



ROLE OF ZOOTECHNICAL ADDITIVES ON PRODUCTIVITY AND HEALTH OF RABBITS

FUNCIÓN DE LOS ADITIVOS ZOOTÉCNICOS EN LA PRODUCTIVIDAD Y SALUD DE LOS CONEJOS

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In Latin America, subtherapeutic antibiotics are commonly used in the production of monogastric animals, although their use in rabbits is less relevant than in poultry and pigs. It is known that their use generates bacterial resistance, and they have proven harmful effects on human health. This review addresses the characterization and use of new nutraceutical additives, emphasizing phytochemicals, probiotics, and prebiotics and their effect on the biological indicators of rabbits in different productive categories. These natural alternatives have antimicrobial, anti-inflammatory, antioxidant, immunomodulatory and hypocholesterolemic effects, which can stimulate the native microbiota, the production of short-chain fatty acids and can provoke microbial eubiosis and, therefore, improve intestinal health, digestibility, productive efficiency and meat quality of rabbits. Also, they can alleviate the harmful effects of some common bacterial diseases and intoxications. However, the effectiveness of these natural alternatives will depend on the probiotic strain used, the main secondary metabolites in phytochemicals and the chemical structure of the prebiotics, as well as the health status, diet, age, and productive category of the rabbits.

Keywords: biological indicator, monogastric herbivore, nutraceutical product, supplement

En Latinoamérica, los antibióticos subterapéuticos se utilizan comúnmente en la producción de animales monogástricos, aunque su uso en conejos tiene menor relevancia que en aves y cerdos. Es conocido que su utilización genera resistencia bacteriana y tienen efectos nocivos comprobados en la salud humana. Esta revisión aborda la caracterización y uso de nuevos aditivos nutracéuticos, con énfasis en los fitobióticos, probióticos y prebióticos y su efecto en los indicadores biológicos de conejos en diferentes categorías productivas. Estas alternativas naturales tienen efectos antimicrobianos, antiinflamatorios, antioxidantes, inmunomoduladores e hipocolesterolémicos, lo que puede estimular la microbiota nativa, la producción de ácidos grasos de cadena corta y puede provocar eubiosis microbiana y, por ende, mejorar la salud intestinal, digestibilidad, eficiencia productiva y calidad de la carne de conejos. Además, pueden paliar los efectos perjudiciales de algunas enfermedades bacterianas e intoxicaciones comunes. No obstante, la eficacia de estas alternativas naturales dependerá de la cepa probiótica utilizada, de los metabolitos secundarios mayoritarios en los fitobióticos y de la estructura química de los prebióticos, así como del estado de salud, dieta, edad y categoría productiva de los conejos.

Palabras clave: herbívoro monogástrico, indicador biológico, producto nutracéutico, suplemento

Introduction

Currently, there is a growing demand for rabbit meat, due to the high concentration of high biological value proteins, essential amino acids, and low concentration of saturated fat, cholesterol, purines, and uric acid (Fang *et al.* 2020). However, these productions are characterized by high

production intensity, which causes changes in the intestinal microbiota, microbial dysbiosis, digestive diseases, immunosuppression, and decreased productive efficiency in rabbits. Due to these recurring problems, many producers and enterprises use antibiotic growth promoters in the diet or drinking water, either at critical stages or during the productive life of the animals (Dumont *et al.* 2020).

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It is known that the European Union and other countries have banned antibiotic growth promoters (Vidovic and Vidovic 2020). However, in Latin America, these synthetic products are constantly used in animal production, even though their indiscriminate use promotes antimicrobial resistance of pathogenic strains and causes changes in the ecological balance of the intestinal microbiota, which affects the appearance of gastrointestinal disorders (Glajzner *et al.* 2023). Also, they can leave traces of chemical residues in products of animal origin, which directly affects human health (Treiber and Beranek-Knauer 2021). Although subtherapeutic antibiotics have been investigated and used more in poultry and pigs than in rabbit breeding, some studies have recommended the oral use of bacitracin Zn to control the population of *Enterobacteriaceae* and alleviate its implications for animal health (Haj-Ayed and Ben Saïd 2008).

Nutraceutical additives are bioactive components present in natural products, with beneficial effects on the health of humans and animals (Chandra *et al.* 2022). In this sense, nutraceuticals can prevent or treat one or more diseases and improve the physiological performance of the host (Mali *et al.* 2022). Multiple studies show that medicinal plants, probiotics, and prebiotics, as the most studied nutraceuticals, are reliable alternatives to replace the indiscriminate use of antibiotic growth promoters in animals (Haj-Ayed and Ben Saïd 2008). Scientific research on alternatives to preventive antibiotics focuses on evaluating different biological indicators, with emphasis on growth, reproduction, intestinal health and possible antimicrobial, anti-inflammatory and antioxidant effects (Colitti *et al.* 2019). Furthermore, dietary components have a direct influence on cecal pH and intestinal integrity, and microbiology, affecting host genetic expression (Sun *et al.* 2016). Nowadays, new natural additives with diverse biological properties are emerging, which demonstrate their beneficial effects *in vivo* in rabbits. The aim of this review was to generate updated information on the role of zootechnical additives (mainly phytobiotics, probiotics, and prebiotics) in the main productive and health indicators in different rabbit categories.

New phytobiotics in rabbit breeding

Phytobiotics are characterized by secondary metabolites, synthesized by plants that perform non-essential functions. These compounds intervene in the ecological interactions between plants and their environment. They also differ from primary metabolites in that each of them has a restricted distribution in the plant kingdom, sometimes to a single species or a group of them (El-Sabrout *et al.* 2023). The most common secondary metabolites are alkaloids, non-protein amino acids, steroids, phenols, flavonoids, glycosides, coumarins, quinones, tannins, and terpenoids, which have defensive functions against insects, bacteria, fungi, and others.

The highest concentrations of these chemical compounds are found in flowers, leaves, and seeds (Akinpelu 2021). Some phytochemicals used in small concentrations have bacteriostatic or bactericidal action or inhibit the adhesion of pathogenic bacteria to the intestinal and urinary mucosa. They also have an antioxidant effect by reducing free radical reactive oxygen species (ROS) produced in the organism, as part of normal cell metabolism, as well as anti-inflammatory and immune effects in the presence of pathogenic microorganisms or inflammatory processes (Chouegouong *et al.* 2021).

Many phytobiotics have been used to promote biological indicators in rabbits and reduce the use of subtherapeutic antibiotics. Recent studies found that the dietary inclusion of 0.6, 0.12, and 0.18 % of thyme (*Thymus* spp.) essential oil improved productivity, semen quality, and serum testosterone concentration and decreased aspartate transaminase, alanine transaminase, urea, and creatinine compared to a diet without additives and another with oxytetracycline. The authors justified these results to the anti-inflammatory, antioxidant, and antimicrobial activity of this natural product (Abdel-Wareth and Metwally 2020).

Ayala *et al.* (2011) reported that carvacrol is the major secondary metabolite in oregano (*Origanum vulgare*) and that the inclusion of 1 % of this medicinal product dried at 60 °C improved viability, feed intake, body weight gain, and feed conversion ratio. In another study by Ayala *et al.* (2012) it is mentioned that the dietary inclusion of 1 % of two species of sage (*S. lavandulifolia* and *S. officinalis*), dried at 60 °C, promoted body weight, weight gain and viability (especially *S. lavandulifolia*), due to the high concentration of camphor and α -thujene in these medicinal plants.

Johnson *et al.* (2022) confirmed that the inclusion of 1 % garlic (*Allium sativum*) and ginger (*Zingiber officinale*) in the diet improves the productive efficiency and carcass yield of fattening rabbits. Also, Liu *et al.* (2019) reported that 3 % of Chinese mugwort (*Artemisia argyi*) in the diet decreased diarrheal syndrome in weaned rabbits and the authors attributed this finding to the increased in immunoglobulin A content and the genetic expression of binding proteins, such as zonula occludin-1 and claudin-1. However, Olorunsogbon *et al.* (2022) stated that the use of the aqueous extract of ginger (*Zingiber officinale*) and almond fruit (*Terminalia catappa*) in drinking water did not change the hematological parameters and blood biochemistry of weaned rabbits.

Other authors reported that aqueous extract of black cumin (*Nigella sativa*) decreased the growth of *Staphylococcus aureus* *in vitro* and *in vivo* tests. The use of 0.125, 0.25 and 0.50 % of this natural product increased digestive enzymes and genetic expression of binding proteins, such as occludin, claudin-1 (CLDN-1),

junctional adhesion molecule-2 (JAM-2) and secretory glycoprotein mucin-2 (MUC2), which provoked a natural growth-promoting effect and decreased the adverse effects of infection with *Staphylococcus aureus* (Elmowalid *et al.* 2022). Moreover, the dietary inclusion of 0.15 and 0.25 % of cinnamon (*Cinnamomum verum*) and clove (*Syzygium aromaticum*) increased body weight, average daily gain, meat quality, and serum concentration of total protein, albumin and globulin in blood plasma and decreased concentration of glucose, cholesterol, triglycerides, aspartate aminotransferase and alanine aminotransferase (Abdel-Azeem and El-Kader 2022). Furthermore, Ingweye *et al.* (2020) found that the inclusion of up to 1 % Aidan pod powder (*Tetrapleura tetraptera*) in diets without antibiotic growth promoters improved the performance of weaned rabbits, however, higher inclusion of this natural product increased abdominal fat yield in these animals.

Dalle-Zotte *et al.* (2016) mentioned that medicinal plants such as fennel (*Foeniculum vulgare*), lupin (*Lupinus albus* L.), fenugreek (*Trigonella foenum-graecum* L.) and khartoum (*Cassia senna* L.), rich in lipid-soluble secondary metabolites (essential oils) and in the hydroalcoholic extract, have antimicrobial effects *in vivo* on *Clostridium coccoides* and *Clostridium leptum* in rabbits and anti-inflammatory, immunomodulatory and antioxidant effects, which benefits the antioxidant capacity of meat and meat products. Likewise, the dietary use of 0.17 and 0.34 % daidzein (isoflavones extracted from soybeans) in breeders improved fertility and increased the weight of the young rabbits at birth and at weaning. The cited authors found a direct relationship between the bioactive compound (isoflavones) and the anti-inflammatory, antioxidant and immune response in breeding animals and their offspring (Xie *et al.* 2022 and Xie *et al.* 2023). The beneficial effects of medicinal plants, considered phytochemicals, will depend on the type, concentration, and inclusion of secondary metabolites in the diets, in addition to the age, raw materials, productive purpose, and health status of the rabbits.

New probiotics in rabbit breeding

Probiotics are zootechnical additives composed of live microorganisms, which can colonize and modify the intestinal microflora or provoke microbial eubiosis and produce enzymes that help the organic functioning of animals (Mancini and Paci 2021). They confer benefits for the health and physiology of the host (FAO/WHO 2006), although it will depend on the type of microbial strain, concentration, and level of inclusion of the probiotic, age, and health status of the host. According to Florido *et al.* (2017), these natural products protect against physiological stress, modulate the intestinal microbiota and the epithelial barrier in the intestine and stimulate the antioxidant capacity and the immune

system. However, there are various scientific contradictions about the beneficial effect of some bacterial strains, demonstrated by studies with inconclusive hypotheses, different effects between animal species and productive categories, low tolerance to feed pelleting and chlorine in drinking water. The most used probiotics are *Pediococcus pentosaceus*, *Lactobacillus casei*, *Enterococcus faecalis*, *Lactobacillus helveticus*, *Lactobacillus lactis*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Enterococcus faecium* and *Lactobacillus acidophilus* (Krysiak *et al.* 2021).

Other beneficial bacteria of the *Bacillus* genus, which are Gram-positive spore-forming bacteria of the *Firmicutes* division that do not colonize the gastrointestinal tract, are frequently used as probiotics in animal production (Lee *et al.* 2019). The most used strains are *B. cereus*, *B. subtilis*, *B. coagulans*, *B. polyfermenticus*, *B. licheniformis*, *B. pumilus*, and *B. clausii*. These probiotics produce enzymes and vitamins and have antioxidant and microbial properties (Florido *et al.* 2017). Some live yeasts, such as *Saccharomyces boulardii* and *Saccharomyces cerevisiae*, can cause microbial eubiosis and improve gut health, also to producing vitamins and enzymes (García-Mazcorro *et al.* 2020).

Suárez-Machín *et al.* (2022) reported that a probiotic mixture (*Bacillus subtilis* B/23-45-10 Nato, *Lactobacillus bulgaricus* B/103-4-1 and *Saccharomyces cerevisiae* L-25-7-12) improved the intestinal health of rabbits by increasing the count of beneficial bacteria in the gastrointestinal tract and the production of volatile fatty acids, which provoked a significant decrease in pH in the small intestine and cecum and, therefore, higher feed efficiency in these animals. Also, Abd El-Hamid *et al.* (2022) informed that a mixture of several bacterial strains increased the productivity, digestibility, genetic expression of binding proteins and viability of rabbits infected with *Listeria monocytogenes*. These authors concluded that these probiotic bacteria have adjuvant and immune effects. Likewise, oral administration of *Aspergillus awamori* improved the health status of intoxicated weaned rabbits with ochratoxin, causing an increase in the height and width of intestinal villi and higher protection against liver damage, which benefited the productive efficiency of these animals (El-Deep *et al.* 2020). The use of lactic acid bacteria and live yeasts can also reduce the serum concentration of harmful lipids, as well as positively modify the immune system and improve the antioxidant capacity of rabbit meat (Adli *et al.* 2023).

Abdel-Wareth *et al.* (2021), using a mixture of probiotics and phytochemicals up to 0.3 % in the diet, found improvements in the digestibility of crude protein, ether extract, and crude fiber. This experimental treatment exacerbated the concentration of testosterone and estrogens in male and female rabbits, respectively, which led to better productive efficiency and higher carcass yield in these

animals. Díaz-Fuente *et al.* (2022) reported that the use of a probiotic bioproduct based on efficient microorganisms, at doses of 10 and 15 mL per liter of drinking water for 70 d, increased body weight and weight gain by 6.98 and 4.34 % compared to the control treatment, respectively.

Nwachukwu *et al.* (2021) found that a probiotic mixture (*Lactobacillus acidophilus*) and prebiotic mixture of fructooligosaccharides promoted growth, nutrient digestibility, villus height, nutrient absorption, and immunoglobulin production in growing rabbits.

Another study recommended oral administration of a mixture of *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* subsp. *Lactis* BB-12 and *Saccharomyces boulardii* CNCM I-745 to improve productivity, electrolyte and immunoglobulin concentrations and decrease harmful lipid concentrations (Kadja *et al.* 2021). Likewise, oral administration of *Pediococcus acidilactici* CNCM I-4622 improved weight gain and feed efficiency and controlled the physiological triad (respiratory rate, rectal temperature, and heart rate) in heat-stressed rabbits, without affecting the relative weight of viscera, digestive tract, and edible portions (Ayyat *et al.* 2018). Despite the availability of several studies on rabbits, these are still limited compared to poultry, and pigs. Further research is needed to elucidate the benefits of probiotics in these herbivorous monogastric at different productive stages, ages, and health statuses.

New prebiotics in rabbit breeding

Prebiotics are chemical compounds that selectively stimulate the growth of some beneficial bacteria in the large intestine, mainly *Bifidobacteria* and *Lactobacilli*. These bacteria use the prebiotics that mainly reach the colon to produce volatile fatty acids and release minerals, which are absorbed and utilized by the host (Wlazło *et al.* 2021). The most widely marketed prebiotics are rich in fructo-oligosaccharide, α galacto-oligosaccharide, transgalacto-oligosaccharide, β -glucans, mannan-oligosaccharide and xylo-oligosaccharide. Thus, changes in the intestinal environment, produced by including prebiotics in the diet, can prevent or reduce the incidence of colibacillosis and other diseases (Zhu *et al.* 2021). Other research has shown that prebiotics have a marked impact on the metabolic activity of the intestinal microbiota (Pogány-Simonová *et al.* 2020). These natural products stimulate the immune system, regulate glucose levels and lipid metabolism, as well as increase the mineral bioavailability and decrease intestinal inflammatory responses (El-Ashram *et al.* 2019). Furthermore, prebiotics modify the antioxidant capacity and intestinal competitive exclusion, which reduces intestinal pH and the population of pathogenic bacteria, directly benefiting animals (Bosscher *et al.* 2006).

Ayyat *et al.* (2018) reported that the dietary use of a commercial prebiotic promoted productive efficiency and increased serum concentrations of hemoglobin, total protein, and globulin. This natural product decreased respiratory and heart rates and body temperature in rabbits under heat stress. Likewise, dietary inclusion with 0.1 % mannan-oligosaccharides and arabinoxylan oligosaccharides improved daily weight gain, cecal volatile fatty acid production, and ileal villus height and decreased cecal coliform population in male rabbits (Bosscher *et al.* 2006). Moreover, the dietary inclusion with 0.5 % *Trigonella foenum-graecum* seed meal rich in dietary fiber and galactomannan decreased intestinal pH and $\text{NH}_3\text{-N}$ concentration and increased total cecal volatile fatty acid production (Zemzmi *et al.* 2020). Oral administration of fructooligosaccharides improved weight gain and feed conversion ratio, without affecting nutrient digestibility and serum harmful lipids in fattening rabbits (Abo El-Maaty *et al.* 2019).

Agaves are plants with a high content of fructans synthesized and stored in the stems and made up of fructose polymers, derived from the sucrose molecule and with glucose as the terminal monomer (Peralta-García *et al.* 2020). Several investigations (Iser *et al.* 2016 and Iser *et al.* 2019) demonstrated that the dietary inclusion of up to 1.5 % of *Agave tequilana* stem powder improved productivity and meat quality, with a hypolipidemic effect, although without changes in the blood count of rabbits. Also, the inclusion of this natural product (*Agave tequilana*) increased the thickness of the muscular and mucosal layers, and the height, width, and number of villi in the duodenum, which provoked a natural non-antibiotic growth-promoting effect (Martínez *et al.* 2021). Furthermore, the dietary use of 1.5 % *Agave fourcroydes* stem powder promoted body weight and feed efficiency, although it decreased the serum concentration of glucose, cholesterol, triglycerides, and atherogenic index, without affecting the concentration of urea nitrogen, creatinine, and very low-density lipoproteins and the relative weight of the digestive organs and viscera of fattening rabbits (Martínez *et al.* 2022).

Dead yeasts and their chemical compounds, mainly β -glucans, mannoproteins, and chitin, have also been used as prebiotics in animal diets (Klassen *et al.* 2023). The inclusion of 0.12 g of yeast/kg of feed improved weight gain, productive efficiency, viability, loin yield, and intestinal histomorphometry. This natural product reduced harmful lipids (triglycerides and cholesterol) and increased proteins produced by the liver in weaned rabbits (Abd El-Aziz *et al.* 2021). β -glucans, derived from yeasts with prebiotic function, demonstrated adjuvant effects, due to antioxidant, immunomodulatory, and anti-inflammatory activity in rabbits intoxicated with *Pythium insidiosum* (Santurio *et al.* 2020).

A study, where rabbit diets were formulated with yeast cell walls, enzymes, and a mixture of both, showed that all treatments improved growth performance, intestinal health, and nutrient digestibility, without causing changes in meat quality (Khan *et al.* 2021). New technologies have recommended the encapsulation of prebiotics to achieve higher resistance to gastric and intestinal digestion. An experiment confirmed that prebiotics and phytobiotics encapsulated in rabbit diets stimulated the population of lactic acid bacteria and yeasts and decreased cecal pathogenic bacteria and proinflammatory and oxidative stress patterns, which promoted phagocytic and lysosomal activity (Hashem *et al.* 2020).

Currently, fungal bioproducts have been recommended to replace preventive antibiotics in animal diets (Morris *et al.* 2018). Lebeque-Pérez *et al.* (2022) identified high concentrations of β -glucans, mannan-oligosaccharide, crude protein, nucleic acids, and total carbohydrates in two biopreparations, based on *Kluyveromyces marxianus* and *Pleurotus ostreatus*. *In vivo* studies confirmed that the inclusion of 0.5 % of both natural products promoted feed efficiency and viability of rabbits, without causing pyrogenicity, due to the absence of endotoxins in the biopreparation.

Considering that rabbit diets, due to their physiological characteristics, require high fiber content, and that many fibrous feeds have high concentrations of chemical compounds with a prebiotic effect, the use of these additives could apparently be a viable alternative to eliminate antibiotic growth promoters. However, it will depend on the chemical structure and distribution of the prebiotics, as well as on the age, health status, diet, and zootechnical management.

Table 1 summarizes the main effects of phytobiotics, probiotics and prebiotics on rabbit breeding.

Conclusions

New phytobiotics, prebiotics, and probiotics represent effective alternatives to the common use of subtherapeutic antibiotics in rabbit production. These nutraceutical additives can modulate intestinal microflora, permeability, and histomorphometry, as well as antioxidant capacity,

immune activity, and the concentration of harmful lipids, which has a positive effect on weight gain, feed conversion ratio, nutrient digestibility, intestinal health, and meat quality. They can also alleviate problems associated with diarrheal syndrome, bacterial infections, and mycotoxin in rabbits.

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Table 1. Effect of zootechnical additive on rabbit breeding

Additives	Productive category	Effects	References
Phytobiotics (alkaloids, phenols, tannins, flavonoids, coumarins and terpenoids)	Young rabbits, growth-fattening and breeders	Antimicrobial, antioxidant, anti-inflammatory, lipid-lowering, hepatoprotective and immunomodulatory	Elmowalid <i>et al.</i> (2022) Johnson <i>et al.</i> (2022) Xie <i>et al.</i> (2023)
Probiotics (<i>Lactobacillus spp.</i> , <i>Aspergillus awamori</i> , <i>Bifidobacterium animalis</i> and <i>Saccharomyces boulardii</i>)	Young rabbits and growth-fattening	Antimicrobial, antioxidant, anti-inflammatory, immunomodulatory and antidiarrheal	Abdel-Wareth <i>et al.</i> (2021) Nwachukwu <i>et al.</i> (2021) Kadja <i>et al.</i> (2021)
Prebiotics (Mannan-oligosaccharides, arabinoxylans, galactomannan, fructooligosaccharides and β -glucans)	Young rabbits and growth-fattening	Production of volatile fatty acids, antiinflammatory, immunomodulatory and lipid-lowering	Abd El-Aziz <i>et al.</i> (2021) Khan <i>et al.</i> (2021) Martínez <i>et al.</i> (2022)

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