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Research article

The effect of a direct-fed microbial and dietary fat inclusion on performance and energy metabolism in broiler chicks and turkey poults

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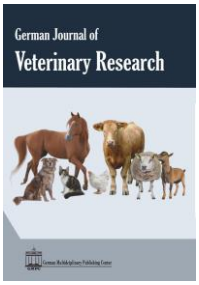

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Abstract

Two battery trials were conducted to determine the effects of dietary direct-fed microbial (DFM) and dietary fat inclusion on broiler chick and turkey poult performance and dietary apparent metabolizable energy, nitrogen corrected (AME n) when fed corn, wheat, and soy diets. For both experiments, a 2 x 2 factorial experimental design was used with supplemental fat at low (1%, LF) or high (6%, HF) levels and DFM inclusion (0 or 0.91 kg/ton) as main effects. Dietary treatments were randomly assigned to 72 cages of birds and fed for 21 days. Growth performance was measured weekly, and cecal contents were collected for volatile fatty acid (VFA) analysis on day 21. Fat supplementation resulted in increased body weight gain (BWG) in both broilers and turkeys. Specifically, in LF diets, DFM inclusion resulted in increased BWG compared to the non-DFM treatments. Cumulative feed conversion ratio (FCR) was improved in HF treatments and the LF with DFM treatment compared to the LF with no DFM. In both trials, as expected, feeding HF diets resulted in increased AME n. However, the DFM inclusion resulted in a greater uplift in AME n in LF diets for both broilers and turkeys. In either trial, diet did not impact cecal VFA concentrations; thus, the impact on DFM on VFA production remains uncertain. In conclusion, the performance of broiler chicks and turkey poults was improved by DFM inclusion in reduced fat diets, which was associated with increased energy digestibility as measured by AME_n.

Keywords: Direct-fed microbials, Probiotics, Lactobacillus, Broiler chicks, Turkey poults, Dietary fat.

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Introduction

Feed is the single largest expense in poultry production, accounting for up to 75% of costs (Sibbald, 1982). Saving in feed costs has become an important strategy for many producers in today's marketplace, especially with increasing ingredient prices. Fat is a major source of energy in the poultry diet, and nutritionists have few other options for adding energy to the diet beyond what is provided by the inclusion of cereal grains (Sanz et al., 2000). The energy value of fats is 2.25 times that of the carbohydrates in grains (USDA-NRCS, 2012), and thus, even when grain prices are low, fats are added to the diet in optimal amounts to meet the animal's energy requirements. While fat addition to poultry diets can be useful, fat can also be expensive (Birk et al., 2016).

Therefore, depending on grain prices, if the amount of fat added to the diet can be reduced without deleterious effects on performance, there is the potential to reduce total feed costs. Improvements in growth have been reported when direct-fed microbials (DFM), also referred to as probiotics, were supplemented in both chickens and turkeys diets (Gadde et al., 2017; Aziz Mousavi et al., 2018; Jha et al., 2020). There are numerous reports where DFM increased apparent metabolizable enērgy and nitrogen-corrected (AME_n) (Nurmi and Rantala, 1973; Patterson and Burkholder, 2003; Chichlowski et al., 2007a; Lutful Kabir, 2009; Lee et al., 2010). Since dietary fat is supplemented to increase AME_n, DFM supplementation may be an opportunity to replace fat in the diet. DFM may also impact the production of volatile fatty acids (VFAs) in the gut, which, together with glucose, are the primary metabolic fuel sources (Markowiak-Kopeć and Śliżewska, 2020).

Two trials were conducted to test the effect of DFM inclusion to replace dietary fat (1 or 6%) on the performance and energy metabolism as measured by AME n and VFA production in a 2 x 2 factorial design treatment.

The first experiment was conducted using male broiler chicks, and the second was conducted using male turkey poults.

Materials and methods

Birds

All bird handling procedures were approved by the North Carolina State University institutional animal care and use committee. In two separate trials, male broiler chicks (504 chicks, Ross 708, Aviagen Group, Huntsville, AL) and male turkey poults (504 poults, Nicholas Select, Aviagen Turkeys, Lewisburg WV) were reared to 21 days.

Birds were randomly placed in 72 Petersime battery cages (Petersime, Gettysburg, OH) in one room (Talley Turkey Education Unit, North Carolina State University Prestage Department of Poultry Science) with 7 birds/cage. There were 6 batteries in the room, each with 12 cages over 6 decks. Each battery was considered a block. Each bird was tagged for identification. One of four dietary treatments was randomly assigned to each cage of birds in each block (18 replicates/treatments). For both trials, all birds were individually weighed at placement and then at days 7, 14, and 21. Birds were offered feed and water *ad libitum*. Feed intake (FI) was determined weekly for each cage of birds. The body weight (BW) of culls and mortalities was recorded daily and was included in calculating the feed conversion ratio (FCR).

Dietary treatments

All feed was manufactured at the North Carolina State University Feed Mill Education Unit and was formulated to broiler and turkey starter diets based on breeder recommendations (Table 1). Birds were fed mash starter diets for the duration of the experiments. One basal ration containing all feed ingredients except DFM and additional fat was blended in a counterpoise mixer (Model TRDB126060, Hayes and Stolz, Fort Worth, TX). In each experiment, the basal diet was split into 4 sub-groups where the DFM or the additional fat were added to the basal and mixed in a double ribbon mixer (Model SRM 304, Scott Equipment Co., New Prague, MN) for an additional two minutes. All the feed was bagged and then transported to the Talley Turkey Education Unit. The four dietary treatments were designed as a 2 x 2 factorial with DFM and additional fat as main effects and were supplemented as follows: low supplemental fat (1%, LF) without DFM, LF with DFM (0.91 kg/ton), high supplemental fat (6%, HF) without DFM, and HF with DFM (0.91 kg/ton). The fat source used was poultry fat, and the DFM used was PrimaLac (Star-Labs/Forage Research, Inc., Clarksdale, MO). All feeds were sampled, coded, and analyzed blindly by a private laboratory (Star-Labs/Forage Research, Inc., Clarksdale, MO) for the presence or absence of PrimaLac. PrimaLac (Star-Labs/Forage Research, Inc., Clarksdale, MO) is a DFM cocktail that contains *Lactobacillus acidophilus*, *Lactobacillus casei* subsp. *rhamnosus*, *Bifi- dobacterium bifdium*, and *Enterococcus faecium*. PrimaLac contains a minimum of $1.0x10^8$ CFU of *Lactobacillus* per Gram.

Sample collection

On days 21 and 16 for trials 1 and 2, respectively, excreta from all cages was collected and frozen at -20°C until analysis for AME_n. On day 21, for both trials, two birds per cage were euthanized for sampling. Using aseptic techniques, the ileum was removed by cutting at the Meckel's diverticulum at the ileocecal junction. Next, the ceca were removed by cutting both sections at the ileocecal junction. A sample of 10-15 g of ileal contents per cage and an 8-10 g sample of cecal contents per cage were collected into labeled 15 mL conical tubes. The tubes were immediately placed on ice and stored until further processing.

Volatile fatty acid analysis

Ileal and cecal samples were prepared for VFA analysis by weighing out one g of sample, adding 2.0 mL diH_2O, vortexing, and spinning for five minutes at 2500 rpm. A portion (1.0-2.0 mL) of supernatant was decanted into a micro-centrifuge tube and centrifuged at 15,000 rpm (21,000 rcf) for 10 minutes. A 1.0 mL portion of supernatant was collected into another micro-centrifuge tube and 200 L of MIS (Meta-phosphoric acid with internal standard: 2-Ethylbutyric acid) in a 5:1 ratio. Samples were then frozen at -70°C, thawed, and centrifuged at 15,000 rpm for 10 minutes to aid in sample cleaning. Samples were analyzed for VFA by gas-liquid chromatography (Varian CP 3380 with NUKOL Fused Silica Capillary Colum 30 m x 0.25 mm x 0.25 µm film thickness).

Chemical analysis

Approximately 200 g of representative excreta sample was dried for approximately 72 hrs at 60°C in a forced air convection oven (Blue-M, Model DC-326F, Serial DC-509, Blue M, Atlanta, GA). Once dried, the excreta was ground into a fine powder and stored at room temperature until further analysis. Approximately 200 g of representative feed sample was dried for 24 hrs at 60°C in a forced air convection oven (Model 725F, Serial 1584070342379, Fisher Scientific, Dubuque, IA) and then ground into a fine powder and stored at room temperature until further analysis.

Ground excreta and feed samples were analyzed via combustion for crude protein (AOAC, 1995). Insoluble ash for Celite recovery was performed with modifications of a previously described method (Vogtmann et al., 1975). Briefly, a 2 g sample of dried excreta and feed, in duplicate, were boiled with 40 mL of 4N HCl in 100 mL beakers for 10 minutes. The slurry was filtered through ash-less filter paper with 50 mL of deionized water to wash residue free of acid and allowed to drain. Using clean, fired, pre-weighed crucibles, the filter paper was folded in and placed in a muffle furnace (BF1700 Series, Thermo Scientific Lindberg/Blue M, Asheville, NC). Samples were ashed at 600°C for approximately 12-14 hrs (AOAC, 2006). The muffle furnace was turned off and allowed to cool.

Table 1: Composition of nutrient content of experimental low or high-fat starter diets with or without a direct-fed microbial (DFM†) fed to chicks or poults for 21 days.

Ingredients (%)	Broiler		Turkeys	
Corn	36.8		33.5	
Soybean meal 48	28.0		32.5	
Distiller's dried grain	7.5		5.0	
Wheat	20.0		10.0	
Poultry meal	0.00		10.0	
Poultry fat*	1.00		1.00	
Calcium carbonate	1.20		1.85	
Dicalcium phosphate	2.10		2.20	
Salt (NaCl)	0.22		0.25	
L-lysine[1]	0.40		0.55	
Dl-methionine[2]	0.30		0.425	
Threoninne	0.125		0.15	
Selenium premix[3]	0.05		0.05	
Choline chloride	0.10		0.20	
Trace mineral premix[4]	0.10		0.10	
Sodium bicarbonate	0.00		0.125	
Celite	2.0		2.0	
Vitamin premix[5]	0.10		0.10	
Nutrient composition				
ME poultry, kcal/kg	1258		1217	
Crude protein, %	21.95		29.47	
Crude fat, %	3.33		4.53	
Calcium, %	1.02		1.46	
Available phosphorus, %	0.78		0.98	
Sodium,%	0.21		0.21	
Total lysine, %	1.36		1.80	
Total met + cys, %	0.92		1.24	
Threonine	0.82		1.12	
Choline, mg/lb	885.48		2397	
Nutrient analysis (%)	Low fat	High fat*	Low fat	High fat*
Crude protein	21.34	20.47	28.24	26.71
Crude fat	3.53	7.64	3.58	7.51

†In the Direct-Fed Microbial (DFM) dietary treatment, the DFM (0.91 kg/ton) replaced corn

[1]Ajinomoto North America.

[2] Evonik North America.

[3] Selenium premix provided 0.2 mg/kg Se.

[4] Mineral premix provided the following per kg of diet: 5.00 mg/kg of Cu, 40.04 mg/kg of Fe, 60.07 mg/kg of Mn, 60.07 mg/kg of Zn, 1.25 mg/kg of I.

[5] Donated by DSM Nutritional Products; vitamin premix provided the following per kg of diet: 13242 IU of vitamin A, 3973 IU of vitamin D, 66 IU of vitamin E, 0.40 mg/kg of vitamin B12, 0.25 mg/kg of biotin, 3.97 mg/kg of vitamin K, 13.24 mg/kg of riboflavin, 22.07 mg/kg of pantothenic acid, 110.35 mg/kg of niacin, 2.21 mg/kg of folic acid.

* fat diets included an additional 5% poultry fat added to the basal ration.

Samples were weighed to obtain ash weight. Excreta samples were prepared for gross energy analysis by weighing 1.000-1.005 g of dried sample, transferring it to a large clean crucible, adding two drops of diH_2O, and mixing into the sample, ensuring that no clumps of water remained. Samples were re-weighed and poured into a clean pellet press to form pellets. The sample was placed into a tared calorimeter crucible and stored in the desiccator for approximately 12-15 hrs. A plain jacket calorimeter (1341 Parr Instrument Co., Moline, Illinois) was used to calculate the gross energy of dried excreta and feed samples. The AME n was calculated according to a previously detailed method (Lammers et al., 2008) using the following equations:

$$N_{\text{retained}} = N_{\text{feed}} - \frac{(N_{\text{excreta}} \times AiA_{\text{feed}})}{AiA_{\text{excreta}}}$$

$$AME_n = GE_{\text{feed}} - \frac{GE_{\text{excreta}} \times AiA_{\text{feed}}}{AiA_{\text{excreta}}} - (8.22 \times N_{\text{retained}})$$

Where: AME_n (Kcal/g) is the nitrogen-corrected apparent metabolizable energy of the diet; GE_{feed} and $GE_{excreta}$ were the gross energy of the diet and excreta, respectively. AiA_{feed} and $AiA_{excreta}$ were the concentrations of Celite recovered as acid-insoluble ash in diet and excreta, respectively. 8.22 (Kcal/g) is the energy value of uric acid and Nretained (g/kg) is the nitrogen retained by the bird per kilogram of diet consumed. Nfeed and Nexcreta (%) were the nitrogen content of the diet and excreta, respectively. All values in this calculation were expressed as grams per kilogram (g/kg) of DM.

Statistical analysis

Data were analyzed using JMP 11. Experiments were 2 x 2 factorial designs. Each cage of birds was considered the experimental unit. Both the broiler and the turkey trial performance and AME_n data were analyzed by 2 x 2 factorial ANOVA, and means were separated using LSMeans. The VFA data were analyzed using 2 x 2 factorial ANOVA with means separated by LSMeans Contrasts. Means were considered significant at $p<0.05$.

Results

Broiler trial-growth performance

The main and interaction effects of dietary DFM and fat inclusion on BW gain (BWG), FI, and FCR for broiler chicks are presented in Table 2. There was low mortality with no differences between treatments. An expected performance improvement was observed due to the main effect of HF vs. LF diets. However, in the LF treatments, DFM inclusion improved BWG by 120 grams, on average, over LF treatments with no DFM inclusion. The same effect was observed for 21 days FI, where LF treatments with DFM consumed more feed relative to LF treatments without DFM and both HF treatments. Cumulative FCR was improved with HF and LF with DFM treatments compared to the LF with no DFM.

Turkey trial–growth performance

The main and interaction effects of dietary DFM and fat inclusion on BWG, FI, and FCR in turkey poults are presented in Table 3. Birds mortalities were low with no differences due to the feed treatments. As with the broiler trial, there was an interaction effect of DFM and fat inclusion. The fat main effect was as expected where birds fed HF diets experienced improved performance compared to birds fed LF diets. The DFM supplemented in the LF diet resulted in improved bird performance. However, there were no improvements observed when DFM was supplemented at the HF level. There were no differences observed in BWG during the first week of the experiment. During the second week of the experiment, birds fed the LF with DFM treatment had significantly higher BWG than birds fed the LF without DFM and HF without DFM treatments. The birds fed the HF with DFM treatment were intermediate in BWG. A similar effect was also observed during the second week and third weeks. For the 21 days cumulative BWG, the birds fed the LF with DFM and HF without DFM treatments had the highest BWG and differed from the birds fed the LF without DFM treatment. The birds fed the HF with DFM treatment had an intermediate value for cumulative BWG (p=0.0002).

There were no differences observed in FI due to the feed treatment. The response for FCR was consistent in that the birds fed the HF diet had reduced (improved) FCR. Except for week 1, birds fed the LF diet without DFM had higher (worse) FCR. The birds fed the LF diet with DFM had improved FCR compared to the birds fed the LF without DFM treatment. However, the addition of DFM to the LF diet did not result in the same FCR as for those birds fed the HF diet. This weekly effect was also observed for the 0-21 days cumulative FCR (p=0.0017).

AME_n

The main and interaction effects of feed treatments on AME_n of broiler chicks and turkey poults are presented in Table 4. In the broiler chick experiment, the main effect of fat resulted in an uplift of 129 kcal/kg in AME n from the low to high-fat diets (p=<0.0001). There was an improvement in AME n at the LF level when DFM was supplemented, resulting in an uplift of 31 kcal/kg in AME_n. However, at the HF level, there was no effect of the addition of DFM. For the turkey poult experiment, the main effect of DFM resulted in an uplift of 69 kcal/kg in AME n from no DFM inclusion to 2 lb DFM/ton inclusion (p<0.0001). The main effect of fat resulted in an uplift of 250 kcal/kg in AME n between the LF and HF diets (p<0.0001). There was an improvement in AME n at the LF level when DFM was supplemented (226 kcal/kg). Again, at the HF level, DFM supplementation did not result in an improvement in AME_n.

Table 2: Effect of dietary DFM[1] and fat[2] inclusion on performance[3] of broiler chicks from placement to 21 days [4].

	Inclusion level				DFM main effect		Fat main effect			Source of variation		
DFM	No	Yes	No	Yes	No	Yes	Low	High	SEM	DFM	Fat	DFMxFat
Fat	Low	Low	High	High								
					BWG (g)					*p*-values		
0-7*	94[b]	117[a]	112[a]	118[a]	103	117	105	115	0.32	<0.0001	0.002	0.003
7-14	219[b]	274[a]	275[a]	269[a]	247	272	247	272	0.89	0.004	0.003	0.0005
0-14	313[b]	391[a]	387[a]	387[a]	350	389	352	387	1.2	0.0004	0.001	0.0004
14-21	317[b]	358[a]	377[a]	369[a]	347	364	337	373	0.93	0.04	<0.0001	0.003
0-21	629[b]	749[a]	764[a]	756[a]	697	753	689	760	1.7	0.0006	<0.0001	0.0001
					FI (g)					*p*-values		
0-7	128	141	139	144	134	143	134	142	0.26	0.0002	0.002	0.1221
7-14	312[b]	373[a]	357[a]	357[a]	335	365	343	357	0.023	0.0007	0.09	0.0005
0-14	441[b]	514[a]	496[a]	501[a]	469	507	477	499	1.1	0.0002	0.03	0.001
14-21	487[b]	528[a]	528[a]	530[a]	507	529	507	529	0.88	0.007	0.006	0.01
0-21	948[b]	1071[a]	1054[a]	1053[a]	1001	1062	1010	1054	2.3	0.006	0.04	0.006
					FCR (g:g)					*p*-values		
0-7	1.373[a]	1.208[b]	1.249[b]	1.235[b]	1.311	1.222	1.290	1.242	0.021	<0.0001	0.01	0.0002
7-14	1.445[a]	1.393[ab]	1.300[b]	1.352[ab]	1.373	1.373	1.419	1.326	0.03	0.99	0.004	0.097
0-14	1.419[a]	1.304[b]	1.285[b]	1.311[b]	1.352	1.308	1.361	1.298	0.02	0.03	0.003	0.0009
14-21	1.732	1.671	1.613	1.641	1.673	1.656	1.701	1.627	0.111	0.9	0.6	0.7
0-21	1.480[a]	1.398[b]	1.346[b]	1.37[b]	1.413	1.386	1.439	1.360	0.016	0.09	<0.0001	0.001

Period (days)

[1] Direct-Fed Microbial (DFM) inclusion rates: No at 0 kg/ton or Yes at 0.91 kg/ton.
[2] Fat inclusion rates: Low at 1%, High at 6%.
[3] Performance parameters: BWG=bodyweight gain, FI=feed intake, FCR=feed conversion ratio (feed/gain).
[4] Values are means of 18 replicate pens of 7 male broiler chicks per pen.
* Average hatching body weight, across all treatments, was 45.5g±0.02 g. [a,b]Means within a row lacking a common superscript differ ($p \leq 0.05$).

Table 3: Effect of dietary DFM[1] and fat[2] inclusion on performance[3] of turkey poults from placement to 21 days [4].

	Inclusion level				DFM main effect		Fat main effect			Source of variation		
DFM	No	Yes	No	Yes	No	Yes	Low	High	SEM	DFM	Fat	DFMxFat
Fat	Low	Low	High	High								
					BWG (g)					*p*-values		
7-14	228[b]	240[a]	234[ab]	224[b]	231	232	234	229	3.3	0.8753	0.0761	0.0003
0-14	343[ab]	356[a]	351[ab]	337[b]	347	347	350	344	5.4	0.906	0.227	0.0041
14-21	324[c]	345[b]	367[a]	357[ab]	345	351	335	362	4.3	0.1593	<0.0001	0.0003
0-21	667[b]	701[a]	718[a]	694[ab]	692	698	684	706	8.7	0.0042	0.4696	0.0002
					FI (g)					*p*-values		
0-7	128	125	124	122	126	124	126	123	2.2	0.2540	0.109	0.6472
7-14	288	294	279	282	283	288	281	291	5.6	0.2915	0.0214	0.6821
0-14	415	419	403	404	409	411	417	404	7.3	0.6767	0.0241	0.8749
14-21	508	520	509	509	509	515	514	509	8.6	0.3904	0.4524	0.3906
0-21	941	956	928	934	934	945	948	931	17.5	0.4904	0.2745	0.773
					FCR (g:g)					*p*-values		
0-7	1.115[a]	1.073[a]	1.061[b]	1.090[ab]	1.088	1.08	1.094	1.074	0.011	0.3239	0.018	0.0002
7-14	1.258[a]	1.226[b]	1.192[c]	1.212[ab]	1.225	1.22	1.242	1.203	0.008	0.3549	<0.0001	<0.0001
0-14	1.020[a]	0.997[b]	0.970[c]	0.982[ac]	0.995	0.989	1.009	0.976	0.005	0.2334	<0.0001	0.0007
14-21	1.585[a]	1.517b	1.400c	1.426[c]	1.491	1.472	1.551	1.411	0.017	0.253	<0.0001	0.0046
0-21	1.268[a]	1.231[b]	1.169[c]	1.184[c]	1.219	1.208	1.249	1.177	0.009	0.166	<0.0001	0.0017

Period (days)

[1] Direct-Fed Microbial (DFM) inclusion rates: No at 0 kg/ton or Yes at 0.91 kg/ton.
[2] Fat inclusion rates: Low at 1%, High at 6%.
[3] Performance parameters: BWG=bodyweight gain, FI=feed intake, FCR=feed conversion ratio (feed/gain).
[4] Values are means of 18 replicate pens of 7 male broiler chicks per pen.
* Average hatching body weight, across all treatments, was 63g±0.32 g. [a,b,c]Means within a row lacking a common superscript differ ($p \leq 0.05$).

Table 4: Effect of dietary DFM[1] and fat[2] inclusion on AME_n of broiler chicks at 16 d and turkey poults at 15 days[3].

		Broiler chicken	Turkey poults
Inclusion level		AME_n	
DFM	Fat	kcal/kg	
No	Low	3235[d]	2885[d]
Yes	Low	3266[c]	3111[c]
No	High	3407[a]	3291[a]
Yes	High	3351[b]	3204[b]
DFM main effect			
No		3321	3088
Yes		3309	3157
Fat main effect			
Low		3250	2998
High		3379	3248
Source of variation		*p*-value	
SEM		12.29	18.32
DFM		0.09	<0.0001
Fat		<0.0001	<0.0001
DFM x Fat		<0.0001	<0.0001

[1] Direct-Fed Microbial (DFM) inclusion rates: No at 0 kg/ton or Yes at 0.91 kg/ton.
[2] Fat inclusion rates: Low at 1%, High at 6%.
[3] Values are means of 18 replicate pens of 7 male broiler chicks per pen. [a,b,c,d] Means within a row lacking a common superscript differ ($p \leq 0.05$).

Table 5: Effect of dietary DFM[1] and fat[2] inclusion on cecal volatile fatty acid concentrations of broiler chicks at 21 days.

		Molar percent					Concentration
Inclusion Level		Acetate	Buytrate	Propionate	Valerate	Isovalerate	Total
DFM	Fat			(%)			mM
No	Low	74.73	24.63	1.320	0.734	0.930	134.16
Yes	Low	75.44	23.91	1.942	0.868	0.941	133.10
No	High	74.20	24.17	2.277	0.834	0.935	124.52
Yes	High	74.49	24.75	2.483	0.764	0.827	124.62
DFM Main Effect							
No		74.97	24.40	1.799	0.784	0.933	129.34
Yes		74.91	24.33	2.212	0.816	0.884	128.86
Fat Main Effect							
Low		75.03	24.37	1.631	0.801	0.936	133.63
High		74.85	24.46	2.380	0.799	0.881	124.57
Source of Variation							
DFM		0.9350	0.9149	0.6722	0.7039	0.8853	0.9198
Fat		0.7983	0.7771	0.4460	0.982	7 0.8706	0.0662
DFM x Fat		0.3611	0.3457	0.8310	0.2442	0.9613	0.9035
SEM		0.738	0.701	0.861	0.107	0.341	6.051

[1] Direct-Fed Microbial (DFM) inclusion rates: No at 0 kg/ton or Yes at 0.91 kg/ton.
[2] Fat inclusion rates: Low at 1%, High at 6%.
[3] Values are means based on representative cecal samples of all birds per pen.

Volatile fatty acids

For the broiler trial, acetate, butyrate, propionate, valerate, and isovalerate were detected on day 21. Acetate was found in the highest molar percentage, followed by butyrate, propionate, valerate, and isovalerate. No significant differences were observed in any individual VFA between dietary treatments. There was no response to DFM or fat inclusion for total cecal VFA concentrations (Table 5). For all ileal samples that were analyzed by gas-liquid chromatography, the VFAs were below detectable levels.

For the turkey trial, butyrate, isobutyrate, propionate, valerate, and isovalerate were detected on day 21. As expected, acetate was found in the highest percentage, followed by butyrate, propionate, and then, in smaller quantities, valerate, isobutyrate, and isovalerate. There were no significant differences found in any individual VFA between dietary treatments. Additionally, there was no observed response to DFM or fat inclusion for total cecal VFA concentrations (Table 6).

In the turkey trial, VFA concentrations were found in the ileum (Table 7). The individual VFA present were acetate, butyrate, isobutyrate, propionate, valerate, and isovalerate. There were no significant differences found in any individual VFA between dietary treatments. However, there was an interaction effect in the total ileal VFA concentration where birds fed LF no DFM and HF with DFM diets had increased VFA compared to birds fed LF with DFM and HF no DFM diets.

Discussion

The objective of these trials was to determine if DFM can partially replace dietary fat for broiler chicks and turkey poults and determine if this improvement is associated with increased dietary energy. As expected, high-fat diets resulted in improved performance compared to low-fat diets for both chicks and poults. This can be attributed to the increased energy digestibility and increased transit time seen with increased supplemental fat levels (Mateos and Sell, 1980). Additionally, the observed increased BWG and improved FCR at higher supplemental fat levels agree with other reports (Biely and March, 1954; Jensen et al., 1970; Pesti et al., 2002).

Strikingly, both trial results demonstrated a positive effect on performance and AME_n at the low-fat level when DFM was added to the diet, but not at the high-fat level. Increased BWG and improved FCR due to DFM are in agreement with other reports (Jin et al., 1998b; Angel et al., 2005; Torres-Rodriguez et al., 2007; Mountzouris et al., 2007; Russell and Grimes, 2009; Mountzouris et al., 2010; Gadde et al., 2017). The proposed modes of action for DFM are varied, including improved maintenance of the epithelial barrier, changes in gut morphology, improved nutrient digestibility, immune function regulation, control of inflammation, decreased ammonia and urea excretion, and protection against pathogens (Gusils et al., 1999; Fooks and Gib- son, 2002; Chichlowski et al., 2007b; Gadde et al., 2017; Aziz Mousavi et al., 2018; Jha et al., 2020).

The positive effects of feeding DFM with enzyme inclusion have also been reported (Nusairat and Wang, 2020). In this study, the dietary fat level was lower than the low-fat diets used herein. The current study results also agree with other reports where AME_n was improved with the addition of DFM to the diet (Mohan et al., 1996; Mountzouris et al., 2010). This could be through increased digesta passage (Schneitz et al., 1998), through increased digestive enzyme activity due to altered pH (Rowland, 1992; Aziz Mousavi et al., 2018), or increased absorptive surface area in the small intestine (He et al., 2019). Therefore, the DFM in the gut may be changing the environment in ways resulting in improved nutrient digestibility.

In this study, the major VFA was measured in the ceca of both the broilers and turkeys. Higher amounts were present in the digestive tract of turkeys than in broilers. Less butyrate was present in the low-fat treatments, possibly meaning that more butyrate was used locally as an energy source for enterocytes (Bergman, 1990; Bloemen et al., 2009). However, the other VFA were observed in approximately constant ratios. It may be important to note the presence of isobutyrate in the broiler trial and isovalerate in both trials, indicating that significant amounts of amino acid breakdown may be occurring in the gut.

While there was no treatment effect on VFA in the current study, in many reports, probiotics generally positively affect VFA production in humans (Markowiak Kopeć and Śliżewska, 2020). For instance, one study found that the administration of three Lactobacillus species encouraged the growth of lactate-consuming bacteria and increased VFAs, especially buytrate (Moens et al., 2019), while a different experiment observed that the administration of *Lactobacillus plantarum* for 4 weeks resulted in a significant increase in acetate and propionate (Wang et al., 2014).

In broilers, administration of DFM may reduce the presence of pathogenic bacteria, such as *Enterobacteriaceae*, in the gut by promoting fermentation of anaerobic bacteria to produce high concentrations of VFAs, which have a bacteriostatic effect in the ceca (van Der Wielen et al., 2000). The relationship of gut VFA and microbiota in broilers can be very important, especially in commercial environments. The feeding of bacterial cultures to birds has a known effect on energy metabolism in the bird, resulting in improved performance (Jin et al., 1998a). In addition, the feeding of *Lactobacillus* to broilers exposed to lightly applied stressors to simulate field conditions resulted in improved performance and VFA compared to stressed birds that did not receive the dietary *Lactobacillus* (Meimandipour et al., 2010). However, the mechanisms of why VFA was not affected by DFM in the trials herein are uncertain, and further study is needed to ascertain the specific impact of DFM on VFAs.

In conclusion, DFM can replace part of the fat in broiler and turkey diets with improvement in performance associated with an increased AME_n, while the association with changes in VFA is less clear.

Table 6: Effect of dietary DFM[1] and fat[2] inclusion on cecal volatile fatty acid concentrations[3] of turkey poults at 21 days.

		Molar percent						Concentration
Inclusion Level		Acetate	Buytrate	Propionate	Isobutyrate	Valerate	Isovalerate	Total
DFM	Fat	(%)						mM
No	Low	56.37	22.42	2.66	13.16	2.93	2.51	170.74
Yes	Low	57.40	22.29	2.61	12.43	2.84	2.46	169.29
No	High	51.89	26.53	2.73	12.18	2.96	2.56	160.80
Yes	High	54.39	24.28	2.87	12.60	3.14	2.71	151.05
DFM main effect								
No		54.13	24.48	2.69	12.67	2.95	2.53	165.77
Yes		55.90	23.28	2.74	12.52	2.99	2.59	160.17
Fat main effect								
Low		56.89	22.35	2.64	12.79	2.89	2.48	170.01
High		53.14	25.40	2.80	12.39	3.05	2.64	155.93
Source of variation								
DFM		0.2545	0.5238	0.7212	0.7883	0.7298	0.6865	0.5316
Fat		0.0177	0.1060	0.2227	0.4802	0.2751	0.2408	0.1190
DFMxFat		0.6332	0.5682	0.4699	0.3230	0.3651	0.4613	0.6428
SEM		1.71	2.46	0.188	0.934	0.107	0.179	6.051

[1] Direct-Fed Microbial (DFM) inclusion rates: No at 0 kg/ton or Yes at 0.91 kg/ton.
[2] Fat inclusion rates: Low at 1%, High at 6%.
[3] Values are means based on representative cecal samples of all birds per pen.

Table 7: Effect of dietary DFM[1] and fat[2] inclusion on ileal volatile fatty acid concentrations[3] of turkey poults at 21 days.

		Molar Percent						Concentration
Inclusion Level		Acetate	Buytrate	Propionate	Isobutyrate	Valerate	Isovalerate	Total
DFM	Fat	(%)						mM
No	Low	46.40	12.05	6.02	23.95	5.98	5.25	67.23
Yes	Low	46.43	12.06	6.03	23.98	5.97	5.53	63.72
No	High	46.36	12.06	6.08	24.00	6.00	5.52	64.25
Yes	High	46.52	12.06	6.03	23.81	5.98	5.50	67.78
DFM main effect								
No		46.38	12.05	6.05	23.97	5.99	5.52	65.74
Yes		46.48	12.06	6.03	23.89	5.97	5.51	65.75
Fat main effect								
Low		46.42	12.05	6.03	23.97	5.98	5.51	66.01
High		46.44	12.06	6.05	23.90	5.99	5.53	65.47
Source of variation								
SEM		0.183	0.064	0.063	0.123	0.026	0.023	1.82
DFM		0.4912	0.8912	0.6248	0.3339	0.4186	0.6077	0.9935
Fat		0.8848	0.9420	0.6340	0.3898	0.5507	0.2880	0.7125
DFMxFat		0.6466	0.9192	0.5073	0.1759	0.8980	0.4659	0.0190

[1] Direct-Fed Microbial (DFM) inclusion rates: No at 0 kg/ton or Yes at 0.91 kg/ton.
[2] Fat inclusion rates: Low at 1%, High at 6%.
[3] Values are means based on representative cecal samples of all birds per pen.

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