

Investigation on the impact of GALLIPRO® Fit on gut-brain axis and performances in broiler production

Prof. Ibiara C.L. Almeida Paz, Universidade Estadual Paulista - Julio De Mesquita Filho - Brazil
 Alberto Yocytaca Inoue, Sales and Marketing Manager South America, Chr. Hansen AHN
 Jean-Christophe Bodin, MSc. Agr., Sr. Technical Product Manager Poultry, Chr. Hansen AHN
 Antoine Meuter, MSc., Product Manager Poultry, Chr. Hansen AHN



ANIMAL WELFARE RAISES QUESTIONS

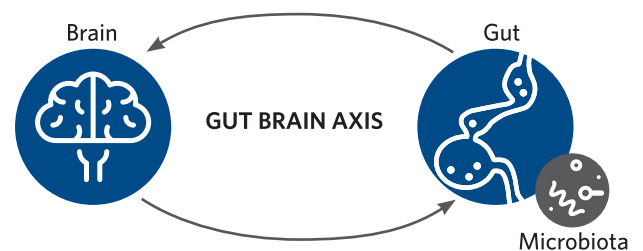
From hatch to harvest, broilers eat, drink, and grow within commercial production systems. The application of biosecurity and responsible animal husbandry is necessary regardless of the scale of production. Commercial broiler chickens have been selected for their rapid rates of growth, high carcass yields, with regard to their breast, and ability to thrive in modern production systems. They are often raised at relatively high stocking densities that range from 30 to 40 kg of live weight/m². An ever-present concern under these intensive conditions is the behavior and welfare of birds. On a worldwide basis, there is an evolving effort to ensure that animals are raised with concern of their natural behavior. A focus on animal welfare is the rule rather than the exception. This desire to improve welfare has led to the establishment of standard processes and practices around such things as stocking density, light regimen, air quality, water hygiene, ammonia emissions, and litter quality, to name a few. Interestingly, there is a growing body of scientific and industry evidence that a beneficially-balanced intestinal microbiome is associated with an improvement in the well-being of poultry and consequently in their performance.

PROBIOTICS PLAY A ROLE IN THE GUT-BRAIN AXIS

The capacity of broilers to digest nutrients from their feed requires a healthy intestinal tract. Facing disease challenges, not only to survive, but to thrive under commercial conditions, will be the biggest drivers of performance and economic success. Daily feeding of an effective probiotic can support all those capacities. It can improve each of the measured outcomes of poultry production, including, but not limited to, rates of growth, feed efficiency, flock uniformity, mortality, and postharvest condemnation. In human health, the study of the microbiota-gut-brain axis has advanced greatly in recent years as investigators seek to understand the interaction of probiotic strains and cognitive functions (Bested *et al.*, 2013; Carabotti *et al.*, 2015). Interestingly, specific strains of probiotic bacteria, including strains of *Bacillus subtilis*, have been shown to affect patterns of

behavior (Hu *et al.*, 2017). In humans and in mice (Ezenwa *et al.*, 2012; Foster *et al.*, 2013), supplementation with probiotics – and their action on commensal microbiome – led to a release of neuroendocrine factors that subsequently affected the relative abundance of neurotransmitters, such as serotonin (involved in the feeling of well-being), and neuronal regulators, such as tryptophan, in the gut and in the brain (Figure 1). Tryptophan is an essential amino acid required in the biosynthesis of serotonin.

Figure 1: Gut brain axis synthetic representation: 90% of serotonin (molecule of happiness) is produced in the intestine.



In the poultry industry, beside improvements on productivity, the daily feeding of probiotics is assumed to positively influence the welfare of broilers and layers (Yano *et al.*, 2015; Almeida Paz *et al.*, 2019). The cellular mechanisms underlying behavioral changes that impact welfare in avian species may be like those described above in mammals.

BEHAVIOR VARIABLES ARE GOOD INDICATORS

A broiler experiment was conducted to determine the effects of a commercially-available probiotic, **GALLIPRO® Fit**, a combination of three strains of *Bacilli* that were initially selected for their capacity to mitigate *Salmonella spp.*, *E. coli*, *Clostridium spp.*, and others key pathogens that plague the poultry industry.

LATENCY TO LIE TEST

Is a behavioral method to assess leg health in broilers by recording the amount of time a bird takes to sit after being placed in a standing position in tepid water.

APPROXIMATION TEST

Evaluation of the relative reactivity of broilers when an assessor enters their pen and attempts to touch them for 3 minutes.

GAIT SCORING: Large problems have been established on walking difficulties among birds, which have welfare, discomfort and mortality repercussions. Gait scoring, being used as a measure of welfare assurance, is a non-invasive method of field assessment of walking ability of commercial broilers and it has significant correlation with latency to lie. The method for gait scoring used in this study is a three category system (0: No obvious signs, 1: Obvious signs, 2: Severe signs).



Experimental Design: 1600 male broilers (Aviagen AP95) were randomly assigned to one of four treatment groups (50 birds per replicate, 8 replications per treatment)

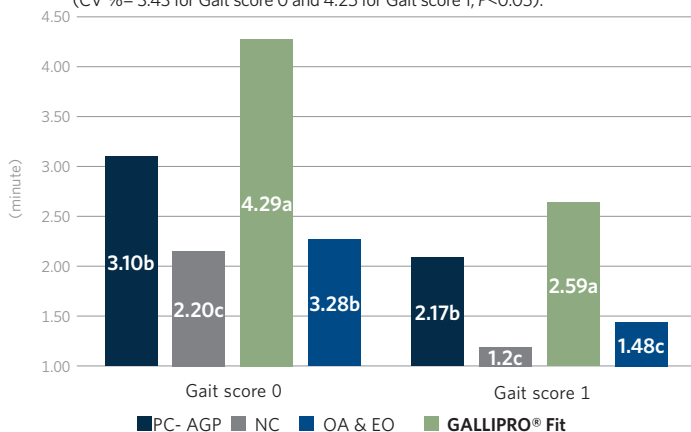
1. Positive control group fed daily with Halquinol, an antibiotic growth promoter (PC-AGP)
2. Negative control group no AGP (Antibiotic growth promoters), neither probiotic/alternative feed additive (NC)
3. Treatment group fed daily with GALLIPRO® Fit at 500 g/T (GPF)
4. Treatment group fed daily with a combination of one Organic Acids and Essential Oils at 300 g/T (OA&EO)

All feed additives were included from day 0 till the end of the trial. Zootechnical performance data, such as feed intake, body weight, feed conversion ratio and mortality (%), were collected on a weekly basis.

In addition, the effects of treatment on behavior were examined using a Latency to lie test and Approximation test at 42 days. Plasma concentrations of serotonin were measured at 40 days of age, sampling 8% of broilers from each treatment.

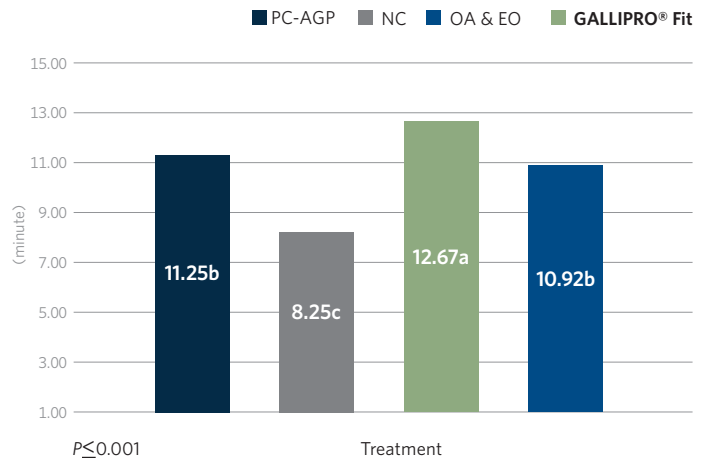
Figure 2: Latency to lie test results

Means followed by different letters differ from each other by Chi square test (CV % = 3.43 for Gait score 0 and 4.25 for Gait score 1, $P < 0.05$).



For both Gait scores 0 and 1, (Figure 2) illustrates a significantly longer time for the Latency to Lie (LTL) in the group of broilers fed with GALLIPRO® Fit in comparison to negative control (NC), to treatment with antibiotic (PC-AGP) and to broilers fed diets containing combination of one organic acid and essential oils (OA&EO). These test data demonstrate a better leg health status with probiotics supplementation.

Figure 3: Approximation Test results



Means followed by different letters differ from each other by Chi square test ($P < 0.05$).

Birds in the GALLIPRO® Fit group were calmer in the Approximation Test (Figure 3), indicative of their relative state of calmness. In contrast, birds fed diets containing antibiotic (PC-AGP) were the most reactive and more stressed. The magnitude of reactivity of the remaining groups was higher when compared to the GALLIPRO® Fit group.

BLOOD CIRCULATING SEROTONIN LEVELS WERE POSITIVELY IMPACTED

Serotonin (5-hydroxytryptamine; 5-HT) is best known as a neurotransmitter critical for the development and proper function of the central nervous system. Remarkably, 90% of the body's serotonin is produced in the intestine. **Several publications report a strong positive correlation between health of the intestinal mucosa and production of serotonin; the healthier the intestinal mucosa, the higher the concentration of serotonin produced by the birds.** In parallel to Latency to lie and harvesting, blood serotonin levels were measured at Day 40 (Table 1).

Table 1: Blood circulating serotonin (5-HT) levels in broilers at day 40.

TREATMENT	SEROTONIN (5-HT) µg/mL
Positive Control (PC-AGP)	91 c
Negative Control (NC)	100 c
GALLIPRO® Fit (GPF)	402 a
Organic Acid+Essential Oils (OA&EO)	316 b
CV %, P value	1.73, 0.001

ZOOTECHNICAL PERFORMANCES OVERVIEW

The zootechnical results for the different treatments are summarized in Table 2 below.

Table 2: Zootechnical performance by treatment group at 42 days of age.

TREATMENT	BODY WEIGHT (g)	FEED CONVERSION	MORTALITY (%)
PC-AGP	3103 c	1.52 a	1.71
NC	3086 c	1.51 a	1.79
GALLIPRO® Fit	3209 a	1.48 b	2.57
OA & EO	3168 b	1.51 a	2.00
CV%, P value	3.25, 0.001	3.15, 0.001	5.55, 0.552

Means followed by different letters differ from each other by Chi square test

Daily feeding of **GALLIPRO® Fit** and mix of one organic acid and essential oils (OA&EO) resulted in significant improvements in final body weight (CV%= 5.55, $P<0.05$), corrected on mortality) as compared to the NC and PC-AGP groups.

However, **GALLIPRO® Fit** demonstrated significantly higher body weight (CV%=5.54, $P<0.05$) in comparison to organic acid and essentials oils (OA&EO). Feed conversion ratio of birds fed with **GALLIPRO® Fit** was significantly higher (CV%=3.15, $P<0.05$, corrected on Mortality) compared to the 3 other treatments NC, OA&EO and PC-AGP groups. No significant differences was observed in mortality rate (%).

WELFARE IMPROVEMENT IS A NEW ERA FOR CHOOSING PROBIOTICS

Based on demonstrated modes of action (protective biofilm creation at the top of the villi, competition for space and nutrients, local production of bacteriocins, immune and microbiome modulation), **GALLIPRO® Fit** significantly and positively impacts the behavior and welfare of broilers.

Combining the outcomes of the Latency to Lie and Approximation Test with the production of serotonin leads to the conclusion that daily feeding of **GALLIPRO® Fit**, and its actions in the gut and on the microbiome, result in better leg health status and calmer birds at harvest. With increased calmness and better leg health status, fewer injuries and better carcass quality would be expected.

These results must be an improvement in the welfare of the birds. All together, these results open a new era for choosing to use probiotics.

Daily feeding of **GALLIPRO® Fit** to commercial broilers was shown to be beneficial in terms of performance and animal welfare.

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Ibiara Correia de Lima Almeida Paz, Ianê Correia de Lima Almeida, Leonardo Thielode La Vega, Elisane Lenita Milbradt, Mariana Rodrigues Borges, Gustavo Henrique Coelho Chaves, Caio César dos Ouros, Marconi Italo Lourenço da Silva, Fabiana Ribeiro Caldara, Raphael Lucio Andreatti Filho, 2019. Productivity and well-being of broiler chickens supplemented with probiotic. *J. Appl. Poultry Res.*, 28: 930-942.

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