

# Use Of Propolis As A Natural Additive In The Production Of Broiler Chickens

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## Summary

*Propolis is a resinous and sticky substance that bees collect from tree shoots and mix it with their own salivary secretions, making it an ideal source of protein for animal growth and its high content of vitamins and other substances such as antioxidants, antimicrobials, anti-inflammatories, immunostimulants. Objective: to characterize the chemical properties of propolis as a natural additive that strengthens the immune system in birds. A quantitative, experimental methodology was used, where 64 chickens of the Cobb 500 line of 1 day of birth were used, with the random distribution of 4 groups made up of 16 chickens and 4 replications per group: T0 base diet (Control), T1 base diet + propolis in drinking water 5%, T2 base diet + propolis in drinking water (10%), Base diet T3 + propolis in drinking water 15%, the experimental units were distributed under a Completely Randomized Design (DCA). As a result, it was obtained that they reached an average weight in week 8 of T2 (10%) of 2855.55g higher than the other treatments and especially the T1 Control, with a feed intake of 4413.30 g and a feed conversion of 1.54; mortality of 4.68% in T0 and T1 in the third week vs. T2 and T3 that mortality was 0%, and a net benefit of 1.05 for T2 (10%). Concluding that the addition of propolis in drinking water for broilers increases weight, promotes the immune system preventing viruses and bacteria from affecting poultry production and obtaining minimum mortality parameters.*

**Keywords:** *recine, propolis, immunomodulator, feed conversion, antioxidant.*

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## INTRODUCTION

In the last 10 years, global production of animal protein has increased by 20%, of which a large part is attributed to poultry farming. Chicken meat is expected to make up more than half of the global share of additional meat produced by 2024. The short production cycle of poultry, compared to other meats, allows the producer to respond quickly to greater profitability, along with the fact that improvements are made in genetics, health and feeding practices (1).

Its production exceeds 100 million tonnes (t) in 2016, of which the Americas are likely to contribute about 44.3 million tonnes (44%). While the Americas are arguably the largest producing region, this is because the growth rate in this region would have averaged less than 3% over the decade, compared to 4% or more in the other major producing regions, and a global average of 3.5%. In 2013, seven countries in the region produced more than 1 million tons per year, including 5 countries in South America: Brazil, Argentina, Colombia, Venezuela and Peru (2).

The reuse of broiler chicken bedding is a common practice in the modern poultry production system, supported by the reduction in environmental impact, scarcity of this material and decrease in production costs. The main risk of these microorganisms is when there is a poor health status in the sheds, and the health of the animals, the quality of food, water and bedding material are neglected, as well as the presence of rodents and insects and the entry of vehicles without biosecurity measures. (3)(4) Natural additives are considered an alternative to replace antibiotics, from a technical, economic and biological point of view due to their zero residuality.. (5)

Antimicrobials are used in poultry diets with the aim of increasing competitive exclusion on the microflora of the gastrointestinal tract (GIT), controlling enteric processes of a subclinical nature, frequent in intensive production, which increases weight gains and conversion ratio between 1 and 5 %. However, antibiotics can increase the number of resistant strains, as well as transfer cross-resistance to other microorganisms not only for birds, but also for humans. (6)

According to the National Corporation of Poultry Farmers of Ecuador (CONAVE) and the National Institute of Statistics and Census INEC, through the Surveys of Area and Continuous Agricultural Production (ESPAC) in our country there is a poultry population of 224 million broilers (around 450 thousand tons of meat) and 9.5 million layers. with a production of 48,000,000 eggs per week, of which the industry contributes 85% and the production of the field contributes the remaining 15%. Per capita consumption in Ecuador is 32 kg person/year of chicken meat and 140 units of eggs person/year. The main producers are concentrated in the provinces of Pichincha, El Oro, Guayas, Imbabura and Manabí. (7)(8)

### Development

In our country there are approximately 1,819 productive poultry farms throughout the territory, with the poultry business being an economically sustainable engine generating approximately 32,000 direct sources of employment, 220,000 indirect sources and around 2000 million dollars a year, that is, 16% of the agricultural GDP and 2% of the total GDP. (9) Current alternatives to the use of antibiotics are natural products. Phytochemical additives have improved animal health and production, among their main beneficial effects are the reduction of pathogens, growth promoters, mitigators of the immune response of hosts to critical stress situations and greater bioavailability of essential nutrients for their absorption in the gastrointestinal tract. (10) (11)

Propolis is collected by bees that are more than fifteen days old and, with their jaws, take the resinous particles that are on the buds of different plants: poplar, willow, birch, alder, wild chestnut, pine and some herbaceous plants. After holding the resinous particle, the bee moves its head backwards until it manages to detach it, storing it with its legs in the pollen baskets. The enzymes in your mouth are also involved in the operation to prevent it from sticking. When he arrives at the hive with the load, other workers help him unload the propolis, a mission that can last several hours. (12)(13)

The flavonoids contained in propolis participate indirectly in the mechanism of cellular immunity, because they stimulate T8 lymphocytes, which receive the message from macrophages producing cytokines and interleukins and other cells, which inform about the presence of antigens in the body, T8 lymphocytes act as a second line of defense of the immune system. acting against invading cells, such as cancer, viruses, and bacterial cells. (14)

Propolis in the diet of broilers prevents digestive disorders, improves feed conversion and stimulates the immune system, when offered in doses of 250 mg/kg of feed (15). In laying birds, doses of 100 and 150 mg of propolis per kilogram of feed are sufficient to improve production and immunity. The use of high doses of propolis and vitamin C supplementation can counteract the depression in broiler carcass performance and quality caused by heat stress (16).

**Table 1. Chemical Components Identified in Propolis**

Class of components	Compound
Resins/Balms (Soluble in ethanol, 40 to 70%)	Phenolic compounds: phenols, phenolic acids, esters, flavonoids, aliphatic acids, alcohols, aldehydes, ketones, benzoic acid, and esters
Waxes (Insoluble in ethanol, 20 to 35%)	Myricil palmitate (approximately 80%), cerotic acid (approximately 15%), myricil cerotate, lignoceric acid, montane acid, psylic acid, among others

Class of components	Compound
Essential oils (7%)	Monoterpenes and sesquiterpenes
Other 5%	Minerals, polysaccharides, proteins, amino acids, amines, amides, trace carbohydrates, lactones, quinones, steroids and vitamins.

**Source: Taken from Vankova (2020) (17)**

Propolis has several beneficial characteristics: it is antioxidant, hepatoprotective, antibacterial, antifungal, antiallergic, immunomodulatory, anti-inflammatory, antiulcerogenic, antimutagenic, antitumor and chemopreventive. Red propolis stands out for its analgesic and antiparasitic properties. Black propolis, obtained by maceration in organic brandy, is the most active and efficient for treating conditions and improving health. On the other hand, white propolis, macerated in spring water, is versatile and alcohol-free, suitable for children. It contains more than 150 chemical compounds, such as polyphenols and flavonoids, which enhance its benefits. (18)

Propolis, part of apitherapy that uses beehive products, is a tradition of more than 10,000 years. Although less well known, its composition is complex, with more than 300 substances identified, including polyphenolic compounds, flavonoids, phenolic acids, terpenes, and minerals such as Mg, Ca, and Zn. It also contains vitamins B1, B2, B6, C, and E, as well as saturated and unsaturated fatty acids. These properties make it a beekeeping product of great medicinal interest. Propolis is a generally harmless product that rarely causes side effects, such as numbness, dry mouth, or allergic reactions related to honey. However, it should not be taken indiscriminately and it is advisable to follow the advice of a qualified professional. In addition, It has been shown to be compatible and complementary to other therapeutic practices. (19)

## METHODOLOGY

The research was carried out in the parish of San José de Poaló, located in the Cotopaxi province of the Latacunga Canton, between 00°51 07" and 00°54 45" south latitude and according to the Quito meridian it is between 00° 08 21" and 00°17 28" west longitude. With a temperate climate that is sometimes windy and cold, the average annual temperature is 11 °C. The study was experimental, with the use of 64 one-day-old birds, using 5%, 10% and 15% of propolis added to drinking water, as a natural additive, immunostimulant, antioxidant, antibacterial and antiviral in chickens. Propolis must be transformed from wax to liquid as follows:

### Procedure

Selection of propolis, harvest of the year, as a solvent 70° ethyl alcohol is used, or it can also be made from 96° alcohol, in addition to using required materials such as container, aluminum foil, funnel and paper filters.

### Instructions

Cut the beeswax resin into small pieces so that they dissolve better. It can be frozen 24 hours before so that the wax is hard and this will facilitate the operation.

Put the resin in the mash container and add the ethyl alcohol needed to complete the tincture.

Store the container in a dark place by covering it with aluminum foil, or use an amber container, to preserve them from light (which damages active components). Shake daily for at least 2 weeks at room temperature, 22 to 25 °C, up to 4 weeks.

1. The tincture will be ready when most of the propolis lumps have been turned into a barrel, which will be placed on top.
2. The propolis macerate is filtered. In the filter are the barrels of wax and non-solubilised propolis. And the tincture remains in the container with the filtrate. All that remains is to pack in amber glass containers, label and use. (20)

According to the treatments, the amount of propolis was added to the chickens' drinking water: T1

(950ml of water + 50 ml of propolis), twice a day, throughout the research. T2 (900ml of water + 100ml of propolis), twice a day, throughout the research. T3 (850ml of water + 150ml of propolis), twice a day, throughout the research.

The management of the experimental units was based on several stages:

Initially, with the reception, where the feeders and drinkers were located equally, a balanced diet corresponding to the initial phase was administered, drinking water with sugar was added in the first two hours of arrival and after that time vitamins plus electrolytes were administered for three consecutive days, at an average temperature of 31°C. The chicks were received and placed in the reception roundabout, 32 of them were weighed to obtain the average weight on arrival.

The initiation stage took place during the first 7 days, they were given initial balanced food powder at will divided into four rations, supplying drinking water with vitamins, with a temperature control every hour for 24 hours gradually decreasing according to the age of the chicks, the washing of the drinkers and the change of beds was carried out periodically. From the 8th day onwards, three study groups were carried out to start with the treatments, the initial balanced feed plus the propolis extract was provided divided into three study rations T0 - (control treatment - base diet), T1- (Base diet - 5% propolis in the drinking water), T2- (Base diet - 10% propolis in the drinking water), T3- (Base diet - 15% propolis in drinking water), also starting with vaccination against New Castle disease (ocular route) at a dose of one drop per chicken. Throughout this stage, the temperature will be strictly controlled, ensuring that the chicks have a constant and appropriate heat source.

The growth stage will comprise from 14 days to 24, where the starter food was maintained, until day 13, from day 14 balanced corresponding to the growth phase plus treatments of 5%, 10% and 15% of propolis in the drinking water, always having control over the temperature. From the 13th, the light control began with the suspension of 5 hours (10 pm - 3 am). Weight was controlled to determine if there were any negative changes related to light management. After that day, vaccination against Newcastle disease + Bronchitis (ocular) was carried out, the dose was one drop per chicken. Light management was suspended. A control of waste and daily consumption was carried out.

Finally, the final stage took place from 25 - 49 days, on day 21 the revaccination of Newcastle + Bronchitis (ocular) was provided with a dose of one drop per chicken, immediately the vitamins were added to the drinking water in order to control the stress produced by the vaccine. On day 28 a fattening feed was provided with the addition of different concentrations of propolis for each treatment distributed as follows: T0 - (control treatment - base diet), T1- (Base diet - 5% propolis in drinking water), T2- (Base diet - 10% propolis in drinking water), T3- (Base diet - 15% propolis in drinking water).

The chickens were weighed on that day, in order to know their weight prior to the implementation of these diets. On the 28th, the vaccine against Gumboro disease will be revaccinated at a dose of one drop (orally). The previously mentioned diets continued to be supplied until day 56, after this day the process of slaughtering the birds was carried out, taking care of animal welfare throughout the process to obtain safety in the product.

#### **Statistical analysis**

The characterization of the chemical composition of propolis was evaluated by applying descriptive statistics. In addition, a Completely Randomized Design (DCA) was applied with four replications for each treatment, which allowed the comparison between two or more treatments in a random manner for the experimental units in a homogeneous way, considering different sources of variability. The data obtained were analyzed using the DUNCAN method for significance testing ( $p < 0.05$ ).

## **RESULTS AND DISCUSSION**

He calculated the main productive parameters to assess the response in chickens after ingesting propolis in drinking water as an immunomodulator in their diet for 56 days. Thus, it has been possible to evaluate the feed efficiency of the diets provided and the validity of each one as an alternative in the

production processes of the poultry industry. In addition, the results obtained in the field of each of the treatments are presented to determine which of the diets provided has a greater effect as a growth promoter, immunomodulator, antibacterial, antiviral and antifungal antioxidant in broiler broiler fattening.

The average feed intake of the chickens in each treatment was recorded weekly for analysis. Table N°1 shows the results obtained from feed intake, expressed as the mean for each treatment, and also presents the parameters of interest of an ANOVA and Duncan test performed for this dataset. Observing that from week 2 there are significant differences between the control treatment and those of propolis inclusion at the different levels, this is typical of the research considering that the respective amount of propolis was added to the drinking water according to the treatment, so that the average consumption of food for the control treatment was 4012.1 g. compared to the T3 treatment which was 4613.9g. which is equivalent to a difference of 601.8 g in the addition of propolis. In the analysis between weeks, it is observed that there is an increase in feed consumption and it is due to the fact that as the chickens grow, feed consumption is higher, that is, age and feed consumption have a direct relationship.

In week 1 the chick feed is 280.5 g, for all treatments, considering that according to the Cobb Chicken Rearing table for the first seven days each chick should feed 34.0 g. In the research each chick consumes 28.05 g. According to the Cobb 500 technical guide manual, 2015, a chick's feed intake at 14 days is 68.18 g, at 21 days it is 111.13 g, at 35 days it is 189.14 g, at 28 days it is 151.95 g, at 42 days it is 215 g. Feed intake was calculated on the basis of weight gain. When comparing these data on feed consumption, it is determined that according to the Ergomix tables and the technical guide for the management of Cobb500 chickens, the values are almost similar, considering that environmental factors, type of feed and infrastructure have not been taken into account.(21)(22)

**Table 2. Average feed intake per treatment**

Means with a common letter are not significantly different ( $P > 0.05$ ) according to Duncan's multi-range test

Weekly Feed Intake(g)										
Parameter	Witness		Kicked. 1		Kicked. 2		Trat.3		Problems.	CV
Week 1	280,5		280,5		280,5		280,5			
Week 2	439,98	d	461,98	c	483,98	b	505,98	HIM	0,0001	1,4
Week 3	979,70	d	1028,60	c	1077,60	b	1126,60	HIM	0,0001	4,2
Week 4	1519,50	d	1595,47	c	1671,40	b	1747,40	HIM	0,0001	4,4
Week 5	1628,40	d	1709,82	c	1791,20	b	1872,66	HIM		
Week 6	2500,00	d	2625,00	c	2750,00	b	2875,00	HIM		
Week 7	3509,30	d	3579,50	c	3860,20	b	4035,70	HIM		
Week 8	4012,10	d	4212,70	c	4413,30	b	4613,90	HIM		

**Source:** Own elaboration

The table shows the confidence intervals presented by the treatments where it is evident that all levels of broccoli flour inclusion differ with the T1 (Control), but when analyzing the results of the treatments in the weeks it is reflected that there is a significant difference with an increasing tendency, with the probability of 95%. showing that feed intake is directly proportional to the age of the birds.

Which indicates that when supplying Zingiber officinale in the diet of chickens they will achieve good indicators of weight gain, on the contrary, indicates that weight gain in birds from 36 to 56 days reaches an average of 1 704 g whose value is lower than those obtained indicates that diets supplemented with ginger flour are used to optimize feed conversion, to the extent represented. This research coincides with the present study as it shows that all treatments containing a natural additive provide a more optimal result in terms of feed consumption compared to the control group.(23)(24)

**Tabla 3. Ganancia de Peso promedio por tratamiento**

Medias con una letra común no son significativamente diferentes ( $P > 0.05$ ) según el test de rango múltiple de Duncan

Weekly Weight Gain(g)										
Parameter	Witness		Trat. 1		Trat. 2		Trat.3		Problems	CV
Week 1	162,26	ONU	162,13	ONU	161,60	ONU	162,38	ONU	0,844	0,81
Week 2	291,88	b	298,04	Ab	306,78	ONU	308,99	ONU	0,067	2,98
Week 3	682,25	b	691,13	b	695,80	b	746,38	ONU	0,0001	1,8
Week 4	1075,78	b	1107,38	b	1199,93	ONU	1234,35	ONU	0,003	4,51
Week 5	1191,53	c	1348,88	b	1424,93	ONU	1439,10	ONU	0,0001	1,94
Week 6	1813,39	a.C	1810,23	c	1852,77	Ab	1889,63	ONU	0,0029	1,41
Week 7	2302,50	d	2346,43	c	2397,75	b	2465,25	ONU	0,0001	0,98
Week 8	2750,12	b	2824,75	ONU	2855,55	ONU	2837,43	ONU	0,0090	1,33

**Source:** Own elaboration

Weight gain in chickens shows variability between treatments, T1 (Control) presents significant differences compared to others with propolis, with T2 (10%) being the highest. Although analysis of variance reveals weekly differences, weight gain increases with the age of the birds. Weight gains of 505.4 g at 28 days of age of the birds with the use of 5% thanks to gingerol and zingerone, with significant differences ( $P < 0.0001$ ), with respect to the weights of the other treatments. (25) When compared with other antioxidants, the values obtained in the research are higher, reaching 703.89 g at the fourth week. According to the Cobb 500 technical guide, 2015, the ideal weight gain that should be reached at 42 days is 782.2 g. and at 49 days 788.6 g. considering very appropriate environmental means, water and food management with strict measures, authors such as Golestan, mentions that the antibacterial and growth promoter effects are closely related since they beneficially affect the intestinal microbial ecosystem by controlling pathogenic bacteria and their toxins and, consequently, improving nutrient digestibility. (26) (27)

In the weekly weight gain there is an increase, observing that if there is a significant difference, when comparing the T0 control treatment with the T2 treatment (10%) there is a difference of 105.43 g and when comparing between T2 (10%) and T3 (15%) there is only a difference of 18g, concluding that the best treatment is T2 with 10% addition of propolis in the drinking water.

**Table 4. Feed conversion by treatment. Means with a common letter are not significantly different ( $P > 0.05$ ) according to Duncan's multi-range test**

Feed Conversion(g)										
Parameter	Witness		Trat. 1		Trat. 2		Trat.3		Problems.	CV
Week 1	1,73	ONU	1,72	ONU	1,72	ONU	1,72	ONU	0,788	0,85
Week 2	1,50	c	1,55	a.C	1,58	Ab	1,63	ONU	0,010	2,86
Week 3	1,43	c	1,48	a.C	1,55	ONU	1,53	Ab	0,003	2,38
Week 4	1,41	ONU	1,45	ONU	1,39	ONU	1,41	ONU	0,727	4,84
Week 5	1,36	ONU	1,26	a.C	1,25	c	1,30	b	0,001	2,14
Week 6	1,38	d	1,45	c	1,48	b	1,52	ONU	0,0001	1,3
Week 7	1,52	c	1,52	c	1,61	b	1,64	ONU	0,0001	0,92
Week 8	1,45	d	1,49	c	1,54	b	1,62	ONU	0,0001	1,3

**Source:** Own elaboration

The feed conversion in each treatment shows significant differences between them, from week 5, the best result of the feed conversion is of the control group with a value of 1.45 compared to the rest of the treatments with the addition of propolis in the drinking water, especially T3 (15%) which reached 1.62. The main indicators of productive behavior in the initial phase, the birds at the opening of the experiment presented similar BW (live weight) ( $P>0.05$ ), but at the different periods evaluated (0 to 15 days of age) the Cobb 500 and Ross 308 hybrids presented differences in weights and a higher feed conversion of 1.19 and 1.15 of the Cobb 500 and Ross 308 hybrids respectively. (28)

At 49 days, the Cobb 500 and Ross 308 lines achieved a feed conversion ratio of 1.50 and 1.66, respectively, (29) observing data superior to those obtained in the research of the different concentrations of propolis in drinking water, but they fully coincide in obtaining a healthy chicken with good weights that is representative in poultry production.

**Table 5. Percentage of mortality per treatment**

Treatments	% mortality
T0	3,12
T1	1,56
T2	0
T3	0

Source: Own elaboration

In the second week the chickens showed signs of asphyxiation, sudden death, at necropsy they show liver congestion with rounded edges and friable to the touch, congested lungs, suspicious of respiratory conditions. Table 4 shows the percentage of mortality of each treatment as follows: 4.68% of dead birds distributed in the T0 Control, 3.12% in T1 (5%) 1.56%, in the rest of the treatments no mortality is recorded to date, corroborating that the use of propolis in different concentrations prevents respiratory diseases due to different causes. whether they are: viral, bacterial or fungal because it is an immunomodulator, antioxidant and natural antibiotic.

Causes of mortality in poultry production include biosecurity, feeding, and handling factors. In a study on the use of organic acids and antibiotics in broiler chickens, the following results were found: T1 (positive control) with Zinc Bacitracin in the feed, showed a 5.4% mortality; T2, with a mixture of organic acids, had a mortality rate of 9.9%; and T3 (negative control), without additives, also had a 5.4% mortality rate. These data reflect the influence of additives on the mortality of chickens during their growth. (30)

## Conclusions

Propolis contains flavonoids such as pinocembrin, pinobanksine, chrysin, galangin, acetone and apigenin as its main component. The resinous fraction is made up of phenolic compounds and flavonoids that are very important at a therapeutic level in both humans and animals, preventing bacterial, fungal, viral infections, and is antioxidant and immunomodulatory. The productive variables considered in this experiment showed relevant differences between them, with the T2 treatment (base diet + 10 % propolis) presenting the best performance in each of these, which shows that the dosage used in this treatment is the most effective to be applied in a broiler farm.

The cost-benefit ratio of T2 (10%) reflects a higher net benefit with 1.05, which shows a greater gain for this treatment, when compared to the T0 Control that obtained a loss of 0.5 cents.

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