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Research Article
Effects of *Megasphaera elsdenii* on Growth Performance and Characteristics of Cecal Digesta in Broiler Chickens

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Abstract

Objective: A study was conducted to evaluate effects of *Megasphaera elsdenii* supplementation on growth performance and characteristics of cecal digesta in broiler chickens. **Materials and Method:** Day-old male Cobb 500 broiler chicks (n = 2520) were separated into 72 pens of 35. Pens were blocked by location within the barn and randomly assigned to 1 of 3 treatments. Treatment 1 consisted of a 0.2 mL oral gavage of *M. elsdenii* culture. Treatment 2 was an aerosolized mist of *M. elsdenii* culture, applied to the body surface at a rate of approximately 1.7 mL/bird. Cultures contained 1.97×10^9 CFU mL^{-1} of *M. elsdenii*. Treatment 3 served as a negative control. Feed intake and pen weight were collected at each feed change (day 16, 30 and 36). **Results:** At day 36, carcass data were collected from 5 birds/pen. Growth, carcass weight and mortality rate were unaffected by treatment ($p>0.10$). Cecal pH and volatile fatty acid concentrations were evaluated weekly. Cecal pH was lower in treated birds than control ($p<0.01$). Cecal contents of orally gavaged birds contained greater volatile fatty acid concentrations than control birds on day 14 ($p<0.01$). No differences in cecal profiles were observed across treatments after day 21 ($p>0.30$). *Megasphaera elsdenii* supplementation appeared to have the greatest effect on cecal characteristics between day 14 and 21 following administration, after which no further response is detected. **Conclusion:** Decreased cecal pH observed in treated birds suggests potential for use as an acidifier, a commonly incorporated antibiotic alternative in poultry production.

Key words: *Megasphaera elsdenii*, direct fed microbial, acidifier, probiotic, poultry feed

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

With the introduction of new federal regulations governing use of growth promoting antibiotics in food animal production due to concern of microbial antibiotic resistance, producers are presented with the challenge of finding alternative methods to maintain health of their flocks while maximizing feed efficiency[1-3]. Growth promotion by antimicrobial compounds occurs through selective manipulation of populations of gastrointestinal (GI) microorganisms [4,5]. Modulation of these microbial populations may improve gut health, prevent establishment of pathogenic organisms or treat clinical or subclinical infection[6]. Several antibiotic alternatives have been investigated in poultry including acidifiers and probiotics.

Administration of live cultures of beneficial microorganisms serve to stabilize the microbial populations and activity of the GI tract, preventing a decline in microbial diversity and promoting population growth and activity of commensal microorganisms while inhibiting pathogen colonization[7]. Stabilization of this activity results in improved performance and resistance to disease through several modes of action. These include suppression of pathogens, strengthening of the intestinal barrier and increasing nutrient retention in the GIT[8-10]. There is ample evidence that supplementation with probiotic cultures containing several different lactate-producing bacterial (LAB) species limits the growth and fecal shedding of pathogenic bacteria like *S. enterica*[11-13].

Sen *et al*.[14] demonstrated enhanced crude protein and energy digestibility in birds administered *Bacillus subtilis.* This probiotic also affected broiler gut morphology, increasing villus height and villus height to crypt depth ratio in the duodenum and ileum of treated birds. Increased villus height is associated with enhanced absorptive capacity of the small intestine which may result in improved feed efficiency and growth performance[14]. Probiotic administration in the poultry industry is typically accomplished via application of an aerosolized spray over newly hatched chicks, followed by supplementation through drinking water[15]. Administration via oral gavage has also proven to be an effective method of delivery for probiotic cultures; however, due to cost and labor constraints it is impractical in commercial production systems[10,16]. Delivery via the feed is also possible; however, this requires the microbial strain be oxygen tolerant or somehow protected from the aerobic environment[10,17]. In the present study, *M. elsdenii* culture was administered via either oral gavage or spray application over the body surface of day old broiler chicks in a manner similar to that described by Corrier *et al*.[18]

Megasphaera elsdenii is a novel microorganism with potential use in poultry production systems. This naturally occurring bacterium colonizes the lower GIT of birds and mammals[19-21]. *Megasphaera elsdenii* is a lactate-utilizing, Gram-negative coccus, regarded as an important bacterial species for the maintenance of normal gastrointestinal health and activity [22]. The present study was conducted to evaluate effects of *M. elsdenii* supplementation on broiler performance and cecal digesta parameters.

MATERIALS AND METHODS

All procedures followed in this study were approved by the Kansas State University Institutional Animal Care and Use Committee. This experiment was performed with 24 replicates of 3 treatments in a randomized complete block design. Treatments consisted of *M. elsdenii* strain NCIMB 41125 (MSBiotec, Wamego, Kansas) administered as either an oral gavage or an aerosolized mist applied to the body surface of birds and a negative control (C; no direct contact with *M. elsdenii).* Pen was used as the experimental unit (n = 72), with each pen containing 35 birds at the onset of the experiment (2,520 birds total).

Facilities: Each of the 72 pens (2.43×1.83 m) used in this experiment was located within a single building. The back and side barriers of each pen were lined with 4 mil polyethylene plastic sheeting to prevent direct contact between birds in adjacent pens, thus limiting the potential for cross contamination between treatments. Fresh pine shavings were used as bedding (approximately 8 cm depth) in each pen. Location within the barn was used as a blocking criterion to account for possible differences in ventilation and temperature. Treatments were randomly assigned to pens within each block. Groups of birds were processed by block, with experimental treatments being assigned randomly within each block.

Animals, diets and treatments: Day-old male Cobb 500 broiler chicks were obtained from Cobb-Vantress in Siloam Springs, Arkansas and transported to the Kansas State University Poultry Research Center in Manhattan, Kansas. Starter, grower and finisher diets (Table 1) were assigned to mimic to normal commercial production stages with starter diet being fed from day 1-16, grower from d 16-30 and finisher from day 30-36.

Administration of treatments: Prior to administration of *M. elsdenii*, 5-L foil bags of fresh culture were vigorously shaken to homogenize contents. Tygon tubing was used to

connect a manually operated dosing device to the bag. The reservoir of the dosing device was repeatedly filled and dispensed to evacuate air. The contents were deemed free of ambient air when the culture, which contains an oxygen indicator, retained its normal color.

Twenty-four pens (35 birds/pen; 840 birds total) were dosed by oral gavage (OG) with 0.2 mL of a fresh probiotic culture containing 1.97×10^9 CFU mL^{-1} of *M. elsdenii* strain NCIMB 41125 using a Scorex Classic 173.05005 auto-filling syringe (Ecublens, Switzerland). Technicians restrained the birds by using the thumb and forefinger to hold the beak open while the contents of the syringe were discharged directly into the birds' oral cavities.

Twenty-four pens (35 birds/pen; 840 birds total) were dosed by aerosolized mist (AM) of a fresh culture containing 1.97×10^9 CFU mL^{-1} of *M. elsdenii* strain NCIMB 41125 applied by a pneumatic drenching device fitted with an atomizing tip. Birds were placed into a plastic tub (50×35×40 cm) and the culture was applied to their body surfaces as an aerosolized mist at a volume of 60 mL per pen (~1.7 mL bird^{-1}).

Twenty-four pens (35 birds/pen; 840 birds total) had no contact with *M. elsdenii* culture and served as controls. To prevent cross contamination with treated birds, control birds were handled only by designated personnel that had no contact with treated birds and placed in designated carriers to be weighed and transferred to pens. In one case the birds were miscounted and pen 51 received 33 birds rather than 35 birds due to technician error.

Fresh water was offered *ad libitum* through sippers (6 sippers/pen) suspended from a water supply line. The sipper height was adjusted throughout the trial to accommodate growth of birds. Diets are shown in Table 2. All diets were fed in gravity feeders suspended in the center of each pen. Feed was added as needed to ensure *ad libitum* access throughout the duration of the study.

The starter diet was removed from the pens on day 16 of the study. Residual feed was weighed, removed from each feeder and placed into numbered bins that corresponded with pen number. Feeders were refilled with the grower diet. This process was repeated on day 30 of the study, this time replacing the grower diet with the finisher diet. On day 36 the experiment was terminated and the residual finisher diet was weighed and recorded for each pen.

Total feed consumption per pen for each phase (starter, grower and finisher) was calculated as:

Feed issued - feed recovered

Table 1: Composition of broiler experimental diets

	Dietary phase†		
Ingredient, percentage of total diet	Starter	Grower	Finisher
Ground corn	55.26	59.74	65.06
Dehulled soybean meal, 47% CP	37.15	32.60	27.90
Soybean oil	3.10	3.35	3.10
Ground limestone	1.45	1.40	1.25
Salt	0.37	0.37	0.37
Monocalcium phosphate, 21%*	1.70	1.60	1.40
Sodium bicarbonate	0.22	0.19	0.17
Vitamin and mineral premix$^{\tau}$	0.25	0.25	0.25
L-lysine hydrochloride	0.33	0.30	0.17
L-methionine	0.13	0.15	0.28
L-threonine	0.04	0.05	0.07

† Diets were pelleted through a 3-mm die, cooled, crumbled and dispensed into paper sacks for storage until feeding. Diets were provided *ad libitum* within each dietary phase, *Biofos®, Mosaic Co., Plymouth MN, $^{\tau}$Nutrablend poultry VTM premix, Neosho, MO

Table2: Effects of *Megasphaera elsdenii* on broiler growth performance

					p-value	
Item*	C[1]	AM[2]	OG[3]	SEM	Treatment$^{\tau}$	Contrast$^{\tau\tau}$
Starter						
Feed intake (kg)	38.80	39.10	39.20	0.430	0.69	0.40
Feed:gain	1.23	1.24	1.23	0.007	0.65	0.58
ADG, kg	31.60	31.60	31.90	0.310	0.57	0.53
Grower						
Feed intake (kg)	142.00	142.40	141.60	0.860	0.67	0.97
Feed:gain	1.46	1.47	1.56	0.005	0.47	0.70
ADG, kg	97.30	97.10	97.20	0.760	0.97	0.80
Finisher						
Feed intake (kg)	179.00	180.70	178.90	1.400	0.27	0.47
Feed:gain	2.19	2.17	2.19	0.043	0.91	0.88
ADG, kg	82.00	84.00	82.40	2.160	0.60	0.51
Overall						
Feed intake (kg)	98.60	99.10	98.80	0.630	0.68	0.49
Feed:gain	1.55	1.55	1.54	0.006	0.92	0.95
ADG total (kg)	63.80	64.00	64.00	0.540	0.85	0.57

[1]Birds had no direct contact with *M. elsdenii*, [2]Birds received *M. elsdenii* as an aerosolized mist applied to their body surfaces at a rate of ~1.7 mL bird^{-1} (1.97×10^9 CFU mL^{-1}). [3]Birds received 0.2 mL *M. elsdenii* as an oral gavage (1.97×10^9 CFU mL^{-1}), $^{\tau}$Effect of treatment, $^{\tau\tau}$Contrast of *M. elsdenii* vs. control

Intake per bird per day was calculated as:

$$\frac{\text{Total feed consumed}}{\text{Daily head count in pen} \times \text{total days on feed}}$$

Pen weights: Pen weights were recorded at the end of each feeding period (starter, grower, finisher). At the end of the starter period (day 16), all birds in each pen were placed into a tub (50×35×40 cm) and weighed. The weight of the tub was subtracted from total weight to determine the weight of the birds in the pen. At the end of the grower period (day 30) all birds in each pen were placed into 2 tubs of equal weight (each 103×55×41 cm), weighed and the weights added together. The weight of each tub (taken prior to the birds being placed into them) was subtracted from total weight to determine the weight of the birds in the pen. At the end of the finisher period (day 36) all birds in each pen were placed into 2 tubs of equal weight (each 103×55×41 cm) and weighed. This time, the scale was tared with the tubs in place. The weight of the birds in each tub were then added to determine total pen weight. The scale was re-tared between pens to account for fecal accumulation. At each of the weighing periods, head count verification was performed as birds were placed into tubs.

Sample collections: Each week (day 7, 14, 21, 28 and 35), 1-3 birds were randomly selected from each pen and euthanized by cervical dislocation. Cecal contents (0.5 g) were collected and mixed with deionized water (2 mL) in a 20 mL HDPE scintillation vial (Fisher Sci.; 03-337-23B) using a vortex mixer (Scientific Industries Vortex-Genie 2 vortex mixer, Houston, TX). A portable pH meter (Thermo Scientific Orion 3-star portable pH meter, Waltham, MA), calibrated using pH 4.0, 7.0 and 10.0 standards was used to determine pH. Four parts of the cecal mixture were added to 1 part 25% w/v metaphosphoric acid solution and homogenized using a vortex mixer. The sample was then transferred into 2 microcentrifuge tubes in 1-mL aliquots and frozen at -18°C to await analysis of VFA.

Laboratory analyses: Previously diluted and acidified cecal samples were thawed, homogenized using a vortex mixer and centrifuged at 24×g for 18 min. The aqueous supernatant was transferred to gas chromatography vials. Volatile fatty acids (VFA) were measured using an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a DB-WAX capillary column (30×0.53×0.5 mm film thickness; Sigma Aldrich, St. Louis, MO) and flame ionization detector. Helium was used as a carrier gas at a flow rate of 22 cm sec^{-1}, with a 1 µL split injection and a split flow of 50:1. Initial oven temperature was 80°C and temperature was increased at 10°C min^{-1} to 220°C. Inlet and detector temperatures were 250°C. Volatile fatty acids were quantified by comparison to known standards (Supelco Volatile Fatty Acid Standard Mix; Sigma-Aldrich, St. Louis, MO) containing acetate, propionate, isobutyrate but yrate, isovalerate, valerate, isocaproate, caproate and heptanoate.

Carcass data: Birds were slaughtered at 5 weeks of age to determine carcass weight. Feed was withheld approximately 4 h prior to slaughter. Five representative birds were selected from each pen and placed into catch boxes for transport to the processing area. The 5 birds were weighed by pen to determine live weight just prior to slaughter by stunning and exsanguination. The birds were bled for 2 min and then placed into a rotary scalder at 63°C for approximately 30 sec. The birds were then transferred to a rotary drum mechanical plucker for 30 sec for feather removal. The feet, head and shanks were removed and carcasses were eviscerated through an incision around the vent. Finally, carcasses were weighed by pen to determine hot carcass yield.

Statistical analyses: Data were analyzed using the Mixed procedure of SAS[23], Version 9.4. The model included fixed effect of treatment, random effect of block and pen as the experimental unit. Significance was declared at $p<0.05$. Differences among least-squares means were determined using the PDiff function of SAS.

RESULTS

Performance: Broilers demonstrated similar feed intake ($p = 0.68$), feed efficiency ($p = 0.92$; FE) and average daily gain ($p = 0.85$; ADG) across treatments (Table 2). Bird weights and mortalities also were unaffected by treatment ($p>0.15$; Table 3). However, dressed yield was less for birds that received *M. elsdenii* as an oral gavage compared to that of birds that received *M. elsdenii* as an aerosolized mist ($p = 0.02$; Table 3).

Characteristics of cecal digesta: A treatment effect was detected for cecal pH (Table 4). Cecal pH was less in birds that received *M. elsdenii* by either mist or oral application, compared to that of control birds ($p<0.01$). The mean cecal pH for C, AM and OG treatment groups were 6.76, 6.63 and 6.60 (SEM = 0.046), respectively. Treatment effect in cecal pH likely

Table 3: Effects of *Megasphaera elsdenii* on bird weights, carcass characteristics and mortalities

					p-value	
Item	C[1]	AM[2]	OG[3]	SEM	Treatment[τ]	Contrast[ττ]
Bird weight (g)						
day 1	40	40	40	0.2	0.48	0.23
day 7	157	155	159	2.2	0.39	0.90
day 14	437	448	443	5.9	0.34	0.20
day 16	559	557	561	3.3	0.71	0.90
day 21	1016	996	1028	12.5	0.18	0.79
day 28	1786	1784	1792	21.2	0.95	0.95
day 30	1948	1948	1950	10.5	0.98	0.93
day 35	2487	2493	2472	33.5	0.89	0.91
day 36	2550	2552	2553	14.5	0.99	0.91
Harvest weight	2580	2568	2591	21.0	0.69	1.00
Carcass weight	1833	1832	1832	15.1	1.00	0.96
Dressed yield (%)	70.99^{AB}	71.38^{B}	70.68^{A}	0.220	0.02	0.84
Mortalities (%)	3.34	3.35	1.95	0.851	0.41	0.50

All bird weights reported in g. [1]Control birds had no direct contact with *M. elsdenii*. [2]Birds received *M. elsdenii* as an aerosolized mist applied to their body surfaces at a rate of ~1.7 mL bird^{-1} (1.97×10^9 CFU mL^{-1}). [3]Birds received 0.2 ml *M. elsdenii* as an oral gavage (1.97×10^9 CFU mL^{-1}). [τ]Effect of treatment. [ττ]Contrast of *M. elsdenii* vs. control. [A,B]Means within a row without a common superscript are different at $p \leq 0.05$

was due in large part to pH differences between treatments on day 14 where pH in C birds was 6.67 compared to 6.25 and 6.11 in AM and OG birds, respectively.

A treatment by day interaction was detected for cecal acetate ($p<0.01$), propionate ($p = 0.03$) but yrate ($p<0.01$), acetate to propionate ratio ($p = 0.01$; A:P ratio), caproate ($p = 0.002$) and total VFA ($p<0.01$) concentrations (Table 2.4). Acetate increased from day 7-14, peaking on day 14. Cecal contents of birds that received *M. elsdenii* as an oral gavage contained greater concentrations of acetate but yrate and caproate than those of control birds on day 14 ($p<0.01$). By day 21, acetate concentration decreased across all treatments; however, concentration of acetate in the ceca was greater in control birds when compared to birds treated with either aerosolized mist or oral gavage ($p<0.01$). Propionate and butyrate concentrations also were greater in cecal contents of control birds than those of birds treated with *M. elsdenii* on day 21 ($p<0.01$). Propionate concentration increased from day 7-21 across treatments and remained elevated until day 35 but did not differ between treatments on day 28 or day 35 ($p>0.05$). The A:P ratio was greater in the cecal contents of birds treated with *M. elsdenii* compared to controls on day 7 ($p<0.01$), with an A:P ratio of 31.96 (C), 41.33 (AM) and 42.03 (OG). On day 14 the cecal A: P ratio of birds treated with an aerosolized mist of *M. elsdenii* (40.44 mM) was greater than that of control birds (29.80 mM) or those that received an oral gavage of *M. elsdenii* (33.69; $p<0.03$). Cecal A: P ratio was not different across treatments on day 21-35 ($p>0.05$). Isobutyrate, valerate, isovalerate, isocaproate and heptanoate concentrations in cecal contents were not affected by treatment ($p>0.10$). Total cecal VFA concentration was greater in orally gavaged birds compared to control birds on day 14 ($p<0.001$). However, total VFA concentration was less ($p<0.05$) in the cecal contents of birds that received *M. elsdenii* as an aerosolized mist or an oral gavage (64.90 and 64.82 mM, respectively) than that of controls (87.57 mM) on day 21. Total cecal VFA concentrations were similar across treatments for days 7, 28 and 35 ($p>0.30$).

DISCUSSION

In the current study, similarities in ADG and FE indicate that *M. elsdenii* administered to broiler chickens had neither a beneficial nor detrimental effect on performance. Although there have been several researchers who have demonstrated improvement in growth characteristics and feed intake of broilers in response to probiotic supplementation in several studies[24-26], efficacy of probiotics in poultry production is dependent upon the environment, external or internal stressors and stage of production. Probiotics tend to be most effective in conditions of environmental stress, such as severe temperature variation, poor hygiene or husbandry practices, or disease challenge[8,24].

Carcass weights also were unaffected by treatment; however, a greater dressed yield percentage of AM birds compared to OG was observed. This may have been a result of increased butyrate production and absorption, which could lead to greater intestinal weight in supplemented birds. Butyrate serves as an energy source for colonic and cecal mucosa, which leads to epithelial proliferation and increased cecal weight[27-29]. This hypothesis is supported by Yoshida *et al.*[30], who observed increased butyrate production,

Table 4: Effects of *Megasphaera elsdenii* on broiler cecal pH and VFA concentrations

						p-value	
Item*	Day	C[1]	AM[2]	OG[3]	SEM	Treatment[τ]	Contrast[ττ]
pH	7	6.870^{Aa}	6.850^{Aac}	6.810^{Aa}	0.103	T,D	0.01
	14	6.670^{Aa}	6.250^{Bb}	6.110^{Bb}			
	21	6.150^{Ab}	6.120^{Ab}	6.250^{Ab}			
	28	6.830^{Aa}	6.810^{Aa}	6.670^{Aac}			
	35	7.260^{Ac}	7.100^{Ac}	7.150^{Ad}			
Acetate	7	53.530^{Aa}	58.660^{Aa}	58.420^{Aa}	3.604	D,I	0.91
	14	61.790^{Aa}	66.200^{ABa}	74.000^{Bb}			
	21	61.480^{Ab}	47.660^{Bb}	46.400^{Bc}			
	28	40.420^{Abc}	38.830^{Ab}	42.360^{Acd}			
	35	39.130^{Abc}	41.830^{Ab}	36.220^{Ad}			
Propionate	7	1.820^{Aa}	1.480^{Aa}	1.520^{Aa}	0.608	D,I	0.51
	14	2.350^{Aab}	2.100^{Aa}	2.690^{Aab}			
	21	6.360^{Ac}	4.010^{Bb}	3.630^{Bbc}			
	28	3.730^{Abd}	4.080^{Ab}	4.210^{Ac}			
	35	4.580^{Ad}	5.880^{Ac}	5.960^{Ad}			
A:P[a]	7	31.960^{Aa}	41.330^{Ba}	42.030^{Ba}	2.168	T,D,I	0.003
	14	29.800^{Aa}	40.440^{Ba}	33.690^{Ab}			
	21	12.360^{Ac}	13.620^{Abcd}	13.570^{Ac}			
	28	13.250^{Ac}	12.960^{Acd}	14.300^{Ac}			
	35	9.810^{Ac}	8.330^{Ad}	7.800^{Ad}			
Butyrate	7	5.600^{Aa}	5.540^{Aa}	5.730^{Aa}	1.094	D,I	0.73
	14	9.450^{Ab}	10.950^{ABb}	13.400^{Bb}			
	21	17.790^{Ac}	11.770^{Bb}	13.450^{Bb}			
	28	6.670^{Aab}	7.020^{Aac}	7.350^{Aac}			
	35	8.150^{Ab}	9.700^{Aab}	8.400^{Aac}			
Isobutyrate	7	0.390^{Aa}	0.360^{Aa}	0.340^{Aa}	0.055	D	0.04
	14	0.370^{Aa}	0.350^{Aa}	0.450^{Aa}			
	21	0.380^{Aa}	0.170^{Bb}	0.150^{Bb}			
	28	0.050^{Ab}	0.000^{Ac}	0.000^{Ab}			
	35	0.370^{Aa}	0.370^{Aad}	0.340^{Aa}			
Valerate	7	0.29^{0Aa}	0.310^{Aa}	0.290^{Aa}	0.078	D	0.89
	14	0.680^{Abc}	0.710^{Ab}	0.900^{Bb}			
	21	1.130^{Ac}	0.910^{Bb}	0.960^{ABb}			
	28	0.320^{Aac}	0.280^{Ac}	0.340^{Aa}			
	35	0.630^{Ab}	0.790^{Ab}	0.680^{Ac}			
Isovalerate	7	0.317^{Aa}	0.292^{Aa}	0.314^{Aa}	0.0592	D	0.96
	14	0.375^{Aa}	0.357^{Aab}	0.505^{Ab}			
	21	0.419^{Aa}	0.396^{ABb}	0.257^{Ba}			
	28	0.038^{Ab}	0.040^{Ac}	0.050^{Ac}			
	35	0.325^{Aa}	0.396^{Aab}	0.355^{Aab}			
Caproate	7	0.150^{Aa}	0.168^{Aa}	0.144^{Aa}	0.0208	T,D,I	0.13
	14	0.150^{Aa}	0.161^{Aa}	0.292^{Bb}			
	21	0.000^{Abc}	0.000^{Ab}	0.001^{Ac}			
	28	0.000^{Ac}	0.000^{Ab}	0.001^{Ac}			
	35	0.000^{Ac}	0.000^{Ab}	0.001^{Ac}			
Isocaproate	7	0.125^{Aa}	0.116^{Aa}	0.142^{Aa}	0.0148	D	0.79
	14	0.085^{Ab}	0.101^{Aa}	0.078^{Ab}			
	21	0.000^{Ac}	0.001^{Ab}	0.001^{Ac}			
	28	0.000^{Ac}	0.001^{Ab}	0.001^{Ac}			
	35	0.000^{Ac}	0.001^{Ab}	0.001^{Ac}			
Heptanoate	7	0.177^{Aa}	0.186^{Aa}	0.146^{Aa}	0.0239	D	0.88
	14	0.104^{Ab}	0.082^{Bb}	0.103^{ABac}			
	21	0.000^{Ac}	0.002^{Ac}	0.003^{Ab}			
	28	0.000^{Ac}	0.001^{Ac}	0.003^{Ab}			
	35	0.000^{Ac}	0.001^{Ac}	0.053^{Abc}			
Total VFA	7	62.400^{Aa}	67.100^{Aa}	67.010^{Aa}	4.806	D,I	0.79
	14	75.340^{Ab}	81.000^{ABb}	92.390^{Bb}			
	21	87.570^{Ab}	64.900^{Ba}	64.820^{Bad}			
	28	51.230^{Aa}	50.250^{Ac}	54.290^{Aac}			
	35	53.180^{Aa}	58.980^{Ac}	51.970^{Ad}			

*All VFA concentrations reported in mM. [1]Control birds had no direct contact with *M. elsdenii*. [2]Birds received *M. elsdenii* as an aerosolized mist applied to their body surface at a rate of ~1.7 mL bird^{-1} (1.97×10^{9} CFU mL^{-1}). [3]Birds received 0.2 mL *M. elsdenii* as an oral gavage (1.97×10^{9} CFU mL^{-1}). [a]Acetate to propionate ratio [τ]T: Effect of treatment, D: Effect of day of sampling, I: Interaction between treatment and day of sampling, $p \leq 0.05$. [ττ]Contrast of *M. elsdenii* vs. Control. [A,B]Means within a row without a common superscript are different at $p \leq 0.05$. [a,b]Means within a column without a common superscript are different at $p \leq 0.05$

improved recovery from mucosal atrophy and increased colonic mucosal thickness in piglets supplemented with a combination of *M. elsdenii* and *L. plantarum* at weaning. In the current study, cecal butyrate concentrations were similar in AM and OG birds but production and absorption rates are unknown.

Mortalities were analogous across treatments, suggesting no ill effects of *M. elsdenii* on bird health. The bacterium does, however, alter fermentation in the distal GIT. Cecal pH was lower for birds treated with *M. elsdenii* compared to controls, likely due to greater VFA and organic acid production by the bacterium. Reduction in pH may indicate a potential use for *M. elsdenii* as an acidifier, which are commonly employed by the poultry industry as antibiotic alternatives. Organic acids such as formic acid, propionic acid and butyric acid have been used as acidifiers in poultry production, primarily to decrease GI colonization and fecal shedding of pathogenic microorganisms[31-35]. Although, Thompson and Hinton[34] noted a decrease in *Salmonella* concentrations in response to the bactericidal activity of fumarate and propionate when these organic acids were added to the diet, this reduction was limited to the anterior GIT of treated chickens. While effective in the crop to reduce pathogen colonization, organic acids are largely absorbed prior to reaching the cecum, the primary site of *Salmonella* colonization[35-37]. In order to reach the distal GIT, organic acids must be protected from prececal digestion which is typically accomplished via encapsulation but may also be accomplished through delivery of probiotics which produce these acidifying compounds in the distal GI tract. As such, lactate producing bacteria are often provided as a probiotic culture with the intention of improving performance and decreasing pathogen colonization by either competitive exclusion or production of lactate, a bactericidal compound[11-13,25].

In the current study, *M. elsdenii* apparently reached the ceca and established sufficient populations to alter fermentation, evidenced by differences in cecal VFA concentrations between treatments. A single dose method of probiotic administration was only effective for about 21 day following administration in this experiment. After day 21, no effect of treatment on VFA production was observed. It is likely that the effects of this probiotic may be extended or enhanced by more frequent administration in either feed or water[16,17,34,35]. *Megasphaera elsdenii* appeared to be most effective in altering the cecal environment around day 14 post-administration, indicated by decreased pH as well as increased cecal butyrate, acetate, caproate and total VFA concentrations in supplemented birds. It is also possible that efforts to control cross-contamination between treatments were not completely effective and colonization of the ceca of control birds by *M. elsdenii* occurred. Unexpected colonization of *M. elsdenii* in the lower GIT of control birds may explain the lack of differences between treatments observed after day 21.

Megasphaera is known to produce butyrate from the fermentation of lactate or glucose[19,22,38-40] which supports our findings at day 14. Increased caproate production, presumably due to saccharolytic and proteolytic activity of *M. elsdenii* also was observed in this study[38,41,42]. Glucose and lactate are also fermented by *M. elsdenii* to produce acetate, which may account for the greater concentrations of this VFA seen in OGchickens[38,41,43]. Cecal propionate concentrations did not increase in AM or OG birds compared to C birds. This may be due to the rapid rate of passage of digesta in chickens, which may not allow sufficient time for lactate to accumulate or be fermented by *M. elsdenii* to produce propionate[44]. Branched-chain fatty acid production by deamination of amino acids has also been observed as a fermentation product of *M. esldenii*[38,41,45], although increased branched-chain fatty acid concentrations were not observed in this experiment.

Cecal VFA profile alone cannot be used to completely explain the differences observed between treatments in pH. Total VFA concentrations were greater on day 14 in OG birds compared to the control, however, differences in individual fatty acids were not substantial enough to account for the observed pH differences. Therefore, we postulate that production of other organic acids not measured in the present study, such as formate, are responsible for the observed decrease in pH. Supporting this, Marounek *et al*.[38] and Shetty *et al*.[42], observed formate production by *M. elsdenii* from the fermentation of glucose.

In this trial, caproate production was increased in the first 14 day after administration of *M. elsdenii* by OG. Caproate, along with butyrate, propionate and formate, have been shown to have antipathogenic effects, decreasing colonization of *S. enterica in vitro* and *in vivo*.[35,46] Because *M. elsdenii* produces these antipathogenic compounds, it would likely be beneficial to measure changes in *S. enterica* colonization and fecal shedding in response to *M. elsdenii* supplementation. Although not evaluated in the current study, inhibition of pathogenic colonization and prevention of disease is the primary purpose of acidifier use in poultry production. Diebold and Eidelsburger[47] indicated that the ability of organic acid or probiotic supplementation to alter the GI environment or improve growth performance in poultry is inconsistent and largely dependent upon the environment in which they are raised. Under ideal conditions of good hygiene and low stress, probiotic or acidifier addition to the diet are less effective[8,47].

CONCLUSION

In summary, *M. elsdenii* did not affect growth performance or mortality rates in broiler chickens. Consideration should be made of this bacterium's potential for use as an acidifier to reduce pH and increase organic acid production in the ceca. Supplementation with *M. elsdenii* appears to be an effective method of increasing production of bactericidal compounds, such as butyrate or caproate in the distal GIT in addition to decreasing the pH of the ceca on day 14. It would likely be beneficial to focus future research on ascertaining this microorganism's ability to improve gastrointestinal health and decrease pathogenic colonization and transmission in broiler chickens.

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