ABSTRACT

ESSICK, SAMANTHA BLACK. The Effect of a Direct-Fed Microbial (DFM) and Fat Inclusion on Broiler Chick and Turkey Poult Performance and Energy Metabolism. (Under the direction of Dr. Jesse Grimes).

Four experiments were conducted to test the effect of DFM inclusion on broiler chick and turkey poult performance and energy metabolism. The first two experiments were designed with DFM inclusion at 0, 1, 2, or 4 lb/ton, in diets with no supplemental fat, fed to broilers and turkeys to 21 days of age. The second two experiments were designed as 2 x 2 factorial treatments with dietary supplemental fat at 1 or 6% and DFM inclusion at 0 or 2 lb/ton fed to broilers and turkeys to 20 or 21 days of age. For all trials body weight (BW) and feed intake (FI) were measured weekly with feed conversion ratio (FCR) calculated. Data were analyzed using JMP 11. Means were considered significant at p<0.05.

In the first broiler experiment, no significant differences were observed in BW gain (BWG), FI or FCR except during week 2 where 2 and 4 lb/ton of DFM resulted in significantly improved FCR over the non-DFM treatment. The DFM inclusion increased total cecal volatile fatty acid (VFA) concentrations on day 22 (p=0.06). Cumulative BWG in the first turkey experiment was improved for the 2 lb/ton DFM treatment over the non-DFM treatment with 1 and 4 lb/ton treatment birds having intermediate responses. Cumulative FI was higher for the 4 lb/ton DFM treatment over the non-DFM treatment with 1 lb/ton treatment birds consuming intermediate amounts. FCR differences were only observed during the second week when the 1 lb/ton DFM treatment was improved over the 4 lb/ton DFM treatment with intermediate results for the 2 lb/ton DFM and non-DFM treatments. A trend (p=0.07) of increased total cecal VFA for the non-DFM treatment over all other treatments was observed.
The second broiler experiment resulted in improved performance parameters when DFM was included at the low fat level but not at the high fat level. This effect was observed for cumulative BWG, FI and FCR. For the turkey experiment, cumulative BWG was improved for the birds fed low fat with DFM and both high fat treatments over the low fat with no DFM fed birds. There were no differences observed for FI. The FCR was improved in both high fat treatments over low fat with DFM, and low fat with DFM was improved over low fat with no DFM. The same effects were observed for apparent metabolizable energy (AMEₙ) in the second set of experiments. High fat diets, regardless of DFM level, resulted in increased AMEₙ. At the low fat level, an uplift in AMEₙ was observed with DFM inclusion; however no effect was observed at the high fat level.

In summary, there were observed improvements in performance with the addition of DFM to the diets of broilers chicks and turkey poults especially in low fat diets. Furthermore, improvements in performance and AMEₙ were observed when DFM is supplemented to low fat diets fed to broiler chicks and turkey poults. In conclusion, dietary DFM results in increased AME and improved performance and can be substituted for dietary fat in poultry diets.
The Effect of a Direct-Fed Microbial (DFM) and Fat Inclusion on Broiler Chick and Turkey Poult Performance and Energy Metabolism

by
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DEDICATION

I would like to dedicate this work to my dear parents, for this would not have happened without your love.
BIOGRAPHY

Samantha Black Essick was born in Annapolis, Maryland. She earned her Bachelors degrees in Poultry Science and Biological Sciences from North Carolina State University. As an undergraduate student she worked at the University Feed Mill, as an undergraduate teaching assistant for introductory Poultry Science classes, and as a student technician at the Turkey Educational Unit. Upon graduation in 2012, Samantha decided to continue her education by pursuing a Master of Science degree in Poultry Science under the direction of Dr. Jesse Grimes. Samantha has decided to continue to work towards her PhD in Animal Sciences and Nutrition at North Carolina State University in 2015 after the completion of her M.S. degree.
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LITERATURE REVIEW
INTRODUCTION

Poultry encompasses 35 percent of the total world meat intake. The intake of poultry products has increased by 104 percent from 1990 to 2012 (FAO, 2014). In 2014 there was 51.4 billion pounds of broiler meat produced in the United States, valued at $32.7 billion (USDA NASS, 2015). The United States is the world’s largest turkey producer, with 238 million turkeys produced in 2014, valued at $5.30 billion (USDA NASS, 2015). Over 7 billion pounds of broiler meat and over 800 million pounds of turkey meat were exported from the United States in 2014 (WASDE, 2015). With this type of financial investment and revenue opportunity, it is important to protect the products produced. Poultry products caused 17 percent of out-break associated illnesses from 1998 – 2008 (CDC, 2013). Outbreaks of common enteropathogenic bacteria, including Salmonella, Camplybacter and enterohaemorrhagic Escherichia coli, are related to contamination of poultry products and/or uncooked or raw poultry meat (Tauxe et al., 1988; WHO, 2014). Producers seek methods to reduce enteric disease caused by pathogenic bacteria and eliminate contamination that could be seen in poultry products after processing (Lee et al., 2010).

Currently, antimicrobials such as antibiotic growth promoters (AGP) are being used to treat infections caused by pathogenic bacteria (WHO, 2014). However, pathogenic bacteria that are resistant to antimicrobials are starting to enter the food chain system (Flint & Garner, 2009) becoming a threat to modern medicine and food manufacturers (WHO, 2014). This has led to the search for alternatives to AGP in the poultry industry.

There is no single treatment or product that has been as successful, or provided such consistent and robust effects on performance as antibiotics (Dibner & Buttin, 2002). An
alternative strategy to treat enteric disease is through the use of direct-fed microbials (DFM) in poultry flocks. However, there is a need to quantify the effects of DFM fed to poultry species. After gaining approval as fermentation products by the Association of American Feed Control Officials (AAFCO, 1999) for use as feed ingredients (FDA, 1995), a great deal of microorganisms have been selected for use as DFM and are classified as Generally Recognized As Safe (GRAS) products for use in any animal feed. This has led to great variation among strains of bacteria, administration doses, and effects seen in performance parameters. There are many known effects of DFM including improved microbial balance to improve gut health and condition (Nurmi & Rantalla, 1973; Patterson & Burkholder, 2003; Chichlowski et al., 2007a; Kabir, 2009; Lee et al., 2010). Yet, there are still many opportunities to explore the impacts of DFM on poultry species.

The objective of this chapter is to review scientific evidence of the use of DFM in poultry diets and discuss the merits of the effects of DFM on various health and performance parameters in poultry species.

**WHAT ARE DIRECT-FED MICROBIALS?**

The term DFM can be used interchangeably with probiotics. Originally, probiotics were considered protozoa that produce substances that lead to growth acceleration in other species (Lilly & Stillwell, 1965). But later the term came to be described by Fuller as “a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance” (1989). DFM can aid in restoring protective gut microflora that is lacking in today’s industrial setting where chicks and poults are not exposed to their mother’s feces at hatch (Kabir, 2009).
According to Chichlowski et al., successful colonization of DFM in the GI tract is dependent on: strain, dose and frequency of administration of DFM, health and nutritional status, age, stress applied, and genetics of the host (2007a). Additionally each host has a unique microbiome which is influenced by the host genotype and physiology, the colonization history, environmental factors, food, and drugs (Zoetendal et al., 2001). The ceca and colon are chambers of microbial action in the GI tract (Bergman, 1990). DFM are not permanently attached to the epithelial surfaces of the GI tract and will be eliminated from the system a few days after cessation of administration due to the reversal of weak attractions between bacterium and epithelium by normal intestinal flow and peristalsis (Chichlowski et al., 2007a; Guslis et al., 1999). Attachment to the surface of epithelial cells (cecal and colonic) allows probiotic organisms to resist peristalsis and mixing with the digesta and mucous layer which are eventually removed from the GI tract (Chichlowski et al., 2007a). *Lactobacilli* adhere to epithelial surfaces through interactions between the GI surface and proteinaceous molecules or lectins (carbohydrate-specific molecules) through various surface determinants such as: passive forces, electrostatic interactions, hydrophobic forces and steric forces (Gusils et al., 1999). DFM can also be found attached to individual feed particles, within the aqueous matrix of digesta, or within the mucous blanket that covers the epithelium of the GI tract (Chichlowski et al. 2007a). DFM are thought to produce optimally balanced microbial populations by maintaining beneficial commensal microorganisms in the gut (Lee et al., 2010). Kabir states that within the GI tract of healthy, non-stressed birds, there is an imbalance of beneficial (probiotic) and non-beneficial (pathogenic) bacteria. When birds become stressed, probiotic bacteria numbers decrease and pathogens will overgrow leading
to disease and reduced performance. However, when the beneficial and non-beneficial bacteria are in balance, via probiotic bacteria, bird performance will be at maximum efficiency (2009).

Characteristics of ideal DFM are listed in Table 1.1. Some concerns with DFM are the production of a product that is improperly manufactured, either at the manufacturing facility or at the feed mill by improper mixing or improper pelleting temperatures (Flint & Garner, 2009; Russell & Grimes, 2007). The resulting product then contains inappropriate organisms for use in animal feeds or is affected by infectious agents that result in decreased performance (Flint & Garner, 2009). Figure 1.1 shows a general overview of the selection process of probiotics in the poultry industry. Various microbial species are being used as DFM, including: *Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium, Bifidobacterium spp. Streptococcus spp., Bacillus spp., Aspergillus spp., Candida spp., and Saccharomyces spp.* (Kabir, 2009). The three phyla that are most abundant in the intestine are Bacteriocidetes (gram-negative), Firmicutes (gram-positive), and Antinobacteria (gram-positive) (den Besten et al., 2013). *Lactobacillus spp.* and *Enterococcus spp.* are Firmicutes, while *Bifidobacterium spp.* are Antinobacteria.

**Lactobacillus spp.**

*Lactobacillus spp.* are lactic acid bacteria that are gram-positive (firmicute), non-sporeforming and non-flagellated rods of coccobacilli (Hammes & Vogel, 1995). They are aerotolerant or anaerobic and strictly fermentative and have characteristically low guanine plus cytosine (G + C) content (Gomes & Malcata, 1999). They ferment glucose predominantly to lactic acid in a homofermentative manner (Gomes & Malcata, 1999). Their
optimal growth temperature range is from 35 to 40°C, they are acid tolerant between 0.3 and 1.9 percent, and their optimal pH is from 5.5 to 6.0 (Gomes & Malcata, 1999). They are often desirable as feed additives because they can form endospores that are resistant to extreme temperatures and process environments, such as pelleting (Flint & Garner, 2009). A suggested effect on metabolism is the lowering of pH for pathogen inhibition and the promotion of volatile fatty acid (VFA) production.

**Enterococcus spp.**

*Enterococcus spp.* are typical lactic acid bacteria that are gram-positive (firmicute), non-sporeforming, facultative anaerobes with low G + C content (Klein et al., 1998). Their major end product is lactic acid, but they have to ability to produce acetate from citrate. *Enterococcus faecium* are vancomycin susceptible, which is in contrast to many within the genera that are resistant to the antibiotic, making them feasible as a probiotic (Klein et al., 1998). Their optimal temperature and pH are 42°C and 7.5 but they will grow within a wide range from 6.5°C to 48°C and a pH of 4.6 – 9.9 (Fisher & Phillips, 2009).

**Bifidobacterium spp.**

*Bifidobacterium spp.* are gram-positive (actinobacteria), non-spore forming, non-motile and catalase-negative anaerobes (Sgorbati et al., 1995). They are characterized by a high G+C content (Sgorbati et al., 1995). They are saccharolytic organisms that produce acetic and lactic acids without the generation of CO₂ (Gomes & Malcata, 1999). They utilize heterofermentation that is initiated by splitting fructose-6-phosphate into one C2 and one C4 moiety, the C2 moiety is converted to acetate and C4 goes towards deriving another fructose-6-phosphate molecule, resulting in a total of three moles of acetate and two moles of lactate.
Their optimum pH for growth is 6 – 7 and their optimal temperature is from 37 to 41°C (Gomes & Malcata, 1999).

**Bacillus spp.**

*Bacillus* strains are gram-positive rod-shaped anaerobic bacteria that produce endospores under aerobic conditions in order to produce catalase and form gas from nitrate and utilize propionate (Sorokulova et al., 2008). Asymmetric division of *Bacillus* during sporulation produces the mother cell and the forespore (Seavers et al., 2001). Spores are advantageous because they have a long shelf life, and some such as *B. subtilis* are avirulent (La Ragione et al., 2001). *Bacillus* genera do not normally inhabit the GI tract (Sorokulova et al., 2008). However, *Bacillus* spores formed from *Bacillus spp.* have been reported to be successful in competitive exclusion of pathogens (La Ragione et al., 2001). Also, many *Bacillus* are heat resistant which make them ideal DFM when used in diets that are pelleted.

**DFM MECHANISMS AND MODES OF ACTION**

The mechanisms of action for DFM are still not fully understood. However, the literature supports several mechanisms in poultry production. Below are discussed some of the known modes of actions for DFM.

**Broiler Performance Impacts**

Some researchers have found that the addition of DFM to broiler diets does not improve performance. When Lactobacillus cultures were administered to broilers via water to 7 weeks of age there were no improvements in body weight or feed conversion ratio (Watkins & Kratzer, 1983). Also, when broiler chicks were fed *Streptococcus faecium* to 7 days of age, there were no significant differences in body weights (BW) or feed conversion r
atios (FCR) (Maioino et al., 1990). Broiler chicks fed two commercially available DFM products via feed or water did not produce any significant differences in BW or FCR from hatch to 6 weeks of age (O’Dea et al., 2006). Alternatively, when supplemented with a single strain or with a mixture of strains of Lactobacillus cultures, BW was increased and FCR was improved in broilers from 0 - 6 weeks (Jin et al., 1998a). Mountzouris et al. found that DFM treatment (multistrain including Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus) resulted in bird performance (BW gain (BWG) and FCR) that was at least as good as the birds provided the antibiotic treatment (2007). In another instance, broilers fed with $10^8$ inclusion level of DFM had higher BW compared to an antibiotic treatment during the growing phase (15 to 28 days) (Mountzouris et al., 2010). When DFM was supplemented in low nutrient diets, an improvement in BWG and FCR in broiler chicks fed pelleted diets during the grower and finisher phases was observed (Angel et al., 2005).

**Turkey Performance Impacts**

There is conflicting evidence about the use of DFM in turkey production. Potter et al., found no significant differences in performance parameters in 16 week old medium white turkeys when fed 0, 0.025, 0.050 or 0.075 percent Lactobacillus acidophilus cultures (1979). Large white male poultls supplemented with Streptococcus strains did not show any improvements in BW at 126 days of age (Owings, 1992). Additionally, only numerical increases in BW were seen with the addition of a Lactobacillus product to the diet of broad breasted large white turkey poultls fed to 3 weeks of age (Francis et al., 1978). However, in recent years, there has been evidence to support the effect of DFM on performance in turkeys. When a Lactobacillus DFM was added to the water in commercial turkey flocks, increased market
BW, a small reduction in FCR, and lower cost of production (cents/kg of live turkey) were observed (Torres-Rodriguez et al., 2007). Using a pelleted DFM, there was improved performance in turkey hens and toms during a university trial, and nominal increases in BW and FCR in field study with tom turkeys (Russell & Grimes, 2007). Grimes et al., saw increased BW and cumulative FCR in poults supplemented with DFM in both mash and crumbled diets fed to 3 weeks of age (2008).

There is great variation in results for DFM trials with performance response in poultry species. This can be attributed to the circumstantial nature of each individual trial. Some trials are conducted with near optimal conditions, while others are conducted in sub-optimal conditions. In some cases, birds might be exposed to an unknown stressor, e.g. heat or disease, therefore resulting in decreased performance. Additionally, it is difficult to compare the general effect of DFM due to the varying use of bacterial strain(s) and dosages across different poultry species.

**Immune System**

Lee et al., suggested that DFM lowers inflammatory status, and increases protective immunity (2010) both through cell-mediated and innate immune responses. DFM interact with GALT (gut-associate lymphoid tissues), including B-1 cells (for the production of natural antibodies). For example, chicks treated with DFM had increased serum and intestinal antibodies against *tetanus toxoid* (TT) and *Clostridium perfringens* alpha toxin, and immunoglobulin A (IgA) reactive to bovine serum albumin (BSA) suggesting induction of natural antibodies and cell-mediated immunity in DFM-treated chicks (Haghighi et al., 2006). Furthermore, the cell-mediated response increases in broilers fed with DFM versus a
control diet. This is characterized by increased intestinal intraepithelial lymphocyte (IEL) expression of surface markers, which is important during enteric infections and leads to the production of T cells (Dalloul et al., 2003).

DFM supplementation down regulated ileal, cecal tonsil, and plenic cytokine production as part of the innate immune response, suggesting that DFM have anti-inflammatory responses in broilers (Waititu et al., 2014). Similarly, when broilers were fed DFM, there was decreased intestinal mRNA levels of pro-inflammatory cytokine IL-6 and increased expression of anti-inflammatory cytokine IL-10 (Chichlowski et al., 2007a) suggesting increased immune response.

**Digestive Enzyme Activity**

DFM alter gastrointestinal pH (Jin et al., 1998b; Kabir, 2009) and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients (Dierick, 1989). It is suggested that DFM stimulate host digestive enzymes or produce a source of those enzymes through their metabolism (Rowland, 1992). They also reduce the redox potential of GI tract, making it an environment suitable for obligate anaerobe bacteria (Cummings & Macfarlane, 1997). Jin et al. found that the addition of a single or multiple strains of Lactobacilli significantly increases the amount of intestinal amylase after 40 days of age and do not significantly increase the amount of intestinal lipolytic or proteolytic activities, suggesting that the addition of DFM does not affect dietary protein digestion (2000). DFM have been found to reduce the enzymatic activity of β-glucuronidase that is involved in the production of carcinogenic metabolites in the lower GI tract (Fooks & Gibson, 2002).

Some anti-nutritional components of poultry diets include the presence of non-starch
polysaccharides (NSP) and phytic acid. Supplementation with phytases and carbohydrases may alleviate these negative effects. NSP are indigestible and reduce nutrient utilization by limiting the amount of nutrients available for digestion and absorption (Bedford & Schulze, 1998; Kim et al., 2005). Exogenous carbohydrases degrade NSP in poultry diets based on viscous grains, such as wheat, into oligo-saccharides and monosaccharides (Bedford, 2000; Selle & Ravindran, 2007). Xylanase, a carbohydrase that is used to break down NSP in wheat or corn based diets, promotes changes in the cell wall by hydrolyzing structural arabinoxylans that encapsulate nutrients (Dourado et al., 2009; Woyengo & Nyachoti, 2011). Two-thirds of phosphorus in plant-based feedstuffs is poorly digested in poultry because it is bound to phytic acid and poultry do not produce enough phytase enzyme to sufficiently hydrolyze it (Bedford, 2000). Exogenous phytase dephosphorylates phytic acid to myo-inositol and inorganic P moieties through two-step cleavage of phytic acid (Selle & Ravindran, 2007). Phytase can effect energy utilization by increasing fat and protein digestibilities. It is proposed that increased ileal fat digestibility occurs when phytase partially prevents the formation of insoluble metallic soaps, which limit fat utilization in the gut (Selle & Ravindran, 2007). Additionally, phytase increases protein and amino acid digestibility through partial prevention of protein-phytate complexes, mainly in the crop, by prior hydrolysis by phytase in the proventriculus (Selle & Ravindran, 2007). Phytase may release starch, enzymes, enzyme cofactors, proteins, and minerals so they can be better digested and absorbed by the bird, thereby improving energy usage (Dourado et al., 2009). It has been reported that enzymes improve the ME of feedstuffs in the diet (Dourado et al., 2009), thereby improving energy utilization in birds. This is evidenced through several
experiments, which show increased apparent metabolizable energy (AME\textsubscript{a}) upon supplementation with phytase (Selle & Ravindran, 2007), 1000 XU/kg xylanase (Wu et al., 2004), and especially with a combination of phytase and carbohydrases (Dourado et al., 2009; Woyengo & Nyachoti, 2011). Similar outcomes have been reported with DFM supplementation, indicating that DFM may have enzyme-like effects in the GI tract of birds.

**Maintenance of Epithelial Barrier Integrity and Gut Morphology**

The GI tract contains three-dimensional compartments that constitute a dynamic fluid surface (Gusils et al., 1999). The two methods of maintaining the epithelial barrier are through the mucous blanket covering the barrier, and the formation of tight junctions (a continuous unbroken biological barrier that prevents the entrance of pathogens) (Chichlowski et al., 2007a). DFM have been shown to increase the number of goblet cells within the epithelial barrier, which leads to increased production of mucus (Chichlowski et al., 2007a). DFM, whether shed from epithelial surfaces or multiplying in ingested food exert their effect through adherence and colonization of the GI tract, essentially protecting and maintaining the epithelial barrier (Gusils et al., 1999). *Lactobacillus* was able to prevent the adhesion of several *Salmonella* strains to the epithelial barrier (Schneitz et al., 1998; Gusils et al., 1999).

DFM (*Lactobacillus, Bifidobacterium, Enterococcus*) treated broilers had increased villus height, crypt depth and muscle thickness in the jejunum and increased muscle thickness in the ileum. Additionally in this study, increased villus size potentially led to a shift in the effective absorptive area, which increases passive nutrient absorption (Chichlowski et al., 2007b). In another study with broilers, birds fed *Enterococcus faecium* had higher villus height than the positive or negative control treatments (Cao et al., 2013).
There was increased villus height in turkey poults supplemented with DFM in a crumble diet over a control diet for both non-challenged and *Salmonella* challenged birds. Also, larger villus heights were observed in the challenged birds over the non-challenged birds (Rahimi et al., 2009). Increased villus height can be associated with increased surface area for greater absorption of nutrients (Cao et al., 2013).

**Pathogen Inhibition**

Nurmi and Rantalla first described that bacteria compete with each other for space and nutrients, and they named the process competitive exclusion (1973). It is suggested that DFM occupy a niche at the expense of potentially harmful organisms, thereby specifically blocking the adherence of enteropathogens (Fooks & Gibson, 2002). A potential mechanism for the reduced incidence and duration of disease are the enhancement of colonization resistance and/or direct inhibitory effects against pathogens (Kabir, 2009). For example Jin et al., suggests that when birds are supplemented with a single strain or with a mixture of strains of *Lactobacillus* cultures, the numbers of coliforms (pathogenic bacteria) in the cecum 10 and 20 days after feeding is significantly decreased (1998a).

It is important to reduce the incidence of enteropathogens, such as *Campylobacter spp.* and *Salmonella spp.* because they cause sporadic cases of food-borne illness (Tauxe et al., 1988) and have the potential to cause declines in performance of the animal. Many DFM have a wide-ranging effect on many pathogenic bacteria, for example, one strain of *Lactobacillus casei* subsp. *rhamnosus* protected against nine bacterial pathogens (Forestier et al., 2001).

Gusils et al. proposed that *Lactobacilli* block the in vitro binding sites for *Salmonella*
by a specific mechanism or by non-specific steric hinderance (1999). Compared to a control group, birds supplemented with a continuous flow mixture (including Enterococcus faecium, Lactobacillus spp., Bifidobacterium spp.) had decreased numbers of Salmonella typhimurium in cecal contents (Corrier et al., 1995). Moreover, when birds are given microbial cultures derived from chicken cecal contents, they not only decreased Salmonella isolated from chick ceca but also reduced the number of chicks colonized by the pathogen when challenged with $10^4$ Salmonella typhimurium (Nisbet et al., 1993). Additionally, in a study where one-day-old chicks were challenged with Salmonella enteritidis, a 70 percent colonization rate 21 days after challenge was observed. None of the chicks receiving a strain of Lactobacillus salivarius as a pathogen challenge were Salmonella positive at 21 days of rearing (Pascual et al., 1999). In turkeys, the administration of DFM lowered Salmonella in the lower GI tract at 3 weeks of age by 1 log (Grimes et al., 2008). However, some have shown that cultures of Lactobacillus and Enterococcus were ineffective in reducing Salmonella cecal colonization; again suggesting the effect of DFM is situation specific (Adler & DaMassa, 1980; Hinton & Mead, 1991).

Addition of Enterococcus faecium decreased cecal Escherichia coli and Clostridium perfringens (the etiologic agent of necrotic enteritis) and increased the populations of Lactobacillus and Bifidobacterium in cecal contents (Cao et al., 2013). McReynolds et al., found reduced log10 values in Clostridium perfringens within the intestinal contents of birds when challenged with C. perfringens and supplemented with probiotic containing Enterococcus spp., Pediococcus spp., Bifidobacterium spp., Lactobacillus spp. (2009). It was also demonstrated that administration of Lactobacillus acidophilus and Enterococcus
faecium to chicks reduced fecal shedding frequency of Campylobacter jejuni by 70 percent (Morishita et al., 1997).

**Impacts on Apparent Metabolizable Energy by DFM**

DFM treated broilers have shown higher AME\textsubscript{n} content compared with a control treatment (Monhan et al. 1996; Mountzouris et al., 2010). DFM treatment increased metabolizable energy (ME) value over a control treatment, which could be attributed to increased nutrient digestibility and decreased observed digesta viscosity (Schneitz et al., 1998). The mechanism of increased nutrient digestibility of DFM has not been elucidated. However, the mechanisms could be similar to those seen with enzymes, or due to the changes in environment produced by the DFM itself. There was an increase in AME\textsubscript{n} when birds were supplemented with DFM; however, this did not result in improved growth performance, suggesting that the basal diet was nutritionally adequate (Waititu et al., 2013). Increased AME\textsubscript{n} in birds offered DFM-supplemented diets might be due to increased total tract apparent retention rate (TTAR) of fat and possibly starch that escaped upper gut digestion (Waititu et al., 2013). Some studies show increased nutrient retention, which is a similar measurement to AME\textsubscript{n}, when birds were supplemented with DFM over control treatments (Angel et al., 2005; Waititu et al., 2013). However, these measurements only give a partial view of metabolism in the gut and are not a direct comparison to the AME\textsubscript{n} because they do not indicate total energy utilization.

**VFA Production**

VFA play a role in the development of the microflora in the ceca of broiler chickens during growth (Van Der Wielen et al., 2000). The concentration of VFAs in the cecal
contents of chicks steadily increases from day of hatch to day 21 and is used as an indicator of the growth and establishment of normal anaerobic bacteria in ceca (Barnes et al., 1979; Barnes et al., 1980; Nisbet et al., 1996). It is suggested that the normal VFA-producing anaerobic bacteria are established in the ceca of the broiler chicks by 10 days of age (Corrier et al., 1990). The concentrations of VFA at different sites of the GI tract are a direct function of anaerobic bacterial population in the gut and are proportional to the time to which digesta are retained (Bergman, 1990). When supplemented with various Lactobacillus cultures, total VFA in the ileum and ceca were significantly increased (Jin et al., 1998b). Likewise, there was increased propionic acid when supplemented with Broilact probiotic, due to the fact that the majority of the anaerobes in the product produce high amounts of propionate (Schneitz et al., 1998). However, some have shown that a Lactobacillus strain was not affected during growth in brain-heart infusion (BHI) broth with volatile fatty acids, suggesting a lack of correlation between Lactobacilli and concentrations of volatile fatty acids in the ceca of broiler chickens (Van Der Wielen et al., 2000). Little data is available on the effect of DFM on VFA production in turkeys.

**WHAT ARE VOLATILE FATTY ACIDS?**

VFA are the principal products of fermentation in the lower GI tract with acetate (C2), propionate (C3) and butyrate (C4) occurring in greatest amounts (Cummings & Macfarlane, 1991). Fermentation is considered the anaerobic breakdown of carbohydrate and protein by bacteria (Cummings & Macfarlane, 1991). Some of the major substrates for fermentation are: complex carbohydrates originating from plant cells, such as cellulose, hemicellulose, pectins, starches, dextrans, and soluble carbohydrates (Bergman, 1990).
Fermentable substrates for VFA production that are not provided by the diet include sloughed cells, mucus, and endogenous secretions, such as proteins and polysaccharides (Bergman, 1990). Branched-chain fatty acids (isobutyrate, isovalerate) are products of protein and amino acid fermentations (Cummings & Macfarlane, 1991; den Besten et al., 2013). Higher chain VFAs, such as valerate (C5), which is produced from the condensation of acetate and propionate, produce small amounts of glucose (Bergman, 1990). VFA are metabolized by the colon and ceca of non-ruminants by the process of absorption and transport to the bloodstream (Bergman, 1990). VFA produced by the microbiota in the cecum and the colon can be found in hepatic, portal (Murase et al., 1995), and peripheral blood (Cummings et al., 1987).

A majority of VFA absorption is by passive diffusion of protonated form across colonocyte cell membrane (Cummings & Macfarlane, 1991). VFA are transported across the apical and the basolateral membranes of colonocytes (den Besten et al., 2013). At the apical membrane by passive diffusion of undissociated VFA and active transport of dissociated VFA anions, VFA are mediated by a number of different transporters (den Besten et al., 2013). Transport systems include coupled import of VFA anion to HCO$_3^-$ secretion into intestinal lumen (Vidyasaga et al., 2005), monocarboxylase transporters (MCTs) that catalyze VFA anion co-transport with H$^+$ dependent cations (Vidyasaga et al., 2005; Teramae et al., 2010), and sodium dependent monocarboxylase transporters (SMCT1) that couples Na$^+$ transport with VFA anion and stimulates Cl$^-$ and water absorption (Teramae et al., 2010). It has been reported that VFA anion transport by MCT dominates over transport by SMCT1 (Gonçalves et al., 2011). The part of VFA that is not consumed by the colonocytes is
transported across the basolateral membrane, mediated via the VFA-HCO$_3^-$ antiport and cation-VFA anion symport (den Besten et al., 2013). Because the intracellular pH is higher than the pH in the intestinal lumen, all intracellular VFA are present in the dissociated form, implying that transport at the basolateral side should be via transporters only because no diffusion can occur (den Besten et al., 2013). 95 percent of the produced VFA are rapidly absorbed by the colonocytes while the remaining 5 percent are secreted in the feces (Topping & Clifton, 2001).

VFA absorption has a significant role in sodium and water conservation by the body (Bergman, 1990). The colonic luminal pH is the result of microbial VFA production and the neutralizing capacity of bicarbonate which is exchanged with VFA when they are absorbed by the host (den Besten et al., 2013). The process of VFA absorption is concentration dependent. VFA transport is associated with the appearance of bicarbonate ions and stimulation of sodium absorption (Argenzio et al., 1975; Cummings, 1981). VFA anion transport stimulates sodium and water absorption as well as deacidification of the cell (Musch et al., 2001). VFA provide a powerful driving force for the movement of water out of the colonic lumen and consequently may be an important protection against diarrhea (Cummings & Macfarlane, 1991).

VFA in the GI tract have also been shown to lower blood cholesterol levels (Bergman, 1990). Cholesterol is synthesized from its precursor unit, acetyl-CoA, via a complex metabolic pathway in which 3-hydroxy-3-methylglutaryl-CoA reductase is the rate-limiting enzyme (Rodwell et al., 1976). Propionate lowers cholesterol synthesis rate by decreasing the enzyme activity of hepatic 3-hydroxy-3-methylglutaryl-CoA synthase
(HMGCS) and 3-hydroxymethylglutaryl-CoA reductase (HMGCR) (Rodwell et al., 1976; Bush & Milligan, 1971). Serum cholesterol levels are also affected by acetate (Fushimi et al., 2006). In a study by Fushimi, rats receiving a diet containing 1 percent (w/w) cholesterol showed significantly less increased serum cholesterol levels when the diet was supplemented with 0.3 percent (w/w) acetate (2006). Cholesterol-lowering effects described are mediated through AMPK activation by VFA similar as the effects of VFA on fatty acid and glucose metabolism (den Besten et al., 2013).

From the host’s viewpoint, the end products of fermentation reactions are important because they are absorbed from the gut and influence many aspects of metabolism (Cummings & Macfarlane, 1991). VFA affect lipid (Demigné et al., 1995), glucose (Todesco et al., 1991), and cholesterol (Fushimi et al., 2006; Demigné et al., 1995) metabolism in various tissues. In humans, VFA provide approximately 10 percent of the daily caloric requirements. And the large intestines of pigs provide approximately 11 percent of daily caloric requirements via VFA (Bergman, 1990). There is very little data available on the contributions of VFA on energy requirements in poultry species.

Epithelial cells of the colonic mucosa obtain 60-70 percent of their energy from bacterial fermentation products generated in the colonic lumen (Roediger, 1982). Generally, VFA are transported from the intestinal lumen into the blood compartment of the host and are taken up by organs where they act as substrates or signal molecules (den Besten et al., 2013). VFA metabolize to CO2 and ketone bodies as precursors for lipid biosynthesis (Roediger, 1980). VFA also beneficially affect glucose metabolism by normalizing plasma glucose levels and increasing glucose handling. These affects could be directly via a hepatic
AMPK regulation pathway, or indirect via the gut derived hormones PYY and GLP-1 (den Besten et al., 2013).

At lower pH, firmicutes produce butyrate as their primary metabolic end product (Macfarlane & Macfarlane, 2003). However, at higher pH, most firmicutes disappear and bacteroides become dominant producing acetate and propionate (Walker et al., 2005). This suggests a production balance between VFA, when pH fluctuates, based on digesta and parasystllic movements. Additionally, VFA production is reduced and more butyrate is produced when pH decreases. But when pH becomes slightly higher, production trends towards acetate and propionate. This indicates that changes in the microbiota and markers of energy harvesting are not in direct association and that energy utilization is held constant in the gut (den Besten et al., 2013).

**Acetate**

Acetate (C2) is formed by hydrolysis of acetyl-CoA or from CO2 via Wood-Ljunddahl pathway where CO2 is reduced to CO and converted with a methyl group and CoASH to acetyl-CoA (Pryde et al., 2002; Ragsdale & Pierce, 2008). Acetate is metabolized by entering the TCA cycle as acetyl-CoA, but because it loses 2 carbons as it enters, there is no net gain of oxaloacetate or glucose, only a gain of ATP (Bergman, 1990; den Besten et al., 2013). Acetate can also yield some ketone bodies (Bergman, 1990).

Up to 70 percent of acetate is taken up by the liver (Bloemen et al., 2009). Acetyl-CoA is readily used for fatty acid synthesis and lipogenesis in the liver of birds (Bergman, 1990). In the liver it is utilized as an energy source, substrate for synthesis of cholesterol and long-chain fatty acids and co-substrate for glutamine and glutamate synthesis (den Besten et
al., 2013). Exogenous acetate formed by colonic bacterial fermentation enters the blood compartment and is mixed with endogenous acetate released by tissues and organs (Ballard, 1972; Knowles et al., 1974). Acetate comprises 90 – 98 percent of VFA present in both arterial and peripheral venous blood (Bergman, 1990). Peripheral tissues including the heart, adipose tissue, kidney and muscle (Bergman, 1990) metabolize the remaining acetate (Knowles et al., 1974; Bergman, 1990). In these tissues, acetate is converted to aspartate and glutamate that go towards protein synthesis (Marty et al., 1985).

With a relatively high colonic concentration of acetate compared with butyrate, acetate is at least as important as butyrate for the energy supply (den Besten et al., 2013). Up to 70 percent of the acetate is taken up by the liver where it is used as an energy source and then transported to other tissues including the heart, adipose tissue, kidney, and muscle for anabolism (Knowles et al., 1974) Acetate is a valuable fuel in the peripheral tissues where it is oxidized as the free fatty acid (Cummings & Macfarlane, 1991).

**Propionate**

Propionate (C3) is formed via the electron transport chain using PEP or from the acrylate pathway, where lactate is reduced to propionate (Miller & Wolin, 1996). Propionic acid may be used as an energy source for chicks (Hume et al. 1993). Propionate is the only VFA that can be a major source of glucose (Bergman, 1990). Propionate is metabolized and enters the TCA cycle as oxaloacetate, and in the liver is converted to glucose therefore becoming an important respiratory fuel for the colonic mucosa and liver (Bergman, 1990).

After being absorbed and metabolized by colonic epithelium, the remaining amount of propionate is transported to the liver via the portal vein (Bergman, 1990). Most of
propionate is removed from portal blood by the liver by propionyl-CoA synthetase (Bergman, 1990) where it acts as a precursor for gluconeogenesis in the liver (Roy et al., 2006). The liver clears a large fraction of propionate and peripheral tissues take up the remainder of propionate (den Besten et al., 2013). Propionate has been shown to lower systemic serum cholesterol levels as it is closely tied to gluconeogenesis throughout the body (Cummings & Macfarlane, 1991).

**Butyrate**

Butyrate (C4) is formed from condensation of two molecules of acetyl-CoA, followed by the reduction to butyryl-CoA which is then converted by butyrate kinase to butyrate and CoASH with simultaneous formation of ATP (Louis & Flint, 2009). Alternatively, lactate-utilizing bacteria can first produce acetyl-CoA from lactate which is then used to form butyrate in the same method described above (Duncan et al., 2004). In a third way, butyrate is produced by the conversion of butyryl-CoA to butyrate by butyryl-CoA:acetate CoA transferase (den Besten et al., 2013). Butyrate metabolism begins by entering the TCA cycle as acetyl-CoA and it loses two carbons as it enters the cycle thereby only gaining ATP but not oxaloacetate or glucose (Bergman, 1990; den Besten et al., 2013). From there, butyrate is readily oxidized to CO₂, or converted to ketone bodies and free amino acids (Roediger, 1982; Bergman, 1990).

A major part of butyrate is used as fuel for colonocytes (Bergman, 1990; Bloemen et al., 2009) as an important respiratory fuel and energy source for the colon epithelium (Bergman, 1990). After being absorbed and metabolized by colonic epithelium remaining amounts of butyrate are transported to the liver via the portal vein (Bergman, 1990). In the
liver, butyrate is converted to butyryl-CoA by butyryl-CoA synthetase (Demigné et al., 1986) and then is rapidly converted to acetyl-CoA, longer-chain fatty acids or ketone bodies (Bergman, 1990). After trace amounts enter the general blood circulation, nearly all peripheral tissues have the ability to metabolize butyrate where it is rapidly oxidized or used in lipogenesis (Bergman, 1990). However, the remainder is mostly oxidized by hepatocytes in order to prevent toxic systemic concentrations (Bloemen et al., 2010). When butyric acid was fed to broilers at 0.2, 0.4 or 0.6 percent, there was reduced pH of the upper GI tract and the villus height in the duodenum was increased thereby potentially increasing nutrient utilization through increased surface area (Panda et al., 2009).

Butyrate enters the TCA cycle in the distal colon indicating that butyrate is the primary respiratory fuel in this region (Cummings & Macfarlane, 1991). Colonocytes are the first cells that absorb VFA and depend largely on butyrate for their energy supply (Topping & Clifton, 2001; Binder, 2010). Butyrate has an additional role in the modulation of nucleic acid metabolism, particularly on regulation of gene expression and cell growth and butyrate’s capacity to modify cell growth in colonic epithelium (Cummings & Macfarlane, 1991).

**Pathogen Inhibition**

VFA have a bacteriostatic effect in the ceca (Van Der Wielen et al., 2000). The bacteriostatic action of VFAs is pH-dependent and exerted only when the acids are present in the undissociated lipophilic state where they increase as the pH of the environment decreases and approaches the specific dissociation constant (pKa) of each fatty acid (Barnes et al., 1979; Corrier et al., 1990; Dibner & Buttin, 2002). The decreased cecal pH, increased by the concentration of pH-dependent undissociated bacteriostatic VFAs produced by intestinal
anaerobic bacteria, are shown to decrease *Salmonella* cecal colonization in market-age broilers exposed to repeated *Salmonella* challenge. However, this low pH (5.0 to 6.0) is not bacteriostatic in itself, and that *Salmonella* growth is not directly inhibited by the low pH present in the cecal contents of the chicken, but rather in combination with undissociated VFAs (Corrier et al., 1990). Undissociated concentrations of VFAs are responsible for the in vivo reduction in number of *Enterobacteriacea* (including *Salmonella*) in the ceca of broilers (Van Der Wielen et al., 2000). It is suggested that the undissociated form of VFAs can diffuse freely across the bacterial membrane into the cell and inside the bacterial cell, where the acid dissociates, thereby reducing the internal pH which will cause internal damage (volatile fatty acid toxicity) (Cherrington et al., 1990; Bearson et al., 1997). Moreover, the lower pH values change the gut microbiota composition and prevents overgrowth by pH-sensitive pathogenic bacteria like *Enterobacteriaceae* and Clostridia in vivo (Cherrington et al., 1991). VFA produced by anaerobic bacteria inhibit *Salmonella* growth and colonization in poultry (Corrier et al., 1990). An effect of feeding a continuous flow mixture of bacteria is the increased VFA production and subsequent reduction of *Salmonella typhimurium* in cecal contents and (Corrier et al., 1995). This coincides with the fact that the concentration of VFA in the cecal contents of chicks increases with the establishment of indigenous anaerobic cecal bacteria (Nisbet et al., 1996). And high concentrations of volatile fatty acids are indicative that fermentations by obligate anaerobic bacteria are important (Van Der Wielen et al., 2000). Interestingly, cecal propionic acid concentrations have a direct relationship to reductions in *Salmonella* (Nisbet et al., 1993), most likely due to a pH effect in the lower GI tract.
Dietary Fat

Diets fed to broilers and turkeys generally include fat as a concentrated source of metabolizable energy (ME) (Sanz et al., 2000). Poultry fat offers relatively high ME and it can have a cost advantage because rendering plants are often located close to vertically integrated operations (Ouart et al., 1992). The average price of poultry fat in North Carolina in 2015 is relatively stable at $0.20 - $0.25 per pound (Feedstuffs, 2015). Comparatively, phosphorus is the third most expensive feed ingredient after energy and amino acids and was priced at $0.04/lb in 2013 (Woyengo & Nyachoti, 2011; Kelly et al., 2013). During least-cost formulation these ingredients are often the first to be limited due to their high costs. Fat inclusion in formulation is also dependent upon other energy source prices, such as corn, which have historically variable prices ranging from under $2 per bushel in 2005 to over $7 per bushel in 2012 (Figure 1.2). If corn prices are low then fat inclusion is limited and corn inclusion is increased. However, if corn prices are high and the price of fat has not changed, then fat in the diet will still be one of the first ingredients reduced. Any additives that increase energy utilization and that might be used as replacements for fat, such as DFM, have the potential to be important in least-cost formulation.

Addition of supplemental fat to the diet has been shown to increase body weights and improve feed efficiency (Jackson et al., 1982; Atteh et al., 1983). Increased body weight with increased supplemental fat plateaued at eight percent at 24 weeks of age in large white turkey toms resulting in improved feed efficiency at higher supplemental fat levels (Jensen et al., 1970). Additionally, increasing the fat level from three to six percent in broiler diets resulted in decreased FCR as a result of birds fed the six percent fat treatment consuming more feed
than the three percent fat treatment (Pesti, 2002).

Cullen et al., suggest that poultry fat has a higher energy value than the theoretical value indicating that the poultry fat influences the utilization of other feed components (1962). Differences in ME do not translate into differences of productive energy (net energy) (Pesti et al., 2002). The net energy is 75, 60, and 90 percent of the ME for carbohydrates, proteins and fats respectively (Scott, 1976; Pesti et al., 2002). Supporting this claim, one study showed approximately 94 percent absorbability of poultry fat in broiler diets (Cullen et al., 1962). Additionally in one turkey study, researchers saw a 32 percent extra caloric effect of added fat where the added fat improved actual ME over calculated ME (Jensen et al., 1970). The mechanism for the extra caloric effect is that supplemental fat increases transit time of ingesta in chickens which may improve digestibility of other dietary constituents and increase the utilization of dietary energy (Mateos et al., 1980).

<table>
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<tr>
<th>Table 1.1. Characteristics of ideal probiotics(^1)</th>
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<tr>
<td>Be of host origin</td>
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<tr>
<td>Non-pathogenic</td>
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<tr>
<td>Withstand processing and storage</td>
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<td>Resist gastric acid and bile</td>
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\(^1\)Adapated from Patterson and Burkholder, 2003 and Simmering and Blaut, 2001.
Figure 1.1 Diagram for the selection of probiotics in the poultry industry (Kabir 2009)
Figure 1.2 Prices Received for Corn by Month – United States (USDA-NASS)
REFERENCES


CHAPTER 2

THE EFFECT OF DIRECT-FED MICROBIALS ON PERFORMANCE AND ENERGY METABOLISM IN BROILER CHICKS AND TURKEY POULTS FED LOW FAT DIETS
ABSTRACT

Two battery experiments were conducted to determine if a DFM was effective in improving broiler chick and turkey poults performance and energy metabolism, and VFA production in corn, wheat, soy diets. For both experiments, no-supplemented, low fat diet (1%) with DFM treatments of 0, 1, 2 and 4 lb/ton were fed to chicks and poults in 48 cages to 21 days of age. Body weight (BW) and feed intake (FI) were measured weekly with FCR calculated. Cecal contents were collected for VFA analysis on d 23 from broilers and d 22 for turkeys. A cage of birds was considered the experimental unit. Data were analyzed using JMP 11. Means were considered significant at p<0.05. There was no improvement in BW gain (BWG) (cumulative: 0=924, 1=908, 2=899, 4=880 ± 18 g) or FI (cumulative: 0=1,394, 1=1,387, 2=1,305, 4=1,312 ± 47 g/bd) for treatments in the broiler experiment. However, cumulative FCR at 2 weeks was improved for birds fed 2 and 4 lb/ton of DFM compared to birds fed the non-DFM treatment (0=1.524, 1=1.513, 2=1.414, 4=1.413 ± 0.018). After week 2 there was no effect on FCR. When DFM was added at any level, there was increased total VFA production in the ceca on d 22 (0=99.98, 1=113.96, 2=110.57, 4=109.69 ± 10.01 mM, p=0.09). For the turkey experiment there was a cumulative effect on BWG where 2 lb DFM/ton resulted in higher BWG over birds-fed the non-DFM treatment, with 1 and 4 lb DFM/ton treatments resulting in intermediate responses (0=612, 1=635, 2=642, 4=637 ± 7.2 g). A FI trend (p=0.004) was observed from week 2 to the conclusion of the experiment where the 4 lb DFM/ton treatment resulted in higher FI compared to the non-DFM and 1 lb/ton treatments, with the 2 lb DFM/ton treatment resulting in an intermediate response.
(Cum, 0=814, 1=832, 2=857, 4=902 ± 17 g). The only FCR effect was observed during the second week of the experiment when the 1 lb DFM/ton treatment resulted in an improved FCR over the 4 lbs/ton treatment with the non-DFM and 2 lb DFM/ton treatment being intermediate (0=0.822, 1=0.749, 2=0.868, 4=0.942 ± 0.03). The opposite trend for VFA was observed in turkeys where higher total amount of cecal VFA concentrations were found in non-DFM treatment over all other treatments (0=164.57, 1=130.20, 2=142.41, 4=130.81 ± 14 mM, p=0.06). In conclusion, there is the potential for improvements in performance with the addition of DFM to the diets of broiler chicks and turkey poults. However, diets with no supplemental dietary fat may not provide the optimal situation or condition for DFM usage.
INTRODUCTION

Poultry encompasses 35 percent of the total world meat intake and intake of poultry products has increased by 104 percent from 1990 to 2012 (FAO, 2014). Broiler meat production in the United States in 2010 had a retail equivalent of $45 billion dollars (USDA, 2012). The United States is the world’s largest turkey producer, with over 230 million turkeys produced each year (USDA, NASS 2015). Outbreaks of common enteropathogenic bacteria, including Salmonella, Camplybacter and enterohaemorrhagic Escherichia coli, are related to contamination of poultry products and/or uncooked or raw poultry meat (WHO, 2014). Currently, antimicrobials such as antibiotics are being used to treat infections caused by bacteria (WHO, 2014). However, resistant bacteria are beginning to enter the food chain, and resistance to antimicrobials has become a threat to modern medicine and food manufacturers (WHO, 2014). DFM is an increasingly more common method used to treat enteric disease (AAFCO, 1999, FDA, 1995). Many of the known modes and mechanisms of actions of DFM support that they aid in gut and overall health of the host organism. DFM not only protect against enteric disease, they have other potential benefits, such as: improved performance, increased nutrient utilization and VFA production. Some even suggest that an improvement in AMEₙ (indicator of energy utilization) can be measured when DFM is supplemented in the diet (Sibbald, 1982).

Two experiments were conducted to determine if the inclusion of a DFM in poultry diets improved performance and increased energy metabolism (increased by AMEₙ or VFA production). A range of DFM inclusion was used to attempt to capture the appropriate DFM
dosage at which both performance and energy parameters are maximized. The first experiment was performed using broiler chicks and the second experiment used turkey poults.

MATERIALS AND METHODS

All bird handling procedures were approved by the institutional animal care and use committee.

Experiment 1 - Broiler Chicks and Husbandry.

Ross 708 male broiler chicks (288 chicks, Aviagen Group, Huntsville, AL) were reared to 21 days of age. Birds were placed in 48 Petersime battery cages in one room (Turkey Educational Unit, NCSU Prestage Department of Poultry Science) with 6 birds per cage. All birds were raised using typical management practices. There were 6 batteries in the room, each with 8 cages spread over 5 decks. Each battery was considered a block. Each chick was tagged and placed in cages, and cages of chicks were assigned to one of four dietary treatments. The four dietary treatments were assigned randomly throughout the 48 cages (12 replicates per treatment).

Experiment 2 - Turkey Poults and Husbandry.

Nicholas 700 male turkey poults (288 poults, Aviagen Turkeys, Lewisburg, WV) were reared to 21 days of age. Birds were placed in 48 Petersime battery cages in one room (Turkey Educational Unit, NCSU Prestage Department of Poultry Science) with 6 birds per cage. All birds were raised to typical management practices. There were 6 batteries in the room; each with 8 cages spread over 5 decks. Each battery was considered a block. Each poul was tagged and placed in cages, and cages of poults were assigned to one of four
dietary treatments. The four dietary treatments were assigned randomly throughout the 48 cages (12 replicates per treatment).

For both experiments, birds were individually weighed at placement, 7, 14, and 21 days of age. Feed consumption was determined by pen. Mortality (and weight) were recorded daily and used to adjust feed conversion. Birds consumed feed and water ad libitum.

**Dietary Treatments**

All feed was manufactured at the NCSU Feed Mill Education Unit and was formulated to standard broiler and turkey requirements, fulfilling all nutrient requirements and amino acid balances based on breeder recommendations (Table 2.1). Birds were fed a mash starter diet for the duration of the experiment. One basal ration containing all feed ingredients with the exception of DFM was blended in a counterpoise mixer (Model TRDB126060, Hayes & Stolz, Fort Worth, TX). The basal was split into 4 sub-groups of 300 lb for broiler diets, or 350 lb for turkey diets and the DFM was added at the expense of corn and mixed in a 500 lb double ribbon mixer (Model SRM 304, Scott Equipment Co., New Prague, MN) for an additional two minutes. All of the feed was bagged and then transported to the Turkey Educational Unit.

For both experiments the 4 treatments were supplemented as follows: PrimaLac® (Star Labs, Clarksdale MO) DFM at, 0 lb DFM/ton, 1 lb DFM/ton, 2 lb DFM/ton, and 4 lb DFM/ton. All feeds were sampled, coded, and analyzed blind by an independent lab (Star Labs/Forage Research, Inc., Clearwater, FL) for the presence or absence of PrimaLac® bacteria. PrimaLac® is a direct fed microbial cocktail that contains *Lactobacillus acidophilus*, *Lactobacillus casei* subsp. *rhamnosus*, *Bifidobacterium bifidium*, and *Enterococcus faecium*. 


PrimaLac® contains a minimum of $1.0 \times 10^8$ CFU of *Lactobacillus* per gram.

**Sample Collection.**

On day 21 and day 17 for broiler and turkey experiments respectively, excreta from all cages was collected and frozen at -20°C until analysis for apparent metabolizable energy. On day 22 for both experiments, two or three birds per cage were sacrificed for sampling. Using aseptic techniques the ileum was removed by cutting at the Meckel’s diverticulum at the ileo-cecal junction and the ceca were removed by cutting both sections at the ileo-cecal junction. 10 to 15 grams of ileal contents per cage and 8 to 10 grams 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 volatile fatty acid analysis by weighing out one gram of sample, adding 2.0 mL diH$_2$O, vortexing the sample, and spinning for five minutes at 2500 rpm. 1.0-2.0 mL of supernatant was pulled off into a micro-centrifuge tube and spun at 15,000 rpm (21,000 rcf) for 10 minutes. 1.0 mL (or all available) supernatant was pulled off into another micro-centrifuge tube and 200 μL 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 spun at 15,000 rpm for 10 minutes to aid in cleaning the sample. Samples were analyzed for volatile fatty acids by gas liquid chromatography (Varian CP 3380 with NUKOL Fused Silica Capillary Colum 30 m x 0.25mm x 0.25 μm film thickness).

**Microbiological Enumeration and Identification.**

Ileal and cecal tissues were collected on day 19 for broilers and day 18 for turkeys.
Serial dilutions of tissue samples were prepared and plated on 3 selected media plates for enumeration and isolation. Isolates were sent to an independent lab for identification (Appendix I).

**Chemical Analysis.**

On day 21 for the broiler experiment and day 17 for the turkey experiment, excreta was collected from pans under cages and stored in -20°C freezer until further processing. Approximately 200 grams of representative excreta sample was dried for approximately 72 hours 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 grams of representative feed sample was dried for 24 hours 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, 2006). Insoluble ash for Celite recovery was performed with modification of the method described by Vogtmann et al. (1975). 2 grams 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 D.I. water to wash residue free of acid and allowed to drain. Using clean, fired pre-weighed crucibles, filter paper was folded and placed in muffle furnace (BF1700 Series, Thermo Scientific Lindberg/Blue M, Asheville, NC). Samples were ashed at 600°C for approximately 12-14 hours (AOAC, 1995). The muffle furnace was turned off and allowed to cool. Samples were pulled out and weighed to obtain
ash weight. Excreta samples were prepared for gross energy analysis by weighing 1.000-1.0050 g of dried sample, transferring it to clean large crucible, adding two drops of diH₂O and working into sample, ensuring that no clumps of water remained. Samples were re-weighed and poured into a clean pellet press to form pellets. Tarring out a clean calorimeter crucible, the sample was placed into the crucible and stored in the desiccator for approximately 12-15 hours. A plain jacket calorimeter (1341 Parr Instrument Co., Moline, Illinois) was used to calculate gross energy of dried excreta and feed samples. The apparent metabolizable energy nitrogen corrected (AME_n) was calculated according to Lammer et al. (2008) using the following equations:

\[
N_{\text{retained}} = N_{\text{feed}} - \left( \frac{N_{\text{excreta}} * A_{\text{I feed}}}{A_{\text{I excreta}}} \right)
\]

and

\[
\text{AME}_n = \text{GE}_{\text{feed}} - \left[ \left( \frac{\text{GE}_{\text{excreta}} * A_{\text{I feed}}}{A_{\text{I excreta}}} \right) \right] - (8.22 * N_{\text{retained}})
\]

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; A_{I feed} and A_{I excreta} were the concentration of Celite recovered as acid insoluble ash in diet and excreta respectively; 8.22 (Kcal/g) is the energy value of uric acid; and N_{retained} (g/kg) is the nitrogen retained by the bird per kilogram of diet consumed, and N_{feed} and N_{excreta} (%) were the nitrogen content of the diet and excreta respectively. All values in this calculation were expressed as grams per kilogram of DM.

**Statistical Analysis.**

Data were analyzed using JMP 11. Experiments were completely randomized block
designs. Each cage of birds was considered the experimental unit. Both the broiler and the turkey experiment performance and AME\textsubscript{n} data were analyzed by one-way ANOVA and means were separated using LSMeans. VFA data were analyzed using one-way ANOVA with means separated by LSMeans Contrasts. Means were considered significant at less than or equal to 0.05.

**RESULTS**

*Broiler Experiment – Growth Performance*

The effects of DFM on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) for broiler chicks are presented in Table 2.3. There was low mortality observed with no differences between treatments. There was no improvement observed in BWG for experiment 1. There were no differences in FI or any improvements in FCR for broiler experiment 1 except for during the 7 to 14 day period, where the cumulative week 2 FCR was affected. Here, there was decreased FI for birds fed 2 and 4 lb/ton treatments over 0 and 1 lb/ton treatments (0=631\textsuperscript{a}, 1=637\textsuperscript{a}, 2=573\textsuperscript{b}, 4=577\textsuperscript{b} ± 18 g), which therefore lead to an improvement in FCR of seven points for birds fed the 2 and 4 lb/ton treatments (0=1.255\textsuperscript{ab}, 1=1.273\textsuperscript{b}, 2=1.195\textsuperscript{a}, 4=1.190\textsuperscript{a} ± 0.02) over birds fed the 0 and 1 lb/ton treatments for these periods.

*Turkey Experiment – Growth Performance*

The effects of DFM on BWG, FI, and FCR for turkey poults are presented in Table 2.4. There was low mortality seen with no differences between treatments. There were no weekly differences observed for BWG by treatments throughout the experiment. However, for the second week cumulative BWG, there was an improvement in BWG for all treatments
supplemented with DFM over that of the control (0=300\textsuperscript{b}, 1=315\textsuperscript{a}, 2=318\textsuperscript{a}, 4=317\textsuperscript{ab} ± 7.2 g). A similar effect was observed for the final cumulative BWG, where birds fed the 2 lb/ton treatment had increased BWG over birds fed the 0 lb/ton treatment, with intermediate values for birds fed 1 and 4 lb/ton treatments (0=612\textsuperscript{b}, 1=635\textsuperscript{ab}, 2=642\textsuperscript{a}, 4=637\textsuperscript{ab} ± 7.2 g).

There was an effect in cumulative FI observed throughout the experiment, where birds fed the 4 lb/ton treatment consumed more feed than birds fed the 0 lb/ton treatment, which was an intermediate amount compared to birds fed the 2 lb/ton treatment (0=814\textsuperscript{b}, 1=832\textsuperscript{b}, 2=857\textsuperscript{ab}, 4=902\textsuperscript{a} ± 17 g). Birds fed the 1 lb/ton treatment were either not different from the 0 lb/ton treatment (d 0 – 14 and d 0 – 21) or intermediate between the 0 and 4 lb/ton treatments (d 0 – 7). The latter effect was also observed during the second week of the experiment (0=152\textsuperscript{b}, 1=151\textsuperscript{b}, 2=178\textsuperscript{ab}, 4=194\textsuperscript{a} ± 9.4 g), but not during the third week.

During the first or last weeks of the experiment or cumulatively, there were no differences observed in FCR. However, during the second week, there was an improvement in FCR for birds fed the 1 lb/ton treatment over birds fed the 4 lb/ton treatment, with intermediate values for birds fed the 0 and 2 lb/ton treatments (0=0.822\textsuperscript{ab}, 1=0.749\textsuperscript{a}, 2=0.868\textsuperscript{ab}, 4=0.942\textsuperscript{b} ± 0.04). This effect was observed for the second week cumulative FCR in which birds fed the 0 and 1 lb/ton treatments had improved FCR over 2 and 4 lb/ton treatments (0=0.804\textsuperscript{a}, 1=0.804\textsuperscript{a}, 2=0.882\textsuperscript{ab}, 4=0.921\textsuperscript{b} ± 0.03).

*Apparent Metabolizable Energy*

There was no consistent response to DFM inclusion on AME\textsubscript{n} for the broiler experiment with variable outputs (Table 2.5). The 0 lb/ton treatment resulted in AME\textsubscript{n} similar to AME\textsubscript{n} of the 2 lb/ton treatment, but different from AME\textsubscript{n} of the 1 and 4 lb/ton
treatments (0 = 3026, 1 = 2901, 2 = 3058, 4 = 2959 ± 14.81 kcal/kg). However, for the turkey experiment an improvement was observed for AME\textsubscript{n} for all treatments supplemented with DFM over that of the control (Table 2.6). Bird fed the 2 lb/ton treatment had the most improved AME\textsubscript{n}, which was different from birds fed 1 and 3 lb/ton treatments. The 2 lb/ton treatment resulted in an uplift in AME\textsubscript{n} of 149 kcal/kg over that of the control treatment.

**Volatile Fatty Acids**

For the broiler experiment, the presence of acetate, propionate, butyrate and valerate in the ceca on day 21 was detected (Table 2.5). Among these, acetate was present in the highest molar percentage, followed by butyrate, propionate, and then valerate. No significant differences were observed in any individual VFA molar percentage between dietary treatments. There was a trend (p = 0.06) in which there was increased total VFA cecal concentrations at any DFM inclusion level over that of the control (0=94.65, 1=124.54, 2=110.57, 4=102.68 ± 7.98 mM). All samples tested from the ileum were below detectable levels when analyzed via gas liquid chromatography.

For the turkey experiment, the presence of only acetate, butyrate and propionate was detected in the ceca on day 17 (Table 2.6). Acetate was again observed in the highest percentage, followed by butyrate and propionate. There were no significant differences observed in any individual VFA molar percentage between dietary treatments. There was a trend (p=0.07) where all treatments supplemented with DFM had lower total cecal VFA concentrations than the control treatment (0=164.57, 1=130.20, 2=142.41, 4=130.81 ± 14 mM). All samples from the ileum were below detectable levels when analyzed via gas liquid chromatography.
Microbiological Identification

By analysis, the concentration of probiotic product administration in the feed was found to be in agreement with the manufacturer’s recommended inclusion levels.

DISCUSSION

The objective of this study was to determine if dietary DFM inclusion in low fat broiler and turkey diets results in improved performance and increased energy digestibility. As with many DFM studies, it is always important to match DFM administration level with the maximum efficiency in these parameters, which may be different for each situation (Mountzouris et al. 2010). In these experiments, the highest inclusion of DFM did not produce the best results. This is supported by other researchers, who suggest that there is a dose dependent response to DFM inclusion, where the highest doses produce results similar to the control group, whereas intermediate inclusions result in the most improved performance (Jin et al., 1998a; Flint & Garner, 2009). However, for the protection against pathogens, it is suggested that large to massive doses of DFM will improve results (Vahjen et al., 2002). For the broiler experiment, the best performance observed was from the 2 lb/ton DFM inclusion rate. For the turkey experiment, there were variable results for the inclusion level; however, all DFM inclusion levels resulted in improved performance compared to the control.

Examining AMEₙ gives an overall assessment of energy utilization in the bird. These two experiments resulted in variable responses of DFM on AMEₙ. In broilers, the effect on AMEₙ was variable; whereas in the turkey experiment, DFM inclusion resulted in a consistent increase in AMEₙ. The broiler results for AMEₙ conflict with other research that
suggests that birds supplemented with DFM had in improved AME\textsubscript{n} (Mountzouris et al., 2010, Monhan et al., 1996, Schneitz et al., 1998). It could be that the effect from the addition of DFM is not impacting AME\textsubscript{n}, but rather impacting other mechanisms of energy metabolism in the body, such as altering the gut morphology to allow for better nutrient absorption (Chichlowski et al., 2007b). In addition, these experiments were conducted in battery cages with near optimal conditions. It has been shown that the effect of DFM is better characterized when birds are reared in sub-optimal conditions (Torres-Rodriguez et al., 2007).

All major VFA were observed through measurement in the ceca of chickens and turkeys. Therefore, one can conclude that the VFA are an important contributor to the energy balance of the birds, although specific mechanisms have not been elucidated. In the broiler experiment, an increased total cecal VFA production was observed in all DFM supplemented treatments over the control. This is supported by others who suggest that supplementation with DFM in the lower GI tract leads to increased amounts of VFA produced (Jin et al., 1998b). Valerate (C5), which is produced from the condensation of acetate and propionate, produce small amounts of glucose (Bergman, 1990). While the total concentrations were greater with DFM supplementation, proportionally the amount of acetate and butyrate were generally lower in DFM supplemented treatments over the control. In addition, propionate was higher in DFM supplemented treatment birds over that of the control fed birds. It is possible that birds were utilizing more acetate and butyrate and less propionate than the control treatment. It could be suggested that more energy was needed locally through the use of butyrate and acetate in colonocytes for the increased absorption of nutrients (Bergman,
The opposite trends were observed in turkeys, with the control birds having increased total concentrations over birds fed the supplemented treatments, and higher amounts of acetate and butyrate in DFM supplemented treatments of the control. It may be that the birds fed the control diet utilized the energy more than the birds fed the DFM supplemented treatments in order to gain nutrients for BWG, compared to birds fed supplemented treatments. It is important to note that with VFA analysis, sampling variation is large, which may lead to pronounced variation in results observed in three ways. First, during sampling, it is difficult to isolate the gut digesta as it passes through the lower GI tract. Second, each bird is unique and will utilize and process each VFA differently, making it difficult to compare bird-to-bird samples. Third, to ensure that there was enough sample material, birds were grouped by pen, and therefore data were not analyzed by bird.

In conclusion, the effect of DFM inclusion in non-fat supplemented turkey poult diets resulted in improved poult performance, which is associated with increased dietary AMEn and lowered total cecal VFA. In broilers, it appears that for birds fed non-fat supplemented diets, the response to dietary DFM is more variable and inconclusive with respect to improved performance and increased energy utilization.
In dietary treatment that did not receive PrimaLac® (0 lb/ton), corn was used as a replacement.

Vitamin premix provided the following per kg of diet: 1324 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.

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.

Selenium premix provided 0.2 mg/kg

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Broiler % of total diet</th>
<th>Turkey % of total diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>36.6</td>
<td>33</td>
</tr>
<tr>
<td>Soybean Meal 48</td>
<td>28</td>
<td>32.5</td>
</tr>
<tr>
<td>Distiller’s Dried Grain</td>
<td>7.5</td>
<td>5</td>
</tr>
<tr>
<td>Wheat</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Poultry Meal</td>
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</tr>
<tr>
<td>Poultry Fat</td>
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<tr>
<td>Calcium Carbonate</td>
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<td>Dicalcium Phosphate</td>
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<td>Salt (NaCl)</td>
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</tr>
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<tr>
<td>Choline Chloride</td>
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<td>Sodium Bicarbonate</td>
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<td>Celite</td>
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<td>2.0</td>
</tr>
<tr>
<td>Vitamin Premix 2</td>
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<td>0.10</td>
</tr>
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</table>

### Nutrient Composition

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Broiler kcal/kg</th>
<th>Turkey kcal/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME Poultry</td>
<td>1277</td>
<td>1269</td>
</tr>
<tr>
<td>Crude Protein, %</td>
<td>22.34</td>
<td>29.55</td>
</tr>
<tr>
<td>Crude Fat, %</td>
<td>2.78</td>
<td>4.58</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.02</td>
<td>1.46</td>
</tr>
<tr>
<td>Available Phosphorus, %</td>
<td>0.48</td>
<td>0.72</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Total Lysine, %</td>
<td>1.38</td>
<td>1.86</td>
</tr>
<tr>
<td>Total Met + Cys, %</td>
<td>0.94</td>
<td>1.28</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.86</td>
<td>1.15</td>
</tr>
<tr>
<td>Choline, mg/lb</td>
<td>889.04</td>
<td>2399</td>
</tr>
</tbody>
</table>

### Nutrient Analysis

| Crude Protein, %                          | 22.78           | 29.10          |

---

1In dietary treatment that did not receive PrimaLac® (0 lb/ton), corn was used as a replacement.
2Vitamin 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.
3Mineral 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.
4Selenium premix provided 0.2 mg/kg
Table 2.2. Effect of dietary DFM inclusion on performance of broiler chicks from placement to 21 days of age for experiment 1.\(^1\)

<table>
<thead>
<tr>
<th>DFM(^2)</th>
<th>Inclusion Level</th>
<th>Source of Variation</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>0-7(^*)</td>
<td>136</td>
<td>124</td>
<td>121</td>
<td>124</td>
</tr>
<tr>
<td>7-14</td>
<td>310</td>
<td>309</td>
<td>301</td>
<td>302</td>
</tr>
<tr>
<td>0-14</td>
<td>447</td>
<td>433</td>
<td>422</td>
<td>427</td>
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<tr>
<td>14-21</td>
<td>478</td>
<td>475</td>
<td>477</td>
<td>454</td>
</tr>
<tr>
<td>0-21</td>
<td>924</td>
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<td>899</td>
<td>880</td>
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<tr>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>BWG (g)</td>
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<td></td>
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</tr>
<tr>
<td>0-7</td>
<td>149</td>
<td>145</td>
<td>136</td>
<td>138</td>
</tr>
<tr>
<td>7-14</td>
<td>483(^{ab})</td>
<td>493(^{a})</td>
<td>437(^{b})</td>
<td>439(^{b})</td>
</tr>
<tr>
<td>0-14</td>
<td>631(^{a})</td>
<td>637(^{a})</td>
<td>573(^{b})</td>
<td>577(^{b})</td>
</tr>
<tr>
<td>14-21</td>
<td>718</td>
<td>704</td>
<td>708</td>
<td>691</td>
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<tr>
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<td>1394</td>
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<td>1312</td>
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<tr>
<td></td>
<td>FI (g)</td>
<td></td>
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</tr>
<tr>
<td>0-7</td>
<td>0.800</td>
<td>0.837</td>
<td>0.797</td>
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</tr>
<tr>
<td>7-14</td>
<td>1.524(^{b})</td>
<td>1.513(^{b})</td>
<td>1.414(^{a})</td>
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<tr>
<td>0-14</td>
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<td>1.273(^{b})</td>
<td>1.195(^{a})</td>
<td>1.190(^{a})</td>
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<td>14-21</td>
<td>1.407</td>
<td>1.385</td>
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<tr>
<td>0-21</td>
<td>1.326</td>
<td>1.324</td>
<td>1.304</td>
<td>1.300</td>
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</tbody>
</table>

\(^1\)Values are means of 12 replicate pens of 6 male broilers per pen.

\(^2\)DFM inclusion rates: 0, 1, 2, and 4 lb/ton, respectively.

\(^*\)Average hatchling body weight, across all treatments, was 45.0 g ± 0.02 g.

\(^+\)Values calculated using body weight gain.

\(^{a,b}\)Means within a row with different superscripts are significantly different \(p<0.05\).
Table 2.3. Effect of dietary DFM inclusion on performance of turkey poults from placement to 21 days of age for turkey experiment 1.1

<table>
<thead>
<tr>
<th>DFM²</th>
<th>Source of Variation</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inclusion Level</td>
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</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0-7*</td>
<td>BWG (g)</td>
<td>102</td>
<td>108</td>
</tr>
<tr>
<td>7-14</td>
<td>108</td>
<td>206</td>
<td>210</td>
</tr>
<tr>
<td>0-14</td>
<td>300⁵</td>
<td>315¹</td>
<td>318⁴</td>
</tr>
<tr>
<td>14-21</td>
<td>312⁵</td>
<td>321⁴</td>
<td>328⁴</td>
</tr>
<tr>
<td>0-21</td>
<td>612⁵</td>
<td>635⁴</td>
<td>642⁴</td>
</tr>
<tr>
<td></td>
<td>FI (g)</td>
<td>144</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>0-7</td>
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<td>151</td>
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<td>7-14</td>
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<td>330</td>
</tr>
<tr>
<td></td>
<td>0-14</td>
<td>518</td>
<td>528</td>
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<tr>
<td></td>
<td>14-21</td>
<td>814</td>
<td>832</td>
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<tr>
<td></td>
<td>FCR+ (g:g)</td>
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<td>1.411</td>
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<td>0-7</td>
<td>0.822</td>
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<td>7-14</td>
<td>0.804</td>
<td>0.804</td>
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<td></td>
<td>0-14</td>
<td>1.637</td>
<td>1.706</td>
</tr>
<tr>
<td></td>
<td>14-21</td>
<td>1.257</td>
<td>1.230</td>
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</table>

1Values are means of 12 replicate pens of 6 male turkeys per pen.
2DFM inclusion rates: 0, 1, 2, and 4 lb/ton, respectively.
*Average hatchling average body weight, across all treatments, was 63.4 g ± 0.57 g.
Values calculated using body weight gain.
Means within a row with different superscripts are significantly different (p<0.05).
Table 2.4. Effect of DFM inclusion on apparent metabolizable energy with nitrogen correction in broiler chicks at 21 days of age for experiment 1 and turkey poults at 17 days of age for experiment 1.\(^1\)

<table>
<thead>
<tr>
<th>DFM Inclusion Level(^2)</th>
<th>Broiler</th>
<th>Turkey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AME(_n) kcal/kg</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3026(^a)</td>
<td>3002(^c)</td>
</tr>
<tr>
<td>1</td>
<td>2901(^c)</td>
<td>3063(^b)</td>
</tr>
<tr>
<td>2</td>
<td>3058(^a)</td>
<td>3151(^a)</td>
</tr>
<tr>
<td>4</td>
<td>2959(^b)</td>
<td>3098(^b)</td>
</tr>
</tbody>
</table>

Source of Variation | p-value |
---------------------|---------|
SEM                  | 14.81   |
P-Value              | <0.0001 |

\(^1\)Values are means of 12 replicate pens of 6 male broilers or turkeys per pen.

\(^2\)DFM inclusion rates: 0, 1, 2, and 4 lb/ton, respectively.

\(^a,b,c,d\) Means within a column with different superscripts are significantly different (p<0.05).

\(^\)Values are means of 12 replicate pens of 6 male broilers or turkeys per pen.
Table 2.5. Effect of dietary DFM inclusion on cecal volatile fatty acid concentrations of broiler chicks at 21 days of age for experiment 1.1

<table>
<thead>
<tr>
<th>DFM Inclusion Level2</th>
<th>Molar Percent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
<td>Buytrate</td>
</tr>
<tr>
<td>0</td>
<td>76.34</td>
<td>21.39</td>
</tr>
<tr>
<td>1</td>
<td>73.95</td>
<td>23.56</td>
</tr>
<tr>
<td>2</td>
<td>74.27</td>
<td>23.26</td>
</tr>
<tr>
<td>4</td>
<td>71.79</td>
<td>26.12</td>
</tr>
</tbody>
</table>

Source of Variation

<table>
<thead>
<tr>
<th>SEM</th>
<th>1.83</th>
<th>1.87</th>
<th>0.707</th>
<th>0.172</th>
<th>7.98</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Value</td>
<td>0.1666</td>
<td>0.1454</td>
<td>0.2149</td>
<td>0.9917</td>
<td>0.0644</td>
</tr>
</tbody>
</table>

1Values are means based on representative cecal content sample of 2-3 birds per cage.
2DFM inclusion rates: 0, 1, 2, and 4 lb/ton, respectively.
Table 2.6. Effect of dietary DFM inclusion on cecal volatile fatty acid concentrations turkey poults at 17 days of age for experiment 1.

<table>
<thead>
<tr>
<th>DFM Inclusion Level</th>
<th>Molar Percent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
<td>Butyrate</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>mM</td>
</tr>
<tr>
<td>0</td>
<td>53.70</td>
<td>27.75</td>
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<tr>
<td>1</td>
<td>56.51</td>
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<td>30.91</td>
</tr>
<tr>
<td>4</td>
<td>45.34</td>
<td>34.82</td>
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</tbody>
</table>

Source of Variation: SEM 3.34 3.46 6.69 14.0

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>p-values</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEM</td>
<td>0.442</td>
<td>0.1484</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.8762</td>
<td>0.066</td>
</tr>
</tbody>
</table>

1Values are means based on representative cecal content sample of 2-3 birds per cage.
2DFM inclusion rates: 0, 1, 2, and 4 lb/ton, respectively.
REFERENCEs


AOAC. 1995. Ether Extract in Animal Feed, AOAC Official method 920.39

AOAC. 2006. Combustion Analysis (LECO), AOAC Official Method 990.03.


62


Jin et al., 1998,


CHAPTER 3

THE EFFECT OF DIRECT-FED MICROBIALS AND FAT INCLUSION ON PERFROMANCE AND ENERGY METABOLISM IN BROILER CHICKS AND TURKEY POULTS
ABSTRACT

Two battery experiments were conducted to determine the effects of dietary DFM and fat inclusion on broiler chick and turkey poult performance and energy metabolism in corn, wheat, soy diets. For both experiments, a 2 x 2 factorial experimental design was used with supplemental fat (1% or 6%) and DFM inclusion (0 or 2 lbs/ton). Treatments were assigned to 72 cages and fed to chicks to 20 days and pouls to 21 days. Body weight (BW) and feed intake (FI) for FCR were measured weekly. Cecal contents were collected for VFA analysis on d 21 for broilers and d 22 for turkeys. Data were analyzed using JMP 11. A cage of birds was considered the experimental unit. Means were considered significant at p<0.05. In both experiments, there was an interaction of treatments. Fat supplementation resulted in increased BWG. However, in low fat diets, DFM inclusion resulted in increased BWG compared to the non-DFM supplemented low fat diet treatment. This effect was observed for the broiler experiment for BWG where there was an increased BWG at the low fat level when DFM was supplemented, but no effect of DFM supplementation at the high fat level (cumulative BWG: High no DFM=764a, High with DFM=756a, Low no DFM=629b, Low with DFM=749a ± 1.7 g). The same effect was observed with FI (cumulative FI: High no DFM=1,054a, High with DFM=1,071a, Low no DFM=948b, Low with DFM=1,071a ± 2.0 g/bd). Cumulative FCR was improved with both high fat treatments and in Low fat with DFM treatment compared to the Low fat with no DFM (High no DFM =1.346b, High with DFM=1.373b, Low no DFM=1.480a, Low with DFM=1.398b ±16). For the turkey experiment, there was improved cumulative BWG for Low fat with DFM compared to the low fat with no DFM inclusion. The high fat diet without DFM treatment resulted in
improved BWG compared to low fat treatments, with High fat with DFM having an intermediate response (High no DFM =718\textsuperscript{a}, High with DFM=694\textsuperscript{ab}, Low no DFM =667\textsuperscript{b}, Low with DFM=701\textsuperscript{a}± 0.3 g). There were no differences observed in feed intake. There was an effect in FCR where high fat treatments resulted in improved FCR over the low fat treatments. In the low fat treatments, there was an improvement in FCR when DFM was supplemented (High no DFM =1.169\textsuperscript{a}, High with DFM=1.184\textsuperscript{a}, Low no DFM =1.268\textsuperscript{c}, Low with DFM=1.231\textsuperscript{b} ± 0.009). The same trend for broilers and turkeys were observed for AME\textsubscript{n} where increased AME\textsubscript{n} was observed for the high fat diets while DFM inclusion resulted in an uplift in AME\textsubscript{n} at the low fat level (Broilers: High no DFM =3407\textsuperscript{a}, High with DFM=3351\textsuperscript{b}, Low no DFM =3235\textsuperscript{d}, Low with DFM=3266\textsuperscript{c} ± 12 kcal/kg, p<0.0001) (Turkeys: High no DFM =3291\textsuperscript{a}, High with DFM=3204\textsuperscript{b} Low no DFM=2885\textsuperscript{d}, Low with DFM=3111\textsuperscript{c} ± 18 kcal/kg, p<0.0001). There was a trend (p=0.07) for higher concentration of total VFA in the ceca of the birds fed the low fat diets compared to birds fed the high fat diets at d 21 (High no DFM=124.52, High with DFM= 124.62, Low no DFM=134.16 mM Low with DFM=133.10 ± 6.05 mM). A similar trend (p=0.12) was observed in the turkey experiment where there was a higher concentration of total VFA in ceca of low fat treatments over high fat treatments, although the trend was not significant (High no DFM=160.80, High with DFM=151.05, Low no DFM=170.74, Low with DFM=169.29 ± 6.05 mM).

In conclusion, 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\textsubscript{n}. 
INTRODUCTION

In 2014, broiler meat production was valued at $32.7 billion and turkey meat production was valued at $5.30 billion. (USDA NASS, 2015). Feed is the single largest production cost in animal production and feed costs account for up to 75 percent of poultry production costs (Sibbald, 1982). Savings in feed costs has become an important strategy to many producers in today’s marketplace, especially in the face of increasing ingredient costs.

Fat is a primary source of energy in the diet, and nutritionists have limited other options for the addition of energy (Sanz et al., 2000). The energy value of fats is 2.25 times more than that of carbohydrates. (USDA NRCS, 2012). The current average price for corn is $5.23/bushel (Feedstuffs, 2015). However, it has ranged from $3.41/bushel to $7.14/bushel over the last five years (Figure 1.2). Even when grain prices are low, fat must be added to the diet in greater amounts in order to meet the energy requirements of the animal. It is even more critical to try and reduce the amount of fat in the diet when grain prices are high, as they were in 2012. Cost of fat in the diet is very high at around $0.22 per pound for poultry grease (Feedstuffs, 2015). If the amount of fat (or total ME) that is added to the diet can be reduced without deleterious effects on performance, there is the potential to reduce total feed costs.

As observed in the previous experiments, there were potential increases in growth parameters when DFM was supplemented in the diets of both chickens and turkeys. There are numerous studies which have reported increased AMEn due to DFM supplementation (Nurmi & Rantalla, 1973; Patterson & Burkholder, 2003; Kabir, 2009; Chichlowski et al., 2007a; Lee et al., 2010). Therefore, there may be a potential that DFM supplementation can
replace fat in the diet.

Two experiments were conducted to test the effect of DFM inclusion at varying fat levels on performance and energy metabolism (through AMEn or VFA production). Based on the previous experiments, it was decided to use 0 and 2 lb/ton of DFM inclusion and fat supplemented at 1% and 6%, for a 2 x 2 factorial design of treatments. The first experiment was conducted using male broiler chicks and the second experiment was conducted using male turkey poults.

**MATERIALS AND METHODS**

All bird handling procedures were approved by the institutional animal care and use committee.

*Experiment 2 – Broiler Chicks and Husbandry.*

Ross 708 male broiler chicks (504 chicks, Aviagen Group, Huntsville, AL) were reared to 20 days of age. Birds were placed in 72 Petersime battery cages in one room (Turkey Educational Unit, NCSU Prestage Department of Poultry Science) with 7 birds per cage. All birds were raised using typical management practices. There were 6 batteries in the room; each with 12 cages spread over 6 decks. Each battery was considered a block. Each chick was tagged and placed in cages, and cages of chicks were assigned to one of four dietary treatments. The four dietary treatments were assigned randomly throughout the 72 cages (18 replicates per treatment).

*Experiment 2 – Turkey Poults and Husbandry.*

Nicholas 700 male turkey poults (504, Aviagen Turkeys, Lewisburg WV) were reared to 21 days of age. Birds were placed in 72 Petersime battery cages in one room (Turkey
Educational Unit, NCSU Prestage Department of Poultry Science) with 7 birds per cage. All birds were raised using typical management practices. There were 6 batteries in the room; each with 12 cages spread over 6 decks. Each battery was considered a block. Each poult was tagged and placed in cages, and cages of poulters were assigned to one of four dietary treatments. The four dietary treatments were assigned randomly throughout the 72 cages (18 replicates per treatment).

For both experiments, birds were individually weighed at placement, 7, 14, and 20/21 days of age. Feed consumption was determined by pen. Mortality (and weight) were recorded daily and used to adjust feed conversion. Birds consumed feed and water ad libitum.

**Dietary Treatments**

All feed was manufactured at the NCSU Feed Mill Education Unit and was formulated to standard broiler and turkey requirements, fulfilling all nutrient requirements and amino acid balances based on breeder recommendations (Table 3.1). Birds were fed a mash starter diet for the duration of the experiment. One basal ration containing all feed ingredients with the exception of DFM was blended in a counterpoise mixer (Model TRDB126060, Hayes & Stolz, Fort Worth, TX). The basal was split into 4 sub-groups of 600 lb for the broiler diets and 500 lb for the turkey diets. The DFM was then added at the expense of corn, supplemental fat was added on top of the basal, and the feed was mixed in a 500 lb double ribbon mixer (Model SRM 304, Scott Equipment Co., New Prague, MN) for an additional two minutes. All of the feed was bagged and then transported to the Turkey Educational Unit.

The four dietary treatments were designed as a 2 x 2 factorial with DFM and fat, and
were supplemented as follows: low supplemental fat (1%) without DFM, low supplemental fat (1%) with DFM (2 lb/ton), high supplemental fat (6%) without DFM, high supplemental fat (6%) with DFM (2 lb/ton). The fat source used was poultry fat and DFM used was PrimaLac® (Star Labs, Clarksdale, MO). All feeds were sampled, coded, and analyzed blind by an independent lab (Star Labs/Forage Research, Inc., Clearwater, FL) for the presence or absence of PrimaLac®. PrimaLac® is a direct fed microbial cocktail that contains \textit{Lactobacillus acidophilus}, \textit{Lactobacillus casei} subsp. \textit{rhamnosus}, \textit{Bifidobacterium bifidium}, and \textit{Enterococcus faecium}. PrimaLac® contains a minimum of $1.0 \times 10^8$ CFU of \textit{Lactobacillus} per gram.

\textbf{Sample Collection.}

On day 20 and day 16 for the broiler and turkey experiments respectively, excreta from all cages was collected and frozen at -20°C until analysis for apparent metabolizable energy analysis. On day 20 for the broiler experiment, two or three birds per cage, and on day 21 for the turkey experiment, all birds per cage were sacrificed for sampling. Using aseptic techniques the ileum was removed by cutting at the Meckel’s diverticulum at the ileo-cecal junction and the ceca were removed by cutting both sections at the ileo-cecal junction. 10 to 15 grams of ileal contents per cage and 8 to 10 grams 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.

\textbf{Volatile Fatty Acid Analysis.}

Ileal and cecal samples were prepared for volatile fatty acid analysis by weighing out one gram of sample, adding 2.0 mL diH$_2$O, vortexing, and spinning for five minutes at 2500
rpm. 1.0-2.0 mL of supernatant was pulled off into a micro-centrifuge tube and spun at 15,000 rpm (21,000 rcf) for 10 minutes. 1.0 mL (or all available) supernatant was pulled off into another micro-centrifuge tube and 200 μL 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 spun at 15,000 rpm for 10 minutes to aid in cleaning the sample. Samples were analyzed for volatile fatty acids by gas liquid chromatography (Varian CP 3380 with NUKOL Fused Silica Capillary Colum 30 m x 0.25mm x 0.25 μm film thickness).

**Microbiological Enumeration and Identification.**

Ileal and cecal tissues were collected on day 21 for broilers and day 22 for turkeys. Serial dilutions of tissue samples were prepared and plated on 3 selected media plates for enumeration and isolation. Isolates were sent to an independent lab for identification (Appendix I).

**Chemical Analysis.**

On day 21 for the broiler experiment and day 17 for the turkey experiment, excreta was collected from pans under cages and stored in -20°C freezer until further processing. Approximately 200 grams of representative excreta sample was dried for approximately 72 hours 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 grams of representative feed sample was dried for 24 hours 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, 2006). Insoluble ash for Celite recovery was performed with modification of the method described by Vogtmann et al. (1975). 2 grams 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 D.I. water to wash residue free of acid and allowed to drain. Using clean, fired pre-weighed crucibles, filter paper was folded and placed into a muffle furnace (BF1700 Series, Thermo Scientific Lindberg/Blue M, Asheville, NC). Samples were ashed at 600°C for approximately 12-14 hours (AOAC, 1995). The muffle furnace was turned off and allowed to cool. Samples were pulled out and weighed to obtain ash weight. Excreta samples were prepared for gross energy analysis by weighing 1.000-1.0050 g of dried sample, transferring it to clean large crucible, adding two drops of diH₂O and working into the sample, ensuring that no clumps of water remained. Samples were re-weighed and poured into a clean pellet press to form pellets. Tarring out a clean calorimeter crucible, the sample was placed into the crucible and stored in the desiccator for approximately 12-15 hours. A plain jacket calorimeter (1341 Parr Instrument Co., Moline, Illinois) was used to calculate gross energy of dried excreta and feed samples. The apparent metabolizable energy nitrogen corrected (AMEₙ) was calculated according to Lammer et al. (2008) using the following equations:

\[
N_{\text{retained}} = N_{\text{feed}} - \left( \frac{N_{\text{excreta}} \times A_i A_{\text{feed}}}{A_i A_{\text{excreta}}} \right)
\]

and

\[
AME_{\text{n}} = GE_{\text{feed}} - \left[ \frac{(GE_{\text{excreta}} \times A_i A_{\text{feed}})}{A_i A_{\text{excreta}}} \right] - (8.22 \times N_{\text{retained}})
\]
Where: AME<sub>n</sub> (Kcal/g) is the nitrogen corrected apparent metabolizable energy of the diet; GE<sub>feed</sub> and GE<sub>excreta</sub> were the gross energy of the diet and excreta respectively; AiA<sub>feed</sub> and AiA<sub>excreta</sub> were the concentration of Celite recovered as acid insoluble ash in diet and excreta respectively; 8.22 (Kcal/g) is the energy value of uric acid; and N<sub>retained</sub> (g/kg) is the nitrogen retained by the bird per kilogram of diet consumed, and N<sub>feed</sub> and N<sub>excreta</sub> (%) were the nitrogen content of the diet and excreta respectively. All values in this calculation were expressed as grams per kilogram 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 experiment performance and AME<sub>n</sub> data were analyzed by 2 x 2 factorial ANOVA and means were separated using LSMeans. VFA data were analyzed using 2 x 2 factorial ANOVA with means separated by LSMeans Contrasts. Means were considered significant at less than or equal to 0.05.

**RESULTS**

**Broiler Experiment – Growth Performance**

Main and interaction effects of dietary DFM and fat inclusion on body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) in broiler chicks are presented in Table 3.2. There was low mortality with no differences between treatments. Throughout the entire broiler experiment, there was an interaction effect between DFM and fat. An expected improved performance was observed due to a main effect of high supplemental fat diets over that of low supplemental fat diets. In low fat diets, DFM inclusion resulted in positive effects.
There was a consistent improvement in BWG due to fat inclusion while at the low fat level, DFM inclusion resulted in improved BWG (cumulative: Low No=629\textsuperscript{b}, Low Yes=749\textsuperscript{a}, High No=764\textsuperscript{a}, High Yes=756\textsuperscript{a} + 1.7 g). There were no consistent effects observed for FI. An interaction was observed consistently throughout the experiment with improved cumulative FCR when DFM was supplemented at the low fat level, resulting in FCR similar to that of high fat diets (Low No=1.480\textsuperscript{a}, Low Yes=1.398\textsuperscript{b}, High No=1.346\textsuperscript{b}, High Yes=1.373\textsuperscript{b} + 0.02).

**Turkey Experiment – Growth Performance**

Main and interaction effects of dietary DFM and fat inclusion on BWG, FI and FCR in turkey poult\textsuperscript{s} are presented in Table 3.3. There was low mortality with no differences between treatments. As with the broiler experiment, there was an interaction effect of DFM and fat inclusion. Fat main effect was as expected, where birds fed high fat diets displayed improved performance over birds fed low fat diets. The DFM supplemented at the low fat level resulted in improved performance. However, there were no improvements observed when DFM was supplemented at the high fat level.

There were no differences observed in BWG during the first week of the experiment. During the second week of the experiment, the low fat with DFM treatment had significantly higher BWG than the low fat without DFM and high fat without DFM treatments, with high fat with DFM having an intermediate BWG. This is similar to the effect seen during second week cumulative BWG where the low fat with DFM treatment still had the highest BWG over high fat with DFM, and intermediate values were observed for the low fat without DFM and high fat without DFM treatments (p=0.0041). During the third week the high fat without
DFM treatment had the highest BWG, and was significantly different from the low fat with DFM treatment with the high fat without DFM treatment having an intermediate BWG. The low fat without DFM treatment had the poorest BWG and was different from all other treatments (p=0.0003). The final cumulative BWG showed that the low fat with DFM and high fat without DFM treatments had the highest BWG and was different from the low fat without DFM treatment. The high fat with DFM treatment had an intermediate value for cumulative BWG (p=0.0002).

There were no differences observed in FI throughout the entire experiment. The trend for FCR observed throughout the entire experiment was that the high fat without DFM had significantly lower FCR than the low fat without DFM treatment. During the first week, the low fat with DFM treatment is not different from the high fat without DFM treatment, and the high fat with DFM had an intermediate FCR (p=0.002). During the second week, the high fat without DFM treatment is significantly lower than the low fat with DFM treatment, which is also different from the low fat without DFM treatment. Here, the high fat with DFM treatment has an intermediate FCR to the high fat without DFM and low fat with DFM treatments (p<0.0001). The same effect is seen for the cumulative FCR at week 2 (p=0.0007). A similar effect is observed in the last week of the experiment with the exception that the high fat with DFM treatment is not different from the high fat without DFM treatment (p=0.0046). This follows for the final cumulative FCR at week 3 (p=0.0017).

**Apparent Metabolizable Energy**

The main and interaction effects of AME\(_n\) on broiler chicks and turkey poult are presented in Table 3.4. 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\textsubscript{n} at the low fat level when DFM was supplemented, resulting in an uplift of 31 kcal/kg in AME\textsubscript{n}. However, at the high fat level, the addition of DFM did not result in an improvement in AME\textsubscript{n} (p<0.0001).

For the turkey experiment, the main effect of DFM resulted in an uplift of 69 kcal/kg in AME\textsubscript{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\textsubscript{n} between the low and high fat diets (p<0.0001). There is an improvement in AME\textsubscript{n} at the low fat level when DFM is supplemented (226 kcal/kg). But at the high fat level, DFM supplementation did not result in an improvement in AME\textsubscript{n}.

**Volatile Fatty Acids**

For the broiler experiment, the presence of acetate, butyrate, propionate, valerate and isovalerate was 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 (p=0.9198). All ileal samples that were analyzed by gas liquid chromatography were below detectable levels.

For the turkey experiment, the presence of acetate, butyrate, isobutyrate, propionate, valerate and isovalerate was detected on day 22. 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 response to DFM or fat
inclusion for total cecal VFA concentrations (p=0.5316).

Additionally in the turkey experiment, VFA concentrations were found in the ileum (Table 3.7). The individual VFA present were acetate, butyrate, isobutyrate, propionate, valerate and isovalerate. As with the other experiments, there were no significant differences found in any individual VFA between dietary treatments. There was an interaction effect in the total ileal VFA concentration where birds fed low fat without DFM and high fat with DFM diets had increased VFA than birds fed low fat with DFM and high fat without DFM diets.

*Microbiological Identification*

By analysis, the concentration of probiotic product administration in the feed was found to be in agreement with the manufacturer’s recommended inclusion levels.

**DISCUSSION**

The objective of these experiments was to determine if DFM can be substituted for dietary fat for broiler chicks and turkey poults and to determine if this improvement is associated with increased dietary energy. Fairly consistently throughout these studies, it was observed that there was an effect at the low fat level when DFM was added, but not at the high fat level. This is observed in broiler and turkey performance (Tables 3.2 and 3.3) and in the AMEₙ values for both experiments (Tables 3.4). It can be noted that in all the scenarios listed above, the high fat level resulted in expected improved performance compared to the low fat treatments. This can be attributed to the increased nutrient digestibility and increased transit time seen with increased supplemental fat levels (Mateos et al., 1980). However, the high fat diets in the broiler experiment were not formulated to industry standards, and were
formulated to capture the extreme high end of the energy spectrum for chickens. The high fat turkey diets were at the upper range of fat inclusion used in the industry. Therefore, the high fat broiler diets would not typically be seen in the broiler industry. Most likely, fat will be added at under 5 percent for broilers (Ross 708 Handbook, 2009) and 3-6 percent for turkeys (Aviagen Nicholas Feeding Recommendations for Commercial Stock, 2010). Additionally, our results of increased BWG and FCR with increased supplemental fat levels are in agreement with other researchers (Biely & March, 1954, Jensen et al., 1970, Pesti, 2002).

At low fat level, an effect of DFM inclusion was observed for both experiments with broilers and turkeys. We observed increased BWG and FCR, which is in agreement with other researchers (Jin et al. 1998a, Angel et al., 2005, Mountzouris et al. 2007, Russell & Grimes, 2007, Torres-Rodriguez et al., 2007, Mountzouris et al. 2010). Along with these growth effects, other modes of action could have been at work in the gut such as maintenance of the epithelial barrier, changes in gut morphology, and protection against pathogens (Gusils et al., 1999, Chichlowski et al., 2007b, Fooks & Gibson, 2002). Comparing the two broiler trial, there was a 5 – 7°F temperature difference from the first, May experiment to the second, August experiment. This may have led to decreased FI and therefore improved FCR in the second experiment compared to the first. Additionally, we observed an uplift in AMEn for both broilers and turkeys at the low fat level when DFM was supplemented. These results are in agreement with other researchers who support that AMEn is improved with the addition of DFM to the diet (Mountzouris et al., 2010, Monhan et al., 1996). Therefore, the DFM in the gut may be changing the environment in one or more of the ways listed above, so that nutrient digestibility is increased. This could be through increased digesta passage
(Schneitz et al., 1998), or through increased digestive enzyme activity (Rowland, 1992). The uplift in AMEn could even be attributed to increased VFA production, which has a known effect on energy metabolism in the body (Jin et al., 1998b).

The major VFAs were measured in the ceca of both the broiler and turkey experiments, although higher amounts were present in the gut of turkeys than broilers (Tables 3.5 & 3.6). In both experiments, there were higher concentrations of total VFAs in the low fat treatments than in the high fat treatments. 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 held in approximately constant ratios. It is important to note the presence of isobutyrate in the chicken experiment and isovalerate in both experiments. Thus significant amounts of amino acid breakdown may be occurring in the gut. Valerate (C5), which is produced from the condensation of acetate and propionate, produce small amounts of glucose (Bergman, 1990). The mechanisms behind why VFA were observed in this experimental design are not clear. However, sampling variation is great and could be affecting the results of the experiment. The same sample procedures were taken as in the previous studies, and the same variation was observed with fluid movement of gut digesta, individual bird differences, and combination of birds within each pen. This could also be the explanation as to why we observed ileal concentrations of VFA in the turkey experiment. In the ileum, we observed the same VFA present as in the ceca, except in smaller concentrations. Therefore, most bacteria fermentation is occurring in the ceca (Cummings & Macfarlane, 1991).

In conclusion, DFM can be substituted for fat in broiler and turkey diets with
moderate amounts of fat included in the diet. This improvement in performance with DFM at lowered fat levels is associated with an increased $\text{AME}_n$. The association with changes in VFA are less clear.
Table 3.1. Composition & nutrient content of experimental starter diets fed to broiler chicks to 20 days of age for experiment 2 and turkey poults to 21 days of age for experiment 2.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Broiler</th>
<th>Turkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>36.8</td>
<td>33.5</td>
</tr>
<tr>
<td>Soybean Meal 48</td>
<td>28</td>
<td>32.5</td>
</tr>
<tr>
<td>Distiller’s Dried Grain</td>
<td>7.5</td>
<td>5</td>
</tr>
<tr>
<td>Wheat</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Poultry Meal</td>
<td>0.00</td>
<td>10</td>
</tr>
<tr>
<td>Poultry Fat</td>
<td>1.00</td>
<td>1</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>1.20</td>
<td>1.85</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>2.10</td>
<td>2.2</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.40</td>
<td>0.55</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.30</td>
<td>0.425</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.125</td>
<td>0.15</td>
</tr>
<tr>
<td>Selenium Premix</td>
<td>0.050</td>
<td>0.05</td>
</tr>
<tr>
<td>Choline Chloride</td>
<td>0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>Trace Mineral Premix</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>0.00</td>
<td>0.125</td>
</tr>
<tr>
<td>Celite</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin Premix</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**Nutrient Composition**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Broiler</th>
<th>Turkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME Poultry, kcal/kg</td>
<td>1258</td>
<td>1217</td>
</tr>
<tr>
<td>Crude Protein, %</td>
<td>21.95</td>
<td>29.47</td>
</tr>
<tr>
<td>Crude Fat, %</td>
<td>3.33</td>
<td>4.53</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.02</td>
<td>1.46</td>
</tr>
<tr>
<td>Available Phosphorus, %</td>
<td>0.78</td>
<td>0.98</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Total Lysine, %</td>
<td>1.36</td>
<td>1.80</td>
</tr>
<tr>
<td>Total Met + Cys, %</td>
<td>0.92</td>
<td>1.24</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.82</td>
<td>1.12</td>
</tr>
<tr>
<td>Choline, mg/lb</td>
<td>885.48</td>
<td>2397</td>
</tr>
</tbody>
</table>

**Nutrient Analysis**

<table>
<thead>
<tr>
<th></th>
<th>Low Fat</th>
<th>High Fat*</th>
<th>Low Fat</th>
<th>High Fat*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>21.34</td>
<td>20.47</td>
<td>28.24</td>
<td>26.71</td>
</tr>
<tr>
<td>Crude Fat, %</td>
<td>3.53</td>
<td>7.64</td>
<td>3.58</td>
<td>7.51</td>
</tr>
</tbody>
</table>

1Vitamin 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.

2Mineral 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.

3Selenium premix provided 0.2 mg/kg Se.

4High fat diets included additional 5 % poultry fat added to basal.
Table 3.2. Effect of dietary DFM and fat inclusion on performance of broiler chicks from placement to 20 days of age for experiment 2.1

<table>
<thead>
<tr>
<th>Period (Days)</th>
<th>DFM Fat</th>
<th>Inclusion Level</th>
<th>DFM Main Effect</th>
<th>Fat Main Effect</th>
<th>Source of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No Low</td>
<td>Yes Low</td>
<td>No High</td>
<td>Yes High</td>
</tr>
<tr>
<td>0-7</td>
<td>94b</td>
<td>117a</td>
<td>112a</td>
<td>118a</td>
<td>103</td>
</tr>
<tr>
<td>7-14</td>
<td>219b</td>
<td>274a</td>
<td>275a</td>
<td>269a</td>
<td>247</td>
</tr>
<tr>
<td>0-14</td>
<td>313b</td>
<td>391a</td>
<td>387a</td>
<td>387a</td>
<td>350</td>
</tr>
<tr>
<td>14-20</td>
<td>317b</td>
<td>358a</td>
<td>377a</td>
<td>369a</td>
<td>347</td>
</tr>
<tr>
<td>0-20</td>
<td>629b</td>
<td>749a</td>
<td>764a</td>
<td>756a</td>
<td>697</td>
</tr>
</tbody>
</table>

Values are means of 18 replicate pens of 6 male broilers per pen.

1DFM inclusion rates: no at 0 lb/ton, yes at 2 lb/ton.
2Fat inclusion rates: low at 1%, high at 6%.
3Average hatchling body weight, across all treatments, was 45.5g ± 0.02g.
4Values calculated using body weight gain.
5Means within a row with different superscripts are significantly different (p<0.05).
Table 3.3. Effect of dietary DFM and fat inclusion on performance of turkey poults from placement to 21 days of age for experiment 2.¹

<table>
<thead>
<tr>
<th>Period (Days)</th>
<th>DFM² Fat³</th>
<th>Inclusion Level</th>
<th>BWG (g)</th>
<th>DFM Main Effect</th>
<th>Fat Main Effect</th>
<th>Source of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DFM No</td>
<td>DFM Yes</td>
<td>Fat No</td>
<td>Fat Yes</td>
<td>SEM</td>
<td>DFM</td>
</tr>
<tr>
<td>0-7*</td>
<td>115</td>
<td>116</td>
<td>117</td>
<td>113</td>
<td>16</td>
<td>115</td>
</tr>
<tr>
<td>7-14</td>
<td>228²b</td>
<td>240¹a</td>
<td>234²ab</td>
<td>224²b</td>
<td>231</td>
<td>232</td>
</tr>
<tr>
<td>0-14</td>
<td>343³ab</td>
<td>356³a</td>
<td>351³ab</td>
<td>337³b</td>
<td>347</td>
<td>347</td>
</tr>
<tr>
<td>14-21</td>
<td>324³c</td>
<td>345³b</td>
<td>367³a</td>
<td>357³ab</td>
<td>345</td>
<td>351</td>
</tr>
<tr>
<td>0-21</td>
<td>667³b</td>
<td>701³a</td>
<td>718³a</td>
<td>694³ab</td>
<td>692</td>
<td>698</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period (Days)</th>
<th>FI (g)</th>
<th>FCR* (g:g)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7</td>
<td>1.115b</td>
<td>1.073a</td>
<td>1.061a</td>
</tr>
<tr>
<td>7-14</td>
<td>1.258⁶c</td>
<td>1.226b</td>
<td>1.192a</td>
</tr>
<tr>
<td>0-14</td>
<td>1.020⁶c</td>
<td>0.997b</td>
<td>0.970a</td>
</tr>
<tr>
<td>14-21</td>
<td>1.585⁶c</td>
<td>1.517b</td>
<td>1.400a</td>
</tr>
<tr>
<td>0-21</td>
<td>1.268⁶c</td>
<td>1.231b</td>
<td>1.169a</td>
</tr>
</tbody>
</table>

¹Values are means of 18 replicate pens of 7m male turkeys per pen.
²DFM inclusion rates: no at 0 lb/ton, yes at 2 lb/ton.
³Fat inclusion rates: low at 1%, high at 6%.
⁴Average hatchling body weight, across all treatments, was 63.7 g ± 0.32 g.
⁵Values calculated using body weight gain.
⁶Means within a column with different superscripts are significantly different (p<0.05)
Table 3.4. Effect of dietary DFM and fat inclusion on apparent metabolizable energy of broiler chicks at 16 days of age for experiment 2 and turkey poults at 15 days of age for experiment 2.\(^1\)

<table>
<thead>
<tr>
<th>Inclusion Level</th>
<th>Broiler AME(_n) (kcal/kg)</th>
<th>Turkey AME(_n) (kcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DFM(^2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Low</td>
<td>3235(^d)</td>
<td>2885(^d)</td>
</tr>
<tr>
<td>Yes Low</td>
<td>3266(^c)</td>
<td>3111(^c)</td>
</tr>
<tr>
<td>No High</td>
<td>3407(^a)</td>
<td>3291(^a)</td>
</tr>
<tr>
<td>Yes High</td>
<td>3351(^b)</td>
<td>3204(^b)</td>
</tr>
<tr>
<td><strong>DFM Main Effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3321</td>
<td>3088</td>
</tr>
<tr>
<td>Yes</td>
<td>3309</td>
<td>3157</td>
</tr>
<tr>
<td><strong>Fat Main Effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>3250</td>
<td>2998</td>
</tr>
<tr>
<td>High</td>
<td>3379</td>
<td>3248</td>
</tr>
<tr>
<td><strong>Source of Variation</strong></td>
<td>-----------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>SEM</td>
<td>12.29</td>
<td>18.32</td>
</tr>
<tr>
<td>DFM</td>
<td>0.0870</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DFM x Fat</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^1\)Values are means of 18 replicate pens of 7 male broilers or turkeys per pen.
\(^2\)DFM inclusion rates: no at 0 lb/ton, yes at 2 lb/ton.
\(^3\)Fat inclusion rates: low at 1%, high at 6%.
\(^a,b,c,d\) Means within a column with different superscripts are significantly different (p<0.05).
Table 3.5. Effect of dietary DFM and fat inclusion on cecal volatile fatty acid concentrations of broiler chicks at 21 days of age for experiment 2.1

<table>
<thead>
<tr>
<th>Inclusion Level</th>
<th>Acetate</th>
<th>Butyrate</th>
<th>Propionate</th>
<th>Valerate</th>
<th>Isovalerate</th>
<th>Total</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFM2</td>
<td>Fat3</td>
<td>-----------</td>
<td>------------</td>
<td>----------</td>
<td>-------------</td>
<td>-------</td>
<td>---------------</td>
</tr>
<tr>
<td>No Low</td>
<td>74.73</td>
<td>24.63</td>
<td>1.320</td>
<td>0.734</td>
<td>0.930</td>
<td>134.16</td>
<td></td>
</tr>
<tr>
<td>Yes Low</td>
<td>75.44</td>
<td>23.91</td>
<td>1.942</td>
<td>0.868</td>
<td>0.941</td>
<td>133.10</td>
<td></td>
</tr>
<tr>
<td>No High</td>
<td>74.20</td>
<td>24.17</td>
<td>2.277</td>
<td>0.834</td>
<td>0.935</td>
<td>124.52</td>
<td></td>
</tr>
<tr>
<td>Yes High</td>
<td>74.49</td>
<td>24.75</td>
<td>2.483</td>
<td>0.764</td>
<td>0.827</td>
<td>124.62</td>
<td></td>
</tr>
</tbody>
</table>

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 | 0.9827 | 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 |

1Values are means based on representative cecal samples of all birds per pen.
2DFM inclusion rates: no at 0 lb/ton, yes at 2 lb/ton.
3Fat inclusion rates: low at 1%, high at 6
Table 3.6. Effect of dietary DFM and fat inclusion on cecal volatile fatty acid concentrations of turkey poults at 22 days of age for experiment 2.¹

<table>
<thead>
<tr>
<th>Inclusion Level</th>
<th>Molar Percent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate</td>
<td>Buoyrate</td>
</tr>
<tr>
<td>DFM² Fat³</td>
<td>(%)</td>
<td>mM</td>
</tr>
<tr>
<td>No Low</td>
<td>56.37</td>
<td>22.42</td>
</tr>
<tr>
<td>Yes Low</td>
<td>57.40</td>
<td>22.29</td>
</tr>
<tr>
<td>No High</td>
<td>51.89</td>
<td>26.53</td>
</tr>
<tr>
<td>Yes High</td>
<td>54.39</td>
<td>24.28</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFM</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>DFM x Fat</td>
</tr>
<tr>
<td>SEM</td>
</tr>
</tbody>
</table>

¹Values are means based on representative cecal samples of all birds per pen.
²DFM inclusion rates: no at 0 lb/ton, yes at 2 lb/ton.
³Fat inclusion rates: low at 1%, high at 6.
Table 3.7. Effect of dietary DFM and fat inclusion on ileal volatile fatty acid concentrations of turkey poults at 22 days of age for experiment 2.1

<table>
<thead>
<tr>
<th>Inclusion Level</th>
<th>Acetate</th>
<th>Butyrate</th>
<th>Isobutyrate</th>
<th>Propionate</th>
<th>Valerate</th>
<th>Isovalerate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFM2 Fat3</td>
<td>%</td>
<td>mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Low</td>
<td>46.40</td>
<td>12.05</td>
<td>6.02</td>
<td>23.95</td>
<td>5.98</td>
<td>5.25</td>
<td>67.23</td>
</tr>
<tr>
<td>Yes Low</td>
<td>46.43</td>
<td>12.06</td>
<td>6.03</td>
<td>23.98</td>
<td>5.97</td>
<td>5.53</td>
<td>63.72</td>
</tr>
<tr>
<td>No High</td>
<td>46.36</td>
<td>12.06</td>
<td>6.08</td>
<td>24.00</td>
<td>6.00</td>
<td>5.52</td>
<td>64.25</td>
</tr>
<tr>
<td>Yes High</td>
<td>46.52</td>
<td>12.06</td>
<td>6.03</td>
<td>23.81</td>
<td>5.98</td>
<td>5.50</td>
<td>67.78</td>
</tr>
<tr>
<td>DFM Main Effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>46.38</td>
<td>12.05</td>
<td>6.05</td>
<td>23.97</td>
<td>5.99</td>
<td>5.52</td>
<td>65.74</td>
</tr>
<tr>
<td>Yes</td>
<td>46.48</td>
<td>12.06</td>
<td>6.03</td>
<td>23.89</td>
<td>5.97</td>
<td>5.51</td>
<td>65.75</td>
</tr>
<tr>
<td>Fat Main Effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>46.42</td>
<td>12.05</td>
<td>6.03</td>
<td>23.97</td>
<td>5.98</td>
<td>5.51</td>
<td>66.01</td>
</tr>
<tr>
<td>High</td>
<td>46.44</td>
<td>12.06</td>
<td>6.05</td>
<td>23.90</td>
<td>5.99</td>
<td>5.53</td>
<td>65.47</td>
</tr>
<tr>
<td>Source of Variation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.183</td>
<td>0.064</td>
<td>0.063</td>
<td>0.123</td>
<td>0.026</td>
<td>0.023</td>
<td>1.82</td>
</tr>
<tr>
<td>DFM</td>
<td>0.4912</td>
<td>0.8912</td>
<td>0.6248</td>
<td>0.3339</td>
<td>0.4186</td>
<td>0.6077</td>
<td>0.9935</td>
</tr>
<tr>
<td>Fat</td>
<td>0.8848</td>
<td>0.9420</td>
<td>0.6340</td>
<td>0.3898</td>
<td>0.5507</td>
<td>0.2880</td>
<td>0.7125</td>
</tr>
<tr>
<td>DFM x Fat</td>
<td>0.6466</td>
<td>0.9192</td>
<td>0.5073</td>
<td>0.1759</td>
<td>0.8980</td>
<td>0.4659</td>
<td>0.0190</td>
</tr>
</tbody>
</table>

1Values are means based on representative ileal samples of all birds per pen.
2DFM inclusion rates: no at 0 lb/ton, yes at 2 lb/ton.
3Fat inclusion rates: low at 1%, high at 6.
REFERENCES


AOAC. 1995. Ether Extract in Animal Feed, AOAC Official method 920.39

AOAC. 2006. Combustion Analysis (LECO), AOAC Official Method 990.03.


MICROBIOLOGICAL SAMPLING METHODS

On the day of sampling, prior to sampling, media agar was prepared in the lab, so that the media would be ready when the samples were finished being processed. All media was sterilized by autoclave at 121°C for 15 minutes. De Man, Rogosa, Sharpe (MRS) media was prepared by adding 31 grams of MRS Dehydrated Culture Media (REF: R454052, Remel Products: Thermo Fisher Scientific, Lenexa, KS) into 500 mL DI water, heated to boiling with agitation to completely dissolve agar, 1 mL 1% methyl blue (M5528-252G, Sigma-Aldrich Corp. St. Louis, MO) was added to mixture, then was autoclaved. To prepare the MRS + vancomycin media, the same procedure was followed, except after autoclaving and cooling to 55°C, 1 mL/100 mL Vancomycin HCL (BP29581, Fisher BioReagents: Thermo Fisher Scientific, Lenexa, KS) was added. RCM media was prepared by adding 19 grams RCM Dehydrated Culture Media (REF: R454182, Remel Products: Thermo Fisher Scientific, Lenexa, KS) and 17.25 grams Nutrient Agar into 500 mL DI water, then autoclaved. KF Strep Media was prepared by autoclaving 500 mL bottle of DI water, 38.2 grams of KF Strep Meida (CM0701, Oxoid Limited, Thermo Fisher Scientific, Hampshire, UK) and bring to a boil for five minutes with frequent agitation to completely dissolve agar, once bottle is cooled to 55°C, then 1 vial TTC (1% 2,3,5-Triphenyltetrazolium chloride, SR0229K, Oxoid Limited, Thermo Fisher Scientific, Hampshire, UK) is aseptically added. All media was held at 45°C in water bath or in incubator until ready to use.

Chilled ileal and cecal samples were taken to the microbiology lab at Scott Hall for further processing. Each ileal section was placed in a sterile weigh dish and a sterile scalpel
was used to make a transverse incision exposing the interior surface and digesta. The sample was then placed with tweezers in a pre-filled sterile dilution bottle labeled for waste and digesta was washed by gently swirling the sample in the bottle. Next the sample was placed into a new sterile weigh dish and 1 gram of tissue was measured. 1 gram of tissue was then placed in a fresh pre-filled sterile dilution bottle containing 99 mL of buffer and set to the side. This procedure was repeated for the remaining ileal samples and all of the cecal samples. The contents of the bottles containing 1 mL of tissue were then transferred to a sterile 16 oz mason jars and sterile blades, gaskets and lids were applied to each jar. The samples were then blended using an oster blender base for 30 seconds on high or until the sample was thoroughly severed. The samples were then replaced into their same dilution bottles and from this point forward were considered the 10-2 dilution. To make the 10-4 dilution, 1 mL of aliquot was transferred using a sterile pipette tip from the 10-2 dilution bottle into a new pre-filled sterile dilution bottle containing 99 mL of buffer and then vortexed to ensure even distribution of aliquot. To create the 10-6 dilution, 1 mL of aliquot from the 10-4 dilution bottle was then transferred using a sterile pipette tip, into another pre-filled sterile dilution bottle containing 99 mL of buffer and then vortexed to ensure even distribution of aliquot. And finally to make the 10-5 dilution, 0.5 mL of aliquot was transferred from the 10-4 dilution bottle using a sterile pipette tip, into a new pre-filled dilution bottle containing 99 mL of buffer and vortexed to ensure even distribution of aliquot. This procedure was repeated for the remaining ileal samples and all of the cecal samples.

To plate the samples, 100 μL from selected dilution bottle was aseptically transferred to the bottom of clean, sterile petri dish. This was repeated a second time to create a duplicate
plate. Next approximately 15 mL of liquid media was poured over the plates and swirled until the media evenly covered the bottom of each dish. This process was repeated for 3 dilutions for each sample. The selected dilutions for all four medias were 10-4, 10-5, 10-6. The remaining liquid agar was poured into sterile petri dishes and allowed to solidify. These plates were then placed in the refrigerator to be used for colony isolation. Samples were left for approximately 30 minutes to solidify and then were flipped for proper growth with the agar side of the dish facing upward. Samples were sorted based on media. MRS + vancomycin and RCM plates were placed in anaerobe jars, AnaeroPaks (Mitsubishi Gas Chemcal Co., Inc., International BioProducts) were opened and placed inside the jars, the jars were sealed and were then placed into a 43°C incubator. MRS plates were also placed in anaerobe jars with AnaeroPaks then sealed. They were placed in a 37°C incubator. KFS plates were placed in plate racks and transferred directly into a 37°C incubator. All samples were incubated for 72 hours.

At 72 hours, plates were removed from the incubators and sorted. From two plates per sample, colonies were selected for isolation. Plates were labeled by drawing a line down the center of the plate. Two colonies per half were plated using single colony isolation method. Colonies were picked using sterile plastic inoculation loop from the source plate. Apply primary streak from loop to selected half of new plate. Using a new sterile plastic loop, make secondary streak by crossing from primary streak into unused area of agar. Plates were sorted by media type and incubation set-up was the same as the initial plating. All colony plates were incubated for 18 hours.

From these re-incubated plates, colonies were selected for isolation and re-plated on
pre-made media plates using streak method. These plates were incubated for 18 hours. At 18 hours, all plates were removed from the incubators. Colonies were selected for gram-staining and long-term storage. Gram staining was performed using gram-staining kit (Remel Products, Thermo Fisher Scientific, Lenexa KS) on BBL Gram Slides (REF 231401 Becton Dickinson Co., Sparks, MD) according to manufacturer specifications. Colonies from plates were also selected and aseptically transferred to Microbank™ (PL.170/M Pro-Lab Diagnostics, Round Rock, TX) vials, which were stored in -70°C freezer until transport. Samples were sent to an independent lab (Star Labs/Forage Research, Inc., Clearwater, FL) for cell recovery and bacterial identification using a Riboprinter® (DuPont®).