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Rahul Sharma
V.E.O. Department of Animal Husbandry, Rithi, Katni, Madhya Pradesh, India

Shivangi Sharma
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, NDVSU, Civil Lines, Jabalpur, Madhya Pradesh, India

PC Shukla
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, NDVSU, Civil Lines, Jabalpur, M.P. India

Versha Sharma
Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, NDVSU, Civil Lines, Jabalpur, M.P. India

RPS Baghel
Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, NDVSU, Civil Lines, Jabalpur, M.P. India

Arpana Raikwar
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, NDVSU, Civil Lines, Jabalpur, M.P. India

Shashi Pradhan
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, NDVSU, Civil Lines, Jabalpur, M.P. India

Vandana Yadav
PhD Scholar, Department of Animal Genetics and Breeding, NDRI, Karnal, Haryana, India

Correspondence
Shivangi Sharma
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, NDVSU, Civil Lines, Jabalpur, M.P. India

Microbial and functional feed supplement to improve livestock and poultry productivity with special reference to synbiotics: A review

Rahul Sharma, Shivangi Sharma, PC Shukla, Versha Sharma, RPS Baghel, Arpana Raikwar ,Shashi Pradhan and Vandana Yadav

Abstract
India is blessed with world's largest livestock population (529.7 million) and stands on first rank in the world. Livestock sector plays an important role in national economy it contribute approximately 4% to GDP and 27% to agriculture GDP but the major problems for Indian livestock industry are gap between demand and supply of feed and fodder and diseases. To overcome these problems antibiotic growth promoters (AGP) are used worldwide in poultry and livestock industry they not only prevent some diseases but improve growth performance also. Continuous and misuses of antibiotic in livestock production resulted in development of drug resistant bacteria, drug residues in milk and body of birds and imbalance of normal micro flora. Therefore importance of alternative growth promoters such as probiotics, prebiotics and synbiotics is evident in place of antibiotic growth promoters. Probiotics are live microorganisms which when administered in adequate amount; confer health benefit on the host. Prebiotics are non-digestible but fermentable food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or limited number of bacteria. Synbiotics are the combination of probiotics and prebiotics. It is a new approach by this a food supplement will include both the live cells of beneficial bacteria and a selective substrate, bacterial cells survive and grow quickly because of selective substrate and establish their predominance.

Keywords: Synbiotics, probiotics, prebiotics, growth promoters, feed supplement

Introduction
India ranks first in having the largest livestock population in the world. India is blessed with bovine population of 199.10 million cattle and 105.30 millions buffalo, accounting 16.24 per cent and 56.90 percent, respectively of world bovine population and buffalo population and stand first in the world in number of bovine population (Livestock census 2007, GOI) [24]. India has highest milk production in the world i.e. 121.8 million tones with per capita availability of 281 gm/day. In dairying, India ranks as the world's largest milk producer with an annual output of 116 million tones approx. With an annual growth rate of 4%, India's milk production accounts for 16% of the total global output. India has 15% of cattle population and 52% of buffalo population. It ranks third in sheep population and second in goat population in the world. India is emerging as the world's 2nd largest poultry market with an annual growth of more than 14%, producing 61 million tones or 3.6 percent of global egg production (Iplexpo 2013) [19]. Apart from this, India ranks 6th in broiler production (125 billion Rupees) with an annual output of 2.39 million tones of broiler meat, as per the estimates of the Ministry of Agriculture, Govt. of India. The total poultry industry is valued at about 350 billion rupees.

India is rapidly emerging as one of the biggest markets in the world. Livestock sector contributes approximately 4% to GDP and 27% to agriculture GDP. Poultry and dairy sectors are the major sectors contributing to economic development (Tanwar *et al.* 2012) [43].

The biggest problem in India for livestock production disease and feed and fodder availability for livestock and this limitation exist in India from centuries, according to a survey the gap between demand and availability for dry fodder, green fodder and concentrate is about 40%, 36% and 57% respectively (DAHD 2012) [18]. To overcome these problems antibiotics were used worldwide in poultry and livestock industry in the past 60 years for preventing diseases and improvement of growth performance. But continuous and misuses of antibiotics in livestock production and poultry industry resulted many concerns about development of drug-resistant bacteria (Sorum and Sunde, 2001) [42], drug residues in the body of the birds (Burgat 1999) [4], and imbalance of normal microflora (Andremont 2000) [2]. Therefore, importance of using alternative growth promoters such as prebiotic, probiotic and synbiotic is evident.

Probiotics

"Probiotics" term comes from the Greek words "pro" (in favour) and "biotic" (life). Probiotics are defined as "living microorganisms in feed which when taken at certain level provide stability of intestinal microflora (Metchnikff 1908) [27]. Fuller defined probiotic as "live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora" (Fuller 1989) [12].

Some commonly used probiotics

Lactobacillus acidophilus, Lactobacillus bifidus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus planterum, Lactobacillus ruminis, Lactobacillus salivericus, Bifidobacterium bifidum, Enterococcus feccium, Streptococcus faecium, Streptococcus thermophilus, Saccharomyces cerevisiae (Simmering and Blaut 2001)[40].

Mechanism of action of probiotics

- **Competitive Exclusion**

Detrimental bacteria such as E. coli need to become attached to the gut wall to exert their harmful effects. Attachment of bacteria achieved by means of hair like structures, called fimbrae, on the bacterial surface. The fimbrae are made up of protein called lectins, which recognize and selectively combine with specific oligosaccharide receptor sites on the gut wall. Due to introduction of live microbial feed number of non-pathogenic bacteria increases, competition occurs between pathogenic and non pathogenic bacteria, attaches with gut wall (Mc Donald *et al.*2002) [26]. Oral administration of suspension of cecal contents or faeces obtained from adult chickens to newly-hatched chicks increase their resistance to oral infection by *Salmonella* and this phenomena is called competitive exclusion (Nurmi and Rantala 1973) [31]. Oral inoculation of day old chick with 1:10 dilution of normal intestinal contents from healthy adult birds one day prior to oral challenge with *Salmonella infentis* resulted in 77% of birds free of infection compared with a 100% infection rate in control birds. It is suggested that the protection primarily results from direct competition between native gut flora and *Salmonella* for the attachment to local site in gut epithelium.

- **Acid production and lowering of pH**

Antagonism by lactic acid bacteria has been associated with major end products of their metabolism. The lactic acid bacteria produce organic acid like acetic and lactic acid in large quantities, which reduce intestinal pH. The organic acids are toxic for the undesirable micro flora in the intestine and this toxicity is increased manifold at low pH, as the un-dissociated form of these organic acids has better penetration into the bacterial cell. The antagonistic effect of *Lactobacillus* against *Enterobacterium* and *Salmonella* is due to lactic acid production (Fox 1988) [11].

- **Excretion of antibiotic like substances**

The antagonistic activity of lactic acid bacteria towards pathogens can be attributed to the production of bactericidal substances. The antibiotic like substances produced by various strains of *Lactobacilli* has been named as acidophilin, acidolin, lactobacillin, lactocidin etc. These antibacterial compounds have been found effective against *Salmonellae, Shigellae, Proteus, klebsiella, E.coli, Pseudomonas,* etc. Hydrogen peroxide produced by some *Lactobacillus* spp. appears to be partially responsible for the antagonistic interaction since it has strong germicidal activity at low pH environment (Gilliland 1990) [15].

- **De-conjugation of bile salts**

The bile salts secreted into the gastrointestinal tract act as detergents and emulsify dietary fats. Most of the lactic acid producing bacteria has the ability to de-conjugate bile acids to free form i.e. cholic acid, deoxylicholic acid and taurocholic acid. These free acids (de-conjugated acids) are inhibitory than the conjugated bile acids and are more effective against anaerobic and gram positive aerobic bacteria. These conjugation of bile acids by the probiotic culture enhances its microbial nature and the production of antimicrobial compound (acidolin and reuterin), which further reduce the population of harmful bacteria in the gut (Juven *et al.* 1991) [20].

- **Digestive enzyme activity**

Microbial enzymes are beneficial to the host because they increase digestion of nutrients especially in the lower intestine (Sissons, 1989) [41]. *Lactobacillus* spp., which was used in cheese products, was found to have lipolytic, amylolytic and proteolytic activities (Lee and Lee 1990) [23].

- **Anti-mutagenic and anti carcinogenic activity**

Microbial feed additives suppress the activity of mutagenic compounds. The activities of carcinogenic enzymes such as nitroreductase, azoreductase and β-glucuronidase in the guts of rats can be reduced by supplementation of *Lactobacillus acidophilus* (Goldin and Gorbach 1977) [16]. Reduction in the fecal level of carcinogenic amines was achieved by feeding the diet supplemented with microbial feed additive (Martini *et al.* 1991) [25].

- **Ammonia Production**

Suppressing ammonia production and urease activity by probiotic culture may be beneficial for improving animal health and enhancing growth because ammonia produced by ureolysis in the intestinal mucosa can be significantly damage the surface cells. Yeo and Kim (1997) [45] reported that feeding of probiotic (*L. casei*) significantly decreased the urease activity in the small intestinal contents of broiler chicks during the first three weeks. Reduction in ammonia production is beneficial for minimizing respiratory problems and reduces environmental pollution from poultry farming.

- **Anti-cholesteremic effects**

Feeding the Lactobacillus decreases the level of cholesterol in serum. The anti-cholesteremic activity of lactic acid bacteria is expressed by three ways, by inhibiting cholesterol synthesis, its absorption in intestine or by de-conjugation of bile salts (De Rodas *et al.* 1996) [8].

- **Immuno-Modulation**

Nahason *et al.* (1994) [30] reported that Lactobacillus supplementation to layer chickens increased cellularity of Peyer's patches in the ileum, which is an indication of stimulation of mucosal immune system that responded antigenic stimuli by secreting immunoglobulin (IgA). DFM activate immune system by increasing the phagocytic activity of lymphocytes, production of T-helper cell mediated cytokines by spleen cells and inhibition of IgE production and by protecting the gut associated lymphoid tissue (GALT) to come in contact with different antigens (Saloffcoste 1995) [39].

Prebiotics

Prebiotics are defined as "a non-digestible but fermentable food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon" (Gibson and Roberfroid 1995) [13].Efficient prebiotic need to induce a specific fermentation in the colon. This process should occur by stimulation of potentially health-promoting indigenous beneficial bacteria but not harmful organism (Gibson and Wang 1994) [14].

Some commonly used prebiotics and their characterstics

Commonly used prebiotics are oligosaccharides i.e. fructo-oligosaccharides (FOS), mannon-oligosaccharides (MOS), lactulose, inulin etc. Sources of prebiotics may be natural or synthetic. Natural sources are legumes (oligosaccharides) i.e. field pea, black gram, chick pea which contains raffinose, staychose, verbascose. Synthetic oligosaccharides are formed by direct polymerization of disaccharides, fractionation of microbial cells to obtain the material from cell wall, fermentation of polysaccharides.

Mechanism of action of prebiotics

- They are not easily digested by host digestive enzymes, compounds such as FOS can be fermented by the favorable bacteria (e.g. Bifidobacteria and lactobacilli), giving them a competitive advantage. This shifts the microbial population towards such microorganisms and away from harmful species.
- The gut microbial population may be altered by the oligosaccharide interfering with the attachment of harmful bacteria to the gut wall. As a means of cell recognition all cell types have a unique configuration of carbohydrate containing compounds (glycoprotein and glycolipids) on their surface. Pathogenic bacteria have surface compounds called 'lectins' which recognize these carbohydrates and by which they attach to the gut cells. Once attach the bacteria are able to multiply and produce their harmful effects. Species such as Salmonella and *E.coli* have mannose specific lectins which bind to mannose residues on the gut mucosal surface. By introducing mannose containing compounds (MOS) into the diet the binding by pathogenic bacteria is disrupted and instead they bind to the oligosaccharide and are carried out of the gut with passage of digesta (Mc Donald *et al.* 2002) [26].
- Dietary oligosaccharide may direct inhibit the growth of certain intestinal pathogens by increasing the concentration of lactic acid and thereby decreasing pH in the lower gut (Choi *et al.,* 1994; Okumara *et al.* 1994) [4, 34]. Like other carbohydrate such as glucose, fructose, sucrose, starch and pectin, fructo-oligosaccharides induce decrease in pH of the culture medium during anaerobic fermentation (Gibson and Wang 1993) [14].
- Saccharides indigestible by the host are fermented by flora into volatile fatty acids (acetate, propionate and butyrate), lactate and several gases including carbon dioxide, methane and hydrogen (Cummings 1981) [7]. Bifido-bacteria ferment FOS and partially utilize the energy released by such metabolism to grow and incorporate carbon atoms from the fermented carbohydrates into their structural and functional molecules. Short chain fatty acids released from the fermentation are metabolically utilized by the epithelial tissue of the intestine and also play a role in modulating endogenous metabolism (Demigne *et al.* 1986) [9].
- Oligosaccharides may stimulate the blood immune system after crossing the intestinal mucosal barrier. Some pathogenic bacteria are able to cause disease only after they had translocated through the intestinal wall and are most common in animals fed fiber free diet. It is possible that oligosaccharides acting as soluble fiber reduce translocation and help to preserve systemic immunity (Monsan and Paul 1995) [28].

Synbiotics

It is a new approach envisaged is to combine both probiotics and prebiotics. This combination will beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in gastrointestinal tract by selectively stimulating the growth and/ or by activating the metabolism of one on limited number of health promoting bacteria. This combination of prebiotic and probiotic is known as "Synbiotics". Thus, a symbiotic in its simplest definition is "a combination of prebiotics and probiotics" (Collins and Gibson 1999) [6].

Example of Synbiotics

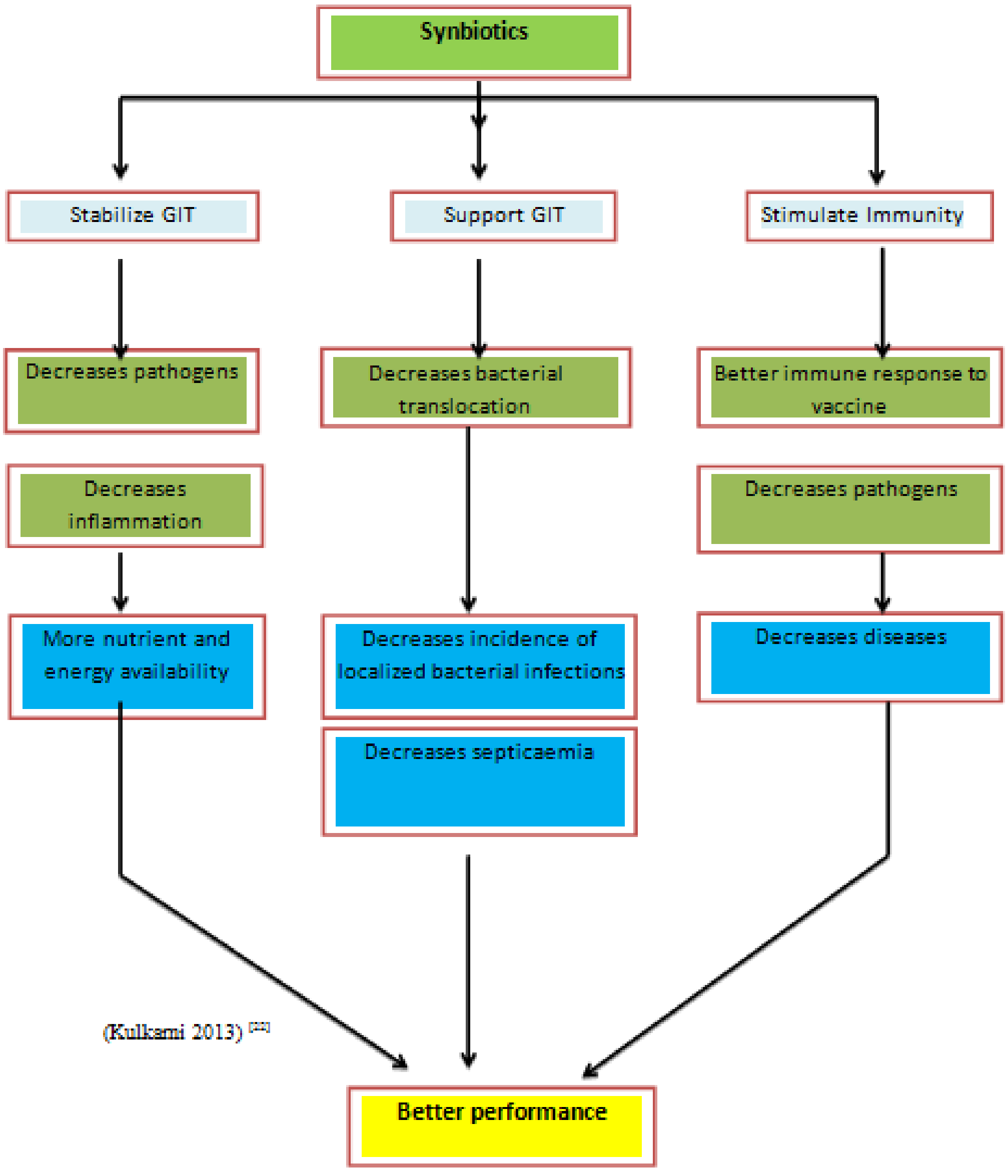
1. Fructo-oligosaccharide and bifidobacteria.
2. Lactitol and lactobacilli.
3. Fructooligosaccharide and lactobacilli.
4. Inulin and bifidobacteria.
5. Inulin and lactobacilli.

Rationale for the concept of synbiotics

According to this approach, a food supplement will include both the live cells of beneficial bacteria and a selective substrate; the idea behind that the bacterial cells that survive their transit through the upper gastrointestinal tract can quickly grow and competitively because of the selective substrate and establish their predominance. Thus a symbiotic food could be more efficient as compared to either probiotic or prebiotic alone (Roberfroid and Delzenne 1998) [37].

Mechanism of Action

Mechanism of action of synbiotics is the combination of probiotics and prebiotics so the following diagram can represent this mechanism of action.



(Kulkarni 2013) [22]

Effects of synbiotics feeding on different species

1. Calves

Before weaning, dairy calves are susceptible to many pathogens and nutritional problems. For several years antibiotics have been used to overcome these problems also to obtain economic benefits in terms of improved calf's performance and reduced medication costs. However, the use of antibiotics in animal husbandry is in question because of antibiotic resistance of microorganisms. Research shows an association between the use of sub-therapeutic dose of antibiotics and antibiotic-resistance organisms (Amabile-Cuevas 1995; Fevier *et al*. 1955; Piddock 1996) [1, 10, 35]. In an effort to replace antibiotics from animal feeds, many additives have been proposed. Probiotic, prebiotics and synbiotics (combination of probiotic and prebiotic) are examples of these additives (Heinrich *et al*. 2003; Morill *et al*. 1995) [17, 29].

Roodposti and Dabiri (2012) [38] conducted an experiment on 32 Holstein calves. They assigned calves randomly into four treatments, including whole milk without additives (control), whole milk containing probiotic, whole milk containing prebiotic and whole milk containing synbiotic (probitic and prebiotic in combination). Average daily gain was greater in calves fed probiotic, prebiotic and synbiotic at weeks 6, 7 and 8 ($p<0.05$). *E. coli* count was significantly lower in calves fed probiotic, prebiotic and synbiotic on d 56 ($p<0.05$). This study showed that addition of probiotic, prebiotic and combination of these additives to milk enhanced average daily weight gain and reduced fecal *E. coli* count in pre-ruminant calves.

2. Milch Cow

Synbiotic is a combination of live bacteria (probiotics) and their specific substrate (prebiotics) (Gibson and Roberfroid 1995). It was reported that *Lactobacillus casei* subsp. *casei* JCM 1134T (Lcc) uniquely and specifically utilizes Dex, and that a new synbiotic of Lcc in conjunction with Dex enhances humoral and cell-mediated immune responses in mice (Ogawa *et al*., 2005) [33] and chickens (Ogawa *et al*. 2006) [32]. The purpose of this study was to evaluate whether the new synbiotic, probiotic Lcc, and its prebiotic Dex in combination, consistently improved milk production in Holstein dairy cows throughout the year.

To evaluate the effects of a new synbiotic consisting of *Lactobacillus casei* subsp. *casei* (Lcc) and dextran (Dex) on milk production Yasuda *et al*., (2007) [44] conducted an experiment on 58 Holstein dairy cows, which became pregnant and gave birth to calves at regular intervals and lactated steadily and continuously, were selected. The study had a completely randomized design, and the animals were divided into two groups. Group A was fed with a basic diet only, and Group B was fed with a basic diet supplemented with the synbiotic consisting of freeze-dried Lcc and mixed feed containing Dex for one year from August 2004. After supplementation with the synbiotic, milk yields and components of Group B were compared with those of Group A in the August, December of 2004, April and August of 2005. Milk yields of Group B were greater than those of Group A. There were significant differences (p<0.01 or 0.05) between these groups for all values. Furthermore, total amounts of fat, protein and solid non-fat in Group B significantly increased in comparison with those of Group A. In addition, the somatic cell counts of Group A significantly increased in August of 2004 and 2005 in comparison with those of Group B. Thus, the new synbiotic consisting of Lcc and Dex can increase the milk production of Holstein dairy cows throughout the year.

3. Broiler

In the poultry industry, antibiotics are used worldwide to prevent poultry pathogens and disease so as to improve meat and egg production. However, the use of dietary antibiotics resulted in common problems such as development of drug-resistant bacteria (Sorum and Sunde, 2001) [42], drug residues in the body of the birds (Burgat, 1999) [4], and imbalance of normal micro flora manipulation, the combination of several strains, and the combination of probiotics and synergistically acting components. This approach seems to be the best way of potentiating the efficacy of probiotics and is widely used in practice. A way of potentiating the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics, which may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract. Those effects are due to activating the metabolism of one or a limited number of health-promoting bacteria or by selectively stimulating their growth, which improved the welfare of the host, or both (Gibson and Roberfroid 1995) [13].

A feeding trial was conducted by Awad *et al*. (2009) [3] to investigate the effects of dietary supplementations of synbiotic and probiotic on broiler performance and carcass yield. Six hundred 1-d-old broiler chicks were randomly assigned to 1 of 3 dietary treatments for 5 wk. The dietary treatments were (1) control, (2) basal diets supplemented with synbiotic (1 kg of Biomin IMBO/ton of the starter diets and 0.5 kg/ton of the grower diets), (3) basal diets supplemented with probiotic (1 kg of a homofermentative and a heterofermentative *Lactobacillus* sp./ton of feed). The BW, average daily weight gain, carcass yield percentage, and feed conversion rate were significantly (P < 0.05) increased by the dietary inclusion of the synbiotic compared with the control and probiotic-fed broilers.

4. Layer

Radu-Rusu *et al*. (2010) [36] conducted an experiment to assess the effects of dietary usage of a synbiotic product (strain of Enterococcus faecium + fructo-oligosaccharide + certain ficofytic compounds from marine algae) on laying hens production parameters and eggs quality. The biological material, represented by 60 ISA Brown females, 57 weeks old, was divided in two groups: control (C) (n=30) and experimental treatment (E) (n=30). C group was fed with layer standard diet, while E group received the same feed, supplemented with 1‰ synbiotic. Certain traits have been assessed during the 4 weeks of the experiment: hens live weight and feed intake dynamics, feed conversion ratio, laying intensity, egg mass production, eggs and eggshell weight, eggshell thickness, shell index, shell breaking strength, occurrence of eggs with unconformities. The laying hens in the experimental group, whose fed was added with 1‰ synbiotic, gave better yield performances (+2.2% laying intensity; +1.7% egg mass production; -2.0% feed conversion) and improved shell quality (+5.31% weight; +3.4% thickness and breaking strength). Therefore, the usage of probiotic-prebiotic- ficofytic compounds mixture in a single commercial product, as feed additive generated better results related to hens performance, feed valorization, eggs yield, and their quality. They found the following results-

- Usage of the synbiotic feed additive (1%) in laying hens feeding induced better production performances in the experimental group (E), compared to the control one.
- External eggs quality revealed improved traits in the eggs produced in hens group fed with 1‰ synbiotic, compared to control. Shell thickness increased with 3.35%, while shell breaking strength (mg/cm2) improved with 3.36%.
- Synbiotic based feed additive led to a decrease in eggs unconformities. Thus, 5.60% more eggs with undamaged shell were produced across the whole period by the hens in the experimental group, compared to the control ones.
- The beneficial effects induced by the synbiotic additive on quantitative and qualitative eggs production also generated increased income, which meant 10.59 % higher revenue in the experimental group than in control.

5. Dog

Kore *et al*. (2012) [21] conducted an experiment to study the effect of prebiotics, probiotics and synbiotics as functional foods on nutrient utilization, hindgut health and faecal microflora in Labrador dogs. They were selected 16 Labrador dogs and divided them into four groups in completely randomized design (CRD) for the period of 11 weeks. The dietary treatments consist of a control (experimental diet without prebiotic or probiotic), prebiotic diet supplemented with chicory (Chicorium intybus) inulin on DM basis. Probiotic diet supplemented with 5% of diet DM providing $1x10^9$ of *L. acidophilus.* Synbiotic supplemented with both means prebiotic and probiotic. The experimental diet was fed twice a day to meet the nutrient requirement of dogs. Prebiotics positively modified the hindgut indices and intestinal microflora. Probiotics positively influence the nutrient utilization, hindgut health attributes and intestinal microbial balance. Synbiotic provide a healthier alternative then their exclusive use individually from all aspect of the functional properties typically ascribed to probiotics or prebiotics.

Conclusion

Considering some evidence that the use of antibiotic growth promoters (AGP) may cause pathogen resistance, the application of antibiotics as animal growth enhancers had already been prohibited in the European Union since 2006. Today’s animal farming, especially the poultry industry have been greatly intensified with respect to both large number of animals and modern feeding systems. Concerns about the losses in animal performance and thus sustainability of production and its profitability coupled with this ban have led to an increase in research on the alternative supplements to AGP and strategies for food-producing animals. Nutritional supplements combining probiotics and prebiotics are referred to as synbiotics, which are a combination of “a probiotic and a prebiotic that beneficially affects the host by improving the survival and establishment of live microbial dietary supplements in the gastrointestinal tract”. The main importance of this form of synergism is that a probiotic alone, i.e. without a source of nourishment which can be represented by a prebiotic, cannot survive well in the digestive system. Synbiotics are gaining popularity and scientific credibility as functional food (feed) supplements at nutritional and therapeutic levels. It is believed that they can ensure a high level of viable probiotic cells once ingested. Some studies have shown the importance and benefits of this kind of synergy between probiotics and prebiotics and the effectiveness in helping young animals to achieve better growth performance.

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