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Evaluating complementary effects of ethanol extract of propolis with the probiotic on growth performance, immune response and serum metabolites in male broiler chickens

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ABSTRACT

The aim of this study was to evaluate the effects of propolis (a natural resinous substance that bees collect from different sources of plants) extract alone or in combination with probiotics on the growth performance and immune response of male broiler chickens. A total of 256 broiler chickens were randomly assigned to 4 treatments: maize-soybean meal basal diet with no supplement as control, the basal diet containing propolis (0.20 g/kg), probiotic (0.45 g/kg), or a combination of propolis (0.20 g/kg) and probiotic (0.45 g/kg). Each treatment consisted of 4 replicated pens with 16 broiler chickens per pen. Broiler chickens had ad libitum access to feed and water and the light program was 23 h light/1 h dark. The inclusion of propolis extract decreased ($P < 0.05$) body weight and feed intake. Propolis extract alone or in combination with probiotic increased ($P < 0.05$) the relative spleen weight in broiler chickens at 42 d of age compared to the control and probiotic. The relative weight of bursa of Fabricius was greater ($P < 0.05$) in broiler chickens receiving probiotics, propolis extract, or their combination compared to those fed the control diet. Supplementation of diet with probiotic, propolis extract, or a combination of both caused an increase ($P < 0.05$) in the concentration of antibody titer to Newcastle Disease virus. In conclusion, supplementation of the diet with propolis extract decreased the body weight of broiler chickens, however, inclusion of propolis, probiotic, and their combination in the diet caused an immunomodulatory effect in broiler chickens.

1. Introduction

Antibiotics are widely used as growth promoters in poultry production. In recent years, administration of these antimicrobial additives as growth promoters in poultry diets has been banned because of the concerns about antibiotic residues in animal tissues and the subsequent induction of emerging antibiotic resistant strains of micro-organisms (Roe and Pillai, 2003; Saleha et al., 2009; Simon, 2005). As a result, additives such as probiotics and natural substances like propolis have been introduced as potential alternatives to antibiotics in poultry diets.

Propolis (also known as 'bee glue') is a natural resinous substance that bees collect from different sources of plants to seal cracks in the hive, mummify the dead bodies of invaders to prevent their decomposition and spreading of disease, and position honeycombs within the hive.

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Propolis is known to have considerable antimicrobial, anti-inflammatory, and immunomodulatory effects (Bankova et al., 2000; Burdock, 1998; Dobrowolski et al., 1991). It is also has been shown that propolis could be a successful additive in poultry production. Khojasteh Shalmany and Shivazad (2006) reported that the inclusion of propolis in broiler chickens diets improved the growth performance. Galal et al. (2008) showed that dietary supplementation of propolis improved both performance and the immune response of laying hens. Ziaran et al. (2005) also reported that diet supplementation with propolis modulated both the humoral and cellular immunity of broiler chickens.

Probiotic is defined as a live microbial feed supplement that beneficially affects the host animal by improving the intestinal microbial balance (Fuller, 2008). Recently, poultry nutritionists have developed an interest in probiotics and have incorporated them into the broiler chicken diets. The beneficial effects of probiotics on broiler chicken growth performance, nutrient digestibility and cecal microflora composition have been reported (Apata, 2008; Gyawali and Ibrahim, 2012; Mountzouris et al., 2010; Song et al., 2012). It also has been shown that supplementation of beneficial microbes enhances antibody responses to Newcastle disease virus (NDV) and infectious bursal disease vaccination (Talebi et al., 2008), and potentially can play an important role as a hypolipidemic agent in broiler chickens (Kalavathy et al., 2003).

According to the aforementioned results, it could be hypothesized that the administration of propolis along with probiotics might boost the beneficial effects of these 2 supplements on growth performance and immune status. Therefore, the present study was conducted to evaluate the effects of the co-administration of probiotic and propolis on growth performance and immune response of broiler chickens.

2. Materials and methods

2.1. Origin, processing and preparation of propolis

Propolis samples were gathered (Najafabad, Isfahan, Iran) and frozen (− 24 °C) immediately. Samples were then extracted using the method of Yaghoubi et al. (2007) with some modifications. The frozen samples were broken into small pieces and extracted with 70% ethanol. The extract was then shaken at 250 Hz at room temperature for 48 h. Next, the extract was passed through a Whatman No. 41 filter paper. After approximately 12 h, the extract was sprayed onto dietary treatments and mixed thoroughly. Propolis samples were analyzed by gas chromatography–mass spectrometry (GC–MS) according to Popova et al. (2004) at the Institute of Organic Chemistry (Centre of Photochemistry, Sofia, Bulgaria), and the results are shown in Table 1.

2.2. Probiotic strains

A commercial product (PrimaLac) containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidium* and *Enterococcus faecium* (PrimaLac Co.; Clarksdale, MO, United State) was used as the source for the probiotic. The viability of the strains included in this product was confirmed using the method developed by Ibrahim and Salameh (2001) prior to use in the experiments.

Table 1
Compositions of the propolis sample[a,b].

Substance	RT	Percentage (% of TIC)
Glycerol	7.62	0.5
Butanedioic acid	10.53	0.3
Hydroxybutanedioic acid	12.60	0.3
Hydroxybenzoic acid	15.98	1.2
Isovanilinic acid	19.00	0.7
Inositol	22.30	2.3
Gluconic acid	24.15	1.5
Hexanoic acid	25.12	0.6
Ferulic acid	25.41	0.3
Caffeic acid	26.40	0.7
Monoterpenic ester of hydroxybenzoic acid	27.11	0.5
Oleic acid	27.45	0.8
Suberosin	27.55	1.8
Monoterpenic ester of isovanilinic acid	29.36	0.1
2-methyl-2-butenyl caffeate	30.65	0.2
Pinostrobin chalcone	32.19	0.4
Pinocembrin	32.88	0.7
Pinobanksin chalcone	33.22	0.7
Pinobanksin acetate	34.96	1.1
Sesquiterpenic ester of hydroxybenzoic acid	35.89	4.8
Phenethyl caffeate	36.58	0.8
Sesquiterpenic ester of isovanillinic acid	38.14	1.3
Triterpenic alcohol	44.15	0.3

[a] RT = retention time (min); and TIC = total ion chromatogram (ion current generation depends on the characteristics of the compound concerned and it is not a true quantization).
[b] Propolis sample gathered from Najafabad (Isfahan, Iran) and analyzed by gas chromatography–mass spectrometry.