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Growth Performance and Nutrient Digestibility in Broiler Chickens Fed with an Encapsulated Blend of a Phytogenic Feed Additive

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ABSTRACT

Phytogenic Feed Additives (PFAs) from herbs, spices, and derived natural or corresponding synthetic chemically defined flavorings have gained momentum due to the rising worldwide ban of Antibiotic Growth Promoters (AGPs) in food animals. The present study evaluated the efficacy of a PFA in broiler chickens' diets on growth performance and digestibility parameters. A total of 880 male one-day-old broiler chickens (Ross 308) were randomly assigned to two dietary treatments, each with 20 replicates and 22 chickens per replicate. A corn-soybean-based diet was fed for 42 days as a control diet without PFA, and a treatment diet contained a blend of Carvacrol, Thymol, Carvone, Methyl salicylate, and Menthol encapsulated (as PFAs) at 65 g/ton of feed. Chickens supplemented with PFA had a 3.6% higher Body Weight Gain (BWG) during the starter phase (0 to 14 days) than those in the control group (25.9 versus 25.0 g/d) and a 2.9% reduced Feed Conversion Ratio (FCR) during the same period, compared to the control group (1.34 versus 1.38). Improved FCR (1.95 versus 2.01) was recorded in the PFA supplemented broiler chickens during the finisher phase (35 to 42 days) as well as throughout the experimental period from 1 to 42 days, compared to the control group (1.60 versus 1.62). In addition, the apparent ileal protein digestibility improved by 3.9% during 42 days, compared to the control group (74.3 vs 71.5%). Enhanced ileal protein digestibility and a reduced FCR suggested a cost-effective potential of PFA to improve broiler chickens' production performance.

Keywords: Broilers, Digestibility, Feed conversion ratio, Performance, Phytogenic feed additive

INTRODUCTION

Dietary feed supplements also known as feed additives or so-called growth promoters in the form of antibiotics have been traditionally used in agricultural livestock feeding since the mid-1940s for maintaining a healthy gut environment and improving performance (Dibner and Richards, 2005). Prompted by stricter regulations regarding the protection of human health, animal welfare and the environment on one side and increasing demand for animal protein on the other side, making alternative adaptations are necessary for the ongoing animal production. Due to the rising worldwide ban on the use of Antibiotic Growth Promoters (AGPs) in food animals, regarding the concerns about the development of antimicrobial resistance and the subsequent transfer of antibiotic resistance genes from animal to human microbiota (Castanon, 2007; Steiner and Syed, 2015), the present trend among poultry producers is to move away from the use of AGP in poultry rations. Plant-derived feed additives known as Phytogenic Feed Additives (PFAs), comprising of herbs, spices, Essential Oils (EOs), plant extracts, and their components have therefore become a growing class of feed additives for food animals, due to consumer preferences for natural and antibiotic-free animal products.

The potential of PFA to improve performance is attributed to their ability to maintain a healthy gut environment (Windisch et al., 2008). In a significant number of scientific studies, EOs containing most of the active substances of the plant have been reported to promote health and enhance the zootechnical performance by increasing nutrient availability for animals due to their antioxidant and anti-inflammatory effects, gut microbiota modulation, beneficial impacts on the gut quality resulting in better performance (Diaz-Sanchez et al., 2015; Upadhaya and Kim 2017; Luna et al., 2019), improved nutrient digestibility (Jamroz et al., 2003; Jamroz et al.,

2005), and gut health (McReynolds et al., 2009) in broiler chickens and poultry. Numerous studies have shown that supplementing broiler chickens' diets with PFAs resulted in positive effects on the performance (Upadhaya and Kim 2017; Luna et al., 2019; Zumbaugh et al., 2020). Direct anti-inflammatory effects have been attributed to essential oils and their blends in a number of scientific studies (Gbenou et al., 2013; Gessner et al., 2013; Kaschubek et al., 2018). PFAs have also been reported to possess antioxidative properties due to their essential oil content (Miguel 2010; Gessner et al., 2013; Oh et al., 2018), which have also been reported to positively influence carcass and meat quality characteristics in animals (Puvača et al., 2016; Syed et al., 2018; Syed, 2019). The specific mode of action of PFA is still being debated although several studies have attempted to explain the potential mechanism of action. Increased apparent ileal crude protein digestibility in broiler chickens at the age of 21, 35, and 42 days was reported by Amad et al. (2011) when broilers' diet was supplemented with an essential oil containing thymol and anethole. Akin effects were observed when broilers' diets were supplemented with an essential oil containing oregano, cinnamon, and pepper in the finisher phase of feeding (Hernandez et al., 2004). Similarly, increased trypsin and lipase activity was noticed in the lumen of the duodenum of the broiler chickens supplemented with Carvacrol and Thymol (Hashemipour et al., 2013). These various beneficial effects of PFAs are attributed to their bioactive molecules like thymol, carvacrol, cineole, and capsaicin (Mountzouris et al., 2011). Regarding all these properties, PFAs can serve as ideal natural alternatives to the traditional AGP diet supplementation.

The objective of the present study was to evaluate the efficacy of supplementing broiler chickens' diets with a PFA (Biomin® DC-P, a blend of five encapsulated compounds; carvacrol, thymol, carvone, methyl salicylate, and menthol) on the growth performance and digestibility parameters.

MATERIALS AND METHODS

Ethical approval

The broiler chickens in the current study were raised and treated according to Directive 2010/63/EU of 22 September 2010, and according to the recommendation of the European Commission 2007/526/CE covering the accommodation and care of animals used for experimental and other scientific purposes.

All the animal procedures were conducted in accordance with the prevailing institutional ethical norms and relevant Standard Operating Procedures described in the Imasde Agroalimentaria, S.L., Madrid, Spain, Quality Manual (version 4). Husbandry, euthanasia methods, experimental procedures, and biosafety precautions were approved by the Ethical Committee of the Faculty of Veterinary Medicine, Murcia University, Spain.

Animals and housing

A total of 880 one-day-old male Ross 308 broiler chickens were obtained from a commercial hatchery. The chickens were weighed and randomly assigned to 40-floor pens of 1.82 m^2 with wood shavings litter. The buildings were supplied with artificial, programmable lights, automated electric heating, and forced ventilation. The temperature inside the buildings was set at 33ºC at the start of the experiment, and was gradually decreased to 22ºC during the first three weeks of the experiment. The lighting program was 18 hours light and 6 hours dark every 24 hours throughout the experiment. Feed and water were available *ad libitum*. During the experimental period, animals were observed daily by the animal supervisor in its pen and any variation of its appearance, the appearance of its excreta or its behaviour was noted. If an animal was in poor condition it was observed more frequently. If it was judged unlikely to survive or to be suffering pain or distress it was euthanized and most probable cause of the poor condition was noted. Culled and dead chickens were weighed and date recorded.

Diets and experimental design

The chickens were allocated to two experimental diets with 20 replicates of 22 chickens each in a completely randomized design. All experimental diets were corn-soybean meal-based. Two treatments were used, including the control diet without any PFA (T1), and a diet supplemented with a PFA (Biomin® DC-P, BIOMIN Holding GmbH, Getzersdorf, Austria), a blend of carvacrol, thymol, carvone, methyl salicylate, and menthol encapsulated at 65 g/t (T2). Three feeding phases were offered, including 0-14 days (starter), 15-28 days (grower), and 29-42 days (finisher). Feeds were presented as mash. All experimental finisher diets had 0.50% titanium dioxide as an indigestible marker. The composition, the calculated analyses of the diets, and the results of the proximate analyses (nutritional) are presented in Table 1.

Table 1. Composition, calculated analyses, and analyzed nutrients of the experimental diets (as-fed basis) of the Ross 308 broiler chickens during the 42-day experiment in the facility of Imasde, Spain

Ingredients (%)	Starter (0-14 days of age)		Grower (15-28 days of age)		Finisher (29-42 days of age)	
Maize	58.089		58.595		63.113	
Soybean meal 47%	35.315		33.694		28.389	
Soy oil	2.479		4.246		4.719	
Calcium carbonate	1.218		1.009		0.940	
Monocalcium phosphate	1.065		0.983		0.921	
Salt	0.299		0.309		0.311	
Sodium bicarbonate	0.150		0.100		0.100	
DL-Methionine	0.346		0.263		0.228	
L-Lysine HCl	0.316		0.156		0.140	
L-Threonine	0.123		0.046		0.039	
Vit &Min Premix[1] (incl. phytase)	0.400		0.400		0.400	
Inert marker	0.000		0.000		0.500	
BIOMIN Product premix	0.200		0.200		0.200	
Calculated analysis[2] (%) unless specified						
AMEn, kcal/kg	3000		3125		3200	
Dry Matter	87.67		87.80		87.75	
Ash	5.35		4.95		4.59	
Crude Protein	21.56		20.58		18.62	
Ether Extract	5.25		7.00		7.55	
Crude Fibre	2.79		2.73		2.64	
Starch	37.07		37.39		40.22	
Calcium	1.00		0.90		0.85	
Total Phosphorus	0.74		0.71		0.68	
Av. Phosphorus	0.45		0.43		0.41	
Sodium	0.17		0.16		0.16	
Digestible Lysine	1.24		1.08		0.95	
Digestible Methionine	0.64		0.54		0.49	
Digestible Met+Cys	0.92		0.82		0.74	
Digestible Threonine	0.81		0.71		0.64	
Digestible Tryptophan	0.22		0.21		0.19	
Analyzed nutrients (%)	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Dry matter	87.90	87.90	87.60	87.80	87.80	87.80
Crude protein	21.60	21.60	21.40	21.90	17.00	17.00
Crude fiber	2.70	2.70	3.40	2.80	3.70	4.20
Ash	5.60	5.60	6.10	5.50	5.50	5.60
Starch	40.60	40.60	38.00	37.50	45.00	45.00
Ether extract	4.60	4.60	6.30	6.00	6.50	6.40
Calcium	0.93	0.93	0.90	0.90	0.74	0.78
Phosphorus	0.61	0.61	0.57	0.58	0.50	0.51

[1]Provided per kilogram of diet: Vitamin A (E 672): 10,000 IU, Vitamin D3 (E 671): 2,000 IU, Vitamin E (a- tocopherol): 30.0 mg, Vitamin K3: 2.0 mg, Vitamin B1: 2.0 mg, Vitamin B2: 5.0 mg, Vitamin B6: 3.0 mg; Vitamin B12: 12.0 µg, Nicotinic acid: 40.0 mg, Calcium pantothenate: 10.0 mg, Folic acid: 1.0 mg, Biotin:0.1 mg, Choline chloride: 400 mg; Cu (CuSO4·5H2O): 8.0 mg; Fe (FeCO3): 60.0 mg; I (IK): 2.0 mg; Mn (MnO): 70.0 mg; Se (Na2SeO3): 0.15 mg; Zn (ZnO): 80.0 mg; Phytase: 6 Phytase EC 3.1.3.26 [2]Based on the values for feed ingredients as per Guidelines of the Spanish Foundation for Development of Animal Nutrition (FEDNA, 2010), T1: Treatment 1 (control), T2: Treatment 2 (PFA), PFA: Biomin® DC-P

Experimental procedures

Chickens' weights per pen were recorded on days 0, 14, 35, and 42 days. Body Weight Gain (BWG), Feed intake (FI), and FCR was corrected for the weight of the dead chickens recorded on the days of mortality.

Excreta were collected twice daily on wax paper from 40 to 42 days being immediately mixed and pooled by two consecutive pens from the same treatment and stored at -20°C until analysis. Previous to analysis, excreta were dried in a forced-air oven at 55°C, and grounded to pass through a 0.5 mm screen.

Intestinal ileal contents were collected from seven chickens per pen at day 42 after euthanasia by cervical dislocation. Ileal digesta were collected from the Meckel's diverticulum to approximately 2 cm cranial to the ileocecal junction. Ileal contents from the seven chickens were flushed with distilled water into plastic containers, pooled by pen, immediately frozen, and stored in a freezer at -20°C until freeze-drying.

Chemical analysis and calculations

Freeze-dried ileal content and feed samples were grounded to pass through a 0.5 mm screen in a grinder. Excreta samples were dried in a forced-air oven at 55ºC and grounded to pass through a 0.5 mm screen in a grinder. Dry Matter (DM) analysis of the samples was performed after the samples were dried in an oven at 105°C for 16 hours (method 930.15; AOAC, 2016). Crude protein (N × 6.25) was determined by Kjeldahl method (method 990.03; AOAC, 2016). Titanium concentration in

the feed, excreta, and ileum samples were determined by ICP-OES assay (Morgan et al., 2014). Calcium and Phosphorus analysis were done using the method 968.08 and 965.17 of AOAC (2016).

Apparent ileal digestibility and apparent fecal digestibility were calculated using the following equation;

$$\text{Digestibility (\%)} : [1 - (Ti_{feed}/Ti_{out}) \times (N_{out}/N_{feed})] \times 100$$

Where, Ti_{feed} represents the concentration of titanium in the feed in g/kg of DM, Ti_{out} denotes the concentration of titanium in the excreta or ileal digesta in g/kg of DM output, N_{feed} stands for the concentration of CP, Ca, or P in the diet in mg/kg of DM, and N_{out} is the concentration of CP in the excreta or Ca and P in ileal digesta in mg/kg of DM output.

Statistical analysis

The experimental design was a completely randomized design. Data were subjected to a one-way ANOVA using the GLM procedure of SPSS (v. 19.0). The model included the experimental treatment as the main effect. Means were separated with Tukey post-hoc comparison test. Statistical significance was declared at $p \leq 0.05$, with $0.05 < p \leq 0.10$ considered as a near-significant trend.

RESULTS

The chickens were healthy during the entire experimental study, and no adverse events were noted. Total mortality ratio during 42 days was 33/880 chickens (3.75%). Zootechnical performance of the animals (BWG, FI and FCR) was in accordance with trial conditions (male broiler chickens fed mash diets and raised in floor pens). At 14 days of age, broiler chickens which received the PFA had 3.2% higher body weight than the chickens in the control group ($p = 0.08$), however, these differences declined during the rest of rearing period thereafter (Table 2). During the starter period (from 0 to 14 days of age), chickens receiving the PFA achieved higher body weight gain (25.8 versus 25.0 g/d, $p = 0.05$) and exhibited better FCR (1.34 vs 1.38 feed/gain, $p < 0.01$) than the broiler chickens in the control group. During the grower period from 15 to 35 days of age, no differences between the treatments were observed in the body weight gain, FI, and FCR. From 36 to 42 days of age, there was a trend towards a better (lower) FCR for the group with the PFA supplementation (1.95 vs 2.01 feed/gain, $p = 0.10$). For the overall study period (0 to 42 days of age), broiler chickens supplemented with the PFA converted feed into gain significantly better than the control group (1.60 vs 1.62 feed/gain, $p = 0.02$, Table 2).

The effect of dietary treatment on the apparent fecal and ileal digestibility of broilers at 42 days of age is summarized in Table 3. No effect of treatment was observed for Calcium and Phosphorus digestibility. However, apparent ileal digestibility of crude protein increased when PFA was included in the diet (+3.9%; 74.7 vs 71.9 %, $p = 0.04$). The observed improvement in crude protein digestibility was reflected in feed conversion from 36 to 42 days, although the difference was only a trend (-3.0%; 2.01 vs 1.95 g feed/gain for Control vs PFA, Table 2).

Table 2. Effect of phytogenic feed additive supplementation on zootechnical performance of Ross 308 broiler chickens from day one to day 42 at the trial facility of Imasde, Spain

Parameter		Treatment		SEM[1] (n = 20)	p value
		T1	T2		
Body weight (g)	Initial	44.0	43.8	0.78	0.86
	14 d of age	393.8	405.5	4.69	*0.08*
	35 d of age	2192	2208	17.6	0.53
	42 d of age	2934	2964	19.8	0.29
Starter phase, 0-14 d of age	ADG (g/d)	25.0	25.8	0.30	0.05
	ADFI (g/d)	34.5	34.5	0.32	0.98
	FCR	1.38	1.34	0.009	< 0.01
	Mortality, %	0.91	1.36	0.449	0.48
Grower phase, 15-35 d of age	ADG (g/d)	85.6	85.8	0.68	0.84
	ADFI (g/d)	129.6	129.6	1.01	0.97
	FCR	1.51	1.51	0.007	0.71
	Mortality (%)	2.28	2.99	0.877	0.57
Finisher phase, 36-42 d of age	ADG (g/d)	105.9	108.0	1.34	0.28
	ADFI, g/d	212.6	210.2	1.65	0.31
	FCR	2.01	1.95	0.027	0.10
	Mortality, %	0.00	0.00	--	--
Whole experiment, 0-42 d of age	ADG (g/d)	68.8	69.5	0.46	0.27
	ADFI (g/d)	111.7	111.3	0.77	0.70
	FCR	1.62	1.60	0.006	**0.02**
	Mortality (%)	3.18	4.32	0.920	0.39
	EPEF	410	415	3.4	0.33

[1]Standard error of the mean (n: number of observations), ADG: Average daily gain, ADFI: Average daily feed intake, FCR: Feed conversion ratio, EPEF: European Production Efficiency Factor, T1: Treatment 1 (control), T2: Treatment 2 (PFA), PFA: Biomin® DC-P, Bold numbers indicate the significance level.

Table 3. Effect of Phytogenic Feed Additive supplementation on apparent fecal and ileal digestibility of Ross 308 broiler chickens on day 42

Parameter		Treatment		SEM[1]	p value
		T1	T2		
Apparent fecal digestibility at 42 days	Dry matter (%)	71.0	70.8	0.79	0.88
	Calcium (%)	35.2	36.3	1.30	0.56
	Phosphorus (%)	28.9	30.7	1.61	0.42
Apparent ileal digestibility at 42 days	Dry matter (%)	69.8	72.6	0.85	0.02
	Crude protein (%)	71.9	74.7	0.93	0.04

[1]Standard error of the mean (n: number of observations, n:10 for fecal digestibility and n:20 for ileal digestibility), T1: Treatment 1 (control), T2: Treatment 2 (PFA), PFA: Biomin® DC-P, Bold numbers indicate the significance level

DISCUSSION

In view of the advancing worldwide ban on the use of AGPs in the diets of food animals, particularly poultry, due to an anticipated risk of evolving microbiota with resistance to the antibiotics used for treating humans and animals (Windisch et al., 2008; Puvača et al., 2013; Steiner and Syed, 2015), PFAs have gained considerable importance in the feeding of agricultural livestock. Due to their multifarious properties, such as anti-inflammatory, antioxidative, antimicrobial, and antiviral activities, reflected in a large number of scientific studies (Oyuntsetseg et al., 2014; Patil and Patil, 2016), PFAs are seen as promising alternatives to AGPs. The PFAs have been reported to enhance the digestibility of nutrients in the gastrointestinal tract (less undigested nutrients excreted), improve carcass and meat traits in broiler chickens, and thus promote a sustainable food production without burdening the environment (Gopi et al., 2014; Syed, 2019; Zumbaugh et al., 2020). Results of the present study revealed that PFAs supplementation to broiler chickens' feeds resulted in significantly higher BW and BWG, and improved FCR during the critical starter phase of rearing from 0 to 14 days of age compared to the control group (Table 2). No differences were observed in the BWG, FI, and FCR between the treatments during the grower period (15 to 35 d of age; Table 2), however, FCR was improved in the finisher period (36 to 42 days of age) without affecting the FI (Table 2). Finally, an improved FCR was recorded for the PFA supplemented broilers for the entire experimental period (0-42 days) without any notable differences in BW or FI (Table 2). These results supported the findings of Windisch et al. (2008) and Alhajj et al. (2015) revealing that PFAs caused reduced feed intake at largely unchanged BWG or final body weight, thereby can improve FCR. This also looks to be in accordance with earlier reports (Alcicek et al., 2003; Guo et al., 2004; Mountzouris et al., 2011) which indicated an improvement in final BW and FCR due to PFA supplementation without any effect on the daily weight gain or FI.

No effect of the treatment was observed for apparent fecal digestibility of Calcium and Phosphorus (Table 3). However, the apparent ileal digestibility of crude protein was increased by 3.9% when PFA was supplemented with the broiler chickens' diet (Table 3). The observed improvement in protein digestibility was also reflected through a reduced FCR (-3.0%) of PFA supplemented broiler chickens during the finisher phase from 36 to 42 days of age (Table 2). The difference reflected only a trend (1.95 vs 2.01 g feed/gain for PFA vs control, Table 2). Improvements in crude protein digestibility by supplementing diets with the PFA have been attributed to their potential of causing a lengthening of the intestinal villi and increasing endogenous secretions (Williams and Losa, 2001; Amad et al., 2013; Giannenas et al., 2014). Furthermore, it has been reported in several studies that PFAs could improve the digestibility of feed nutrients especially protein digestibility (Maenner et al., 2011; Steiner and Syed, 2015), which resulted in better utilization of amino acids, and accordingly, reduced the excretion of nitrogenous compounds in the slurry. The current results are in agreement with the findings of El-Deek et al. (2012) and Zentner et al. (2012) who reported that PFAs have the potential to reduce emissions from animal houses. By maintaining good litter quality in poultry houses, producers can reduce economic losses, and improve the welfare of chickens (Taira et al., 2014).

CONCLUSION

From the present study, it can be concluded that supplementation of broiler chickens diets with the commercially available phytogenic feed additive (Biomin® DC-P) can improve growth during the starter period, feed conversion ratio during the overall experimental period, and apparent ileal digestibility of crude protein at 42 days of age. This advantageous effect of the phytogenic feed

additives could be cost-effective, and bring more value to broiler chicken producers. Further studies could be done to explore the exact mode of action of the phytogenic feed additives.

DECLARATIONS

Authors' contribution

The experimental study was conceived and designed by Basharat Syed and Marta Gracia in consultation with Jutta Kesselring and Jaime Sánchez. Jaime Sánchez and Marta Gracia supervised the experimental study, collection of data, and analysis. The manuscript was written and drafted by Basharat Syed. All authors read, reviewed, and approved the final manuscript for submission and publication.

Competing interests

The authors declare that they have no competing interests.

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Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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