Review on application of probiotics /prebiotics and symbiotic in livestock production.

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Summary: Livestock provides a major source of disposable income for disadvantaged and marginal populations in developing countries, and provides a major entry point to fight against rural poverty. Among the livestock ruminants are mostly used as direct and indirect food source of human so that improving their production may solve food scarcity. Ruminants are restricted to grazing on low-quality forages, crop residues and agro-industrial by-products with very little or no concentrate diets, which adversely affect the animals in exhibiting their full production potential. So animal production can be increased by using feed additives like prebiotics probiotic and synbiotics. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host, mainly through the process of replacing or including beneficial bacteria in the gastrointestinal tract. The most common genera that have been used and possess probiotic characteristics are the lactic acid bacteria, Bifidobacterium and Lactobacillus. It is accepted that intake of probiotics contributes to the enhancement and maintenance of well-balanced intestinal micro flora. The improvements in productive performance of all animal species fed with probiotics are mostly due to the fact that probiotics promoted the metabolic processes of digestion and nutrient utilization. Many evidences support the use of these probiotics in increasing animal performance and health; such as increase growth rate, protect host from pathogen, increase digestibility and nutrient absorption, modulation of gut flora, production of antimicrobial substances and improve immunity. This feed additives are characterized by low pathogen to the host, resistance to low PH, can compete with the resident pathogen, non toxic to the host, normal inhabitant to the host and metabolically active. Strain identity is important to link a strain to a specific health effect.
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Introduction

Livestock provides a major source of disposable income for disadvantaged and marginal populations in developing countries, and provides a major entry point to fight against rural poverty (Smith *et al*., 2013). Ruminant feeding and production in the tropics are restricted to grazing on forages, crop residues and agro-industrial by products with very low allowances of concentrates (Adegoke and Abioye, 2016). These forages are rich in neutral detergent fiber and deficient in nitrogen, and during their ruminal fermentation fewer amounts of volatile fatty acids and microbial biomass (microbial protein) are synthesized (Santra and Karim., 2003), which always results in productivity loss. Hence, manipulation of the digestion process by feed supplementation is imperative to improve the utilization of the available feed resources and increase the productivity of ruminants. Nutritionists have developed many methods of feed supplementation such as the use of antibiotic growth promoters to enhance production by limiting the effects of pathogenic infection on ruminant productivity (Reti *et al*., 2013; Valero *et al*., 2014).

However, their usages have been significantly reduced on the basis of its health and environmental implications (Gaggia *et al*., 2010) because of the emergence of resistant pathogenic bacteria and the possible contamination of animal products that may pose health challenges to the consumers and resulting in searching more natural feed additives as alternative to antibiotics (Khan *et al*., 2016). So that to alleviate this problem several feed supplements have been used to improve animal performance either by manipulation of the rumen environment or by directly altering the composition and metabolic activities of rumen microorganisms (Azzaz *et al*., 2015a). Among these feed additives, probiotic micro-organisms have become increasingly important owing to their functional potentialities (Mantovani, A. *et al*., 2006).

Therefore the objective of this term paper is:

- To collect scientific evidences regarding prebiotic, probiotic and synbiotics.
- To know about the effect using prebiotic, probiotic and synbiotics on animal growth, production and health.

Description of Prebiotics, Probiotics and Symbiotics

The term "probiotic" comes from the Greek word "pro bios" which means "for life". Many definitions have been proposed for the word "probiotic" at different times. The more widely accepted one is Probiotics is defined as 'live microorganism which when administered in adequate amounts confers a health benefit on the host (Mack farland, 2015).'Viable microorganisms that can be consumed separately or with foods and assist dietary and microbial balance by regulating the mucosal and systemical immunity and beneficially affect the consumer's health (Lollo *et al*., 2013). Also defined as mono or mixed cultures of "live microorganisms which, when administered in adequate amounts confer a health benefit on the host" (Sekhon and Jairath, 2010).

Prebiotic is "indigestible fermented diet substrates that selectively stimulate the composition, growth and activity of microflora in gastrointestinal tract" (Hamasalim, 2015). Whereas Synbiotics refer to nutritional supplements combining probiotic and prebiotic together. When two nutritional ingredients or supplements (prebiotics and probiotics) are given together, the resulting positive effect generally follows one of the three patterns: potentiation, synergism and additivity (Nekoubin and Sudagar, 2012).

Many studies have evaluated the effects of different synbiotic preparations. Though, the use of synbiotics may possibly produce greater benefits rather than the application of individual portions (Merrifield. *et al*., 2010).

Merits Of Probotics

Increase Growth Rate

Probiotics can increase the weight gain of animals in case those Hydrocarbons are broken down by the bacteria which mean the food is being split into its most basic elements. This allows almost total absorption through the digestive system (Apas. *et al.,* 2010). In this way, they dramatically increase overall nutrition and enhance rapid cellular growth and development. For instance, Lactobacillus and Bifidobacteria increased weight gain and reduced mortality in young piglets (Frizzo *et al.,* 2011)

Protection of Host from Pathogen The intestinal microflora is an ecosystem shaped by a diversity of ecological niches, made of several bacterial species and a very large amount of strains (Aureli, 2011) that are important for health (Kamada *et al*., 2013). Their major function includes metabolic activities that result in salvage of energy and absorbable nutrients, trophic effects on the intestinal epithelium and reduced intestinal colonization by pathogenic *E. coli* and prevented or reduced the severity of the intestinal infection (Le Bon *et al*., 2010). However, reports of shifts in the composition of intestinal micro flora with age, will increased numbers of bacteria (mainly Enterobacter) and a decrease in the number of beneficial organisms such as Lactobacilli and Bifidobacteria (Vyas and Ranganathan, 2012). Healthy microbial populations in the gastrointestinal tract are often associated with enhanced animal performance, reflecting more efficient digestion and improved immunity (Hung *et al*., 2012).

Increase Digestibility and Absorption of Nutrients

Improvements in productivity of animals by using probiotics can be associated with an increase in digestion and absorption of nutrient that is as a result of increased enzyme activity in the intestine (Zhang and Kim, (2014). The increase in digestive enzyme activities would permit the host degrades more nutrients, enhancing digestion and promoting a probable increase in the weight gain rate and/or feed efficiency (Cerezuela *et al*, 2011). Probiotics also increase the height of intestinal villi and villus height: crypt ratio in poultry thus increasing the surface area for nutrient absorption (Biloni *et al*., 2013; Jayaraman *et al*., 2013; Afsharmanesh and Sadaghi, 2014).

Improve the Innate Immunity

Epithelial cells in the gastro-intestinal mucosa create a selectively permeable barrier between the intestinal lumen (which contains harmful substances such as foreign antigens, micro-organisms and toxic materials, as well as beneficial nutrients) and the internal environment of the body (Willing *et al*., 2012; Bajagai *et al*., 2016; Lee *et al*., 2016). This barrier is the first line of defense against the microbes in the gastro intestinal tract (Peterson and Artis, 2014). Probiotic in the organism of a healthy animal stimulate non-specific immune response and enhance the system of the immune protection, increased intestinal IgA, secretion both in sows and piglets and elevated IgG and IgM in turkey (Marshall-Jones *et al*., 2006).

Modulation of Gut Micro Flora

Several studies on weanling pigs and reported that probiotics increased the counts of Lactic acid bacteria and decreased Clostridium, *E. coli*, and Enterobacterium Species in swine gut (Bajagai *et al*., 2016).

Competitive Exclusion

Competitive exclusion is the action of normal microbiota that protects the gut against the establishment of harmful microorganisms and decreases the risk of intestinal infections and disorders. growth of *E. coli* was successfully inhibited by different strains of *lactobacilli, P*. acidi lactici or S. cerevisiae boulardii (Daudelin *et al*., 2011 and Yirga, (2015). Thereby blocking receptor sites against the pathogen attachment so, the probiotic bacteria exclude pathogens and thus prevent them from causing infection (Yang *et al*., 2015a).

Production of Antimicrobial Substances

Probiotic bacteria produce antimicrobial compounds that may inhibit harmful microbes in the gastrointestinal tract (Arrebola *et al*., 2010). Lactic acid bacteria produce Bacteriocin that inhibits the growth of pathogenic micro-organisms by inhibiting cell wall synthesis, with the formation of pores in the bacterial surface (Hassan *et al*., 2012).

Increase in Milk Production

Probiotics are incorporated in livestock feeding in order to improve the health of the animal and also to ensure food safety (Song *et al*., 2012). Furthermore, yeast is said to optimize rumen function resulting into more nutrient bioavailability, which consequently improve the milk production performance while ensuring digestive comfort of the animal (Maamouri *et al*., 2014). Supplementation with yeast probiotic (*Sacchacromycets cerevisiae*) improved the production of milk in cows, and the lactation peak of the cows was stretched longer by 1 week than that of control cows (Ayad *et al*., 2013).

Probiotic microorganisms and commercial products

There are wide arrays of microorganisms that have been studied as probiotics, which leads to numerous commercial products that are being promoted and marketed as food supplements for humans or feed additives for farm animals (Ahasan *et al*., 2015).

Table 1. List of some probiotic, prebiotic and synbiotic applied or studied for application in animal feed (Cruz *et al*., 2012: Song *et al*., 2014).

perbiotics (substrates)	**probiotics (products)**	**probiotics (microbes)**	**synbiotics (probiotics +prebiotics)**
inulin	Yogurt, cheese	Bifidobacterium spp.	Bifidobacterium spp+inulin
garlic, rye	kefir	lactobacillus spp.	lactobacillus spp.+inulin
onions root,	Yakult Dairy Drink	pediococcus spp.	lactobacillus spp. +FOS
chicory root	Juice, chocolate	saccharomyce spp	lactobacillus spp.+lactitol
Asparagus, Lactiol	Ricera Rice Yogurt	Bacillus spp.	Bifidobacteriumspp+ lactobacillus spp+inulin
wheat, barley	tempeh	propionibacterium	Bifidobacteriumspp+ lactobacillus spp+FOS
Galactoolygosaccharide (GOS)		lactococus *spp*	Bifidobacterium spp+GOS

Characteristics Of Probiotics

Non-toxic and non-pathogenic, normal inhabitant of the targeted species, colonization and being metabolically active in the targeted site, which implies: Resistance to gastric juice and bile Persistence in the gastrointestinal tract, Adhesion to epithelium or mucus, Competition with the resident micro flora, Production of antimicrobial substances, Antagonism towards pathogenic bacteria, modulation of immune responses, ability to exert at least one scientifically-supported health-promoting properties, genetically stable, amenability of the strain and stability of the desired characteristics during processing, storage and delivery, Viability at high populations, Desirable organoleptic and technological properties when included in industrial processes (Gaggìa *et al*.,2010).

Fermentation Types, Processes And Harvesting Methods

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi. In the course of this metabolic breakdown, they also release several additional compounds apart from the usual products of fermentation, such as carbon dioxide and alcohol (Dharmaraj, 2010).

There are two techniques of fermentation that are used either to produce microbial cells in large quantity or to produce extracellular microbial products or commercially available probiotics (Shim Y. *et al*., 2012).

Submerged liquid Fermentation (SLF): involves growth of microbes in an aqueous medium and solid substrate fermentations (SSF) are characterized by the growth of microorganisms on moist solid substrates in the absence of free flowing water (Hu *et a., l* 2008). Currently solid state fermentation (SSF) process is being employed for the production of probiotics (Patel *et al*., 2004); while submerged state fermentation are used in biotech industrial process such as production of enzymes (Battan *et al*., 2006) and bio-pesticides (El-bendary, 2006). Solid state fermentation utilizes solid substrates, like bran, bagasse, and paper pulp. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrate. It is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content (Muller *et al*., 2009).

In case of SLF first the probiotic microbe (mother culture) can be selected and the general purpose media (culture broth) containing the desired nutrient for the microbe can be autoclaved before use. Equal amount of mother culture and culture broth is mixed then subject to fermentation for the desired time. Incubate it with appropriate temperature and power of hydrogen (PH). the microbe grown in the culture broth can be sprayed in to the appropriate carrier (corresponding substrate of the used microbe) with equal proportion. Lastly dry it by comfortable temperature for the limited time then probiotic is produced (Shim *et al*., 2010).

Probiotic microbes like Lactobacillus acidophilus (KNU No. 31), Saccharomyces cerevisiae (KNU No. 55), Bacillus subtilis (KNU No. 42), Aspergillus oryzae (KNU No. 48) can be maintained in the laboratory as mother cultures. A culture broth medium containing required composition (6% corn steep liquor, 4% molasses, 0.3% yeast extract, 0.5% KH2PO4 and 0.25% K2HPO4) in distilled water can prepared and autoclaved before being used.

In the solid fermentation method, 2 L of autoclaved culture broth could inoculated with 2 ml of mother culture of each microbe separately and subjected to fermentation for 48 h. L. acidophilus and B. subtilis could incubated at 37°C at pH 7.0, whereas S. cerevisiae and A. oryzae could incubated at 32°C at pH 4.0. The microbes grown on culture broth could directly sprayed on 13 kg of corn-soybean meal (1:1) used as carrier followed by drying at 40°C for 72 h. This is termed the SLF probiotic product and can composed of 1.1×109 cfu/g L. acidophilus, 1.1×109 cfu/g B. subtilis, 1.5×107 cfu/g S. cerevisiae and 2.6×107 cfu/g A. oryzae.

The microbes grown on culture broth can be used as starter to produce probiotic product by the SSF method. Corn and soybean meal (1:1) was used as the substrate and water was added to maintain a 30% moisture level followed by pasteurization. Then the substrate (13 kg) was inoculated with 2 L of starter and fermented for 7 days. The conditions maintained during fermentation for different microbes were as follows: L. acidophilus starter+5 L CB at 37°C and pH 6.8; B. subtilis starter+5 L water at 37°C and pH 7.0; S. cerevisiae starter+5 L CB at 32°C and pH 4.0; A. oryzae starter+5 L water at 32°C and pH 4.0. After 7 days fermentation, the microbial biomass was dried at 40°C for 72 h and mixed to obtain the SSF probiotic product. The microbial count in the SSF probiotic product was 4.0×108 cfu/g L. acidophilus, 4.8×109 cfu/g B. subtilis, 1.0×104 cfu/g S. cerevisiae and 4.3×107 cfu/g A. oryzae. The cells produced are counted by plate count method and spectrophotometric (turbidmetric) analysis.

Futuretrendsandcurrentchallenges Of Probiotics

Strain identity is important to link a strain to a specific health effect. The trend for future could be focus on basic research to identify and characterize existing probiotics strains, determine optimal doses needed for certain strain and asses their stability through processing and digestion (Ben Ameor,2007). For the probiotics to represent a real and effective alternative to antibiotics and chemotherapeutics it is absolutely necessary to ensure their consistently high efficacy. The efficacy of probiotics may be enhanced by selection of more efficient strains of microorganism, gene manipulations, combination of a number of strains of microorganism and combination of probiotics and synergistically acting components. Genetic engineering techniques can be use to insert one or more antigen from a pathogen into probiotic strains with good colonizing capacity for use in immunotherapeutic applications, such as vaccination and delivery of immunoregulatory (Zhu, 2009).

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