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## The Effects of Probiotics Supplementation on Milk Yield and Composition of Lactating Dairy Cows

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### ABSTRACT

The effects of microbials dietary supplements on performances and health of the animals, in recent is becoming very critical. Consequently, direct-feds microbials (DFM), is an area of interest of several authors, since last few decades. The probiotics usually, used as DFM for animals are lactic acid bacteria; *lactobacillus*, *streptococci*, *bifidobacterial*, *enterococcus* and anaerobic fungi species; *saccharomyces* and *aspergillus*. Normally, the mechanisms of action of DFM, are modulation of microflora balance in gastrointestinal tract and improvements in digestion and nutrients absorption, sustaining the health of the animals (i.e., through competitive exclusion, secretion of the substances that inhibit the growth or kill and altering gene expression of pathogenic agents) and stimulates the immunity of the animals. Generally, though the effects of host species, types of diet, animal physiological conditions, dosage of probiotics or strain, time of probiotic supplementation and variant strains used, are amongst important factors to be considered, DFM often plays an important role in improvements of milk yield and composition of lactating dairy cows. The DFM of *Propionibacterium*, *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, the mixture of yeast products and *Enterococcus* and combination of *L. acidophilus*, *L. casei* and *Enterococcus faecium* to dairy cows, significantly improves milk yield and well as the composition. Therefore, DFM, is one of the promising areas of ruminant's nutrition in general or dairy cows in specific, not only because of its nutritional and health benefits to animals, but also due to its negligible residual effects to the animal and animal products.

**Keywords:** Lactating cows, Milk yield and composition, Probiotics.

### INTRODUCTION

The nutritional value of fibrous feeds to the ruminant is basically attributed to the unique digestive system of ruminants involving an intensive preliminary ruminal fermentation step prior to a more classical enzymatic phase. The reticulo-rumen hosts a highly specialized anaerobic microbial community responsible for the fiber breakdown, which is influenced by biochemical and microbial characteristics of the rumen. However, in intensive farming practices, due to an excessively, higher fermentable carbohydrate supply which is important for enhancing the performance of animal's usually, disturbs the rumen microbial balance, eventually led to severe metabolic disorders and impair the production<sup>[1]</sup>. Hence, the role of direct microbial feed (DMF) of identified species were associated with rumen pH regulation, prohibition of pathogenic bacterial growth, maintaining health and enhancing production have been so far considered as a promising strategy in sector over the past few decades<sup>[2]</sup>. Consequently, at present time there is an increased interest in the use of probiotics basically, as animal feed and modifier rumen<sup>[3]</sup>.

Thus, the term 'probiotics' was first used by Lilly and Stillwell<sup>[4]</sup> to designate unknown growth promoting substances produced by a ciliate protozoan that stimulated the growth of another ciliate. Now a days, the term covers a much broader group of organisms. The joint Food and Agricultural Organization (FAO) and World Health Organization (WHO) of United Nations Working Group defined probiotics as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host"<sup>[5,6]</sup>. The most commonly used organisms in probiotic preparations are the lactic acid bacteria (*Lactobacillus*, *Streptococci*, *bifidobacterial*), which are found in larger quantity in the gut of healthy animals and do not appear to adversely affect them. The Anaerobic fungi yeast (*Saccharomyces cerevisiae*, *Saccharomyces boulardii*) and filamentous fungi (*Aspergillus oryzae*) were also used for probiotic preparations<sup>[7]</sup>. In general, the<sup>[8,9]</sup> have pointed out the potential benefits of DFM in ruminants are; maintaining rumen pH and population of microbiota, enhancing fiber digestibility, decreasing methane production, modulation of immune system of ruminant animals.

The beneficial effects of probiotics to the animals can be affected by a myriad of factors such as host species, type of diet, animal conditions, dosage, timing and strain of probiotic however, several research outcomes shown that DFM have a positive response in improving animal production and health performance [10, 11, 12, 13]. Similarly, in dairy cattle, probiotics are commonly used to improve ruminal and intestinal microflora populations, consequentially enhance the performance and health of animal, and boosts synthesis of protein and vitamins, as well as milk production and compositions [14]. Accordingly, [15] study on the multiparous Polish Black and White cows indicated the fat corrected milk yield, milk fat yield, milk protein yield, casein yield, lactose percentage, total solid and solid-not-fat were significantly improved. Additionally [16], reported the addition of lactic acid bacterial inoculants to maize at ensiling has improved the palatability, intake and the aerobic stability of maize silage compared to the control. In other hand, Sablik [17], conducted two studies to analyze the effect of Yeast probiotic (i.e. *Saccharomyces cerevisiae*) and bioplex mineral mixture on milk yield and composition of dairy cows. And, therefore, it was observed that, there is significant increase in milk yield, milk fat yield, protein yield and FCM when 15 g of Yea-Sacc1026 strain was given to the cows in both experiments.

However, in some other literatures the outcomes were contrasting. Hence, Krishnamoorthy and Krishnappa [18], found no differences in milk yield and milk composition when yeast was added to finger millet straw for lactating crossbred cattle. Similarly, Swartz *et al.* [19] Oetzel *et al.* [20] Maamouri *et al.* [21], did not observe significant difference in milk yield and compositions in their studies.

Therefore, in this article the effect of probiotics supplementation on milk yield and composition of dairy cows and their modes of actions has been reviewed.

## PROPERTIES AND COMMON SPECIES OF PROBIOTICS

### Essential properties of probiotics

Probiotics are products containing either live or dead micro-organisms and the substances they produce. It consists one or more strains of microorganisms that can be administered to animals in the form of powder, tablets, granules or paste [22]. Microorganisms that are used in the manufacture of preparations should be isolated from the animals of the same species in which they are to be used, so that the resulting microbial material can adapt to the conditions that are most likely to occur in the digestive tracts of certain animal species [23]. In the digestive tract of animals, they should contribute for stabilization of microbial populations and increasing enzymatic activity of the system, thus create a positive effect on the development of the animals. According, to Toma & Pokrotnieki [24] Ohashi & Ushida [25] Grela *et al.* [26] the probiotic microorganisms (or strain) to be used in ruminant nutrition have to possess the following important properties:

- Be able to live in low stomach pH and show resistance to bile acids and also, should be tolerated by the immune system of the animal.
- Have a beneficial effect on the animal's organism and should not be pathogenic, allergic or mutagenic/carcinogenic to the animal.
- Have the ability to adhere to intestinal epithelial cells, and permanently or periodically proliferate and colonize gastrointestinal tract (or active in its presumed site).
- Have the ability to sustain under unfavorable storage and living conditions in the digestive tract.

### Common genera or species of probiotics

Most of commercial products uses multi-strain probiotics, although the benefits of incorporating more a single strain and/or species in one product has not been clearly determined [27]. Hence, those microorganisms, that are frequently used as probiotics in animal feeds includes, lactic acid bacteria (such as *Lactobacillus*, *Streptococci*, *bifidobacteria*) and anaerobic fungi (yeast) (i.e. *Saccharomyces*

*cerevisiae*, *Saccharomyces boulardii* and *Aspergillus oryzae*) that are normally, found in the gut of the animal.

## EFFECTS OF PROBIOTICS ON MILK YIELD AND COMPOSITIONS

### Effects of probiotics on milk yield

Modern dairy farms are targeting high milk production utilizing feed composed of high concentrates to meet the metabolic demand of the higher milk production. Such feeding system is associated with metabolic dysfunction like rumen acidosis especially during poor feeding condition and composition. Therefore, probiotics are suggested as an effective mechanism of preventing or treating ruminal acidosis and/or improve animals' performances [28]. Thus, according to Nocek, & Kautz, [29] Rossow, *et al.*, [30] Leicester, *et al.*, [31] and Stella *et al.*, [32], the supplementation of probiotics (i.e. Yeast and *Enterococcus* mix, *Propionibacterium* (strain-P169), Live Yeast Product (LYP), Yeasture DFM and *Saccharomyces Cerevisiae* (strain-CNCMI-1077) to dairy cows have significantly improved milk yield in comparison to control (see Table 1).

**Table 1:** The effect of direct-fed microbial1 (DFM) supplementation on milk yield

DFM (Spp. or Strain)	Control	Treatment	SEM	P	References
Yeast and <i>Enterococcus</i>	36.9	39.2	0.5	0.01	[29]
Live Yeast Product	32.3	33.0	0.41	0.0014	[30]
Yeasture DFM	47.77	48.93	0.412	0.01	[31]
SCC(CNCMI-1077)	2.08	2.38	0.14	0.03	[32]

On the other study, Hossain, *et al.*, [33], have reported a 0.3 liter / day / animal increase (which is 8.8 %) in average daily milk yield after feeding probiotics (*Saccharomyces Cerevisiae*) (P<0.05). Lehloenya, *et al.*, [34] reported, 9 % increase in milk yield when the mixture of yeast and *Propionibacterium* is fed dairy cows 2 weeks from pre-partum to 30.0-weeks post-partum. Similarly, Williams *et al.*, [35], Wohlt, *et al.*, [36], Piva, *et al.*, [37], Dutta, & Kundu, [38], Yalcin, *et al.*, [39] & Vibhute *et al.*, [40]. In other study, Bruno *et al.*, [41], reported that, the cows fed from yeast culture has produced more than 1.2kg milk per day. Also, Jacquette, *et al.*, [42] and Ware, *et al.*, [43] reported a 1.8 kg/day milk yield increase when the cows fed *Lactobacillus acidophilus* (2 x 10<sup>9</sup> cells/day) when compared with the control group. Gomez-Basauri, *et al.*, [44] observed an increase in milk production (0.73 kg/day) when feeding cows, form the mixture of *L. acidophilus*, *L. casei* and *Enterococcus faecium*. More recently, Stein, *et al.*, [45] reported, 8.5% yield increase (4% fat correction) in cows receiving, 6 x 10<sup>10</sup> *Propionibacterium* per day from two weeks' pre-partum to 30 weeks. Post-partum.

Nonetheless, other authors presented a contradicting result; were there was no significant improvement in milk yield from probiotics supplementation [46, 19, 47, 21]. But, in another hands, Arambel, and Kent, [48], found that milk yield of dairy cows has increased with addition of yeast, but only when the protein content is deficient in the diet.

In large animal studies, Oetzel, *et al.*, [20], did not observe any effect of *Enterococcus faecium* and *Saccharomyces cerevisiae* on milk yield or composition when fed to cows from 10-days prepartum to 23 days of postpartum. However, Nocek, *et al.*, [49], observed an increased dry matter intake (2.6 kg/day) and increased milk yield (2.3 kg/day) with the same combination of probiotics offered from 3 weeks prepartum to 10 weeks post-partum. Similar results were obtained by Nocek and Kautz [29] in a very similar trial using 44 Holstein cows.

### Effects of probiotics in milk compositions

Like milk yield, the effects of probiotics in milk composition were also inconsistent across different literatures. Consequently, the authors such

as [50, 51, 37, 52, 40, 29, 30, 32] were reported significant improvement in milk fat % after supplementation of probiotics. In contrary, Erdman and Sharma [53] Arambel and Kent [48] Swartz, *et al.*, [53] Dutta & Kundu [38]

Weiss, *et al.*, [47] Maamouri, *et al.*, [21] Hossain, *et al.*, [33] reported negligible improvement of milk fat % in the cows with probiotics supplementation (Table 2).

**Table 2:** The effect of direct-fed microbial1 (DFM) on milk composition (MC)

MC	DFM (Spp. or Strain)	Control	Treatment	SEM	P	References
Fat (%)	YE	4.76	4.44	0.09	0.01	[29]
	PB (P169)	4.12	4.00	0.11	0.17	[47]
	LYP	3.68	3.61	0.037	.048	[30]
	SCC	3.59	3.37	0.300	0.20	[21]
	SCC(CNCMI-1077)	4.46	4.32	0.09	0.001	[32]
	SCC	4.57	4.52	--	0.614	[33]
Protein (%)	YE	3.12	3.13	0.06	NS	[29]
	PB (P169)	2.72	2.73	0.03	0.9	[47]
	LYP	3.25	3.31	0.017	0.002	[30]
	SCC	2.94	2.94	0.005	0.90	[21]
	SCC(CNCMI-1077)	3.65	3.65	0.07	NS	[32]
	SCC	3.29	3.43	--	0.032	[33]
Lactose (%)	YE	4.59	4.65	0.02	0.05	[29]
	PB (P169)	4.69	4.72	0.04	0.6	[47]
	SCC	4.55	4.54	0.010	0.60	[21]
	SCC(CNCMI-1077)	4.99	4.94	0.13	NS	[32]
	SCC	28.9	30.1	--	0.008	[33]
Ash (%)	SCC	7.85	7.81	0.160	0.60	[21]
SNF%	SCC	8.28	8.57		0.014	[33]

NB: - PB: *Propionibacterium*; SCC: *Saccharomyces Cerevisiae*; LYP: Live Yeast Production; YE: Yeast & *Enterococcus*

In similar ways, different authors reported that probiotic supplementation have significantly affected milk protein (%) [30, 33, 54, 20, 41, 39, 40], milk lactose (%) [29, 33] and SNF% [33]. While, some authors have showed that, there was no significant influence of probiotics on yield of milk protein % [29, 47, 21, 32, 46, 48, 53, 38], lactose % [29, 47, 21, 32] and Ashe % [21] (see Table 2).

### Probiotics Mode of Action

Different probiotics exert their effects in various mechanisms, which is not yet fully understood, presumably, due to their actions was either carried out in gastro-intestinal lumen or in the GIT wall. Although, probiotics are being promoted as a substitute for Antibiotic Growth Promoters (AGP), the mechanisms of action of these feed additives appears to be different [55] and some of them are described in the following section.

### Modification of GIT's microbial population

Maintaining gut health in animals, particularly in the context of AGP being gradually phased out, though the manipulation of the diet was crucial to maintain or improve the animal production performance [56]. One of the basic determining factors of a healthy gastro-intestinal tracts (GIT) is the composition of the microbial population. The probiotics can change the microbial population dynamics in the GIT, by creating a shift in balance between the beneficial and harmful microbes. favorable [57]. Further, healthy microbial populations in GIT, are often associated with enhanced animal performance, reflecting more efficient digestion and improved immunity [58]. The reduction in pathogenic micro-organisms in GIT may be attributed to the production of antimicrobial substances such as [59] and adhesion of the probiotic microbes to the intestinal epithelium, thereby, excludes competitiveness of pathogens or by inducing immune system response.

The most common modulation of the GIT microflora by probiotics is an increase in the populations of *Lactobacillus* and *Bifidobacteria* [60, 61, 62] while populations of coliforms particularly *Escherichia coli* [61] and *Clostridium* spp. [59] become decreased. *Lactobacilli* and *bifidobacteria* produces protein or polypeptide bacteriocins which reduce the growth of closely related bacterial species [63], and may reduce the number of harmful micro-organisms in GIT.

### Production of antimicrobial substances

Some probiotics produce antimicrobial substances that may inhibit the growth of pathogenic micro-organisms in the intestine. Many bacterial species, including lactic acid producing bacteria [64], *bifidobacterial* [65] and *bacillus* [66], can produce several types of thermostable bacteriocins which possesses antimicrobial properties against a wider range of potential pathogens including, *Bacillus*, *Staphylococcus*, *Enterococcus*, *Listeria* and *Salmonella species* [67, 64, 68, 69]. In another study, the probiotic *L. salivarius* (strain UCC118 and Abp118) produced a broad spectrum bacteriocin and protected mice against pathogenic *listeria monocytogenes* [68]. But, a mutant of the same probiotic unable to produce bacteriocins and unable to protect the mice against pathogenic *Listeria monocytogenes*, confirming that the bacteriocins were the active agent. Bacteriocin produced by LAB (i.e. Nisin) inhibits the growth of pathogenic micro-organisms by inhibiting cell wall synthesis, with the formation of pores in the bacterial surface [70]. To achieve this, the bacteriocin bonded to the cell wall precursor, the lipid-II, forming a complex, which can form a pore onto the bacterial cell membrane leading to the death of the bacterium [71].

The DFM Yeast as probiotic has been found effective in decreasing pathogenic effects of infectious bovine rhinotracheitis virus in calves [72]. Probiotics strains were found to prevent and reduce mastitis and metritis in cattle by reducing adhesion of pathogenic bacteria, producing antimicrobial substances and with other modes of action [73, 74]. *Lactobacillus acidophilus* has been reported to provide protection

from *E. coli* O157:H7 infection in cattle owing to their bacteriostatic/bactericidal effects [75, 76].

### Alteration of gene expression in pathogenic micro-organisms

Bacteria communicate cell to cell through the secretion of chemical signals, called auto-inducers, which affect the behavior of bacteria [77]. This process of bacterial communication, called quorum sensing, is also used for communication between bacteria and their host [78]. Probiotics may affect quorum sensing in pathogenic bacteria, thus influencing their pathogenicity. Extracellular secretion of a chemical signal (autoinducer-2) by human enterohaemorrhagic *E. coli* (serotype O157:H7) was substantially, inhibited by fermentation products from *L. Acidophilus* La-5, resulting the suppression of the virulence gene (LEE – locus) expression in vitro. This would disrupt the quorum sensing and eventually prevent GIT colonization by *E. coli* (serotype O157:H7) [79].

### Colonization resistance

The GIT of neonatal animals and birds are naturally colonized with micro-organism, generally originating from the adult mother. These micro-organisms provide protection from enteric pathogens. Intensification of animal agriculture has reduced the opportunity for natural colonization of GIT, making animals more susceptible to intestinal pathogens infection. However, probiotics could mimic a natural colonization in neonates, or colonize an adult animal, thus, preventing pathogenic organisms from colonizing the intestinal mucosa. Certain strain of *Lactobacillus* and *Bifidobacterium* possess hydrophobic surface layer proteins which help the bacteria to non-specific adherence to the animal's cell surface [80, 81]. Such adhesion of probiotic bacteria to the intestinal epithelium covers the receptor binding sites, hence, prevent the pathogenic micro-organisms (such as *E. coli*, *Salmonella* and others) from attaching into the epithelium [81].

### Increase in digestion and absorption of nutrients

Improvement in productivity of animals due to probiotics can be associated with an increase in digestion and absorption of nutrients. A combination of *L. acidophilus* (NP52) and *P. freudenreichii* (NP24) improved the digestibility of crude protein, neutral detergent fiber and acid detergent fiber in lactating Holstein cows resulting in increased milk production per day by 7.6% without increasing dry matter intake (DMI) [12] and it was suggested that this was due to a change in the rumen microbial ecology. Similarly, supplementation of dairy cows with Probios TC (containing 2 strains of *Enterococcus faecium*) at the rate of  $5 \times 10^9$  cfu per day as well as  $2 \times 10^9$  viable yeast cells per day from 21 days prior to expected calving date through 10 weeks of postpartum, has increased milk yield by 2.3 kg per cow per day, no difference in 3.5% fat corrected milk. The *E. faecium* strains were thought to act by producing lactic acid, which supports a rumen microbial population, in enhancing digestion of roughages (such as maize silage and haylage) and increase DMI [29].

Increased enzyme activity in the GIT of animals supplemented with probiotics could be due to either production of enzyme by the probiotics itself or induced change in the microbial population and hence enzyme production. The other possible reasons are, the probiotics can increase the height of intestinal villi and villus [82], which can, thus increase their surface area for nutrient absorption.

### CONCLUSION AND RECOMMENDATIONS

With advancement in the knowledge of gastro-intestinal microbial ecology, their mode of action and species (or strains) isolation techniques, the uses of probiotics in animal nutrition was became an area of interest in very recent years. Hence, DFM is a promising in the future ruminant nutrition not because of its beneficial effects, but also, due to its negligible residual effect to the animal and animal products. More specifically, apart from a wider variation of the effects and uncertainty in its reproducibility across a wide range of probiotic

species (or strains), livestock species (or breeds) and husbandry (or environments), DFM can be the potential inputs in dairy cattle feeds biotechnology and thereby, improves milk production and productivity as well as milk composition.

And, a substantial species and subspecies of microorganisms were used as probiotics in animal nutrition, of which lactic acid bacteria (*Lactobacillus*, *Streptococci*, *bifidobacteria*) and anaerobic yeast (*Saccharomyces cerevisiae*, *Saccharomyces boulardii* and *Aspergillus oryzae*) were frequently used among others. Inexplicit, the details of DFM modes of action was yet not noticed very well. However, most of the literatures enlists that, modification of rumen microbial populations, enhancing feed digestibility and nutrient absorption, rumen pH regulation, colonization of gastro-intestinal tracts and competitive exclusion of pathogenic agents, production of antimicrobial substances and altering gene expression of pathogenic microorganisms were among common modes of action.

Therefore, in accordance to the above conclusions the following recommendations have been forwarded;

- ✓ The strict regulatory procedures should have to be set in place and accordingly, implemented to insure the DFM products used in animal feed which possibly, enter human food chain should be free of harmful substances and were, strictly gone through the standard procedures and contents in production.
- ✓ Further studies are necessitated in determining the factors influencing the effects of probiotics supplements to dairy cows on milk yield and compositions.

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