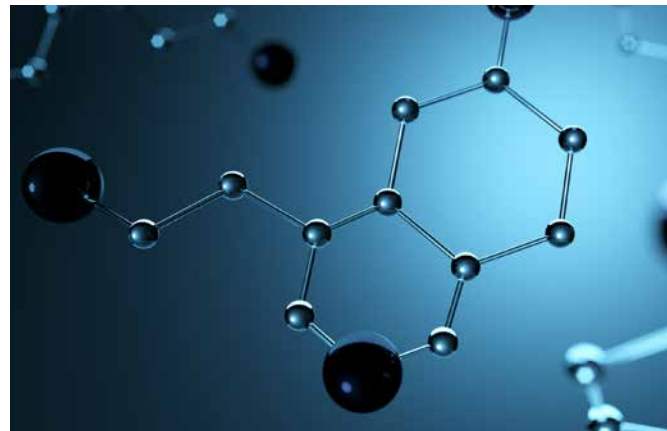


Gut-Brain-Microbiome Axis: Promoting Health and Well-Being with probiotics and the Role of the Gut-Brain-Microbiome Axis in broiler chickens



BACTERIA AND THEIR HOSTS

The earliest life form to appear on the land masses of Earth may have been bacteria approximately 3.22 billion years ago¹. It only makes sense that all later-arriving mammals and birds evolved in the presence of those innumerable living organisms. The tens of trillions of bacteria that we carry with us, on our skin, in our airways, in our mouths, and throughout our digestive systems are referred to as our microbiome. Each animal's, bird's, and human's microbiome is unique and the composition and actions of that unique set of organisms has a significant, demonstrable impact on the health and well-being of their host.

As the majority of the microbiome lives in the digestive system of their host, it's no surprise that we tend to speak about the role of those organisms in supporting the normal functions of the gut, i.e., digestion, absorption, barrier, and immune functions. However, there is now irrefutable evidence that the microbiome can effectively communicate with the central nervous system of its host through at least three parallel and interacting channels involving nervous, endocrine, and immune signaling mechanisms². Likewise, via the autonomic nervous system, the brain can impact the community structure and function of the gut microbiome. This is accomplished by sending nervous messages to modulate regional gut motility, intestinal transit and secretion, gut permeability, and potentially through the luminal secretion of hormones². It is important to note that these circular communication loops between the brain, gut, and microbiome are essential for proper function of the individual and breakdown at any point can result in dysfunction, disease, and death.

THE ROLE OF SHORT-CHAIN FATTY ACIDS

When it comes to signaling from the microbiome to the brain, short-chain fatty acids (SCFAs) are the key³ (Figure 1). SCFAs are the main metabolites produced by the gut microbiome through the fermentation of dietary fibers and starches, the most abundant of which are acetate, propionate, and butyrate. Following their production, SCFAs are absorbed by cells that line the intestines via specialized cell-surface transporter molecules. Interaction of SCFAs with their receptors on enteroendocrine cells promotes indirect signaling to the brain via the systemic circulation or vagal pathways by inducing the secretion of gut neurotransmitters, such as glucagon-

like peptide 1 and peptide YY, as well as γ -aminobutyric acid, and serotonin (5-HT). SCFAs can cross the blood-brain barrier and influence the integrity of that essential barrier by upregulating the expression of tight junction proteins. Finally, in the central nervous system, SCFAs also influence neuroinflammation by affecting glial cell morphology and function as well as by modulating the levels of neurotrophic factors, increasing neurogenesis, contributing to the biosynthesis of serotonin, and improving neuronal homeostasis and function. Together, the interaction of SCFAs with these gut-brain pathways can directly or indirectly affect emotion, cognition, and pathophysiology of brain disorders³.

This document focuses on the impact of effective probiotics on the gut-brain-microbiome axis and draws links between the capacity to produce short-chain fatty acids in the gut and the health, performance, and mental well-being of the individual - in this case, a broiler chicken.

SCFAS AND PERFORMANCE - A CLUE

A study was conducted to determine the relationship between the compositional features of the microbiome and the physical performance of broiler chickens⁴. Eggs from similarly aged breeder flocks were incubated in one incubator, transferred to one hatcher, and when hatched, 218 male birds of the same genetics were selected. These birds were transported to and placed into a single pen (13.2 m²) into which extra feeders had been provided and raised conventionally. At 37 days of age, 211 birds remained. Each bird was weighed individually and the 25 lightest (small birds) and the 25 heaviest (big birds) were selected. The cecal tonsils from each bird were collected and DNA was extracted from their contents. The big birds were significantly heavier and less variable than the small birds (2,891 \pm 100 g versus 1,808 \pm 122 g, respectively). Analyses of the microbiomes revealed that the big birds were more even in their distribution of microorganisms and richer in species of bacteria compared to those of the small birds. Taken together, the set of microbiomes from the big birds were more similar one to another compared to those of the small birds. Higher individual richness and evenness and lower variation among the microbiomes of a group are associated with eubiosis and general good health. For our purposes here, the most interesting finding was the composition of the

¹ Martin Homann, Pierre P Sansjofre, Mark van Zuijen, Christoph Heubeck, Jian Gong, et al. Microbial life and biogeochemical cycling on land 3,220 million years ago. *Nature Geoscience*, 2018, 11 (9), pp.665 - 671. f10.1038/s41561-018-0190-9f. f10.1038/s41561-018-0190-9f

² Martin, C.R., Osadchiy, V., Kalani, A. and Mayer, E.A., 2018. The brain-gut-microbiome axis. *Cellular and molecular gastroenterology and hepatology*, 6(2), pp.133-148.

³ Parladore SY, Andressa B, Luiz FR. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication *Frontiers in Endocrinology* Volume 11 2020 URL=<https://www.frontiersin.org/articles/10.3389/fendo.2020.00025>

microbiomes of the big birds. There were 31 species of bacteria that were more abundant in the big versus the small birds and each of them is associated with the production of SCFAs⁴. It is tempting for us to conclude that the big birds had the healthiest, best-functioning digestive systems, as evidenced by their size, and that they felt the best because of the capacity of their gut microbiomes to produce SCFAs that positively impacted cognition and behaviors.

PROBIOTICS, PERFORMANCE, AND BEHAVIOR – SCFAS AND SEROTONIN

In a study conducted at São Paulo State University, 1,600 male broiler chicks were separated into four groups: Negative Control (NC, standard diet), Positive Control (PC, standard diet + AGP Halquinol), Probiotics (**GALLIPRO® FIT** 500 g/ton; 1.6x10⁶ cfu/g feed), and Organic Acid + Essential Oils (OA+EO). These birds were raised in a conventional manner until 42 days of age. Many variables of performance, welfare, blood chemistry, gene expression, and gut morphology were assessed.

In brief, the birds in the Probiotics group were heavier, with better feed conversion and European Performance Efficiency Factor, compared to the birds of the other three groups. Regardless of gait score (normal or slight-affected), the birds in the Probiotics group showed significantly better comfort and significantly less skittishness compared to birds of the other three groups.

Concentrations of serotonin, the neurotransmitter of well-being, was four times higher in the Probiotics group compared to that in birds of the NC group. Concentrations of corticosterone, the molecule of stress, in the Probiotics group was half the concentration of that in the birds of the NC group.

The expression of genes for three proteins involved in the production and transport of serotonin was assessed; the values observed in the PC group were set to 100% and each of the other groups were compared to that standard. The expression of the gene for the Serotonin Transporter Protein, which transports serotonin from synapses to presynaptic neurons, was 135% of the standard for the Probiotics group compared to 70% and 38% for the NC and OA+EO groups, respectively. The expression of the gene for the Solute Carrier Family 6 Member 4 protein, that codes for an integral membrane protein involved in the synaptic movement of serotonin, was 345% of standard for the Probiotics group compared to 50% and 200% for the NC and OA+EO groups, respectively. Lastly, the expression of the gene for the Tryptophan Hydroxylase 1 enzyme, which is responsible for the first-rate limiting step in the synthesis of serotonin, was 1,700% of standard for the Probiotic group compared to 50% and 395% for the NC and OA+EO groups, respectively.

For all measures of gut morphology, response values were highest in the Probiotics group compared to birds of the other three groups. In summary, 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. We believe that this myriad of advantages enjoyed by the birds that were fed daily with probiotics is a direct consequence of the development of robust microbiomes enriched with organisms that produce SCFAs – the positive cascade starts there.

GOING FORWARD

We have been saying for some time now that the greater the percentage of healthy individuals in any production system, the greater the likelihood of a positive outcome. We've shown that the daily feeding of **GALLIPRO® FIT** is associated with a shift towards positive with a notable reduction in variation in the variables of interest in that type of production system. We've associated probiotic-influenced "health" with a robust microbiome and a demonstrable ability to utilize nutrients from feed and to defend oneself against physiological challenges. Now, with our current appreciation for the role of SCFAs and their capacity to positively impact a huge array of functions, including cognition, behaviors, and pathophysiology of the brain, we must expand our set of metrics to include those variables. If possible the variables that establish the correct shift in the microbiome, towards SCFA producers, that demonstrate a positive shift in the hormonal milieu of the recipients, and a positive change in behaviors.

Figure 1. From Parladore SY, Andressa B, Luiz FR The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication *Frontiers in Endocrinology* Volume 11 2020

