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**Effective alleviation of heat stress by using probiotics in laying chickens**

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**Abstract:**

Heat stress in animals can lead to immunity reduction and morbidity increase, prompting the use of therapeutic and prophylactic antibiotics. Particularly for laying hens with a long rearing periods, it is urgent to develop alternatives to reduce antibiotic use and alleviate the adverse effects of heat stress, such as reduced production performance and increased mortality. The aim of this study was to examine the efficacy of probiotics in alleviating heat stress and improving the health of chickens. Sixty 40-week-old chickens were randomly divided into a basal diet group and a probiotic group supplemented with probiotics. The chickens were exposed to 35°C for 6 hours per day for 2 weeks, and the laying rate and survival rate were monitored. Fecal samples were collected during the final phase of the study for the identification of bacterial taxonomic analysis. The results showed that after two weeks of heat stress exposure, the chickens supplemented with probiotics exhibited significantly higher survival rates by 7%, and increased egg production rates by 30% compared to those fed a basal diet. For taxonomy annotation analysis of fecal microbiota at the phylum level, the probiotic group had a similar *Firmicutes* composition compared to the basal diet group. Furthermore, the composition of *Proteobacteria* in the probiotic group was reduced to that of the basal diet group. The ratio of *Firmicutes* to *Bacteroidetes* and Shannon diversity were slightly increased in the probiotic group compared to the basal diet group. In summary, probiotic supplementation of chickens under heat stress has been shown to have beneficial effects. This strategy shows potential as a natural and effective method to support poultry health and productivity under challenging environmental conditions.

**Keywords:** Chicken, Fecal Microbiota, Heat Stress, Probiotic