

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

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Dose-Responses of Broiler Chicks, Given Live *Coccidia* Vaccine on Day of Hatch, to Diets Supplemented with Various Levels of Farmatan® (Sweet Chestnut Wood Tannins) or BMD®/Stafac® in a 42-Day Pen Trial on Built-Up Litter

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Abstract: Dietary Farmatan® Powder (~73% tannins) was used to determine live performance dose-responses of male Cobb broiler chicks on built-up litter top dressed with wood shavings in a summer trial. Dietary Farmatan® concentrations used were: 0 (negative control), 250, 750, or 1,000 ppm. Positive control (antibiotic) diets had BMD® 55 ppm (0-35 days) and Stafac® (35-42 days). Feeds were steam pelleted and fed as crumbles or pellets. There were 45 chicks/pen initially and 8 replicate pens/treatment (8 blocks of 6 pens each; Randomized Complete Block Design; LSD $p = 0.05$). The body weight (BW) gains were not significantly different from 0-21 d or 0-35 days but from 0-42 days ($p = 0.002$) were, respectively (kg): 2.238^b, 2.238^b, 2.299^a, 2.282^{ab}, 2.290^a, 2.316^a. Mortality-adjusted Feed Conversion Ratios (MAFCR) from 0-21 days ($p = 0.002$) were, respectively: 1.513^a, 1.488^{ab}, 1.476^{bc}, 1.469^{bc}, 1.454^c and 1.442^c. The MAFCR from 0-35 days ($p = 0.001$) were, respectively: 1.666^a, 1.657^{ab}, 1.646^{abc}, 1.626^{cd}, 1.621^d and 1.641^{bcd}. The MAFCR from 0-42 days ($p = 0.004$) were, respectively: 1.694^a, 1.698^a, 1.685^{ab}, 1.665^{bc}, 1.661^{bc} and 1.655^c. Stafac® 22 ppm in finisher (35-42 days) gave the best BW gain and feed conversion ratio. Litter moisture % at 21 days was lower ($p = 0.032$) using Farmatan® 500, 750, or 1,000 ppm than BMD® 55 ppm and at 42 days was lower ($p = 0.046$) for each Farmatan® level than for negative control. Farmatan® 750 or 1,000 ppm improved ($p = 0.008$) 42-d litter score (0 driest to 5 wettest) compared to negative control. Mortality % from 0-42 days and litter nitrogen % at 42 days were unaffected by treatment. Farmatan® improved BW gain and MAFCR and promoted drier litter.

Key words: Broiler, chestnut tannins, dose-response, Farmatan, litter

INTRODUCTION

The use of antibiotic feed additives has been banned in the European Union (EU) and antibiotic growth promoters are being phased out in the United States because of the potential risk for generating antibiotic resistance in pathogenic microbes. Consequently, many alternative approaches have been attempted to control or prevent the clinical diseases and maximize growth performance in broiler chickens. At present, natural plant extracts containing hydrolyzable tannins from sweet chestnut wood are approved as astringent feed flavorings and are being evaluated and used commercially due to their natural antibiotic properties. Southern European sweet chestnut wood (*Castanea sativa*) water-soluble extract contains several categories of antimicrobial phytochemicals that have been shown in research trials to give beneficial effects on broiler performance including increased body weight gain, lower feed conversion ratio, better intestinal health, drier manure and litter and/or pathogen reduction.

Hydrolyzable tannins: The term "tannin" is widely applied to any large polyphenolic compound containing sufficient hydroxyls (-OH) and other suitable groups such as carboxyls (-COOH) to form strong complexes with proteins and other macromolecules. Hydrolyzable tannins are hydrolyzed by weak acids or weak bases to produce carbohydrate and phenolic acids. Examples of gallotannins are the gallic acid esters of glucose in tannic acid (C₇₆H₅₂O₄₆), found in the leaves and bark of many plant species (Wikipedia, Hydrolysable Tannins). Condensed tannins such as those found in sorghum grain (milo) typically have adverse effects in animal nutrition whereas hydrolyzable tannins may produce beneficial effects. Condensed tannins from sorghum grain do not seem to be absorbed but completely excreted (Jimenez-Ramsey *et al.*, 1994). The wood of sweet chestnut (*Castanea sativa*) is particularly rich in esters of gallic acid with monosaccharides. The molecular weight of different tannins can vary between 500-3000 Daltons (Da). The vescalagin and castalagin in hydrolyzable tannin can be broken in the digestive tract

of animals to the ellagic acid, then to castalin and vescalin and finally to the gallic acid and glucose or other monosaccharides (Hagerman *et al.*, 1992; Karasov *et al.*, 1992).

Antimicrobial effects: Tannins have antimicrobial properties (including against antibiotic-resistant bacteria; Yoshida *et al.*, 2009) and, when used in appropriate doses, may prevent the development of an undesired intestinal microflora (Scalbert, 1991; Chung *et al.*, 1998a). In an *in vitro* trial, Bole-Hribovsek *et al.* (2012) observed that Farmatan® tannin concentrations of 0.05, 0.025 and 0.0125% decreased the number of *Clostridium perfringens* colony forming units (cfu) by more than 54-fold (under the detection limit) whereas 0.00625% allowed a minimal increase, 0.003125% reduced growth by 2.7-fold and 0.0015625% reduced growth by 1.7-fold. Therefore, Farmatan® tannins at concentrations of 0.0125% or higher had bactericidal activity against *C. perfringens*. For *E. coli*, 0.05% tannins had little effect, 0.5% increased cfu by 4- to 91-fold depending on agar and 2.5% reduced cfu by 218- to 1090-fold (pH decreased with increased tannins). For *Salmonella*, 0.5% tannins reduced cfu to 36 to 64% of control count.

In Trial 1 Graziani *et al.* (2009), using Farmatan® at 0.1% in culture media, found that with 6 hours of pre-incubation *Campylobacter jejuni* was inhibited and with 24 hours of pre-incubation *Pasteurella multocida*, *Staphylococcus aureus* and *Campylobacter jejuni* were inhibited. The bacterial strains were used at a concentration of 1.2×10^9 cfu/mL. Using Farmatan® at 0.25%, with 6 or 24 hours of pre-incubation *Salmonella gallinarum*, *Pasteurella multocida*, *Staphylococcus aureus* and *Campylobacter jejuni* were inhibited. In Trial 2 by Graziani *et al.* (2009), *Salmonella enteritidis* (9.2×10^5 cfu/mL), *Escherichia coli* (1.8×10^6 cfu/mL) and *Clostridium perfringens* type A (1.8×10^6 cfu/mL) strains were used. Farmatan® was used at 2 different concentrations, 0.15 and 0.5%. Before the inoculation on culture media every bacterial strain was preincubated with Farmatan® for 6 hours in Brain Heart Infusion (BHI) broth at room temperature. After 24 hours of incubation at 37°C the antimicrobial activity of Farmatan® was evaluated by observing the presence or the absence of bacterial growth on specific culture media. All 3 bacterial strains were inhibited at both Farmatan® concentrations.

Lewis and Papavizas (1967) tested the effects of gallotannin, wattle, canaigre, and chestnut tannins in a complete medium on *in vitro* spore germination and mycelial growth of *Fusarium solani* f. sp. *phaseoli* and *Verticillium albo-atrum*. The pH values and tannin concentration were important factors determining the effectiveness of tannins as inhibitors. Under acidic conditions (pH 5), gallotannin, canaigre, and chestnut

tannins at concentrations varying from 39 to 625 mg/kg inhibited spore germination of both fungi. At a concentration of 1,000 mg/kg these tannins completely prevented growth at pH 5 of both fungi during a 20-day period. Gallotannin prevented growth of both fungi under alkaline as well as acidic conditions.

Antioxidant properties: Most plant-derived polyphenols exhibit strong antioxidant properties (Bors and Michel, 2002; Koleckar *et al.*, 2008) and inhibit lipid peroxidation and peroxygenases, thus scavenging free radicals such as hydroxyl, superoxide, and peroxy molecules which are known to be important in cellular pro-oxidant states (Masaki *et al.*, 1994). Dietary polyphenols have been reported to be capable of modulating *in vivo* oxidative damage in the gastrointestinal tract of some rodents. These data support the hypothesis that dietary polyphenols might have both protective and therapeutic potential in oxidative damage related pathologies (Giovannelli *et al.*, 2000).

Protein-tannin complex protects mucosa: In The EFSA Journal (2005; 222:1-20), it was stated that the consumption of Farmatan® results in the precipitation of a protein/tannin complex which forms a thin layer of insoluble proteins on the surface of the digestive tract. This thin layer is said to protect the mucous membrane from irritation and reduce the absorption of toxic substances. Consequently, less fluid is eliminated in the digestive tract, reducing the danger of dehydration. Bleeding is also reduced, as the tannins also coagulate blood proteins. However, it should be noted that hydrolyzable tannins react with protein at low pH and the complexes formed are reversible at the pH conditions found in most of the digestive tract (i.e., pH>5.0). In this way, chestnut tannins can help control the effect of pathogenic microflora proliferation due to their ability to create an insoluble tannin-protein layer on the surface of bacterial cell membranes. Farmatan® at a level of 1% in drinking water of Wistar rats for 8 hours showed an antidiarrheal effect by delaying the appearance and reducing the intensity and duration of diarrhea experimentally induced by oral gavage with ricinus oil.

Reduction in excreta and litter moisture: In a broiler chicken feeding trial by Schiavone *et al.* (2007), with further details and results by Schiavone *et al.* (2008), using 14-day old Cobb 508 males, litter dry matter contents were 50.6% for negative control, 52.5 for 0.15% chestnut extract, 49.6% for 0.20% chestnut extract and 45.2% for 0.25% chestnut extract. This indicated an excreta and litter drying effect of chestnut extract at the 2 highest levels of inclusion in the diets. Litter total nitrogen contents were 4.03% for negative control, 3.17% for 0.15% chestnut extract, 2.90% for 0.20% chestnut extract and 3.73 for 0.25% chestnut extract, with

differences for negative control vs. 0.15% or 0.20% chestnut extract being significantly different ($p = 0.05$).

Enhanced protein and fiber digestibility: In rats, polyphenols to be absorbed in the small intestine (Carbonaro *et al.*, 2001) may be bound in protein-polyphenol complexes which are modified by intestinal microflora enzymes (Skrabanja *et al.*, 2000), allowing resulting derivative compounds formed by ring-fission to be better absorbed (Saura-Calixto *et al.*, 2007; Del Rio *et al.*, 2010). Polyphenols may also interact with fibers like pectins and have a positive effect in large intestine accessibility. Apple pectin and a polyphenol-rich apple concentrate are more effective together than separately on cecal fermentations and plasma lipids in rats (Aprikian *et al.*, 2003).

Martinez and Moyano (2003) used the pH-stat system to assess the effect of tannic acid on solubility and *in vitro* enzyme hydrolysis of different proteins. Added tannic acid (from 10 to 50 g/kg) increased the enzymatic hydrolysis of casein, pea meal, soybean meal and hemoglobin but decreased the extent of hydrolysis of bovine serum albumin, as measured by total amino acids released and by the degree of hydrolysis. SDS-PAGE confirmed the results of the *in vitro* enzymatic hydrolysis. Improved digestibility of proteins may account for lower fecal nitrogen contents found in some broiler chicken trials.

Anticoccidial effects: McCann *et al.* (2006) used 0 or 500 mg sweet chestnut tannins per kg of diet to determine efficacy against coccidian by reducing intestinal lesions. The birds were challenged with coccidia on day 28 by oral administration of a solution containing sporulated oocysts of *Eimeria acervulina*, *Eimeria tenella* and *Eimeria maxima*. On day 35, birds were slaughtered and dissected and assessed for lesion scores at a number of regions along the intestinal tract. The chestnut tannin treatment did not significantly affect 35-day body weight, feed/gain ratio, or ileal viscosity compared to negative control. The tannin treatment gave a slight improvement in lesion scores with each strain of *Eimeria* used in the challenge compared to negative control scores, but the reduction in lesion scores was less than that with BayCox® live coccidia vaccination by water and avilamycin in feed (positive control). In other words, the tannin treatment was intermediate in effect; however, it was competing against a live coccidia vaccine and an in-feed antibiotic combination.

Broiler feeding trials with chestnut tannins: Salobir *et al.* (2008) conducted 2 repetitions of a small-scale nutrient balance (retention) trial with 5 Ross 308 broiler chickens per treatment (20 total) housed in individual cages with excreta collection to determine effects of

Farmatan® (~73% tannins) at 0, 500, or 1,500 mg/kg of feed on digestibility of selected nutrients. The trials began at 23 to 25 days of age and about 1.2-1.3 kg body weight per bird using diets with 75% of Ross 308 recommended levels for protein, calcium and phosphorus. Mean weight gains were 284, 373 and 354 g/bird, respectively (not analyzed statistically). Feed/gain ratios were 2.30, 2.29 and 2.33, respectively ($p = 0.79$). Excreta dry matter contents were 19.8, 22.1 and 21.7%, respectively ($p=0.60$). Neither nutrient digestibilities nor mineral bioavailabilities were significantly different by treatment ($p = 0.19$ or higher).

Jamroz *et al.* (2009) conducted a feeding trial with 950 one-day-old male chicks to evaluate the effects of Farmatan® tannin supplementation (0, 250, 500, or 1,000 mg/kg) to 28 or 41 days old. Supplementation of 250 or 500 mg of sweet chestnut tannin per kg of feed had nonsignificant beneficial effects on body weight (+3.0% and +2.6%, respectively) and feed conversion ratio (-1.6 and -2.0%, respectively) of 41-day-old chickens compared to negative control birds. The highest tannin supplement (1,000 mg/kg) gave performance similar to the negative control. Effects on the intestinal tract such as decreased number of mucogenous goblet cells and destabilization of enterocytes indicated that the tannins were definitely present at the highest level. No effects of tannin supplementation on carcass quality were found; sampled birds were somewhat different in body weight. Addition of tannin to the diets increased dry matter content of the litter by 8.8% (250 and 7.7% (500 mg/kg) when compared to negative control results. For 28-day old chickens, higher doses of tannins (500 or 1,000 mg/kg) significantly reduced the number of *E. coli* and coliform bacteria in the small intestine compared to negative controls, with 250 mg/kg tannin treatment results intermediate. For other microorganisms, great variability of microbial populations in small intestine and colon were observed. The histologies of jejunal walls in chickens of negative control (0 mg/kg), 250 mg/kg and 500 mg/kg groups were similar. The structure was characteristic of correctly developed and functioning tissues and the villi were formed correctly. Tannin applied at the highest dose (1,000 mg/kg) showed some degradation processes and disturbances in intestinal wall morphology and function.

In an Egyptian paired-house field trial (2010), there were 3 houses with 54,000, 28,410 and 26,900 chicks (109,310 total) that received positive (lincomycin) control diets and 2 houses with 27,550 and 53,400 broilers that received Farmatan® (500 mg/kg) supplemented diets. Average age of broilers in the control houses was 36.12 days whereas the ages in the 2 Farmatan® houses were 35.61 and 35.14 days. Data by house was not provided; therefore, no statistical analysis was possible. Using Farmatan® in the diets, body weight was

increased by 0.035 kg (+2.17%), feed conversion ratio was decreased by 0.08 (-4.44%) and European broiler performance index was improved by 22.0 (+9.25%) at similar mortalities (4.01 vs. 3.97%, respectively).

The Panvita Group, Murska Sobota, Slovenia (April-May, 2011) conducted a commercial broiler field trial and collected intestinal samples from birds at the time of slaughter to compare effects of 2 dietary treatments, either negative control or Farmatan® (500/250/500 mg/kg feed). Color pictures taken under high-powered microscope were examined and it was found that broilers fed dietary Farmatan® had longer (1,211 vs. 503 µg), healthier villi and more prominent blood vessels than negative control broilers and these intestinal mucosa changes were associated with a reduced feed conversion ratio for the Farmatan® group. On average during the growout, the Farmatan®-fed broilers consumed about 0.111 liter less water per bird per day than negative control broilers.

Yellow-feathered broilers were used in a Farmatan® feeding trial from 11 to 55 days of age at a research farm in China (2012). There were 3 replicate pens of 80 chickens each/ treatment. The dietary treatments were: 1) positive control diets with virginiamycin 22/22/11 mg/kg; 2) positive control + Farmatan® 400 mg/kg; 3) Farmatan® 500 mg/kg; Farmatan® 1,000 mg/kg; Farmatan® 1,500 mg/kg; and Farmatan® 750/400/1,300 mg/kg. There were no significant differences by treatment but feed/gain ratios were better with Farmatan® supplementation.

The purpose of pen trial described in this report was to evaluate the effects of different doses of dietary Farmatan® on live performance responses of broilers and on litter characteristics.

MATERIALS AND METHODS

A 42-day Cobb×Cobb 500 male broiler pen trial on built-up litter (~10 cm), top dressed with pine shavings, was conducted at Southern Poultry Research, Inc., Athens, Georgia, USA from June 6 to July 18, 2012 under summer conditions. The trial was conducted under Standard Operating Procedures of the test facility to assure that animal welfare was acceptable. The objectives of the trial were: 1) to determine the optimal level(s) of Farmatan® in broiler diets for improving body weight and feed conversion ratio using 0 to 1,000 ppm (mg/kg) inclusion levels compared to basal diets and antibiotic diets and 2) to confirm the moisture reduction in excreta and litter by analyzing litter moisture and doing subjective scoring of litter in pens when Farmatan® is fed.

At the hatchery, the chicks received routine vaccinations and were sexed. The diets were provided *ad libitum* in one tube-type feeder per pen. From day 0 until day 7 feed was also supplied on trays directly placed on the litter. Water was provided *ad libitum* from one per pen Plasson-type (bell shaped) automatic watering fount.

Stocking density was estimated to be approximately 0.077 sq. m/bird (0.83 sq. ft/bird) at the end of the trial (42 days), with space occupied by equipment being subtracted out. Illumination was provided by fluorescent bulbs placed above the pens. The lighting scheme was 24 hours of light per day. Pens were checked daily for mortality. A bird was culled only to relieve suffering. When a bird was culled or found dead, the date and removal weight (kg) were recorded. A gross necropsy was performed on all dead or culled birds to determine the sex and probable cause of death.

There were 6 dietary treatments as shown in Table 1. The experiment consisted of 48 pens with 45 newly hatched male broiler chicks per pen initially. The 6 treatments were replicated within 8 blocks of pens. All chicks were spray vaccinated at Southern Poultry Research, Inc. with label recommended dosage of Coccivac-B® on day of hatch using a Spraycox® machine. Commercial type (corn-soy-meat meal) chicken starter, grower and finisher diets made in large batches (Table 2) and experimental diets were prepared by treatment using aliquots from the larger batches. All feeds were steam pelleted using a California Pellet Mill and fed as crumbles or pellets. Feed phases were 0-21, 21-35 and 35-42 days of age.

On day 42, litter was taken by hand from 5 locations and pooled together in drying containers to make a composite sample of about 100 g per pen. Litter condition was visually scored (0 driest to 5 wettest). The following point scale and definitions were used to grade the quality of the litter/bedding:

- 0 = Dry, friable material throughout the pen
- 1 = Predominantly dry material but with some evidence of crusting around drinkers and feeders
- 2 = Litter material mostly acceptable but with some areas of wet shavings or capped material
- 3 = Poor quality litter material with a large proportion of wet areas and capping of the litter
- 4 = Unacceptable litter quality-wet and capped but with a few areas of dry material remaining
- 5 = All litter wet and soggy, no dry areas left

Means for live weight, weight gain, feed conversion ratio, mortality-adjusted feed conversion ratio, mortality, litter moisture and litter scores were calculated. The raw data was analyzed statistically (ANOVA) using a Random Complete Block Design with 6 treatments and 8 replicate pens per treatment (Statistix 8, Analytical Software, Tallahassee, FL, USA). LSD test ($p = 0.05$) was used to separate means when ANOVA F values were significant ($p = 0.05$).

RESULTS AND DISCUSSION

The results from the Farmatan® dose-response trial are presented in Table 3-6. The BW gains (kg) were not significantly different from 0-21 days or 0-35 days but

Table 1: Experimental design including dietary treatments, pens/treatment and chicks/pen

Treatment	Dietary treatment	Replicate pens/treatment ¹	Chicks/pen No.
1	Negative control (0 ppm Farmatan®)	8	45
2	Farmatan® 250 ppm (0.025%)	8	45
3	Farmatan® 500 ppm (0.050%)	8	45
4	Farmatan® 750 ppm (0.075%)	8	45
5	Farmatan® 1,000 ppm (0.100%)	8	45
6	BMD® 55 ppm (0-35 d); Stafac ppm (35-42 d)	8	45

¹There were 8 blocks of 6 replicate pens each.

Table 2: Ingredients and calculated nutrient contents of experimental broiler chicken basal diets (starter, 0-21 days; grower, 21-35 days; and finisher, 35-42 days)

Ingredient or nutrient	Starter (%)	Grower (%)	Finisher (%)
Corn	59.62	63.93	68.87
Soybean meal	30.83	26.51	21.94
Fat, animal	2.82	3.07	2.97
Meat meal	4.00	4.00	4.00
Dicalcium phosphate	1.08	1.03	0.79
Limestone	0.70	0.57	0.62
Salt	0.44	0.41	0.41
Standard micromix ¹	0.45	0.41	0.34
Choline Cl (70%)	0.06	0.07	0.06
Total	100.00	100.00	100.00
Calculated analysis:			
Metabolizable energy (kcal/kg)	3,100	3,190	3,243
Crude protein (%)	21.86	20.09	18.22
Lysine (%)	1.22	1.10	0.96
Methionine (%)	0.58	0.53	0.46
Met. + cys. (%)	0.95	0.88	0.78
Crude fat (%)	6.00	6.00	6.00
Crude fiber (%)	3.00	3.00	3.00
Calcium (%)	0.95	0.91	0.82
Phosphorus total (%)	0.69	0.67	0.60
Sodium (%)	0.22	0.21	0.21

¹Standard micromix at 0.45% in starter provides per kg feed: DL-methionine, 2.292 g; copper, 2.4 ppm from copper sulfate; thiamine, 1.1 ppm; riboflavin, 6.6 ppm; L-lysine, 0.273 g; D-pantothenic acid, 11 ppm; vitamin B12, 11 mcg/kg; niacin, 38.5 ppm; biotin, 0.055 ppm; vitamin E, 13.23 IU/kg; vitamin A, 6,613.8 IU/kg; folic acid, 0.715 ppm; MSBC (source of vitamin K), 3.85 ppm; pyridoxine, 1.92 ppm from pyridoxine HCl; vitamin D3, 2,204.6 IU/kg; manganese, 80.4 ppm from manganous oxide; zinc, 64.2 ppm from zinc oxide; iodine, 0.6 ppm from calcium iodate; and selenium, 0.24 ppm from sodium selenite.

Table 3: Farmatan® broiler dose-response pen trial live performance results and litter moisture (%) by treatment during the starter period, 0-21 days of age (LSD p = 0.05)¹

Treatment	No.	Day 21 BW, kg	0-21d BW Gain, kg	0-21d Feed Conv. Ratio	0-21 d MAFCR	0-21 d Mortality, %	21-d Litter Mortality, %
Neg. Control	1	0.711	0.665	1.537a	1.513a	2.778	25.29b
Farmatan 250	2	0.703	0.656	1.504ab	1.488ab	2.222	26.38ab
Farmatan 500	3	0.720	0.674	1.495b	1.476bc	2.500	23.01b
Farmatan 750	4	0.720	0.674	1.495bc	1.469bc	1.389	23.19b
Farmatan 1000	5	0.718	0.673	1.478bc	1.454c	2.222	25.05b
BMD/Stafac	6	0.716	0.670	1.450c	1.442c	1.111	29.44a
SEM		0.004	0.004	0.007	0.006	0.287	0.623
P-value		0.803	0.789	0.002	0.002	0.570	0.032

¹Farmatan levels are in ppm (mg/kg). BMD® at 55 ppm was used. BW is body weight. MAFCR is mortality-adjusted feed conversion ratio.

from 0-42 days (p = 0.002) were, respectively: 2.238^b, 2.238^b, 2.299^a, 2.282^{ab}, 2.290^a, 2.316^a. The 42-day BW was significantly heavier for the Farmatan 500, 750 and 1000 groups and for BMD/Stafac group than for negative control. The BW gain from 0-42 days was significantly better for Farmatan 500 and 1000 and for BMD/Stafac than negative control group. Farmatan 500 ppm and higher groups were statistically equivalent to BMD/Stafac treatment for 42-day BW and 0-42 day BW gain.

During the grower phase (21-35 days), there appeared to be a peak in body weight gain with Farmatan 250 and 500 with a slight decline at higher levels or with BMD/Stafac but a minimum with regard to mortality-adjusted feed conversion ratio with Farmatan 750 and 1000 (they were best; better than BMD/Stafac which was slightly better than Farmatan 500 result). Farmatan clearly exceeded BMD 55 ppm in body weight gain and feed conversion ratio improvement during the grower period.

Table 4: Farmatan® broiler dose-response pen trial live performance results by treatment during the grower period, 21-35 days, and from 0-35 days of age (LSD p = 0.05)¹

Treatment	No.	21-35d BW Gain, kg	21-35d Feed Conv. Ratio	Day 35 BW, kg	0-35d Feed Conv. Ratio	0-35 d MAFCR	0-35d Mort., %
Neg. Control	1	0.980	1.803	1.690	1.689	1.666a	4.167
Farmatan 250	2	0.995	1.803	1.697	1.678	1.657ab	3.889
Farmatan 500	3	1.003	1.779	1.722	1.660	1.646abc	3.333
Farmatan 750	4	0.975	1.769	1.695	1.645	1.626cd	3.056
Farmatan 1000	5	0.964	1.783	1.682	1.648	1.621d	4.444
BMD/Stafac	6	0.962	1.825	1.678	1.665	1.641bcd	3.333
SEM		0.007	0.009	0.006	0.006	0.004	0.436
P-value		0.064	0.492	0.112	0.130	0.001	0.949

¹Farmatan levels are in ppm (mg/kg). BMD® at 55 ppm was used from 0-35 days. BW is body weight. Conv. is conversion. MAFCR is mortality-adjusted feed conversion ratio. Mort. % is mortality %.

Table 5: Farmatan® broiler dose-response pen trial live performance results by treatment during the finisher period, 35-42 days, and from 0-42 days of age (LSD p = 0.05)¹

Treatment	No.	35-42d BW Gain, kg	35-42d Feed Conv. Ratio	Day 42 BW, kg	0-42d BW Gain, kg	0-42d Feed Conv. Ratio	0-42d MAFCR
Neg. Control	1	0.593c	1.791	2.284c	2.238b	1.715	1.694a
Farmatan 250	2	0.610bc	1.830	2.307bc	2.238b	1.718	1.698a
Farmatan 500	3	0.623bc	1.811	2.346ab	2.299a	1.698	1.685ab
Farmatan 750	4	0.633bc	1.786	2.328abc	2.282ab	1.683	1.665bc
Farmatan 1000	5	0.654ab	1.796	2.336ab	2.290a	1.688	1.661bc
BMD/Stafac	6	0.684a	1.704	2.362a	2.316a	1.676	1.655c
SEM		0.008	0.015	0.008	0.008	0.006	0.004
P-value		0.012	0.190	0.035	0.004	0.949	0.898

¹Farmatan levels are in ppm (mg/kg). BMD® at 55 ppm was used from 0-35 days and Stafac® at 22 ppm was used from 35-42 days. Mort. is mortality. BW is body weight. Conv. is conversion. MAFCR is mortality-adjusted feed conversion ratio.

Table 6: Farmatan® broiler dose-response pen trial live performance results by treatment overall, 0-42 days, or at 42 days (LSD p = 0.05)¹

Treatment	No.	0-42d Mort., %	42d Litter Moisture, %	42d Litter Score (0-5)	42d Litter Nitrogen, %	42d Litter crude Protein, %
Neg. Control	1	4.722	29.52a	3.625a	2.921	18.6
Farmatan 250	2	4.167	24.05b	3.500a	3.098	19.36
Farmatan 500	3	3.611	22.11b	3.625a	3.060	19.13
Farmatan 750	4	3.333	23.59b	2.625b	2.890	18.06
Farmatan 1000	5	5.000	23.18b	2.625b	2.659	16.62
BMD/Stafac	6	3.611	25.15ab	3.250ab	2.700	16.87
SEM		0.460	0.755	0.111	0.069	0.429
P-value		0.898	0.046	0.008	0.355	0.355

¹Farmatan levels are in ppm (mg/kg). BMD® at 55 ppm was used from 0-35 days and Stafac® at 22 ppm was used from 35-42 days. Mort. % is mortality %. Litter score, 0 driest to 5 wettest. Litter crude protein % = litter nitrogen % x 6.25.

The BW gain was significantly greater for Farmatan 1000 and BMD/Stafac groups than negative control from 35-42 days (finisher phase). Farmatan 1000 and BMD/Stafac were statistically equivalent and highest in 35-42 day BW gain. Stafac® 22 ppm in finisher (35-42 days) gave the best BW gain and feed conversion ratio, indicating its beneficial effects.

From 0-21 days, Feed Conversion Ratio (FCR) and Mortality-adjusted Feed Conversion Ratio (MAFCR) values were significantly reduced by Farmatan 500 and higher and by BMD/Stafac compared to negative control results. Farmatan 750 and 1000 were statistically equivalent with BMD/Stafac for 0-21 day FCR. Mortality-adjusted Feed Conversion Ratios (MAFCR) from 0-21 days (p = 0.002) were, respectively: 1.513^a, 1.488^{ab}, 1.476^{bc}, 1.469^{bc}, 1.454^c and 1.442^c. Farmatan 1000 performed best among Farmatan® levels with regard to

MAFCR, although no plateau was reached indicating that higher doses may have continued to give beneficial responses. Farmatan 500 and higher were statistically equivalent with BMD/Stafac in MAFCR. The MAFCR from 0-35 day (p = 0.001) were, respectively: 1.666^a, 1.657^{ab}, 1.646^{abc}, 1.626^{cd}, 1.621^d and 1.641^{bcd}. The MAFCR from 0-42 day (p = 0.004) were, respectively: 1.694^a, 1.698^a, 1.685^{ab}, 1.665^{bc}, 1.661^{bc} and 1.655^c. The 0-42 day MAFCR was significantly reduced by Farmatan 750 and 1000 and by BMD/Stafac compared to negative control values.

By interpolation, Farmatan® at ~561 ppm was equivalent to BMD® 55 ppm for improving 0-35 day MAFCR. Using the Farmatan 500 and 750 values for 0-35 day MAFCR (1.6456 and 1.6264, respectively; difference 0.0192) and BMD/Stafac value (1.6409; difference of 0.0047 from Farmatan 500), it can be estimated that Farmatan at 561

ppm would have equaled BMD® 55 ppm (that is, $0.0047/0.0192 \times 100 = 24.479\%$ of difference of 250 ppm between 500 and 750 ppm, or +61) during starter and grower period combined.

During the finisher phase (35-42 days), Stafac® (Virginiamycin) at 22 ppm gave the highest body weight gain (not significantly different from Farmatan 1000 ppm) and the lowest feed conversion ratio although there were no significant difference between treatments. Stafac® clearly performed very well during the finisher phase. Stafac® had 35-42 day gain of 0.6844 kg/bird whereas other treatments had 0.5934 to 0.6439 kg/bird. Similarly, Stafac® had 35-42 day finisher period feed conversion ratio of 1.7036 compared to 1.8302 to 1.7863. A study of Farmatan® and Stafac® combined during the finisher period may be advisable to determine whether there is any beneficial interaction due to their different modes of action.

Mortality % were not significantly different by treatments at any age. Although not shown in Table 3-6, the mortality % for grower (21-35 days) and finisher (35-42 days) periods were not significantly different by treatments either.

Litter moisture % at 21 days was lower ($p = 0.032$) using Farmatan® 500, 750, or 1,000 ppm than BMD® 55 ppm and at 42 days was lower ($p = 0.046$) for each Farmatan® level than for negative control. Farmatan® 750 or 1,000 ppm improved ($p = 0.008$) 42-day litter score (0 driest to 5 wettest) compared to negative control. Farmatan® promoted drier litter.

Farmatan 750 and 1000 gave significantly lower (better) 42-day litter scores than either negative control or Farmatan 250 or 500, with BMD/Stafac litter scores being intermediate. The 42-day litter nitrogen % and crude protein % were numerically lower in the Farmatan 1000 and BMD/Stafac groups.

Overall, because the Farmatan® doses were constant by treatment during the trial, it is useful to look at 42-day results. The Farmatan 250 inclusion rate did not offer much benefit overall with regard to 0-42 day live performance results which were identical or very close to negative control results. Benefits in live performance started accruing at Farmatan® level of 500 ppm and tended to improve further with 750 and 1,000 ppm. The results here agree with those of Jamroz *et al.* (2009) who fed Farmatan® at 0, 250 and 500 ppm to broiler chickens from 0-41 days and found improved BW, BW gain and FCR for the Farmatan® diets compared to negative controls. Those researchers found that Farmatan® at 1,000 ppm gave similar results to negative control except that there was some degradation and morphological changes in the intestinal villi due to this level of tannins. Salobir *et al.* (2008) conducted 2 five-day nutrient digestibility (balance) trials (Exp. 1 and 3) in which Farmatan® at 500 or 1,500 ppm increased BW gain, without any significant changes in nutrient

digestibility or mineral bioavailability and increased excreta dry matter content (reduced moisture) compared to negative control group.

In conclusion, regarding Farmatan® dose-responses, during the starter period (0-21 days) Farmatan 1,000 ppm performed best especially with regard to MAFCR. The MAFCR can only be calculated from day 0 and not for interim phases such as 21-35 or 35-42 days. During the grower period, BW gain from 21-35 days was curvilinear peaking with Farmatan® at 500 ppm, and FCR for all Farmatan® levels (including 0 ppm) were numerically lower than BMD® 55 ppm. Using MAFCR results for the combined starter and grower period (0-35 days), it was interpolated that Farmatan® at 561 ppm was equivalent to BMD® 55 ppm. In the finisher period, Stafac® gave the best BW gain and FCR improvements. From 0-42 days, Farmatan® showed growth and feed conversion benefits, as well as drier litter at the higher levels of Farmatan®, using male Cobb broiler chickens on built-up litter top dressed with new shavings in a summer pen trial.

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