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## **DIETARY SUPPLEMENTATION OF PROBIOTICS IN POULTRY EXPOSED TO HEAT STRESS – A REVIEW**

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### **Abstract**

Heat-related stress has become a serious problem in poultry industry along with the global temperatures rise. Heat stress causes detrimental effects on physiology, immunology and microbiology resulting in abnormalities and impaired performances of birds. Several nutritional strategies have been conducted to counteract the detrimental effects of heat stress in poultry, including dietary supplementation of probiotics. This strategy has been proposed to ameliorate the intestinal ecosystem, physiological conditions and immune system, leading to the improved performance and health of birds subjected to heat stress. This review presents the potential benefits of probiotics against heat stress in poultry from the viewpoint of intestinal microbial ecology, morphology and structure, physiological conditions, immune system and production performances. The possible mechanisms through which probiotics may give beneficial impacts on heat-stressed birds are also discussed along with the data reporting the possible drawbacks of using probiotics in heat-stressed poultry.

**Key words:** poultry, heat stress, probiotics, performance, health

Poultry industry is an important subsector of livestock production and plays an important role in economic growth. In some poultry-producing countries, high environmental temperature is one of the most important inhibiting factors to poultry production. Heat stress is known to impair the performance, productivity and health of the birds by reducing feed intake, decreasing nutrient utilization, disrupting intestinal structure and compromising the immune systems (Sahin et al., 2009). Besides improving environmental management, nutritional strategies have been developed to partially alleviate the negative impacts of heat stress in birds (Lara and Rostagno, 2013), including feeding diet with increased energy density, addition of salts, antioxidant vitamins and minerals in heat-stressed poultry diets (Sahin et al., 2009; Das et al., 2011). Recently, dietary supplementation of probiotics, prebiotics and synbiotics has also been implemented in poultry to counteract the negative effects of

heat stress (Lara and Rostagno, 2013). This review aimed at elucidating the dietary supplementation of probiotics in ameliorating the deleterious effects of heat stress in poultry. To prepare the review, we conducted a literature search with focus on the potential benefits and drawbacks of probiotics on heat-stressed poultry (broiler, hen, duck and quail) using the following criteria: (1) peer-reviewed journal articles in English were included; (2) chapters in an edited book were selectively included; (3) studies on humans and rodents were selectively included to verify and/or support the data on poultry. The key words used during literature search included probiotics, heat stress, chicken, microbe, immune system, physiology, growth. For collection of the related articles, we employed several scientific portals including Elsevier ScienceDirect, EBSCO E-journal, Proquest Research Library, Cambridge University Press E-Journal and Springerlink E-Journal. We also used Google Scholar when we did not find any intended articles through the above mentioned scientific portals.

### **Heat stress in poultry**

Stress is a term describing the responses of the body to abnormal conditions that potentially interrupt homeostasis or normal physiological equilibrium (Sahin et al., 2009; Lara and Rostagno, 2013). In poultry, stress is characterized by the changes in behaviour, biochemistry and physiology that are all addressed to re-establish homeostasis (Sahin et al., 2009). At molecular level, stress may change the expression of genes like heat-shock protein (HSP) including HSP 40 and HSP 90 that are involved in self-regulation and compensation to maintain homeostasis (Sun et al., 2015). High ambient temperature is one of the important factors inducing stress in birds. Due to the global warming, high temperature has recently become one of the most important stressors affecting poultry industry worldwide (Lara and Rostagno, 2013). Hence, effort has to be undertaken to cope with the detrimental effects of heat stress on poultry.

### **Impacts of heat stress on intestinal morphology and microbiology**

Heat stress has been shown to impair the intestinal morphology and barrier integrity of chickens (Song et al., 2013, 2014), leading to impaired digestive and absorptive capacity and increased permeability to luminal antigens and toxins. A number of factors have been attributed to the impaired intestinal morphology and integrity of birds exposed to heat stress, including the diversion of systemic blood flows from internal organs to peripheral circulation (in order to dissipate heat), which may cause ischemia and hypoxia in the intestinal epithelial cells (Al-Fataftah and Abdelqader, 2014; Song et al., 2014). Corticosterone has also been inferred to be responsible for the damage of intestinal mucosa in birds exposed to high temperature (Quinteiro-Filho et al., 2010), as this hormone can delay proliferation of the intestinal epithelial cells that in turn lowers intestinal villus height and crypt depth (Hu and Guo, 2008). Moreover, corticosterone may induce the production of proinflammatory agents (Yang et al., 2015), that can act in the intestinal epithelium's tight junctions of birds leading to the increased mucosal permeability to pathogenic antigens (Song et al., 2013, 2014). Heat stress has been implied in the alteration of normal microbiota composition in the intestine of birds (Burkholder et al., 2008). According to Yu et

al. (2012), this unbalanced intestinal microbiota may distort the regulation of intestinal epithelial cell turnover, epithelial restitution and reorganization of tight junctions. Such distortion may in turn provoke the changes in intestinal morphology and integrity.

### **Impacts of heat stress on physiology and immune system**

It has been reported that heat stress can adversely change the metabolic status and physiological equilibrium in birds (Donkoh, 1989; Rhoads et al., 2013), leading to health problems and high mortality rate (Lara and Rostagno, 2013). Indeed, heat stress decreased blood Na, K and partial pressure of carbon dioxide ( $p\text{CO}_2$ ), which may disturb acid-base balance and cause respiratory alkalosis, respectively (Borges et al., 2004). Moreover, heat stress increased serum concentrations of corticosterone (Sohail et al., 2012), glucose, triglycerides, total cholesterol and low-density lipoprotein (LDL)-cholesterol (Habibian et al., 2014), while decreasing the concentration of haemoglobin (Borges et al., 2004), triiodothyronine ( $T_3$ ) (Tollba and Sabry, 2004), plasma protein (Donkoh, 1989; Tollba and Sabry, 2004), uric acid (Sun et al., 2015) and high-density lipoprotein (HDL)-cholesterol of broilers (Habibian et al., 2014). Unlike the above mentioned studies, Tollba and Sabry (2004) reported that heat stress decreased plasma glucose, while Sun et al. (2015) did not find any significant difference in plasma corticosterone between heat-stressed and control chickens. The extent and duration of heat exposure may be the reason for these discrepancies, as Zulkifli et al. (2006) reported that blood glucose levels of birds declined with long-term heat exposure.

Heat stress has been shown to compromise immune functions of poultry and therefore increase the susceptibility of birds to infections and increase the mortality and morbidity (Habibian et al., 2014; Akhavan-Salamat and Ghasemi, 2015; Hosseini-Vashan et al., 2015). The increased and decreased production of corticosterone (Sohail et al., 2012; Deng et al., 2012) and antioxidant enzymes (Sahin et al., 2009; Hosseini-Vashan et al., 2015), respectively, have been attributed to the compromised immune functions in birds exposed to heat stress. The increase in corticosterone level induced lymphoid organ involution (Quinteiro-Filho et al., 2010; Yang et al., 2015), changed the characteristics of heterophil and lymphocyte (Shini et al., 2008; Akhavan-Salamat and Ghasemi, 2015) and affected the levels of tumor necrosis factor (TNF) alpha, interleukin (IL)-2 and immunoglobulin (Ig) G (Yang et al., 2015). The decreased level of antioxidant enzymes may be associated with the insufficient protection of immune cells from the oxidative stress which increase during heat stress (Sahin et al., 2009).

### **Impacts of heat stress on performance**

The deleterious effects of heat stress on the growth performance of broilers have been reported (Song et al., 2013; Al-Fataftah and Abdelqader, 2014; Song et al., 2014; Akhavan-Salamat and Ghasemi, 2015). In laying hens, heat stress was also found to decrease egg production (Deng et al., 2012). The impaired performances of poultry subjected to heat stress have been associated with a number of factors including poor appetite and reduced feed intake (as a mechanism to decrease heat increment; Sohail et al., 2013), impaired digestion (due to damage of intestinal mor-

phology and lowered digestive enzyme activity; Song et al., 2013, 2014; Chen et al., 2014) and metabolism (due to lowered activity of thyroid hormones; Tollba and Sabry, 2004; Sohail et al., 2010), altered endocrine status (increased corticosterone hormone; Deng et al., 2012; Sohail et al., 2012), metabolic shifts at the systemic and cellular levels and changes in body composition (Rhoads et al., 2013).

### **Probiotics and heat stress**

As part of the nutritional strategies, inclusion of feed additives in the diet has been conducted for ameliorating the negative effects of heat stress in poultry (Lara and Rostagno, 2013). Among feed additives, probiotics have gained more attention from poultry nutritionists as this additive is reported capable of improving the physiological conditions, intestinal morphology and structure, immune system and thus performance and well being of heat-stressed poultry (Al-Fataftah and Abdelqader, 2014; Jahromi et al., 2015). Apart from these beneficial effects, some studies reported no effect of probiotics on heat-stressed poultry (Sandikci et al., 2004; Sohail et al., 2013, 2015). This will be the subject of discussion in the following section of this review paper.

### **Probiotics and intestinal microbial ecology of heat-stressed poultry**

A number of studies have reported the potential benefits of probiotics on the intestinal microbial diversity and population in poultry subjected to high ambient temperature (Table 1). The mechanisms through which probiotics elicit beneficial impacts and/or re-establish the balanced intestinal microbial diversity and populations in birds have been elucidated elsewhere (Sugiharto, 2016). In contrast to the above mentioned studies, Sohail et al. (2013) reported no effect of probiotic mixture (*L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus*, *E. faecium*, *A. oryzae* and *C. pintolopesii*) on the populations of *Clostridium perfringens*, total coliforms and *Escherichia coli* in the jejunum and cecum of heat-stressed broilers. Likewise, Sohail et al. (2015) demonstrated that probiotic mixture (*L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus* and *E. faecium*) had no positive effect on the abundance of probiotic bacteria in the cecum and trachea of heat-stressed broiler. The different type and dose of probiotic microorganisms used as well as the different part of gastrointestinal organs observed may explain the above discrepancy.

### **Probiotics and intestinal morphology of heat-stressed poultry**

Several studies have revealed the potential benefits of probiotics in improving the intestinal morphology and integrity of birds subjected to heat stress (Table 1). Probiotics may reverse the impaired villus-crypt structure of heat-stressed birds, for example, by controlling the corticosterone level (Sohail et al., 2012; Lei et al., 2013) and the excessive release of proinflammatory agents that can cause intestinal tissue injuries and increase the permeability of the intestine (Deng et al., 2012). The presence of mucus layer is vital for maintenance of intestinal integrity of birds. The mucus may cover the intestinal absorptive surface and act as a barrier against bacterial invasion. A study in quails showed that heat stress decreased the number of mucus-

producing goblet cells located in the ileal villi (Sandikci et al., 2004). Conversely, probiotic *B. licheniformis* was able to maintain (comparable to those in birds reared under thermoneutral) goblet cell counts in the ileum and cecum of heat-stressed hens in the study of Deng et al. (2012). Likewise, *Lactobacillus*-based probiotics enhanced goblet cell counts in the duodenum and jejunum of heat-stressed broilers (Ashraf et al., 2013). One possible mechanism by which probiotics enhanced goblet cell counts was that probiotics may regulate mucin mRNA expression and accelerate the differentiation of goblet cells (Smirnov et al., 2005). Different from the latter studies, Sandikci et al. (2004) reported that probiotic *S. cerevisiae* had no impact on the goblet cell counts along the small intestinal segments of quails. These discrepancies were perhaps due to the different type of probiotic, animals or intestinal segments used in the experiments.

Table 1. Examples of probiotic treatments to improve intestinal microbiology and morphology of heat-stressed poultry

Strains or types of probiotics	Biological activities	References
1	2	3
Probiotic mixture ( <i>L. pentosus</i> ITA23 and <i>L. acidophilus</i> ITA44)	Increased the population of bifidobacteria, <i>Lactobacillus</i> and <i>Enterococcus</i> in the intestine of heat-stressed broilers. Increased antioxidant capacity of the liver in birds at high ambient temperature.	Jahromi et al., 2015
Probiotic <i>B. subtilis</i>	Enhanced the colonization of beneficial intestinal bacteria ( <i>Lactobacillus</i> and <i>Bifidobacterium</i> ). Restored the impaired villus-crypt structure.	Al-Fataftah and Abdelqader, 2014
Probiotic mixture ( <i>B. licheniformis</i> , <i>B. subtilis</i> and <i>L. plantarum</i> )	Increased the viable counts of small intestinal <i>Lactobacillus</i> and <i>Bifidobacterium</i> , and decreased coliforms in heat-stressed broilers. Increased jejunal villus height and ameliorated intestinal barrier function.	Song et al., 2014
<i>Lactobacillus</i> -based probiotics ( <i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. bulgaricus</i> , <i>L. rhamnosus</i> , <i>B. bifidum</i> , <i>S. thermophilus</i> , <i>E. faecium</i> , <i>A. oryzae</i> and <i>C. pintolopesii</i> )	Reversed the reduced villus height, crypt depth and surface area in duodenum and ileum of broilers due to heat stress. Maintained the activity of goblet cells.	Ashraf et al., 2013
Probiotic mixture ( <i>L. acidophilus</i> , <i>L. casei</i> , <i>E. faecium</i> and <i>B. bifidum</i> )	Increased and decreased populations of <i>Lactobacilli</i> spp. and coliforms, respectively, in the intestine of broilers under cyclic heat stress condition.	Landy and Kavyani, 2013
Probiotic <i>B. licheniformis</i>	Maintained normal villi in the heat-stressed hens. Prevented the intestinal epithelial damage and maintained gut integrity of heat-stressed hens by countering the excessive increase in mast cells (play an important role in the inflammatory process). Stabilized the pattern of mucin-secreting cells and thus improved protective barrier against harmful intraluminal substances.	Deng et al., 2012

Table 1 – contd.

1	2	3
Probiotic mixture ( <i>L. plantarum</i> , <i>L. delbrueckii</i> ssp. <i>Bulgaricus</i> , <i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>B. bifidum</i> and <i>S. salivarius</i> ssp.)	Improved intestinal microarchitecture (villus width and surface area) of heat-stressed broilers.	Sohail et al., 2012
Probiotic <i>S. cerevisiae</i>	Decreased the number <i>Salmonella</i> and <i>E. coli</i> in the intestine and excreta of heat-stressed broilers. Increased the villus height in the duodenal mucosa of heat-stressed broilers relative to the control.	Haldar et al., 2011
<i>Lactobacillus agilis</i> JCM 1048 and <i>Lactobacillus salivarius</i> subsp. <i>salicinius</i> JCM 1230	Enriched the diversity of <i>Lactobacillus</i> flora in chicken jejunum and cecum by increasing the abundance and prevalence of <i>Lactobacillus</i> spp. inhabiting the intestine.	Lan et al., 2004
<i>Lactobacillus</i> sp. and yeast culture	Decreased total counts of <i>E. coli</i> and <i>Salmonella pullorum</i> . Decreased the pH of intestine (duodenum, jejunum, ileum and cecum) in heat-stressed broilers.	Tollba and Sabry, 2004

In the study of Song et al. (2014), there was a tendency that administration of probiotics mixture (*B. licheniformis*, *B. subtilis* and *L. plantarum*) in the diet alleviated the increased intestinal mucosal permeability of heat-stressed chickens as indicated by the improvement on transepithelial electrical resistance (TER) and permeability of fluorescein isothiocyanate dextran 4 kDa (FD4) in jejunal mucosa. In addition to proinflammatory agents, reactive oxygen species (ROS) generated during heat stress can increase intestinal permeability in birds (Lara and Rostagno, 2013). Interestingly, Sohail et al. (2011) found that probiotic mixture (*L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus*, *E. faecium*, *A. oryzae* and *C. pintolopesii*) decreased serum total oxidants concentration in heat-stressed broilers, comparable with that in thermoneutral, and thus may reverse the increased intestinal permeability. Concomitant with this, Jahromi et al. (2015) reported that feeding a mixture of *L. pentosus* ITA23 and *L. acidophilus* ITA44 prevented the adverse effects of heat stress on the antioxidant capacity of liver. It was hypothesized that probiotics might produce some bioactive substances with free radical chelating ability (such as glutathione) that potentially prevents oxidative damage (Sohail et al., 2011). In line with this hypothesis, Lutgendorff et al. (2009) reported that probiotic pre-treatment prevented oxidative stress in Sprague-Dawley rats by increasing mucosal glutathione biosynthesis.

### Probiotics and physiological variables of heat-stressed poultry

Several works have indicated the potential benefits of probiotics in ameliorating the impaired physiological conditions in poultry due to heat stress. Zulkifli et al. (2006) reported that probiotic-enhanced water acidifier (Acid-Pak 4-Way™, combination of sorbic acid, citric acid, sodium chloride, sodium citrate, potassium

chloride, zinc sulfate, ferrous sulfate, magnesium sulfate, cellulase, *S. faecium* and *L. acidophilus*) helped in restoring serum Na and K levels of broilers following one day of heat exposure. However, it is not clear whether probiotic microorganisms or electrolytes (sodium chloride, sodium citrate, potassium chloride) which played dominant roles in restoring serum Na and K levels in heat-stressed broilers, as limited information is available to support our view. Probiotic treatment has been demonstrated to increase serum concentration of  $T_3$  (Tollba and Sabry, 2004) and  $T_4$  (Sohail et al., 2010) in broilers subjected to heat stress. Considering that thyroid hormones play important roles in stimulating the synthesis of many structural proteins, enzymes and hormones, the increased levels of thyroid hormones following probiotic supplementation are reasonably expected to improve digestion and metabolism in heat-stressed chickens (Aluwong et al., 2013). One possible factor that might be responsible for enhancing the concentrations of  $T_3$  and  $T_4$  in probiotics-supplemented heat-stressed birds was the reduced circulating level of corticosterone (Sohail et al., 2012), as elevated concentration of corticosterone may result in hypothyroid activity (Ganong, 2005).

It has been reported that probiotic (Protexin® Boost) treatment increased uric acid level in the serum of heat-stressed birds (Hasan et al., 2015), which may indicate the reduced protein digestibility in birds (Saki et al., 2005). This seems to be contradictory with the study of Tollba and Sabry (2004) reporting that probiotics (*Lactobacillus* sp. and yeast culture) increased total plasma protein (indicator of high protein digestibility). Considering that uric acid is an important antioxidative agent (de Oliveira and Burini, 2012), the increased level of uric acid may be a mechanism of probiotic in alleviating the oxidative damage following heat stress in birds. The increased level of total serum glucose in response to heat stress has been reported to be alleviated by provision of probiotic-enhanced water acidifier in the study of Zulkifli et al. (2006). In this case, probiotics might decrease the concentration of corticosterone that in turn decreased gluconeogenesis in heat-stressed birds (Zulkifli et al., 2006). Heat stress is associated with the decreased concentration of some haematological variables such as haemoglobin (Borges et al., 2004). Hasan et al. (2015) showed that probiotics (Protexin® Boost) increased haemoglobin concentration in birds subjected to heat stress. In contrast, Rahimi and Khaksefidi (2006) did not find any effect of probiotics (Bioplus 2B containing of *B. subtilis* CH201 and *B. licheniformis* CH200) on haemoglobin concentration in heat-stressed chickens. The different type of probiotics used in the diets of heat-stressed birds seemed to be the reason for the discrepancy.

### **Probiotics and immunity of heat-stressed poultry**

Probiotics have been reported to be useful to improve the immune system of birds subjected to heat stress (Deng et al., 2012; Landy and Kavyani, 2013). Table 2 shows some of the probiotic treatments and their potential effects to ameliorate the compromised immune system in heat-stressed poultry. Apart from the suggested mechanism in Table 2, probiotics may reduce the circulating level of corticosterone leading to lowered heterophil to lymphocyte (H/L) ratio and improved immune responses (Hassan et al., 2007; Beski and Al-Sardary, 2015). Yang et al. (2015) suggested that corticosterone possess immunosuppressive effects in poultry, and therefore the re-



duced corticosterone level may be beneficial for restoring the normal function and development of immune system.

The lowered antibody response to infectious agents is usually believed to increase the mortality of heat-stressed chickens. Probiotic administration has been found to enhance antibody responses (Zulkifli et al., 2000; Asli et al., 2007; Haldar et al., 2011; Deng et al., 2012; Landy and Kavyani, 2013) as well as leukocytes count (Rahimi and Khaksefidi, 2006) in birds reared under hot temperature. However, some studies reported no effect of probiotic treatment on the antibody level in heat-stressed birds. Sohail et al. (2010) reported that *Lactobacillus*-based probiotics (*L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus*, *E. faecium*, *A. oryzae* and *C. pintolopesii*) did not affect the antibody titers against Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV) in broilers subjected to heat stress. Similar report was revealed by Rahimi and Khaksefidi (2006), in which Bioplus 2B containing *B. subtilis* CH201 and *B. licheniformis* CH200 did not influence antibody production against sheep red blood cells (SRBC) antigen and NDV vaccination in heat-stressed broilers at different ages. The exact rationale for this discrepancy was not clear, but the different type and dose of probiotics used in the experiments seemed to be responsible.

Intraepithelial lymphocytes (IEL) are important components of the host immune system that can rapidly respond to infection (Sheridan and Lefrançois, 2010). Heat stress is known to lower the number of IEL in the ileum and cecum of laying hens at 61 (but not at 62) weeks of age (Deng et al., 2012). Indeed, probiotic *B. licheniformis* reversed the decreased number of IEL due to heat stress in the latter study. In contrast to the above data, Ashraf et al. (2013) reported that IEL in all the segments of small intestine of broilers increased with heat stress. This increase was probably associated with initiation of the inflammatory response by heat stress (Quinteiro-Filho et al., 2010). It should be noted that exaggerated inflammatory responses may be deleterious (induce immunopathology) for poultry. Treatment with *Lactobacillus*-based probiotics (*L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus*, *E. faecium*, *A. oryzae* and *C. pintolopesii*) indeed alleviated the increased count of IEL in all intestinal segments of heat-stressed broilers (Ashraf et al., 2013), and therefore the balanced mucosal immune responses could be maintained. In laying hens, the increase in serum corticosterone due to heat stress was associated with the increased mast cells in the ileum and cecum and TNF- $\alpha$  and IL-1 in the serum (Deng et al., 2012). Probiotic *B. licheniformis* reversed the increased count of mast cells as well as TNF- $\alpha$  and IL-1 in the study of Deng et al. (2012), and thus the excessive inflammatory responses in the small intestine of heat-stressed hens could be prevented.

In most cases, the relative weight of immune organs can represent the capability and functionality of the immune system in poultry. Hassan et al. (2007) reported that lymphoid organ involution due to heat stress in poultry can be prevented by probiotic *B. subtilis* administration. Again, probiotics seemed to reduce the level of corticosterone (Lei et al., 2013) responsible for lymphoid organs involution under heat stress (Quinteiro-Filho et al., 2010). It is well known that intestinal microbial diversity and population affect the development of immune system of poultry. Dietary



probiotic administration has been reported by several authors able to improve the intestinal microbial diversity and population, and thus immune system of poultry under heat stress condition (Al-Fataftah and Abdelqader, 2014; Song et al., 2014). The mechanisms through which intestinal microbial ecosystem affects the immune system of birds have extensively been reviewed elsewhere (Sugiharto, 2016). Recently, dyslipidemia (elevation of plasma cholesterol, triglycerides, or both, or a low HDL level) has been believed to be a marker of inflammation in human studies (Aulinas et al., 2015). Concomitant with the enhanced levels of the conventional inflammatory marker such as TNF- $\alpha$  and IL-1 (Deng et al., 2012), heat stress in poultry was also associated with the dyslipidemia (Habibian et al., 2014). There is growing evidence that probiotics can control dyslipidemia in birds subjected to heat stress as reported by Tollba and Sabry (2004) when providing *Lactobacillus* sp. and yeast culture. Corresponding report was shown by Sohail et al. (2010) when feeding *Lactobacillus*-based probiotic to heat-stressed broilers.

Table 2. Examples of probiotic treatments to improve immune system of heat-stressed poultry

Strains or types of probiotics	Biological activities	References
1	2	3
<i>B. subtilis</i> and <i>B. licheniformis</i>	Increased expression levels and enzyme activity of <i>LXR<math>\alpha</math></i> which controls the <i>functional</i> specialization of splenic <i>macrophages</i> in ducks.	Huang et al., 2015
<i>Lactobacillus</i> -based probiotics ( <i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. bulgaricus</i> , <i>L. rhamnosus</i> , <i>B. bifidum</i> , <i>S. thermophilus</i> , <i>E. faecium</i> , <i>A. oryzae</i> and <i>C. pintolopesii</i> )	Ameliorated the excessive inflammatory response (decreased excessive numbers of IEL) in all intestinal segments of heat-stressed broilers. Increased the count of goblet cells (producing mucins which is component of innate immunity) in the duodenum and jejunum of heat-stressed broilers.	Ashraf et al., 2013
Probiotic mixture ( <i>L. acidophilus</i> , <i>L. casei</i> , <i>E. faecium</i> and <i>B. bifidum</i> )	Improved antibody responses to Newcastle disease, bronchitis and Gumboro disease in broilers under cyclic heat stress condition.	Landy and Kavyani, 2013
Probiotic <i>B. licheniformis</i>	Improved mucosal immunity (IgA-secreting cells) of heat-stressed hens. Reversed the increased levels of serum TNF- $\alpha$ and IL-1 due to heat stress. Reversed the decrease in IEL counts in the ileum and cecum of heat-stressed hens. Reversed the increased number of mast cells in the ileum and cecum of birds due to heat stress.	Deng et al., 2012
Probiotic <i>S. cerevisiae</i>	Increased the hemagglutination inhibition (HI) titer against NDV in heat-stressed broiler 14 days past the second vaccination.	Haldar et al., 2011
Multi strain probiotics ( <i>L. plantarum</i> , <i>L. bulgaricus</i> , <i>L. acidophilus</i> , <i>L. rhamnosus</i> , <i>B. bifidum</i> , <i>S. thermophilus</i> , <i>E. faecium</i> , <i>A. oryzae</i> and <i>C. pintolopesii</i> )	Increased antibody titer against SRBC.	Asli et al., 2007

Table 2 – contd.

1	2	3
Probiotic <i>B. subtilis</i>	Increased immune organs/body weight ratio in heat-stressed broilers. Reduced H/L ratio and excessive inflammatory reaction of chicks. Increased natural agglutinin response.	Hassan et al., 2007
Acid-Pak 4-Way™	Reduced H/L ratio of chickens exposed to heat stress.	Zulkifli et al., 2006
Bioplus 2B containing <i>B. subtilis</i> CH201 and <i>B. licheniformis</i> CH200	Increased leukocytes counts. Reduced H/L ratio.	Rahimi and Khaksefidi, 2006
<i>Lactobacillus</i> cultures	Increased antibody response against NDV in broilers after heat stress.	Zulkifli et al., 2000

### Probiotics and performance of heat-stressed poultry

Probiotics have been exploited to ameliorate the detrimental impacts of heat stress on poultry performance (Lara and Rostagno, 2013). Jahromi et al. (2015) reported that incorporation of a mixture of *L. pentosus* ITA23 and *L. acidophilus* ITA44 into diets improved the growth and feed conversion ratio (FCR) of broilers exposed to heat. Similarly, Al-Fataftah and Abdelqader (2014) showed that dietary supplementation of *Bacillus subtilis* was effective in improving the growth performance, while Song et al. (2014) reported that supplemental probiotics (contained *B. licheniformis*, *B. subtilis* and *L. plantarum*) improved feed to gain ratio of broiler chickens reared under hot temperature. In heat-stressed laying hens, administration of multi strain probiotics (*L. plantarum*, *L. bulgaricus*, *L. acidophilus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus*, *E. faecium*, *A. oryzae* and *C. pintolopesii*) (Asli et al., 2007) or probiotic *B. licheniformis* (Deng et al., 2012) increased egg production and feed intake. Apart from the potential of probiotics in improving the performances of heat-stressed birds, Lara and Rostagno (2013) suggested that the effect of feed additives including probiotic on the performance of heat-stressed chickens is not always discernible. This can be seen in the study of Sohail et al. (2012) and (2013), in which probiotic mixture containing *L. plantarum*, *L. delbrueckii* ssp. *Bulgaricus*, *L. acidophilus*, *L. rhamnosus*, *B. bifidum* and *S. salivarius* ssp. *Thermophilus* and *E. faecium* as well as probiotic mixture containing *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *L. rhamnosus*, *B. bifidum*, *S. thermophilus*, *E. faecium*, *A. oryzae* and *C. pintolopesii*, respectively, could not significantly improve the growth performance of broilers under heat stress condition. The type or species and the dose of probiotics used and the ambient temperature applied during the study may be responsible for the above discrepancy.

One of the predisposing factors for the impaired growth performance in heat-stressed chickens is the reduced activities of digestive enzymes ( $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ -adenosine triphosphatase [ATPase],  $\text{Na}^{+}$ - $\text{K}^{+}$ -ATPase, maltase, sucrase, and alkaline phosphatase) which impede digestion and hence limit the nutrient utilization by birds (Chen et al., 2014). To date, the definite mechanism through which probiotics could improve the performance of poultry exposed to heat remains unclear. However, Sugiharto (2016) suggested that probiotics may improve the metabolism of poultry

by increasing the digestive enzyme activity and decreasing bacterial enzyme activity. It has been known that heat stress can decrease feed digestibility of the different components of the diets including starch, fats and proteins (Bonnet et al., 1997; Lara and Rostagno, 2013). With regard to protein, administration of probiotics may improve protein digestibility and decrease ammonia production, and hence improve the growth performance of poultry exposed to heat (Sugiharto, 2016). Apart from the enhanced digestive enzyme activity, probiotic microorganisms have been found to produce several enzymes that are not produced by host to help hydrolyzing complex macromolecules (Nayak, 2011). Taking this into view, it is therefore reasonable to expect that probiotics may be useful to improve the digestibility in birds which have reduced dietary digestibility during heat stress.

The reduced thyroid hormones activities due to heat stress could be a major problem for the growth performance of broilers (Sohail et al., 2010). Tollba and Sabry (2004) revealed that *Lactobacillus* sp. and yeast culture increased plasma  $T_3$  while Sohail et al. (2010) reported that *Lactobacillus*-based probiotics enhanced serum  $T_4$  concentrations in heat-stressed broilers. There is evidence that thyroid hormones possess enterotrophic effects in rats (Tutton, 1976). Hence, the increased level of thyroid hormones (after probiotic administration in heat-stressed chickens) is reasonably expected to increase villus surface area, which eventually improves the absorptive capacity of the chickens. In addition, the increased level of thyroid hormones would be beneficial for alleviating the impaired growth performance in heat-stressed chickens, given that thyroid hormones play important roles in regulating the metabolic processes essential for normal growth and development (Mullur et al., 2014).

### Conclusions

Due to global temperatures rise, heat stress has recently become a serious threat to poultry production in most poultry producing countries. Heat stress can impair physiology, immunology and microbiology of poultry and thus performances of birds. Probiotics seem to be useful to ameliorate the detrimental effects of heat stress in poultry, given that probiotics may improve intestinal microbial ecology and morphology, physiological conditions, immune system and performance of birds reared under heat stress condition. However, probiotics should be used with caution as some studies reported no effect.

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