

ABSTRACT:

Plant tissues are rich in a wide variety of secondary metabolites which have been found to have *in-vitro* antimicrobial properties. Tannins, a type of plant polyphenol, are widely distributed among several plant species where they play a protective role (i.e. pathogen attack). Tannins of different plant species have specific physical and chemical properties which enclose very different biological activities. A series of detailed *in vitro* and *in vivo* studies from our laboratory have been performed to characterize the valuable properties of different natural polyphenols, resulting in the selection of some of them to design a blend competent to replace and improve the use of antibiotics as growth promoters. These selected tannins showed not only bacteriostatic and bactericidal activities against different pathogens, including *Clostridium perfringens*, but also antitoxin properties, without evident induction of bacterial resistance against themselves. Also, this combination of selected natural compounds was able to regulate the intestinal microbiota, the physiologic host function, the morphology of the gastrointestinal tract and the development of different infectious diseases including necrotic enteritis. The weight gain was improved when compared to non-growth promoter control under all the different conditions tested: experimental farm (1.8%), experimental pathogen challenge (5-22%) and commercial farm (4.8%). A two years evaluation in large commercial settings of different countries corroborated our laboratory findings. In all of the farms analyzed, comparing to controls using or not antibiotic growth promoters, an improving of intestinal health, podal lesions, weigh gain, reduction of mortality was measured, producing an improvement of productive parameters with a cost reduction. The available information supports the use of specific mixture of polyphenols as alternative to antibiotic growth promoters.

INTRODUCTION:

Plant tissues are rich in a wide variety of secondary metabolites which have been found to have *in-vitro* antimicrobial properties. Tannins, a type of plant polyphenol, are widely distributed among several plant species where they play a protective role (i.e. pathogen attack). Tannins of different plant species have specific physical and chemical properties which enclose very different biological activities. A series of detailed *in vitro* and *in vivo* studies from our laboratory have been performed to characterize the valuable properties of different natural polyphenols, resulting in the selection of some of them to design a blend competent to replace and improve the use of antibiotics as growth promoters.

MATERIALS, METHODS AND RESULTS:

In-vitro antimicrobial activity:

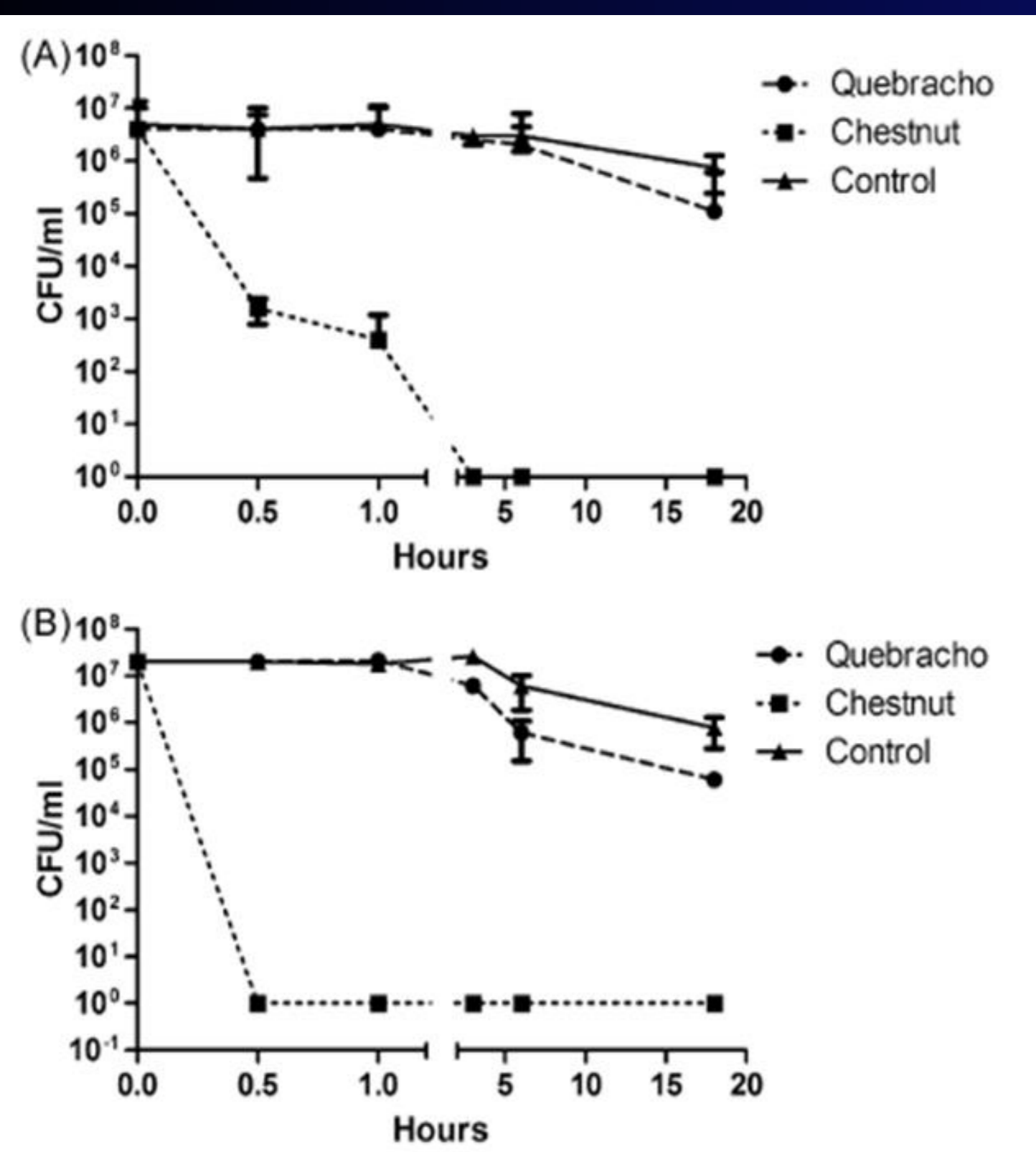


Fig 1: Antimicrobial effects tannins treatment on different strains (A and B) of *C. Perfringens*. Chestnut tannins shows bactericidal effect, while quebracho tannins shows bacteriostatic effect.

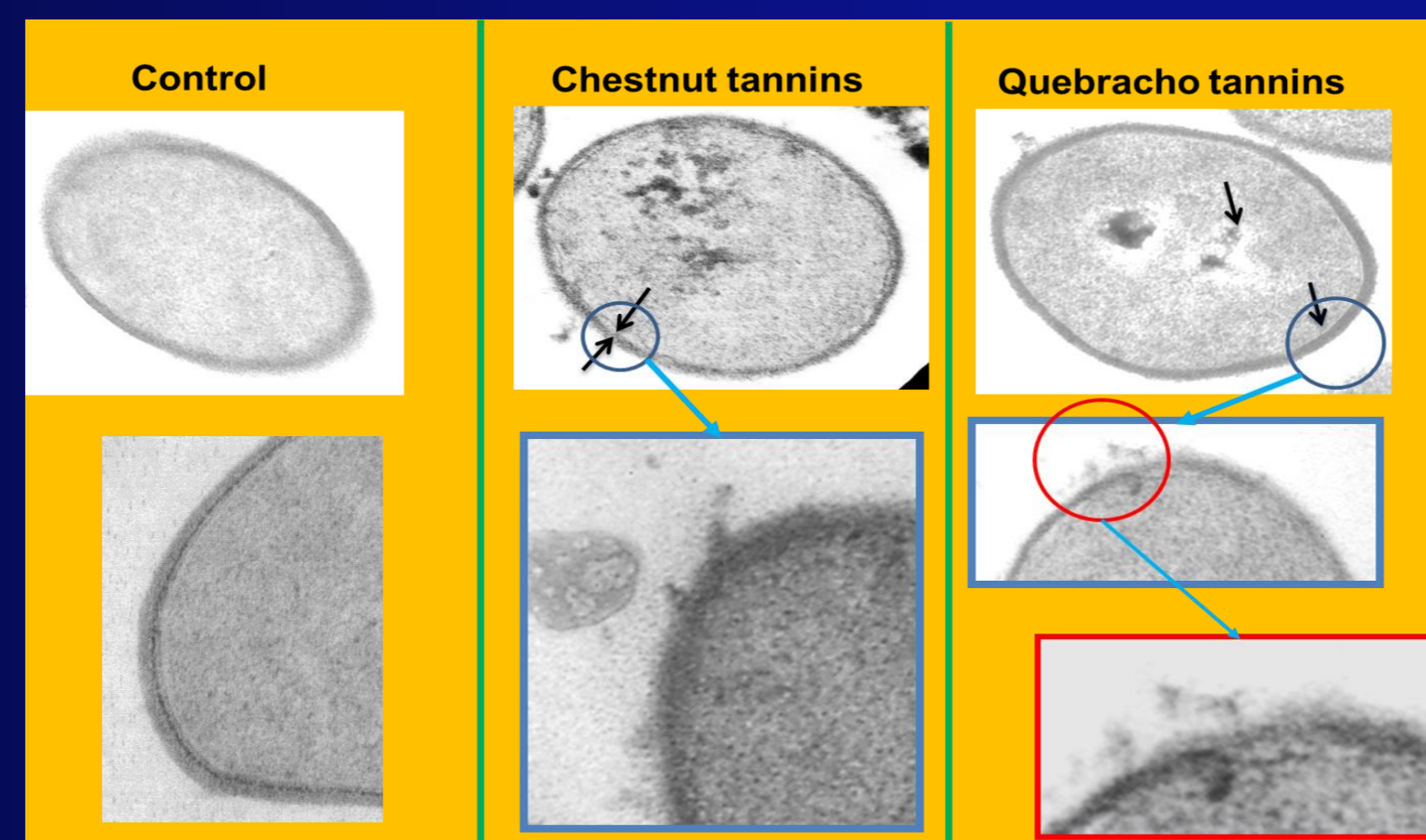


Fig 2: Tannins effects on *C. perfringens* wall structure. TEM results: (A) Control; (B) Vegetative cells grown with 0.5×MIC of chestnut, or (C) quebracho.

Table 1: MIC for selected resistant.

Treatment	Generation Time	Cattle strain		Poultry strain
		MIC (mg/L)		
Avilamycin	0	4	4	32
	400	8	8	
Bacitracin	0	4	8	32
	400	16	16	
Quebracho	0	16	0.2	0.2
	400	16	0.2	
Chestnut	0	4	32	32
	400	4	32	

In-vivo trials (*C. perfringens* challenge):

- Birds were weighed once a week until day 30.
- On days 15, 16 and 17 birds were challenged with *C. perfringens*

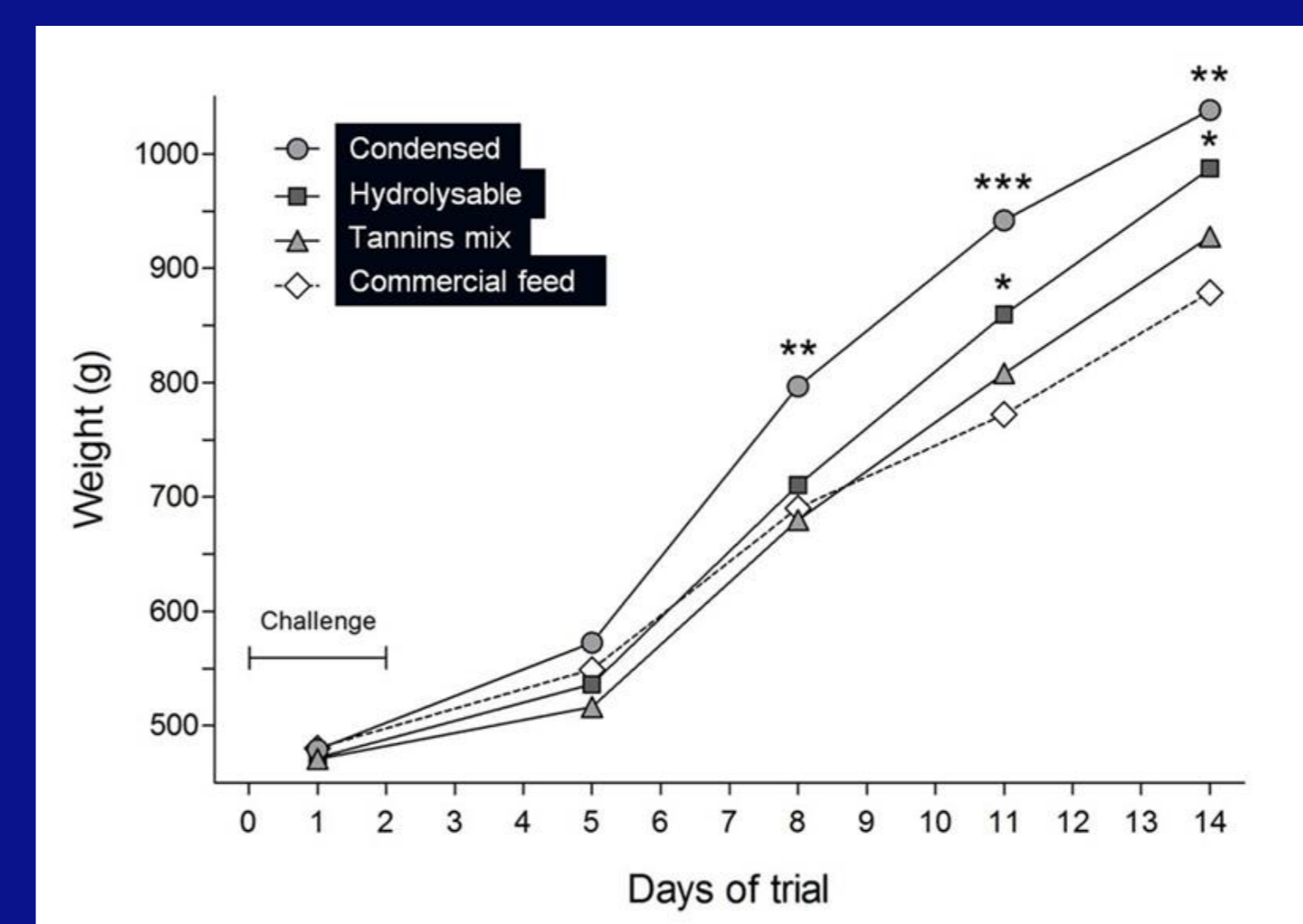


Fig 4: Average body weight in birds challenged with *C. perfringens* and supplemented with tannins Asterisks above symbols represent levels of significance at $p < 0.10.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

In-vivo trials (commercial conditions):

- 1 commercial broiler farm was monitored during one year.
- Birds in 3 houses were fed with tannin added diet
- Birds in 3 houses were fed with antimicrobial added diet

Table 2: Effects of tannins based additive on broiler productive performance compared with antimicrobial growth promoter (AGP).

Parameter	Tannin vs AGP
Weight	+4,02% (p>0,05)
Conversion rate	-0,05 (NS)
Mortality	-0,12% (NS)
EPEF	+19,7

In-vivo trials (experimental conditions):

- One-day old Cobb Broiler chicks
- Commercial feed (3200 Kcal/kg EM, 20% total protein) added with bacitracin or tannins.
- Birds were weighed once a week until day 30.
- Cecum samples were collected for 16S rRNA metagenomics studies.

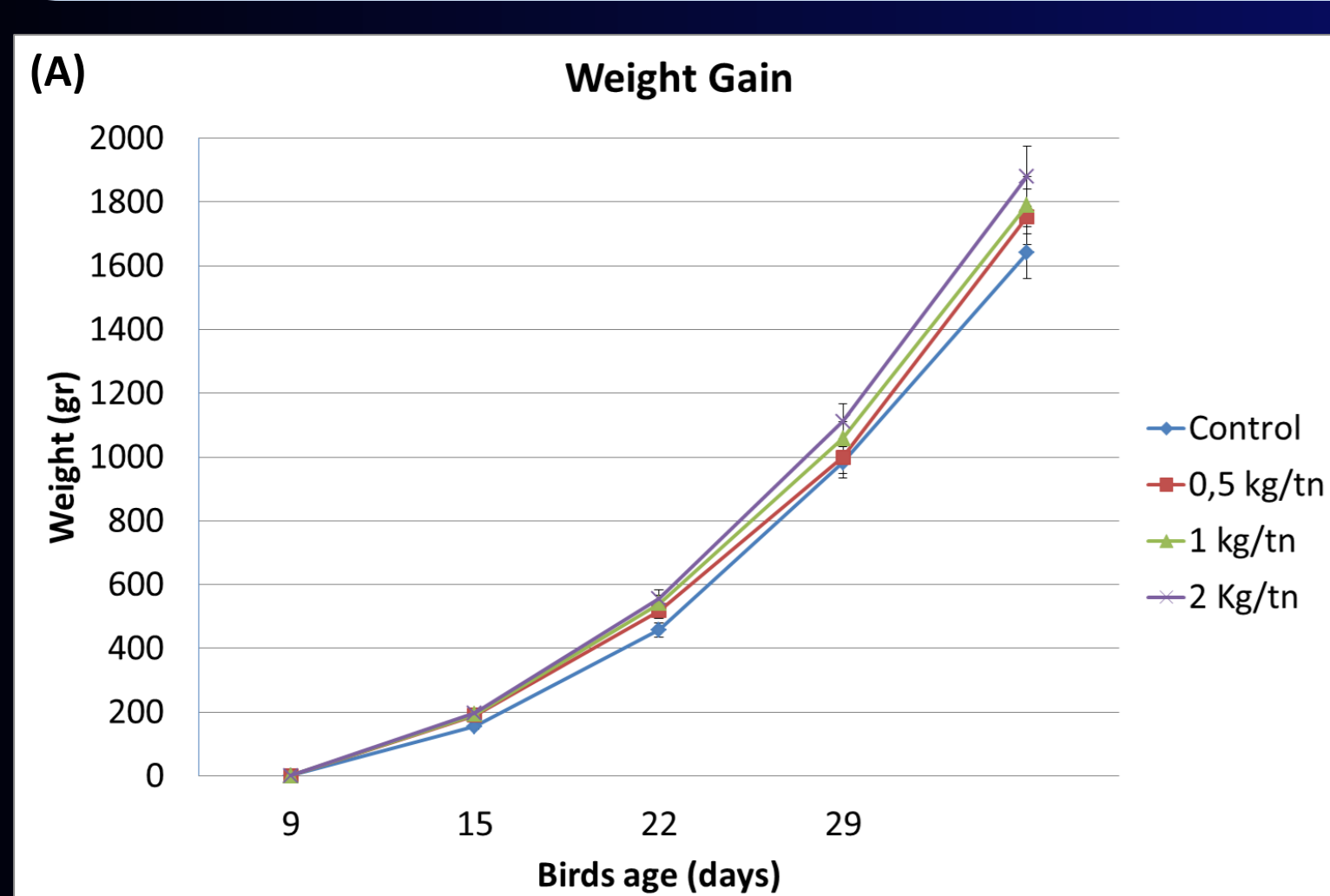


Fig 3: (A) Effects of different doses of tannins based additive on broiler weight gain. Fed additives effects on broiler microbiota. (B) Changes in the population of *Lactobacillus* spp. (C) Ratio between phyla Firmicutes and Bacteroidetes.

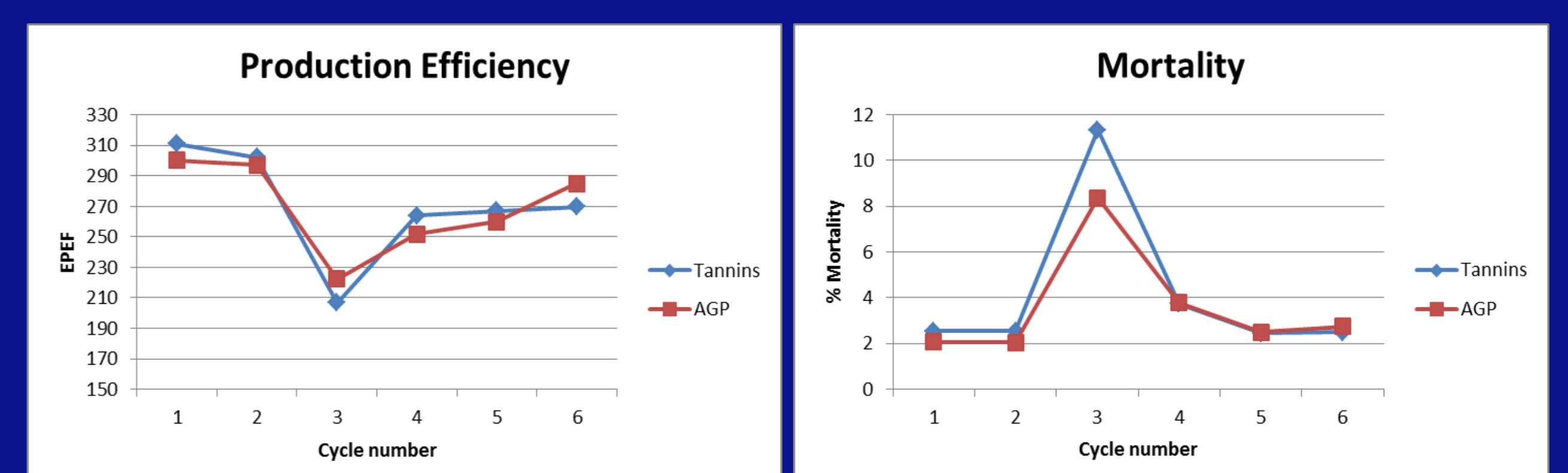
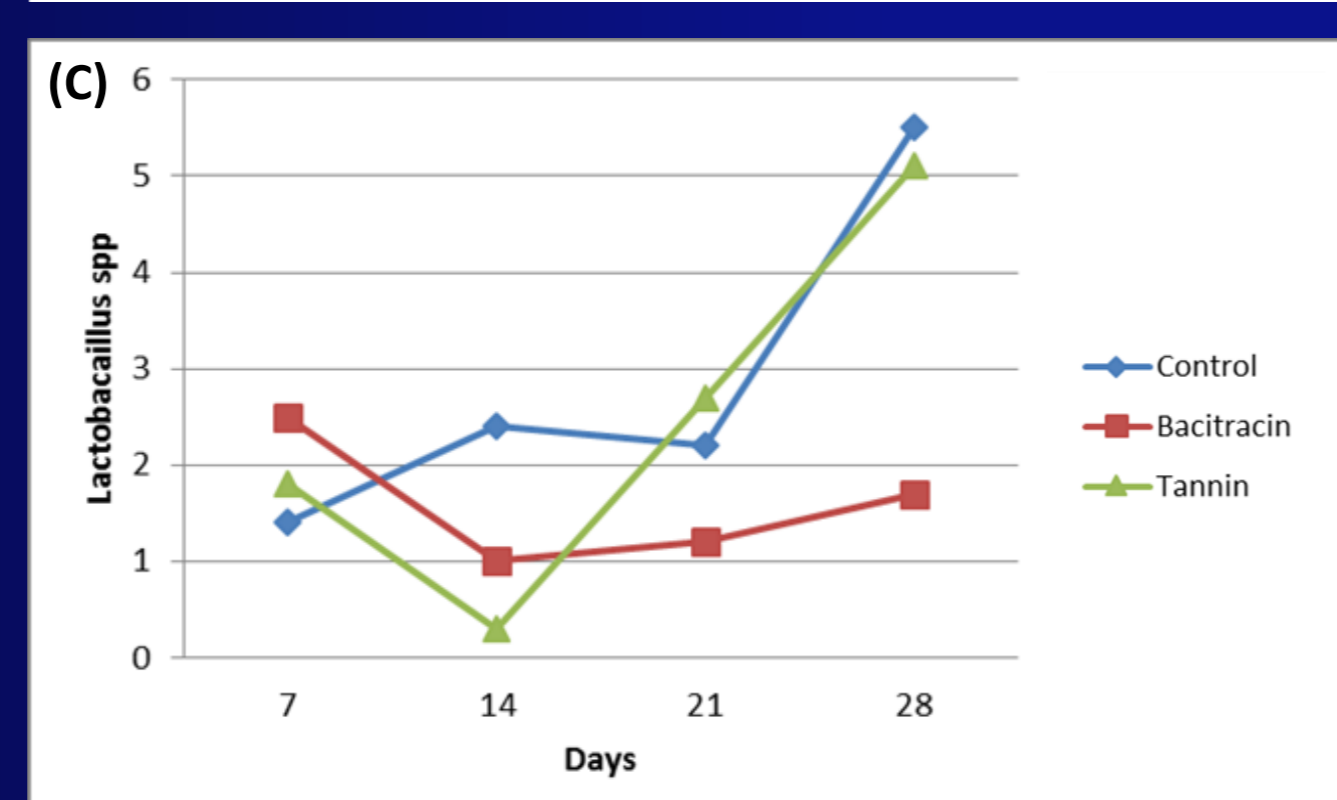
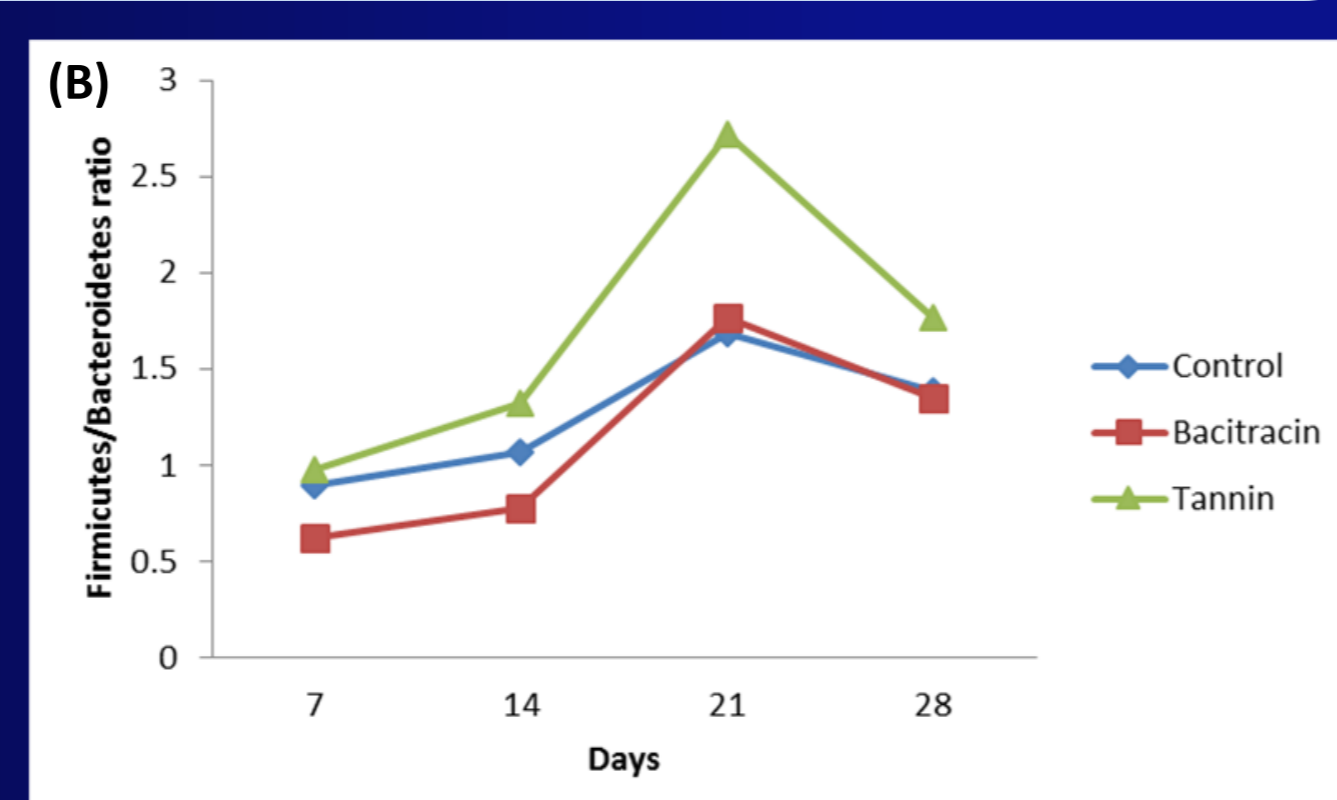


Fig 5: Comparative productive performance of birds fed with antimicrobial growth promoter (AGP) and tannins mix through different production cycles.

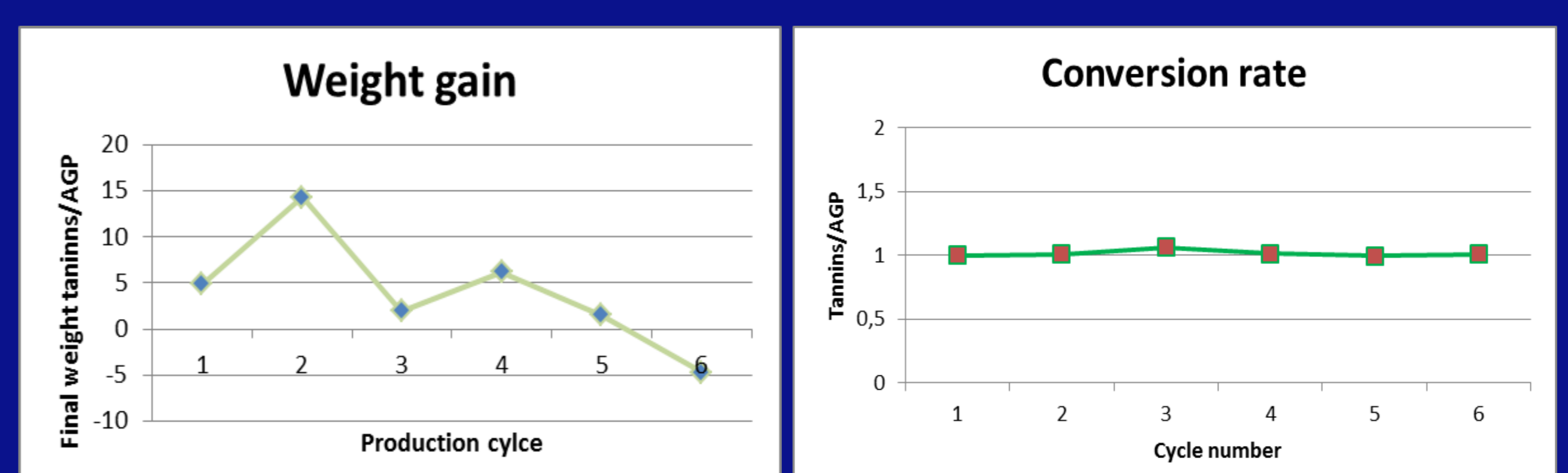


Fig 6: Relative effects of tannins based additive on broiler productive performance compared with an antimicrobial growth promoter (AGP).

CONCLUSIONS:

The present work shows a brief of the process to develop a product based in polyphenols as alternative to antibiotic growth promoters in poultry chickens. Selected natural compounds showed *in-vitro* antimicrobial activity against poultry pathogens like *C. perfringens*, modifying intestinal microbiota, and increasing productive efficiency probably by regulating physiologic host function, morphology of the gastrointestinal tract and controlling different infectious diseases including necrotic enteritis. The use of tannins rich plant extracts appears as an economically viable and sustainable alternative to the use of antimicrobial growth promoter factors. The use of tannins in different commercial farms under.