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Probiotics and Ruminant Health

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Abstract

Probiotics are viable microorganisms with beneficial health effects for humans and animals. They are formulated into many functional foods and animal feed. There is a growing research interest in the application and benefits of probiotics in ruminant production. Several recent studies have evaluated the potential of probiotics in animal nutrition and health. In this chapter, we have reviewed current research on the benefits of probiotics on gut microbial communities in ruminants and their impact on ruminant production, health and overall wellbeing.

Keywords: probiotic, ruminant health, gut microbiota, immune response

1. Introduction

The gastrointestinal tract of domestic ruminant animals mainly cattle, sheep and goat are inhabited by diverse and complex microbial communities including bacteria, protozoa, fungi, archaea and viruses. In the last three decades, there have been numerous research studies to characterize the gut and rumen microbiota population and understand their importance on ruminant nutrition and health. In dairy cows, the rumen, which is the main fermentation chamber contains different microbial communities; about 100 billion bacteria, protozoa, methanogens and other anaerobic fungi [1, 2]. The major microbial groups in the rumen include *Prevotella*, *Selenomonas*, *Streptococcus*, *Lactobacillus* and *Megasphaera*. The rumen is also predominately inhabited by fiber-degrading bacteria such as *Fibrobacter*, *Ruminococcus*, *Butyrivibrio* and *Bacteroides* [2]. These native microbial groups have important function in the digestion and fermentation of dietary polysaccharides by the host [3]. In addition, the rumen microbial population must be balance and healthy for efficient digestion of feed and impact animal health [4]. In ruminants, variation in the rumen microbiota between individual animals has



been reported. This variation is dependent on animal age, health status and environmental factors [5–8]. There is a growing research interest in the application of beneficial microbes/probiotics in ruminant production to help balance the gut microbiota, and as possible alternative to antibiotic use through improved gut health.

Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [9]. Probiotics are widely recognized as non-pathogenic microbes with health benefits [10]. The beneficial health effects of probiotics are related to their immunomodulatory activity in the gut by stimulating the secretion of immune modulators such as cytokines and IgA in intestinal mucosa [11]. In ruminants, probiotics are administered to target the rumen (main site of feed digestion) where they have an effect on rumen fermentation especially on feed digestibility and degradability and rumen microbiota [12]. Probiotic positively affect celluloysis and synthesis of microbial protein during digestion [13], and stabilizes rumen pH and lactate levels. In addition, probiotics are able to enhance nutrient absorption [14]. Direct-fed probiotic have been shown to reduce ruminal acidosis [15].

Lactic-acid bacteria strains such as *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Saccharomyces* and *Enterococcus* are commonly used as probiotics in functional foods and animal feed [16–20]. *Lactobacillus* and *Bifidobacterium* species have been shown to provide protection against enteric infection. These beneficial microbes consist of different species of microorganisms such as bacteria and yeast and they may be used as single or multi-strain. The multi-strain probiotics have a broad spectrum effect from the different strains against infections [21], and could increase their beneficial effects of probiotics due to their synergistic adhesion effect [22].

Probiotics are typically used to improve gastrointestinal health, reduce diarrhea, bloating and protect against infectious diseases [23]. Several researchers have reported the benefits of oral administration of probiotics to ruminants. Probiotics regulate and balance gut microbes, promote growth and development of animals, and improve the host resistance to diseases [24]. Recent studies suggest that utilization of probiotics as feed supplement for ruminants improves growth performance, production, and enhance health and overall wellbeing of the animals. Applications of probiotics have been shown to reduce the negative environmental impact such as methane emission associated with ruminant production. In this chapter, we have reviewed current research on the benefits of probiotics on gut microbial communities in ruminants and their impact on ruminant production, health and overall wellbeing.

1.1. Selection of probiotic strain

It is important to select the suitable strain of a microorganism for use as probiotic. The suitable potential probiotic strain is considered as an inhabitant of the host organism and has the ability to adhere and colonize the epithelial cells of the gut. Also, the potential probiotic microbe should be able to grow and survive in the host [25]. Microbial strains used as probiotics are required not to affect the indigenous gut microbiota population of the host. Other important requirement for the potential probiotic strain is to be able to adapt to the environment of the gut and locate a suitable niche in the rumen (such as epithelium, fluid or feed), and exerts positive effects on the host [8]. Other Safety criteria and characteristic of probiotics to consider

include, non-pathogenic, resistance to gastric juice and bile, anatgonize pathogenic bacteria, genetically stable, and exhibit stable qualities during processing, storage and delivery, viable at high populations [16]. In the USA, there are regulatory considerations by the Food and Drug Authority for safety evaluation of microorganisms used as probiotic. The specific microorganism should have "Generally Regarded As Safe" (GRAS) status [26].

1.2. Different types of probiotic microorganisms

There are different microbial species used as probiotics in ruminants which include bacteria, yeast, etc. Table 1 presents a list of microorganism targets commonly used as probiotics in ruminants feeds and this includes bacteria species belonging to the genera Bacillus, Enterococcus, Lactobacillus, Pediococcus, Streptococcus and yeast strains such as Saccharomyces cerevisiae and Kluyveromyces [29]. The most common commercial probiotics products for ruminants consist of live yeast (Saccharomyces cerevisiae). Although, majority of these strains are nonpathogenic and safe, others especially Bacillus cereus produces enterotoxins which may not be safe [29]. The use of yeast and fungal probiotics are more effective in adult ruminants, whereas probiotic containing bacteria species have high efficacy in pre-ruminant

Lactobacillus species	Bifidobacterium species	Enterococcus species	Other species	
L. reuteri	B. longum	E. faecalis	Sporolactobacillus inulinus	
L. paracasei	B. breve	E. faecium	Bacillus cereus	
L. brevis	B. infantis		Saccharomyces boulardii	
L. lactis	B. adolescentis		Streptococcus Salivarius subsp. thermophilus	
L. johnsonii	B. lactis		Clostridium botyricum	
L. crispatus	B. animalis		Escherichia coli	
L. fermentum	B. bifidum		Lactococcus lactis subsp. lactis	
L. amylovorus			Lactococcus lactis subsp. cremoriss	
L. delbrueckii subsp.			Pediococcus acidilactici	
L. rhamnosus			Propionibacterium freudenreichii Leuconostoc mesenteroides subsp. dextranicum	
L. helveticus				
L. acidophilus			исанинент	
			Aspergillus oryzae	
L. gallinarum			Aspergillus niger	
L. plantarum			Kluyveromyces fragilis	
L. casei			Kluyveromyces marxianus	
L. salivarius			Saccharomyces pastorianus	

Table 1. Common probiotic microorganisms use for ruminant (Adapted from [16, 17, 27, 28]).

calves. In pre-ruminants since their rumen is not yet developed, probiotic species administered targets the small intestine, help balance the gut microbiota, and reduce pathogen colonization of the host [30].

Prebiotic are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth/or activity of one or a limited number of bacteria in the colon" [31]. Prebiotics are commonly dietary fiber and have effect on both the upper and lower GI tract. In the upper GI tract, prebiotics are able to withstand digestion, delay gastric removal, decrease glucose absorption and stimulate the release of intestinal hormonal peptides. The main prebiotics used in animal diet are carbohydrates and oligosaccharide. Non-digestible oligosaccharides used include oligofructose, inulin, lactulose, galactooligosaccharide, transgalactooligosaccharide [16, 32].

Synbiotics on the other hand are products that contain a mixture of probiotic and prebiotics. The host benefit from the synergistic effect of probiotic and prebiotic. Results from studies done have demonstrated the promising effect of synbiotics in reducing the numbers of food borne pathogens [33].

1.3. Administration of probiotics

There are different route of administration of probiotics. These sites include the oral cavity, intestines, vagina and the skin [34]. In ruminants, probiotics are usually administered orally [35–43]. A study by Deng et al. [44, 45] utilized intravaginal infusion as mode of administering probiotics (containing a lactic acid bacteria mixture) to periparturient cows.

2. Probiotics and ruminant growth and production performance

2.1. Effect of probiotic on growth performance

Utilization of probiotics (either dry or live) as natural feed additives have been shown to favorably improve animal performance and welfare, via modulation of gut microbial community which is essential in ensuring host homeostasis [46]. Probiotic have positive effect on growth rate and production performance of animals when administered as single or multi-strain feed supplement (**Table 2**). Oral administration of probiotic has been shown to improve feed intake, daily weight gain and overall weight gain in sheep, goats, and cattle [38–43, 47, 49, 51, 52]. The population of beneficial microbes such as *Lactobacillus* and *Bifidobacteria* are low in neonatal calves, but studies have shown that supplementation with probiotics containing these microbes increases their growth [55]. In dairy cows, probiotic composed of live yeast increased food intake, improved feed efficiency, improved average daily gain and overall total weight. Additionally, probiotic increased milk yield and quality [51, 52].

In small ruminants such as goats and sheep, treatment with commercial probiotic improved average daily gain [35]. Gyenai et al. [36], Ekwemalor et al. [37] and Ekwemalor et al. [41], reported contrary results where there was no effect of probiotics on body weight. These different

Probiotics type	Ruminant	Performance effect	References	
Lactobacillus casei ssp casei	Calves	Weight gain	[47]	
Lactate-utilizing/or lactate-producing bacteria	Cattle	Improve feed efficiency, increase in daily gain (2.5%)	[48]	
Calf-specific probiotic (six <i>Lactobacillus</i> species)	Veal calves	Reduced diarrhea	[49]	
		Decreased fecal coliform counts		
Live yeast	Beef cattle	Improve average daily gain, final weight, feed intake, feed to gain ratio	[50]	
Yeast	Dairy cows	Increased milk yield and quality	[51, 52]	
		Increase feed efficiency		
		Reduced ruminal acidosis		
Lactobacillus casei Zhang and Lactobacillus plantarum P-8	Dairy cows	Improve quality and quantity of milk production	[24]	
FasTrack Microbial pack (Lactobacillus acidophilus, Saccharomyces cerevisiae, Enterococcus faecium, Aspergillus oryzae, fructooligosaccharide, active dry yeast culture	Dairy cows	Improve body weight	[42, 43]	
Lactobacillus acidophilus, Saccharomyces cerevisiae, S. boulardii, Propionibacterium freudenreichii	Lactating cows	Increased milk production	[53]	
Bacillus licheniformis and Bacillus subtilis	Sheep	Reduced lamb mortality rate	[54]	
		Increased average daily milk yield per ewe		
		Improved fat and protein content of milk		
Probiotic mixture (Bifidobacteriumlongum, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus reuteri and Lactobacillus rhamnosus)	Goat	No effect on body weight, PCV, White blood cells differential count	[36]	
Mushroom-based probiotic (Coriolus versicolor)	Goats	No effect on body weight, PCV, White blood cells differential count	[37]	
Commercial probiotic	Meat Goats	Improved average daily gain	[35]	
Multi-strain Probiotic (Lactobacillus acidophilus, Saccharomyces cerevisiae, Enterococcus faecium, Aspergillus oryzae, fructooligosaccharide, active dry yeast culture)	Goats	Improved Packed cell volume and FAMACHA scores	[41]	

Table 2. Summary of benefits of probiotics on growth and production performance of ruminants.

observations reported may be due to difference in the probiotic composition used, amount use, specific activity of the probiotic strains and variation in the breeds of goats used in their individual studies. This because studies have shown that different probiotic strains may have different effects depending on their capabilities and enzymatic activities different host species [34]. In the study by Gyenai et al. [36] Spanish Boer kid-goats were drenched with a probiotic mixture consisting of *Bifidobacterium longum*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus reuteri* and *Lactobacillus rhamnosus*. The commercial probiotic used by Ekwemalor et al. [41] composed of *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, *Enterococcus faecium*, *Aspergillus oryzae*, *fructooligosaccharide*, active dry yeast culture. Whitley et al. [35] used a commercial probiotic containing active dry yeast and lactic acid-producing bacteria, including *Lactobacillus acidophilus* and *Enterococcus faecium*. In addition, Whitley et al. [35] tested the probiotic on Boer crossbred meat goats (50–75% Boer of genetic background) however, Spanish Boer goats were used in the studies by Gyenai et al. [36] and Ekwemalor et al. [41]. Furthermore, two indicators of anemic condition in goats, Packed cell volume and FAMACHA have been reported to be affected by probiotic treatments [41].

2.2. Effects of probiotics in milk

Use of probiotics as feed supplements for ruminants have beneficial influence on milk production, milk quality and functional components such as protein and fat content [24, 54]. Studies have shown that probiotic dairy products are safe for large-scale consumption [56]. A study conducted by Yu et al. [57] showed that dairy cows treated with probiotic species Aspergillus oryzae ad Saccharomyces cerevisiae increased milk production and milk proteins. Also Sun et al. [58] and Qiao et al. [59] found that probiotics containing Bacillus subtilis improved the milk yield and rumen fermentation of dairy cows. Stein et al. [51] and Stella et al. [60] reported that probiotics improved the feed utilization rate, the milk yield and component profiles, and increase the dry matter intake in dairy cow. Xu et al. [24], also reported that probiotic application could reduce udder inflammation and increase milk yield while suppressing somatic cell count. Sun et al. [58] and Lehloenya et al. [61] reported that probiotic administration to dairy cows increased the milk production and simultaneously improved the milk fat, protein and lactose yield, accompanied by a decrease in milk somatic cell count. These positive effects of probiotics on milk production and milk quality characteristics are attributed to the subsequent effects of probiotics on the number of cellulolytic and fiber-degrading bacteria as well as changes in the volatile fatty acid in the rumen [54].

3. Molecular mechanism of action of probiotics

The mode of action of probiotics in the host organism include: regulation of intestinal microbial homeostasis, stabilization of the gastrointestinal barrier function, expression of bacteriocins enzymatic activity inducing absorption and nutrition, immunomodulatory effects, inhibition of procarcinogenic enzymes and interference with the ability of pathogens to colonize and infect the mucosa [62]. In ruminants, the mechanism of probiotics metabolism is dependent on the strain of microorganism used. Probiotic bacteria can serve to decrease the severity of infection via a number of mechanisms including competition for receptors and

nutrients, and/or the synthesis of organic acids and bacteriocins that create an environment unfavorable for pathogen development [49, 63–66].

4. Probiotics and immunity

The beneficial health effect of probiotics have been partly attributed to the ability of probiotic bacteria to modulate the immune system, increasing both innate and adaptive immune response [67, 68]. Research evidence obtained from various in vivo and in vitro studies have demonstrated that probiotic promote gut health via stimulation of the innate immune response [69]. Different probiotic bacteria including Lactobacillus casei, Lactobacillus casei strain Shirota, Streptococcus thermophilus, Lactobacillus fermentum and yeast have been tested to elicit an immune response [67, 70]. Oral administration of Lactobacillus casei activated immune cells of the innate immune response, and increased the expression of innate immune receptor, TLR2 [70]. In a similar study in mice, administration of Lactobacillus casei strain Shirota (LcS) enhanced innate immune response by stimulating or inhibiting the production of TH1/ TH2 cytokines [67, 71]. Ghadimi et al. [71] reported that probiotic enhanced secretion of IFN-γ (a TH1 cytokine) and inhibited the stimulation of TH2 cytokines such as IL4 and IL5. Research findings by Yan and Polk [72] showed that immunomodulatory effect of a probiotic (Lactobacillus rhamnosus GG) in preventing cytokine-induced apoptosis in intestinal epithelial cells. Results from their study indicated that the probiotic inhibited activation of the p38/mitogen activated protein kinase which is a pro apoptotic kinase induced by cytokines TNF, IL- 1α , or IFN-γ. Furthermore, the Lactobacillus rhamnosus GG probiotic activated Akt/protein kinase B (anti-apoptotic) in colon cells from mice and humans. In an in vitro study, a multiple probiotic formulation has been demonstrated to activate NF- κ B and stimulate production of TNF- α in epithelial [69]. Studies done in vivo and ex vivo have demonstrated the effect of probiotics treatment on the inflammasome [73]. The inflammasome found in various immune cells (macrophages and dendritic cells) and intestinal epithelial cells consists of cytosolic proteins such as NOD-like receptors, apoptosis-associated speck-like protein containing a CARD domain and caspase-1 (the serine protease). Activation of inflammasome receptors further leads to activation of caspase-1 and Interleukin (IL)-1β and IL-18. Activation and secretion and of the inflammatory cytokines Interleukin (IL)-1β and IL-18 stimulates and enhance the antimicrobial effect of immune cells against intracellular pathogens infection and also activates cell death of inflammasome-activated cells [74-76]. Studies have reported a potential role of the inflammasomes in the development of chronic intestinal inflammation [73, 76]. In ruminants such as bovine, the probiotic Lactobacillus rhamnosus GR-1 have been shown to amend E. coli induced inflammation in primary bovine mammary epithelial cells. Findings from their study showed that probiotic pretreatment impaired the activation ASC-independent NLRP3 inflammasome, and decreased protein expression of NLRP3 (NOD -like receptor family member pyrin domain-containing protein 3) and caspase 1 induced by *E. coli* [77].

The molecular impact of oral probiotic supplementation on systemic expression of genes associated with innate immune response in blood have been reported for ruminant species; cows [38, 42, 43], and goats [41] as shown in **Table 3**. Probiotics have been reported to activate pathways immunity and homeostasis including Toll-like receptor pathway, Wnt signaling pathway,

Innate immune response parameters	Genes	Ruminant type	References
Toll-like receptors	TLR2	Goats	[37]
	TLR8	Dairy cow	[38, 43]
	TLR6		
	TLR7		
Cytokines	IL4	Goats	[37]
	IL6	Dairy cow	[43]
	IL1B		
	IFNB1		
	CCL2		
	CCL3		
	CCL19		
	IL16		
	IL10RA		
Chemokines	CXCR2	Cattle	[43]
	CXCR1		
	CCL2		
	CXCL8		
Γh1 marker	STAT4	Goats	[37]
	CXCR3		
Nnt signaling	WNT8A	Dairy cow	[37, 42, 43]
	WNT5A	Goats	
	WNT10B		
	KREMENS		
	DVL1		
	PRICKLE3		

Table 3. Effect of probiotics on innate immune response gene expression in ruminants.

innate and adaptive immune response pathway [38, 41-43]. A study by Ekwemalor et al. [41] showed that oral probiotic administration may exhibit systemic effect in goat blood, by modulating the expression of genes associated with immunity and homeostasis. In goats, probiotic treatments induced the expression of 32 innate immunity genes and 48 genes in the Wnt signaling pathway. Furthermore, treatment of goats with a mushroom based probiotic in an vivo study trial resulted in serum increase in pro-inflammatory cytokines such as interferon production regulator (IFNr), Rantes and Granulocyte-Colony Stimulating Factor (GCSF). But the level of granulocyte macrophage colony stimulating factor (GM-CSF) reduced [37].

In dairy cows, oral probiotic supplementation had systemic effect on differential global gene expression. Probiotic treatment targeted 87 bovine pathways including Wnt signaling pathway, inflammatory response pathway, toll-like receptor signaling pathway, prostaglandin synthesis and regulation pathway and B cell receptor signaling pathway. Probiotic treatment modulated the expression of genes associated with innate immunity and homeostasis such as receptors TLR2, TLR6, TLR7, TLR8; cytokines, IL16, IL10RA; Wnt signaling genes Wnt8A, Wnt5A, Wnt10B, Kremens; and transcription regulators MAP4K3 and MAP3K8 [38, 42, 43].

5. Application of probiotic in ruminant

5.1. Probiotics and cattle

Probiotic have been widely used in cattle production for both dairy and beef cows at all developmental stages and growth. Studies have shown the beneficial effect of direct-fed microbials or probiotic bacteria including *Lactobacillus* and *Bifidobacteria* on growth, production performance (milk production, milk functional components and milk composition) and immune response of dairy cows, beef cattle, neonatal calves, and periparturient cows [38, 43, 51, 52, 55]. Furthermore, probiotic supplementation showed potential effect to decrease ruminal acidosis in feedlot cattle and dairy cows, and also improved immune response in stressed calved [48]. In dairy cows, probiotic increased food intake, improved feed efficiency, and improved average daily gain. Additionally, probiotic increased milk yield and quality [51, 52]. There are limited studies on the effect of probiotics on beef cattle compared to the research conducted on dairy cows. However, the use of probiotic yeasts to improve beef production has been variable, possibly due to the diet composition, strain of yeast or yeast viability [78].

Studies by Krehbiel et al. [48] have shown that probiotics are effective decreasing fecal shedding of Escherichia coli O157:H7 in infected calves. Research findings reported by Sherman et al. [79], demonstrated that treatment of intestinal cells with lactic acid producing bacteria reduced epithelial injury due to *E. coli* O157:H7 and *E. coli* O127:H6 exposure. Therefore, probiotic is used as one of the many strategies to reduce shedding of *E. coli* o157:H7 and non-O157:H7 in ruminants [80].

5.2. Probiotics and goats

Utilizing probiotics as functional food supplement have been encouraged in goat production [81]. Various commercial probiotic products consisting of either single strains or mixture of strains such as *Lactobacillus reuteri* DDL 19, *Lactobacillus alimentarius* DDL 48, *Enterococcus faecium* DDE 39 and *Bifidobacterium bifidum* DDBA have been tested in goats [35, 81]. Probiotic administration significantly increased body weight, and modified microflora by increasing the number of lactic acid bacteria and *Bifidobacteria* of goats. In addition, probiotic treatment reduced fecal mutagenicity by 60%, which is an indication of the protective influence of probiotics in goats [81]. Whitley et al. [35], observed no effect of probiotics on growth performance, diet digestibility, carcass traits, or fecal microbial populations in meat goats, although

an effect on the average daily gain was observed. Research findings by Gyenai et al. [36] supports the use of probiotics in goats to enhance microbial retention in the rumen.

There is an increasing market demand for nonfat goat milk and milk products such as yoghurt containing probiotics. A nonfat yoghurt has been developed from goat milk and is enriched with probiotic strains *Lactobacillus acidophilus* and *Bifidobacterium spp.* [82]. Other probiotic microbes have been used to develop different types of fermented drinking milk form goat milk. *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 novel putative probiotic *Propionibacterium jensenii* 702 co-culturing in goat milk affected their viability and physico-chemical properties of the milk [83]. In a similar study, goat milk fermented with *Lactobacillus fermentum* ME-3 and tested in healthy human subject reduced peroxidized lipoproteins levels, decreased 8-isoprostanes, improved total antioxidant activity and demonstrated an anti-atherogenic effects. The population and activity of lactic acid bacteria in milk was affected after fermentation with *Lactobacillus fermentum* ME-3 [84].

5.3. Probiotics for sheep

In sheep production, probiotics have been applied to improve feed digestion and gut health. Two probiotics, Saccharomyces cerevisiae and Aspergillus oryzae tested in sheep had no effect on Nitrogen digestibility and net microbial protein flow in the duodenum [85]. In another study, a probiotic mixture containing Bacillus licheniformis and Bacillus subtilis administered in ewes at late pregnancy and lactation reduced mortality in young lambs. In addition, probiotic treatment increased daily milk yield per ewe and fat and protein content of milk were also increased [54]. In a study by Rigobelo et al. [86], administration of a probiotic mixture containing Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermophilus and Enterococcus faecium to sheep infected with a non-O157 Shiga toxin-producing Escherichia coli (a foodborne pathogen of humans) reduced the fecal shedding of the pathogen. Probiotic treatment in sheep has beneficial effect on rumen methanogenesis, energy retention and Nitrogen utilization. In particular adding yeast culture and β1-4 galacto-oligosaccharides decreased methane emission in sheep [87] Propionibacteriaand lactobacilli-based probiotics were tested in sheep modified the bacterial population have been suggested to be useful to reduce the incidence of butyric and propionic subacute ruminal acidosis in sheep [88].

A study conducted in sheep showed that probiotic microorganisms are been used to improve food safety for consumers. Delcenserie et al. [89], found in their study that the presence of *Bifidobacteria choerinum* may be used as an indicator of fecal contamination of mutton. The study findings suggest that detection and identification of Bifidobacteria correlated with *E. coli* numbers can be used to improve hygienic quality during mutton processing.

Conflict of interest

The authors declare no conflict of interest.

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