Animal Nutrition 1 (2015) 213–219

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: www.www.keaipublishing.com/en/journals/aninu

Original research article

# Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens

Toshi Wati [a], Tapan K. Ghosh [a], Basharat Syed [b], Sudipto Haldar [a,*]

[a] Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata 700037, India
[b] BIOMIN GmbH, Getzersdorf 3131, Austria

ARTICLE INFO

*Article history:*
Received 3 June 2015
Accepted 11 August 2015
Available online 19 August 2015

*Keywords:*
Broilers
Phytogenic feed additives
Performance
Nutrient retention
Enteric challenge
Intestinal microbiology

ABSTRACT

The aim of this work was to compare the efficacy of a commercially available phytogenic feed additive (PFA) and an antibiotic growth promoter, which was bacitracin methylene disalicylate (BMD), on performance, nutrient retention, caecal colonization of bacteria and humoral immune responses against Newcastle disease in broiler chickens challenged orally with *Salmonella enteritidis* and *Escherichia coli*. One-day-old male Cobb 400 broiler chicks ($n = 120$) were fed with 1) a negative control (NC) diet, which is the basal diet without any added growth promoter, 2) a positive control (PC) diet, the basal diet supplemented with BMD, 500 mg/kg and 3) a diet supplemented with PFA (150 mg/kg) for 39 days and the birds were inoculated with *S. enteritidis* and *E. coli* on d 28. Supplementation of PFA improved body weight, feed conversion ratio, retention of N and crude fiber, increased fecal moisture content and decreased digesta transit time as compared with the NC and PC groups ($P < 0.01$). Both the PC and the PFA was found to be equally effective in controlling the surge in numbers of *Salmonella* and *E. coli* following oral inoculation of these bacteria as compared with the NC group ($P < 0.05$) at 24 h past inoculation. Caecal content analysis on d 39 indicated lower numbers of *Salmonella*, *E. coli* and *Clostridium* in the PC and PFA groups as compared with the NC group ($P < 0.05$). The number of *Lactobacillus* in the PFA group was higher than those in the NC and PC groups ($P < 0.05$). Humoral immune response, measured as hemagglutination inhibition titer against Newcastle disease, was better in the PC and PFA groups compared with the NC group ($P < 0.05$) at d 21 but the difference did not last till d 39. The heterophil to lymphocyte ratio was narrower ($P < 0.001$) and alkaline phosphatase activity was higher ($P < 0.01$) in the PFA group as compared with the NC and PC groups on d 39. It was concluded that the PFA, which is animal, environment and consumer friendly, may be used as an effective replacement for common in-feed antibiotics like BMD to enhance broiler performance especially when the birds are exposed to heavy infections on fields.



## 1. Introduction

Phytogenic feed additives (PFA) are the plant derived products used to improve performance of livestock and poultry (Windisch et al., 2008; Jacela et al., 2010). The PFA comprise of a wide variety of herbs, spices and products derived thereof and are mainly essential oils. This class of feed additives is at present used to a great extent as alternatives to the antibiotic growth promoters (AGP) in poultry and swine nutrition. Obviously the ban imposed on the usage of the AGP in diets of food animals is the main driving force that has caused the surge in the use of PFA. Ideally, an

* Corresponding author.
*E-mail address:* sudipto.haldar@gmail.com (S. Haldar).
Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2015.08.003

alternative to the AGP should have the same beneficial effects when included in diets. The dubious mechanism of action of the in-feed antibiotics notwithstanding (Huyghebaert et al., 2011), it is generally accepted that the AGP elicit some antibacterial action and thereby reduce the incidence and severity of subclinical infections. In doing so, they reduce the microbial usage of nutrients and improve their absorption from the intestine by thinning the intestinal mucosa (Brennan et al., 2003; Snyder and Wostmann, 1987). The indirect impact of all these actions is translated into higher growth rate. The basis of this mechanistic explanation is that the AGP do not exert growth promoting effects in germ-free animals. The general industry practice is to feed the livestock with doses of antibiotics at sub-therapeutic levels which is unlikely to exert any growth inhibitory action on the resident bacteria (Niewold, 2007) although a clear shift in the intestinal microbiota was observed when antibiotics were added to broiler diets at levels below the minimum inhibitory concentration (Pedroso et al., 2006; Wise and Siragusa, 2007) and this, at least partly, explains the effects of the AGP. Furthermore, microbiota shifts affect morphology of the gut wall and induce immune reactions, which by affecting energy expenses of the host animal may promote their growth (Teirlynck et al., 2009). Based on the hypothesized mechanism of action of the AGP, the alternative products should have modulatory effects on the gut microbiota and the immune system. Although antimicrobial and growth promoting effects of PFA have been reported by several workers the mode of action of most of the PFA is still not very clear. Possibly, the essential oils present in the PFA promote gut functions by stimulating secretion of digestive enzymes, bile and mucus (Platel and Srinivasan, 2004), the terpenoids and phenolic compounds help the animals to combat with the oxidative stress the animals come across (Aeschbach et al., 1994) and compounds like carvacrol, other terpenoids and aldehydes present in most of the PFA exert substantial antimicrobial effects (Baratta et al., 1998; Burt, 2004; Mathlouthi et al., 2012) thus establishing eubiosis in the gut.

In this experiment a commercially available preparation of PFA was compared with a conventional in-feed antibiotic, bacitracin methylene disalicylate (BMD), as a growth promoter for broilers. The PFA under study contained extracts of fennel, Melissa balm, pepper, anise, oak clove and thyme. Since the efficacy of an antimicrobial agent cannot be assessed properly in absence of a real bacterial challenge (Moralez-Lopez et al., 2009), the chickens were inoculated with pathogenic strains of *Salmonella enteritidis* and *Escherichia coli*. It was hypothesized that the infection induced stress (Geraert et al., 1996; Tankson et al., 2001) would facilitate both the growth promoters to elicit their effects more discernibly. The objective of the study was to gauge the performance differences in the birds supplemented the antibiotic or the PFA vis-à-vis a negative control in terms of body weight gain, feed conversion efficiency, nutrient metabolism and colonization pattern of some of the major intestinal bacteria in presence of artificially inoculated gut pathogens.

## 2. Materials and methods

### 2.1. Bird husbandry and measurement of performance traits

A 39-d experiment was conducted with 120 one-day-old male Cobb 400 broiler chicks (average body weight 45 g at 6 h post-hatch) according to the prevailing institutional ethical norms. The chicks were weighed individually and assigned to three treatments each consisting of 8 replicate cages ($n = 5$ chicks on d 0 and $n = 4$ chicks from d 8 onwards) following a completely randomized design to minimize the effects of the cages. Immediately after arrival, swabs from the cloacae were collected from all chicks and analyzed for *Salmonella* and *E. coli* counts. The chicks were found negative for *Salmonella* but *E. coli* was detected in 2 chicks and they were not included in the experiment. Each cage (0.5 × 0.75 m × 0.75 m) was fitted with a feeder, drinker and an excreta collection tray. Feed (starter mash from d 1 to 7, grower mash from d 8 to 21 and finisher mash from d 22 onwards) and water were offered ad libitum. Lighting program was 23 h of light for the first 7 d, 20 h until d 15 and 18 h afterward. The birds were vaccinated against Marek's disease (d 0), Newcastle disease (ND live B1 at d 7 and La Sota at d 21) and infectious bursal disease (d 14). Temperature was maintained around 32 to 34°C during the first week and at 25 to 27°C subsequently.

The chicks were individually weighed for their empty body weight following a 16-h fast at weekly intervals and live weight (LW) and live weight gain (LWG) was calculated cage wise. Cage average for feed intake (FI) was determined every week and feed conversion ratio (FCR) was calculated for each cage as the ratio between feed intake and LWG.

### 2.2. Experimental diets

The dietary treatments included feeding a corn-soybean based negative control (NC) diet devoid of any added growth promoter, a positive control diet (PC) supplemented with BMD (containing 450 mg active BMD/g) 500 mg/kg, and the PFA diet which was supplemented with the phytogenic feed additive (150 mg/kg). The ingredients and chemical composition of the experimental diets are presented in Table 1. The PFA used in this study was obtained from Biomin Phytogenics GmbH, Germany and was included in diet according to the manufacturer's recommendation. The PFA contained extracts from fennel (*Foeniculumvulgarae* var. dulce mil), Melissa balm (*Melissa officinalis* L.), peppermint (*Mentha arvensis* L.), anise (*Pimpinella anisum* L.), oak (*Quercus cortex*), clove (*Syzygium aromaticum* L.), and thyme (*Thymus vulgaris* L.). All the diets were analyzed (AOAC, 1990) for dry matter (DM, method 934.01), N and crude protein (CP, method 968.06; protein-nitrogen determination, Kelplus, Pelican Equipments, Chennai, India), crude fiber (CF, Foss Fiber Cap 2021 Fiber Analysis System, Foss Analytical, Hilleroed, Denmark) and crude fat (petroleum ether extraction; method 920.39; Socsplus, Pelican Equipments, Chennai, India).

### 2.3. Measurement of nutrient retention and digesta transit time

Retention of N and CF was determined through a metabolism trial by total excreta collection method performed between days 36 and 38. During this period excreta were collected daily per cage at every 2 h intervals during 0600 to 2200 h and at 4 h intervals during 2200 to 0600 h and preserved at −20°C. From the pooled excreta a 10% aliquot was preserved for final analysis. Feed samples were collected daily and pooled to produce a single composite of each diet. Both diets and excreta samples were analyzed for N (AOAC, 1990).

Digesta transit time was determined (Afsharmanesh et al., 2010) on d 34 before commencing the metabolism trial. All birds were fasted overnight and force-fed with the respective treatment diets for a period of 15 min. The diets were mixed with chromic oxide (1 g/kg). The transit time was determined as the time from the introduction of the diets to the first appearance of green-colored droppings.