Chapter

The Domino Effects of Synbiotic: From Feed to Health

Flávia Pelá

Abstract

Around of 60,000 tons per year of antibiotics are consumed to produce our food through subtherapeutic dosage usage which aim is improve healthy and performance of animal in intensive system production. If the use of antibiotics allowed greater access to food, on the other hand, it allowed a selective pressure of antimicrobial resistant strains, the superbugs. Considered a worldwide public health problem, this ultimately led to the prohibition of antibiotics as growth enhancers in animal production and the synbiotic, prebiotic and probiotic, is claimed to be effective alternative to withdraw of antibiotics in poultry farm. Hence, in this chapter, an antimicrobial resistance, animal health regulatory affairs and synbiotic influences will be summarized. The results of scientific assays and field trials from our synbiotics commercial formulations will be described to concerning the effect of zootechnical performance and sanitary control in the poultry production.

Keywords: synbiotics additive, antimicrobial resistance, poultry production, quality food, human health

1. Introduction

The human health is intrinsically associated from health and nutrition to animal and plants. This direct proportionality stems from the fact of that animals and plant, as food, can be by a direct source of contamination by pathogens, is been a common strain or an antimicrobial resistance strain [1].

The constant growth of human population rise the food demand which imply in a better intensive animal productivity. The intensive system has several challenges to produce eggs, meat, milk, fish and others, with high productivity, low costs and a quality and safety standard conditions. One of the most practices to improve the animal production is a use of subtherapeutic dosages of antibiotics for animal growth performance and sanitary control [1].

However, since 1970, the international agencies like as World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), U.S. Food and Drug Administration (FDA), World Organization for Animal Health (OIE) are doing severe appointments through by global public campaign for limit and/or ban the use of antibiotics as feed additive, because, this subtherapeutic practical for growth performance is one of the causes that triggers antimicrobial resistance from the selective pressure carried out by antibiotics [2–4].

Antimicrobial resistance, nowadays, is one of public health problem in the world. Each year it causes the death of more than 700,000 people worldwide, which the most common serotypes of infections are being Salmonella ssp., followed by

Escherichia coli and *Staphylococcus aureus*. Spending on patient care is high, reaching costs of around $29,000 per patient in the United States.

Efforts to substitute the antibiotics are occurring and the synbiotics additive has been one of potential alternative feed additives for the banned antibiotic-based stimulators [2–4].

Synbiotic is described by "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" [5]. Refers to nutritional supplements combining prebiotic and probiotic in complementary or synergism form which will beneficially affect the host by improving the implantation of live microbial dietary supplements in gastrointestinal (TGI) tract by selectively stimulating the growth and/or by activating the metabolism of one on limited number of health promoting bacteria [6, 7].

The mechanisms of synbiotic influence the host is the prebiotic stimulates growth of probiotic bacteria or the prebiotic and probiotic act independently in the GIT tract, both stimulating the intestinal microbiota. Non-digestible elements (prebiotics) are fermented in the GIT, while beneficial live microorganisms (probiotics) colonize it [5].

In this chapter, an antimicrobial resistance, animal health regulatory affairs and synbiotic influences will be summarized. The results of scientific assays and field trials from our synbiotics commercial formulations will be described to concerning the effect of zootechnical performance and sanitary control in the poultry production.

2. The domino effect

2.1 Antibiotic resistance

Antibiotics are in fact one of the best drugs developed. Initially applied for the treatment of infections, these drugs revolutionized modern medicine and changed the therapeutic paradigms [8]. Discovered in 1928 by Alexandre Fleming with penicillin, successive antimicrobials were developed and applied in the period between 1930 and 1960, the golden age [9–11]. However, concurrently with the findings, resistance to antibiotics has been identified, with the marked increase in patients relapsing to the infection of common bacterial pathogens. The truth is that every molecule used in the treatment of bacteria, fungi, parasites, viruses and, still, chronic diseases, by biochemical and physiological mechanisms favor the potential development of tolerance or resistance to the compound since the first use [10, 11]. This resistance associated with factors such as: overpopulation, improved global migration, indiscriminate use of antibiotics, as well as, the incorrect use neglecting the prescribed treatment, the intensification of animal production and the underdosing used as a zootechnical additive, selective pressure and basic sanitation precarious conditions have accentuated antibiotic resistance by living beings, making it one of the most important threats to public health in the 21st century, according to the WHO [2, 8, 12].

Results of genomic studies indicate the existence of more than 20,000 potential resistance genes (r genes) with about 400 different types [10], which mutation, horizontal gene transfer, conjugation and transduction are key hypothesis of the selective pressure that contributes to the distribution and co-selection of resistance and virulence genes. The impact of this selective pressure is reflected in the mechanisms of action of pathogenic strains that, in general, can modify the target site of antibiotics on the chromosome, promote the efflux of the molecule and degrade or modify the conformation of the compounds through enzymatic actions, thus

favoring colonization and invasion of pathogens and, consequently, causing damage to the host by expressing clinical picture of infection [9, 12–14].

Li *et al*. in a metagenomic assay identified profiles of a wide spectrum of multiresistance genes from different environments. These profiles were correlated and grouped according to incidence, and the results showed a higher prevalence of multiresistance genes in animal feces and in residual water from farms, followed by sewage and human feces, STP effluents, STP ADS and STP AS & BF and, finally, in drinking water, rivers, soils and sediments [15].

The result by Li *et al*. corroborates with the identification of multiresistance genes in animal production environments systems from the practice of indicating subtherapeutic amounts of antibiotics to animals for the purpose of improving performance [8, 9, 15]. In addition to the zootechnical performance, the increase in the intensive practice of animal husbandry, with overcrowding of the sheds, absent or precarious hygiene and disinfection practices, increased the prophylactic use of antibiotics. [8]. It is estimated that 60,000 ton per year are consumed on agricultural farms, and 80% of antibiotics consumed in the USA are used in livestock, with around 27 different antimicrobial classes being used in animals [9–11]. Antibiotics depending on the drug and the species treated will have an absorption or metabolization range between 10–80%, with the remainder being excreted as active compounds in the urine and feces to the environment. Thus, soils, water, effluents are contaminated and the selective pressure on the microbiota of these environments is selected, increasing the resistance to antibiotics [9–11]. In the holistic analysis of the ecosystem, each environment and living being serves as a regulator or regulated agent of selective pressure to multiresistance genes, serving an evolutionary cycle.

Unfortunately, global resistance to antibiotics has no tendency to decline. Data show that around 700,000 deaths per year worldwide are due to antimicrobial resistance. In Europe this number is 33,000 and the estimative of resistance to antibiotics represents a costs of €1.5 billion per year with healthcare expenses and productivity losses. In the US, the of deaths is 99,000, estimated cost with patients treatment is about $20 billion and the social costs reach $35 billion. In the Americas, about 77 million people per year fall ill after consuming contaminated food. Out of these, nine thousand die. In Brazil, from 2007 to 2016, 90.5% of the cases of foodborne diseases were caused by bacteria, mostly *Salmonella spp* (7.5%), followed by *Escherichia coli* (7, 2%) and *Staphylococcus aureus* (5.8%). Therefore, coordinated efforts to implement new policies to regulate the use of antibiotics, stimulate the research efforts and seek measures to manage the crisis are necessary to maintain intensive livestock productivity, animal and human health, and the ecosystem balance [2–4].

2.2 Animal health regulatory affairs

As an effort to reduce the antimicrobial resistance promoted by antibiotics used asr growth promoter, international agencies are searching to regulate a tolerance levels to antibiotics used for animals. The problem has been obtain similar commitments by the WHO, FAO and OIE in which measures of banned or establishment of minimum tolerance level of the drug shall be evaluated for mitigated noise and, consequently, avoid opportunities to inappropriate use of antimicrobial [16].

Despite the divergences, countries have been establishing regulatory measures regarding the use of antibiotics as growth promoters. The Europe (EU), in 2006, finished the progressive elimination of antibiotics program, used as growth promoters, banning sodium monensin, sodium salinomycin, avilamycin and flavophospholipol. These final measures aim to combat the emergence of superbugs, due to antibiotics overexploitation or misuse [17–19]. In 2017, the European

Commission adopted a "EU AMR Action Plan" which the key objectives are to make EU an example practice region; improve the research, development and innovation; and, shape the global agenda. Nowadays, since the plan implementation, updates have been made in order to further strengthen EU's response to AMR, such as, Pharmaceutical Strategy for Europe, creation of a new EU authority named Health Emergency Response Authority (HERA), creation of Commission Implementing Decision (EU) 2020/1729 for monitor and report antimicrobial resistance; adoption a tool Farm to Fork Strategy for sustainable food systems, implementation of Regulation (EU) 2019/6 on Veterinary Medicinal Products (VMP Regulation) and Regulation (EU) 2019/4 on Medicated Feed (MF), an implementation of better animal welfare, and others [20, 21].

In United States (USA), the antibiotic reforms were difficult, marked by constant clashes with the industries. Only from 2000, some formal procedures were started to withdrawal the antibiotics in animals for growth promoters. In 2013, FDA published a guidance for industry to phase out antibiotic growth promotion via label changes [22, 23]. In 2017, the completed implementation of guidance represented a changed of antimicrobial drugs used in the feed animal production. Of the 292 animal drug applications, 84 were banned and 208 remaining applications were converted from over the counter to prescription status or to veterinary feed directive status [23].

In Brazil, the Ministry of Agriculture, Livestock and Supply, through Normative Instruction N° 45, of November 22, 2016, prohibited the import and manufacture of the antimicrobial substance colistin sulfate with a performance-enhancing zootechnical additive throughout the territory in animal feeding [24]. Ordinance N°.195, of July 4, 2018 establishes good management practices in commercial farms, in order to obtain sustainable production, preserving health and well-being [25]. Furthermore, Ordinance N° 171, of December 13, 2018, informed that the use of the antimicrobials tylosin, lincomycin, bacitracin and tiamulin is prohibited for the purpose of performance-enhancing additives in farm animals [26].

Despite the alarming situation that resistance to antimicrobials has triggered in public health worldwide and the repeated appeals to reduce the inclusion of antibiotics in animal production by internationals agencies, many low- and middle-income countries do not include these recommendations in their national commitments. China is a country example: considered one of the largest consumers of antibiotics in livestock animals, elaborated a National Action Plan to Combat Antimicrobial Resistance from Animal Resources which regulates the withdraw all antibiotics used as feed additive; revised indicative use that antimicrobials are used only for prevention or treatment and stablished that new approvals of antimicrobials are only indicate for veterinary medicine [27]. South Africa, in 2018, by Africa Centers for Disease Control and Prevention (Africa CDC) has also developed a national framework plan which aimed detect to respond the infectious diseases in country [28].

In summary, the overview commitments of the recommendations are: i) implement a global public campaign to awareness about the importance to reduce antimicrobial used; ii) improve practical of hygiene and disinfections in daily routine either for human health or for animal management; iii) reduce the indiscriminate use of antimicrobials; iv) develop new diagnostics tools for rapid and reliable assay, including for accuracy monitoring antimicrobial development; v) improve management procedures for disease prevention and control; vi) develop sustainable and effective substitutes for antibiotics in animal production system [29].

During recent years, efforts focused to develop and work on providing novel and alternative supplements for growth performance and therapeutics to prevent diseases and enhance animal immunity. One of the potential substitutes evaluate is the synbiotic additive.

2.3 Synbiotic mode of action: an overview

The synbiotic concept is "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host". The symbiotic term is a Greek word compound of prefix 'syn', meaning 'together' and the suffix 'biotic', meaning 'pertaining to life' [5]. The prebiotic and probiotic combination product might not have any co- dependent function, acting trough by complementary and synergistic mechanisms. Both, independently promote an eubiosis, a maintain physiology homeostasis, modulating the digestive and immune system, and others functions in the host. The synbiotic product can be applied to intestinal or extra-intestinal microbial ecosystems in human, animals and agricultural species by regulatory categories, such as, feed additive, foods, non-foods, nutritional supplements or drugs [5].

The symbiotic formulation performs its function in a gastrointestinal tract, where more than 100 trillion (10^{14}) microorganisms inhabit. The resident microbial groups are affected by endogenous factors, such as, temperature, pH, oxygen concentration, diet, secretions, and others. Particularly, diets rich in non-digestible ingredient can highly modify the composition and function of gut microbiota by selectively influence [5, 30].

These non-digestible food ingredient as named prebiotics was described as "a non-digestible components of food, fiber or non-carbohydrate digestible, that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" [5, 30–32]. The criteria for prebiotic classification are: i) resist acidic pH, digestion action and adsorption by their host; ii) should be metabolized or fermented by microorganism residing in the TGI tract; iii) should promote a microbiota selectively stimulation, conferring beneficial physiological effect on the host; iv), not to all or poorly metabolized by pathogenic organism in gut bowel [5, 30, 31]. Most commonly known and characterized prebiotics include inulin, fructooligosaccharide (FOS), glucooligosaccharide (GOS), mannanoligosaccharide (MOS) [5, 30–33].

Prebiotics are considered a specific fuel that indigenous probiotic bacteria can utilize to grow. The selective fermentation of prebiotic occurs through correlation between chemical oligosaccharide structure and biochemicals metabolites of gut microbiota. The presence of carbon anomeric, the molecular weight and the number of branching present in prebiotic structure select microbiome preferences. For example, *Bifidobacterium sp* prefer to ferment low weight molecular of trisaccharides and tetrasachhrides in a series of oligosaccharides with reduced number of branching [30]. Beside this, the prebiotic metabolization by gut microbiome are influenced by secretion of a wide range of specific enzymes such as polysaccharidases, aminopeptidases, proteases, glycosidases, glycanases and others that will digest the prebiotics in a monomeric constituent. These parameters influence results the microbiome selective fermentation and explain the a non-digestion of prebiotic by host enzymes and the non-metabolization of them by pathogens strains, such as, *Salmonella spp*., *E. coli*, and *Clostridial* population [30, 32].

Furthermore, the metabolic fermentation results in a lactic acid, short-chain fatty acid (SCFA), or some antibacterial substances, such as bacteriocins, leading to a reduction of the metabolic activity of potentially harmful bacteria [30–34]. In general, the SCFA acts acidifying the luminal pH which suppresses the growth of pathogens, influence intestinal motility and acts stimulating enterocytes proliferation and mucin secretion. The rapidly absorption of SCFA by the enteric mucosa contributes to the quickly supply of host's energy requirements. Furthermore, they can be recognized by protein coupled receptors (GPR) expressed on polymorphonuclear immune cells, enterocytes and enteroendocrine cells stimulating the

chemokine and cytokines expression, such as, pro-inflammatory IL-2 and interferon (IFN)-γ and immuno-regulatory IL-10 production [30, 32, 33].

In addition, to modulating the immune system by SCFA, prebiotics can be direct recognized by toll-like receptors (TLRs) and NOD-like receptors (NLRs), both a pattern recognition receptor (PRR) present in immune membrane cells. This recognition will modulate the innate immune response inducing an overexpression of innate immune cells such as epithelial cells, macrophages, mast and dendritic cells. Another way of prebiotic action in immune systems is promoting the recognition of PAMPs signs by PRR, activating innate immune cells and the production of cytokines [30, 32, 33].

Finally, new scientific results correlate show that prebiotics also accelerate uptake of various micronutrients like iron, zinc, and calcium and significantly reduces or prevent the chances of colon-associated cancers, cholesterol, and elevated levels of triacylglycerols [30, 32, 33].

For establishment of health microbiota in host, probiotics play an important role enhancing the gut balance. Several studies revealed that supplementation of probiotics has positive impacts on TGI tract development and on the immune system modulation, consequently, improving the feed efficiency ratio, nutrient absorption, growth performance and the animal productivity. Probiotics are defined as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [35].

There are several species with probiotics abilities such as live bacteria, *Bacillus sp., Lactobacillus sp, Bifidobacterium sp,* yeast, *Saccharomyces cerevisiae* and *Saccharomyces boulardii*, fungi, *Aspegillus*, which are isolated from fermented products and human and animal body like as gut, breast milk, feces and other [36]. A good probiotic should have the following characteristics: i) the fermentation process should result in a minimum $1x10^9$ CFU culture; ii) the stain should be specie specify with high ability to survive and multiply fast in TGI tract host; iii) should be stable and safe to the host, GRAS 0, resisting an acid and bile action; iv) should have an ability in maintaining the normal physiology of host animals by strong adhesive capability in TGI tract, an effective competitive exclusion to reduce pathogenic microorganisms, and others; v) should have a durable shelf-life of commercial manufacturing, processing and distribution [36, 37].

The mode of action of probiotics in animal includes: i) maintaining normal intestinal microflora by competitive exclusion and antagonism; ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production; iii) improving feed intake and digestion; iv) and neutralizing enterotoxins and stimulating the immune system [38].

The main major mechanisms triggered by probiotics described are: i) modulation of the physical–chemical environment; ii) synthesis of biologically active molecules with antimicrobial properties; iii) and, modulation of the immune system.

The modulation of the physical–chemical environment of the enzymatic activities through the gastrointestinal tract and enzymatic activities catabolism stimulate the food's energy and protein digestibility which favors, the absorption of nutrients promoting the growth of the probiotic microbiota in detriment of the pathogenic one by the establishment of competition between them. This dynamic is called competitive exclusion. Concomitant to competitive exclusion, probiotics are also able to decrease the gut pH, though fermentation of carbohydrates providing an inhospitable environment for pathogenic bacteria, which are more susceptible to acidic pH. This is called growth modulation by pH. Still, the consumption of lactic acid by lactic bacteria and yeast strains can occur, which will result in the buffering of the TGI tract and the production of organic acids and vitamins [39–41].

Regarding the synthesis of biologically active molecules, the production of bacteriocins, antibiotics, free fatty acids, hydrogen peroxide is mentioned, among others, in particular, when there is the establishment of the probiotic microbiota to the gastrointestinal mucosa. These biologically active molecules control the proliferation and/or survival of the surrounding microorganisms. For example, bacteriocins are cited as peptides or proteins that kill related bacteria by permeabilizing their cell membranes or by interfering with the structure of their essential enzymes [42–44]. Another benefit comes from the increase in the concentration of propionate, succinate, valerate which, as precursors of gluconeogenesis, favor the availability of glucose to the animal, favoring the increase production, as well as its quality [45].

Concerning the intestinal homeostasis, there are literature describing the commensal intestinal microbiota as the main modulator of host physiology. The presence of probiotics adhered to the intestinal mucosa forms the so-called intestinal barrier capable of reducing the installation of pathogenic microorganisms, interfering with intestinal permeability, increasing the degradation of enteric antigens, as well as altering their immunogenicity. The repercussion of probiotic activity in the intestine implies an immunological homeostasis which in adverse contexts will favor immunological tolerance through the development of tolerogenic dendritic cells, regulatory T cells, Toll-Like Receptors (TLR), production of cytokines, according to immunological balance patterns [46–50].

In summary, the ability of synbiotics to do a protective effect on the intestinal microbiota may be dependent of multiple factors regulations such as formulation composition, indicative use dosage, host's genetic background, age and health status, hygiene and disinfections ambient conditions and treatment condition and duration.

2.4 From feed to health: the influence of Synbiotic commercial formulations in the poultry farm

In 2019, around of 100 thousand tons of poultry meat were produced in worldwide, being the U.S the world production leader followed by China and Brazil. The combined production of these countries represents half of the world poultry meat production [51]. In the exports, Brazil is a largest exporter with 4,200 ton shipped to more than 150 countries [52]. In 2020, this number increased on4% in production due to the national consumption increase and due to continuity of Chinese demand for animal protein. Also, the consumption of eggs increased as well [53].

This rising in the poultry production impacted in increase of 3.6% in feed production and, consequently, in a higher consumption of macronutrients and micronutrients that compose them. Around 16,494 tons of zootechnical additives were consumed in 2020, in which 10,144 tons were enzyme consumption, 4,947 tons were prebiotics and probiotics and 1403 tons were performance enhancers [53].

Through a comparative analysis of this data to the same parameters rescued from 2011, it is possible to of almost 50% in the consumption of performance enhancers and an increase of 1649% in the consumption of prebiotics and probiotics. In 2011, 5,628 tons of additives were consumed in poultry production, distributed in 2,434 tons of enzymes, 2895 tons of antibiotics growth promoter and 300 tons of prebiotics and probiotics [54].

The expressive increase of prebiotics and probiotics consumption is a consequence of the guidelines of the international agencies about antimicrobial resistance, the prohibition of the use of certain antibiotics as a growth promoter, the elaboration and execution of the National Action Plan on Antimicrobial Resistance

in Agriculture and the adjustments in the production chain in order to comply with the requirements of the foreign market.

The significant changes in the growth of commercial poultry have focused on intestinal development from two related but different directions. The tremendous genetic progress for largely grown poultry at ever decreasing ages turn recognize the first week posthatch represent a significant period of avian development and have a critical influence for intestinal growth. Immediately posthatch, the small intestine has proportional weight as body weight and will increase around 30% at 3 days. The contents of the residual yolk nutrients can be transferred to blood and intestine up 72 h, it represents a faster fed in chicks supplying their energy demand. At 7 days-old, the intestine will be twice as heavier weight than at day 1. Significant differences in villus height and crypt depth at day 3 from hatch noted, emphasizing the importance of intestinal development related to supporting accelerated growth and the importance of the intestinal given by histological measurements. A critical point in posthatch is the logistics of the chicks to the farm. During this period the birds are not feed with specific food, so they are susceptible to the environmental microbiota and, as a consequence, to a pathogen colonization [55, 56].

In this scenario of posthatch, in our trial research to evaluation of a commercial probiotic product, dispersive powder, composed by $3.5x10^7$ CFU/g *Bifidobacterium bifidum*, $3.5x10^7$ CFU / g *Enterococcus faecium*, 3, $5x10^7$ CFU/g *Lactobacillus acidophilus*, $4x10^7$ CFU / g *Bacillus subtilis* and $4x10^7$ CFU/g *Bacillus licheniformis*, indicated for application via spray, in the hatchery, on the chicks at a final concentration of $1.23x10^7$ CFU/ml, was applied in commercial layers to evaluate the microbial profile also too the product efficacy reduce the vulnerability that can occur by pathogen colonization in the gastrointestinal tract. Swabs from intestinal fragment, jejunum and ileum junction, were realized at times zero (D0), 7 days (D7) and 32 days (D32) and analyzed by next-generation sequencing technique, for evaluated the dynamic microbiome during the development of the gastrointestinal tract, also too, the better eubiosis establishment when probiotic intake is provided to the hens in the first moment of life.

At D0, hours after supplied the hens with probiotic supplement, were identified 12 bacterial species in the samples of jejunum and ileum junction, which 3189 reads (121 reads in treated group - SG; 3068 reads in control group - CG). The bacteria species identified are *Aeromonas hydrophila* (10 reads SG; 0 read CG), *Bacillus foraminis* (9 reads SG; 0 read CG), *Bacillus persicus* (5 reads SG; 0 read CG), *Brevundimonas bullata* (0 read SG; 185 reads CG), *Deftia acidovorans* (5 reads SG; 0 read CG), *Enterococcus faecalis* (82 reads SG; 1959 reads CG), *Noviherbaspirilum canariense* (0 read SG; 137 reads CG), *Ochrobactrum anthropi* (5 reads SG; 0 read CG), *Pantoea agglomerans* (0 read SG; 288 reads CG), *Pseusomonas koreensis* (0 read SG; 275 reads CG), *Pseudomonas putida* (5 reads SG; 129 CG), *Stenotrophomonas maltophilia* (0 read SG; 95 reads CG). These microbial profiles had a statistically significant difference to the treatment variable ($f(1) = 4.56$; *p-value* = 0.0353), and to the microbial diversity variable ($f(11) = 2.04$; *p-value* = 0.0329); and statistical trend for the interaction between the variables treatment and microbial diversity (*p-value* = 0.0765).

In start of feed consumption, at the first day of the birds' life (D1), hens of treated group were supplied with commercial product composed by $5x10^7$ CFU/g *Bacillus coagulans*, $5x10^8$ CFU/g *Bacillus subtillis*, $5x10^8$ CFU / g *Bacillus licheniformis*, $5x10^7$ CFU/g *Lactobacillus acidophilus* and $2x10^7$ CFU/g of *Saccharomyces cerevisae* and 2 g/kg Mannan oligosaccharides (MOS) was insert into the extruded feed at a final concentration of $2.24x10^5$ CFU/g of feed, to continue the gastrointestinal and immune system modulation. The analysis of the microbiome profile, at D7, had have a quantification of 88989 reads (18360 reads SG; 70629 reads CG) with

identification of 17 bacterial strains distributed in *Bacillus cereus* (5 reads SG; 0 read CG), *Butyticicoccus pullicaecorum* (0 read SG; 385 reads CG); *Clostridium beijerinckii* (9 reads SG; 0 read CG), *Clostridium difficile* (0 read SG; 25 reads CG), *Clostridium innocuum* (0 read SG; 26 reads CG), *Clostridium spiroforme* (0 read SG; 243 reads CG), *Enterococcus faecalis* (0 read SG; 157 reads CG), *Enterococcus gallinarum* (0 reads SG; 38 reads CG), *Erysipelatoclostridium ramosum* (0 reads SG; 135 reads CG), *Kurthia gibsonii* (0 reads SG; 27 reads CG), *Lactobacillus gasseri* (0 read SG; 7293 reads CG), *Lactobacillus helveticus* (13869 reads SG; 1526 reads CG), *Lactobacillus intestinalis* (0 reads SG; 2029 reads CG), *Lactobacillus johnsonii* (0 read SG; 36340 reads CG), *Lactobacillus reuteri* (2070 reads SG;4471 reads CG); *Lactobacillus vaginalis* (6 reads SG; 659 reads CG) and *Lactobacillus oris* (0 read SG; 1076 reads CG). These results in a statistical trend for the treatment variable ($f(1) = 3.50$; *p-value* = 0.0657) and a statistically significant difference for the microbial diversity variable ($f(16) = 2.42$; *p-value* = 0.0031), and to the interaction of these variables (*p-value* = 0.0071). In addition, at D7, greater intestinal length was observed in the hens of the treated group (X = 110.77 cm, Min = 100 cm, Max = 123 cm) compared to the control group (X = 103.5 cm, Min = 91 .5 cm, Max = 115.5 cm) resulting in a statistically significant difference, *p-value* = 0.0168.

At D32, end period of the microbial profile evaluation, there were a total quantification of 85069 reads (53042 reads SG; 32027 reads CG), with 37 bacterial strains identified distribute in *Acinetobacter junii* (0 read SG; 73 reads CG), *Acinetobacter ursingii* (0 reads SG; 106 reads CG), *Brachbacterium articum* (18 reads SG; 292 reads CG), *Brachbacterium faecium* (17 reads SG; 106 reads CG), *Brevibacterium epidermidis* (985 SG reads; 1557 CG reads), *Brevibacterium senegalense* (142 SG reads; 490 CG reads), *Brevundimonas diminuta* (0 SG reads; 283 CG reads), *Clostridium ruminantium* (18790 SG reads; 4844 CG reads), *Comamonas kerstersii* (121 SG reads; 0 CG reads), *Corynebacterium stationis* (1035 SG reads; 767 CG reads), *Corynebacterium casei* (997 SG reads; 915 CG reads), *Corynebacterium nuruki* (24 SG reads; 0 CG read), *Corynebacterium terpenotabidum* (0 SG reads); 75 reads CG), *Corynebacterium variabile* (227 reads SG; 382 reads CG), *Dietzia maris* (549 reads SG; 0 read CG), *Enterococcus cecorum* (506 reads SG; 290 reads CG), *Escherichia coli* (0 read SG; 10248 reads CG), *Facklamia tabacinalis* (148 reads SG; 0 read CG), *Fusobacterium mortiferum* (62 reads SG; 0 reads CG), *Fusobacterium necrogenes* (82 reads SG; 0 read CG), *Globicatella sanguinis* (35 reads SG; 0 reads CG), *Lactobacillus agiis* (0 read SG; 142 reads CG), *Lactobacillus aviarius* (611 reads SG; 0 read CG), *Lactobacillus helveticus* (21816 reads SG; 6348 reads CG), *Lactobacillus salivaris* (6061 reads SG; 1852 reads CG), *Ochrobactrum pseudogrignonense* (0 read SG; 47 reads CG), *Pantoea aeptica* (0 read SG; 121 reads CG), *Providencia rettgeri* (95 read SG; 0 read CG), *Pseudomonas veronii* (0 read SG; 41 read CG), *Staphylococcus gallinarum* (170 read SG; 178 read CG), *Staphylococcus lentus* (216 read SG; 0 read CG), *Staphylococcus saprophyticus* (229 read SG; 899 reads CG), *Staphylococcus sciuri* (18 reads SG; 0 read CG), *Streptococcus infantarius* (0 read SG; 192 reads CG), *Streptomyces rectiviolaceus* (17 reads SG; 0 reads CG), *Subdoligranulum variabile* (71 reads SG; 0 reads CG) and *Veillonela magna* (0 read SG; 1425 reads CG). There was no statistical difference for the treatment variable ($f(1) = 0.81$; *p-value* = 0.3692), there was statistical difference for microbial diversity variable ($f(36) = 2.53$; *p-value* < 0.0001), and no difference statistics for the interaction between variables (*p-value* = 0.4220).

As can be seen, immediately after posthatch, colonization of the gastrointestinal tract of the bird begins, whose quantitative and qualitative composition presents distinct microbial dynamics and profiles according to the influence of the zoogenic conditions of the environment, the components of the diet supplied to the animal, the interaction of microorganisms the physiology, metabolism and immunology of

the host, and the dynamics of interaction between microorganisms to achieve the complex and dynamic establishment of the microbiota [57].

When analyzing the results of the microbial profile, at D0, the quantitative discrepancy of the microbial load present between the experimental groups is observed. Hypothetically, it is suggested that there was competitive exclusion between bacterial species: the environmental microbiota and the probiotic multi-strains supplied through the commercial product. This hypothesis is based on the analysis of the microbial distribution profile in the intestinal fragment in which an approximate percentage of pathogenic bacteria colonizing both experimental groups is observed, but with a lower microbial load in the treated group. As an example, there was a prevalence of colonization of *Enterococcus faecalis* strains (82 reads SG, 68%; 1959 reads CG, 61%; FC = 23.89) followed by *Pseudomonas putida* (5 reads SG, 4%; 129 reads CG, 4%; FC = 25.8) in both groups, however, in the control group the presence of 23.89 more *Enterococcus faecalis* and 25.8 more *Pseudomonas putida* is observed in relation to the treated group.

These same pathogenic strains prevalent at D0 are suppressed from the microbial profile at D7, at distribution of *Enterococcus spp* being 0.27% in the control group and 0% in the treated group. The genus *Pseudomonas spp* is absent in both experimental groups, which shows the occurrence of competitive exclusion in the colonization of the intestinal fragment. Still, at D7, when analyzing the microbial profile of the experimental groups, the control group showed the greatest diversity and quantity of bacterial strains colonizing the intestinal fragment, with a prevalence of 98.53% of lactic strains and the presence of pathogenic strains with 0.41% *Clostridium spp* and 0.27% *Enterococcus spp*. Meanwhile, the treated group had lower microbial diversity, but higher prevalence of lactic strains (99.92%).

This dynamic microbiota in the first life stage of chicken was also reported by Śliżewska *et al.* [58] since the posthatch, in which they observe a prevalence of coli, enterococcus and lactic bacteria genera present in the crop, duodenum and jejunum. In the first and second weeks of life, they described the prevalence of the Lactobacillus spp. genera in the composition of the gastrointestinal tract and, in the third week, the microbial constitution was distributed in *Lactobacillus spp.* (70%), *Clostridium spp.* (11%), *Streptococcus spp*. (6.5%), *Enterobacteriaceae* family bacteria (6.5%), *Enterococcus spp.* (6%), corroborating a distribution of a microbial profile close to that identified in our results. Another common correlation identified was the significant reduction of potential pathogenic bacteria such as *Escherichia coli* and *Clostridium spp* when adding the symbiotic in the feed. In summary, both results show the beneficial effects of the consumption of the synbiotic in favoring sanitary control by establishing the balance of the intestinal microbiota.

At D32, the period reported in the literature for the establishment of eubiosis, effective bacterial diversity is observed in both experimental groups, and in eubiosis the group treated with commercial synbiotic product had a higher and better microbial profile. It should be noted that the prevalence of probiotic strains in the treated group throughout the experiment, even with a smaller amount of reads identified at D0 and D7, favored the establishment of eubiosis with the proliferation of other lactic strains that benefited the development and maturation of the treatment. Gastrointestinal tract of birds. While the control group had 26% probiotic strains (*Lactobacillus agiis, Lactobacillus helveticus and Lactobacillus salivaris*), 32% *Escherichia coli*, 4% *Staphylococcus spp* and other environmental strains, the treated group had 54% probiotic strains (*Lactobacillus aviarius, Lactobacillus helveticus and Lactobacillus salivaris*), absence of *Escherichia coli*, 1% *Staphylococcus ssp* and other environmental strains, showing the impact of consumption of the synbiotic for the establishment of eubiosis with a better microbial profile in hens.

Adhikari [59] described the distribution of lactic strains along the gastrointestinal tract of birds correlated with what was identified in our results. This reports the identification of greater abundance of *Lactobacillus salivarius* and *Lactobacillus johnsonii* in all intestinal fragments analyzed: cecal lumen, cecal mucosa and ileum mucosa, with the highest concentration of lactic strains identified in the ileum mucosa and cecal lumen followed by the cecal mucosa. Similar colonization profiles of lactic strains were described by Ranjitkar *et al*. [60] and Wang et al. [61].

Dunislawska *et al.* [62] corroborate the benefits of consumption of synbiotics by describing effects of consumption of microflora-promoting bioactive compounds, even in a single dose of prebiotic or synbiotic *in ovo* and immediately posthatch, in interfering with the dynamics of microbiota colonization as well as across the entire spectrum of phenotypic characteristics in the broiler development stages, including zootechnical performance, development and modulation of the immune system, development and histological composition of the gastrointestinal tract, change in molecular expression in cecal tonsils, spleen and liver, change in the composition of meat quality.

The reflection of this dynamics of colonization of the gastrointestinal tract of birds has repercussions in various field scenarios in the results of zootechnical performance, in sanitary control and in the reduction of antimicrobial pulses administered to the birds. To report this scenario of the reality of the field, whose management variables are diverse and often distinct from each other, one of our field trials is presented. This assay was carried out on a commercial poultry farm producing broilers, which houses about 7,000,000 birds per month. Two farms, Farm 1 and Farm 2, composed of 15 and 16 sheds respectively, housed Ross lineage birds. Farm 1 had in its ambience a cepillo's bed, dating back to 1st and 2nd, conventional lighting, side plates and an oven per aviary. Farm 2 had a cepillo's bed, dating back to n°. 2, dark lighting, side and front plates and two ovens per aviary. As for the treatment, the commercial synbiotic product was composed of $5x10^7$ CFU/g *Bacillus coagulans*, $5x10^8$ CFU/g *Bacillus subtillis*, $5x10^8$ CFU/g *Bacillus licheniformis*, $5x10^7$ CFU/g *Lactobacillus acidophilus* and $2x10^7$ CFU/g of *Saccharomyces cerevisae* and 2 g/kg Mananooligosaccharide was administered on extruded feed mixture at a final concentration of 1.02x105 CFU/g feed at farm 1, while poultries from farm 2 received the probiotic product consisting of $1x10^8$ CFU CFU/g *Bifidobacterium animalis*, $6x10^8$ CFU CFU/g *Enterococcus faecium*, $2{,}5x10^7$ CFU/g *Lactobacillus reuteri*, $2{,}5x10^7$ CFU/g *Lactobacillus salivarius*, $2{,}5x10^8$ CFU/g CFU/g *Pediococcus acidilactici* added to the extruded feed with final concentration of $1.00x10^5$ UFC/g of feed. Farm 1 will be named as the treated group and farm 2 as the control group.

In terms of zootechnical performance, there were no statistical differences between both treatments, at the seventh day (D7), regarding weight gain, *p-value* = 0.966 (control group X = 183.2 g, Min = 159 g, Max = 203 g; treated group X = 185.4 g, Min = 167 g, Max = 228 g) and as for intestinal length, *p-value* = 0.977 (control group X = 106.2 cm, Min = 90 cm, Max = 122 cm; treated group X = 107.2 cm, Min = 94 cm, Max = 124 cm); at D14, as for weight gain, *p-value* = 0.6111 (control group X = 510.3 g, Min = 400 g, Max = 572 g; treated group X = 510.2 g, Min = 473 g, Max =542 g) and as for intestinal length, *p-value* = 0.114 (control group X = 137.9 cm, Min = 115 cm, Max = 166 cm; treated group X = 144.1 cm, Min = 125 cm, Max = 174 cm); at D21, as for weight gain, *p-value* = 0.368 (control group X = 1014 g, Min = 969 g, Max = 1118 g; treated group X = 1019 g, Min = 878 g, Max = 1145 g) and as for intestinal length, *p-value* = 0.160 (control group X = 164.2 cm, Min = 148 cm, Max = 178 cm; treated group X = 169.8 cm, Min = 153 cm, Max = 198 cm); at D28, as for weight gain, *p-value* = 0.989 (control group X = 1596 g, Min = 1435 g, Max = 1702 g; treated group X = 1600 g, Min = 1441 g, Max = 1763 g) and as for intestinal length,

p-value = 0.808 (control group X = 187.6 cm, Min = 160 cm, Max = 220 cm; treated group X = 185.7 cm, Min = 166 cm, Max = 207 cm).

Despite the absence of significant statistics in the above results, at the end of the management, the treated group showed better performance in relation to the zootechnical results, as they had greater daily weight gain (control group = 68.82 g; treated group = 71.34 g), greater corrected slaughter weight (control group = 2573 g; treated group = 2853 g), better feed conversion (control group = 1.593; treated group = 1.571), consequently, better productivity factor (control group = 413.19; treated group = 430. 89). The only zootechnical results of the treated group with lower performance than the control group were the mortality parameter (control group = 4.27%; treated group = 5.17%) whose established hypothesis refers to the ambience, the presence of a single oven in the aviary, and the thermal challenges, variations from 8–25°C throughout the day, that the birds in the treated group went through in the first week of bird life.

As for sanitary control, it is routine in the management of farms to carry out at D21 the evaluation of the identification of the presence/absence of salmonella in the sheds using a drag swab, performing both polymerase chain reaction (PCR) methodology and the conventional method of microbial cultivation. The results for the PCR assay showed 4 positive samples for the control group and 2 positive samples for the treated group, while, for the conventional method of microbial cultivation, the control group showed 2 positive samples while the treated group did not show characteristic culture growth. These results show the best sanitary control of the synbiotic product to the sanitary control for salmonella. It is noteworthy that this drag swab is carried out in the bed of the sheds and does not necessarily reflect the presence of salmonella in the cecal content of the poultries. Further tests carried out in other poultry farms whose house received the treatment of the synbiotic product, despite showing identification of salmonella in the house and outside areas, did not show identification of salmonella in the cecal content of poultries.

As for the consumption of antibiotics, there was a significant reduction in the consumption of antibiotics in the treated group compared to the control group (FC = 0.37). The treated group consumed throughout the management three types of antibiotics which total of 33 administration pulses, while the control group consumed four types of antibiotics, totaling 89 administration pulses. The description of antibiotic consumption in the control group was, at D1, four aviaries received pulses of the antibiotic Cipronil for five days; at D7, seven farms received Trimoxil pulses for three days; at D9, 1 aviary also received Trimoxil for three days; at D13, 3 farms received Farmaxilin pulses for three days; at D20, three farms received Cipronil pulses for five days, two farms received Farmaxilin pulses for three days; and, at D28, one house received Farmaxilin pulses for three days and four houses received Amprol Base pulses for two days. While the birds in the treated group, distributed in 15 aviaries, at D7, three aviaries received Farmaxilin pulses for three days; at D18, two farms received three Farmaxilin pulses; at D26, two aviaries received three pulses of Amprol Base; at D29, one aviary received three pulses of Cipronil and three aviaries received three pulses of also Amprol Base.

There is an adversity in comparing the results of zootechnical performance obtained on the commercial farm with results published in the literature, as the indications for use of synbiotic products and the experimental environment variables are distinct and extrapolate the behavior of commercial products in the development of the gastrointestinal tract of birds. In short, when evaluating the results described by Syed *et al*. [63] whose treatment 4 (T4) used the same commercial synbiotic product present in the control group, but with an indication

for use $5.00x10^5$ CFU/g of feed, if the control group had a lower performance in body weight gain than reported by the authors, but with a performance similar to that observed by the treated group whose inclusion of synbiotic product was five times more lower (BWG = 2573 g CG, BWG = 2983.9 g T4 and BWG = 2853 g SG). The feed conversion rate also differs in the experimental and field settings, in this variable, the corrected feed conversion rate was better in the treated group, followed by the control group and treatment 4 (FCR = 1.57 SG, FCR = 1.59 CG and FCR = 1.87 T4). Finally, mortality, whose treatment 4 showed better results compared to the control group and the treated group (Mortality (%) = 1.11 T4, Mortality (%) = 4.27 CG and Mortality (%) = 5.17 SG). In conclusion, the treated group prevailed with better results in 2 of the 3 variables compared in zootechnical performance. The zootechnical results obtained in both experiments are a reflection of several variables such as management and ambience protocols, nutritional quality of the feed as well as the composition and indication of use of zootechnical additives, environment and sanitary challenges. And, these results are reproduced and can also be compared to the results described by Śliżewska *et al*. [58], Abdel-Wareth *et al*. [64] and others.

Synbiotic products in sanitary control promote resistance to infections by favoring morphological changes to the intestinal mucosa, developing longer villus, smaller crypts and better villus/crypt ratios, also by reducing the gastrointestinal pH due to higher lactic acids and by mitigating frequency and histopathological lesions [64]. Results described by Mora et al. [65] report the sanitary control of *Salmonella Typhimurium* and *Clostridium Perfringens* when birds are supplemented with symbiotics. Shanmugasundaram et al. [66], Markazi et al. [67], Luoma et al. [68], Asahara et al. [69], also report a reduction in salmonella proliferation in the cecum of birds.

It was not possible to carry out a comparative evaluation of the reduction in the consumption of antibiotics in therapeutic dosages, as a result of the use of synbiotics in the animals' diet and also of different compositions of synbiotics. The results presented are unprecedented and effectively report the benefits that the consumption of certain synbiotics influences on the modulation of animal health and reflects on the residual reduction of these antimicrobial agents in meat and the environment, as well as on the operational result of the creation.

It is in this dual scenario between science and the reality of the field that it is important to highlight the equalization between basic science and the application of development and innovation carried out in research centers, because although experimental tests are essential for the development and proof of new products, the reality of management on commercial farms presents adverse variables and challenges, often even unpredictable, that will compromise the zootechnical performance of the birds, the final quality of this food and, consequently, the operating result of the farm, on the health of the final consumer that consumes the food and in the environment that receives the residues from the handling operation.

The complexity of correlating the mode of action of synbiotic effects in poultry production demonstrates the wide spectrum of opportunities that science has to develop to understand all pathways influenced by prebiotics and probiotics in the TGI tract. Scientific results showed a specific interaction with the environment, the host and the synbiotic formulation. In addition, it demonstrated that the synbiotic participates in metabolic pathways little described in the scientific literature.

In summary, the results of scientific and field tests have shown a beneficial effect of all elaborated synbiotics on the balance of the intestinal microbiota, its metabolism and the performance of broiler chickens. Supports the ability of commercial synbiotic products to replace the use of antibiotics as a growth performance in order to mitigate rising antimicrobial resistance.

3. Conclusion

Synbiotic formulations are a potential choice to withdraw antibiotic as growth promoter. The complementary or synergic action of synbiotic improve the poultry production and control infections disease. Further studies should be developed to identify target microorganism's species according to farm management conditions. The hope is that, going forward, the prebiotic, probiotic or synbiotic will have greater representativeness among feed additive, reducing the use of antibiotics and the selective pressure of microorganism. Advances in symbiotic research will promote better understanding of interested parties, enabling better communication with consumers.

Acknowledgements

The author thanks Vanderson Camilo, João Ito, Marissol Cardoso, Kelly Pereira, Alisson Rotter and Filipe Ribeiro for their input and assistance in preparation of this review. This research was supported by PolySell Chemical Products LTDA.

Conflict of interest

The author declares no conflict of interest.

Author details

Flávia Pelá
Poly Sell Chemical Products LTDA, Brazil

*Address all correspondence to: flavia@polysell.com.br

References

[1] Markowiak P. & ŚliżewskaK. The role of probiotics, prebiotic and synbiotics in animal nutrition Gut Pathog.2018,10:21. DOI: https://doi.org/10.1186/s1

[2] Antimicrobial resistance and foods of plant origin [Internet]. Food and Agriculture Organization of United Nations, 2018. Available from: http://www.fao.org/3/BU657en/bu657en.pdf. Accessed on March 20, 2021.

[3] Bondad-Reantaso, MG., Lavilla-Pitogo CR., Karunasagar, I., Arthur, J.R., Hao, B., Irde, E., Garrido Gamarro, E. and Peñarubia, O.R. Outputs and activities of FAO Project FMM/RAS/298/MUL on antimicrobial resistance in fisheries and summary of FAO's recent work on antimicrobial resistance in aquaculture. FAO, 2020. Fisheries and Aquaculture Circular No. 1215. Rome, FAO.https://doi.org/10.4060/cb1209en

[4] Caruso G. Antibiotic Resistance in *Escherichia coli* from Farm Livestock and Related Analytical Methods: A Review. J AOAC Int. 2018; 1;101(4):916-922. DOI: 10.5740/jaoacint.17-0445.3099-018-0250-0

[5] Swanson KS., Gibson GR., Hutkins R., Reimer RA., Reid G., Verbeke K., Scott KP., Holscher HD., Azad MB., Delzenne NM., Sanders ME. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotic. Nat Rev Gastroenterol Hepatol. 2020;17(11):687-701. DOI: 10.1038/s41575-020-0344-2.

[6] Hamasalim HJ. Synbiotic as feed additives relating to animal health and performance. Advances in Microbiology. 2016. 6, 288-302. http://dx.doi.org/10.4236/aim.2016.64028

[7] Sharma R., Sharma S., Shukla PC., Sharma V., Baghel RPS., Raikwar A., Pradhan S., Yadav V. The Pharma Innovation Journal 2018; 7(7): 62-68. DOI:

[8] Aminov RI. The role of antibiotics and antibiotic resistance in nature. Environ Microbiol. 2009; 11(12):2970-2988. DOI: 10.1111/j.1462-2920.2009.01972.x.

[9] Aslam B., Wang W., Arshad MI, Khurshid M., Muzammil S., Rasool MH., Nisar MA., Alvi RF., Aslam MA., Qamar MU., Salamat MKF., Baloch Z. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 2018; 11: 1645-1658. DOI: 10.2147/IDR.S173867.

[10] Davies J & Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Ver 2010;74(3):417-433. DOI: 10.1128/MMBR.00016-10.

[11] Ventola CL. The Antibiotic Resistance Crisis. Part 1: Causes and Threats. P T. 2015; 40(4): 277-283. PMC4378521.

[12] Martinez JL. General principles of antibiotic resistance in bacteria. General principles of antibiotic resistance in bacteria. Drug Discovery Today: Technologies, 2014. 11, 33-39. DOI:10.1016/j.ddtec.2014.02.001.

[13] Munita J.M. & Arias C. A. Mechanisms of Antibiotic Resistance. Microbiol Spectr. 2016; 4(2):10.1128/microbiolspec. VMBF-0016-2015. DOI: 10.1128/microbiolspec.VMBF-0016-2015.

[14] Marshall BM., Levy SB. Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev. 2011;24(4):718-733. DOI: 10.1128/CMR.00002-11.

[15] Li, B. et al. Metagenomic and network analysis reveal wide

distribution and co-occurrence of environmental antibiotic resistance genes. ISME J. 2015;9(11):2490-2502. DOI: 10.1038/ismej.2015.59.

[16] Innes GK., Randad PR., Korinek A., Davis MF, Price LB., So AD., Heaney CD. External Societal Costs of Antimicrobial Resistance in Humans Attributable to Antimicrobial Use in Livestock. Annu Rev Public Health. 2020; 41: 141-157. DOI:10.1146/annurev-publhealth-040218-043954.

[17] European Comission. Regulation 1831/2003/EC on additives for use in animal nutrition, replacing Directive 70/524/EEC on additives in feeding-stuffs. Downloaded from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32003R1831. Accessed on March 20, 2021.

[18] Millet S & Maertens L. The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities. Vet J. 2011;187(2):143-144. DOI: 10.1016/j.tvjl.2010.05.001.

[19] Castanon JIR. History of the use of antibiotic as growth promoters in European poultry feeds. Poult Sci. 2007;86(11):2466-2471. DOI: 10.3382/ps.2007-00249.

[20] European Comission. EU Action on Antimicrobial Resistance. Downloaded from: https://ec.europa.eu/health/antimicrobial-resistance/eu-action-on-antimicrobial-resistance_en. Accessed on March 20, 2021.

[21] European Comission. Farm to folk strategy. 2020. Downloaded from: https://ec.europa.eu/food/farm2fork_en. Accessed on March 20, 2021.

[22] Kirchhelle C. Pharming animals: a global history of antibiotics in food production (1935-2017). Palgrave Communications. 2018. Vol. 4, Nº.96. DOI: 10.1057/s41599-018-0152-2.

[23] U.S. Food and Drug Administration (FDA). Timeline of FDA Action on Antimicrobial Resistance. Downloaded from: https://www.fda.gov/animal-veterinary/antimicrobial-resistance/timeline-fda-action-antimicrobial-resistance. Accessed on March 20, 2021.

[24] Ministry of Agriculture, Livestock and Supply (MAPA). Normative Instruction No 45, of November 22, 2016. Downloaded from: https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-pecuarios/alimentacao-animal/arquivos-alimentacao-animal/legislacao/instrucao-normativa-no-45-de-22-de-novembro-de-2016.pdf/view. Accessed on March 20, 2021.

[25] Ministry of Agriculture, Livestock and Supply (MAPA). Ordinance No.195, of July 4, 2018. Downloaded from: https://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb/content/id/29305677/do1-2018-07-10-portaria-n-195-de-4-de-julho-de-2018-29305658. Accessed on March 20, 2021.

[26] Ministry of Agriculture, Livestock and Supply (MAPA). Ordinance No 171, of December 13, 2018. Downloaded from: https://www.in.gov.br/materia/-/asset_publisher/Kujrw0TZC2Mb / content/id/55878469/do1-2018-12-19-portaria-n-171-de-13-de-dezembro-de-2018-55878239. Accessed on March 20, 2021.

[27] Hu YJ & Cowling BJ. Reducing antibiotic use in livestock, China. Downloaded from: https://www.who.int/bulletin/volumes/98/5/19-243501/en/. Accessed on March 20, 2021.

[28] Selaledi LA., Hassan ZM., Manyelo TG., Mabelebele M. The Current Status of the Alternative Use to Antibiotics in Poultry Production: An African Perspective. Antibiotics (Basel). 2020;11;9(9):594. DOI: 10.3390/antibiotics9090594.

[29] Marquardt RR., LI S. Antimicrobial resistance in livestock: advances and alternatives to antibiotics. Anim Front. 2018. 19;8(2):30-37. DOI: 10.1093/af/vfy001.

[30] Roberfroid M., Gibson GR, Hoyles L, McCartney AL., Rastall R., Rowland I., Wolvers D., Watzl B., Szajewska H., Stahl B., Guarner F., Respondek F., Whelan K., Coxam V., Davicco MJ, Léotoing L., Wittrant Y., Delzenne NM., Cani PD, Neyrinck AM, Meheust A. Prebiotic effects: metabolic and health benefits. Br J Nutr. 2010;104 Suppl 2:S1-63. DOI: 10.1017/S0007114510003363.

[31] Gibson GR. Dietary modulation of the human gut microflora using prebiotics. British Journal of Nutrition.1998; 80(S2), S209–S212. DOI:10.1017/S0007114500006048.

[32] Althubiani AS., Al-Ghamdi SB., Qais SFA., Khan MS., Ahmad I., Malak HA. Chapter 4 - Plant-Derived Prebiotics and Its Health Benefits. Advancements in Herbal Products as Novel Drug Leads. 2019, 63-88. DOI: https://doi.org/10.1016/B978-0-12-814619-4.00004-5

[33] Teng PY. & Kim WK. Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. Front. Vet. Sci. 5:245. DOI: 10.3389/fvets.2018.00245

[34] Davani-Davari D., Negahdaripour M., Karimzadeh I., Seifan M., Mohkam M., Masoumi SL., Berenjian A., Ghasemi Y. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. Foods. 2019; 8(3): 92. DOI: 10.3390/foods8030092.

[35] Food and Agriculture Organization of the United Nations (FAO). Probiotics in animal nutrition: production, impact and regulation. 2016. ISBN 978-92-5-109333-7

[36] Patterson JA. & Burkholder KM. Application of Prebiotics and Probiotics in Poultry Production. Poultry Science. 2003; 82:627-631. DOI: https://doi.org/10.1093/ps/82.4.627

[37] Khan RU, Naz S. The applications of probiotics in poultry production. World's Poultry Science Journal. 2013. 69(3), 621-632. DOI:10.1017/s0043933913000627

[38] Jin LZ., Ho YW, Abdullah N., Jalaludin S. Probiotics in poultry: modes of action. World's Poultry Science Journal. 1997. 53 (4) 351-368. DOI: https://doi.org/10.1079/WPS19970028

[39] Fong W., Li Q. & Yu J. Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer. Oncogene. 2020;39, 925-4943. DOI: https://doi.org/10.1038/s41388-020-1341-1

[40] Yirga, H. The use of probiotics in Animal Nutrition. Journal of Probiotics & Health. 2015. 3 (2) DOI: 10.4172/2329-8901.1000132

[41] Kim CH, Park J, Kim M. Gut microbiota-derived short-chain Fatty acids, T cells, and inflammation. Immune Netw. 2014; 14:277-288 DOI: 10.4110/in.2014.14.6.277

[42] Gillor O., Etzion A. & Riley MA. The dual role of bacteriocins as anti- and probiotics. Appl Microbiol Biotechnol. 2008 Dec; 81(4): 591-606. DOI: 10.1007/s00253-008-1726-5

[43] Dobson A, Cotter PA, Ross RP & Hill C. Bacteriocin Production: a Probiotic Trait? Appl Environ Microbiol. 2012 Jan; 78(1): 1-6. DOI: 10.1128/AEM.05576-11

[44] Vieco-Saiz N., Belguesmia Y., Raspoet R., Auclair, E., Gancel, F., Kempf, I., Drider, D. Benefits and Inputs From Lactic Acid Bacteria and Their Bacteriocins as Alternatives to

Antibiotic Growth Promoters During Food-Animal Production. Frontiers in Microbiology. 2019. 10(), 57–. DOI:10.3389/fmicb.2019.00057

[45] Engevik MA & Versalovic J. Biochemical features of beneficial microbes: foundations for therapeutic microbiology. Microbiol Spectr. 2017. 5(5) DOI: 10.1128/microbiolspec.BAD-0012-2016

[46] La Fata G, Weber P & Mohajeri M.H. Probiotics and the Gut Immune System: Indirect Regulation. Probiotics & Antimicro. Prot. 2018. 10:11-21. DOI: 10.1007/s12602-017-9322-6.

[47] Ashraf R & Shah NP. Immune system stimulation by probiotic microorganisms, critical. Reviews in Food Science and Nutrition. 2014. 54:7, 938-956, DOI: 10.1080/10408398.2011.619671

[48] Belkaid Y. & Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014; 157(1): 121-141. DOI: 10.1016/j.cell.2014.03.011

[49] Hardy H., Harris J., Lyon E., Beal J, Foey AD. Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients. 2013; 5(6): 1869-1912. DOI: 10.3390/nu5061869

[50] Calder PC. Feeding the immune system. Proc Nutr Soc. 2013;72(3):299-309. doi: 10.1017/S0029665113001286.

[51] Brazilian Agricultural Research Corporation (Embrapa). Poultry and swine intelligence center - Production performance statistics. 2021. Downloaded from: https://www.embrapa.br/suinos-e-aves/cias/estatisticas. Accessed on March 21, 2021.

[52] Brazilian Agricultural Research Corporation (Embrapa). Poultry and swine intelligence center - Statistics | World | Broilers. 2021. Downloaded from: https://www.embrapa.br/suinos-e-aves/cias/estatisticas/frangos/mundo. Accessed on March 21, 2021.

[53] Sindirações. Boletim informativo do setor. 2021. Downloaded from https://sindiracoes.org.br/produtos-e-servicos/boletim-informativo-do-setor/. Accessed on March 21, 2021.

[54] Ministério do desenvolvimento, indústria e comércio exterior. Estudo de Viabilidade Técnica e Econômica destinado à implantação do Parque Produtivo Nacional de Aditivos da Indústria de Alimentação de Animais de Produção. 2012. Downloaded from: http://www.asbram.org.br/wp3/wpcontent/uploads/2015/11/Estudo_de_aditivos_da_industria_de_alimentacao_de_anima is_de_producao.pdf Accessed on March 21, 2021.

[55] Lilburn MS. & Loeffler S. Early intestinal growth and development in poultry. Poultry Science. 2015. 94 (7) 1569-1576. DOI: 10.3382/ps/pev104

[56] Rubio LA. Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. Poultry Science. 2019; 2(1) 695-706. DOI: https://doi.org/10.3382/ps/pey416

[57] Pan D & Yu Z. Intestinal microbiome of poultry and its interaction with host and diet. Gut Microbes. 2014; 5(1), 108-119. DOI: http://dx.doi.org/10.4161/gmic.26945

[58] Śliżewska K, Markowiak-Kopeć P, Żbikowski A & Szeleszczuk P. The effect of synbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens. Scientific Reports. 2020; 10:4281 DOI: https://doi.org/10.1038/s41598-020-61256-z

[59] Adhikari B. Investigation of Microbiota in Health and Disease of

Poultry. Theses and Dissertations. 2019, 3371. DOI: https://scholarworks.uark.edu/etd/3371

[60] Ranjitkar S, Lawley B, Tannock G & Engberg RM. Bacterial Succession in the Broiler Gastrointestinal Tract. Appl Environ Microbiol. 2016, 82(8): 2399-2410. DOI: 10.1128/AEM.02549-15.

[61] Wang Y, Sun J, Zhong H *et al*. Effect of probiotics on the meat flavour and gut microbiota of chicken. Scientific Reports. 2017, 7 6400. DOI:10.1038/s41598-017-06677-z.

[62] Dunislawska A, Slawinska A, Stadnicka K *et al*. Synbiotics for Broiler ChickensÐIn Vitro Design and Evaluation of the Influence on Host and Selected Microbiota Populations following In Ovo Delivery. PLoS ONE. 2016, 12(1): e0168587. DOI: 10.1371/journal.pone.0168587.

[63] Syed B, Wein S & Ruangapanit Y. The Efficacy of Synbiotic Application in Broiler Chicken Diets, Alone or in Combination with Antibiotic Growth Promoters on Zootechnical Parameters. J. World Poult. Res. 2020, 10(3): 469-479. DOI: https://dx.doi.org/10.36380/jwpr.2020.54

[64] Abdel-Wareth A. A.A, Hammad S, Khalaphallah, Rafat. et al. Synbiotic as eco-friendly feed additive in diets of chickens under hot climatic conditions. Poultry Science, 2019, 98, 4575-4583. DOI: 10.3382/ps/pez115.

[65] Mora ZV, Nuño K, Vázquez-Paulino O, *et al. Effect* of a synbiotic mix on intestinal structural changes, and *Salmonella typhimurium* and *Clostridium perfringens* colonization in broiler chickens. Animals. 2019, 9, 777.DOI:10.3390/ani9100777

[66] Shanmugasundaram R, Mortada M, Cosby DE *et al*. Synbiotic supplementation to decrease Salmonella colonization in the intestine and carcass contamination in broiler birds. PLoS ONE. 2019, 14(10): e0223577. DOI: https://doi.org/10.1371/journal.

[67] Markazi A, Luoma A, Shanmugasundaram, R. *et al.* Effects of drinking water synbiotic supplementation in laying hens challenged with Salmonella. Poultry Science, 2018, 97:3510-3518. DOI: http://dx.doi.org/10.3382/ps/pey234

[68] Luoma A, Markazi A, Shanmugasundaram, R. *et al.* Effect of synbiotic supplementation on layer production and cecal Salmonella load during a Salmonella challenge. Poultry Science. 2017, 0:1-9. DOI: http://dx.doi.org/10.3382/ps/pex251

[69] Asahara T, Nomoto K, Shimizu K. *et al.* Increased resistance of mice to *Salmonella enterica* serovar Typhimurium infection by synbiotic administration of Bifidobacteria and transgalactosylated oligosaccharides. Journal of Applied Microbiology. 2001, 91, 985±996. DOI: 10.1046/j.1365-2672.2001.01461.x