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The Role of Direct-Fed Microbials in Conventional Livestock Production

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Abstract

Supplementation of direct-fed microbials (DFM) as a means to improve the health and performance of livestock has generated significant interest over the past 15+ years. A driving force for this increased interest in DFM is to reduce or eliminate the use of low-dose antibiotics in livestock production. This increased attention toward DFM supplementation has generated an extensive body of research. This effort has resulted in conflicting reports. Although there has been considerable variation in the design of these studies, one of the main causes for this lack of consistency may be attributed to the variation in the experimental immune challenge incorporated to evaluate DFM supplementation. Taking into account the experimental immune challenge, there is strong evidence to suggest that DFM supplementation may have an impact on the immune response, overall health, and performance of livestock.

INTRODUCTION

The past 15 years have seen a significant increase in the marketing of direct-fed microbials (DFMs) to all sectors of livestock agriculture (beef, pork, and poultry). A major reason for this expansion in DFMs is directed toward replacement of low-dose antibiotics (LDAs). The use of LDAs for improving animal health in livestock agriculture has been a common practice for over 30 years; the first documented research trial took place in 1946 (1). LDAs are used to improve the overall health of livestock. A benefit of this improved health is an increase in body weight (BW) and increased feed efficiency. However, this production practice has received a great deal of scrutiny owing to (*a*) an increase in the number of consumers demanding products produced without LDAs and (*b*) increased attention to the issue of antibiotic-resistant bacteria strains in human medicine.

With the increased attention to LDA use in livestock production, a great deal of research has been devoted to investigating DFMs as a replacement for LDAs. This increased interest in DFMs has resulted in an enormous amount of research worldwide. In researching this review, we identified 1,246 scientific articles simply by searching the generic terms "direct-fed microbial," "performance," "health," and livestock species. These 1,246 articles were from just 7 highly/moderately ranked journals (impact factor $\geq$1.4) in the area of animal agriculture (*Journal of Animal Science*, *Journal of Dairy Science*, *Journal of Poultry Science*, *Journal of the Science of Food and Agriculture*, *Livestock Science*, *World's Poultry Science Journal*, and *Journal of Dairy Research*). In addition to these journals directly related to livestock agriculture and numerous other animal/livestock journals, several journals in the microbiology field also have published studies related to DFMs. Thus, as a conservative estimate, there could be well over 3,000 scientific articles regarding the use of DFMs to enhance livestock production. In such a large, extensive collection of research studies, there have been conflicting reports. Numerous studies have reported significant benefits for the use of DFMs (regardless of species), whereas others have reported results that indicate little to no effect. In this review, we have focused on some of the prevailing research, conflicting data, and future directions relating to DFM use in the livestock health industry and, particularly, whether DFMs can adequately substitute for LDAs.

CURRENT DIRECT-FED MICROBIALS

The use of DFMs in livestock has been a research focus for well over 25 years. During this period, numerous DFMs have been investigated as a means to improve animal health. In 1999, the official publication of the American Association of Feed Control Officials listed 42 of these reagents that are accepted as "food" products (i.e., not labeled or promoted with any therapeutic or structure/function claims). The majority of DFMs that have been investigated are bacteria (39 different bacteria; see **Table 1**) (2). However, there have also been numerous studies on using molds and yeast as DFMs, the majority of which centered around the yeast *Saccharomyces cerevisiae* and the mold *Aspergillus niger* and *Aspergillus oryzae* (among others). Using just this list, we identified over 400 scientific studies that directly investigated the use of an "accepted" DFM in livestock production.

EFFECT OF DIRECT-FED MICROBIALS ON THE IMMUNE RESPONSE OF LIVESTOCK

Unlike antibiotics (which have either a direct bactericidal or bacteriostatic effect on bacteria), DFMs must function through indirect mechanisms, such as alterations to the intestinal microbiome, enhancement of intestinal efficiency, and modulation of the host innate immune response.

Table 1 The Association of American Feed Control Officials 1999 official publication list of organisms that are accepted as food products (i.e., not labeled or promoted with any therapeutic or structure/function claims)

Lactobacillus	***Enterococcus***
L. acidophilus	*E. faecium*
L. reuteri	*E. intermedius*
L. casei	*E. lactis*
L. fermentum	*E. thermophiles*
L. plantarum	*E. cremoris*
L. brevis	*E. diacetylactis*
L. bunchner[a]	***Pediococcus***
L. delbrueckii	*P. acidilactici*
L. helveticus	*P. cerevisiae* (*damnosus*)
L. lactis	*P. pentosaceus*
L. bulgaricus	***Bacillus***
L. cellobiosis	*B. coaglunas*
L. curvatus	*B. lentus*
L. farciminis[b]	*B. licheniformis*
Bifidobacterium	*B. pumilus*
B. adolescentis	*B. subtilis*
B. animalis	***Bacteroides***
B bifidum	*B. amylophilus*
B. infantis	*B. capillosus*
B. longum	*B. ruminocola*
B. thermophilum	*B. suis*
Propionbacterium	**Yeast**
P. freudenreichii	*Saccharomyces cerevisiae*
P. shermanii	**Molds**
	Aspergillus niger
	Aspergillus oryzae

[a] Cattle only.
[b] Swine only.

The innate immune response is the immunity present in all animals at birth. It consists of rapidly responding chemical and cellular defense mechanisms (e.g., macrophages, dendritic cells, neutrophils, cytokines, and natural killer cells) that need not be induced by prior exposure to an infectious agent (3). All segments of the livestock industry (cattle, swine, and poultry) have acknowledged the possible health benefits of DFMs. Thus, a major focus of DFM research has been the impact of specific or multistrain DFMs on innate immune responses of livestock.

When reviewing the literature on the impact of DFMs on animal health, one must also evaluate the experimental immune challenge model incorporated to investigate the alterations to health. In terms of investigating animal health/immune response studies, several models are used: (*a*) pathogen challenge, or experimental challenge of live pathogens (bacterial or viral); (*b*) direct immune challenge, or experimental challenge with a substance that initiates an immune response (immunization or endotoxin); and (*c*) natural exposure, or introduction to an environment similar to a production setting, in which animals are exposed to pathogens similar to those in the real

world. Each method is vital for evaluation of animal health and the immune system, and each contributes to our understanding of the impact of DFMs on the immune response. Thus, by taking into account the method of challenge, we may be able to shed some light on the role of DFMs in animal health.

Swine

Pathogen challenge. In terms of DFM research for swine, a great deal of attention has been directed toward enterotoxigenic *Escherichia coli* (ETEC). ETEC infection causes postweaning diarrhea, which is one of the most economically devastating diseases afflicting the swine industry (4). Several researchers have used the ETEC pathogen challenge model to evaluate the response of DFM supplementation to control diets without LDAs (control$^{-DFM-LDA}$; **Table 2**).

Using an ETEC challenge, researchers (5–7) reported that DFM supplementation resulted in significant alterations in severity and duration of diarrhea in weaned pigs. Pigs supplemented with *S. cerevisiae* presented reduced daily diarrhea scores and reduced duration of diarrhea when compared with control$^{-DFM-LDA}$ pigs (5). Pigs supplemented with *Bacillus subtilis* were reported to have fecal scores at both 6 h and 24 h post ETEC challenge that were lower than those in control$^{-DFM-LDA}$ pigs (6). Furthermore, mortality was decreased in *B. subtilis*–supplemented pigs when compared with control$^{-DFM-LDA}$ pigs, and supplementation of *Lactobacillus plantarum* appeared to reduce the incidence of diarrhea in pigs, compared with control$^{-DFM-LDA}$ pigs (7).

Researchers have also reported significant alterations to the microbial community of ETEC-challenged pigs supplemented with a DFM. Supplementation of *Pediococcus acidilactici* and/or *S. cerevisiae* reduced bacterial translocation to the mesenteric lymph nodes when compared with control$^{-DFM-LDA}$ pigs (8). *B. subtilis* supplementation resulted in a microbial profile that was richer and more diverse than that of control$^{-DFM-LDA}$ pigs (6). Supplementation of *P. acidilactici* or *S. cerevisiae* during an ETEC challenge limited the attachment of ETEC F4 to the ileal mucosa (4). Furthermore, DFM supplementation during an ETEC challenge altered intestinal morphology (4). Supplementation of *L. plantarum* resulted in an increased ileum height and villous/crypt ratio when compared with control$^{-DFM-LDA}$ pigs (7).

In terms of immune response, ETEC challenge studies have also reported significant alterations when DFMs were supplemented. Supplementation of *L. plantarum* resulted in a decrease in the cytokine tumor necrosis-α (TNFA) and a suggested association for a decrease in pig-major acute phase protein/ITIH4 (7). *P. acidilactici* supplementation tended to increase CD8^{+low} T lymphocyte concentrations in the ileum and CD8^{+high} cells in the mesenteric lymph nodes when compared with control$^{-DFM-LDA}$ pigs (8). These alterations to lymphocyte subpopulations within the gastrointestinal tract could be the result of *P. acidilactici* stimulation of the local immune system. A combination of *P. acidilactici* and *S. cerevisiae* supplemented to pigs during an ETEC challenge resulted in an increase in interleukin (IL)-6 in ileal tissue when compared with control$^{-DFM-LDA}$ pigs (4). Supplementation with *P. acidilactici* alone also indicated a suggested relationship for increasing IL-6 concentrations compared with control$^{-DFM-LDA}$ pigs (4). There was also a tendency for increased TNFA (*TNFRSF1A*) expression in *P. acidilactici*– and *S. cerevisiae*–supplemented pigs and increased IL-12p35 (*IL-12A*) and antimicrobial peptide porcine β-defensin 2 (*DEFB1*) expression in *P. acidilactici* when compared with control$^{-DFM-LDA}$ pigs. There was no effect of treatment on interferon-γ (IFNG), IL10, or IL8 (4).

Within ETEC challenges, the alterations to animal performance are not as clear due to variation in experimental design, and in many instances, animal performance was not evaluated/reported. Supplementation of *S. cerevisiae* to pigs during an ETEC challenge resulted in an increase in BW following the challenge compared with control$^{-DFM-LDA}$ pigs (5). However, supplementation of

Table 2 Impact of direct-fed microbial (DFM) supplementation on swine immune response, animal health, and performance

DFM	Dosage	Challenge[a]	Neg/Pos Con[b]	Immune[c]	Health	Performance	Reference
Pediococcus acidilactici *Saccharomyces cerevisiae*	10^9 CFU/500 g/feed 10^9 CFU/500 g/feed combination	PC	Neg & Pos	+	+	NR	4
S. cerevisiae	Sows = 1 g/kg/diet Pigs = 1 g/kg/diet to 21 days Pigs = 5 g/kg/diet to 22–42 days	PC	Neg	NI	+	+	5
P. acidilactici *S. cerevisiae*	10^9 CFU/kg/diet 10^9 CFU/kg/diet combination	PC	Neg & Pos	+	NR	NI	8
Lactobacillus plantarum	2×10^{10} CFU/hd/days combination[d]	PC	Neg	+	NR	NI	7
Bacillus subtilis	1.2×10^9 CFU 0.5 g/kg/feed combination[e]	PC	Neg & Pos	NI	+	NI	6
S. cerevisiae	182 g/ton/diet	DC	Neg	+	+	+	10
S. cerevisiae	2 g/kg/diet	DC	Neg	NI	NI	+	9
Lactobacillus brevis	5×10^9 CFU/pig/day[f]	NE	Neg	+	+	NI	15
Enterococcus faecium	Sows = 10^6 g/diet[g] Pigs = 10^5 g/diet	NE	Neg	NR	+	NI	13
S. cerevisiae	3. 2.5 g/kg/diet 5 g/kg/diet 10 g/kg/diet 20 g/kg/diet	NE	Neg & Pos	+	NR	+	12
S. cerevisiae	0.125% of diet combination[h]	NE	Neg & Pos	+	NR	+	11
Lactobacillus acidophilus *E. faecium* *Bacillus licheniformis* *B. subtilis*	Bolus = 10^9 CFU/L[i] 0.05% of diet combination	NE	Neg & Pos	NR	NR	+	14

[a]Challenge type: DC, direct challenge; NE, natural exposure; PC, pathogen challenge.
[b]Control used for the studies: Neg, negative control group containing no DFM and no low-dose antibiotics (LDAs); Pos, positive control (provided LDAs).
[c]Impact of the study: +, positive impact; NI, no impact; NR, not reported.
[d]Fed as a combination of *L. plantarum* and lactulose at 15 ml/kg diet.
[e]Fed as a combination of *B. subtilis* and spray-dried porcine plasma at 1.0 g/kg of diet.
[f]DFM supplemented via added milk.
[g]Viable cells/g of diet, supplemented for 90 days of gestation and 56 days following parturition.
[h]DFM supplemented at 0.125% of diet in conjunction with yeast cell wall supplement at 0.2% of diet.
[i]*L. acidophilus* and *E. faecium* were administered as a one-time bolus, whereas *B. licheniformis* and *B. subtilis* were supplemented via the diet.

L. plantarum (7), *P. acidilactici* and/or *S. cerevisiae* (8), or *B. subtilis* (6) did not result in an improvement in animal performance [BW, average daily gain (ADG), gain:feed ratio (G:F), and rate of diarrhea] during an ETEC challenge.

Direct immune challenge. Although limited, a few studies have evaluated the supplementation of *S. cerevisiae* or derivatives of *S. cerevisiae*. Significant alterations were observed in the immune response of pigs supplemented DFMs relative to control$^{-DFM-LDA}$ pigs. Although not statistically significant, there was a tendency ($P \leq 0.09$) for supplementation of hydrolyzed yeast (*S. cerevisiae*) to increase IgG and IgM antibodies in serum-binding keyhole limpet hemocyanin, suggesting an activation of the innate immune response; there was no difference in the cytokine pig-major acute phase protein/ITIH4 (9). Supplementation of live *S. cerevisiae* to pigs during a lipopolysaccharide (LPS) challenge reduced peak production of IL1B and IL6, increased peak production of TNFA, and increased amplitude persistence of IFNG when compared with control$^{-DFM-LDA}$ pigs (10). There was also a decrease (no statistical information presented) in mortality between the *S. cerevisiae*–supplemented pigs when compared with control$^{-DFM-LDA}$ pigs (one pig in the *S. cerevisiae* treatment group and four pigs in the control$^{-DFM-LDA}$ treatment group) (10).

Natural exposure. Weaning exposes pigs to multiple stressors (new environment, new cohorts, new diet, and new pathogens) that can weaken the immune system. Because this transition period is a major health hurdle, numerous trials have investigated the role of DFMs as a means to supplement/boost the immune response and animal performance prior to and/or during weaning. Again, with numerous trials come conflicting results. Supplementation of *S. cerevisiae* to newly weaned pigs for a period of 5 weeks resulted in growth performance (ADG), average daily feed intake, and G:F similar to that of pigs fed an LDA and greater than that of control$^{-DFM-LDA}$ pigs (11). Although there were enhancements to animal performance (ADG, average daily feed intake, and G:F), there was little to no effect on variables of complete blood cell count between the treatments and no difference between the LDA treatment group and controls (11). Supplementation of the diets with yeast culture containing *S. cerevisiae* and numerous DFM fermentation products to nursery pigs resulted in ADG similar to that seen with LDA supplementation, and both were greater than in the control$^{-DFM-LDA}$ pigs, with no impact on immune response (12). *Enterococcus faecium* supplemented to weaned pigs had no impact on growth performance when compared with control$^{-DFM-LDA}$ pigs (13). There was (though not statistically different) a tendency in *E. faecium*–supplemented pigs toward improved G:F and a reduction in overall incidence of diarrhea when compared with control$^{-DFM-LDA}$ pigs. Dietary supplementation with *Lactobacillus acidophilus* at the time of weaning resulted in no difference in performance when compared to both the LDA-supplemented pigs and the control$^{-DFM-LDA}$ pigs (14). Supplementation of *Lactobacillus brevis* (dam and piglet) during lactation had no effect on weaning performance when compared with control$^{-DFM-LDA}$ pigs. Aside from performance variables, trials have also reported numerous/a lack of alterations to the gastrointestinal morphology, intestinal integrity, immune response, and bacterial community. *L. brevis* supplementation did decrease *E. coli* concentrations in the jejunum when compared with control$^{-DFM-LDA}$ pigs (15), whereas *S. cerevisiae* supplementation to newly weaned pigs did not result in alterations to the gastrointestinal morphology or the ileal bacterial community (11).

Overall, results of the pathogen and endotoxin challenge studies suggest that supplementing DFMs to pigs can bolster health during the stressful period of weaning (**Table 2**). These studies rather consistently indicate that supplementation of DFMs does modulate the immune response. Whereas there is consistency in the modulation of the immune response, there was an

enormous amount of variation in the immune system variables analyzed. Regarding the natural challenge, these studies provide a somewhat more convoluted picture (**Table 2**). Although there are conflicting reports, the natural challenge studies do provide some very intriguing results. Three of the natural exposure studies incorporated a positive control group (supplemented LDA-supplementation). This allowed for the comparison between DFM and LDA supplementation (11, 12, 14). Across all three studies, the DFM treatment groups were similar to the positive control groups. Based upon these results, across the three different challenge models, the data strongly indicate that DFM supplementation to swine may be a viable replacement for LDAs.

Poultry

The poultry industry has also devoted a great deal of time, energy, and resources to evaluating the use of DFMs. Thus, a great deal of data related to the impact of DFMs on the innate immune response in poultry has been reported. The majority of these studies used natural exposure and pathogen challenge models to define the role of DFMs in poultry; few studies have incorporated the direct immune challenge model (**Table 3**).

Pathogen challenge. The majority of the pathogen challenge research in poultry health has been directed toward avian coccidiosis, which is the major parasitic disease within the poultry industry, causing reduced growth, reduced egg production, decreased feed efficiency, and mortality. DFM supplementation has produced consistent results in terms of decreased fecal shedding of coccidiosis oocysts (*Eimeria acervulina* or *Eimeria tenella*). Supplementation of *Lactobacillus*-based DFM reduced *E. acervulina* oocysts following an oral challenge (10,000 and 20,000 sporulated oocysts) (16, 17). Supplementation of *P. acidilactici*– and *S. cerevisiae boulardii*–based DFM reduced *E. acervulina* or *E. tenella* oocysts following an oral challenge (5,000 or 10,000 sporulated oocysts) (18, 19). *P. acidilactici* and *S. cerevisiae boulardii* supplementation also resulted in an increase in BW gain when compared with broilers in the control$^{-\text{DFM-LDA}}$ group (19). Also, DFM supplementation during a coccidiosis oral challenge can modulate the immune response. *P. acidilactici*– and *S. cerevisiae boulardii*–based DFM supplemented at a rate of 0.1% of diet resulted in elevation of antibody response to the recombinant coccidial antigen 3-1E when compared with a control$^{-\text{DFM-LDA}}$ group (18); however, there was no improvement in growth performance between the groups (17). Supplementation of *Lactobacillus*-based DFM resulted in an increase in intestinal intraepithelial lymphocyte expression of surface markers CD3, CD4, CD8, and αβTCR during a coccidiosis oral challenge when compared with a control$^{-\text{DFM-LDA}}$ group (16). In addition to these alterations, *Lactobacillus*-based DFM supplementation increased IFNG (3 days postinfection) and intestinal IL-2 during an *E. acervulina* challenge when compared with in control$^{-\text{DFM-LDA}}$ chickens, suggesting an enhanced resistance to *E. acervulina* (17).

Direct immune challenge. DFM supplementation during a direct immune challenge has also had a significant impact on performance and immune response (**Table 3**). Supplementation of *Lactobacillus casei*, *L. acidophilus*, or *Scytalidium acidophilum* during an immunization challenge resulted in improved BW, BW gain, and feed intake (20). *L. casei* and *L. acidophilus* supplementation improved BW and BW gain when compared with control$^{-\text{DFM-LDA}}$ and was similar to positive control containing LDA (20). *S. acidophilum* supplementation improved BW, BW gain, and feed intake when compared with a control$^{-\text{DFM-LDA}}$ group and was similar to positive control containing antibiotics (20). In terms of the immune response, supplementation of *L. casei* (inclusion rate of 425 mg/kg of dry matter in starter diet and 332 mg/kg of dry matter in finisher diet) and

Table 3 Impact of direct-fed microbial (DFM) supplementation on poultry immune response, animal health, and performance

DFM	Dosage	Challenge[a]	Neg/Pos Con[b]	Immune[c]	Health	Performance	Reference
Pediococcus acidilactici *Saccharomyces cerevisiae*	0.1 g/kg/feed 1.0 g/kg/feed 10 g/kg/feed	PC	Neg	NI	+	NI	18
P. acidilactici *S. cerevisiae*	*Exp. 1* 0.1% of diet	PC	Neg	+	+	+	19
	Exp. 2 0.1% of diet 0.2% of diet						
Lactobacillus *Streptococcus*	2. 1 g/kg/diet	PC	Neg	+	+	NR	16
Lactobacillus	1 g/kg/diet	PC	Neg	+	+	NR	17
Lactobacillus casei	75/56 mg/kg/DM (low) 425/332 mg/kg DM (high)	DC	Neg + Pos	+	NR	+	20
Lactobacillus acidophilus	77/62 mg/kg/DM (low) 457/368 mg/kg/DM (high)						
Scytalidium acidophilum	68/54 mg/kg/DM (low) 412/326 mg/kg/DM (high)						
Lactobacillus reuteri *Enterococcus faecium* *Bifidobacterium animalis* *P. acidilactici*	7×10^8 CFU/kg/diet	DC	Neg	NR	NR	+	21
L. reuteri *E. faecium* *B. animalis* *P. acidilactici* *Lactobacillus salivarius*	10^8 CFU/kg/diet 10^9 CFU/kg/diet 10^{10} CFU/kg/diet	NE	Neg & Pos	NI	NR	+	25
Lactobacillus *Bacillus cereus*	DFM 1×10^{12} CFU/kg/diet combination of DFM + ASP[d]	NE	Neg	+	NR	NR	27
Lactobacillus bulgaricus	20 mg/kg/feed- 2×10^6 40 mg/kg/feed- 4×10^6 60 mg/kg/feed- 6×10^6 80 mg/kg/feed- 8×10^6	NE	Neg	+	NR	+	26
S. cerevisiae	2.5 g/kg/feed 5 g/kg/feed 7.5 g/kg/feed	NE	Neg	+	NR	+	24

(*Continued*)

Table 3 (*Continued*)

DFM	Dosage	Challenge[a]	Neg/Pos Con[b]	Immune[c]	Health	Performance	Reference
L. reuteri *Bacillus subtilis* *S. cerevisiae*	0.1% of feed (*L. reuteri*) 0.1% of feed (combination)	NE	Neg + Pos	+	NR	NI	23
L. acidophilus *Bifidobacterium bifidum* *Streptococcus faecalis*	0.5 mL bolus 10^6	NE	Neg	+	NR	NR	22

[a]Challenge type: DC, direct challenge; NE, natural exposure; PC, pathogen challenge.
[b]Control used for the studies: Neg, negative control group containing no DFM and no low-dose antibiotics (LDAs); Pos, positive control (provided LDAs).
[c]Impact of the study: +, positive impact; NI, no impact; NR, not reported.
[d]*Astragalus membranaceus* polysaccharide (ASP).

L. acidophilus (inclusion rate of 77 mg/kg of dry matter in starter diet and 62 mg/kg of dry matter in finisher diet matter) increased KLH-specific antibody concentrations when compared to the control$^{-DFM-LDA}$ group (20). During a repeated LPS challenge (21), supplementation of a multistrain DFM was able to lessen the negative impact of LPS-induced anorexia compared with an LPS-challenged control$^{-DFM-LDA}$ group (21). Although not statistically different, broiler chicks supplemented with the multistrain DFM had a tendency for increased BW gain and total BW following the LPS challenge when compared with LPS-challenged control$^{-DFM-LDA}$ chicks (21).

Natural exposure. DFM supplementation also can cause significant alterations to the immune response in the absence of a controlled immune insult (**Table 3**). Supplementing chicks with a three-strain DFM containing *L. acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus faecalis* increased serum and gut antibodies reactive to foreign antigens (tetanus toxoid, α-toxin of *Clostridium perfringens*, and bovine serum albumin) (22). Specifically, the three-strain DFM increased serum IgG and IgM antibodies (reactive to both the foreign antigens tetanus toxoid and α-toxin) when compared to chicks within the control$^{-DFM-LDA}$ group. Supplementation of a combination of *Lactobacillus reuteri*, *B. subtilis*, and *S. cerevisiae* to broiler chicks increased white blood cell counts when compared with both positive control (with LDA) and control$^{-DFM-LDA}$ broiler chicks and increased monocyte concentration when compared with positive control chicks (23). A combination of the three strains also resulted in increased concentrations of IgA, IgG, and IgM antibodies when compared with controls, suggesting a possible stimulation of the humoral immune response. The supplementation of *S. cerevisiae* and fermentation products to broiler chicks resulted in increased serum lysozyme content, possibly improving resistance to bacterial infection. Furthermore, supplementation increased antibody titers to Newcastle disease antibodies and IgM antibodies when compared with control$^{-DFM-LDA}$ broiler chicks, which may be beneficial to the humoral and mucosal immunity (24). Supplementation of *Lactobacillus bulgaricus* to broiler chicks increased antibodies to Newcastle disease antigen and white blood cells when compared with the control$^{-DFM-LDA}$ broiler chicks (25). When supplementing broiler chicks with *Astragalus membranaceus* polysaccharide and a combination of *Lactobacillus* and *Bacillus cereus*, there was an increase in Newcastle disease antibody titer and increased ANAE+ T lymphocytes when compared with control$^{-DFM-LDA}$ broiler chicks (26). This combination supplementation also resulted in increased immune organ

relative weights when compared with the control$^{-DFM-LDA}$ broiler chicks (26). In contrast to these alterations in IgA, IgG, and IgM, supplementation of a five-strain DFM (*L. reuteri*, *E. faecium*, *Bifidobacterium animalis*, *P. acidilactici*, and *Lactobacillus salivarius*) to broiler chicks did not alter the concentrations of IgA, IgG, and IgM when compared with control$^{-DFM-LDA}$ broiler chicks (27).

As with the swine DFM studies, the pathogen and direct immune challenge studies provide rather consistent results in regard to immune response and animal health. All pathogen and direct immune studies indicated some benefit to DFM supplementation in comparison to the control$^{-DFM-LDA}$ groups. Regardless of the challenge model, the improvement in animal performance was rather mixed; however, some of this could be attributed to several studies not reporting information related to animal performance (**Table 3**) (16, 17, 22, 26). For the studies that reported animal performance, 75% (6 of 8 studies; **Table 3**) reported that DFM supplementation did result in an improvement when compared with control$^{-DFM-LDA}$ broilers. Three of the studies used both a positive and a control$^{-DFM-LDA}$ group; within these studies, there was little to no difference between DFM-supplemented broilers and the positive control. Overall, the results of the poultry DFM immune studies are the most convincing in terms of DFM supplementation as a replacement for LDAs.

Beef

Bovine respiratory disease (BRD) is the most devastating disease facing the beef industry, with an estimated annual economic impact of ~$600 million (28). BRD has a multifactorial etiology that includes stress, viral pathogens, and bacterial pathogens, making it very difficult to combat. The majority of BRD cases occur during the receiving period at the feedlot. Thus, the main area of interest for DFMs is during the receiving period. For cattle, the receiving period is the first 28–56 days at the feedlot. During this time, cattle that have endured the stress of relocation are exposed to additional stressors, such as comingling with unfamiliar cattle, changes in environment, diet modification, and exposure to new/novel pathogens. However, unlike in the poultry and pork industry, no LDAs are used to mitigate the effects of BRD in the beef industry; thus, although research has been directed at the use of DFMs to improve the health status of cattle during this feedlot receiving period, this research effort is not as diverse as that for swine and poultry.

Pathogen challenge. In terms of BRD challenges to investigate the role of DFM viability, only one trial was identified (29). During an infectious bovine respiratory virus challenge, steers supplemented with *S. cerevisiae* demonstrated an increase in dry matter intake (DMI) on days 3, 10, and 13 postinfection compared with control steers. This increase resulted in the tendency for *S. cerevisiae*–supplemented steers to have less BW loss when compared with the controls (29).

Direct immune challenge. Very few beef cattle trials have incorporated the direct immune challenge model to evaluate the impact of DFM supplementation on the immune response of cattle. Although limited in number, these studies have reported significant alterations to both immune and metabolic responses during a direct immune challenge. Supplementation of *S. cerevisiae* to newly received steers results in a modification of metabolism (30). Heifers supplemented with *S. cerevisiae* for a period of 36 days had greater concentrations of insulin, increased blood urea nitrogen (BUN), and, although not statistically different, a tendency toward decreased non-esterified fatty acids (NEFA) prior to LPS challenge when compared with control heifers (2 h prior to LPS challenge). Furthermore, modification of the metabolic response was observed following the LPS challenge, and heifers provided with the supplement had decreased NEFA

concentrations and increased BUN concentrations when compared with control heifers (30). In the same study, researchers also reported a suggested relationship between DFM supplementation and improved ADG from arrival until LPS challenge when compared with control heifers. In two similar trials, during an LPS challenge, supplementation of *S. cerevisiae* to newly received steers resulted in an increase in glucose and BUN concentrations and decreased NEFA concentrations compared with controls (31, 32). A few studies have reported the effect of *S. cerevisiae* supplementation on the innate immune response. Though not statistically significant, it was reported that supplementation of *S. cerevisiae* to newly received cattle resulted in a tendency for decreased sickness scores (33), cortisol (33–35), and white blood cells and lymphocytes (33). In terms of the proinflammatory cytokines, two trials indicated decreased concentrations of IFNG and TNFA compared with control cattle (34, 35). However, another trial indicated that supplementation of *S. cerevisiae* increased the proinflammatory cytokines TNFA and IFNG compared with control steers (34). All three trials are fairly similar in design, LPS challenge (source and dose), duration, and experimental units. However, the possible variation in results could be a result of animal-to-animal variation, difference in management of cattle prior to the trial, plane of nutrition prior to the trials, and differences in the specifics and dosage of *S. cerevisiae*.

Natural exposure. Similar to the direct immune challenge, few DFM studies have investigated the role of DFMs in improving cattle health during the receiving period (defined as the first 28 days after arrival at the feedlot). Supplementation of a multistrain DFM to newly received steers during the receiving period had no effect on percentage of steers treated for sickness compared with control steers (36). Furthermore, DFM supplementation alone did not improve growth performance. However, there was a connection between DFM supplementation and increasing concentrations of degradable intake protein (portion of dietary protein that can be degraded in the rumen by microbial fermentation). As degradable intake protein increased from 80% to 120% of recommended degradable intake protein (10% increments), there was a cubic increase in ADG. Supplementation of *S. cerevisiae* to steers upon arrival at the feedlot did not improve the percentage of cattle identified as morbid but did increase DMI over the 56-day receiving period when compared with that of controls; there was no difference in BW, ADG, or G:F ratio (35). In another study, *S. cerevisiae* supplementation in conjunction with administration of antibiotics upon arrival at the feedlot did result in decreased rates of cattle treated once or more for BRD and a decreased odds ratio for "likely to treat" for BRD when compared with control heifers (37). However, there was no impact of supplementation on DMI, ADG, or G:F when compared with the control cattle (37). In a three-part study, *S. cerevisiae* had no impact on morbidity, mortality, or growth performance when compared with control cattle (29).

The results of the direct immune challenges and the sole pathogen challenges provide strong evidence that there is some alteration to both the immune and metabolic response of supplemented cattle when exposed to an immune challenge. These alterations include changes in hormone concentrations (insulin and cortisol), metabolite concentrations (glucose, BUN, and NEFAs), proinflammatory cytokines (TNFA, IFNB, and IL-6), and cattle performance (increased DMI and tendency for decreased BW owing to challenge). However, the data are not nearly as clear in the studies that used natural exposure. The majority of the natural exposure trials indicated that DFM supplementation had little to no effect on animal performance and animal health (morbidity and mortality). Although these natural exposure trials do not provide a clear picture of the role of DFMs in improving health, there is one major difference between cattle studies and those performed on swine and poultry: the source of the animals. Unlike swine and poultry studies, in which all of the animals for the studies originated from a single source (herd) with known genetics, nutrition, and management background, the cattle used in all of these trials were purchased from

sale barns or order buyers. Thus, very little is known about the prior management, health, and genetic background of these cattle. Even the cattle's chronological age is likely unknown.

UNDERSTANDING THE LACK OF CONSISTENCY IN DIRECT-FED MICROBIAL TRIALS

So, one must ask, why are there conflicting reports related to DFM supplementations? To explain this lack of consistency, we must look at LDA function in the gastrointestinal tract (GIT) of livestock and how this differs from that of DFMs. We must also look at the difference between the distinct digestive systems represented (monogastric, modified-monogastric, and ruminant). And finally, we must look directly at the DFM studies and the amount of variation within them.

Livestock agriculture uses LDAs as a means to improve animal health via disease prevention and treatment. The improvement in animal performance is driven by this improvement in health, as supplementation of LDAs in a germ-free environment itself does not enhance animal performance (38, 39). The current explanation for LDA improvement in livestock health is via a direct effect on the GIT microbiota. This direct interaction with the microbiota is proposed to occur via three modes of action: (*a*) prevention of subclinical infection (opportunistic pathogens), (*b*) retardation of the production of growth-depressing microbial products, and (*c*) regulation of microbial use of nutrients (40). Furthermore, research has also suggested that LDA supplementation enhances absorption of nutrients (38). This reduction/enhancement of the GIT microbiota and alteration of the GIT are believed to enhance animal health and performance upon LDA supplementation.

Unlike LDAs, which have a direct impact on the GIT microbiota (antimicrobial or bacteriostatic), DFMs must improve animal health via an indirect modulation. The mode of action for DFMs includes (*a*) competitive exclusion, (*b*) improved digestion and nutrient utilization, (*c*) immunostimulation, and (*d*) enhancement of GIT microbiota. Competitive exclusion is defined as the protective effect of nonpathogenic bacteria limiting the colonization of pathogens. The DFMs can achieve this competitive exclusion via competitive attachment to the intestinal wall, thereby preventing colonization by pathogens (41, 42). Supplementation of DFMs may also improve host digestion. DFMs can provide digestible proteins, enzymes, vitamins, and other important cofactors to the host GIT. These coproducts can reduce the GIT pH and enhance digestion of carbohydrates, proteins, and fats (42). Several studies have also reported that supplementation of DFMs can have a beneficial effect on commensal bacteria within the GIT (43). Supplementation of a multistrain DFM significantly increased *Bifidobacterium* spp., *Lactobacillus* spp., and Gram-positive cocci counts (27). Given all of these roles, there is strong evidence that DFMs may be an alternative to LDA supplementation.

There are three unique digestive systems within livestock agriculture: the monogastric (swine), modified-monogastric (poultry), and ruminant (cattle) systems. Swine are a true monogastric, with a system similar to the digestive system of humans. Poultry, although classified as a monogastric, actually have a unique digestive system (modified-monogastric or avian system), which is drastically different from that of swine. And finally, the ruminant digestive system is distinct from the monogastric owing to its four-chambered stomach. This four-chambered stomach allows for the digestion via bacterial fermentation of feedstuffs that are high in cellulose. With three distinct digestive systems comes a unique and complicated GIT microbiome. With the recent development of next-generation sequencing, scientists have been able to unlock a greater part of the microbiome diversity. The swine GIT is a rich microbiome with approximately 1,000 different bacterial species (44). Evaluations of the pig microbiome from two commercial facilities identified 171 genera from fecal samples (45). Firmicutes and Bacteroidetes phyla accounted for 90% of the total sequences. Of these 171 genera, 15 represented over 59% of the

total sequences (*Prevotella*, *Anaerobacter*, *Streptococcus*, *Lactobacillus*, *Coprococcus*, *Sporacetigenium*, *Megasphaera*, *Subdoligranulum*, *Blautia*, *Oscillibacter*, *Faecalibacterium*, *Pseudobutyrivifrio*, *Dialister*, *Sarina*, and *Roseburia*). The poultry GIT is also very rich, with over 900 different bacterial species represented (46). Phylogeneric and statistical analysis of 16S rRNA gene sequences of the intestinal microbiome has revealed 13 phyla of bacteria; Firmicutes, Bacteroidetes, and Proteobacteria accounted for 90% of the intestinal bacteria (47). The most prominent genera identified were *Clostridium*, *Ruminococcus*, *Lactobacillus*, and *Bacteroides*. The complexity of the swine and poultry microbiome can be overshadowed by the complexity of the ruminant. The rumen is a complex microbial ecosystem containing bacteria ($\sim 10^{10}$), phages ($\sim 10^{9}$), protozoa (10^{8}), archaea ($\sim 10^{7}$), and fungal spores (10^{3}) (48, 49). Estimates of microbial species present within the rumen range from 300–400 (50) to as high as 12,000 (49). Fecal samples for dairy cattle displayed a large diversity within the bacteria species and genera (51). The prominent genera identified from all the cattle sampled were *Clostridium*, *Bacteroides*, *Porphyromonas*, *Ruminococcus*, *Alistipes*, *Lachnospiraceae–like*, *Prevotella*, *Lachnospira*, *Bacteroidales*, *Akkermansia*, and *Enterococcus* spp. (51).

Regardless of the species, this microbiome is dynamic, constantly changing in response to age, exposure to microbes, diet components, and numerous other factors. Furthermore, we are still trying to unlock the nature of the symbiotic relationship existing between the GIT microbiome and the host. Research has indicated that the GIT microbiome plays a vital role in nutrient utilization, regardless of the digestive system. Intestinal bacteria are capable of converting otherwise indigestible polysaccharides, oligosaccharides, and disaccharides into short chain fatty acids via fermentation of the compositional sugars (52). The microbiome plays an important role in the early development of the GIT. Broilers raised in a germ-free environment had reduced small intestine and cecum weights and thinner organ walls when compared with broilers raised in a conventional system (53, 54). There is also very strong evidence that the microbiome has a significant effect on immune system development. Mice raised in a germ-free environment exhibited defects in gut-associated lymphoid tissue (55, 56) and BOX 1 antibody production as well as altered Peyer's patches and mesenteric lymph nodes when compared with mice raised in pathogen-free conditions (57, 58). Also, germ-free animals have impaired development and maturation of isolated lymphoid follicles, which appear to develop normally when GIT microbes are added (59).

This rich and ever-changing microbiome will be a major component in determining the efficacy of DFMs. Scientists are just recently starting to gain a better understanding of the role of the microbiome and its symbiotic relationship with the host animal. As we gain insight into the relationship between the GIT microbiome and the host, we will be better able to identify and determine appropriate dosage, route of administration, and duration of supplementation of DFMs.

Aside from the difference between LDAs and DFMs and the diversity of the GIT microbiome, the other major factor affecting the consistency of DFM research is the variability in the studies conducted. Within all species, this lack of consistency includes the DFM used, as well as its administration route, duration, and dosage/concentration. Even within the studies evaluated in this review, there is an enormous amount of variability in the DFM used (**Tables 5–7**).

Fourteen trials used a combination (2 DFMs) or a multistrain (3 or more) DFM. The yeast *S. cerevisiae* was used in 20 studies, either as the sole DFM or in combination with other DFMs/coproducts. The most prominent genus of bacteria used was *Lactobacillus* (used in 11 studies). For many studies there was also uncertainty as to the specific genus and species of the bacteria used. Several simply reported a genus, and a few studies mentioned only the trade name of the DFM (occasionally making it very difficult to identify the specific DFM).

Although the majority of the studies within this review provided the DFMs via feed (**Tables 2–4**), there was variability. The method for delivery ranged from supplementation of the dam with a DFM, direct single-dose administration (oral bolus), direct supplementation via

Table 4 Impact of direct-fed microbial (DFM) supplementation on beef cattle immune response, animal health, and performance

DFM	Dosage	Challenge[a]	Neg/Pos Con[b]	Immune[c]	Health	Performance	Reference
Saccharomyces cerevisiae	*Exp. 3* 0.75% of DM	PC	Neg	NR	+	+	29
S. cerevisiae	0.5 g/hd/day 5.0 g/hd/day	DC	Neg	+	NR	NR	34
S. cerevisiae	0.5 g/hd/day 5.0 g/hd/day	DC	Neg	NR	NR	+	31
S. cerevisiae	4 g 45 kg/diet	DC	Neg	NR	NR	+	32
S. cerevisiae	4 g 45 kg/diet	DC	Neg	+	NR	NR	33
S. cerevisiae C-wall[d]	2.5 g/hd/day 2.5 g/hd/kg	DC	Neg	NR	NR	+	34
S. cerevisiae	*Exp. 1* – mass med. 1 g/hd/day *Exp. 2* – mass med. 1.0 g/hd bolus (one time) 0.5 g/hd/day *Exp. 3* – No mass med. 0.5 g/hd/day	NE	Neg	NR	+	NI	37
S. cerevisiae	*Exp. 1* 10^{10} CFU/g at 5 g/hd/day combination[e]	NE	Neg	+	NR	+	35
	Exp. 2 10^{10} CFU/g at 5 g/hd/day combination[e]	DC	Neg				
Lactobacillus acidophilus *Enterococcus faecium* *Pediococcus acidilactici* *Lactobacillus brevis* *Lactobacillus plantarum*	80% DIP[f] + 10^9 CFU/hd/day 90% DIP + 10^9 CFU/hd/day 100% DIP + 10^9 CFU/hd/day 110% DIP + 10^9 CFU/hd/day 120% DIP + 10^9 CFU/hd/day	NE	Neg	NR	NI	+	36
S. cerevisiae	*Exp. 1* 0.75% of DM *Exp. 2* 0.75% of DM 1.25% of DM 1.50% of DM	NE NE	Neg Neg	NR	NI	NI	29

[a]Challenge type: DC, direct challenge; NE, natural exposure; PC, pathogen challenge.
[b]Control used for the studies: Neg, negative control group containing no DFM and no low-dose antibiotics (LDAs); Pos, positive control (provided LDAs).
[c]Impact of the study: +, positive impact; NI, no impact; NR, not reported.
[d]Proprietary product: Lesaffre Feed Additives.
[e]Combination treatment included supplementation of *S. cerevisiae* at 1,010 CFU/g at 5 g/hd/day in addition to 5 g/hd/day of yeast cell wall supplementation.
[f]DFM supplementation was evaluated in combination with varying concentrations of digestible intake proteins. For each concentration of degradable intake protein (DIP) + DFM, there was a control with only elevated concentrations of DIP.

Table 5 Dosage, duration of direct-fed microbial (DFM) supplementation, age of animals investigated, number of animals (n), type of immune challenge, and inclusion of negative and/or positive control (Neg/Pos Con) groups of studies investigating the role of DFMs in the health of swine

DFM	Dosage	Duration (days)	Age (days)	n	Challenge[a]	Neg/Pos Con[b]	Reference
Pediococcus acidilactici *Saccharomyces cerevisiae*	10^9 CFU/500 g/feed 10^9 CFU/500 g/feed combination	57	1[c]	80[d]	PC	Neg & Pos	4
S. cerevisiae	Sows = 1 g/kg/diet Pigs = 1 g/kg/diet to 21 days Pigs = 5 g/kg/diet to 22–42 days	70	1[e]	38[f]	PC	Neg	5
P. acidilactici *S. cerevisiae*	10^9 CFU/kg/diet 10^9 CFU/kg/diet combination	42	1	150[g]	PC	Neg & Pos	8
Lactobacillus plantarum	2×10^{10} CFU/hd/day combination[h]	18	25	72	PC	Neg	7
Bacillus subtilis	1.2×10^9 CFU 0.5 g/kg/feed combination[i]	14	17	108	PC	Neg & Pos	6
S. cerevisiae	182 g/ton/diet	17	25	30	DC	Neg	10
S. cerevisiae	2 g/kg/diet	28	25	120	DC	Neg	9
Lactobacillus brevis	5×10^9 CFU/pig/day[j]	28	1	190	NE	Neg	15
Enterococcus faecium	Sows = 10^6 g/diet[k] Pigs = 10^5 g/diet	146	1	176[10]	NE	Neg	13
S. cerevisiae	3. 2.5 g/kg/diet 5 g/kg/diet 10 g/kg/diet 20 g/kg/diet	21	28	192	NE	Neg & Pos	12
S. cerevisiae	0.125% of diet combination[l]	35	27	320	NE	Neg & Pos	11
Lactobacillus acidophilus *E. faecium* *Bacillus licheniformis* *B. subtilis*	Bolus = 10^9 CFU/L[m] 0.05% of diet combination	34	21	188	NE	Neg & Pos	14

[a]Challenge type: DC, direct challenge; NE, natural exposure; PC, pathogen challenge.

[b]Control used for the studies: Neg, negative control group containing no DFM and no low-dose antibiotics (LDAs); Pos, positive control (provided LDAs).

[c]DFM was supplemented to gilts 28 days prior to parturition, and then supplemented to the pigs until 29 days parturition.

[d]Used 40 gilts and then 40 pigs from the litters of the gilts.

[e]DFM was supplemented to sows 28 days prior to parturition, then supplemented to pigs and sows for 21 days after parturition.

[f]Used 4 sows and 34 pigs from the 4 litters.

[g]Used 30 sows and 120 pigs from the 30 litters.

[h]Fed as a combination of *L. plantarum* and lactulose at 15 ml/kg/diet.

[i]Fed as a combination of *B. subtilis* and spray-dried porcine plasma at 1.0 g/kg/diet.

[j]DFM supplemented via added milk.

[k]Viable cells/g of diet, supplemented for 90 days of gestation and 56 days following parturition.

[l]DFM supplemented at 0.125% of diet in conjunction with yeast cell wall supplement at 0.2% of diet.

[m]*L. acidophilus* and *E. faecium* were administered as a one-time bolus, whereas *B. licheniformis* and *B. subtilis* were supplemented via the diet.

Table 6 Dosage, duration of direct-fed microbial (DFM) supplementation, age of animals investigated, number of animals (n), type of immune challenge, and inclusion of negative and/or positive control (Neg/Pos Con) groups of studies investigating the role of DFMs in the health of poultry

DFM	Dosage	Duration (days)	Age (days)	n	Challenge[a]	Neg / Pos Con[b]	Reference
Pediococcus acidilactici *Saccharomyces cerevisiae*	0.1 g/kg/feed 1.0 g/kg/feed 10 g/kg/feed	10	1	90	PC	Neg	18
P. acidilactici	*Exp. 1* 0.1% of diet	20	1	70	PC	Neg	19
S. cerevisiae	*Exp. 2* 0.1% of diet 0.2% of diet	20	1	120	PC	Neg	
Lactobacillus *Streptococcus*	2.1 g/kg/diet	42	1	100	PC	Neg	16
Lactobacillus	1 g/kg/diet	33	1	100	PC	Neg	17
Lactobacillus casei	75/56 mg/kg/DM (low) 425/332 mg/kg DM (high)	42	1	920	DC	Neg + Pos	20
Lactobacillus acidophilus	77/62 mg/kg/DM (low) 457/368 mg/kg/DM (high)						
Scytalidium acidophilum	68/54 mg/kg/DM (low) 412/326 mg/kg/DM (high)						
Lactobacillus reuteri *Enterococcus faecium* *Bifidobacterium animalis* *P. acidilactici*	7×10^8 CFU/kg/diet	21		308	DC	Neg	21
L. reuteri *E. faecium* *B. animalis* *P. acidilactici* *Lactobacillus salivarius*	10^8 CFU/kg/diet 10^9 CFU/kg/diet 10^{10} CFU/kg/diet	42	1	500	NE	Neg & Pos	25
Lactobacillus *Bacillus cereus*	DFM 1×10^{12} CFU/kg/diet combination of DFM + ASP[c]	42	1	240	NE	Neg	27
Lactobacillus bulgaricus	20 mg/kg/feed- 2×10^6 40 mg/kg/feed- 4×10^6 60 mg/kg/feed- 6×10^6 80 mg/kg/feed- 8×10^6	36	1	210	NE	Neg	26
S. cerevisiae	2.5 g/kg/feed 5 g/kg/feed 7.5 g/kg/feed	42	1	960	NE	Neg	24
L. reuteri *B. subtilis* *S. cerevisiae*	0.1% of feed (*L. reuteri*) 0.1% of feed (combination)	35	1	800	NE	Neg + Pos	23
L. acidophilus *Bifidobacterium bifidum* *Streptococcus faecalis*	0.5 mL bolus 10^6	Not reported	1	14	NE	Neg	22

[a]Challenge type: DC, direct challenge; NE, natural exposure; PC, pathogen challenge.
[b]Control used for the studies: Neg, negative control group containing no DFM and no low-dose antibiotics (LDAs); Pos, positive control (provided LDAs).
[c]*Astragalus membranaceus* polysaccharide (ASP).

Table 7 Dosage, duration of direct-fed microbial (DFM) supplementation, age of animals investigated, number of animals (n), type of immune challenge, and inclusion of negative and/or positive control (Neg / Pos Con) groups of studies investigating the role of DFMs in the health of beef cattle

DFM	Dosage	Duration (days)	Age	n	Challenge[a]	Neg / Pos Con	Reference
Saccharomyces cerevisiae	*Exp. 3* 0.75% of DM	56	Unknown	24	PC	Neg	29
S. cerevisiae	0.5 g/hd/day 5.0 g/hd/day	28	~6 months	18	DC	Neg	34
S. cerevisiae	0.5 g/hd/day 5.0 g/hd/day	28	~6 months	18	DC	Neg	31
S. cerevisiae	4 g 45 kg/diet	28	~6 months	18	DC	Neg	32
S. cerevisiae	4 g 45 kg/diet	28	~6 months	18	DC	Neg	33
S. cerevisiae C-wall[b]	2.5 g/hd/day 2.5 g/hd/kg	36	Unknown	24	DC	Neg	34
S. cerevisiae	*Exp. 1* – mass med. 1 g/hd/day	21	Unknown	81	NE	Neg	37
	Exp. 2 – mass med. 1.0 g/hd bolus (one time) 0.5 g/hd/day	35	Unknown	277	NE	Neg	
	Exp. 3 – No mass med. 0.5 g/hd/day	35	Unknown	200	NE	Neg	
S. cerevisiae	*Exp. 1* 10^{10} CFU/g via 5 g/hd/day combination[c]	56	Unknown	184	NE	Neg	35
	Exp. 2 10^{10} CFU/g via 5 g/hd/day combination[c]	56	Unknown	24	DC	Neg	
Lactobacillus acidophilus *Enterococcus faecium* *Pediococcus acidilactici* *Lactobacillus brevis* *Lactobacillus plantarum*	80% DIP[d] + 10^9 CFU/hd/day 90% DIP + 10^9 CFU/hd/day 100% DIP + 10^9 CFU/hd/day 110% DIP + 10^9 CFU/hd/day 120% DIP + 10^9 CFU/hd/day	56	Unknown	192	NE	Neg	36
S. cerevisiae	*Exp. 1* 0.75% of DM	56	Unknown	160	NE	Neg	29
	Exp. 2 0.75% of DM 1.25% of DM 1.50% of DM	56	Unknown	101	NE	Neg	

[a]Challenge type: DC, direct challenge; NE, natural exposure; PC, pathogen challenge.

[b]Proprietary product: Lesaffre Feed Additives.

[c]Combination treatment included supplementation of *S. cerevisiae* at 1,010 CFU/g at 5 g/hd/day in addition to 5 g/hd/day of yeast cell wall supplementation.

[d]DFM supplementation was evaluated in combination with varying concentrations of digestible intake proteins. For each concentration of DIP + DFM, there was a control with only elevated concentrations of DIP.

mixture of diet, and complex combinations of supplementation. There was also variability in the duration of the DFM being supplemented. Beef cattle studies provided the most consistent timeframe, with an average length of supplementation of 40 days (minimum = 28 days; maximum = 56 days); this is mainly because the primary period of concern for beef is the receiving period (the first 28–56 days at the feedlot). The average length of supplementation for poultry trials was 32 days (minimum = 10 days; maximum = 42 days) and for swine was 45 days (minimum = 14 days; maximum = 146 days).

The dosage/concentration of DFMs was also highly variable (**Tables 5–7**). Within the reviewed studies, DFM dosage was reported as CFU/head/day, CFU/kg of the diet, DFM g/kg/feed, and even DFM mg/kg of the diet. Within several studies, the concentration of DFMs was also variable, with DFMs classified as "low concentrations" and "high concentrations" within the same studies.

There was also a large amount of variability in age at time of supplementation, in the animals themselves (from those in vertically integrated systems to those purchased at sale barns), and in variables of interest (primarily in terms of immune response). For the majority of the poultry studies, animal age was rather consistent, with the majority being within 24 h to 48 h of hatching. There was a little more inconsistency within the swine studies, with the age of supplementation ranging from 90 days prior to parturition to 21 days of age (the age at weaning). The cattle had the greatest age variation at the time of supplementation. Because of the structure of the beef industry, most of the trials simply identify the gender of the cattle, with no mention of actual age, and with three independent segments (cow/calf, stocker/backgrounder, and feedlot), the actual known age of cattle is limited at best. Thus, for the majority of these trials, one can only assume that the age ranged from 6 to 10 months.

The source and background of the animals used can be a large source of the variability of DFM trials. For the poultry and swine studies, all animals were sourced from vertically integrated (commercial or university) systems, in which genetics, nutrition, and management are similar for all animals studied. However, the beef industry lacks consistency in this regard as well. Most animals were procured from a sale barn or order buy. Thus, there is little information on genetic background, prior nutritional status, prior health status, and management prior to arrival (e.g., some cattle may have been exposed to a preconditioning program, which includes vaccinations, whereas others may have received no prior vaccinations). This extreme variability may explain a great deal of the variation in DFM supplementation when compared with supplementation for poultry and swine.

The specific parameters that each trial investigated also account for some of the variability observed. For several studies, the variable of interest was a simple production measurement, such as animal performance or rate/incidence of diarrhea, morbidity, or mortality. However, other studies investigated specific aspects of DFM modulation via evaluation of the immune (cytokines, antibodies, lymphocyte proliferation) and endocrine (cortisol and insulin) systems, direct impact on metabolic alterations (metabolites), and impact of GIT physiology.

CONCLUSION

There is strong evidence that DFM supplementation can have an impact on the immune response of livestock (regardless of the species) and potentially improve animal health and performance. However, we must also realize that no DFM will be a 1:1 replacement for LDAs, and there will be no one-size-fits-all solution; we must evaluate not only the DFMs but also the best management practices in conjunction with DFM supplementation. A common statement within most of the DFM studies is "further research is needed." This is a very accurate statement, but with one major caveat: This future research must use a systematic approach that investigates DFMs in

response to a direct immune challenge and a pathogen challenge, and then, in the natural exposure environment, must employ consistent dosages, durations, and routes of administration. Once we have a comprehensive and consistent evaluation of individual DFMs, we will be able to determine the effect of DFMs as a means to improve animal health.

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