Multistrain Probiotics in Livestock Production

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Multistrain probiotics comprise two or more species or strains of important microorganisms as a consortium beneficial to the administered animal. Several studies are being carried out to explore their potency or efficiency. They have proven to be a promising alternative to antibiotics growth promoters and were responsible for enhancing gut health, growth performance, maintaining a balance in gut microbiota, stimulating immunity against pathogenic organisms, improving digestion, and overall production efficiency in ruminants, poultry, and swine production.

Keywords: gut microbes ; feed additives ; growth performance ; cattle ; chicken ; pigs

1. Introduction

Probiotics preparations come in various forms, and their efficacy sometimes varies depending on whether they are mono- or multistrain. The new approach in probiotics utilization has been to use a combination of probiotics strains. This strategy is presumed to have highly influenced animal nutrition, exerted increased health benefits, and created an even more favorable balance of intestinal metabolism, animal welfare [1], and performance than single-strain cultures [2]. They can be administered via several routes (**Figure 1**), but the oral method is most common in animal husbandry.

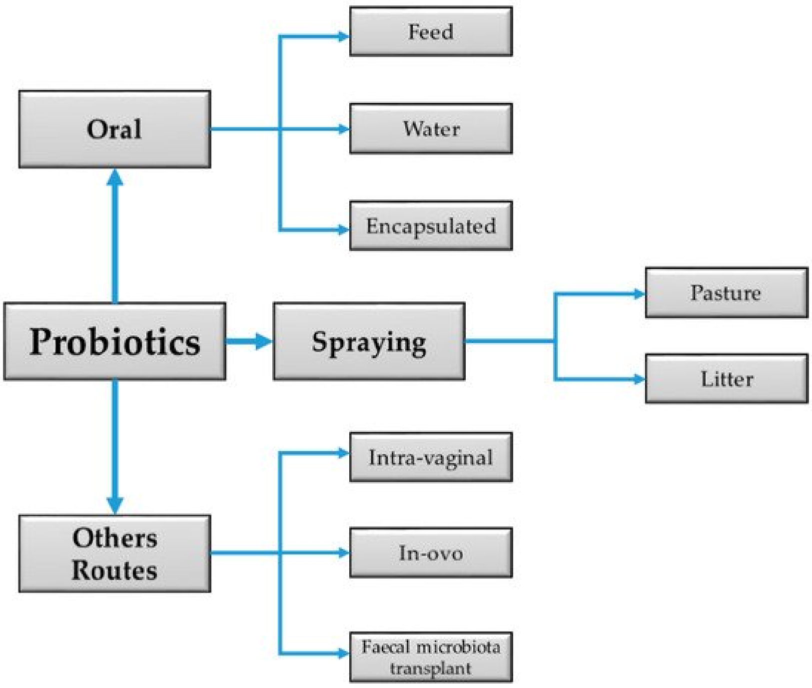

Figure 1. Diagram summarizing the common routes of administering probiotics in livestock.

2. Common Probiotic Strains and Their Mode of Action

Bacteria, bacteriophages, microalgae, and yeasts are all examples of probiotics [3]. Although numerous microorganisms have probiotic potential, Lactobacillus, Streptococcus, Enterococcus, Lactococcus, and Bifidobacteria remain the most commonly used probiotic agents in livestock to date [2][3][4]. Saccharomyces (S. cerevisiae and S. bourlardii), Candida pintolopesii, and Aspergillus oryzae are typical non-bacterial probiotics [4][5][6]. There are currently numerous commercially available mono- and multistrain probiotics [7].

Even though probiotics are considered a possible replacement for antibiotic growth promoters, their mode of action appears to be distinct [8]. Probiotics impacts are species-specific [9] and may also rely on the physiological and immunological condition of the administered animal. Different probiotics exert their benefits via mechanisms yet to be fully understood but are presumed to be related to their gastrointestinal lumen or wall activities. Their primary function results from the production of a range of antibacterial and bacteriostatic substances, such as organic acids, bacteriocins, diacetyl, antibiotics, and hydrogen peroxide [10], which exert beneficial effects through three primary pathways [11]:

(1) Competitive exclusion,
(2) Bacterial antagonism, and
(3) Immune system stimulation.

Probiotics also impact the health of the administered host via competition between beneficial bacteria and pathogens, replacement of pathogens by probiotic bacteria, and regulation of innate and adaptive immunity [12]. Due to their antagonistic effect, probiotics can hinder the growth of noxious bacteria by altering the gut microbiome, reduce the spread of pathogens and their emission during infection, decrease gut permeability, ameliorate clinical symptoms in livestock, boost immunity, and improve disease resistance and health [13][14][15]. In addition, they appear to be effective in foodborne pathogen reduction, for example, Salmonella, Escherichia coli, Campylobacter, Clostridium, Staphylococcus aureus, and perfringens [16][17], hence improving intestinal digestion and nutrient absorption and supporting a healthy micro ecological state. They can even aid pollution reduction by preventing the accumulation of harmful chemicals and lowering ammonia emissions in animal manure [18][19].

3. Multistrain Probiotic Use in Ruminants

Several studies have shown that probiotics can help increase milk quality, improve growth performance, increase average daily weight gain, improve feed efficiency, and reduce diarrhea in ruminants [20][21][22][23][24][25].

At the onset of diarrhea in dairy calves, a multispecies probiotic containing five bacteria strains (Bifidobacterium bifidum, Pediococcus acidilactici, Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium), peptide extract, dead yeast extract, dried whey, an enzyme blend, and natural flavor rapidly resolved the condition by reducing the duration of symptoms. The calves' daily weight gain improved with the combination as well [26]. Buffaloes supplemented with a multistrain probiotic-containing six bacterial strains (Streptococcus faecium, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus reuteri, Lactobacillus lactis) and two yeast strains (Aspergillus oryzae, Saccharomyces cerevisiae) had no improvement with respect to body condition score and dry matter intake but had a higher average daily milk yield, and reduced feed conversion ratio [27].

Furthermore, Kembabazi et al. [28] discovered that a mixture of Lactobacilli plantarum and Saccharomyces cerevisiae could operate as a probiotic. According to the findings, the mechanism by which they exert their probiotic function involves producing a low and stable lactate concentration in the rumen, resulting in a low pH medium suitable for the activity of S. cerevisiae, which usually amplifies the rumen bacteria population and competes against starch-utilizing bacteria. Owing to the potentiality of yeast to regulate pH and scavenge oxygen, they limit lactate build-up, creating a more conducive habitat for the cellulolytic activity of bacteria. Therefore, resulting in enhanced fodder consumption [29] as indicated by improved dry matter intake in nursing dairy cows.

In another study, Olchowy et al. [30] top-dressed pasture with a liquid commercial probiotic product containing a mixture of multispecies constituting four bacteria strains (Lactobacillus rapi, Lactobacillus parafarraginis, Lactobacillus zeae, and Lactobacillus buchneri with a minimum concentration of each strain, 10^6 CFU/mL), Acetobacter fabarum (minimum concentration of 10^5 CFU/mL) and yeast from the environment (Candida ethanolica; minimum concentration of 10^6 CFU/mL). Based on the result, cows that grazed pasture treated with the product produced a significantly higher volume of milk and a higher quantity of milk protein with tendencies towards producing more milk fat. Similarly, when dairy cows were directly fed the pasture from paddocks treated with the same probiotic mixture, the treatment group still produced more milk and higher milk protein content than the control group. In addition, Deng et al. [31] used an intravaginal infusion to give transition dairy cows a lactic acid bacteria cocktail containing Lactobacillus sakei, P. acidilactici FUA3138, and P. acidilactici FUA3140 combinations around parturition. The result revealed lower non-esterified fatty acids, higher cholesterol, and higher lactate levels, indicating that the concentrations of specified metabolites in the blood serum of transition dairy cows had been altered. A summary of several other combinations used in cattle, sheep, and goat of different physiological status and age are presented in **Table 1**.

Table 1. Various combinations of multistrain probiotics and their effect on ruminant production.

Multistrain	Cell Count	Mode of Administration/Dose	Host	Duration	Effect	No Effect	Ref.
Bacillus foraminis, B. firmus B. licheniformis, Staphylococcus saprophyticus bovis	**10^7 CFU/g**	**Oral inoculant using a syringe (1 mL/day at 1–2 weeks, 2 mL/day at 3–9 weeks)**	**Neonate lamb**	**9 weeks**	• Reduced feed intake • Lower acetate to propionate ratio	**No effect on BWG and wool quality**	[32]
P. acidilactici 3G3 L. plantarum BS S. cerevisiae 2030	**5 × 10^9 CFU/mL**	**Orally using a syringe (6 mL)**	**Dairy goats**	**9 weeks**	• Improved BWG, total milk fat yield, solid-non-fat, and lactose, PUN and triglyceride concentration, economic profit, and reduced somatic cell count	**No effect on total milk yield, glucose, hemoglobin, and RBC count**	[33]
E. faecalis L. rhamnous	**2 × 10^9 CFU/mL**	**Orally using dosing gun (5 mL)**	**South African goats**	**30 days**	• Improved weight gain • Lowered gut pH • Maintain the ecology ruminal microbiota	**No effect on feed intake**	[34]
L. acidophilus L. casei B. thermophilum E. faecium	**10^7 CFU/g**	**(Orally) mixed with concentrate**	**Lactating Ewes**	**8 weeks**	• Increased milk fat, butyric, and caproic acid	**Rumen conversion pathway of Fatty acid was not altered**	[35]
L. acidophilus L. plantarum B. bifidum, B. subtilis, A. oryzae	**1 × 10^8 9.8 × 10^7 2 × 10^6 CFU/g**	**Orally (3 g or 20 g/cow/day mixed with diet)**	**Pre-partum dairy cow**	**6 months**	• Increased DMI, milk yield and composition, serum albumin, and reduced globulin during postpartum	**No effect on BW, birth weight of calves, blood biochemical concentrations**	[36]
(Locally produced probiotic bacteria) containing: L. farraginis L. reuteri L. rhamnosus	**10^8 CFU/g DM**	**Orally (mixed with diet)**	**Pre-partum dairy cows**	**3 months**	• Increased feed: milk ratio, DMI, milk yield, % milk fat, and protein • Enhanced postpartum uterine and cervical involution, and conception rate	**No effect on milk lactose, solid non-fat, and ash**	[37]

Multistrain	Cell Count	Mode of Administration/Dose	Host	Duration	Effect	No Effect	Ref.
L. casei Zhang L. plantarum P-8	**1.3×10^9 (50 g/head/day)**	**Orally (mixed with basal diet)**	**Lactating primiparous dairy cows**	**4 weeks**	• Improved milk production and milk IgG content, lactoferrin, lysozyme, and lactoperoxidase, • An increased population of rumen fermentative and beneficial bacteria • Reduced somatic cell count	**No effect on milk fat, protein, and lactose**	[38]
L. acidophilus S. cerevisiae E. faecium A. oryza B. subtilis	**50 mL/day**	**Orally (mixed with endotoxin-free water)**	**Dairy cows**	**60 days**	• Increased % lymphocyte • Decreased neutrophil • Influence genes associated with immunity and homeostasis	**No effect on BW, PCV, and total protein concentration in plasma**	[39]
L. fermentum L. plantarum M. elsdenii S. cerevisiae	**4.5×10^8 4.5×10^8 4.5×10^8 1.4×10^{10}**	**Orally (dosing of 50 mL microbial suspension)**	**Fattening lamb**	**63 days**	• Improved nutrient digestibility, rumen fermentation characteristics, and nitrogen retention.	**No effect on feed intake and blood metabolite**	[40]

BWG, Body weight gain; PCV, packed cell volume; DMI, Dry matter intake; RBC, Red blood cell; PUN, Plasma urea nitrogen; n. s, not stated by the author.

4. Multistrain Probiotic Use in Poultry

Pathogenic bacteria including E. coli, Clostridium, and Salmonella appear to be a severe concern in chicken production, causing mortality, lowered growth rate, and low output. Antibiotics had previously played an important role in combating or regulating this problem; however, their prohibition has resulted in the use of probiotics to fill the void. Generally, because of their high fermentation utilization activity, probiotics promote protein and lipid digestion and interacts with enzymes to break down dietary molecules into simpler forms for digestion and absorption. They stimulate the production of digestive enzymes for carbohydrate metabolism, lower cholesterol, help in the synthesis of nutrients such as vitamins, influence the pH level in the poultry gut, and improve the productive performance, intestinal flora, and histomorphometry in heat-stressed chickens [18][41][42][43].

When broiler chickens were experimentally challenged with Pasteurella multocida, a highly contagious poultry disease that causes fowl cholera [44][45], supplementing dietary multistrain containing Saccharomyces cerevisiae, Lactobacillus fermentum, Pediococcus acidilactici, Lactobacillus plantarum, and Enterococcus faecium improved feed efficiency, growth performance, and intestinal health. It mitigated clinical signs, inflammatory reactions, and mortality-related symptoms [46]. In previous studies, successes have been recorded on probiotics' potency in attenuating the colonization of avian pathogens in the chicken gut [47][48][49][50][51]. These antimicrobial effects are traceable to bacteriocins, organic acids, hydrogen peroxide, and short-chain fatty acids secreted by probiotic bacteria [52]. Besides, the transcriptional profiles of anti-inflammatory genes in the intestinal mucosa of probiotic-fed birds were elevated, haemato-biochemical markers such as packed cell volume, total cholesterol, glucose, proteins, white blood cells, and lymphocytes were also improved. There is a possibility that perhaps the synergy between lactic acid bacteria and yeast strains resulted in higher antimicrobial activity against P. multocida and enterobacteria in the guts of supplemented birds, as well as the ability of the combination to out-compete pathogens, thereby preventing them from attaching to the intestinal walls and as a result improve intestinal microbial balance [53].

5. Multistrain Probiotic Use in Swine

Feed prices contribute to almost two-thirds of overall swine production expenses; hence, to ensure profitability in the pig industry, efficiency in converting feed into pig body mass is essential [54]. Moreover, improved metabolic utilization of dietary nutrients is dependent primarily on a healthy gut, which can lead to improved feed digestion and nutrient absorption [55]. Research has shown that multistrain probiotics could enhance growth performance, feed efficiency, and nutrient digestibility [56][57][58]. It has also been effective in maintaining a balance in the intestinal microbial flora [59][60], stimulating immunity [32][61], increasing litter size, vitality, and weight, and reducing fecal noxious gas emission in pigs [57][58]. A summary of the effects of some multistrain probiotics on pigs of different physiological statuses is presented in **Table 2**.

Table 2. Various combinations of multistrain probiotics and their effect on swine production.

Multistrain	Cell Count	Mode of Administration/Dose	Host	Duration	Effect	No Effect	Ref.
L. acidophilus **B. subtilis** **S. cerevisiae**	**1×10^7** **1×10^7** **1×10^7** **CFU/g**	**Orally** **(0.1% and 0.2% mixed with basal diet)**	**Finishing pigs**	**10 weeks**	• Improves ADWG and feed: gain, nutrient digestibility, growth performance, and gut microbiota • Reduced serum creatinine and noxious gas emission	**No effect on meat quality parameters**	[62]
Product A: **L. plantarum L21** **L. plantarum L80** **L. paraplantarum L103** **Product B:** **B. subtilis** **L. acidophilus** **S. cerevisiae**	**1×10^9** **1×10^9** **1×10^9** **1×10^{12}** **1.5×10^7** **1×10^9** **CFU/mL**	**Oral gavage** **(0.25 g/day)**	**Weaned pigs**	**28 days**	• Increased growth performance, fecal lactobacillus population • Reduced fecal E. coli Increased	**n.s**	[63]

Multistrain	Cell Count	Mode of Administration/Dose	Host	Duration	Effect	No Effect	Ref.
B.coagulans B. licheniformis B. subtilis C. butyricum	1 × 10^9 5 × 10^8 1 × 10^9 1 × 10^8 CFU/g	Orally (0.1 or 0.2 g/kg mixed with basal diet)	Growing-finishing pigs	16 weeks	• Improved BW, ADWG, feed: gain ratio, nutrient digestibility, fecal lactobacilli, and meat quality • Reduced E. coli and incidence of diarrhea	No effect on average daily feed intake and meat color	[64]
L. amylovorus L. reuteri LAB 26 L. reuteri LAB 49 L. johnsonii L. salivarius L. mucosae	1.7 × 10^{19} CFU/mL	Orally (1 mL mixed with PBS and 13% glycerol, aliquots added to feed)	Piglets	3 weeks	• Increased bacteria population in the jejunum • Influenced the expression of specific intestinal mucosa cytokines	No effect on the population of lactobacilli and bacteria in the large intestine digesta and growth enhancement	[65]
B. subtilitis B. licheniformis	1 × 10^9 CFU/g	Orally (0.1 and 0.2% inoculated into limestone and maltodextrin as carriers)	Lactating sow and their suckling piglets	28 days	• Increased piglets birth weight and ADWG • Improved nutrient digestibility in sows • Reduced fecal NH3, total mercaptans, and E. coli population in sows	No effect on reproductive performance, H2S concentration, and fecal score in sows	[66]

BWG, Body weight gain; ADWG, Average daily weight gain; n.s, not stated by the author.

6. Conclusions

In ruminants, poultry, and swine, multistrain probiotics have proven to be a viable alternative to antibiotics, and their usage in animal husbandry continues to grow. The effect on and responses of host animals, however, differs among literature. The variability in results might be due to the microorganism type or strains combined, as different species could possess distinct metabolic effects. The survivability of all the strains until delivery to the gut may also be difficult to ascertain. Probiotic dosage, the number of viable organisms in each dose, host animal physiological status and age, environment, diet composition, production procedures, and the mode of administering to the animal could all have a role. There were also limited reports on the greater benefits of multistrain probiotics over single strains in livestock. As a result, further research is needed to understand the interaction mechanisms among the combined microbes and the host's gut microbiota and the unique role played by the individual microbe. In addition, comparison among the investigated animals and direct comparisons between the mono- and multispecies probiotics should be considered. Finally, stringent recommendations for optimal benefits should be provided.

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