Nutraceuticals
An Alternative Strategy for the Use of Antimicrobials

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KEYWORDS

- Health • Immune • Nutraceutical • Probiotic • Prebiotic • Phytonutrient
- Polyunsaturated fatty acid

KEY POINTS

- Nutraceutical is a term derived from nutrition and pharmaceutical. There are many compounds that improve immune responses and reduce the risk of disease through different mechanisms of action.
- Probiotics are the supplementation of viable microorganisms that offer potential health benefits to the animal. Probiotics are predominately supplemented to improve gastrointestinal health, rumen fermentation, or nutrient utilization.
- Prebiotics are a group of indigestible carbohydrates that function to improve the growth of commensal bacteria. Some fractions (eg, β-glucans and mannanoligosaccharides) have direct immunomodulatory, gram-negative pathogen binding, or hydrophobic mycotoxin absorption capacities.
- Phytonutrients are a group of compounds isolated from plants with potential therapeutic applications given their intrinsic antioxidative, anti-inflammatory, and antimicrobial properties.
- Polyunsaturated fatty acids influence the degree of inflammation. Generally, increasing the supplementation of omega-3 fatty acid sources decreases inflammation, whereas supplementation of omega-6 fatty acids increases inflammation. How much inflammation is needed at any specific physiologic stage is less understood in ruminant species.

INTRODUCTION

Public concerns regarding the potential association between zoonotic multidrug-resistant bacterial strains and antibiotic use in food animals has prompted significant regulatory changes to on-farm antimicrobial availability and use.[1] Producers and

veterinarians alike face increasingly limited options to therapeutically treat diseased animals. As a result, microbial and plant-based compounds and their derivatives received increased research and commercial attention in the past few decades. Nutraceuticals, as many of them are known, is a hybrid of nutrition and pharmaceuticals. They consist of natural compounds and/or microbes that offer potentially advantageous effects related to ruminant health and productivity, including improved feed efficiency, milk production, and disease resistance through immune modulation or decreased disease pressure.[1–3]

Nutraceuticals are a broad group of compounds that can be classified in several ways. Some of the more common classifications are based on the mechanism of action, chemical nature, or the feed source of the compound. In this review, we discuss the general mechanisms of action of common nutraceutical classes that are currently available to be used in ruminants. We classify and discuss each nutraceutical as either a *probiotic*, *prebiotic*, *phytonutrient* (eg, polyphenol, spices/essential oils), or *polyunsaturated fatty acid*. Finally, nutraceuticals are a rapidly developing field and it is important to note that these compounds lack governmental regulatory oversight by the Food and Drug Administration given their classification as "dietary supplements." Therefore, product label statements regarding composition, dosage, effectiveness, and quality are not necessarily independently validated or standardized.

PROBIOTICS

Probiotics, also known as direct-fed microbials, can improve health in many animal species. Common probiotic microorganisms currently used commercially include *Lactobacillus* sp. and other lactic acid-producing bacteria, *Bifidobacterium* sp., *Bacillus* sp., and *Saccharomyces cerevisiae*. Important considerations when supplementing probiotics include the dose (colony-forming units [CFU]), the duration of supplementation, and the age of the animal. A significant portion of the research on probiotic supplementation in ruminants focused on the health benefits to the gastrointestinal tract (GIT), which makes teleologic sense because these microorganisms may directly influence microbial communities and cellular function within the GIT. Most of the health benefits described in **Table 1** report reduced incidence or duration of scours. Ancillary benefits of probiotics, both direct and indirect, in other organ systems including the respiratory tract, urogenital tract, and mammary gland have also been investigated, but are only briefly discussed in this review.

The dynamics of the GIT vary between individual animals, diets, ages, environment, and management factors. Therefore, there is a significant amount of complexity involved in evaluating and comparing the efficacy of these probiotic strategies on their ability to affect health and performance. Typically, a probiotic must contain live microorganisms (usually bacteria and/or yeast) and produce one or more of the following desirable outcomes[66,67]:

- Regulate GIT microbial communities
- Prevent adherence or colonization of potential pathogens in the GIT
- Produce antimicrobial or bactericidal products against potential pathogens
- Maintain host GIT integrity including improved barrier defenses
- Improve mucosal adaptive immune responses
- Regulate GIT inflammation
- Improve ruminal fermentation and nutrient utilization

The application of probiotics in neonates is more common, which makes sense because the GIT and immune system are both developing rapidly and the risk for

Table 1
Effects of probiotics and prebiotics on the immune function, health, and performance of calves, dairy cows, and feedlot steers

Animal	Strain/ Type	Dose	Duration/ Frequency	Health/Production Status	Outcome	Reference
Calf	Probiotic	10^9 CFU/d multistrain	8 wk	High risk	Decreased scours and therapy	Timmerman et al,[4] 2005
Calf	Probiotic	10^9 CFU/d LAB 6 strains	8 wk	High risk	Decreased scours and therapy	Timmerman et al,[4] 2005
Calf	Probiotic	10^8 CFU/d LAB, *Enterococcus faecium*, *B bifidum*, *S thermophilus*	90 d	Low risk	Decreased scours	Mokhber-Dezfouli et al,[5] 2007
Calf	Probiotic	10^7 CFU/d *Lactobacillus acidophilus* and *L plantarum*	15 wk	Low risk	Increased white blood cell counts, increased IgG concentration	Al-Saiady,[6] 2010
Calf	Probiotic	Meta-analysis on LAB	Range 14–187 d	Low risk	Decreased scours	Signorini et al,[7] 2012
Calf	Probiotic	2.0×10^9 CFU/d *L acidophilus* and *Enterococcus faecium*	21 d	Moderate *Salmonella enteria* challenge	Reduced systemic inflammation, decreased mucosal damage, increased villi height:crypt depth in the duodenum and ileum	Liang et al,[8]
Calf	Probiotic	10^{10} CFU challenge EHEC *E coli* then 10^{10} CFU probiotic *E coli*	35 d	8–10-wk-old calves	Decreased *E. coli* O157:H7 GIT growth and fecal shedding	Tkalcic et al,[9] 2003
Calf	Probiotic + yeast	10^9 CFU/d *L acidophilus, E faecium, Saccharomyces cerevisiae*	56 d	High risk	Decreased neutrophil oxidative burst, increased lymphocyte counts, decreased haptoglobin, decreased scours	Davis,[10] 2018
Calf	Probiotic + yeast	10^8 CFU/d LAB, 10^6 CFU/d *S cerevisiae*, 10^8 CFU/d LAB	24 wk	Low risk	Decreased scours	Agarwal et al,[11] 2002
Calf	Yeast	10^9 CFU/d *S cerevisiae*	2×/d for 84 d	High risk	Decreased scours	Galvao et al,[12] 2005

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Table 1
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Animal	Strain/Type	Dose	Duration/Frequency	Health/Production Status	Outcome	Reference
Calf	BG	5 mL/d 50% BG extract	56 d	High risk	Increased neutrophil counts, decreased neutrophil functionality, decreased haptoglobin	Davis,[10] 2018
Calf	BG	113 g/d of a 1.8% BG + vitamin C	28 d	Transport stress	Increased neutrophil counts at d 28	Eicher et al,[13] 2010
Calf	BG	150 g/d of a 70% BG + vitamin C	28 d	Transport stress	Decreased white blood cells and neutrophil phagocytosis	Eicher et al,[13] 2010
Calf	MOS + Bs	3 g/hd/d MOS + 10^9 CFU *Bacillus subtilis*	56 d	High risk	Decreased lymphocyte counts and haptoglobin	Davis,[10] 2018
Calf	MOS	7 g/hd/d MOS	From 5 to 56 d	Low risk	Reduced antibiotic treatments and cost of scours	Kara et al,[14] 2015
Calf	MOS	4 g/hd/d MOS	30 d	Low risk	Decreased fecal scores and fecal coliform counts	Ghosh & Mehla,[15] 2012
Calf	MOS	4 g/hd/d MOS	5 wk	Low risk	Decreased probability of scours	Heinrichs et al,[16] 2003
Calf	MOS	4 or 6 g/hd/d MOS	56 d	Low risk	No changes	Hill et al,[17] 2008
Calf	FOS	4 or 8 g/hd/d FOS	56 d	Low risk	No changes	Hill et al,[17] 2008
Calf	FOS	3 or 6 g/hd/d FOS	168 d	Low risk	Increased carcass weight	Grand et al,[18] 2013
Calf	GOS	3.4% GOS DM in milk replacer	84 d	Low risk	Increased days with high fecal scores, increased epithelium growth in the SI, increased LAB and *Bifidobacterium* abundance in the SI	Castro et al,[19] 2016

Dairy cow	Probiotic	50 g/d *Lactobacillus casei* and *L plantarum* of 1.3×10^9 CFU/g	30 d	Lactating	Decreased SCC, no change in milk components but increased overall milk production at 15 and 30 d of the study (75 and 90 DIM)	Xu et al,[20] 2017
Dairy cow	Probiotic	10, 15, or 20 g/d *Saccharomyces cerevisiae* and LAB, no CFU listed	60 d	Early to mid lactation	Increased milk yield, FCM, solids, and profit	Shreedhar et al,[21] 2016
Dairy cow	Probiotic	10 g/hd/d probiotics, no CFU listed	21 d	Early lactation	Increased total milk yield and fat	Musa et al,[22] 2017
Dairy cow	Probiotic + yeast	10, 15, or 20 g/hd/d *Lactobacillus*, *S cerevisiae*, *Propionibacterium*, no CFU listed	6 wk	Lactating	Increased milk production with 20 g/d (most cost-effective), tendency to increase milk fat and protein	Vibhute et al,[23] 2011
Dairy cow	Yeast	9 mL/hd/d commercial yeast product in the water, no CFU listed	10 wk	30 DIM	Increased rumen pH, decreased BHBA, increased milk production, decreased milk protein yield	Rossow et al,[24] 2014
Dairy cow	Yeast	3 g/hd/d of 6.0×10^9 CFU live yeast	5 periods of 45 d	Lactating	Increased nutrient utilization in the rumen, increased milk yield, protein, and fat	Rossow et al,[25] 2018
Dairy cow	Yeast	2.5 g/d of 2.5×10^{10} CFU *S cerevisiae*	105 d	Lactating	Increased milk production	Maamouri et al,[26] 2014
Dairy cow	Yeast	40 g/d of 5.0×10^{11} CFU *S cerevisiae*	90 d	3rd lactation, early in lactation	Increased milk yield and fat, decreased SCC	Dailidaviciene et al,[27] 2018
Dairy cow	Synbiotic	1.0×10^7 CFU/kg diet *L casei* + 10 g/hd/d Dextran	1 y	Lactating	Decreased SCC, decreased mastitis, decreased adverse impacts of heat stress, increased milk production and components	Yasuda et al,[28] 2007

Table 1
(*continued*)

Animal	Strain/Type	Dose	Duration/Frequency	Health/Production Status	Outcome	Reference
Dairy cow	Synbiotic	10 g/d of 5.0×10^9 CFU/g *Saccharomyces cerevisiae* and a mix of 10^7 CFU *L casei*, *Streptococcus faecium*, *L acidophilus*	75 d	Lactating	Increased milk fat, decreased SCC	Sretenovic et al,[29] 2008
Feedlot steer	Probiotic	10^9 CFU/d LAB	2 y	Feedlot	Decreased fecal shedding of *E coli* O157:H7	Peterson et al,[30] 2007
Feedlot steer	Probiotic	10^8 CFU/d *E coli*	90 d	Feedlot	Decreased fecal shedding of *E coli* O157:H7	Schamberger et al,[31] 2004
Feedlot steer	Probiotic	10^9 LAB+ 10^9 *Propionibacterium freudenreichii*	9 mo	Feedlot	Decreased fecal shedding of *E coli* and *Salmonella*	Tabe et al,[32] 2008
Feedlot steer	Probiotic	10^9 CFU/d LAB	141 d	Feedlot	Decreased fecal shedding of *E coli*, decreased shedding by 80% at slaughter, only 3.4% of hides positive at harvest	Younts-Dahl et al,[33] 2005
Feedlot steer	Probiotic	10^8 CFU/d, 10^7 CFU/d LAB	141 d	Feedlot	Decreased fecal shedding of *E coli*	Younts-Dahl et al,[33] 2005
Feedlot steer	Probiotic	10^9 CFU/d LAB NPC 747	45–108 d	Feedlot	Decreased fecal prevalence of *E coli*, 1.6% prevalence on hides	Brashears et al,[34] 2003
Feedlot steer	Probiotic	10^9 CFU/d LAB NPC 750	45–108 d	Feedlot	Decreased fecal prevalence of *E coli*, 0% prevalence on hides	Brashears et al,[34] 2003
Feedlot steer	MOS	0.2% MOS in diet, DM	24 d	Feedlot	Decreased haptoglobin and endotoxin translocation from the GIT to circulation	Jin et al,[35] 2014

Concentrations of prebiotics are not standardized, so comparisons among studies are not recommended. Some prebiotics included a carrier that may or may not influence the outcome.

Abbreviations: BG, β-glucan; CFU, colony-forming units; EHEC, enterohemorrhagic *E coli*; FCM, fat corrected milk; FOS, fructooligosaccharide; GOS, galactoo-ligosaccharide; LAB, lactic acid-producing bacteria; MOS, mannanoligosaccharide; SI, small intestine

GIT disease is greater in the neonate. There is a shift in the microbial ecology in the GIT in early life. The neonatal GIT is colonized by many facultative anaerobic bacteria that are common in the environment, including a lot of the family of *Enterobacteriaceae* that include many strains of pathogenic *Escherichia coli* and *Salmonella* sp. There is a shift to more strict anaerobic bacteria as the animal ages with reduced abundance of *Enterobacteriaceae*.[68] Many of the probiotic microorganisms supplemented are either strict anaerobes or facultative anaerobes that commonly increase in abundance as an animal increases in age and are more often lactic acid-producing bacteria. Some of the logic is that speeding up this microbial progression will reduce GIT disease, especially by *Enterobacteriaceae*, because the GIT environment is less conducive to their colonization.

Probiotics can help maintain a beneficial host relationship and colonize the lumen and outer epithelium of the lower GIT creating competition for space on the mucosal surface as well as for nutrients on the mucosa that allow for bacterial adherence and growth (**Fig. 1**). The more "beneficial" bacteria present on the mucosa taking up space and nutrients the less that is available for pathogenic bacteria and potentially other enteric disease-causing microorganisms such as rotavirus, coronavirus, *Cryptosporidium*, and *Eimeria*. This mechanism is often referred to as competitive exclusion. Furthermore, increased lactic acid concentrations in the lower GIT reduces lumen pH and may help limit establishment of pathogenic *Enterobacteriaceae*.[69]

Another mechanism of action through which probiotics may influence health and performance of animals is via interaction with the GIT mucosal immune system (see **Fig. 1**). There are specialized cells in the GIT epithelium known as M cells that constantly sample contents from the GIT and deliver antigens to the lymphocyte-rich Peyer's patches. Maintenance of the commensal microbial structure of the GIT
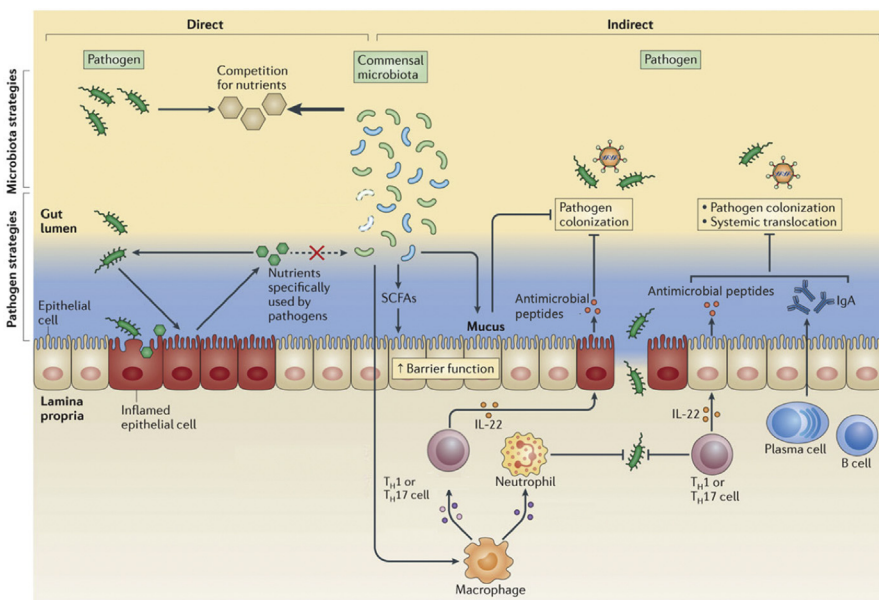
Fig. 1. Mechanisms of action of commensal microbiota and putative probiotics on immune defenses in mucosal tissues. (*From* Kamada et al. Role of the gut microbiota in immunity and inflammatory disease. Nature Reviews Immunology 2013; 13:321-335; with permission.)

helps regulate the inflammatory response in the small intestine to prevent an overreaction to the commensal microbiome and potentially other enteric pathogens.[70] The presence of secretory immunoglobulin A (IgA), antimicrobial peptides, and other regulatory leukocyte responses at the GIT mucosa is an important mechanism that regulates proinflammatory responses, which are essential to maintaining GIT integrity and function. Liang et al. (in press) supplemented Jersey bull calves with 2 strains of lactic acid-producing bacteria and challenged them at 7 days of age with a moderate dose of *Salmonella enterica* serotype Typhimurium. Calves supplemented with probiotic bacteria had reduced systemic inflammation and less mucosal damage as demonstrated by greater villi height:crypt depth in both the duodenum and ileum compared with the unsupplemented, challenged calves.[8] Localized proinflammatory responses are considered beneficial in most tissues; however, in the GIT mucosa, an excessive proinflammatory response may compromise the integrity of the GIT barrier and further exacerbate the pathogenesis of the disease.

Most of the mechanisms of action of probiotics discussed are to decrease the colonization of pathogenic microorganisms or directly improve GIT mucosal immune responses. However, supplementing probiotic species that either produce or stimulate the production of more butyrate have the potential to alter GIT mucosal defenses (see Fig. 1). Butyrate is one of the short-chain fatty acids produced by bacteria in the rumen and intestines. Butyrate is a preferred energy source for GIT epithelium that results in the proliferation and differentiation of epithelial cells in the rumen and both the small and large intestines. Butyrate can affect barrier development of the small intestine by increasing tight junction protein expression and exhibiting immunomodulatory effects on enterocytes and mucosal surfaces of the GIT by increasing neutrophil migration.[71,72] Current research suggests that butyrate has antiinflammatory, antioxidant, and anticancer functions along with promoting epithelial cell proliferation and differentiation.[73] Supplementation of butyrate to dairy heifers increased average daily gain and GIT development.[74,75] Supplementing butyrate-producing bacteria as probiotics is a relatively novel concept. Furthermore, a process known as cross-feeding has been recently described in which butyrate-producing bacteria are stimulated to proliferate in the presence of lactic acid originating from lactic acid-producing bacteria. Therefore, some of the benefits observed from supplementing common probiotic strains currently used in ruminants may partially be explained by changes in butyrate production in the GIT.

The use of probiotics in mature ruminants (eg, feedlot cattle or lactating dairy cows) can affect the rumen and lower GIT. Some probiotic microorganisms bypass the reticuloruminal and abomasal compartments and exert beneficial effects in the lower GIT, likely through similar mechanisms of action as discussed above for preweaned calves. Supplementing feedlot cattle with lactic acid-producing bacteria decreased *E coli* O157:H7 and *Salmonella* sp. fecal shedding, as well as contamination on carcass hides postharvest.[9,30–34] Supplementing probiotics to feedlot cattle as a preharvest food safety strategy is now a common industry practice. Most of the data currently available regarding probiotic supplementation in dairy cow rations have assessed its effect on performance and milk quality (eg, somatic cell count). Fecal pathogen shedding or manure consistency were not commonly reported variables in these studies. It is conceivable that many of the probiotic species discussed previously in neonates may have some lower GIT benefits in mature dairy cows. In fact, some of the performance benefits such as increased milk production may be partially attributable to improvements in GIT health. Furthermore, supplementing probiotics to mature dairy cows seems to have the greatest health and economic benefits during stressful events, most notably the transition period.

The mechanisms of action of orally delivered probiotics on GIT health are more straightforward than how oral probiotics may improve immunity against infections in nonmucosal tissues (eg, mammary gland). The mucosal immune system is a complex network of mucosal surfaces spanning the gastrointestinal, respiratory, and urogenital tracts. Immune responses generated at one mucosal location can transfer to other mucosal surfaces through trafficking and recirculation of effector and memory leukocytes; however, this likely does not include the mammary gland. The paucity of data and the lack of a clear mechanism of action do not support the notion that oral administration of probiotics improves mammary gland health. Rainard and Foucras[76] discussed the potential direct effects of either intramammary infusion or topical application of probiotics, either lactic acid-producing bacteria or *Bacillus* sp., that can inhibit the growth of mastitis causing pathogens in a teat dip as a more promising route of administration to improve mammary gland health. Intramammary infusion of a live lactic acid-producing bacteria culture increased the secretion of proinflammatory cytokines interleukin-8 (IL-8) and IL-1 and attracted neutrophils to the mammary gland.[77] The authors suggested application of this intramammary infusion for the treatment of mastitis because of the production of a broad-spectrum bacteriocin against gram-positive bacteria and a sustained innate immune response.

Finally, a common probiotic application in ruminants is to supplement probiotics, either bacterial or fungal, to improve rumen function and health. The primary mechanisms of action are to stimulate total rumen bacterial population densities, shift or stabilize the balance of rumen bacterial populations, utilize lactic acid, and reduce the risk for ruminal acidosis, and maintain a low oxygen rumen environment.[78–81] Newbold and colleagues[78] designed well-controlled experiments to elucidate the mechanisms by which live yeast supplements may increase rumen bacterial population densities. Oxygen utilization by live yeast supplements seems to be an important mechanism improving the growth of rumen bacteria. Silberberg and colleagues[81] supplemented a live yeast during repeated rumen acidosis challenges and reported improvements in rumen pH, a more stabilized rumen bacteria population, and less systemic inflammation. Another microbial strategy to aid in the prevention of rumen acidosis is a bolus inoculation with lactic acid-utilizing bacteria such as *Megasphaera elsdenii*. Arik and colleagues[82] administered a bolus of lactic acid-utilizing bacteria to the rumen of Holstein heifers with *M elsdenii* for 2 consecutive days during a subacute ruminal acidosis challenge. They observed the *M elsdenii*-treated heifers had a slowed decrease in rumen pH, decreased concentrations of lactic acid-producing bacteria (*Streptococcus bovis*), and increased protozoa counts. These studies support the notion that microbial supplementation strategies can improve rumen function and health.

PREBIOTICS

Prebiotics are indigestible carbohydrates, including many oligosaccharides and fructans, that can improve animal health through a variety of mechanisms. One common mode of action is that they serve as an energy source for commensal or probiotic bacteria in both the rumen and the lower GIT.[83] Synbiotic is the term used when a prebiotic is fed with a probiotic and the effect together is greater than if either is fed alone (see **Fig. 1**). However, the focus of prebiotics in this review is more on their immunomodulatory effects, ability to bind gram-negative bacterial pathogens, and adsorbing certain mycotoxins.

Prebiotics are commonly found in plants, milk, or the cell wall of fungi (yeast and mushrooms). They are found in natural feedstuffs as well as in various extracts. Food sources for galactooligosaccharides include soybeans and milk, whereas

fructooligosaccharides are found in vegetables and plants. Prebiotics isolated from yeast cell walls include mannan-oligosaccharides (MOS) and β-glucans (BGs). The composition, availability, and physical chemistry of a prebiotic in a feedstuff or in an extract influences its ability to improve the health of an animal. For example, a prebiotic extract of yeast cell wall may be very different from another extract of yeast cell wall, even if they are extracted from the same strain of yeast.[84] Methods of extraction (eg, enzymatic, solvents, temperature, and time) and additional processing techniques (eg, deproteination) can significantly influence the functional properties of an extract. Making comparisons among various prebiotics are difficult in that many extracts are blended with other ingredients, so 1 g of "extract A" is likely not equivalent to 1 g of "extract B." Therefore, caution should be taken when reviewing the dose of a prebiotic in **Table 1** and when making comparisons among published studies involving different extracts.

The in vitro binding of yeast cell wall extracts with MOS showed that MOS binds to the type 1 fimbriae expressed on many gram-negative bacteria such as pathogenic *Salmonella* sp. and *E coli*.[84] These mannose -specific filaments, or type 1 fimbriae, bind to MOS and prevent pathogenic bacteria from binding to the epithelium and colonizing the GIT. Thus, supplementing yeast cell wall extracts with MOS may be a useful prevention strategy for those animals impacted by greater exposure to gram-negative pathogenic bacteria.[10] Because adhesion of pathogenic bacteria to host mucosal surfaces is generally required for an infection in the GIT, the binding of MOS to these fimbriae decreases potential pathogenic adherence to the host GIT mucosa. The impacts of MOS on specific immune responses are not well characterized. Furthermore, complicating interpretation of data is that MOS extracts are predominately yeast cell wall extracts that also contain various glucan fractions including BGs. Therefore, many of the immunomodulatory effects described in the next paragraph are possible when yeast cell wall extracts are supplemented and likely attributed to the BG fraction of the extract.

β-Glucans are cell wall fractions usually isolated from fungal sources and have potential immunomodulatory effects. The β-1,3-glucans are able to bind to Dectin-1 receptors on monocytes, macrophages, and neutrophils, and, to a lesser extent on dendritic and T-cell surfaces.[85] Ligation of the Dectin-1 receptor when animals are supplemented a fungal extract can cause a slight activation of various leukocyte responses and is currently considered the mechanism of action for a positive immune response. Oral supplementation of BG fractions or yeast cell wall extracts can increase phagocytosis and oxidative burst capacities of immune cells as well as activate an inflammatory response through a nuclear factor κB-mediated pathway. Oral supplementation of BG also affects systemic immune responses in addition to local GIT immune responses. The mechanism of action of BGs is thought to be mediated through specialized M cells that sample intestinal lumen contents. This increased awareness of the immune system allows for mediated inflammation and an enhanced ability of macrophages, neutrophils, and dendritic cells to identify, engulf, and destroy pathogenic bacteria before they have the opportunity to establish an infection in the host.[10,86]

As discussed previously, yeast cell wall extracts contain varying levels of both MOS and BG, and are commonly supplemented to ruminants and other livestock for potential immunomodulatory effects, binding of gram-negative bacteria, or adsorbing hydrophobic mycotoxins. The quantity of MOS and BG that bypasses the rumen is not well understood. However, it does seem that an appreciable, biological amount of MOS and BG does influence lower GIT because supplementing yeast cell wall extracts reduced stress-related disease, improved mammary gland health, and

increased milk production.[87,88] Supplementation strategies of yeast cell wall extracts seem to be most effective during periods of stress or increased pathogen exposure. Another application for yeast cell wall extracts is to alleviate some of the negative effects of hydrophobic mycotoxins such as zearalenone. Jouany and colleagues[89] reported that BG interacted with zearalenone, in an "adsorption type" chemical interaction. Both Pereyra and colleagues[90] and Fruhauf and colleagues[91] reported that commercial yeast cell wall extracts with the greatest MOS and BG content adsorbed zearalenone to the greatest extent but were not effective at adsorbing aflatoxin, a hydrophilic mycotoxin. Therefore, a common mycotoxin strategy is to combine a mineral clay and a yeast cell wall extract to gain a more broad-spectrum mycotoxin adsorption capability.

PHYTONUTRIENTS

Phenolic compounds represent a family of phytonutrients with potential therapeutic food animal applications given their intrinsic antioxidative and anti-inflammatory properties (Table 2). In nature, plants synthesize polyphenols as both a defense mechanism against exogenous pathogens and for cellular protection against ultraviolet irradiation. Rich sources of phenolic compounds fed to ruminants include a multitude of industrial fruit and vegetable coproducts, such as citrus, pomegranate, green tea, grape, and green vegetable processing residues. Following consumption, the rumen microbiome can significantly degrade polyphenols and decrease host bioavailability. However, a proportion of dietary polyphenols will bypass ruminal degradation and enter the GIT. Following intestinal absorption, phenolic compounds enter the peripheral circulation where they may exert their bioactive effects on inflammation, oxidative status, and immune function (Fig. 2).[92,93]

Chemically, flavonoids are hydroxylated polyphenolic compounds consisting of a 15-carbon chain bound to an oxygenated heterocyclic ring structure.[92,93] Published studies on the immune effects of feeding flavonoid metabolites specifically to ruminants were limited predominantly to grape, pomegranate, and green tea derivatives.

Byproducts from wine and fruit-juicing industries include pomace, which consists of residual grape pulp, seeds, skin, and stems. Grape pomace contains high concentrations of flavonoid derivatives and tannins, thus acting as a potential reservoir of dietary antioxidant, anti-inflammatory, and antimicrobial phenolic compounds.[45] Supplementing grape seed and pomace extracts can reduce mRNA abundancy of fibroblast growth factor 21 (FGF21), a stress hormone associated with hepatic fatty acid oxidation and ketogenesis, in transition dairy cows.[50] Grape polyphenols lowered leukocyte mRNA expression of superoxide dismutase, an endogenous free radical scavenger, in postpartum dairy cows. This effect suggests the ability of grape polyphenols to decrease superoxide dismutase expression as a consequence of their endogenous antioxidant capacity.[46]

Pomegranate byproducts (eg, seeds, pulp, and peels) contain a potent source of polyphenols, predominantly flavonoids, phenolic acids, and tannins, as well as stereoisomers of vitamin E. Together, these compounds reduce reactive oxygen species and chelate metal ions to induce potentially beneficial immunomodulatory effects in ruminants.[49,94] Dairy calves supplemented with pomegranate extracts had increased in vitro synthesis of the lymphocyte-derived cytokines interferon-γ and IL-4, as well as greater total immunoglobulin G (IgG), in response to ovalbumin vaccination. By improving aspects of both humoral and cell-mediated immunity, calves supplemented with polyphenols may have an improved immune response following vaccination.[42] Hydrolysable tannins may be able to reduce intraluminal oxidation thereby increasing

Table 2
Effects of phytonutrients on the immune function, health, and performance of calves, dairy cows, sheep, and goats

Animal	Strain/Type	Dose	Duration/Frequency	Health/Production Status	Outcome	Reference
Calf	Oregano	1%, 1.5%, or 2% oregano oil in MR	4 d to weaning	Low risk, 4 d of age to 5 mo	Increased IgG concentrations, decreased fecal score, reduced *Enterobacteriaceae* shedding	Ozkaya[36] 2018
Calf	Oregano	100 ppm/hd/d	120 d	Low risk, 30–150 d of age calves	No reduction in *Eimeria* oocyst shedding	Grandi et al,[37] 2016
Calf	Oregano	12.5 mg/kg	15 d	Preweaned	Decreased incidence, severity, and duration of scours	Katsoulos et al,[38] 2017
Calf	EO	Multiple doses 0–281 mg/calf/d	24 wk	Low risk	Reduced health scores, scours, and antibiotic treatment	Soltan,[39] 2009, and Oh et al,[40] 2017
Calf	Pomegranate	140 mg polyphenols/g DM; about 5%–20% total DMI	8 wk	Postweaned; apparently healthy; low risk; 11 mo old	Increased DMI, tendency for increased weight gain	Shabtay et al,[41] 2008
Calf	Pomegranate	5 and 10 g/d top-dressed onto starter	70 d	Apparently healthy; low risk; 0–70 d old	Increased peripheral cytokine synthesis (IFN-γ, IL-4), improved IgG response to ovalbumin vax, no effect on fecal scores or rectal temperatures	Oliveira et al,[42] 2010
Dairy cow	EO (garlic, oregano)	3 mL intravaginally, 12 mL intramammary, 25 mL topical teat dip	Once daily for 3 d, 2× daily for 1 d, and 1 application	Mid to late lactation multiparous Holstein cows, *Streptococcus uberus* mastitis challenge	No cure of mastitis	Mullen et al,[43] 2108

Dairy cow	EO	0, 100, and 200 mg/d	28 d	Lactating	Increase CD4+ T-cell response to vaccine/immune challenge, tendency to increase milk production	Oh et al,[44] 2016
Dairy cow	Grape	4.5 g/hd/d	75 d	Apparently healthy, mid lactation; low risk	No effect on SCC, tendency to increase milk yield	Nielsen & Hansen,[45] 2004
Dairy cow	Grape	10 g/d mixed into pellets	3 wk pre-/postcalving; 44 total day approx.	Apparently healthy; primiparous, 7 mo pregnant	Lowered leukocyte mRNA expression of SOD during initial 3 wk postpartum; no effect on glutathione peroxidase expression	Colitti & Stefanon et al,[46] 2006
Dairy cow	Green tea	100 μg/mL	12 h	In vitro study; mammary epithelial cells isolated from lactating Holstein cows	Greater cell viability, protein, mRNA abundance of NFE2L2; lower intracellular ROS accumulation in response to H_2O_2 challenge	Ma et al,[47] 2018
Transition dairy cow	Mixture	150 g/d prepartum; 170 g/d postpartum	25 d prepartum/26 d postpartum	Apparently healthy; multiparous, primiparous Holstein transition cows	Lower serum NEFA, lower NEFA:insulin postpartum, improved insulin sensitivity pre-/postpartum, higher total antioxidant capacity prepartum; lower malondialdehyde pre-/postpartum	Hashemzadeh-Cigari et al,[48] 2015

Table 2
(*continued*)

Animal	Strain/Type	Dose	Duration/Frequency	Health/Production Status	Outcome	Reference
Transition dairy cow	Pomegranate	350 g DM/d for seeds only; 1350 g DM/d pulp (seeds + peels blend)	25 d pre-/postpartum	Apparently healthy; multiparous, primiparous Holstein transition cows	Higher total plasma antioxidant capacity, lower TAG/FFA/BHBA at both pre-/postpartum, pulp blend increased SOD, decreased MDA postpartum, higher FCM yield	Safari et al,[49] 2018
Transition dairy cow	Grape	1% of DM	3 wk prepartum until 9 wk postpartum	Multiparous, primiparous Holstein transition cows	Reduced mRNA expression of FGF21 (liver stress hormone) postpartum, no effect on hepatic inflammatory gene expression, increase daily milk yield, increase daily milk protein yield	Gessner et al,[50] 2015
Transition dairy cow	Green tea	0.175 g/kg feed DM	3 wk prepartum until 9 wk postpartum	Primiparous, multiparous Holstein transition cows	Trend for reduced mRNA (haptoglobin), reduced mRNA (FGF21) postpartum; no difference (TNF), (CRP), higher ECM wk 2–9 postpartum; lower hepatic TAG, cholesterol concentrations wk 1 and 3 postpartum	Winkler et al,[51] 2015

Sheep	Mixture (4 compounds)	Single ruminal infusion; 10% DMI	1 d	Apparently healthy; 18-mo-old, castrated males	Grape-enhanced total plasma antioxidant capacity; reduced plasma susceptibility to liperoxidation	Gladine et al,[52] 2007
Sheep	Green tea	2, 4, or 6 g/kg feed DM	8 wk	Lambs infected with *Haemonchus contortus* GIT parasites	Decreased serum APPs at all dosages (Hpt, LBP, a1AGP), regulate SAA in dose-dependent manner, higher ADG in infected, supplemented lambs vs. infected only lambs; reduced adult worm burden to uninfected levels at 6 g/kg group	Zhong et al,[53] 2014
Goat	Green tea	2, 3, or 4 g TC/kg DM feed	60 d	Low risk	Reduced plasma glutathione most efficaciously at 2 g dosage; over 3 g dosage reduced plasma protein and globulins (bad)	Zhong et al,[54] 2011
Goat	Green tea	2.0% on weight:weight ratio	90 d	Low risk, castrated male goats	Linear increase average weight gain and feed intake, increased splenic cell growth, reduced intramuscular TBARS	Ahmed et al,[55] 2015

Abbreviations: ADG, average daily gain; APP, acute-phase proteins; BHBA, beta-hydroxybutyric acid; DIM, days in milk; DM, dry matter; DMI, dry matter intake; ECM, energy-corrected milk; EO, essential oil; FCM, fat corrected milk; FFA, free fatty acids; MDA, malondialdehyde; MR, milk replacer; NEFA, nonesterified fatty acid; ROS, reactive oxygen species; SAA, serum amyloid A; SCC, somatic cell count; SOD, superoxide dismutase; TAG, triacylglycerol; TBARS, thiobarbituric acid reactive substances.
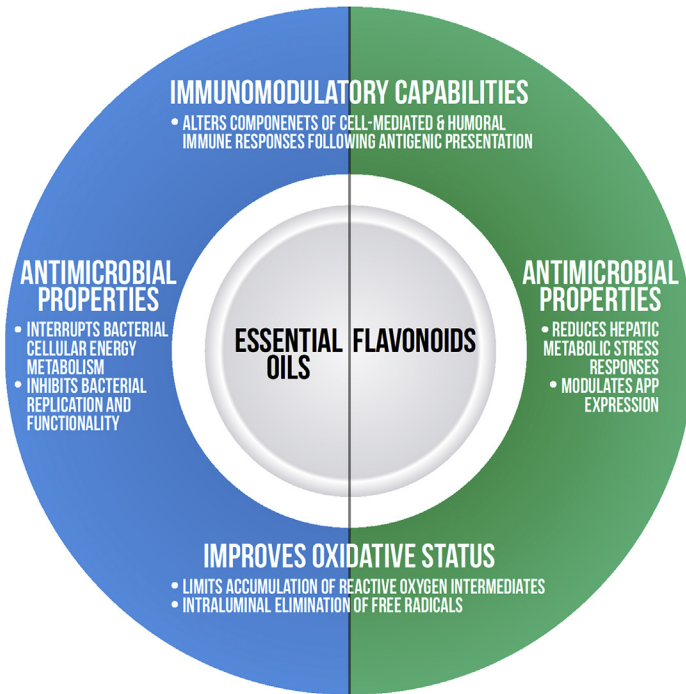
Fig. 2. Mechanisms of action of phytonutrients on immune defenses.

dietary vitamin E bioavailability, suggesting a potential mechanism for antioxidants to improve health.[41]

Green tea leaf extracts, particularly catechins, represent another rich source of phenolic compounds with antioxidant potential. Tea polyphenols reduced accumulations of reactive oxygen intermediates in bovine mammary epithelial cells following a hydrogen peroxide challenge in vivo.[47] Green tea polyphenols also seem to modulate acute-phase protein expression upon parasitic challenge in small ruminant species.[53] Reduced hepatic metabolic stress, coupled with decreased intrahepatic lipid concentrations and acute-phase protein expression, may indicate improved energy status, ultimately leading to improved ruminant health and performance.[51]

Essential oils represent another class of phytonutrients with potential antimicrobial and immunomodulatory capabilities. Essential oils (EOs) provide a plant with its unique color and fragrance. Chemically, EOs are composed of a lipophilic mixture of terpenoids, phenolics, and a variety of low-molecular-weight compounds.[1,95] The antibacterial mode of action of volatile oils is likely multifactorial with hypothesized mechanisms including disruption of biochemical pathways associated with cell membranes and passage of low-molecular-weight EO derivatives across inner plasma membranes of both gram-positive and gram-negative bacteria. By interrupting cellular energy metabolism, EOs can partially inhibit bacterial growth and functionality and have demonstrated microbicidal effects against common food-borne pathogens, rumen bacteria, and gastrointestinal parasites (see **Fig. 2**).[1] Most of the immunologic effects of EOs were investigated in monogastric species. Receptor-mediated responses upon EO supplementation can result in improved mucosal blood flow, altered cytokine and neuropeptide release, and modified immune cell functionality. Volatile

oils undergo significant ruminal biodegradation, likely limiting the immunomodulatory and antibacterial effects.[44] Of the published studies of EOs performed specifically on ruminants, oregano, garlic, and *Capsicum oleoresin* oils were shown have potential health and immunomodulatory effects.

Oregano (*Origanum onite* L) oil and its 2 main phenolic derivatives, carvacrol and thymol, offer a potential potent source of antimicrobial and antioxidant capabilities. Oregano oil increased hematologic variables in dairy calves including hemoglobin, packed cell volume, and mean corpuscular volume.[96] Initial experiments have demonstrated potential benefits of using oregano to minimize scours and improve health scores in neonatal ruminants.[38] Dairy calves supplemented with oregano water had increased fecal firmness, reduced *Enterobacteriaceae* shedding, and increased immunoglobulin concentrations.[36] However, adult dairy cattle supplemented with EO products containing either garlic, thyme, or oregano failed to cure experimentally induced *Streptococcus uberis* mastitis following either topical or intramammary administration.[43] Oregano supplementation also failed to reduce *Eimeria* oocyst shedding in dairy heifers.[37]

Garlic (*Allium sativum*) and garlic oils have the capacity to eliminate free radical species and reduce lipid peroxidation in vivo. The effects of garlic oil on immunologic variables in mature ruminants are inconclusive. However, Oh and colleagues[97] recently described the broad immuno-stimulatory effects of garlic oil following intra-abomasal infusion via an increased neutrophil to lymphocyte ratio as well as increased helper ($CD4^+$) T-cell proliferation.

Capsaicinoids, bioactive compounds sourced from flowering *Capsicum* plants, demonstrated an ability to modulate the acute-phase response in dairy cows, including in response to an intravenous lipopolysaccharide (LPS) challenge.[3,98] Furthermore, postruminal supplementation of capsaicinoids increased $CD4^+$ T-cell proliferation.[97] By potentially affecting both the innate and adaptive branches of the immune system, capsaicinoid supplementation may be particularly beneficial during vaccination events.[99] However, more research is needed to validate observations using these EO-based compounds in ruminant species.

POLYUNSATURATED FATTY ACIDS

Dietary fat is more than just calories. The composition of fatty acids in fat sources can influence many cellular functions (**Table 3**). The fatty acid composition of cellular membranes reflects the fatty acid composition of the diet. The physical chemistry of the various fatty acids differs and increasing the incorporation of polyunsaturated fatty acids (PUFAs) can increase the membrane fluidity. Increasing the fluidity of leukocytes can disrupt the organization of cell surface receptors in the plasma membrane, and ultimately alter cellular function.[100–104]

In addition to influencing membrane fluidity, the fatty acid composition of cellular membranes can alter the biosynthesis of various lipid mediators (**Fig. 3**). The eicosanoid family are some of the most well-known bioactive lipid mediators and include prostaglandins, leukotrienes, and thromboxanes. The balance of omega-6 to omega-3 PUFA in cellular membranes influences the quantity and biological potency of the eicosanoids produced. Generally, eicosanoids from omega-3 PUFAs have a reduced affinity for cyclooxygenase enzymes. They also produce many eicosanoids that have a lower biological activity than those produced from omega-6 PUFAs. This led to the concept that omega-3 PUFAs have a greater anti-inflammatory capacity than omega-6 PUFAs.

Preweaned calves fed milk replacer with either 1% or 2% of the total dry matter (DM) replaced as fish oil, which is rich in omega-3 PUFAs, reduced the

Table 3
Effects of polyunsaturated fatty acids on the immune function, health, and performance of calves, dairy cows, and feedlot steers

Animal	Strain/Type	Dose	Duration/Frequency	Health/ Production Status	Outcome	Reference
Calf	Fish oil	1% and 2% DM of the milk replacer	42 d	High risk	Reduced inflammatory response to LPS challenge, quadratic effect on secondary antibody response, no effect on fecal scores	Ballou et al,[56] 2008; Ballou & DePeters,[57] 2008
Calf	Fish oil	2% of the DM	14–55 d of life	Low risk	No effect on serum haptoglobin or mitogen-induced IFN-γ secretion, no difference in fecal scores, fish oil decreased starter intake and ADG	McDonnell et al,[58] 2019
Calf	Coconut oil	Replaced 20 or 40% of the lard in milk replacer	56 d	Low risk	Quadratic decrease in fecal scores, no difference in starter intake or ADG	Bowen Yoho et al,[59] 2013
Calf	Linoleic acid	0.48% or 9.0% of the fatty acids in milk replacer as linoleic acid	30 d	Low risk	Caused differential expression of many immune-related hepatic genes	Garcia et al,[60] 2016
Calf	Blend of short- and medium-chain fatty acids and α-linolenic acid	0.46%, 0.51%, 0.19%, and 0.18% of DM as butyric, lauric, myristic, and linolenic acids, respectively, in milk replacer	56 d	Low risk	No difference in fecal score or medication, improved DM digestion and ADG	Hill et al,[61] 2016

Animal	Supplement	Amount	Duration	Status	Results	Reference
Dairy cow	Ca salts of palm oil, safflower oil, or fish oil	n-6 to n-3 ratios of 4, 5, or 6	14 d postpartum to 105 d postpartum	Early lactation	Lower n-6 to n-3 decreased plasma IL-6 and haptoglobin concentrations after a moderate intramammary LPS challenge, no effect on neutrophil phagocytosis or oxidative burst, greater DMI and 3.5% FCM in the n-6 to n-3 ratio of 4	Greco et al,[62] 2015
Dairy cow	Whole flaxseed	10.4% of DM	Calving to 105 DIM	Early lactation	Transient reduction in mitogen proliferation of PBMC, no effect on humoral response, decreased prostaglandin E$_2$	Lessard et al,[63] 2003
Transition dairy cow	Fish oil	250 g prepartum and 0.92% DM postpartum	3 wk prepartum until 10 d postpartum	Apparently healthy, multiparous Holstein cows	No effect on inflammatory response to a high dose of LPS intramammary, no difference in DMI or milk production	Ballou et al,[64] 2009
Transition dairy cow	Ca salts of palm oil, safflower oil, or fish oil	1.5% of DM	Safflower oil from 30 d prepartum to 35 d postpartum; fish oil 35–160 d postpartum	Apparently healthy, multiparous Holstein cows	Safflower oil increased plasma acute-phase proteins, neutrophil proinflammatory cytokines, neutrophil L-selectin, and neutrophil phagocytic and oxidative burst activities, fish oil decreased neutrophil proinflammatory cytokine	Silvestre et al,[65] 2011

Abbreviations: DIM, days in milk; DM, dry matter; n-3, omega-3 polyunsaturated fatty acids; n-6, omega-6 polyunsaturated fatty acid; PBMC, peripheral blood mononuclear cell.
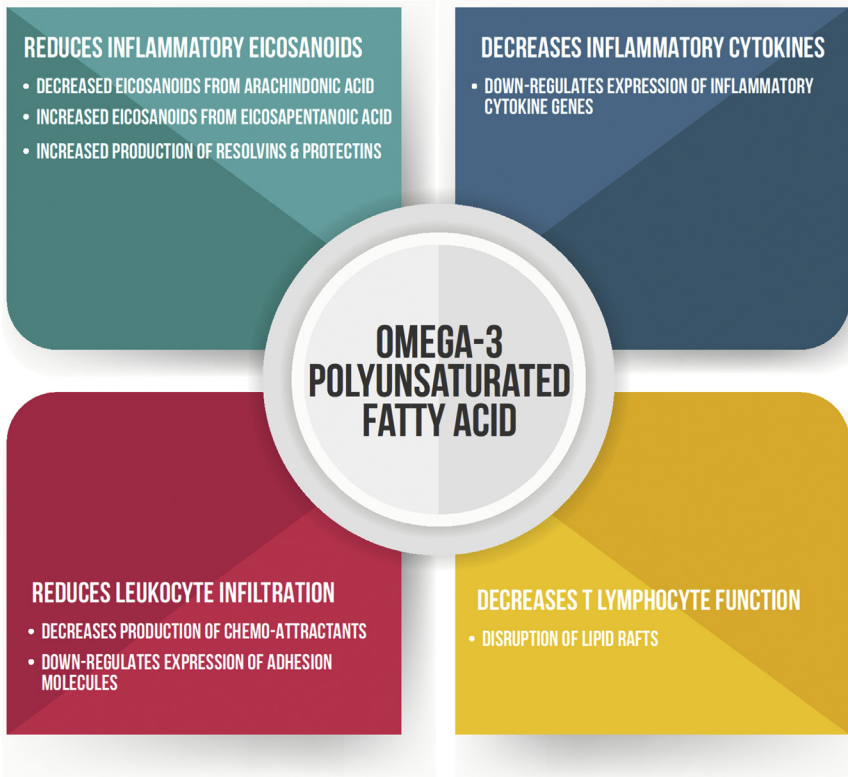
Fig. 3. Mechanisms of action of omega-3 long-chain polyunsaturated fatty acids.

proinflammatory response when challenged with a large dose of LPS to mimic sepsis.[56] In a recent study replacing 2% of the total DM with fish oil fed to preweaned calves did not reduce the serum haptoglobin concentrations or mitogen-induced interferon-γ secretion.[58] In contrast, Garcia and colleagues[60] supplemented preweaned calves with either a low or high omega-6 linoleic acid milk replacer (0.46% or 9.0% of the total fatty acids, respectively) and reported that the high linoleic acid treatment had many differentially expressed genes in the liver that should predict a reduced risk of infection. The linoleic fatty acid compositions used in the study of Garcia and colleagues likely reflect milk diets based on milk fat (low linoleic acid) and those in which the primary fat source is based on lard (high linoleic acid). The exact PUFA composition or balance of omega-6 to omega-3 PUFAs in milk solids that improves immune responses and overall health remains to be determined. Finally, although shortchain fatty acids are not PUFAs it is important to emphasize their importance in GIT development as we discussed previously in the section on probiotics. Supplementing short-chain fatty acids such as butyrate can improve GIT development and calf performance.[61] Furthermore, the effect may be more pronounced in milk replacer diets in which the primary fat source is not milk fat. Milk fat already contains a significant quantity of butyrate; however, the exact concentrations of these short-chain fatty acids to supplement in milk replacer are not known.[59,61]

Supplementing omega-3 PUFAs and altering the omega-6 to omega-3 PUFA ratio to transition and early lactating cows was investigated as a strategy to improve health

and production performance. Feeding whole flaxseed as a source of the omega-3 PUFA, specifically α-linolenic acid, from calving until 105 days in milk (DIM) decreased the serum concentrations of the proinflammatory eicosanoid, prostaglandin E_2.[63] Furthermore, decreasing the omega-6 to omega-3 PUFA ratio using calcium salts of fish oil from 14 to 105 DIM decreased the proinflammatory response following a mild intramammary LPS challenge.[62] In contrast, there was no difference in the proinflammatory response in cows when challenged with 10 times the dose of LPS in the mammary gland at 7 DIM whether they were supplemented with fish oil or not.[64] The authors attributed the lack of an effect on the severity of the LPS challenge. Silvestre and colleagues[65] supplemented 1.5% of the total DM as safflower oil as a source of omega-6 PUFA from 30 d prepartum to 35 DIM. Observations included increased plasma acute-phase proteins, neutrophil proinflammatory cytokines, neutrophil L-selectin, and neutrophil phagocytic and oxidative burst capacities during the periparturient period. Furthermore, cows were switched to a breeding diet at 35 DIM that contained 1.5% of the total DM as calcium salts of fish oil and fed through 160 DIM. Supplementing cows with the calcium salts of fish oil decreased neutrophil proinflammatory cytokine secretion. These data further support that the balance of omega-6 to omega-3 PUFAs in the diets of both calves and cows can influence various immune responses. However, it remains to be determined at what age, physiologic stage, and conditions more inflammation is desirable and vice versa when less inflammation is preferred.[105,106]

SUMMARY

Nutraceuticals represent a group of compounds that may help fill that void because they exert some health benefits when supplemented to livestock. There remains significant ambiguity regarding nutraceuticals because this field is evolving at a rapid pace without regulatory oversight. In this review, we broadly classify and discuss nutraceuticals as either probiotics, prebiotics, phytonutrients, or PUFAs. These compounds work through various mechanisms of action including stabilizing commensal microbial communities, improving mucosal immune responses and barrier function, binding or adsorbing potential pathogens or toxins, improving antioxidant capacity, direct antimicrobial activity, and increasing or decreasing systemic immune responses.

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