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Comparative Effects of Blended Herbal Extracts and Mixed Prebiotics on Growth Performance, Carcass Yields and Intestinal Morphology of Broiler Chickens

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Abstract: An experiment was conducted to determine the effect of blended herbal extracts (BHEs; *Origanum vulgare* L., *Cinnamomum zeylanicum* Nees. and *Capsicum frutescens* L.) and mixed prebiotics (MPs; Mannanoligosaccharide and beta-glucan) in feed on the growth performance, carcass yield and intestinal morphology of broilers. Two hundred and seventy male broiler chickens (Arbor Acres) were divided into 3 groups and each group consisted of 5 replicates of 18 chicks each. The experiment was established as a completely randomized design. There were 3 experimental diets: 1) a control diet; 2) the control diet supplemented with blended herbal extracts (BHEs; 150 g/t); and 3) the control diet supplemented with mixed prebiotics (MPs; 2,000 g/t). At age 35 days, the results indicated that the feed conversion ratio (FCR) was significantly improved by supplementation with BHEs compared to MPs ($P<0.05$), while supplemental MPs seemed to increase the feed intake ($P=0.08$). Supplementation of BHEs or MPs significantly improved the breast meat yield and increased the villi height in the segment of the duodenum ($P<0.05$). However, the breast meat yield of the group fed MPs was higher than that of BHEs ($P<0.05$). In conclusion, supplementing mixed prebiotics or blended herbal extracts showed potential to promote the breast meat yield and improve the small intestine morphology by different mechanisms.

Key words: Herbal extracts, prebiotics, growth performance, intestinal morphology, broiler

INTRODUCTION

Generally, supplemental antibiotics in animal feed improve the health and growth performance of an animal. Due to increases in bacterial resistance and the risk of antibiotic residues in animal products, the use of antibiotics as a growth promoter is being prohibited (Hong *et al*., 2012; Bento *et al*., 2013). Subsequently, alternative feed additives have been intensively investigated (Casewell *et al*., 2003; Koiyama *et al*., 2013). Among the alternatives, phytogenics and prebiotics as feed additives to promote growth performance have been discovered and are being implemented in the broiler feed industry (Gibson *et al*., 2004; Windisch *et al*., 2008; Yang *et al*., 2015).

Essential oils and various extracts of plants have been of interest in the consideration of safe and healthy feed additives from natural resources. Hammer *et al*. (1999); Tabak *et al*. (1999); Bassett (2000); Windisch *et al*. (2008) reported that some chemical extracts from plants such as thymal and carvacrol from oregano (*Origanum vulgare* L.), cinnamaldehyde from cinnamon (*Cinnamomum zeylanicum* Nees) and capsaicin from peppers (*Capsicum frutescens* L.) potentially inhibit pathogenic bacterial growth, stimulate the digestive system and improve the growth performance of broiler chickens. Comparison effects of a single herbal extract (SHE) and blended herbal extracts (BHEs) on the growth performance of broiler chickens have been observed. Langhout (2000) found that growth performance of chicks fed a diet supplemented with BHEs seems to be better than that of the SHE. Furthermore, studies involving supplementation with a mixture of capsaicin, cinnamaldehyde and carvacrol (Rostagno *et al*., 2001) and a combination of oregano, cinnamon, thyme and chili pepper essential oils (Zhang *et al*., 2005) in broilers showed a better FCR compared to birds fed a control diet. Moreover, synergistic, beneficial effects of multi-herbal extracts components on the inhibition of microbial growth were observed (Moleyar and Narasimham, 1992; Montes-Belmont and Carvajal, 1998). Therefore, various BHEs are formulated and applied in the feed industry.

In addition, a prebiotics-mannanoligosaccharide (MOS) and beta-glucan derived from the cell wall of *Saccharomyces cerevisiae*-have been introduced and used as an alternative feed additive to antibiotics (Spring, 1999). It is generally known that MOS and beta-glucan are undigested by animal endogenous enzymes and reach the large intestine, consequently improving gut functions and inhibiting the activity of harmful bacteria in the intestinal tract (Gibson, 1998; Hooge, 2004; Zhang *et al*., 2005; Cheled-Shoval *et al*., 2011; Attia *et al*., 2014). In order to increase the efficacy of prebiotics, mixed prebiotics (MPs) have also been presented to the market (Gibson *et al*., 2004).

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Since the mode of action between herbal extracts and prebiotics is different, the effects of these alternative feed additives on the growth performance, carcass quality and gut functions may also differ. Therefore, the objective of this study was to compare the effects of supplemental BHEs and MP at the commercial recommended levels on the growth performance, carcass yield and intestinal morphology of male broiler chickens.

MATERIALS AND METHODS

Animals and management: The experimental animals were kept, maintained, treated and handled in accordance with accepted standards for the humane treatment of animals. Two hundred and seventy male broiler chickens (Arbor Acres) were used in this experiment. Birds were divided into 15 pens (18 birds/pen) and located in an evaporative cooling system during the 35 days of the feeding trial. Management and vaccination were provided according to commercial practices. Water and feed were offered *ad libitum* throughout the experiment.

Experimental diets: A completely randomized design was used. At age 1 day, chicks were divided into 3 treatments with 5 replications: 1) a control diet; 2) the control diet supplemented with a blend of herbal extracts (BHEs) at 0.015% (150 g/t of diet; combination of *Origanum vulgare* L., *Cinnamomum zeylanicum* Nees. and *Capsicum frutescens* L.); and 3) the control diet supplemented with mixed prebiotics (MPs) at 0.200% (2,000 g/t of diet; MOS and beta-glucan derived from the cell wall of *Saccharomyces cerevisiae*).

All the chicks were fed a starter diet until age 10 days, followed by a grower diet (age 11-24 days) and a finisher diet (age 25-35 days). The experimental diets were formulated to meet the recommended nutritional requirements of the Arbor Acres broiler strain as shown in Table 1. All diets were analyzed for protein, fat, calcium and total phosphorus according to the methods of the Association of Official Analytical Chemists (AOAC, 1990).

Determinations

Growth performance: The body weight gain (BWG), average daily gain (ADG), feed intake (FI) feed conversion ratio (FCR) and flock uniformity (CV%) were determined. Birds were weighed at age 10, 24 and 35 days. Feed consumption was determined during age 1-10, 11-24 and 24-35 days. Mortality was checked twice daily; dead birds were weighed to adjust the FCR.

Carcass yields measurements: At age 35 days, feed was removed for 12 hours before processing. Three broilers from each replication (5 replications) were killed using CO_2 asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO_2). The abdominal fat, breast meat, thigh, wing and drumstick were collected, weighed and calculated as a percentage of the live body weight.

Intestinal morphology: At age 35 days, 5 broilers from each treatment were killed using CO_2 asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO_2) and samples were taken at the median part of each of the 3 intestinal segments (duodenum, jejunum and ileum) for histological measurements. About a 0.5 cm portion of each sample was cleaned in phosphate buffer saline and fixed in 10% neutral buffer formalin for 24 h. The tissue samples were embedded in a paraffin box and sliced using a microtome, stained with hematoxylin-eosin and mounted on slides. The villous height, crypt depth and villous height per crypt depth ratio were measured under a light microscope in accordance with Nunez *et al.* (1996). Measurement of intestinal morphology used a computer-assisted image-analysis system (Biowizard; Thaitec, Thailand). The microscopic observation of the histological sections was randomized and assessment was made of the height of 10 villi and the depth of 10 crypts in each sample.

Statistical analysis: Data were evaluated using analysis of variance procedures appropriate for a completely randomized design and by comparing the differences of means for each experimental group using Duncan's new multiple range tests (Duncan, 1955) according to the following model:

$$Y_{ij} = \mu + A_i + \varepsilon_{ij}$$

where; Y_{ij} is the observed response, A_i is the effect of diet and ϵ_{ij} is experimental error; ϵ_{ij} ~NID $(0,\delta^2)$. Statements of statistical significance were based on $p<0.05$ All statistical analyses were done in accordance with the method of Steel and Torrie (1980).

RESULTS AND DISCUSSION

Growth performance: The effects of supplementing BHEs and MPs in the diets on the growth performance of broiler chicks are presented in Table 2. During the starter and the grower periods, the BWG, feed intake, FCR, mortality and flock uniformity were not affected by supplementations of BHEs or MPs. For the overall period (age 1-35 days), supplementing with PMs tended to increase the feed intake (P=0.08), and resulted in a poorer FCR than for the BHEs group (P<0.05), while supplementing with BHEs slightly improved the FCR compared to the control group.

Beneficial effects have been reported from supplementation using essential oils on the feed utilization and growth performance (Amerah *et al.*, 2012;

Table 1: Feed ingredients and nutrients composition of the experimental diets

Items	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-35 days)
Yellow corn	53.51	54.33	59.60
Soybean meal (48% CP)	36.11	34.47	29.44
Fat	5.03	6.75	6.75
Salt	0.16	0.16	0.17
L-Lysine-HCl	0.32	0.15	0.13
L-Threonine	0.12	0.04	0.03
DL-Methionine	0.39	0.29	0.24
Antioxidant	0.05	0.05	0.05
Premix, Broiler	0.50	0.50	0.50
Calcium carbonate	1.35	1.08	1.06
Monodicalciumphosphate (21% P)	2.46	2.18	2.03
Total	100.00	100.00	100.00
Nutrient composition (calculation)			
ME for poultry Kcal/Kg	3025.00	3151.66	3200.33
Protein %	22.00	21.01	19.00
Fat %	7.49	9.21	9.32
Fiber %	2.58	2.54	2.45
Lysine %	1.43	1.24	1.09
Met+Cys %	1.07	0.95	0.85
Methionine %	0.71	0.60	0.53
Threonine %	0.94	0.83	0.74
Arginine%	1.46	1.41	1.25
Tryptophan%	0.24	0.23	0.20
Calcium %	1.05	0.89	0.85
Available Phosphorus %	0.50	0.45	0.42
Sodium %	0.20	0.19	0.20

Vitamin and mineral premix content (composition per kg): Vitamin A 12,000,000IU, Vitamin D 3,000,000IU, Vitamin E 15,000 IU, Vitamin K 1500 mg, Thiamine 1,500 mg, Riboflavin 5,000 mg, Pyridoxine 2,000 mg, Niacin 25,000 mg, Vitamin B 504 mg, Pantothenic acid 8,000 mg, Folic acid 3,000 mg, Biotin 120 mg, Choline chloride 160 mg, Antioxidant 30 g, Manganese 80 g, Zinc 60 g, Iron 40 g, Copper 8 g, Iodine 0.50 g, Selenium 100 mg, Cobalt 100 mg

Pirgozliev *et al*., 2015; Bozkurt *et al*., 2014). In the current study, it seemed that supplemental BHEs potentially improved the FCR of the broiler chicks compared to the supplementation with MPs. Essential oils or various extracts from herbs or spices can stimulate the 3 peripheral sensing mechanisms (somatosensing, smell and taste) found in the oral and nasal cavities (Tominaga and Julius, 2000; Platel and Srinivasan, 2004; Srinivasan, 2007), and subsequently stimulate salivary, gastric, biliary and pancreatic secretions and the terminal digestive enzymes on the mucosa of the small intestine (Platel and Srinivasan, 1996; 2000; 2004). Accordingly, Lee *et al*. (2003) reported that mixed essential oils (cinnamaldehyde and thymol) increased the secretion of pancreatic digestive enzymes such as amylase, lipase, trypsin and chymotrypsin in broiler chickens. Consequently, Amad *et al*. (2011) stated that the addition of essential oils increased the apparent ileal digestibility of nutrients. Since the feed intake of chicks fed BHEs declined slightly and then the FCR was significantly improved, BHEs may influence the sensing mechanisms involved in voluntary feed intake and in the improvement of digestive enzymes activities. On the other hand, supplementation of MPs seemed to increase the feed intake. Toghyani *et al*. (2011); Bozkurt *et al*. (2009, 2014); Spring *et al*. (2000) and Samli *et al*. (2007) also showed that supplementations of prebiotics increased the feed intake and growth rate through gut fermentation processes. Without having a significant effect on the growth rate, it can be implied that the mechanism controlling the feed intake differed between supplementations with BHEs and MPs. Consequently, there was a difference in the FCR between these feed additives.

Carcass yields: The carcass yields of broiler chickens at age 35 days are shown in Table 3. Supplementation with both BHEs and MPs significantly promoted the breast meat (pectoralis major and pectoralis minor) yield ($P<0.05$), and the breast meat weight of the MP group was more than that of the BHEs group. There were no significant effects of the feed additives on the carcass yield, wing, thigh, drumstick and abdominal fat. Supplementation with both BHEs and MPs significantly increased the breast meat yield of broiler chickens. In terms of the BHEs supplementation, Isabel and Santos (2009) found that the breast meat was increased by adding clove and cinnamon oils. In addition, Nasir and Grashorn (2010) reported that the broiler fed mixture of plant extracts (*Echinacea purpurea* and *Nigella sativa*) in the diet showed a higher crude protein content in the breast meat, which indicated a probable positive effect on protein metabolism. Furthermore, Khattak *et al*. (2014) reported that a supplemental blend of essential

Table 2: Effects of dietary supplementation with BHEs and PMs on growth performance of broiler chicks

Item	Control	BHEs	MPs	SEM	P-value
Starter (age 0 - 10 d)					
Body weight gain (g)	251.85±4.62	253.43±7.69	247.63±3.46	1.48	0.27
Average daily gain (g)	25.18±0.46	25.34±0.77	24.76±0.35	0.15	0.27
Feed intake (g)	328.81±4.24	323.89±6.33	319.92±10.77	2.06	0.22
Feed conversion ratio	1.31±0.01	1.28±0.03	1.29±0.04	0.01	0.40
Mortality (%)	1.11±2.48	0.00±0.00	1.11±2.48	0.50	0.61
Flock uniformity (CV %)	8.91±1.89	9.94±1.78	8.82±1.43	0.43	0.53
Grower (age 11 - 24 d)					
Body weight gain (g)	1148.40±18.74	1126.41±44.58	1141.92±21.28	7.70	0.52
Average daily gain (g)	82.03±1.34	80.46±3.18	81.57±1.52	0.55	0.52
Feed intake (g)	1440.32±67.78	1383.18±18.76	1438.94±41.28	13.31	0.13
Feed conversion ratio	1.25±0.05	1.23±0.06	1.26±0.02	0.01	0.53
Mortality rate (%)	0.00±0.00	1.11±2.48	2.22±3.04	0.59	0.33
Flock uniformity (CV %)	9.83±1.37	10.32±1.46	9.58±1.56	0.36	0.72
Finisher (age 25 - 35 d)					
Body weight gain (g)	1395.71±62.79	1427.93±29.63	1420.69±49.92	12.36	0.57
Average daily gain (g)	126.88±5.71	129.81±2.69	129.15±4.54	1.12	0.57
Feed intake (g)	2150.49±76.60	2125.85±56.53	2211.57±35.22	17.01	0.09
Feed conversion ratio	1.54±0.03	1.49±0.05	1.56±0.04	0.01	0.06
Mortality rate (%)	3.39±5.01	1.17±2.63	0.00±0.00	0.87	0.28
Flock uniformity (CV %)	7.96±1.70	7.25±1.23	7.63±0.97	0.33	0.71
Overall (age 1-35 d)					
Body weight gain (g)	2751.35±64.22	2763.28±47.92	2765.75±36.53	12.27	0.89
Average daily gain (g)	78.61±1.83	78.95±1.37	79.02±1.04	0.35	0.89
Feed intake (g)	3919.19±130.49	3832.92±63.89	3970.43±52.41	26.16	0.08
Feed conversion ratio	$1.43±0.03^{AB}$	$1.39±0.03^{B}$	$1.44±0.03^{A}$	0.01	0.03
Mortality rate (%) (1-35 d)	4.44±6.09	2.22±4.97	3.33±3.04	1.36	0.77
Flock uniformity (CV %)	7.96±1.70	7.25±1.23	7.63±0.97	0.33	0.71

Values presented as mean ± SD

^{A,B}Means within a row with different letters indicate a significant difference (P<0.05)

BHEs = Blend herbal extracts; MPs = Mixed prebiotics

Table 3: Effects of dietary supplementation with BHEs and MP on carcass yields of broilers

Item	Control	BHEs	MPs	SEM	P-value
Carcass yield%	81.16±0.83	81.81±0.57	81.54±0.90	0.20	0.45
Breast%	$21.66±0.60^{C}$	$23.14±0.49^{B}$	$24.28±0.86^{A}$	0.33	<0.05
Wing%	6.99±0.21	6.50±1.49	7.01±0.24	0.22	0.60
Thigh%	12.65±0.26	12.72±0.42	12.55±0.24	0.08	0.68
Drumstick%	9.81±0.46	9.54±0.28	9.54±0.21	0.09	0.36
Abdominal fat%	2.05±0.36	2.07±0.13	2.08±0.36	0.07	0.98

Values are presented as mean ± SD

^{A,B}Means within a row with different letters are significantly different (P<0.05)

BHEs = Blend herbal extracts; MPs = Mixed prebiotics

oils (Tecnaroma Herbal Mix PL) promoted the breast meat yield and increased the villus width and surface area. This increase in the villus surface area indicated an improvement in nutrients absorption which then promoted the breast meat yield (Geyra *et al*., 2001).

In addition, several studies showed an improvement in the breast meat yield in broiler chickens caused by the supplementation of prebiotics in the diet (Piray *et al*., 2007 and Khaksar *et al*., 2008; Falaki *et al*., 2011; Albino *et al*., 2006). Khaksar *et al*. (2008) illustrated that the improvement in the breast meat yield may be associated with the partial replacement of intestinal beneficial microflora and the more efficient uptake of essential amino acid. Since the supplementation with PMs promoted the breast meat yield and feed intake rather than with BHEs, it appears that the higher nutrients consumption due to supplementing with PMs may support higher breast meat production. However, in this study, the exact mechanisms related to breast meat development between BHEs and MPs were unclear.

Intestinal morphology: The effects of supplementing with BHEs and MP on the villi height, crypt depth and villi height per crypt depth ratio of broiler chickens at age 35 days are presented in Table 4. Supplemental BHEs and MP significantly increased the villi height in the segment of the duodenum (P<0.05). The villi height in the jejunum and ileum, crypt depth and villus height per crypt depth ratio in all parts of the small intestine were not significantly influenced by dietary treatments.

The villi height and crypt depth of the small intestine play a crucial role in nutrient digestion and absorption

Table 4: Effects of dietary supplementation with BHEs and MP on intestinal morphology of broilers

Item	Control	BHEs	MPs	SEM	P-value
Villus height (µm)					
Duodenum	974.31±39.36[B]	1144.19±114.41[A]	1057.69±32.24[A]	25.34	0.01
Jejunum	1095.86±82.47	1041.82±110.94	1005.17±126.24	27.69	0.43
Ileum	903.75±214.87	637.32±199.02	748.27±111.78	52.20	0.10
Crypt depth (µm)					
Duodenum	240.11±81.45	288.09±83.19	216.50±34.94	18.57	0.29
Jejunum	248.46±64.44	203.53±50.12	172.99±41.85	15.13	0.11
Ileum	175.00±40.28	138.83±21.48	141.30±9.75	7.81	0.21
Villus height/Crypt depth ratio					
Duodenum	4.42±1.33	4.27±1.29	5.02±1.08	0.31	0.60
Jejunum	4.59±0.96	5.28±0.38	6.10±0.80	0.35	0.21
Ileum	5.23±1.11	4.49±0.81	5.28±0.58	0.23	0.31

Values are presented as mean ± SD

[A,B]Means within a row with different letters are significantly different ($P < 0.05$)

BHEs = Blend herbal extracts; MPs = Mixed prebiotics

(Lenhardt and Mozes, 2003); therefore shortening the villi would decrease the nutrient absorption area in the small intestine (Xu *et al.*, 2003). Moreover, a deeper crypt may indicate faster tissue turnover to permit renewal of the villus (Yason *et al.*, 1987; Gao *et al.*, 2008). In the current study, supplementation with BHEs and MP clearly improved the villi height in the duodenum. It seems that herbal extracted products directly impacted the cell function of the small intestine. Hong *et al.* (2012); Petrolli *et al.* (2012); Sarica *et al.* (2014) reported that supplementations of herbal extractions in the diet increased the villi height in the segment of the duodenum. Dietary essential oil from herbal extract supplementation reduced the number of harmful bacteria and their adhesion to the epithelium in the intestinal wall, hence reducing the production of toxic compounds and damage to the intestinal epithelial cells of broilers (Sarica *et al.*, 2014). Moreover, Jamroz *et al.* (2006) reported that chickens fed a diet with plant extracts (containing 5%, carvacrol, 3% cinnamaldehyde and 2% capsicum) increased mucus release and the thickness of the mucus layer on the glandular stomach and the wall of the jejunum.

MPs also improve the morphology of the duodenal segments, but the mode of action may be different from that of BHEs. Baurhoo *et al.* (2007); Zhang *et al.* (2005) reported a significant increase in the villus height in a yeast cell wall group compared to a control group. It is generally accepted that supplementation with prebiotics increased populations of Lactobacillus and Bifidobacteria in the gut and stimulated vascularization or development of the intestinal villi (Stappenbeck *et al.*, 2002; Baurhoo *et al.* 2007). Moreover, prebiotics may improve dietary energy availability by reducing the microflora-host competition for available starches (Ghasemian and Jahanian, 2016), which can then improve the morphological indices (taller villi) of the intestinal epithelial cells (Baurhoo *et al.*, 2007).

While both BHEs and MPs can improve the morphology in the duodenum, a slightly higher nutrients intake was found in the MPs group which resulted in a greater breast meat yield than that of the BHEs group. On the other hand, BHEs had a higher FCR compared to the MPs group. It was concluded that BHEs and MPs are valuable feed additives and they have different mechanisms to improve performance.

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