

## ORIGINAL PAPER

# Development of broiler chickens after treatment with thymulin 5cH: a zoo technical approach

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**Modulation of immune response due to thymulin 5cH has been previously observed. The aim of the present study is to evaluate the development of broiler chickens treated with thymulin 5cH by conventional zoo technical indices, phytohemagglutinin induced inflammation test and histomorphometric analysis of lymphoid organs (thymus, Fabricius bursa and spleen). Animals were divided in two groups: (a) test: birds with free access to thymulin 5cH diluted into the drink water and (b) control: birds with free access to water only, from the 1st to the 42nd day of life. All experimental procedures were done in blind. The results show that thymulin 5cH treated group had increased productivity index compared to control (391.45 versus 261.93) associated with higher viability in the 7th week ( $p = 0.013$ ), and a possible shunt to B lymphocyte activity. The data suggest that thymulin 5cH could be a viable method to improve productivity in poultry production due to its immune modulation properties. Homeopathy (2012) 101, 68–73.**

**Keywords:** Thymus hormones; Homeopathy; *Gallus gallus*; Poultry; Immune system

## Introduction

The lack of animal protein availability to a significant proportion of the world's population has forced the adoption of techniques and procedures in increased meat production, with reduced costs and time. Birds are very efficient in converting vegetable products into protein.<sup>1</sup> Brazil is a major producer and exporter of poultry meat. Broiler hens are bred in confinement, at high concentration, with high level of stress and high probability of infectious diseases. For these reasons, growth promoters and antibiotics are used to prevent disease and improve productivity, reducing the feeding time up to slaughter.<sup>2</sup> Modern technologies, however, involve chemical substances during all phases of production, inducing resistant microorganisms. Some consumption markets, such as Europe, have increased the search for alternative organic products. In veterinary medicine, the use of homeopathic medicines added to water, food or semen represents a potential zoo technical resource, mainly for treatment of animals in big farms.<sup>3–7</sup>

In previous studies, the use of bovine thymus extract in broilers produced modulation of humoral and cell immune response after vaccination against Newcastle disease.<sup>8</sup> Thymulin is an important component of these extracts due to its role in lymphocyte maturation. It is a zinc dependent nonapeptide (GLP–Ala–Lys–Ser–Gln–Gly–Gly–Ser–Asn–OH) produced by thymus epithelial cells.<sup>9</sup> Several studies have demonstrated the usefulness of thymulin in high and low levels in immunity control in birds.<sup>10–23</sup>

In this work, the aim was to evaluate the effect of treatment with thymulin 5cH on the development of commercial chicken broilers, using the following parameters:

- Conventional zoo technical indices (alimentary conversion, weight gain, weight average, mortality, viability and productive efficiency index – PEI).
- Intensity of inflammation (local immune cell reaction) induced by phytohemagglutinin.
- Gross pathology and histopathology of lymphoid organs, including the thymus, spleen and bursa of Fabricius.

## Material and methods

### Animals and treatment

The study was performed in the experimental unit of a commercial farm specialized in chicken breeding (*Granja Santana*), in Tietê, São Paulo state, Brazil. One-day old

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*Gallus gallus* Cobb birds, of both genders, were randomized before the beginning of the study. Each bird was identified by a plastic ring with a number. These rings were changed weekly, according to animal growth. The birds were immunized against Marek and Gumboro diseases (Ceva<sup>®</sup>, France). 72 birds were maintained in two brick barns, with impermeable flooring and roof. Lateral protections consisted of wire screens and rolling doors. Each barn measured 3 square meters and was equipped with poultry drinkers, manual feeders and gas heating. The chicken barns were disinfected by spray of quaternary ammonia and glutaraldehyde. The floor was covered with a 10 cm layer of *pinus* powder. One-day old chickens were housed at the usual commercial density of 12 birds per square meter.

The birds received food (Flamboyant<sup>®</sup>, Brazil) and tap water 'ad libitum'. Food was made from corn and soybean, with protein content adjustment according to the growth phase of birds (from 23 to 18%). No additive or growth promoter was used. All water offered was supplied from a 50 L reservoir which was filled regularly when the water volume reached 10%. The water supply was the same for both barns; water purity and mineral standards were controlled by a governmental agency (Companhia de Saneamento Básico do Estado de São Paulo - SABESP).

The treatment consisted of adding thymulin 5cH or vehicle (70% ethanol) to the respective reservoir, blind, using the codes 'A' or 'B'. These codes were broken only after statistical analysis. Thymulin 5cH was prepared in distilled water on the day of use, according to the Brazilian Homeopathic pharmacopeia (2nd Edition), from the commercial Thymulin 4cH (Boiron<sup>®</sup>, France). According to information obtained from the supplier, the Thymulin 4cH is prepared from 98.66% of pure peptide solution, without coupled zinc. Additional chemical analysis (CBO analyses – Brazil) revealed that no Zinc was present in the 5cH vial. Thus, the mother solution was the pre-thymulin peptide. Control was prepared by the same method from the same vehicle. The entire content of the flasks (20 mL) was added to the respective reservoirs every time they were filled. The final expected concentration was  $4 \times 10^{-14}$  M, according to the technical information supplied by the manufacturer.

### Zoo technical indices

During the experiment, birds were weighed and food consumption was calculated at 1, 7, 14, 21, 28, 35, 42 days, and just before slaughter (46 days). Weight gain, feed conversion, mean weight, mortality and viability were calculated for both groups. The PEI was calculated by the following formula:<sup>24</sup>

$$\text{PEI} = [(\text{weight gain} \times \text{viability}/\text{age at slaughter} \\ \times \text{feed conversion})] \times 100$$

The PEI calculation was performed twice by two different observers.

### Local cellular immunity evaluation

At the age of 45 days, the end of experimental period, local cell immunity was evaluated using the *cutaneous*

*basophilic response method*,<sup>25–27</sup> using a subcutaneous injection of phytohemagglutinin (Cultilab<sup>®</sup>, Brazil). The inter-digital skin test (between the 3rd and 4th right digits) was performed by measuring just before the injection of 0.1 mL of sterile 1% phytohemagglutinin. The inter-digital membrane thickness of the corresponding part of the left foot was also measured and inoculated with 0.1 mL of sterile NaCl 0.9% solution. A digital micrometer (Mitutoyo<sup>®</sup>, Brazil) was used for these measurements. After 12 h, another inter-digital skin test measurement was made and the difference between the last and the first measurement was calculated, considering each foot side separately. The difference between the right foot thickness and the left foot was considered as the basophilic specific cell reaction for each animal.

### Necropsy and anatomopathologic examination of lymphoid organs

At 46 days of age, birds were killed by cervical dislocation and were submitted to necropsy for macroscopic evaluation of lymphoid organs. From each bird, thymus, spleen and Bursa of Fabricius bursa were analyzed considering their general appearance, size and color. Representative fragments were harvested, labelled and fixed in 10% formaldehyde for about 72 h, for subsequent histopathological examination. Slides were viewed with an optical microscope (1000×) and digital photomicrographs were taken with additional magnification of 4.8×. Histometric evaluation was performed using the software Image Tool 3.0 for Windows. To evaluate the inter-digital skin inflammation, the number of inflammatory cells per 5 randomly chosen fields was counted, per slide. For the lymphoid organs, the diameters of lymphoid follicles were measured in spleen and Bursa of Fabricius. Five randomly chosen follicles per slide were analyzed. Thymus was analyzed in a magnification of 400× for qualitative – descriptive evaluation, since the limits of its structures – medulla and cortex – were not defined enough to allow quantitative analysis.

### Statistical analysis

The methods used were Student 't' test for parametric variables and Mann–Whitney test for non-parametric variables. Viability was analyzed by a contingency table and the Fisher's test was used in this case. The values of  $p \leq 0.05$  were considered significant. All statistical analysis was done using the software INSTAT for Windows 3.

### Bioethics

This study was approved by the Bioethical committee of Universidade Paulista (UNIP) – CEP/ICS/UNIP protocol 006/07 – and was conducted according to the Brazilian laws for animal use in scientific procedures.

## Results

### Weight gain

The females from the group treated with thymulin 5cH had a higher weight gain than females in the control group,

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