Pathol. 31:49–58.

Frankic, T., M. Voljg, J. Salobir, and V. Rezar. 2009. Use of herbs and spices and their extracts in animal nutrition. Acta. Agr. Slov. 92:95–102.

Garcia, V., P. Catala-Gregori, F. Hern ández, M. D. Megias, and J. Madrid 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. J. Appl. Poult. Res. 16:555–562.

Gunal, M., O. Yayli, N. Kaya, N. Karahan, and O. Sulak. 2007. The effects of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. Int. J. of Poult. Sci. 5:149–155.

Guo, F., R. P. Kwakkel, and M. W. A. Verstegen. 2000. The use of Chinese herbs as alternative for growth promoters in broiler diets. Proc. XII World's Poult. Cong. 20–24 Aug., 2000, Montreal, Canada.

Hedemann, M. S., P. K. Theil, and K. E. Bach-Knudsen. 2009. The thickness of the intestinal mucous layer in the colon of rats fed various sources of non-digestible carbohydrates is positively correlated with the pool of SCFA but negatively correlated with the proportion of butyric acid in digesta. Br. J. Nutr. 102:117–125.

Hernández, F., J. Madrid, V. García, J. Orengo, and M. D. Megías. 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult. Sci. 83:169–174.

Hinton, M., and A. H. Linton. 1988. Control of *Salmonella* infections in broiler chickens by the acid treatment of their feed. Vet. Rec. 123:416–421.

Ito, H., M. Satsukawa, E. Arai, K. Sugiyama, K. Sonoyama, S. Kiri-yama, and T. Morita. 2009. Soluble fiber viscosity affects both goblet cell number and small intestine mucin secretion in rats. J. Nutr. 139:1640–1647.

Jamroz, D., and C. Kamel. 2002. Plant extracts enhance broiler performance. Poult. Sci. Assoc. 91stAnnu. Meeting, 11–14 Aug., 2002, Newark, Delaware. Poult. Sci. 80:41(Abstr).

Jang, I. S., Y. H. Ko, S. Y. Kang, and C. Y. Lee 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed Sci. Technol. 134:304–315.

Jukes, T. H. 1972. Antibiotics in animal feeds and animal production. Bioscience 22:526–534.

Kamel, C. 2000. A novel look at a classic approach of plant extracts. Feed mix. 8:19–23.

Liu, Q., X. Meng, Y. Li, C. N. Zhao, G. Y. Tang, and H. B. Li. 2017. Antibacterial and antifungal activities of spices. Int. J. Mol. Sci. 18:1283.

Luna, C. M., C. A. Gonzalez, and V. S. Trippi. 1994 Oxidative damage caused by an excess of Copper in oat leaves. Plant Cell Physiol. 35:11–15.

Manafi, M. 2015. Comparison study of a natural non-antibiotic growth promoter and a commercial probiotic on growth performance, immune response and biochemical parameters of broiler chicks. J. Poult. Sci. 52:274–281.

Manafi, M., M. Hedayati, S. Khalaji, and M. Kamely. 2016. Assessment of a natural, non-antibiotic blend on performance, blood biochemistry, intestinal microflora, and morphology of broilers challenged with *Escherichia coli*. R. Bras. Zootec. 45: 745–754.

Manafi, M., M. Hedayati, S. Khalaji, and N. Pirany. 2017. Efficacy of *Bacillus subtilis* and bacitracin methylene disalicylate on growth performance, digestibility, blood metabolites, immunity, and intestinal microbiota after intramuscular inoculation with *Escherichia coli* in broilers. Poult. Sci. 96:1174–1183.

Mellor, S. 2000. Antibiotics are not the only growth promoters. World Poult. 16:14–15.

Mitsch, P., K. Zitterl-Eglseer, B. Kohler, C. Gabler, R. Losa, and I. Zimpernik. 2004. The effect of two different blends of essential oil components on the proliferation of Clostridium perfringens in the intestines of broiler chickens. Poult. Sci. 83:669–675.

Moore, P. R., A. Evension, T. D. Luckey, E. McCoy, C. A. Elvehjam, and E. B. Hart. 1946. Use of sulfasuxidine, streptothricin and streptomycin in nutritional studies with the chick. J. Biol. Chem. 165:437–441.

Naumann, C., and R. Bassler. 1993. Chemical Analysis of Feedstuffs (in German). VDLUFA-Verlag, Darmstadt, Germany.

Platel, K., and K. Srinivasan. 2004. Digestive stimulant action of spices: a myth or reality? Indian J. Med. Res. 119:167–179.

Pereira, R., J. F. M. Menten, C. Bortoluzzi, G. S. Napty, F. A. Longo, J. Vittori, M. C. Lourenco, and E. Santin. 2015. Organic acid blend in diets of broiler chickens challenged with *Clostridium perfringens*. J. Appl. Poult. Res. 24:387–393.

Puvača, N., V. Stanaćev, D. Glamočić, J. Lević, L. Perić, V. Stanaćev, and D. Milić. 2013. Beneficial effects of phytoadditives in broiler nutrition. Worlds Poult. Sci. J. 69:27–34.

Ricke, S. C. 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poult. Sci. 82: 632–639.

SAS Institute. 2007. SAS/STAT User's Guide. Version 9.2. SAS Institute Inc., Cary, NC.

Schröder, R., R. G. Atkinson, and R. J. Redgwell. 2009. Re-interpreting the role of endo-β-mannanases as mannan endotransglycosylase/hydrolases in the plant cell wall. Ann. Bot. 104:197–204.

Smith, T. N., G. M. Pesti, R. I. Bakalli, J. Kilburn, and H. M. Edwards, Jr. 2001. The use of near-infrared reflectance spectroscopy to predict the moisture, nitrogen, calcium, total phosphorus, gross energy, and phytate phosphorus contents of broiler excreta. Poult. Sci. 80:314–319.

Suganya, T., S. Senthilkumar, K. Deepa, J. Muralidharan, G. Gomathi, and S. Gobiraju. 2016. Herbal feed additives in poultry. Int. J. Sci. Env. Tech. 5:1137–1145.

Vandemaele, F., B. M. Goddeeris, and M. Vereecken. 2002. Incidence and antibiotic resistance of pathogenic *Escherichia coli* among poultry in Belgium. Vet. Rec. 151:355–356.

Valenzuela-Grijalva, N. V., A. Pinelli-Saavedra, A. Muhlia-Almazan, D. Domínguez-Díaz, and H. González-Ríos. 2017. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J. Anim. Sci. Technol. 59:8.

Van Immerseel, F., J. B. Russell, M. D. Flythe, I. Gantois, L. Timbermont, F. Pasmans, F. Haesebrouck, and R. Ducatelle. 2006. The use of organic acids to combat Salmonella in poultry: A mechanistic explanation of the efficacy. Avian Pathol. 35:182–188.

Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, W. J. Pacheco, and J. Brake. 2015. Effect of dietary coarsely ground corn on broiler live performance, gastrointestinal tract development, apparent ileal digestibility of energy and nitrogen, and digesta particle size distribution and retention time. Poult. Sci. 94:53–60.