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Research Article
Performance of Broilers Exposed to Used Litter and Fed Diets with Agolin® Poultry, BMD®/Stafac® or No Additive from 0-42 Days

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Abstract

Background and Objective: Agolin® Poultry is an encapsulated blend of essential oil compounds. A 42 day trial was conducted with 1,800 broiler chicks using 3 dietary treatments and 20 pens/treatment (30 chicks/pen) to evaluate live performance. **Methodology:** Chicks received live coccidia vaccine at placement (day 0). Pelleted basal diets (CON) or diets with Agolin® Poultry (200 mg kg^{-1}, 0-14 days and 100 mg kg^{-1} 14-42 days) or BMD® (55 mg kg^{-1}, 0-28 days) and Stafac® (22 mg kg^{-1}, 28-42 days) were fed. Litter was new wood shavings initially and used litter was added on day 4 to provide *Eimeria* and bacterial pathogens. Daily light: dark cycle was 16:8 h. Litter was sampled at 14, 28 and 42 days for moisture and ammonia (by analyzing for nitrogen, then calculating theoretical ammonia). Oocysts per gram (OPG) feces were counted and foot pad lesion severity scores (0-2) were taken at 42 days. **Results:** The 28 and 35-day body weights were heavier on Agolin® or antibiotic diets than CON diets. Weight gain from 14-28 or 14-35 days was greater on Agolin® diets than CON with antibiotic diets intermediate. The 0-35 days mortality-adjusted feed conversion ratios (MAFCR) were lower on Agolin® or antibiotic diets than CON diets. Mortality (2.46-3.18%) was unaffected by treatment. Mortality percentage was unaffected by treatment. Litter moisture, OPG in feces and foot pad lesion scores were not different but 28 day litter ammonia tended to be lower on Agolin® diets. **Conclusion:** The 35-day BW and 0-35 day MAFCR were significantly improved by Agolin® or antibiotic diets compared to CON diets.

Key words: Agolin, antibiotic, broiler, essential oil, litter

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Essential oil blend: Agolin® Poultry is a blend of high quality plant active ingredients designed to improve "Gut health" by optimizing feed intake, daily gain and feed utilization of broilers and turkeys. All ingredients are of food grade or pharmaceutical quality and Joint Expert FAO/WHO Committee on Food Additives (JEFCA) accepted. Its microencapsulation provides a physical form (0.5 mm particle size) that is excellent for feed processing and handling, with a pleasant smell. The unique double coating provides optimal stability during feed manufacturing and storage but is readily soluble in intestinal juice.

The main compounds in Agolin® Poultry are eugenol ($C_{10}H_{12}O_2$) for control of pathogens[1-3] and as an antioxidant[4], nerolidol ($C_{15}H_{26}O$) for control of pathogens[3] and piperine ($C_{17}H_{19}NO_3$) for stimulation of digestive enzymes[5,6], villi length[7], gut motility[8] and intestinal permeability and nutrient bioavailability[7,9-11]. Piperine is a pungent alkaloid substance from black pepper (*Piper nigrum* L.) and long pepper (*Piper longum*) with diverse physiological effects[12,13] such as improving the bioavailability of beta-carotene, iron and selenium, increasing blood supply to the intestinal tract, increasing emulsifying in the gut and increasing active nutrient transport across the gut wall.

Another component is thymol ($C_{10}H_{14}O$), a monoterpene phenol with a strong aromatic odor, produced by thyme (*Thymus serpyllum*) and horsemint (*Monarda punctata*). It promotes the motility or normal movement (peristalsis) of the intestinal tract by smooth muscle contractions[14,15] and it promotes permeability[16]. Thymol is known to have antibacterial effects against certain pathogens *in vitro* and *in vivo*[1,2,16-27] and to stimulate innate immune response[28].

Broiler chicken studies: Ocak *et al.*[29] supplemented broiler diets with a level of 0 or 0.2% dried thyme leaves (providing thymol at 0 or 70 mg kg^{-1} feed) from 7-42 days to evaluate performance. Live performance parameters were unaffected by treatment but thymol addition to the diets significantly increased abdominal fat pad on processing.

Cardoso *et al.*[30] administered piperine daily by oral dose at 0, 1.12, 2.25 or 4.5 mg kg^{-1} body weight to newly hatched male broiler chicks for 14 days and found normal body weight gain, relative liver weight unchanged and normal size and color of other organs including the intestinal tract were observed. Liver histopathological changes were noticed in a dose-dependent manner. At 1.12 or 2.25 mg piperine kg^{-1} body weight, there was an increase in the number of heterophils to total leukocytes compared to controls. Cordoso *et al.*[31] added piperine at 0, 60, 120 or 180 mg kg^{-1} of feed for male broiler chickens from 0-35 days and observed that diets with 60 mg kg^{-1} piperine numerically improved 8-42 day weight gain improved by 76 g/bird (2.663 vs. 2.587 kg) and feed/gain ratio by 0.12 (1.70 vs. 182) without affecting mortality or carcass yield compared to control. Piperine at all levels of inclusion were most effective at improving growth and feed conversion from 36-42 days.

Kollanoor-Johny *et al.*[2] added eugenol or thymol at different concentrations (ranging from 10-75 mM for *Salmonella enteritidis* and 10-30 mM for *Campylobacter jejuni*) to autoclaved chicken cecal contents inoculated with approximately 7.0 log10 CFU mL^{-1} of *Salmonella enteritidis* or approximately 5.0 log10 CFU mL^{-1} of *Campylobacter jejuni*. The pathogen populations in the cecal contents after 15 sec, 8 and 24 h of incubation at 40°C were determined. *Campylobacter jejuni* was more sensitive to each of the molecules than *Salmonella enteritidis* ($p \leq 0.05$). Eugenol decreased ($p \leq 0.05$) *Salmonella enteritidis* and *Campylobacter jejuni* counts to <1.0 log10 CFU mL^{-1} at 75 and 30 mM, respectively. Although, not as effective as eugenol, thymol also reduced the counts of both pathogens. Kollanoor-Johny *et al.*[32] challenged straight-run broiler chickens at 30 days of age with a 4-strain mixture of *Salmonella enteritidis* (8 log10 cfu/bird). Birds from each group were killed after 24 h (day 31) to check for colonization of *Salmonella enteritidis* in the cecum and cloaca. Birds were given feed supplemented with eugenol (1%) for 5 days before slaughter on day 42 for determination of *Salmonella enteritidis* populations in the cecum and cloaca. The experiment was repeated 2 times. There was ~1.5-2 log10 reduction in *Salmonella enteritidis* counts in ceca and cloaca with eugenol supplementation.

In a feeding trial with male broiler chickens[33] observed that eugenol at 500 mg kg^{-1} of diet from a microencapsulated source significantly increased 21 day body weight and weight gain compared to control diet. Eugenol showed equivalent antioxidant activity to butylated hydroxyanisole (BHA). Eugenol had minimum inhibitory concentrations in the range of 0.4-0.6 mg mL^{-1} against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Clostridium perfringens*.

In an unpublished (Agolin S.A.) small-scale commercial broiler chicken feeding trial with 4 pens (treatments) of 125 chickens each given either 20 mg oxytetracycline per kg or 40, 80, or 160 mg Agolin® Poultry per kg diets from 0-43 days, blood glutathione peroxidase levels were significantly higher at 21 days for the 80 or 160 mg kg^{-1} Agolin® Poultry diets and at 43 days for all three of the Agolin® diets compared

to oxytetracycline control. The main role of glutathione peroxidase is to protect the birds from oxidative damage. Lactobacillus spp. proliferated in the ileum and ceca contents at 21 and 43 days in the Agolin®-fed birds to a significantly ($p<0.05$) greater extent than in the oxytetracycline-treated birds (with the exception that 43 day old, 40 mg kg^{-1} Agolin®-fed birds had intermediate results). Cecal *Escherichia coli* counts were significantly ($p<0.05$) lower at 21 days for birds fed 160 mg kg^{-1} Agolin® diets compared to oxytetracycline diets.

The objective of this broiler feeding trial was to compare the efficacy of Agolin® Poultry supplemented diets with basal diets or diets supplemented with antibiotics (BMD®, Stafac®) for improving live performance, reducing foot pad lesion scores and lower litter moisture and ammonia contents.

MATERIALS AND METHODS

Location and study dates: The broiler chicken pen trial was conducted at a facility managed by Virginia Diversified Research Corporation, 1866 East Market Street, Harrisonburg, Virginia, USA (investigator, Michael D. Sims). Study dates were October 13 (day 0) to December 12 (day 42), 2017.

Broiler chicks: Newly hatched, straight-run (as hatched) Ross 308 chicks were used. About 2,000 chicks were obtained to assure that 1,800 healthy chicks could be placed in the study.

Study design: A randomized complete block design with 3 dietary treatment groups having 20 replicate pens of 30 chicks each was used (Table 1). Pens were located in 20 blocks of 3 pens each which represented each treatment. Data were subjected to analysis of variance and when significant at $p \leq 0.05$, means were separated by Tukey's test at $p = 0.05$ (Statistix® 8, Analytical Software, Tallahassee, Florida).

Dietary treatments: A series of basal diets (starter, grower and finisher, Table 2) served as Control and basal diets supplemented with bacitracin methylene disalicylate (BMD®, Parsippany, New Jersey, USA) at 55 mg kg^{-1} from 0-28 days and virginiamycin (Stafac®, Phibro Animal Health Corporation, Teaneck, New Jersey, USA) at 22 mg kg^{-1} from 28-42 days or Agolin® Poultry (Agolin S.A., Bière, Switzerland) at 200 mg kg^{-1} from 0-14 days and 100 mg kg^{-1} from 14-42 days were test feeds. *Ad libitum* feeding was practiced so that feed was available at all times with the quantity and frequency of consumption being the free choice of the birds. Water was available at all times.

Experimental feeds: All feeds were based on corn, soybean meal, meat and bone meal, corn dried distiller's grains with solubles and soybean oil. All feeds were prepared on site by Virginia Diversified Research Corporation personnel. Each feed type (starter, grower and finisher) was prepared from a single large basal diet and were provided by age of bird (starter feed from 0-14 days, grower feed from day 14-28 days and finisher feed from day 28-42 days). All basal feeds were mixed in a 2,722 kg capacity mixer, subdivided into equal aliquots for each treatment, then blended with supplements for 8 min in tumble mixers (136 or 907 kg capacity). Each aliquot was steam pelleted (in crumbled form for starter feeds) using a GH300 PTO pellet mill with accompanying steam generator adding between 2 and 3 percent moisture to each mix. The temperature during pelleting was between 79.4 and 85 degrees Celsius (175 and 185 degrees Fahrenheit) for each experimental feed. Each pelleted aliquot was allowed to cool and dry for at least 30 min prior to storing in storage containers to prevent molding and spoilage.

Study pens: Each pen contained one bell-type water fountain and one 34 kg capacity tube feeder. The dimensions of each pen were 1.22×1.52 m which provided a stocking density of 0.0618 m^2 per bird when there are 30 broilers per pen (based on pen dimensions). New wood shavings used as bedding was present at placement (day 0) in each pen and used litter was introduced in an equal amount per pen on day 4. The added litter at day 4 was ground to remove clumps and mixed to make it physically uniform in texture prior to placement and contained low enough levels of coccidia (primarily *Eimeria acervulina* and *Eimeria maxima*), *E. coli*, salmonella and clostridia to allow for a mild disease challenge to naïve chickens.

Lighting: An 18 h light and 6 h dark regimen was provided daily during the feeding trial.

Table 1: Experimental design (randomized complete block) and dietary treatments (three)

Dietary treatments	Inclusion by feed phases (mg kg^{-1})	Replicate pens/treatment	Chicks/pen at placement	Blocks of 3 pens each
Control (none)	0/0/0	20 (19)[1]	30	20
BMD®/Stafac®	50/50/20	20 (19)	30	20
Agolin® Poultry	200/100/100	20 (19)	30	20

[1]Design was 20 replicate pens/treatment but one outlier pens was removed per treatment so raw data from 19 pens/treatment was used for statistical analysis

Table 2: Composition and calculated nutrient values of basal diets (by age of birds, days)

	Percentage		
Ingredient or Nutrient	Starter (0-14 d)	Grower (14-28 d)	Finisher (28-42 d)
Ingredients			
Corn, yellow	58.26	61.32	63.60
Soybean meal, dehulled	32.12	27.37	23.00
Corn distillers dried grains w/sol	4.00	6.00	8.00
Meat and bone meal	2.00	2.00	2.00
Soybean oil	0.67	0.66	0.90
Limestone	0.77	0.72	0.68
Dicalcium phosphate	1.01	0.88	0.76
Salt	0.48	0.475	0.467
Choline chloride (60%)	0.08	0.075	0.075
Vitamin premix[1]	0.04	0.04	0.04
Trace mineral premix[2]	0.085	0.085	0.068
L-lysine HCl	0.18	0.156	0.16
D,L-methionine	0.263	0.199	0.195
L-Threonine	0.024	0.002	0.037
Phytase	0.018	0.018	0.018
Calculated nutrients			
Crude protein	22.14	20.57	19.23
Crude fat	3.80	4.06	4.55
Crude fiber	3.00	3.00	3.00
Metabolizable energy (kcal kg^{-1})	3,053	3,086	3,131
Lysine	1.34	1.20	1.09
Methionine+Cysteine	0.99	0.89	0.84
Threonine	0.89	0.80	0.78
Calcium	0.85	0.80	0.75
Phosphorus, available	0.42	0.40	0.38
Sodium	0.23	0.23	0.23
Potassium	0.90	0.84	0.78

[1]Vitamin premix contained per kg: Vitamin A: 22,046,000 IU, Vitamin D3: 9,920,700 IU, Vitamin E: 88,184 IU, Niacin: 176,368 mg, Pantothenic acid: 8,165 mg, Riboflavin: 19,841 mg, Pyridoxine: 10,362 mg, Thiamine: 6,614 mg, Folic acid: 4,850 mg, Biotin: 441 mg and Vitamin B12: 33,069 mcg, [2]Trace mineral premix contained per kg: Copper: 5,900 mg, Iodine: 1,850 mg, Iron: 21,500 mg, Manganese: 103,000 mg, Selenium: 352.94 mg and Zinc: 81,800 mg

Daily observations and mortality: The broilers were observed at least once daily to assure adequacy of feed, water, ventilation and environmental temperature and to monitor health condition. Mortality was recorded by pen daily. Each time a dead bird was found and recorded, along with weight to calculate mortality-adjusted feed conversion ratio.

Live bird and feed weights: All broilers were weighed by pen when bird ages were 0, 7, 14, 28, 35 and 42 days. Feeds were weighed in as needed and weighed back each time birds were weighed. Feed/gain ratio, feed conversion ratio and mortality-adjusted feed conversion ratio were calculated.

Litter moisture and nitrogen (ammonia): On days 14, 28 and 42 one litter sample (~120 g each) per pen was collected into pre-labeled (study id-sample type-date) sample bags which were sealed and placed on ice as soon as collected and then frozen. These samples were analyzed for nitrogen at ANALAB in Fulton, Illinois, USA and that was used to calculate "Theoretical ammonia". Nitrogen (ammonia) was extracted from the litter by allowing it to steep in a 0.2 N (normal) sulfuric acid solution for 18 h (overnight) with mild agitation. Then the solution was filtered through a 0.45 micron filter. This filtrate was analyzed for nitrogen (corrected for the dilution factor of the sulfuric acid solution) and used to calculate theoretical ammonia (NH_3) in ppm (mg kg^{-1}).

Coccidia oocyst counts: On the morning of day 42, 20 g of fresh feces was collected from each pen for coccidia oocyst counts per gram (OPG). Samples were homogenized and a 1 g aliquot was placed in a 14 mL Falcon® tube followed by the addition of 9 mL of a 25% Magnesium sulfate/water solution. The tubes were shaken, a lid applied and then placed into a centrifuge for 3 min at 3,000 rpm. Tubes were removed and oocysts counted using a hemacytometer and microscope.

Foot pads: On Day 42, 10 birds per pen were randomly caught and foot pads scored as follows:

- No lesions or very small superficial lesions, slight discoloration on a limited area, mild thickening of the skin
- Mild lesion, discoloration of the foot pad, superficial lesions, dark papillae
- Severe lesion, ulcers or scabs, signs of hemorrhages or swollen foot pads.

RESULTS AND DISCUSSION

Used litter microbiological assay: A sample of the recently used litter from a healthy flock added to each pen on day 4 to give a natural disease challenge to naive birds was analyzed to obtain its microbial profile (Table 3).

Body weight and weight gain: As shown in Table 4, body weights were not significantly different between treatments at 7, 14, 21, or 42 days. The 28 and 35-d body weights were significantly greater for BMD®/Stafac® or Agolin® treatments than for Control treatment.

Body weight gain is listed by interim periods in Table 5. The antibiotic diets gave significantly heavier weight gain from 7-14 days than the Control diets with Agolin® diets intermediate. The statistical patterns for 14-28 days and 14-35 days were similar and indicated that Agolin® diets had significantly greater gains than Control diets with antibiotic diets intermediate. In Table 6, body weight gain is listed by other interim periods (from 21, 28, or 35 days). There were no significant differences between treatments.

This agrees with Cordosa *et al.*[31] who added piperine alone at 0, 60, 120, or 180 mg kg^{-1} of feed for male broiler chickens from 0-35 days and observed that diets with 60 mg kg^{-1} piperine numerically improved 8-42 days weight

Table 3: Used litter microbiological assay (ANALAB, Fulton, Illinois, USA)[1]

	Colony forming units (CFU g^{-1}) litter, "as received basis"			
Description	*Clostridium perfringens*	Total coliforms	*Escherichia coli*	*Salmonella* spp.
Litter	620,000	5,100,000	<100	<100; negative

[1]Coccidia oocyst counts done by personnel at Virginia Diversified Research Corporation were determined to be 2,250 oocysts per gram of used litter

Table 4: Body weight (kg) by age (7, 14, 21, 28, 35 and 42 days) and treatment

	Body weight in kg by age (days)					
Dietary treatments	7	14	21	28	35	42
Control (none)	0.170	0.422	0.816	1.190[b]	1.749[b]	2.106
BMD®/Stafac®	0.172	0.432	0.833	1.248[a]	1.806[a]	2.146
Agolin® Poultry	0.169	0.429	0.841	1.256[a]	1.820[a]	2.169
p-value RCBD[1]	0.347	0.077	0.087	0.005	0.011	0.273

[1]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at $p = 0.05$

Table 5: Body weight gain (kg) by age periods (from 7 or 14 days) and treatment

	Body weight gain in kg by age periods in days (d)					
Dietary treatments	7-14	7-21	14-21	14-28	14-35	14-42
Control (none)	0.0995[b]	0.646	0.394	0.777[b]	1.438[b]	1.806
BMD®/Stafac®	0.1085[a]	0.661	0.401	0.815[ab]	1.483[ab]	1.834
Agolin® Poultry	0.1081[ab]	0.671	0.412	0.826[a]	1.501[a]	1.860
p-value RCBD[1]	0.034	0.081	0.274	0.015	0.035	0.403

[1]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at $p = 0.05$

Table 6: Body weight gain (kg) by age periods (from 21, 28, or 35 days) and treatment

	Body weight gain in kg by age periods in days (d)					
Dietary treatments	21-28	21-35	21-42	28-35	28-42	35-42
Control (none)	0.383	0.933	1.301	0.550	0.919	0.368
BMD®/Stafac®	0.415	0.973	1.324	0.558	0.909	0.351
Agolin® Poultry	0.415	0.980	1.338	0.565	0.924	0.359
p-value RCBD[1]	0.136	0.136	0.629	0.812	0.946	0.882

[1]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at $p = 0.05$

gain improved by 76 g/bird (2.663 vs. 2.587 kg) compared to control. Piperine at all levels of inclusion were most effective at improving weight gain from 36-42 days. Scherer *et al.*[33] observed that eugenol at 500 mg kg^{-1} of diet from a microencapsulated source significantly increased 21 day body weight and weight gain compared to control diet.

Feed conversion: In Table 7, antibiotic diets had significantly lower FCV 0-35 d than Control diets with Agolin® diets intermediate. In Table 8, the 0-35 days mortality-adjusted feed conversion values for the BMD®/Stafac® or Agolin® diets were significantly lower than the Control diets. Similarly, Cordosa *et al.*[31] added piperine alone at 0, 60, 120 or 180 mg kg^{-1} of feed for male broiler chickens from 0-35 days and observed that diets with 60 mg kg^{-1} piperine numerically improved 8-42 days feed/gain ratio by 0.12 (1.70 vs. 182).

Feed consumption: As shown in Table 9, there were no significant differences in feed consumed per survivor by treatment for any of the periods evaluated.

Cumulative mortality percentage and foot pad lesion scores. In Table 10, cumulative mortality percentage were not significantly different for any of the periods evaluated. In Table 10 also, the 42-day foot pad scores were not affected by supplementing diets with Agolin® or the antibiotic shuttle. The

Table 7: Feed conversion ratio (feed weight/weight of survivors) by age period in days (d) and treatment

	Feed conversion ratio (FCR) by periods in days (d) and treatment					
Dietary treatments	0-7	0-14	0-21	0-28	0-35	0-42
Control (none)	0.980	1.120	1.315	1.506	1.701[a]	1.844
BMD®/Stafac®	0.960	1.116	1.330	1.478	1.639[b]	1.811
Agolin® Poultry	0.982	1.123	1.306	1.477	1.644[ab]	1.826
p-value RCBD[1]	0.556	0.931	0.537	0.430	0.039	0.882

[1]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at p = 0.05

Table 8: Mortality-adjusted FCR (feed weight/weight of survivors+dead) by age period in days (d) and treatment

	Mortality-adjusted FCR (MAFCR) by period in days and treatment (d)					
Dietary treatments	0-7	0-14	0-21	0-28	0-35	0-42
Control (none)	0.981	1.118	1.314	1.498	1.684a	1.827
BMD®/Stafac®	0.909	1.109	1.320	1.459	1.618b	1.784
Agolin® Poultry	0.983	1.121	1.302	1.465	1.625b	1.801
p-value RCBD[1]	0.235	0.751	0.702	0.213	0.010	0.421

[1]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at p = 0.05

Table 9: Feed consumption per survivor (kg) by age period in days (d) and treatment

	Feed consumption per survivor (kg) by period in days (d) and treatment					
Dietary treatments	0-7	0-14	0-21	0-28	0-35	0-42
Control (none)	0.166	0.471	1.071	1.793	2.973	3.842
BMD®/Stafac®	0.165	0.479	1.095	1.819	2.956	3.820
Agolin® Poultry	0.166	0.481	1.095	1.838	2.991	3.897
p-value RCBD[1]	0.844	0.264	0.185	0.326	0.748	0.237

[1]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at p = 0.05

Table 10: Cumulative mortality (%) by age period in days (d) and treatment; foot pad lesion scores (0 = normal to 2 = severe) at 42 days

	Cumulative mortality (%) by period in days and treatment (d)						
Dietary treatments	0-7	0-14	0-21	0-28	0-35	0-42	42-d foot pad lesion score (0-2)
Control (none)	0.000	0.675	0.655	1.310	2.319	2.463	1.52
BMD®/Stafac®	0.000	0.585	0.925	1.851	2.500	3.135	1.62
Agolin® Poultry	0.000	0.405	0.835	1.670	2.679	3.183	1.50
p-value RCBD[1]	0.801	0.868	0.715	0.946	0.836	0.927	

[1]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at p = 0.05

Table 11: Coccidia oocyst counts per gram (OPG) of feces and "as received" litter moisture (H_2O) and "theoretical ammonia" (NH_3, calculated from nitrogen[1]) by age in days and treatment

Dietary treatments	42-Day Oocysts/g feces	14-Day Litter H_2O (%)	14-Day Litter NH_3 (ppm)	28-Day Litter H_2O (%)	28-Day Litter NH_3 (ppm)	42-Day Litter H_2O (%)	42-day Litter NH_3 (ppm)
Control (none)	41.42	52.22	6,960	57.08	8,387	55.55	11,503
BMD®/Stafac®	52.61	55.29	7,538	60.67	9,835	59.95	14,627
Agolin® Poultry	43.55	53.27	7,109	54.29	8,009	56.94	11,940
p-value RCBD[2]	0.478	0.521	0.374	0.161	0.096	0.144	0.311

[1]Weight of nitrogen/0.822442649: Wight of ammonia (NH_3), This is based on atomic weight of nitrogen (14.0067) and hydrogen (1.00797) which gives molecular weight of ammonia (17.03061) and nitrogen/ammonia: 0.822442649. The unit ppm is equivalent to mg kg^{-1}, [2]p-value RCBD is from ANOVA and when significant at $p \leq 0.05$, means were separated by Tukey's test using alpha: 0.05. Any means within a column (age) having a same individual letter superscript as another treatment are not significantly different by Tukey's test at p = 0.05

foot pad lesion score of the BMD®/Stafac® shuttle was also highest (numerically) in this comparison consistent with the numerically higher litter moisture values shown in Table 11.

Coccidia oocysts/g feces; litter moisture and ammonia: As presented in Table 11, the 42-day oocysts/gram (OPG) of feces did not differ significantly between treatments. Litter moisture percentage did not differ significantly between treatments at 14, 28 or 42 days and neither did the litter ammonia (NH_3) levels. However, worthy of note is that BMD®/Stafac® diets resulted in numerically higher litter moisture and ammonia at each age.

CONCLUSION

In this pen trial with high replication (20 pens/treatment by design and 19 pens/treatment used for statistical analysis with outliers removed) on new litter at placement (day 0) and with used litter applied per pen on day 4, birds on BMD®/Stafac® diets or Agolin® Poultry diets had significantly improved 28 and 35-day body weight compared to Control diets. Body weight gain from 14-28 and 14-35 days was significantly greater for birds fed Agolin® diets than for Control diets, with BMD®/Stafac® diets intermediate. The 0-35 days mortality-adjusted feed conversion ratio values were significantly lower for birds fed BMD®/Stafac® or Agolin® diets compared to Control diets. No significant differences in cumulative mortality percentage were observed between treatments for any of the periods evaluated in this study and mortalities in the three treatment groups were quite acceptable at 2.46-3.18% for 0-42 days. Litter moisture, oocysts/gram of feces and foot pad lesion scores were not different but 28 day litter ammonia tended (p = 0.096) to be lower in the group fed Agolin® diets.

SIGNIFICANCE STATEMENT

Use of growth promoting levels of antibiotics in broiler chicken feeds is becoming more unpopular with consumers due to concern about development of antibiotic resistance and alternative natural compounds of plant origin are favored. In this trial, a commercial essential oil blend (Agolin®Poultry) was evaluated as a replacement for antibiotics (BMD®, Stafac®) and found to support similar levels of body weight gain and efficiency of feed conversion from 0-35 days without affecting livability, foot pad lesion scores, or litter moisture or ammonia.

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