

Effect of dietary supplementation of probiotic, *Bacillus subtilis*, on performance and immune parameters in the brain of broiler chickens under heat stress

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The intestinal microbiota affect health and behavior via the gut-brain axis in humans and rodents; however, few studies have been conducted in chickens. The objective of this study was to evaluate if dietary supplementation of *Bacillus subtilis* can reduce the heat stress (HS) response via ameliorating brain inflammation. Based on body weight (BW), 240 1-day-old broiler chicks were randomly assigned to 48 pens with 4 treatments (n=12): TN (thermoneutral)-RD (a regular diet), TN-PD (the regular diet mixed with probiotic), HS (heat stress)-RD, and HS-PD. Probiotic (Sporulin® at 10⁶ CFU/g feed) was administered from d 1; and heat stress, 32 °C for 10 h daily, was initiated on d 15. Feed intake and BW were measured at wk 2 and 6. Blood and hippocampus samples were collected at wk 6. The data showed that final BW, average daily weight gain, and feed conversion efficiency were improved in PD groups as compared to RD groups regardless of ambient temperature ($P < 0.01$). Hippocampal interleukin (IL)-10 mRNA levels were affected by treatments in the order of HS-PD > HS-RD > TN-PD > TN-RD ($P < 0.05$). Hippocampal IL-6 and Toll-like receptor 4 (TLR4) mRNA levels were higher in HS groups compared to TN groups ($P < 0.0001$). Within HS groups, HS-PD had lower mRNA levels of IL-6 ($P = 0.01$) and TLR4 ($P = 0.01$) than HS-RD. Compared with RD groups, hippocampal IL-8 mRNA levels were lower in PD groups regardless of temperature ($P < 0.05$). Within PD groups, HS-PD had higher levels of IL-8 mRNA ($P < 0.01$) than TN-PD. Heterophil to lymphocyte ratio was significantly affected by treatments in the order of HS-RD > HS-PD > TN-

RD > TN-PD ($P < 0.01$). These results indicate that dietary supplementation of the probiotic, Sporulin (*Bacillus subtilis*), is able to reduce broiler heat stress response via regulating brain anti-inflammatory reactions.

Key words: probiotic, heat stress, gut-brain axis, inflammation, broiler chicken