

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

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ANIMAL WELFARE RAISES QUESTIONS

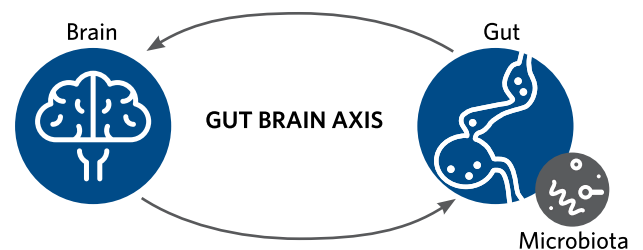
From day-of-hatch to harvest, broilers are reared in commercial production systems. This necessitates responsible animal husbandry regardless of the scale of production. Commercial broilers have been selected for rapid growth rate, increased carcass yield, and ability to thrive in modern production systems. Globally, there is an evolving effort to ensure that birds are reared with concern of their natural behavior. 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 incidence of footpad dermatitis, 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 improved performance and well-being of poultry via the gut-brain-microbiome axis.

PROBIOTICS PLAY A ROLE IN THE GUT-BRAIN AXIS

Daily feeding of an effective microbiota-impacting probiotic can improve the performance and health of poultry. More specifically, dietary probiotic supplementation can improve growth rate, feed efficiency, flock uniformity, mortality, and condemnations at slaughter. Previous investigations in humans have shown bi-directional interactions within the gut-brain-microbiome axis (Bested *et al.*, 2013; Carabotti *et al.*, 2015). Gut microbes communicate to the central nervous system through at least three parallel and interacting channels involving nervous, endocrine, and immune signaling mechanisms. In a study conducted by Hu *et al.* (2018), dietary supplementation of a probiotic, *Bacillus subtilis*, reduced aggressive behaviors in laying hens. Additionally, in humans in mice, supplementation with probiotics, and their action on the microbiome, led to a release of neuroendocrine factors that subsequently affected the relative abundance of

serotonin (the hormone of well-being) and tryptophan (Ezenwa *et al.*, 2012; Foster *et al.*, 2013). Tryptophan, an essential amino acid, is a precursor in the bio-synthesis for serotonin and melatonin.

Figure 1: Gut brain axis synthetic representation: Enterochromaffin cells in the intestine produce more than 90% of the body's serotonin.



Other than improvements on productivity, the daily feeding of an effective probiotic is assumed to positively influence the welfare of poultry (Yano *et al.*, 2015; Almeida Paz *et al.*, 2019). The cellular mechanisms underlying behavioral changes that impact welfare in avian species may be similar to those described in mice and humans.

BEHAVIOR VARIABLES ARE GOOD INDICATORS

A broiler experiment was conducted to determine the effects of **GALLIPRO® Fit**, a commercial triple-strain *Bacillus*-based probiotic that were initially selected for their capacity to inhibit *Salmonella spp.*, *E. coli*, *Clostridium spp.*, and others key pathogens that plague the poultry industry.

LATENCY TO LIE TEST

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 broiler reactivity. An assessor enters the barn, crouched down, and waited two minutes. After this period, the assessor reached out and tried to touch the broilers, it was counted how many broilers could be touched by the assessor in three minutes.

GAIT SCORE

Measure of welfare assurance, it is a non-invasive method of field assessment of walking ability of commercial broilers and is significantly correlated 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).



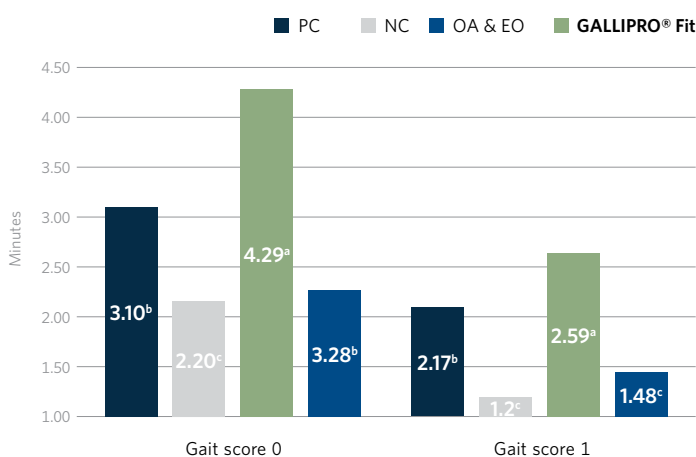
Four treatments were tested with eight replicate groups, each containing 400 day-of-hatch Aviagen AP95 male broilers.

- Positive Control (PC, Halquinol),
- Negative Control (NC, no supplement),
- GALLIPRO® Fit (500 g/T)
- Organic Acid & Essential Oil (OA&EO, 300 g/T).

Halquinol, GALLIPRO® Fit and a mix of Organic Acid and Essential Oils combination were included in all diets throughout the entire duration of the trial. Zootechnical performance data, such as feed intake, body weight, feed conversion ratio and mortality (%), were collected on a weekly basis.

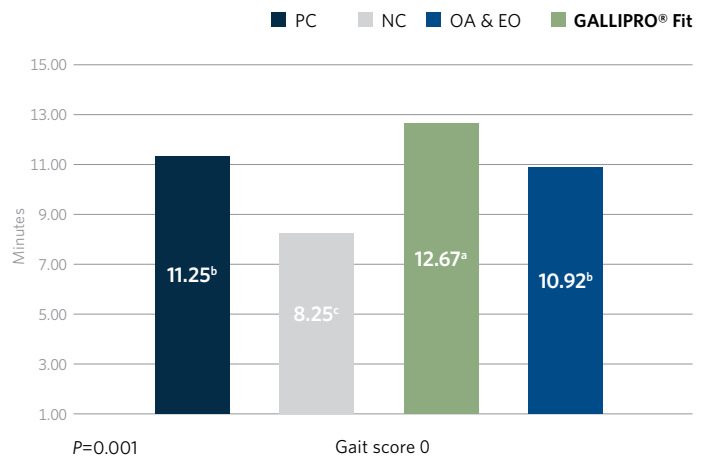
Effects of treatment on behavior were examined using a latency-to-lie test and approximation tests at 42 days of age. Plasma concentrations of serotonin were measured as indicators of stress at 40 days of age, sampling 8% of broilers from each treatment.

Figure 1: Latency-to-lie x Gait score results by treatment group at 42 days of age.



Birds in the GALLIPRO® Fit were more comfortable compared with other treatments, as evidenced by longer standing times (Figure 1).

Figure 2: Approximation test results by treatment group at 42 days of age.



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

Birds in the GALLIPRO® Fit group were less skittish compared with other treatments (Figure 2), indicative of their relative state of calmness. 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 by enterochromaffin cells. 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.

Table 1: Blood circulating concentrations of serotonin (5-HT) in broilers at 40 days of age.

TREATMENT	SEROTONIN (5-HT) µg/mL
(PC)	91 ^c
(NC)	100 ^c
GALLIPRO® Fit	402 ^a
OA&EO	316 ^b
P-value	0.001

PROBIOTICS IMPROVE ZOOTECHNICAL PERFORMANCE

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

TREATMENT	BODY WEIGHT (g)	FEED CONVERSION	MORTALITY (%)	EPEF
PC	3.103 ^c	1.52 ^a	1.71	478 ^c
NC	3.086 ^c	1.51 ^a	1.79	478 ^c
GALLIPRO® Fit	3.209 ^a	1.48 ^b	2.57	503 ^a
OA & EO	3.168 ^b	1.51 ^a	2.00	489 ^b

All growth performance variables are corrected for mortality. EPEF, European Production Efficiency Factor

Daily feeding of **GALLIPRO® Fit** resulted in significant improvements ($P < 0.05$) in final body weight, feed conversion, and EPEF compared with NC, PC, and OA&EO.

No significant differences was obtained on mortality rate (%).

IMPLICATIONS OF PROBIOTICS ON ANIMAL WELFARE

There are circular communication loops between the brain, gut, and gut microbiome that can be demonstrably impacted by daily feeding of effective probiotics. When fed diets containing GALLIPRO FIT, birds are significantly more comfortable (stand longer) and are significantly less skittish (easier to touch). Moreover, the hormone of well-being, serotonin was significantly higher compared with those in other treatment groups.

Daily feeding of an effective microbiota-impacting probiotic improves the performance, and more importantly from the perspective of animal welfare and sustainability, the well-being of broilers via the gut-brain-microbiome axis.

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OIE, <https://www.oie.int/en/animal-welfare/animal-welfare-at-a-glance>

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