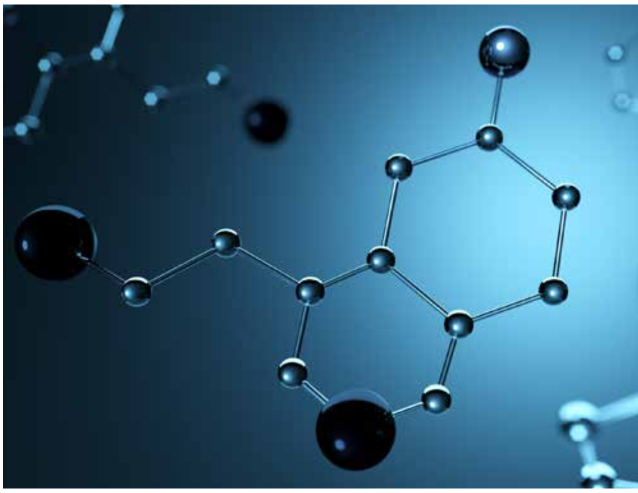
Gut-Brain-Microbiome Axis:
Linking microbiome and animal behavior



THE MICROBIOME IS CRITICAL FOR ANIMAL HEALTH, WELFARE, AND PRODUCTIVITY

As we begin to realize the immense importance of a healthy, robust microbiome on animal health, welfare, and production efficiency of chickens and other food-producing animals, we are finding new ways to effectively move the microbiome from an unhealthy, unstable state to a healthy state. We have preliminary evidence of the impact that effective probiotics can have on the gut-brain axis and the behavior of broiler and layer chickens subject to chronic stress, including heat stress. We are eager to continue discovering the full potential of probiotics on the gut-brain axis of chickens and other food-producing animals.

ANIMAL WELFARE ON THE AGENDA

The interconnected trio of nutrition, intestinal health and animal welfare plays a vital role in efficient production of poultry meat and eggs. With a high degree of awareness, the vast majority of commercial producers of poultry have implemented standard processes and practices to properly manage many important factors such as diet, stocking density, light regimen, air quality, water hygiene, and environmental ammonia, to name a few. However, despite these good efforts, stress and its many consequences are still a major issue in modern poultry production systems. Stress negatively affects the health, welfare, and performance of birds, which can lead to significant economic losses (Nawaz *et al.*, 2021). Like all animals do when exposed to stressful conditions, chickens react on a multitude of levels, including, but not limited to social, behavioral, and physiological responses. Some of the changes in social interactions and individual behaviors are an increase in pecking, feather pulling, and fighting and decreases in intake of feed (de Haas, E. N. & van der Eijk, J. A. J. 2018). These aberrant changes can result in a drastic deterioration of welfare and significantly reduced physical performance. In addition, stress can negatively impact the quality and robustness of the gut microbiome, can compromise intestinal integrity, and can reduce an individual's capacity to mount immune responses (de Haas, E. N. & van der Eijk, J. A. J. 2018). All of these negative impacts can lead to the development of a variety of performance-limiting pathologies. On the other hand, recent advances in microbiological science have shown that proper development and maintenance of a healthy, robust microbiome, particularly early in life, may eliminate the impacts of stress in growing chickens, thereby preventing the breakdowns in welfare and performance (van der Eijk *et al.*, 2020, Ramírez *et al.*, 2020).

GUT-BRAIN AXIS AND THE MICROBIOME

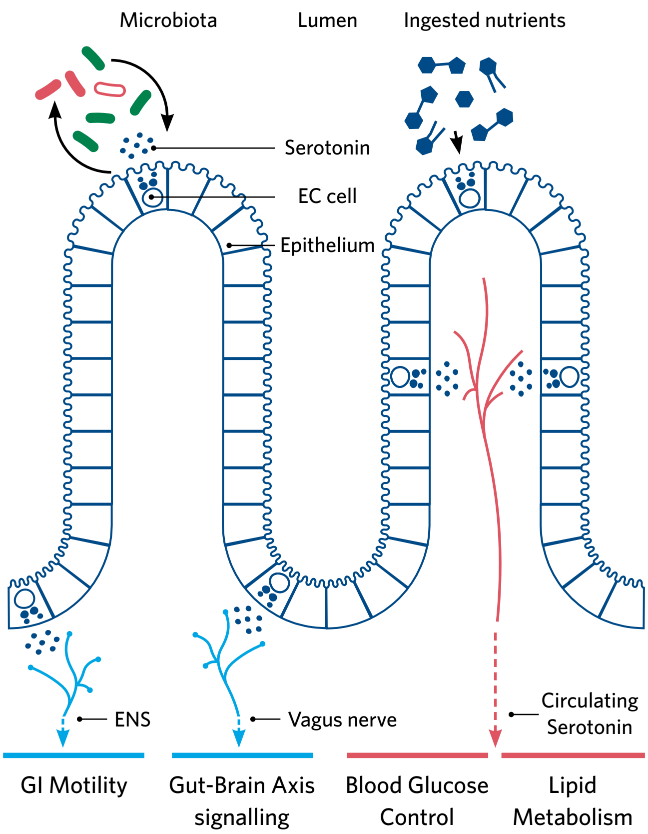
The gut-brain axis is the bidirectional signaling system that exists between the gut and the brain of animals and humans. A link between the gut and our brain seems intuitive as we have all felt the rumbling in our stomachs associated with the awareness and feeling of hunger. However, the existence of a gut-brain axis was first demonstrated in the early 1900's with the Nobel-Prize-winning research of Ivan Pavlov. In his famous dog experiments, Pavlov would ring a bell just prior to feeding the dogs. After a while, the dogs would salivate and release gastric acid whenever they heard the bell ring. With 120 years of research under our belts, we now know that the gut-brain axis is a highly complex system that uses a multitude of physical and chemical pathways to communicate. Studies from a couple of decades ago taught us that effects on the brain, like chronic stress and depression, can negatively affect the gut, can alter the gut microbiome, and are strongly associated with enteric diseases such as functional gastrointestinal disorders and inflammatory bowel disease in humans (Mayer, 2000; Mawdsley et al., 2005). Other studies taught us that effects on the gut, such as a healthy and robust microbiome, can affect the brain. An early indication of this was accomplished using germ-free mice. Mice without a gut microbiome were significantly more negatively affected by stress than normal mice, which was reversible by giving probiotic bacteria to the germ-free mice (Sudo *et al.*, 2004). This clearly showed that the mice's ability to cope with stress was influenced by their microbiome. Since these early mouse studies, we have understood many aspects of the highly complex gut-brain axis across species, and many appear to be universal in all animals and people.

MANY PATHWAYS OF THE GUT-BRAIN AXIS ARE INFLUENCED BY THE MICROBIOME

The digestive tract has its own nervous system that extends from the esophagus to the anus or cloaca. This system is known as the enteric nervous system (ENS) and is one of the essential physical elements of the gut-brain axis. In concert with the physical pathways, there are also signaling pathways that are chemical in nature, including molecules like short-chain fatty acids (SCFAs), metabolites of tryptophan, and blood-borne sugars, like glucose. Each of these is derived from or impacted by the gut microbiome. SCFAs, such as acetate, butyrate, and propionate, are the most widely studied gut microbiome-derived substances and have been shown to regulate appetite, lessen stress, and even have anxiety dampening effects (O'Riordan *et al.*, 2022). Similarly, the microbiome's influence on the hosts' metabolism of tryptophan has been highlighted in recent papers (Jones, *et al.*, 2020). Tryptophan is an essential amino acid obtained from the diet and metabolized in the gut. A major metabolite of tryptophan is serotonin, which functions as both a neurotransmitter and a regulatory hormone. 95% of all serotonin in the body is produced by specialized enterochromaffin (EC) cells that are located throughout the gastrointestinal tract. Gut bacteria interact with EC cells and, through that interaction, can influence important metabolic processes of their host, such as blood glucose control and lipid metabolism (Jones *et al.*, 2020) (See Figure 1).

Figure 1. Gut microbiome interactions with host

The gut microbiome interacts with several parts of the gut-brain axis, including Enterochromaffin Cells (ECs) sensory and the enteric nervous system (ENS) which includes the vagus nerve. The release of serotonin from EC cells ignites a bi-directional relationship with gut microbiota, whereby serotonin increases the colonization of specific bacteria, and specific bacteria increase the number of EC cells. Serotonin produced in the gut affects the brain via the ENS. Modified from Jones, *et al.*, 2020

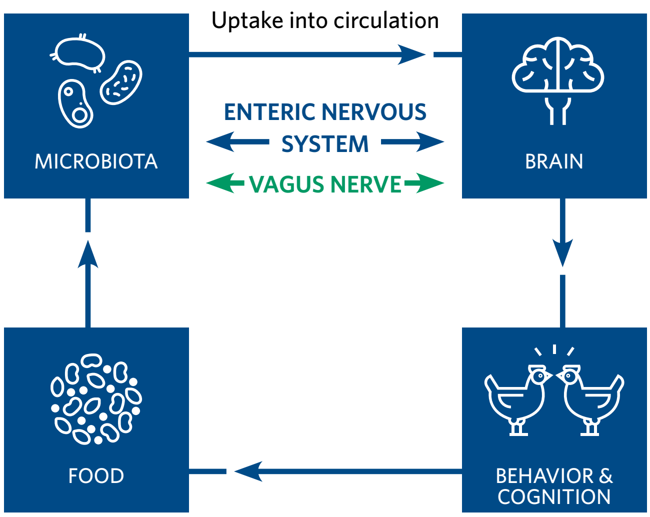


While peripherally produced serotonin is unable to cross the blood-brain barrier, recent studies suggest that the vagus nerve that runs from the brain to several organs, including the gut, plays a major gut-to-brain signaling role in the serotonergic system. Serotonin release also stimulates intestinal motility and augments gastric emptying. On release, serotonin can also enter the circulation and exert effects on glucose homeostasis and lipid metabolism. This was partly discovered in experiments using rodents in which specific gut bacteria were able to increase the concentration of serotonin in the brain, but only when the vagus nerve was intact (Bharwani *et al.*, 2020).

THE IMPACT OF PROBIOTICS ON ANIMAL WELFARE AND HEALTH

The complexity of the gut-brain axis makes it somewhat complicated to study and difficult to fully comprehend. Happily, we continue to advance our understanding of this highly interconnected system (See Figure 2). We are also starting to better understand exactly how gut microorganisms exert their effects on the gut-brain axis.

Figure 2. A hypothetical gut-brain axis in chickens. Modified from Villageliu *et al.*, 2017



Heat stress in poultry has been shown to negatively affect body weight gain and feed efficiency and can lead to enteric diseases, such as coccidiosis and necrotic enteritis (Adhikari *et al.*, 2020; Liu *et al.*, 2020). With stress, birds show decreased concentrations of neurotransmitters such as serotonin in their brains (Calefi *et al.*, 2019). Luckily the gut-brain axis is two-directional and several studies in poultry have shown a positive effect of a healthy gut microbiome on behavior, health, and efficiency of production (Diaz Carrasco *et al.*, 2019). In chickens, transplanting the cecal microbiome from healthy birds, including specific bacteria, resulted in significantly increased bodyweights among the recipients. It was reported in that study that an improved stress response likely played a role in the gains in performance. Similarly, our studies have shown that probiotic products from Chr. Hansen have a positive effect on behavior, health, and production efficiency, likely via the gut-brain axis.

Broilers fed daily with **GALLIPRO® FIT** probiotic had better performance and better social behaviors associated with significantly higher circulating concentrations of serotonin than birds in the control group (Chr. Hansen Trial 80827). In another study, laying hens fed daily with **GALLIPRO® MS** probiotic also had better production efficiency and behavioral patterns, including less feather picking and fewer cracked eggs than those in the control group (Chr. Hansen Trial 80679).

THE FUTURE INCLUDES TAKING CONTROL OF THE GUT-BRAIN AXIS

Today, we are beginning to realize the immense importance of a healthy, robust microbiome on animal health, welfare, and production efficiency. We are finding new ways to effectively move the microbiome from an unhealthy, unstable state to a healthy state. We have preliminary evidence of the impact that effective probiotics can have on the gut-brain axis and the behavior of broiler and layer chickens subject to chronic stress including heat stress. We are eager to continue discovering the full potential of probiotics on gut-brain axis of chickens and other food-producing animals.

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