AMERICAN UNIVERSITY OF BEIRUT

IMPACT OF DIETARY *LACTOBACILLUS PLANTARUM* POSTBIOTICS ON PERFORMANCE OF LAYER HENS UNDER HEAT STRESS CONDITIONS

by
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ABSTRACT
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Title: Impact of Dietary *Lactobacillus plantarum* Postbiotics on Performance of Layers Hens under Heat Stress Conditions

It has been reported that probiotic bacteria may acquire and transfer antibiotic resistance genes between organisms. Subsequently, postbiotics, which are metabolites of probiotics, have been used as feed additives as a potential replacement for antibiotics and probiotics. For this purpose, this experiment was conducted to determine the performance of heat-stressed layers fed a diet containing the probiotic *Lactobacillus plantaru*m RS5 or its products of fermentation. A total of 192 twenty-week-old pullets of an Isa White strain, were equally subdivided into six treatments of 32 birds individually caged.

Half of the birds were reared under regular temperature conditions, while the other half was subjected to cyclic daily heat stress gradually reaching about 30°C. Layers were offered one of three different diets: 1) Control, or 2) Control + *Lactobacillus plantarum* RS5 probiotic, or 3) Control + *Lactobacillus plantarum* RS5 postbiotic products. The liquid probiotics (RS5 in MRS broth) and postbiotics (CFS in MRS broth) were mixed with 100 kg of the feed at a concentration of 200 ml and 300 ml of solution respectively. The birds were tested for performance parameters and visceral organ development and the feeding trial lasted for 5 months.

The study demonstrated that heat stress negatively affected the birds feed intake especially during the first month (79.1g vs 84.2g for the control; $p<0.05$) resulting in a numerical decrease in egg production, however the birds quickly adapted to the elevated temperature. Furthermore, high cyclic temperature showed a negative impact ($p<0.05$) on the egg weight, percent shell weight, Haugh unit, shell thickness, and yolk color in addition to the birds' weight and percentage spleen weight.

Postbiotic supplementation in feed showed a faster effect on percentage egg production than probiotic supplementation. Hens with dietary postbiotic showed a higher ($p<0.05$) egg production percentage than the control and the probiotic feed group (94.8% vs 92.6% vs 93.1%, respectively). In addition, birds under probiotic or postbiotic diet showed a significantly higher ($p<0.05$) feed intake. Probiotic and postbiotic treatments had a positive impact ($p<0.05$) on egg weight, although probiotic had a more pronounced and gradual effect. The Haugh Unit was significantly higher ($p<0.05$) in probiotic group due to the increase in albumen weight percentage; however, the percentage of egg white weight was significantly lower in the postbiotic group (59.3% vs 59.7% for the control; $p<0.05$). Specific gravity, yolk weight percentage and shell thickness didn't show differences among dietary groups. Nevertheless, probiotic showed a significantly lower percentage shell weight ($p<0.05$); and both probiotic and postbiotic groups showed a significantly lower yolk color ($p<0.05$). The different feed treatments did not have any effect on the bird's visceral organ weight, except for the ileum that showed a significantly lower percentage weight ($p<0.05$) under postbiotic supplementation and a slight decrease in ileum weight under probiotic supplementation to feed.

In conclusion, both probiotics and postbiotics could be used as a potential alternative antibiotic growth promoter and might alleviate the impact of heat stress in the poultry industry.

Keywords: *Lactobacillus plantarum*, layers, heat stress, postbiotic, probiotics, performance

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CHAPTER I

INTRODUCTION

Heat stress is one of the most important environmental stressors challenging poultry production worldwide. Having adverse effects on animal health and productivity, heat stress can result in heavy economic losses due to increased mortality and reduced productivity (Ajakaiye, 2011). Furthermore, birds' physiology and behavioral response to heat stress negatively affects productivity owing to lower feed intake and digestive capacity and alteration of the intestinal mucosa and microbiota ecology (Quinteiro-Filho, 2010).

To combat some of the adverse effects of heat stress on poultry specifically on health and growth performance, the inclusion of feed additives such as antibiotics in the diet at sub-therapeutic levels is a common practice. The inclusion of antibiotics as growth promoters in layers' feed have been shown to alleviate the effect of heat stress and improve performance (Loh et al., 2014). However, excessive and prolonged use of antibiotics in animal feeds has raised concerns regarding antibiotic residues in animal products and the development of antibiotic-resistant bacteria (Shazali el al., 2014). This has led to the banning of dietary growth promoter for animals in several countries (Van et al., 2015).

To replace the use of antibiotics, probiotics have been used as feed additives in poultry to promote a healthy gut environment and improve growth performance (Loh et al., 2014). It has been reported that probiotic strains can help maintaining the microbial balance in the gastrointestinal tract (GIT) as well as making changes in the composition of the intestinal microflora by increasing beneficial bacteria and decreasing harmful pathogens. This could be due to competitive exclusion by which beneficial bacteria compete with harmful ones for nutrients and attachment sites on the intestinal epithelial wall (Lokapirnasari et al., 2019); and/or produce of antimicrobial substances, such as organic acids, diacetyl, acetoin, hydrogen

peroxide and bacteriocins (Prabhurajeshwara, 2019). In addition, some probiotic cultures have been reported to be able to improve the morphology of chicken intestine toward increasing nutrient absorption and endogenous digestive enzymes secretion surface (Lokapirnasari et al., 2019).

The use of probiotic supplementation containing beneficial bacteria, such as *Lactobacillus* spp., has a positive effect on the intestinal microbial population (Loh et al., 2014). *Lactobacillus* strains have a high ability to attach to the intestinal epithelium and are able to establish in the chicken intestine within a day, so they are considered to be normal bacterial flora of the GIT of chickens (Shokryazdan, 2017). *Lactobacillus* plantarum is classified as lactic acid bacteria categorized under probiotic microbial groups living in the digestive tract to improve its condition (Lokapirnasari et al., 2019).

The possible mechanisms of probiotic action include, but are not limited to. (1) Competitive exclusion of pathogenic micro-organisms, (2) production of antimicrobial substances, (3) competition for growth factors and nutrients, (4) enhancement of adhesion to intestinal mucosa to protect the gut lining from any damage, (5) improvement of epithelial barrier function by increasing mucin expression and secretion, thereby limiting bacterial movement across the mucous layer, (6) improvement of secretion of IgA the principal weapon protecting the body from pathogens and toxins that might otherwise penetrate mucosal surfaces (Julio et al., 2019).

In poultry, the administration of probiotics could improve the feed conversion ratio (FCR) and feed intake (FI), increase egg production, and stimulate growth rate (Loh et al., 2014). However, it has been stated that probiotic bacteria may acquire and transfer antibiotic resistance genes between organisms (Shazali el al., 2014).

Subsequently, postbiotics, which are metabolites of probiotics, have been used as feed additives in livestock as a potential replacement for antibiotics and probiotics (Loh et al., 2014).

Postbiotics have a similar mechanism of action and capacity as probiotics owing to the presence of secondary metabolites from probiotics but without a living cell (Thanh et al., 2009). The presence of antimicrobial metabolites, such as organic acids and bacteriocins, in postbiotics can reduce the gut pH and inhibit the proliferation of opportunistic pathogens in the feed and gut of animals (Human et al., 2019). It has been demonstrated that the application of postbiotics as a feed additive in livestock promotes the growth performance and health of broilers (Human et al., 2019), layers (Loh et al., 2014) and pigs (Fajardo, 2012), as well as enhancing rumen fermentation and health in ruminants (Izuddin, 2019). In addition, apart from their ability to promote a healthy gut environment, the potential antioxidant capacity of postbiotics obtained from *Lactobacillus* has been found to be particularly strong under heat-stress conditions (Ji, 2015).

Previous study showed that postbiotics obtained from *Lactobacillus plantarum* exhibit inhibitory action on various pathogenic bacteria, including Listeria monocytogenes, Salmonella typhimurium, Escherichia coli and vancomycin-resistant Enterococci (Kareem et al., 2014). In addition, postbiotics obtained from *L. plantarum* has been found to be particularly strong under heat-stress conditions (Loh et al., 2014). In heat-stressed broilers, postbiotics from *L. plantarum* are expected to provide similar benefits to those from probiotic bacteria (Loh et al., 2014).

In layers, postbiotic dietary supplementation improves hen-day egg production, reduces the fecal pH and fecal Enterobacteriaceae population, increases the fecal lactic acid bacteria, reduces the plasma and yolk cholesterol, and increases the fecal volatile fatty acids content. Postbiotic metabolite combinations can be used as an alternative feed additive to achieve high productivity and better poultry health (Loh et al., 2014).

Since probiotic/postbiotic effect is strain dependent and may also depend on the host and its immunologic state, this study aims to evaluate the effect of dietary *L. plantarum* RS5

postbiotic preparations on performance and immunity parameters of layers under heat stress conditions. Performance parameters include live body weight; feed intake; egg production; egg quality namely specific gravity, yolk color shell thickness, HU score, percent white weight, percent yolk weigh and percent shell weight; and visceral organ indices namely liver, spleen, gizzard, proventriculus, duodenum, jejunum, ileum, and abdominal fat.

CHAPTER II

LITERATURE REVIEW

A. Impact of Heat Stress

Heat stress is one of the most important environmental stressors challenging animal production worldwide, especially in tropical climate (Sinha et al., 2017). Air temperature, relative humidity, radiation and wind speed are primary environmental factors that determine the heat stress level in livestock. These factors affect the mechanism of thermoregulation and rates of heat exchange by all animals (NRC, 1981).

In general, stress represents the biological response of the animal organism to external factors that disturb its normal physiological equilibrium. Heat stress results from a negative balance between the net amount of energy flowing from the animal's body to its surrounding environment and the amount of heat energy produced by the animal (Lara et Rostagno, 2013). The detrimental effect of heat stress applies to all species; however, commercial poultry stains seem to be particularly sensitive. Studies have shown that poultry genotypes produce more body heat, due to their greater metabolic activity that reduces their ability to adapt to the changes in environment conditions (Deeb et Cahaner, 2022).

1. Avian defense mechanism under heat stress

The thermoneutral zone of the chicken is generally between 18- 25°C. For avian species heat can be lost in a variety of ways ((Getachew, 2021):

i- First radiation, electromagnetic waves transfer heat through the air to a distant object, body heat is radiated to cooler objects in the house.

ii- Second convection, body heat lost to cooler surrounding air, birds will increase exposed surface area by drooping and spreading wings. Providing moving air can assist

convection. Vasodilation-Blood-swollen wattles and comb bring internal body heat to the surface to be lost to the cooler surrounding air.

iii- Third conduction, body heat loss to cooler objects indirect contact with the bird (i.e. litter, slats, cage wire). Chickens will seek cooler places in the house.

iv- After a bird can no longer maintain its body heat balance by one of these three methods, it must use “evaporative heat loss”, or panting, which is rapid, shallow, open mouth breathing to increase heat loss by increasing the evaporation of water from the mouth and respiratory tract. Evaporative cooling is aided by lower air humidity. Evaporation is important at high temperatures as poultry do not sweat but depend on panting.

As a defense mechanism, under heat stress conditions, birds alter their behavior and physiological homeostasis seeking thermoregulation. It has been suggested that birds subjected to high temperature conditions spend less time feeding, more time drinking and panting, as well as more time with their wings elevated, less time moving or walking, and more time resting, they seek cooler surfaces (Mack et al., 2013).

Avian species utilize a special mechanism to maintain thermoregulation and homeostasis, which are air sacs. Air sacs are very useful during panting, as they promote air circulation on surfaces contributing to increase gas exchanges with the air, and consequently, the evaporative loss of heat (Lara et Rostagno, 2013). Nonetheless, it is known that increased panting, in other terms hyperventilation, leads to increased carbon dioxide levels and higher blood pH (i.e., alkalosis), which in turn hampers blood bicarbonate availability for eggshell mineralization and induces increased organic acid availability, also decreased free calcium levels in the blood. This process is very important in breeding and laying hens, as it affects eggshell quality (Marder et Arad, 1989).

2. Effect of heat stress on production parameters

Heat stress has a detrimental effect on animal welfare. It entails lower feed intake and negative energy balance in the bird (De Rensis and Scaramuzzi, 2003). Studies have shown that for every 1°C rise in the temperature range of 22-32°C feed intake will be reduced by 1.2%; and for 1°C rise in the temperature range of 32-38°C feed intake will be reduced by 5% (Ashish et al., 2019). In broilers, growth rate decreases due to decline feed digestibility such as proteins, fats, starch (Bonnet et al., 1997). Also, in layer hens, egg production decreases due to decrease in the uptake of available nutrients and decreased digestibility of many components of the diet (Allahverdi et al., 2013). In breeder hens, heat stress might affect the reproductive efficiency of the birds due to alterations in acid-base balance and hormonal system (Mahmoud et al., 1996).

Decreased feed intake might lead to decreased body weight, feed efficiency, egg production and quality (Deng et al., 2012). In addition, it has been shown that heat stress leads to reduced dietary digestibility, and decreased plasma protein and calcium levels (Zhou et al., 1998). Many studies were published supporting the harmful effects of heat stress on layer hen production, although many effect variation was observed, it can be noted that the impacts of heat stress on egg quality and production is significant. The impact of heat stress on birds depends on the age or genetic background, as well as the intensity and duration of the heat stress treatments applied (Lucas and Marcos, 2013).

Studies shows, heat stress causes a reduction in feed intake resulting in a decrease egg production (Star et al., 2009; Deng et al., 2012, Mack et al., 2013), reduced feed conversion, decreased egg weight (Star et al., 2009; Mack et al., 2013), decreased production performance, increased egg breakage (Lin et al., 2004), reduced eggshell thickness, lower eggshell weight (Ebeid et al., 2012; Mack et al., 2013), yolk weight, albumen weight, specific gravity, Haugh unit and yolk index (Wiernusz et al., 1998; Zaviezo et al., 1999).

3. Effects of Heat Stress on Physiology of Chickens

Under high temperature conditions, chickens alter their physiological homeostasis seeking thermoregulation, thereby decreasing body temperature. Heat stress can affect the reproductive function of poultry in different ways. In females, heat stress can disrupt the normal status of reproductive hormones at the hypothalamus, and at the ovary, leading to reduced systemic levels and functions (Elnagar et al., 2010). Moreover, negative effects caused by heat stress in males have been shown in different studies. Semen volume, sperm concentration, number of live sperm cells and motility decreased when males were subjected to heat stress (McDaniel et al., 2004).

It is well kwon that heat stress leads to delays in the synthesis of most proteins except heat shock proteins (Piestun et al., 2008). A study on the thermal manipulation on broiler embryogenesis results in significant increases in plasma total proteins and albumin in thermal manipulated chicks. This demonstrated that thermal manipulation modulates the thermoregulation process during embryogenesis and post hatching stages. As reported in the study, the increment of total proteins and albumin concentrations in thermal manipulation birds can be considered as a sort of protection of muscle mass against injury induced by thermal challenge (Al-Zghoul et al., 2015).

4. Effects of Heat Stress in Genomics of Chickens

Literature reports that in general poultry react similarly to heat stress, but express individual variation of intensity and duration of responses, which may also be affected by intensity and duration of the elevated temperature. Another potential factor may be that heat stress might also be accompanied by other stressors, such as limited housing space, insufficient ventilation, unbalanced feed ration and/or pathogens contamination (Lara et Rostagno, 2013).

Additionally, the animal age, genetics namely species, metabolism rate, and thermoregulatory mechanisms may cause this variation in response to heat stress (Mack et al., 2013).

5. Effects of Heat Stress on the Immune Response

Studies show an immunosuppressant effect of heat stress on both broilers and laying hens. A study demonstrated that elevated temperature suppresses immune function by inhibiting total white blood cell counts and antibody level; thereby increasing mortality in laying hens (Mashaly et al., 2004). Other studies showed that plasma calcium and phosphorous concentrations were reduced by heat stress in laying hens. For instance, lower relative weights of the thymus and the spleen in these subjects (Ghazi et al., 2012). Moreover, reduced lymphoid organ weights have also been reported in broilers under heat stress conditions (Barnett et Hemsworth, 2003). Additionally, studies observed that broilers subjected to heat stress had lower levels of total circulating antibodies (Bartlett et Smith, 2003), and a reduced liver weight (Felver-Gant et al. 2012). In addition, broilers subjected to heat stress had lower levels of total circulating antibodies, as well as lower specific IgM and IgG levels, both during primary and secondary humoral responses. Moreover, they observed significantly reduced thymus, bursa, spleen, and liver weights (Bartlett and Smith, 2003)

Environmental stressors can modify the biological defense systems, such as antibody and cell-mediated immune responses, thereby increasing susceptibility to pathogens (Bozkurt et al., 2012). Also, the bird's gastrointestinal tract is particularly responsive to stressors, which can cause an alteration of the protective microbiota as well as decreased integrity of the intestinal epithelium (Collins et al., 2012). Studies reported that mucosal attachment of Salmonella Enteritidis increased in heat-stressed birds. These birds will not only show a higher bacterial level in their feces, but also might have an expanded duration and a higher level of

contamination in the environment where their feces are deposited, potentially leading to faster spreading (Traub-Dargatz et al., 2006).

6. Effect of heat stress on food safety

Regarding food safety, environmental stress has been shown to be a factor that can lead to colonization of farm animals by pathogens, mostly due to immunosuppression. Consequently, the risk of animal by-product contamination, such as meat and egg, might rise due to the increase of fecal shedding and horizontal transmission (Humphrey, 2006). Many studies have demonstrated that bacteria, such as Salmonella and Campylobacter, can exploit the neuroendocrine alterations due to the stress response in the host to promote growth and pathogenicity (Verbrugghe, et al., 2012).

7. Management approaches to reduce heat stress

Some management approaches help to reduce the heat stress in poultry. Farmers can modify the surrounding environment by installing ventilation system, reducing bird density, feeding during the evening time, and managing the nutrition (Dayyani and Bakhtiyari, 2013).

The change in energy: protein ration in the diet minimizes the adverse effect of heat stress. Increasing the ME content of feed improves energy intake. The concentration of energy should be increased by 10% during heat stress to cope with the reduction of feed intake (Daghir, 2008). Decreasing protein in diet helps reduce heat increment, and increases feed intake due to deficiency of amino acids (Gous and Morris, 2005). However, the low protein diet should maintain a balanced critical amino acid namely methionine and lysine (Lin et al., 2005).

Furthermore, studies determined that Vitamins and mineral supplementation help the animals during stress time because heat stress increases excretion of mineral from body (Sahin et al., 2009). More specifically, vitamin A, D, E, C, and folic acid are shown to be very efficient

to animals under heat stress conditions (Sahin et al., 2002). Another feed supplementation that was shown to be effective are probiotics such as *Lactobacillus strains*. These probiotics help to enrich the diversity of *Lactobacillus* flora in chicken jejunum and caecum, and therefore restoring the microbial balance and maintaining the natural stability of jejunal and caecal microbiota of chicken suffered with heat stress (Loh et al., 2014).

B. Probiotic effect

In all animal sectors, feed is one of the largest expenditures, accounting for 70% of total production costs. Like any other sector of agricultural industry, the major aim of poultry industry is to reach optimum production with minimum input. Therefore, balanced, and effective feeding is important for optimal economic poultry production. In the avian industry, several feed additives were used as growth promoters, to increase animal meat or egg production. More specifically, antibiotics have been extensively used for enhancing poultry production. However, the emergence of bacterial resistance to therapeutic antibiotics and the residual effect of these medicines in eggs and meat triggered scientists to find a safer substitute (Salim et al., 2018).

Abundant research has been conducted on numerous feed additives to substitute the Antibiotic growth promoter (AGP) in poultry industry; one of which is probiotics, i.e bacterial microorganisms. used for improved but also safe production in livestock (Bidarkar et al., 2014). Other researchers worked on phytobiotics, classified as plant-based feed additives to improve the health of farm animals (Hashemi et al., 2011). Biosurfactants, which are surfactants of biological origin, were also evaluated by many scientists as they are characterized by low toxicity and high biodegradability. Surfactants, such as hydrogen peroxide, have a bactericidal and bacteriostatic effect; they show positive effects on poultry production and health (Keener et al., 2004). Probiotics may form part of surfactants called microbiological biosurfactants

(Krysiak et al., 2021). Moreover, organic acids, such as lactic acid, showed significant impact on reducing pathogenic microorganisms existing in bird's digestive tract (Araujo et al., 2019).

The name probiotic was first coined in 1974, and it was described as "microorganism or substance, which contributes to the intestinal microbial balance" (Parker, 1974). In 1989 Probiotic was defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal balance" (Fuller, 1989). According to the Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) definition, probiotics are "living microorganisms which when administered in adequate amount confer a health benefit on the host" (hotel, 2014). The term 'probiotic' is derived from a Greek word 'probios' means' 'for life'. Probiotics have been described as the opposite of antibiotics; while antibiotics destroy life, probiotics build up or promote life (Jadhav et al., 2015).

For the probiotic to be considered functional, it must be a bacterium that is a component of the intestinal microflora, resistant to the acid environment, and easily adheres to the intestinal epithelium (Kabir et al., 2009). In addition, the probiotic should maintain the intestinal microflora at the appropriate physiological level (Krysiak et al., 2021). The most common types of microorganisms used in the preparation of probiotics are bacteria such as *Bifidobacterium* spp., *Lactococcus* spp., *Lactobacillus* spp., *Bacillus* spp., *Streptococcus* spp., as well as yeasts such as *Candida* spp. (Park et al., 2016).

The benefits of feeding probiotics to poultry are diverse (figure 1). They stimulate immune system (Sanders, 1984), improve utilization of proteins, intestinal tract health, feed conversion ratio, strengthen beneficial microbial populations and suppress harmful bacterial growth in the digestive system, counteract adverse effect of antibiotic treatment by sustaining the population of beneficial bacteria, and in nutrient synthesis (Jadhav et al., 2015).

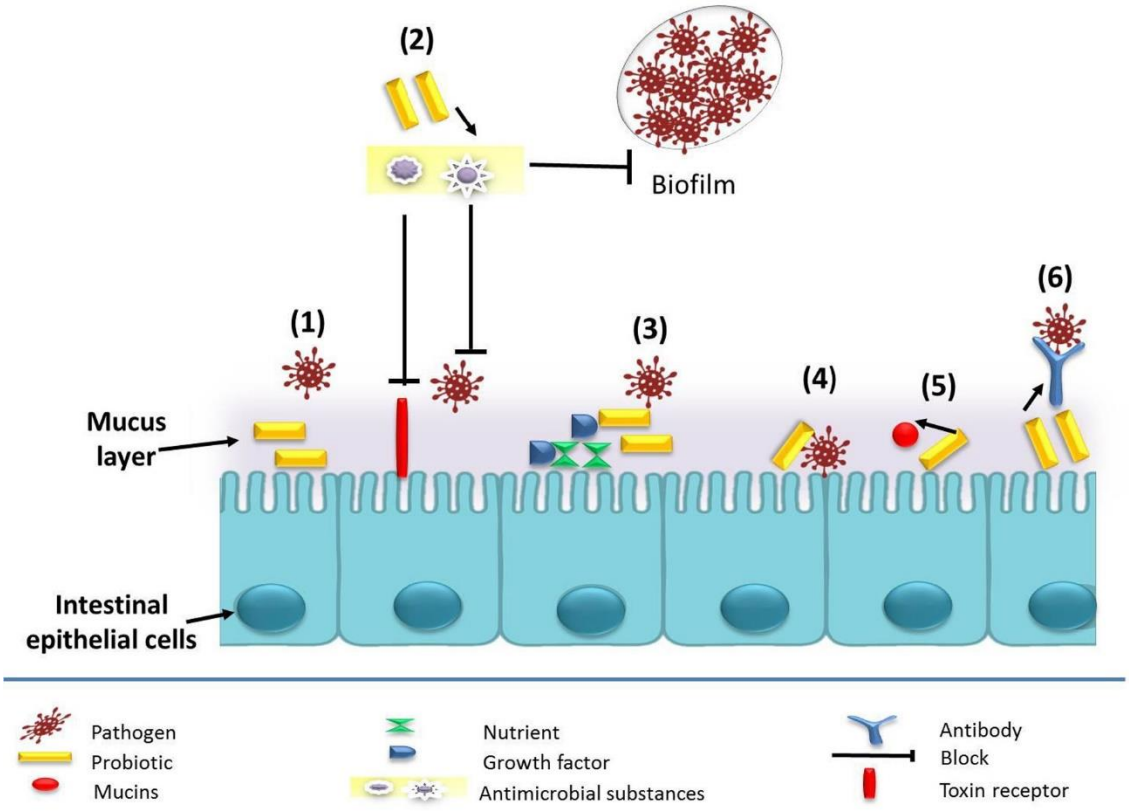

Figure 1 Mechanism of action of Probiotics

1. The advantages of dietary Probiotics for chickens

The poultry digestive tract contains many microorganisms, commonly referred to as microbiota. The most common bacteria are *Lactobacillus* spp., *Bifidobacterium* spp., *Ruminococcus* spp., *Clostridium* spp., and *Bacteroides* spp. Although each of these bacteria has and individual function, they all aim to preserve homeostasis in the poultry body. One of their roles is to increase the energy efficiency of feed by fermentation. This gives a better energetic use of the feed, and the assimilation of important nutrients is easier. It is estimated that 10% of the energy from feed is derived from intestinal bacteria (Krysiak et al., 2021). Bacteria have an influence on the structure of the intestine and it's functioning as intestinal microorganisms enlarge the villi and intestinal crypts. The intestinal microbiome can affect its morphology, particularly regulating the immune processes that occur (Menconi et al., 2014a).

Probiotics are preparations that have a positive effect on the gastrointestinal tract and immune system. These feed additives provide antimicrobial substances that show a similar level of efficacy as antibiotic, organic acids, bacteriocin, or hydrogen peroxide (Alloui et al., 2013). The administration probiotics as feed additives has a positive effect on the level of immunoglobulins M and A and on the percentage of total antioxidant capacity in serum (Wang et al., 2018). Moreover, research indicates that *Lactobacillus* spp. can activate the receptors that are responsible for epithelial growth in the intestine. This results in a reduction in intestinal epithelial apoptosis, which is an important component in the fight against gastrointestinal diseases (Menconi et al., 2014a).

Probiotics lead to variability in bacterial microflora's composition, namely an increase in the number of *Bifidobacterium* spp. and *Lactobacillus* spp. that help in eliminating pathogens. The composition of the microbiome is also influenced by other factors such as the quantity and quality of nutrients and/or the composition and balance of the feed itself (Freter et al., 1992). Other effects include decreased activity of bacterial enzymes and decreased stool pH (Ashraf and sahah, 2014). These factors are caused by increased concentrations of acetic acid, lactic acid, and volatile fatty acids (VFA). The acidified environment is favorable to the development of intestinal microbiome, supporting the immunity against pathogens, and supporting the organism's natural defense mechanisms (Park et al., 2016).

The statement "immunity comes from the intestines" has become more significant in the poultry industry with the emergence of probiotics. This is due to the bacterial interaction, in which the microorganisms compete with each other to survive (Hernandez-Patlan et al., 2020). The addition of probiotics affects the predominance of the intestinal probiotic bacteria in the microflora, reducing the number of pathogenic microorganisms. The use of probiotics improves the quality of meat and eggs, providing healthy and safer goods (Birmani et al, 2019).

In some studies, the use of probiotics displayed an increase in the weight of certain internal organs such as the spleen and thymus (Pourakbari et al. 2016). Furthermore, a diet enriched with a probiotic preparation causes the development of the intestines. It helps improving the quality of the intestinal villi, as well as increasing the crypts which improves nutrient absorption and allow proper colonization of bacteria (Park et al., 2016). Probiotic preparations help also increasing the weight index of some gastrointestinal tract sections including the cecum (Cean et al.,2015), and the thickness of the mucosa (Ghasemi-Sadabadi et al, 2019). In addition, a diet supplemented with probiotic increase the length of the bowel (Harimurti and Hadisaputro, 2015), helps in bones strengthening by increasing calcium and phosphorus retention (Khan et al., 2013), positively affects tibial index, size and mass, and femur density (Yan et al,2018) and decreases the frequency of lameness in broiler chicks (ali et al., 2018).

In broilers, probiotics have a positive effect on animals' physical properties of meat, namely improved poultry carcass quality by increasing overall carcass weight and reducing abdominal fat (Hidayat et al., 2016). The increase in carcass weight is mainly due to the increase in nutrients absorption, including amino acids needed to build tissues (Aziz et al., 2020). The protein content of thigh and breast meat as well as the oxidative stability of the meat have been improved by the use of probiotics, as demonstrated in the work of Salaj et al. (2013). Probiotics also have a moderate effect on cohesiveness, firmness, chewiness and elasticity of cooked breast meat (Duskaev et al, 2020) and A significant improvement of taste, smell, and color of poultry meat (Saleh et al., 2013).

In layer hens, probiotic supplementation improves the egg production percentage (Menconi et al., 2014b). It also ameliorates the quality of the eggs through increasing the eggshell strength and thickness leading to a decrease in the number of broken eggs (Krysiak et al., 2021). Furthermore, probiotics increases egg weight due to the increase in albumen weight percentage (Neupane et al., 2019). In breeder flocks, fertility and hatching capacity of eggs improved after

probiotic administration (Mazanko et al., 2018). However, Soltan et al. (2008) reported a difference in eggshell quality that maybe a consequence of the increased mineral and protein absorption by the birds.

2. Probiotics under heat stress condition

Probiotics were shown to be effective under heat stress conditions in the poultry industry. Regarding the animal's productive capacity, feed additives containing *Lactobacillus* strains had a positive effect on growth and feed conversion factor (Jahromi et al.,2016), also an improved feed intake in broiler (Song et al.,2014). Moreover, in laying flocks, the administration of probiotic has resulted in increased laying (Deng et al., 2012). Probiotics increase the activity of thyroid hormones, whose secretion decreases during heat stress. Thyroid hormones have a significant impact on the metabolism of the body and normal growth and development. Returning T3 (triiodothyronine) and T4 (thyroxine) hormones to the correct level could reduce the number of abnormal changes in the intestinal tissues and increase the growth of birds (Sohail et al, 2010).

In a study on broilers exposed to a temperature of up to 35 ◦C, probiotic addition in feed increased final body weight and carcass weight; and improved absorption of sugars (Jahromi et al., 2016). In another study, dietary probiotic contributed to an increase in the thoracic muscle weight. It also helped in alleviating heat stress effects, such as reducing the water content of broiler carcasses. This is important because high ambient temperature causes a decrease in pH which leads to denaturation and impairment of protein function. Probiotic lowers the water loss in the pectoral muscle immediately after slaughter and at retail (Cramer et al., 2018).

The effective probiotics against heat stress is also confirmed by studies conducted on laying hens. It had a positive effect on the performance parameters of heat-stressed hens, such as average daily feed intake and egg weight which were higher. This was due to the increased

thickness and strength of the shell and the increased albumen percentage in the eggs. In addition, the administration of probiotic bacteria resulted in the improvement of intestinal microflora by reducing pathogenic microorganisms (Zhang et al., 2016; Neupane et al., 2019). Other study, on the probiotic effect on egg traits in laying hens reported that probiotic supplementation may play an important role in altering the lipid metabolism of chickens and subsequently reduce the cholesterol content of egg yolk (Mikulski et al. 2012).

C. Postbiotic effect

Antibiotic use in poultry production has raised immense public health concerns. These include the presence of drug residues in poultry products, and emergence and spread of antibiotic-resistant strains of bacteria (Nhung et al., 2017). Moreover, increasing people awareness of the need to consume safe and healthy foods especially of animal origin, has led to the ban of the use of AGPs in the EU in 2005 (EPC, 2005), and subsequent restriction in America and other countries (Editors, 2017). This probation of the antibiotic use requires the development of generally acceptable and sustainable feed additive(s) that will favorably impact overall poultry performance and health while also improving farmers' income.

Implementing some farming practices may help reduce antibiotic use. Some of these practices include good hygiene, adequate vaccination, improved breeding, animal welfare and husbandry practices. These practices may not totally reduce the risks of infection or pathogens carriage (Cervantes 2015), but will definitely increase the cost of production (Rine et al., 2021).

Several agents have been tested including prebiotics, probiotics, enzymes, antimicrobial peptides (AMP), organic acids, bacteriophages, symbiotic, metal, clay, hyperimmune egg yolk IgY, phytogenics and most recently, postbiotics (Humam et al. 2018). Although most of these alternatives have continuously generated increased attention over the years, studies have been

primarily centered on prebiotics, probiotics and lately postbiotics that gained more global interest.

The essential point in probiotics effectiveness is the viability of the microbial strains, which consequently confers health benefits for the host. Nevertheless, new evidence shows that probiotics' viability is not necessary for exerting the desired health benefits. Various probiotic components such as cell free supernatant, purified cell wall and non-viable whole-cell are able to mimic probiotics benefits (Mohamadshahi et al. 2014). Furthermore, the probiotic health benefits may be exerted by the non-viable components, which are now known as postbiotics (Rad et al. 2020).

In addition, research demonstrated numerous emerging concerns associated with probiotic supplementation. These concerns include virulence factors in microbial strains used as probiotics, distribution of undesired antimicrobial resistance genes in the gut bacterial community, eruption of inflammatory response, emergence and spread of fungemia, the formation of a persistent microbial colony that may prevent colonization by normal gut microflora, development of endocarditis and translocation to blood and different tissues (Kothari et al., 2019). Furthermore, Marteau and Shahanan (2003) highlighted in the association between the application of probiotics and the occurrence of antibiotic resistance genes, especially those encoded by plasmids which can be easily transferred between organisms. Gene transfer between bacteria can occur in three different types (figure 2)

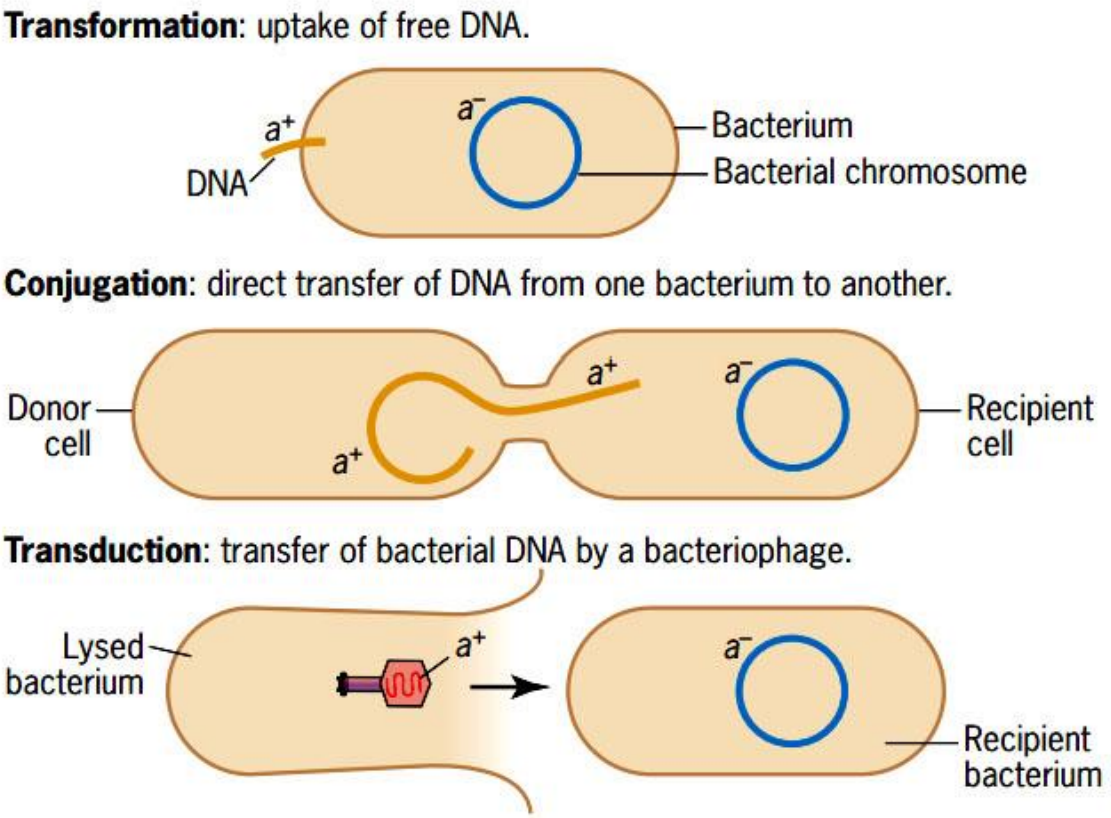

Figure 2 Three types of gene transfer between bacteria

Another study detected virulence genes from bacterial strains used in probiotic preparations (Wassenaar and Klein, 2008). This suggests that undesired traits may be horizontally spread into the gut microflora and can result in the acquisition of resistance or virulence genes in microbial strains (Kataria et al. 2009). Furthermore, it has been reported that an over-aggressive inflammatory response in probiotics (Zhang et al. 2005). These concerns necessitated the use of a safer alternative in sustainable poultry production, namely postbiotics (Haileselassie et al. 2016).

1. Postbiotics: concept and classes

Postbiotic is a moderately new term also regarded as either cell-free supernatants (CSF), metabiotics, biogenics, or simply metabolites. Postbiotics are defined as" non-viable bacterial products or metabolic by-products produced from probiotic microorganisms that have biologic activity in the host" (Patel and Denning 2013). These microbial products also called soluble factors are often produced either by live microorganisms or released after their inactivation or lysis (De Almada et al. 2016). These soluble factors synthesized by different microbial strains include enzymes, short chain fatty acids, peptides, organic acids (e.g. lactic acid, acetic acid), plasmalogens, endo- and exo-polysaccharides, ethanol, polyphosphates, teichoic acids, diacetyl, lactocepins, vitamins (e.g. B-group vitamins), cell surface proteins, muropeptides, hydrogen peroxide and teichoic acids (Rad et al. 2020).

Nonetheless, parabiotics, known as inactivated or non-viable microbial cells which confer health and nutritional benefits when administered to the host (de Almada et al. 2016) may also be referred as postbiotics in case non-viable microbial cellular structures exert beneficial biological function (Patel and Denning 2013).

Furthermore, postbiotics have several impressive features including distinctive chemical structures, shelf-life longevity, and safety dose (Tomar et al. 2015). Postbiotics have shown positive absorption, distribution, and excretion abilities, which could consequentially impact a wide range of host organs and tissues thereby exerting different biological functions (Shenderov, 2013).

2. Effects and bioactivities of postbiotics

Until recently, the most widely studied postbiotics are those obtained from strains of *Lactobacillus* and *Bifidobacterium* (Rine et al., 2021). However, other recent studies have also reported *Faecalibacterium* and *Streptococcus* spp. as good postbiotic sources (Iweala and

Nagler 2019). Studies conducted on postbiotics reported their positive effect on the intestinal microbiota modulation, immune system stimulation, pathogen antagonism and anti-inflammatory effects among others (Compare et al. 2017).

Several studies have emerged on the postbiotics' positive effect on the immune system. These microbial products were shown to be effective on immunity in vitro (Haileselassie et al. 2016). Moreover, postbiotics were beneficial against the invasive Salmonella inflammatory response in the intestinal mucosa of mice (Tsilingiri et al. 2012); and in patients with post-infectious bowel syndrome (Compare et al. 2017). This indicates that postbiotics have an immunomodulatory effect just like probiotics (Rine et al., 2021). Moreover, it has been proposed that a correlation exists between the degree of immune-stimulatory activity of microbial strains and their postbiotics (De Almada et al. 2016).

Postbiotics can inhibit potential pathogens. Postbiotics obtained from different strains *L. plantarum* including RI11, RG11, RG14, RS5, TL1 and UL4 successfully inhibited pathogens such as Salmonella enterica S-1000, Listeria monocytogenes L-MS, vancomycin-resistant Enterococci, and E. coli E − 30 (Kareem et al. 2014). Another study on poultry demonstrated that postbiotic supplementation inhibits or lyses potential pathogens and also mitigate toxin production and virulence expression (Homayouni Rad et al. 2020). Additionally, postbiotics play and important role in the digestive system, it fortifies endogenous beneficial microorganisms within the gut of the host; and also have hepatoprotective ability (Aguilar-Toalá et al. 2018). This activity by postbiotics is suggested to surpass the one resulting from the supplementation with probiotic (Iweala and Nagler 2019).

Postbiotics have a similar mechanism of action and capacity as probiotics (Thanh et al., 2009). Since these microbial by-products contain antimicrobial metabolites, such as organic acids and bacteriocins, they can reduce the gut pH and inhibit the proliferation of opportunistic pathogens in the feed and gut of animals (Aguilar-Toalá et al. 2018). Studies have shown that

postbiotics prepared out of *Lactobacillus plantarum* exhibit inhibitory action on various pathogenic bacteria, including Listeria monocytogenes, Salmonella typhimurium, Escherichia coli and vancomycin-resistant Enterococci (Aguilar-Toalá et al. 2018). The application of postbiotics as a feed additive in livestock promotes the growth performance and health of broilers (Kareem et al., 2016), layers (loh et al., 2014) and piglets (loh et al., 2013), as well as enhancing rumen fermentation and health in ruminants (Izuddin et al., 2019).

3. Postbiotics under heat stress conditions

In broilers under normal condition, postbiotic supplementation improves growth performance and health by promoting the immune status and gut health through the improvement of intestinal villi and increased lactic acid bacteria population and reduction of Enterobacteriaceae population and fecal pH (Kareem et al., 2016). In addition, the potential antioxidant capacity of postbiotic, especially the one obtained from *L. plantarum*, has been found to be particularly strong under heat-stress conditions (Ji et al., 2019).

Different postbiotics produced from different *Lactobacillus plantarum* strains, and defined as RI11, RS5 and UL4 were tested on broiler under heat stress conditions. The study concluded that supplementation with postbiotics, especially RI11, improved growth performance, namely weight gain, feed conversion rate. It also improved intestinal morphology, enhanced lactic acid bacteria count and intestinal villi height, and reduced the Enterobacteriaceae count. In addition, postbiotics significantly ameliorated immune response in heat stressed chickens (Humam et al., 2019).

The postbiotic metabolite combinations have been demonstrated to be more effective than using a single metabolite in broiler (Loh et al., 2010) and pig's diets (Thu et al., 2011). Furthermore, different combinations of postbiotic metabolites of *L. plantarum* strains had shown inhibitory activities against Escherichia coli, Listeria monocytogenes, Salmonella

typhimurium, vancomycin resistant enterococci (Thanh et al., 2010) and Pediococcus acidilactici (Choe et al., 2013).

A study conducted on layer hens, using five strains of *L. plantarum* (TL1, RS5, RG14, RG11 and RI11) demonstrated the positive effect of postbiotic metabolite combinations supplementation on performance parameters. Result showed an increase in hen-day egg production, and also a reduction in the fecal pH and fecal Enterobacteriaceae population. These combinations also increase the fecal lactic acid bacteria, reduce the plasma and yolk cholesterol, and increase the fecal volatile fatty acids content. However, it showed no significant differences in the feed intake, egg weight, egg mass and feed conversion efficiency (Loh et al., 2014). In contrast, Mahdavi et al. (2005) reported that the inclusion of lactic acid bacteria cultures did not affect any egg production parameters. The variations in the results were most probably due to the difference in bacteria strains, concentration and form which was used (Mahdavi et al., 2005). The supplementation of metabolite in the diets of laying hens may exert different effects compared to live probiotic cultures (Loh et al., 2014).

CHAPTER III

MATERIALS AND METHODS

A. Field Evaluation of the preparation of dietary supplements

1. Lactobacillus Plantarum strain

The *Lactobacillus plantarum* strain RS5 (NCIMB 701088) is obtained from NCIMB laboratory in the United Kingdom. This strain was isolated by A A Nichols from cheese.

The bacterial culture was resuspended in Man, Rogosa and Sharpe (MRS) broth and incubated at 37◦C for 48 hours. The solution was subjected to Gram staining for confirmation. Gram positive, non-spore-forming rods were inspected under microscope. The suspension was sub-cultured on MRS agar growth medium for 48 hours at 37°C. A couple of white round colonies were randomly selected, part of which was sub-cultured on MRS agar (streaking for isolation), and the other part was inspected by Gram staining procedure for confirmation. Colonies were re-suspended in sterile 0.85% (w/v) saline solution. Transmittance of this bacterial suspension was adjusted to 3% at 450 nm wavelength. After a serial dilution, culture on MRS agar, and colonies count it has been shown that this mother solution contains 10^{15} CFU/ml. The initial bacterial cultures were preserved at −80 ◦C in MRS broth.

2. Preparation of Postbiotics from L. plantarum Strains

Working cultures of *L. plantarum* were prepared by inoculating 10% (v/w) 10^9 CFU/mL active bacterial cells into MRS media and incubated at 30°C for 10 h, followed by centrifugation (Eppendorf 5810 centrifuge, Eppendorf, Maryland, USA) at 10,000× g and 4°C for 15 min. The cell-free supernatant (CFS) was then collected by filtration through a cellulose acetate membrane of 0.22 microns pore size (Loh, et al. 2014). The CFS was stored at −20 ◦C until the feeding trial was conducted. The liquid postbiotics were mixed with the feed using the

three-way mixing technic in a horizontal feed mixer, at a concentration of 300 ml of solution (CFS in MRS broth) per 100 kg of feed.

3. Preparation of Probiotics from L. plantarum Strains

The culture medium used for bacterial growth was MRS agar. The overnight culture of *Lactobacillu*s isolate was than inoculated for 24 to 48h. The colonies were harvested and resuspended in phosphate buffered saline (PBS, pH 7.4) and count was adjusted to 3.10^9 CFU/mL using spectrophotometry. The suspension was mixed with the basal diet at a concentration of 200 ml of solution (RS5 in MRS broth) in every 100 kg of feed, using the three-way mixing technic in a horizontal mixer.

4. Birds Housings & Treatments

This experiment was conducted at the research facilities (AREC) of the American University of Beirut in the Beqaa region in four identical environmentally controlled poultry houses. The trial was completed over a period of 6 months including one month of adaptation and 5 months of experimental phase. Birds egg production an initial body weight was recorded during the adaptation phase, based on these results birds were allocated into homogenous groups. A total of 192 twenty-week-old pullets of an Isa white strain, were equally subdivided into six groups of 32 birds individually caged, where each bird was considered a replicate. Birds in each group were subdivided into two houses, each pen holed 16 birds. Birds in the first two houses were reared under regular temperature, while those in the second two houses were reared under cyclic heat stress conditions, where the temperature gradually reached about 30°C for 4 consecutive hours daily. Temperature was monitored daily at 10am, 1pm & 4pm, and once a week at 4am. Birds in each house were equally divided into 3 categories according to the offered diet: control, control+probiotic and control+postbiotc.

The birds were given water and feed ad libitum, provided as per the Manual recommendations (Institut de Sélection Animale BV, Villa 'de Körver', Boxmeer, Netherland). At arrival, birds were granted two weeks of adaptation. Afterwards, hens were allocated to different treatments according to live body weight and egg production to ensure homogeneous grouping at the beginning of the experiment.

Birds were assigned to six different treatments as detailed in table 1. The experimental design is described in figure 3.

Table 1 Control and experimental groups

Treatment	**Temperature**	**Diet**	**Cages**	**Replication**
1	Regular	Control	32	32 birds
2	Regular	Probiotic	32	32 birds
3	Regular	Postbiotic	32	32 birds
4	Cyclic heat stress	Control	32	32 birds
5	Cyclic heat stress	Probiotic	32	32 birds
6	Cyclic heat stress	Postbiotic	32	32 birds

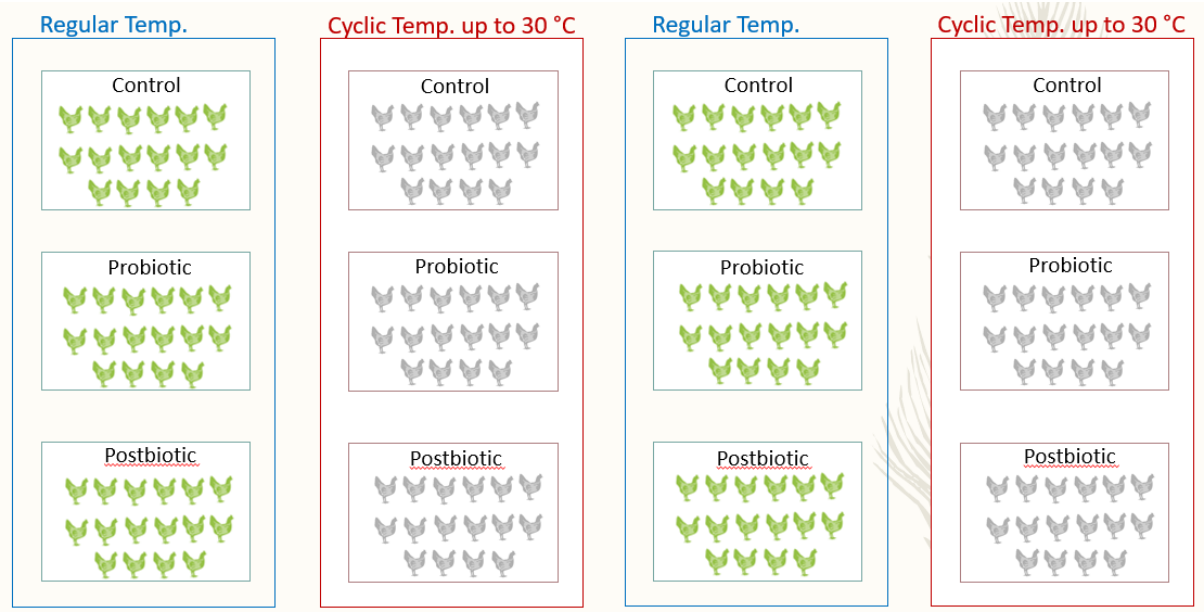

Figure 3 Experimental Design

5. Evaluation of hens' production parameters

The initial live body weight and the egg production were recorded for all birds at the end of the adaptation phase in order to allocate birds into different treatment homogeneously. Afterwards, egg production was recorded on a daily basis, and the live body weight of 4 birds per treatment was measured at sacrifice, i. e. at the middle and the end of the experimental phase.

The feed intake was measured once weekly. Twelve eggs per treatment were randomly collected to evaluate egg quality namely egg weight, Haugh unit, eggshell thickness, yolk color, density, white weight, yolk weight and shell weight. The egg quality was measured monthly for 3 consecutive days.

6. Evaluating hens' visceral organs weight index

Four birds were sacrificed from each treatment in order to measure visceral organ indices namely: liver, spleen, gizzard, proventriculus, duodenum, jejunum, ileum, and abdominal fat. This process was carried out 2 times during the whole experimental phase at middle and again at the end of the trial.

7. Statistical Design and Analyses

The design of the trial is a factorial arrangement of treatments in a randomized block design, factorial 2*3 with 6 treatments and 32 birds/replicate per treatment. Univariate analyses were used to analyze the data and mean comparison at 95% confidence level. Analysis was performed using SPSS software (Statistical Package for the Social Sciences, V. 25).

CHAPTER IV

RESULT AND DISCUSSION

A. Egg production

Eggs from individual birds or cages were collected daily. The hen-day egg production was calculated as the percentage of production per treatment per month during the 5 months of the experiment. Results are presented in Table 2.

A numerical decrease in egg production was observed in heat stressed animals. Other reports show that heat stress significantly reduces egg production due to decrease in feed intake and the uptake of available nutrients and decreased digestibility of many components of the diet (Deng et al., 2012; Allahverdi et al., 2013). Variable results may be explained by the use of birds of different age or genetic background, as well as due to variable intensity and duration of the heat stress treatments applied (Lucas and Marcos, 2013), knowing that temperature reached a maximum of 30°C in this study. Another potential factor is that heat stress might also be accompanied by other stressors, such as limited housing space, insufficient ventilation, unbalanced feed ration and/or pathogens contamination (Lara et Rostagno, 2013) that were not observed in this experiment.

Postbiotic supplementation in feed showed a faster effect on percentage egg production than probiotic supplementation in this experiment. Overall, hens with supplemented postbiotic in their diet showed a significantly higher ($p<0.05$) egg production than the control group and a numerically higher value ($p>0.05$) than probiotic group. Especially for month 2 of the experiment, postbiotic diet had a significant impact ($p<0.05$) in comparison to the other 2 diets (control and probiotic). This might be due to a slower effect of probiotics on egg production. Other research is in agreement with our findings knowing that probiotic supplementations increase laying, so it improves the egg production

percentage (Menconi et al., 2014b; Deng et al., 2012). In other studies, postbiotics were shown to increase hen- day egg production (Loh et al., 2014) due to the increased feed conversion rate but also the improved immune response in chickens (Humam et al., 2019).

None of the interactions between the different feed and temperature parameters was significant for egg production or percent broken and shelless eggs for the entire experimental phase. A lower percentage of broken eggs was observed in heat stressed birds due to bad cage structure in the control group. However, probiotics were shown to ameliorate the quality of the eggs through increasing the eggshell strength and thickness leading to a decrease in the number of broken eggs (Peralta-Sánchez et al., 2019).

Table 2 Percentage egg production and percentage broken or shelless eggs of layer hens under different feed and temperature parameters during the 5 months of the experiment.

Treatment	Percentage egg production						Percentage Broken & Shelless eggs				
	Month 1	Month 2	Month 3	Month 4	Month 5	ALL	Month 2	Month 3	Month 4	Month 5	ALL
Feed											
Control	96.8	94.0[a]	92.4	93.1	90.6	92.6[a]	0.2	1.1	1.5	2.9	1.3
Probiotic	96.3	94.1[a]	93.1	91.7	91.1	93.1[ab]	0.3	1.6	1.6	2.3	1.2
Postbiotic	97.4	97.2[b]	94.8	92.6	93.8	94.8[b]	0.1	1.5	1.3	2.4	1.1
SEM	1.23	1.26	1.83	1.32	1.65	1.10	0.15	0.46	0.52	0.73	0.26
Temperature											
Control	97.7	95.4	93.9	92.7	91.8	93.9	0.3	1.6	1.8	3.0	1.4[a]
Heat Stress	95.9	94.9	93.0	92.3	91.9	93.2	0.2	1.3	1.2	2.1	0.9[b]
SEM	1.00	1.02	1.49	1.08	1.35	0.89	0.12	0.38	0.42	0.59	0.22
Variables											
Feed	.649	.017	.387	.578	.109	.105	.266	.497	.802	.636	.782
Heat Stress	.082	.670	.544	.701	.900	.410	.413	.439	.115	.138	.029
Feed * HS	.291	.643	.252	.674	.614	.189	.225	.267	.748	.409	.337

[a–b] Means within a column in each comparison group with no common superscripts differ significantly (P<0.05).[1]

[1] Total of 192 birds, 32 birds/ treatment

B. Feed intake

Feed intake was measured by subtracting the balance of feed from the quantity originally supplied to the laying hens. Results are presented in Table 3.

The heat stress negatively affected the birds during the first month (79.1g vs 84.2g for the control; $p<0.05$) however they quickly adapted to the elevated temperature. In other studies, birds exposed to high ambient temperature show a significantly lower feed intake and a decreased digestibility of many components of the diet (De Rensis and Scaramuzzi, 2003; Deng et al., 2012; Allahverdi et al., 2013). This might be due to the intensity of the heat in this experiment, reaching only 30°C, and the adaptive capacity of the birds (Lucas and Marcos, 2013).

As per the changes in the diet, the individuals that were under probiotic or postbiotic diet showed a significantly higher feed intake (FI), especially during the first (82.9g vs 82.2g vs 79.7 for the control; $p<0.05$) and third month (99.1 vs 99.8 vs 94.6 for the control; $p<0.05$). Abundant research shows that probiotics prepared from *Lactobacillus* improved feed intake in chickens (Song et al.,2014; Zhang et al., 2016; Neupane et al., 2019). As for postbiotic, research reported a higher, though no significant, increase in feed intake (loh et al., 2014; Humam et al., 2019) which correlates with the overall result. However, the probiotic diet showed an unusual decrease in FI during the fourth month which might be consistent with the small decrease in egg production in probiotic groups during this month. Overall neither the temperature nor the feed had a significant effect on the birds feed intake.

Table 3 Birds average feed intake under different temperature and feed parameters during the 5 months of the experiment

Treatment	Average daily Feed Intake (g)					
	Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**ALL**
Feed						
Control	79.7[a]	94.8	94.6[a]	105.6[a]	112.1	93.8
Probiotic	82.9[b]	93.1	99.1[b]	101.4[b]	112.2	94.4
Postbiotic	82.2[b]	94.4	99.8[b]	106.5[a]	112.8	95.7
SEM	1.01	1.06	1.63	2.09	1.68	2.06
Temperature						
Control	84.2[a]	94.4	97.4	100.9	111.4	94.8
Heat Stress	79.1[b]	93.8	98.5	108.1	113.2	94.6
SEM	0.82	0.86	1.33	1.71	1.37	1.67
Variables						
Feed	.004	.268	.004	.031	.888	.648
Heat Stress	.000	.450	0289	.000	.192	.901
Feed * HS	.998	.166	.909	.003	.463	.875

[a–b] Means within a column in each comparison group with no common superscripts differ significantly (P<0.05).[2]

[2] Total of 192 birds, 32 birds/ treatment

C. Egg quality

Twelves eggs were collected from each treatment monthly for 3 consecutive days. The eggs were tested on the same day. After weighing, the gravity was measured than the egg was broken and placed on to measuring plates. The yolk color, thickness of the shell, Haugh unit, yolk weight and the shell weight were measured, calculated, and recorded. Results are presented in Tables 4 & 5.

The heat stress showed a negative impact ($p<0.05$) on the egg weight, percent shell weight, Haugh unit, shell thickness, and yolk color. Other studies agree with our findings, whereby decreased egg weight under high ambient temperatures has been reported extensively, and low egg weight is correlated with reduced feed intake. This might be an adaptive stress response to conserve metabolic energy (Star et al., 2009; Mack et al., 2013). Additionally, it has been previously reported that exposure to high temperature negatively affects yolk weight, albumen weight, specific gravity, Haugh unit and yolk index (Wiernusz et al., 1998; Zaviezo et al., 1999). This might be due to the decline in feed digestibility such as proteins, fats, and starch (Bonnet et al., 1997). Other reports show the negative effect of birds panting under heat stress on eggshell. Accelerated panting increases carbon dioxide levels and higher blood pH, alter acid- base balance (i.e., alkalosis). This will hamper blood bicarbonate availability for eggshell mineralization, induces increased organic acid availability, and decreases free calcium and phosphorus concentration in the blood (Marder et Arad, 1989; Mack et al., 2013).

The study showed that high ambient temperature did not have a significant effect on the specific gravity probably because the eggs were freshly tested. It did not have a significant impact on percentage yolk weight ($p>0.05$) which could be due to the high impact heat stress had on the percentage shell weight.

Remarkably, a positive effect was demonstrated on egg white percentage. Although it contradicts other study findings (Wiernusz et al., 1998; Zaviezo et al., 1999), the increment of

total proteins and albumin concentrations in heat stressed birds was reported and can be considered as a sort of protection of muscle mass against injury induced by thermal challenge (Al-Zghoul et al., 2015). As reported this study, the percentage white weight increased however Haugh unit decreased which indicates a higher albumen content and a low albumen quality in the produced eggs.

As per the different diets, the supplementation of metabolites in the diets of laying hens may exert different effects compared to live probiotic cultures (Loh et al., 2014). This study shows that both treatments, probiotics and postbiotics had a positive impact ($p<0.05$) on egg weight, although probiotics had a more significant and gradual effect. This agrees with other studies that show an increase in egg weight in birds consuming probiotics. This increase could be due to the increase in albumen weight percentage (Zhang et al., 2016; Neupane et al., 2019). However, for birds consuming postbiotics, other research revealed an increase in egg weight that wasn't significant (Loh et al.,2014). The fluctuating results might be due the bacterial strain, concentration, and route of administration being used (Mahdavi et al., 2005).

Probiotics showed a significantly ($p<0.05$) lower percentage of shell weight; a different outcome in comparison to other studies (Zhang et al., 2016; Neupane et al., 2019; Peralta-Sánchez et al., 2019). That may be a consequence of the variation in mineral and protein absorption (Soltan et al., 2008). It can be also due to the harmful effect of heat stress on blood pH (Mack et al., 2013), the digestibility of many components of the diet (Allahverdi et al., 2013), and the decreased plasma protein and calcium levels (Zhou et al., 1998)

The percentage egg white weight was significantly lower ($p<0.05$) in postbiotic group. In Contrast, Mahdavi et al. (2005) reported that the inclusion of lactic acid bacteria cultures did not affect any egg production parameters. There is a scarcity of reports documenting the effect of postbiotics on egg white weight. However, as previously stated, the variations in the results

were most probably due to the difference in bacterial strains, concentration, and route of administration being used (Mahdavi et al., 2005).

The Haugh Unit was significantly higher in probiotic group (91.7% vs 89.9% for the control; $p<0.05$) due to the increase in albumen weight percentage (Neupane et al., 2019). The Haugh Unit can be associated with the increase in percentage white weight in the probiotic group.

Egg yolk color intensity has been correlated with cholesterol amount in the yolk (Hajati & Zaghari, 2019). In this study, the yolk color was significantly lower in probiotic group in comparison to the control group (7.0% vs 7.2%, respectively; $p<0.05$). As demonstrated in other studies, probiotic supplementation may play an important role in altering the lipid metabolism of chickens and subsequently reduce the cholesterol content of egg yolk (Mikulski et al. 2012). Also, postbiotic group showed a lower yolk color that might be due to a reduced plasma and yolk cholesterol (Loh et al.,2014)

Specific gravity didn't show differences among groups, as mentioned before, probably because the eggs were freshly tested. Also, the percentage yolk weight did not show significant differences ($p>0.05$) and neither the shell thickness. However, other studies reported an increase in eggshell thickness and strength under probiotic supplementation (Krysiak et al., 2021).

In conclusion, the study showed probiotic supplementation under heat stress condition had a positive effect on the egg weight and Haugh unit. Also, postbiotic improved percentage white weight and the egg weight.

Table 4 Percentage egg weight, shell weight, white weight and yolk weight of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed.

TREATMENT	EGG WEIGHT (G)					% SHELL WEIGHT					% WHITE WEIGHT					% YOLK WEIGHT				
	Jul	**Aug**	**Sep**	**Oct**	**ALL**	**Jul**	**Aug**	**Sep**	**Oct**	**ALL**	**Jul**	**Aug**	**Sep**	**Oct**	**ALL**	**Jul**	**Aug**	**Sep**	**Oct**	**ALL**
FEED																				
CONTROL	54.2^{a}	55.3	57.6^{a}	60.1^{a}	56.8^{a}	14.9^{ab}	14.9	15.4^{a}	15.1	15.1^{a}	60.4^{ab}	60.6^{a}	58.9^{a}	59.0	59.7^{a}	24.7	24.7^{a}	25.9	26.1	25.4
PROBIOTIC	55.5^{b}	55.8	59.8^{b}	62.1^{b}	58.2^{b}	14.6^{a}	14.9	14.5^{b}	15.1	14.8^{b}	60.7^{a}	60.1^{ab}	60.2^{b}	59.3	60.1^{a}	24.7	25.1^{ab}	25.8	25.7	25.3
POSTBIOTIC	54.4^{ab}	55.7	58.6^{ab}	61.4^{b}	57.5^{ab}	15.3^{b}	15.2	15.2^{a}	15.5	15.2^{a}	59.9^{b}	59.7^{b}	59.1^{a}	58.5	59.3^{b}	24.8	25.4^{b}	25.9	26.3	25.6
SEM	.65	.58	.63	.57	.37	.27	.24	.26	.25	.13	.44	.43	.46	.42	.23	.33	.33	.32	.33	.17
TEMPERATURE																				
CONTROL	55.2^{a}	56.8^{a}	59.3^{a}	61.0	58.0^{a}	15.1	15.2	15.4	15.3	15.3^{a}	60.5	59.9	59.1	58.4^{a}	59.5^{a}	24.4^{a}	24.9	25.9	26.3^{a}	25.4
HEAT STRESS	54.1^{b}	54.4^{b}	57.9^{b}	61.3	56.9^{b}	14.8	14.9	14.7	15.1	14.9^{b}	60.1	60.4	59.6	59.4^{b}	60.0^{b}	25.1^{b}	25.2	25.8	25.8^{b}	25.5
SEM	.53	.48	.52	.47	.31	.22	.20	.21	.21	.11	.36	.36	.38	.34	.18	.27	.27	.26	.27	.14
VARIABLES																				
FEED	.096	.643	.004	.002	.001	.021	.540	.002	.245	.001	.131	.091	.016	.210	.002	.925	.094	.794	.182	.191
HEAT STRESS	.045	.000	.012	.530	.001	.227	.122	.492	.263	.000	.270	.168	.159	.003	.024	.015	.414	.639	.046	.681
FEED * HS	.269	.212	.093	.000	.012	.827	.525	.147	.527	.524	.714	.581	.128	.165	.079	.320	.706	.016	.105	.020

[a–b] Means within a column in each comparison group with no common superscripts differ significantly (P<0.05).[3]

[3] Total of 192 birds, 32 birds/ treatment

Table 5 Specific gravity, Haugh Unit score, shell thickness and yolk color of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed.

TREATMENT	GRAVITY					HU SCORE					SHELL TICKNESS					YOLK COLOR				
	Jul	**Aug**	**Sep**	**Oct**	**ALL**	**Jul**	**Aug**	**Sep**	**Oct**	**ALL**	**Jul**	**Aug**	**Sep**	**Oct**	**ALL**	**Jul**	**Aug**	**Sep**	**Oct**	**ALL**
FEED																				
CONTROL	1.095	1.093^{a}	1.091	1.091	1.091	96.0^{ab}	88.2^{a}	85.9	89.4	89.9^{a}	.36	.37	.38	.38	.37	7.7	7.7^{a}	6.6	6.9^{a}	7.2^{a}
PROBIOTIC	1.095	1.091^{b}	1.093	1.090	1.090	98.2^{a}	91.3^{b}	87.0	90.5	91.7^{b}	.37	.36	.38	.38	.37	7.4	7.3^{b}	6.5	7.1^{b}	7.0^{b}
POSTBIOTIC	1.091	1.091^{b}	1.092	1.090	1.090	94.6^{b}	89.6^{ab}	86.6	89.4	90.1^{a}	.37	.37	.37	.38	.37	7.5	7.3^{b}	6.7	7.0^{ab}	7.1^{ab}
SEM	.001	.001	.001	.001	.001	1.24	1.37	1.31	1.28	.72	.004	.004	.004	.006	.002	.17	.12	.01	.09	.07
TEMPERATURE																				
CONTROL	1.096	1.093	1.092	1.091	1.091	96.4	91.0^{a}	87.2	90.8	91.3^{a}	.37	.37^{a}	.38	.38	.38^{a}	7.8^{a}	7.4	6.7	7.0	7.2^{a}
HEAT STRESS	1.095	1.091	1.092	1.090	1.091	96.4	88.4^{b}	85.9	88.7	89.8^{b}	.36	.36^{b}	.37	.38	.37^{b}	7.3^{b}	7.5	6.5	6.9	7.1^{b}
SEM	.001	.001	.001	.001	.000	1.01	1.12	1.07	1.05	.59	.004	.003	.003	.005	.002	.14	.09	.08	.08	.06
VARIABLES																				
FEED	.557	.010	.287	.452	.689	.017	.094	.702	.590	.020	.317	.753	.222	.979	.881	.279	.000	.168	.030	.114
HEAT STRESS	.344	.059	.644	.611	.106	.747	.021	.547	.052	.008	.156	.028	.077	.999	.020	.000	.849	.253	.492	.003
FEED * HS	.0.97	.635	.527	.754	.289	.444	.670	.031	.636	.837	.687	.803	.096	.857	.365	.003	.002	.783	.147	.897

$^{a–b}$ Means within a column in each comparison group with no common superscripts differ significantly (P<0.05).[4]

[4] Total of 192 birds, 32 birds/ treatment

D. Visceral organ indices

Four birds per treatment were sacrificed at the middle and the end of the experiment in order to evaluate the visceral organ indices. Results are presented in Tables 6 & 7.

Heat stress showed a significant effect ($p<0.05$) on birds' weight and percentage spleen weight. This effect is initiated by the reduced plasma calcium and phosphorous concentrations under heat stress in laying hens which lowers relative weights of the thymus and the spleen (Ghazi et al., 2012). In addition, growth rates decrease due to the decline of feed digestibility such as proteins, fats, starch (Bonnet et al., 1997; Deng et al., 2012).

However, heat stress didn't affect any of the other visceral organ; liver, gizzard, proventriculus, duodenum, jejunum, ileum, and abdominal fat. In contrast, Felver-Gant et al. (2012) reported reduced liver weights in laying hens subjected to chronic heat stress conditions. The difference in the reported results may be explained, as mentioned before, by the fact that birds of different age or genetic background were used, as well as due to variable intensity and duration of the heat stress treatments applied (Lucas and Marcos, 2013). Additional factors might have aggravated the situation such as limited housing space, insufficient ventilation, unbalanced feed ration and/or pathogens contamination (Lara et Rostagno, 2013).

The different feed treatments did not have any effect on the bird's visceral organ weight, except for the ileum that showed a significantly lower percentage weight ($p<0.05$) under postbiotic supplementation and a slight decrease in ileum weight under probiotic supplementation to feed. In agreement with our findings, it's reported by Dizaji et al. (2012) that weight of Proventriculus, Gizzard, Liver and Bursa did not show any significant difference by addition of probiotics. Moreover, probiotics have a positive effect on animals' physical properties of meat, namely poultry carcass quality by increasing overall carcass weight and reducing abdominal fat (Hidayat et al., 2016). It is supposed that postbiotics mimic the impact of the microbial strain (Mohamadshahi et al. 2014). Furthermore, dietary probiotic did not

affect the relative weight of the duodenum, jejunum, ileum, or small intestine in broilers, although ileum weight was numerically lower at day 40 (Wang et al. 2016). In this experiment, the reduced ileum size may reflect a more efficient absorption and utilization of nutrients (Dibner and Richards, 2005).

Table 6 Birds weight, percentage liver weight, spleen weight, gizzard weight and proventriculus weight of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed

TREATMENT	BIRDS WEIGHT (G)			% LIVER WEIGHT			% SPLEEN WEIGHT			% GIZZARD WEIGHT			% PROVENTRICULUS WEIGHT		
	Mid	End	ALL	Mid	End	ALL	Mid	End	ALL	Mid	End	ALL	Mid	End	ALL
FEED															
CONTROL	1598	1567	**1582**	3.01	3.30	**3.14**	.102	.095	**.099**	1.35^{ab}	1.37	**1.36**	.36^{a}	.41	**0.39**
PROBIOTIC	1535	1572	**1553**	2.99	3.47	**3.23**	.099	.093	**.096**	1.44^{a}	1.33	**1.39**	.41^{b}	.41	**0.41**
POSTBIOTIC	1511	1551	**1530**	2.99	3.29	**3.13**	.091	.093	**.092**	1.29^{b}	1.39	**1.34**	.39^{ab}	.40	**0.39**
SEM	46.5	48.6	**35.7**	.177	.217	**.149**	.009	.009	**.006**	.074	.062	**.050**	.021	.025	**.017**
TEMPERATURE															
CONTROL	1584	1579	**1581^{a}**	3.03	3.23	**3.12**	.100	.103^{a}	**.101^{a}**	1.42	1.37	**1.39**	.39	.41	**0.40**
HEAT STRESS	1512	1547	**1529^{b}**	2.97	3.47	**3.21**	.095	.084^{b}	**.090^{b}**	4.29	1.36	**1.33**	.38	.40	**0.39**
SEM	37.9	39.7	**29.2**	.145	.179	**.123**	.007	.007	**.005**	.060	.051	**.040**	.017	.021	**.014**
VARIABLES															
FEED	.187	.904	**.367**	.991	.651	**.752**	.453	.951	**.554**	.127	.701	**.594**	.065	.898	**.423**
HEAT STRESS	.076	.431	**.084**	.726	.195	**.482**	.500	.024	**.028**	.055	.820	**.114**	.638	.782	**.613**
FEED * HS	.016	.516	**.410**	.745	.742	**.822**	.889	.269	**.621**	.579	.422	**.738**	.490	.344	**.497**

Means within a column in each comparison group with no common superscripts differ significantly (P<0.05).

a–b

Table 7 Birds weight, percentage liver weight, spleen weight, gizzard weight and proventriculus weight of hen under heat stress condition with supplementation of probiotic and postbiotic in their feed

TREATMENT	% DUODENUM WEIGHT			% JEJUNUM WEIGHT			% ILEUM WEIGHT			% ABDOMINAL FAT		
	Mid	**End**	**ALL**	**Mid**	**End**	**ALL**	**Mid**	**End**	**ALL**	**Mid**	**End**	**ALL**
FEED												
CONTROL	.56	.53	**.54**	1.6	1.7	**1.6**	1.42	1.42	**1.41[a]**	2.5	2.3	**2.4**
PROBIOTIC	.58	.52	**.55**	1.5	1.6	**1.6**	1.35	1.39	**1.37[ab]**	2.5	2.0	**2.2**
POSTBIOTIC	.51	.54	**.52**	1.4	1.5	**1.5**	1.26	1.26	**1.26[b]**	2.3	2.3	**2.3**
SEM	.041	.046	**.030**	.13	.16	**.09**	.090	.114	**.071**	.43	.34	**.27**
TEMPERATURE												
CONTROL	.55	.53	**.53**	1.5	1.5	**1.5**	1.36	1.37	**1.37**	2.4	2.1	**2.2**
HEAT STRESS	.55	.54	**.54**	1.6	1.6	**1.6**	1.32	1.35	**1.34**	2.5	2.3	**2.4**
SEM	.034	.038	**.025**	.11	.13	**.08**	.075	.093	**.059**	.35	.28	**.22**
VARIABLES												
FEED	.233	.920	**.656**	.520	.690	**.438**	.271	.356	**.114**	.792	.694	**.848**
HEAT STRESS	.917	.948	**.912**	.790	.412	**.504**	.585	.888	**.606**	.716	.341	**.380**
FEED * HS	.841	.886	**.924**	334	.693	**.560**	.079	.748	**.566**	.504	.433	**.726**

[a–b] Means within a column in each comparison group with no common superscripts differ significantly (P<0.05).[5]

[5] Total of 192 birds, 32 birds/ treatment

CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The present study demonstrated that the heat stress negatively affected the birds feed intake especially during the first month (79.1g vs 84.2g for the control; $p<0.05$) resulting in a numerical decrease in egg production, however the birds quickly adapted to the elevated temperature. Furthermore, high cyclic temperature showed a negative impact ($p<0.05$) on the egg weight, percent shell weight, Haugh unit, shell thickness, and yolk color in addition to the birds' weight and percentage spleen weight. However, it did not have a significant effect on the specific gravity, percentage yolk weight or any of the other visceral organ weight; liver, gizzard, proventriculus, duodenum, jejunum, ileum, and abdominal fat ($p>0.05$)

In addition, this study showed the positive effects of probiotic and postbiotic metabolite supplementation in laying hens under heat stress conditions. Although, the individuals that were under probiotic or postbiotic diet showed a higher feed intake. Postbiotic supplementation in feed showed a faster positive effect on percentage egg production than probiotic supplementation. In addition, the heat stress affected the birds' feed intake during the first month, however they quickly adapted to the elevated temperature.

Furthermore, and regarding egg quality, heat stress increased percentage egg white weight and reduced Haugh unit which indicates a higher albumen concentration and a low albumen quality. Probiotic supplementation under heat stress condition had a positive effect on both percentage egg weight and Haugh unit; and postbiotic improved percentage egg white weight and percentage egg weight. Probiotic treatment also showed a lower

percentage of shell weight, while postbiotic treatment lowered the percentage egg white weight. Both postbiotic and probiotic groups resulted in a lower yolk color that might be due to reduced plasma and yolk cholesterol. Moreover, the different feed treatments have an effect only on the bird's ileum weight percentage. The reduced ileum size may reflect a more efficient absorption and utilization of nutrients following the application of pro- or postbiotics.

Postbiotic metabolite can be an alternative feed additive to achieve high productivity while reducing the use of conventional chemotherapeutic agents such as in-feed antimicrobials under heat stress conditions. Further research is needed to study the changes induced by pro- or postbiotics at the molecular level. This will give a better insight into the role of such products in mitigating heat stress impact and explore in depth the interactions between these products with intestinal pathogens and epithelial cells. In addition, further study is needed to investigate the correlation between egg yolk color intensity and cholesterol volume in the yolk. And another should be conducted on the economic benefits of the use of postbiotics as a replacement feed additive in layer hens.

REFERENCES

Aguilar-Toalá, J. E., Garcia-Varela, R., Garcia, H. S., Mata-Haro, V., González-Córdova, A. F., Vallejo-Cordoba, B., & Hernández-Mendoza, A. (2018). "Postbiotics: An Evolving Term within the Functional Foods Field." Trends Food Science and Technology 75: 105–114.

Ali, N., & Abdelaziz, M. (2018). Effect of feed restriction with supplementation of probiotic with enzymes preparation on performance, carcass characteristics and economic traits of broiler chickens during finisher period. Egypt. J. Nutr. Feed, 21, 243–254.

Allahverdi, A., Feizi, A., Takhtfooladi, H.A., & Nikpiran, A. (2013). Effects of Heat Stress on Acid-Base Imbalance, Plasma Calcium Concentration, Egg Production and Egg Quality in Commercial Layers. Global Veterinaria., 10(2): 203-207.

Alloui, M.N., Szczurek, W., & Swiatkiewicz, S. (2013). The Usefulness of Prebiotics and Probiotics in Modern Poultry Nutrition: A Review. Ann. Anim. Sci. 13, 17–32.

Al-Zghoul, M. B., El-Bahr, S. M., Al-Rukibat, R. K., Dalab, A. S., Althnaian, T. A., & Al-ramadan S.Y. (2015). Biochemical and molecular investigation of thermal manipulation protocols during broiler embryogenesis and subsequent thermal challenge. BMC Vet Res, 11: 292. doi: 10.1186/s12917-015-0609-0

Araujo, R., Polycarpo, G., Barbieri, A., Silva, K., Ventura, G., & Polycarpo, V. (2019). Performance and Economic Viability of Broiler Chickens Fed with Probiotic and Organic Acids in an Attempt to Replace Growth-Promoting Antibiotics. Braz. J. Poult. Sci. 21, 21

Ashish, R., Ranjana, S., Indu, D., Abdul, R., & Shiwani T. (2019). Effect of Heat Stress on Poultry Production and their Managemental Approaches. Int.J.Curr.Microbiol.App.Sci 8(2): 1548-1555

Ashraf, R., & Shah, N.P. (2014). Immune System Stimulation by Probiotic Microorganisms. Crit. Rev. Food Sci. Nutr., 54, 938–956.

Aziz, N. H., Khidhir, Z. K., Hama, Z. O., & Mustafa, N. A. (2020). Influence of Probiotic (Miaclost) Supplementation on Carcass Yield, Chemical Composition and Meat Quality of Broiler Chick. J. Anim. Poult. Prod., 11, 9–12.

Barnett J., & Hemsworth P. (2003) Science and its application in assessing the welfare of laying hens in the egg industry. Aust Vet J 81(10): 615-624.

Bartlett J. R., & Smith M. O. (2003) Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult Sci 82(10): 1580-1588.

Bidarkar V. K., Swain P. S., Ray, S., & Dominic, G. (2014). Probiotics: Potential Alternative to Antibiotics in Ruminant Feeding. Trends in Veterinary and Animal Sciences, 1: 01-04.

Birmani, M.W., Nawab, A., Ghani, M.W., Li, G., Wu, J., Liu, W., & An, L. (2019). Probiotic Supplementation in Poultry Production as an Alternative to Antibiotic Feed Additive. Anim. Rev., 6, 5–16.

Bonnet, S., Geraert, P.A., Lessire, M., Carre, B., & Guillaumin, S. (1997). Effect of high ambient temperature on feed digestibility

Bozkurt, M., Kucukvilmaz, K., Catli, A.U., Cinar, M., Bintas, E., & Coven, F. (2012). Performance, egg quality, and immune response of laying hens fed diets supplemented with manna-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions. Poult. Sci., 91, 1379–1386.

Cean, A., Stef, L., Simiz, E., Julean, C., Dumitrescu, G., Vasile, A., Pet, E., Drinceanu, D., & Corcionivoschi, N. (2015). Effect of Human Isolated Probiotic Bacteria on Preventing Campylobacter jejuni Colonization of Poultry. Foodborne Pathog. Dis., 12, 122–130.

Cervantes, H. M. (2015). "Antibiotic-free Poultry Production: Is It Sustainable?" Journal of Applied Poultry Research 24 (1): 91–97.

Choe, D. W., Foo, H. L., Loh, T. C., Hair-Bejo, M., & Awis, Q. S. (2013). Inhibitory property of metabolite combinations produced from Lactobacillus plantarum Strains. J Trop Agric Science, 36:79–88

Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. Nature Rev. Microbiol., 10, 735–742.

Compare, D., Rocco, A., Coccoli, P., Angrisani, D., Sgamato, C., & Iovine, B. (2017). "Lactobacillus Casei DG and Its Postbiotic Reduce the Inflammatory Mucosal Response: An Ex Vivo Organ Culture Model of Post-infectious Irritable Bowel Syndrome." BMC Gastroenterology 17: 53.

Cramer, T., Kim, H., Chao, Y., Wang, W., Cheng, H., & Kim, Y. H. B. (2018). Effects of probiotic (Bacillus subtilis) supplementation on meat quality characteristics of breast muscle from broilers exposed to chronic heat stress. Poult. Sci., 97, 3358–3368.

Daghir, N. J. (2008). Broiler feeding and management in hot climates, in: Daghir, N.J. (Ed.) Poultry production in Hot Climate, pp. 227-260 (CAB International. Cromwell Press, Trowbridge)

Dahiya, J., Wilkie, D., Van Kessel, A., & Drew, M. (2006). “Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era.” Animal Feed Science and Technology 129: 60–88.

Dayyani, N., & Bakhtiyari, H. (2013). Heat stress in poultry: background and affective factors. International journal of Advanced Biological and Biomedical Research. 1(11): 1409-1413

De Almada, C. N., Almada, C. N., Martinez, R. C. R., & Sant’Ana A. S. (2016). “Paraprobiotics: Evidences on Their Ability to Modify Biological Responses, Inactivation Methods and Perspectives on Their Application in Foods.” Trends in Food Science and Technology 58: 96e114.

De Rensis, F., & Scaramuzzi, R. J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow-a review. Theriogenology., 60: 1139e51

Deeb, N., & Cahaner, A. (2002). Genotype-by-environment interaction with broiler genotypes differing in growth rate. 3. Growth rate and water consumption of broiler progeny from weight-selected versus nonselected parents under normal and high ambient temperatures. Poult. Sci., 81, 293–301.

Deng, W., Dong, X. F., Tong, J. M., & Zhang, Q. (2012). The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poult. Sci., 91, 575–582.

Dibner J. J., & J. D. Richards (2005). Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci., 84, pp. 634-643

Dizaji, B. R., Sajjad, H., & Afshin, Z. (2012). Effects of dietary supplementations of prebiotics, probiotics, synbiotics and acidifiers on growth performance and organs weights of broiler chicken. European Journal of Experimental Biology, 2 (6):2125-2129

Duskaev, G., Rakhmatullin, S., Kvan, O. (2020). Effects of Bacillus cereus and coumarin on growth performance, blood biochemical parameters, and meat quality in broilers. Vet. World, 13, 2484.

Ebeid, T. A., Suzuki, T., & Sugiyama, T. (2012). High temperature influences eggshell quality and calbindin-D28k localization of eggshell gland and all intestinal segments of laying hens. Poult. Sci., 91, 2282–2287.

Editors, A. (2017). “U.S. Bans Antibiotics Use for Enhancing Growth in Livestock”. McGraw-Hill Education.

Elnagar, S. A., Scheideler, S. E., & Beck, M. M. (2010) Reproductive hormones, hepatic deiodinase messenger ribonucleic acid, and vasoactive intestinal

polypeptideimmunoreactive cells in hypothalamus in the heat stressinduced or chemically induced hypothyroid laying hen. Poult Sci 89: 2001-2009.

EPC. (2005). "Ban on Antibiotics as Growth Promoters in Animal Feed Enters into Effect. European Commission—IP/05/1687.

Fajardo P., Lorenzo P., Jesús M., Isabel R., Clara F., & Nelson, P. G. (2012). Effects of Feeding of Two Potentially Probiotic Preparations from Lactic Acid Bacteria on the Performance and Faecal Microflora of Broiler Chickens. World journal, 562635.

FAO. (2016). "Probiotics in Animal Nutrition – Production, Impact and Regulation" by Yadav S. B., Athol V. K., Peter J. D. & Wayne L. B. FAO Animal Production and Health Paper No. 179. Rome.

Felver-Gant, J. N., Mack, L. A., Dennis, R. L., Eicher, S. D., & Cheng, H. W. (2012). Genetic variations alter physiological responses following heat stress in 2 strains of laying hens. Poult. Sci., 91, 1542–1551.

Freter, R. (1992). Factors affecting the microecology of the gut. In Probiotics: The Scientific Basis; Springer: Dordrecht, The Netherlands,; pp. 111–144.

Fuller, R. (1989). Probiotic in man and animals. Journal of Applied Bacteriology, 66: 365-378.

Getachew, B. (2021). Review on the Effect of Heat Stress on Poultry Production and Productivities. Food science and nutrition technology, Volume 6 Issue 2

Ghasemi-Sadabadi, M., Ebrahimnezhad, Y., Shaddel-Tili, A., Bannapour-Ghaffari, V., Kozehgari, H., & Didehvar, M. (2019). The effects of fermented milk products (kefir and yogurt) and probiotic on performance, carcass characteristics, blood parameters, and gut microbial population in broiler chickens. Arch. Anim. Breed., 62, 361–374.

Ghazi, S. H., Habibian, M., Moeini, M. M., & Abdolmohammadi, A. R. (2012) Effects of different levels of organic and inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biol Trace Elem Res 146: 309-317.

Gous, R. M. & Morris, T. R. (2005). Nutritional interventions in alleviating the effects of high temperatures in broiler production. World's Poult. Sci. J., 61:463–466.

Guerra N. P., Bernárdez, P. F., Méndez J., Cachaldora P., & Pastrana C. L. (2007). Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. Animal Feed Science and Technology, 134, (1-2), 89–107.

Haileselassie, Y., Navis M., Vu, N., Qazi, K. R., Rethi, B., & Sverremark-Ekstrom E. (2016). "Postbiotic Modulation of Retinoic Acid Imprinted Mucosal-like Dendritic Cells by Probiotic Lactobacillus Reuteri 17938 in Vitro." Frontiers in Immunology 7: 1–11.

Hajati, H., & Zaghari, M. (2019). Spirulina in poultry nutrition. United Kingdom: Cambridge Scholars Publishing.

Hargis, B., Tellez, G., Latorre, J. D., & Wolfenden, R. (2018). Compositions, Probiotic Formulations and Methods to Promote Digestion and Improve Nutrition in Poultry. U.S. Patent 10,959,447.

Harimurti, S., & Hadisaputro, W. (2015). Probiotics in Poultry. In Beneficial Microorganisms in Agriculture, Aquaculture and Other Areas; Springer: Berlin/Heidelberg, Germany,; pp. 1–19.

Hashemi, S. R., & Davoodi, H. (2011). Herbal plants and their derivatives as growth and health promoters in animal nutrition. Vet. Res. Commun., 35, 169–180

Hernandez-Patlan, D., Solis-Cruz, B., Hargis, B. M., & Tellez, G. (2020). The Use of Probiotics in Poultry Production for the Control of Bacterial Infections and Aflatoxins. Prebiotics Probiotics, 1–21

Hidayat, M. N., Malaka, R., Agustina, L., & Pakiding, W. (2016). Abdominal Fat Percentage and Carcass Quality of Broiler Given Probiotics Bacillus spp. Metabolism, 22, 3–60.

Homayouni Rad, A., Aghebati Maleki, L., Samadi Kafil, H., Fathi Zavoshti, H., & Abbasi., A. (2020). "Postbiotics as Novel Health-promoting Ingredients in Functional Foods." Health Promotion and Perspectives 10 (1): 3–4.

Hotel, A. (2014). Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria; World Health Organization: Geneva, Switzerland.

Humam, A. M., Loh, T. C., Foo, H. L., Samsudin, A. A., Alshelmani, M. I., & Noordin M. M. (2018). "Effects of Feeding Different Postbiotics on Growth Performance of Broilers under Heat Stress." World's Poultry Science Association (Malaysia Branch), Scientific Conference, Enhancing Poultry Health and Production for Sustainable Poultry Industry. Faculty of Veterinary, University Putra Malaysia, Malaysia. 18 – 19.

Humam, A. Z., Loh, T. C., Foo, H. L., Samsudin, A. A., Noordin, M. M., Zulkifli, I., & Izuddin, W. I. (2019). Effects of Feeding Different Postbiotics Produced by Lactobacillus plantarum on Growth Performance, Carcass Yield, Intestinal Morphology, Gut Microbiota Composition, Immune Status, and Growth Gene Expression in Broilers under Heat Stress. Animals, 9, 644.

Humphrey, T. (2006). Are happy chickens safer chickens? Poultry welfare and disease susceptibility. Br. Poult. Sci., 47, 379–391.

Iweala, O. I., & C. R. Nagler (2019). “The Microbiome and Food Allergy.” Annual Review in Immunology 37 (1): 377–403.

Izuddin, W. I., Loh, T. C., Foo, H. L., Samsudin, A. A., & Humam, A. M. (2019). Postbiotic Lactobacillus plantarum RG14 improves ruminal epithelium growth, immune status and upregulates the intestinal barrier function in post-weaning lambs. Sci. Rep., 9, 9938.

Jadhav K., Sharma, K. S., Katoch, S., Sharma V. K., & Mane B. G. (2015). Probiotics in Broiler Poultry Feeds: A Review. international journal of animal and veterinary sciences.

Jahromi, M. F. Altaher, Y. W., Shokryazdan, P. Ebrahimi, R., Ebrahimi, M., Idrus, Z., Tufarelli, V., & Liang, J. B. (2016). Dietary supplementation of a mixture of Lactobacillus strains enhances performance of broiler chickens raised under heat stress conditions. Int. J. Biometeorol., 60, 1099–1110.

Ji, K., Jang, N. Y., Kim, Y. T (2015). Isolation of Lactic Acid Bacteria Showing Antioxidative and Probiotic Activities from Kimchi and Infant Feces. J. Microbiol. Biotechnol, 25, 1568–1577.

Julio, P. D., Francisco, J. R. O., Mercedes, G. C., & Angel, G. (2019). Mechanisms of Action of Probiotics. PMCID: PMC6363529; PMID: 30721959.

Kabir, S. M. L. (2009). The Role of Probiotics in the Poultry Industry. Int. J. Mol. Sci. 10, 3531–3546.

Kareem, K. Y., Ling, F. H., Chwen, L. H., Foong, O. M., & Asmara S. A. (2014). “Inhibitory Activity of Postbiotic Produced by Strains of Lactobacillus Plantarum Using Reconstituted Media Supplemented with Inulin.” Gut Pathogens 6 (1): 23.

Kareem, K. Y., Loh, T. C., Foo, H. L., Akit, H., & Samsudin, A. A. (2016). Effects of dietary postbiotic and inulin on growth performance, IGF1 and GHR mRNA expression, faecal microbiota and volatile fatty acids in broilers. BMC Vet. Res., 12, 163.

Kataria, J., Li, N., Wynn, J. L., & Neu, J. (2009). “Probiotic Microbes: Do They Need to Be Alive to Be Beneficial?” Nutrition Review 67 (9): 546–550.

Keener, K., Bashor, M., Curtis, P., Sheldon, B., & Kathariou, S. (2004). Comprehensive Review of Campylobacter and Poultry Processing. Compr. Rev. Food Sci. Food Saf. 3, 105–116.

Khan, R., & Naz, S. (2013). The applications of probiotics in poultry production. World's Poult. Sci. J., 69, 621–632.

Kothari, D., Patel S., & Kim, S. K. (2019). "Probiotic Supplements Might Not Be Universally-effective and Safe: A Review." Biomedicine and Pharmacotherapy 111: 537–547.

Krysiak, K., Konkol, D., & Korczy ´nski, M. (2021). Overview of the Use of Probiotics in Poultry Production. Animals 11, 1620

Lagha, A. B., Haas, B., Gottschalk, B., & Grenier, D. (2017). "Antimicrobial Potential of Bacteriocins in Poultry and Swine Production." Veterinary Research 48: 22.

Lin, H., Zhang, H. F., Du, R., Gu, X. H., Zhang, Z. Y., Buyse, J., & Decuypere, E. (2005). The thermoregulation response of broiler chickens to humidity at different ambient temperatures I. Four-week-age. Poult. Sci., 84: 1173-1178.

Lin, H., Mertens, K., Kemps, B., Govaerts, T., De Ketelaere, B., De Baerdemaeker, J., Decuypere, E., & Buyse, J. (2004). New approach of testing the effect of heat stress on eggshell quality: Mechanical and material properties of eggshell and membrane. Br. Poult. Sci., 45, 476–482.

Loh, T. C., Thanh, N. T., Foo, H. L., Bejo, M. H., & Kasim, A. (2010). Feeding of different levels of metabolite combinations produced by Lactobacillus plantarum on growth performance, faecal lactic acid bacteria and Enterobacteriaceae count, volatile fatty acids and villi height in broilers. Anim Sci J, 81:205–214

Loh, T. C., Choe, D. W., Foo, H. L., Sazili, A. Q., & Bejo, M. H. (2014). Effects of feeding different postbiotic metabolite combinations produced by Lactobacillus plantarum strains on egg quality and production performance, faecal parameters and plasma cholesterol in laying hens. BMC Vet. Res., 10, 149.

Loh, T. C., Thu, T. V., Foo, H. L., & Bejo, M. H. (2013). Effects of different levels of metabolite combination produced by Lactobacillus plantarum on growth performance, diarrhoea, gut environment and digestibility of postweaning piglets. J. Appl. Anim. Res., 41, 200–207.

Lokapirnasari, W. P., Teguh, B. P., Anam, A. A., Soeharsono, S., Sri, H., Nenny, H., Rifqy, N., Khoirul, H., Hana, C. P. W., Nabil, F. N. R., & Andreas, B. Y. (2019). Potency of probiotics Bifidobacterium spp. and Lactobacillus casei to improve growth performance and business analysis in organic laying hens. Vet world 12(6): 860–867.

Lucas, J. L., & Marcos, H. R. (2013). Impact of Heat Stress on Poultry Production. Animals, 3, 356-369.

Mack, L. A., Felver-Gant, J. N., Dennis, R. L., & Cheng, H. W. (2013). Genetic variation alter production and behavioral responses following heat stress in 2 strains of laying hens. Poult. Sci., 92, 285–294.

Mahdavi, A. H., Rahman, A. R., & Pourreza, J. (2005). Effect of probiotic supplements on egg quality and laying hen's performance. Int J Poult Sci, 4:488–492.

Mahmoud, K. Z., Beck, M. M., Scheider, S. E., Forman, M. F., Anderson, K. P., & Kachman, S. D. (1996). Acute high environmental temperature and calciumestrogen relationship in the hen. Poult. Sci., 75: 1555-1562.

Marder, J., & Arad, Z. (1989). Panting and acid-base regulation in heat stressed birds. Comp. Biochem. Physiol. A Comp. Physiol., 94, 395–400.

Marteau, P., & Shanahan, F. (2003). Basic aspects and pharmacology of probiotics: An overview of pharmacokinetics, mechanisms of action and side effects. Best Practice and Research in Clinical Gastroenterology, 17:725–740.

Mashaly, M. M., Hendricks, G. L., & Kalamz, M. A. (2004) Effect of Heat Stress on Production Parameters and Immune Responses of Commercial Laying Hens, Poultry Science 83: 889-894.

Mazanko, M. S., Gorlov, I., Prazdnova, E. V., Makarenko, M. S., Usatov, A. V., Bren, A. B., Chistyakov, V. A., Tutelyan, A. V., Komarova, Z. B., Mosolova, N. I., et al. (2018). Bacillus Probiotic Supplementations Improve Laying Performance, Egg Quality, Hatching of Laying Hens, and Sperm Quality of Roosters. Probiotics Antimicrob. Proteins, 10, 367–373.

McDaniel, C.D., Hood, J. E., & Parker, H. M. (2004) An attempt at alleviating heat stress infertility in male broiler breeder chickens with dietary ascorbic acid. Int J Poult Sci 3: 593- 602.

Menconi, A., Bielke, L. R., Hargis, B. M., & Tellez, G. (2014a). Immuno-modulation and anti-inflammatory effects of antibiotic growth promoters versus probiotics in the intestinal tract. J. Microbiol. Res. Rev., 2, 62–67.

Menconi, A., Kallapura, G., Latorre, J. D., Morgan, M. J., Pumford, N. R., Hargis, B. M., Tellez, G. (2014b). Identification and Characterization of Lactic Acid Bacteria in a Commercial Probiotic Culture. Biosci. Microbiota Food Health, 33, 25–30.

Mikulski, D., Jankowski, J., Naczmanski, J., Mikulska, M., & Demey, V. (2012). “Effects of Dietary Probiotic (Pediococcus Acidilactici) Supplementation on Performance, Nutrient Digestibility, Egg Traits, Egg Yolk Cholesterol, and Fatty Acid Profile in Laying Hens.” Poultry Science 91 (10): 2691–2700.

Mohamadshahi, M., Veissi, M., Haidari, F., Shahbazian, H., Kaydani, G. A., & Mohammadi, F. (2014). “Effects of Probiotic Yogurt Consumption on Inflammatory Biomarkers in Patients with Type 2 Diabetes.” BioImpacts 4: 83.

Neupane, D., Nepali, D., Devkota, N., Sharma, M., & Kadaria, I. (2019). Effect of Probiotics on production and egg quality of dual purpose chicken at Kathmundu in Nepal. Bangladesh J. Anim. Sci., 48, 29–35.

Nhung, N. T., Chansiripornchai, N., & Carrique-Mas, J. J. (2017). “Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review.” Frontiers in Veterinary Science 4: 126.

NRC. (1981). Effect of environment on nutrient requirements of domestic animals. Committee on Animal Nutrition. Subcommittee on Environmental Stress. National Academy Press, Washington DC

Park, Y. H., Hamidon, F., Rajangan, C., Soh, K. P., Gan, C. Y., Lim, T. S., Abdullah, W. N. W., & Liong, M. T. (2016). Application of Probiotics for the Production of Safe and High-quality Poultry Meat. Food Sci. Anim. Resour. 36, 567–576

Parker, R. B. (1974). Probiotics, the other half of the antibiotic story. Animal Nutrition and Health, 29: 4-8.

Patel, R. M., & P. W. Denning (2013). “Therapeutic Use of Prebiotics, Probiotics, and Postbiotics to Prevent Necrotizing Enterocolitis. What Is the Current Evidence?” Clinical Perinatology 40 (1): 11e25.

Piestun, Y., Shinder, D., Ruzal, M., Halevy, O., Yahav, S. (2008). The effect of thermal manipulations during the development of the thyroid and adrenal axes on in-hatch and post-hatch thermoregulation. J Therm Biol.;33(7):413–8.

Pourakbari, M., Seidavi, A., Asadpour, L., & Martínez, A. (2016). Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. An. Acad. Bras. Cienc., 88, 1011–1021.

Prabhurajeshwara, C., & Kelmani, C. (2019). Evaluation of antimicrobial properties and their substances against pathogenic bacteria in-vitro by probiotic Lactobacilli strains isolated from commercial yoghurt. Journal 23, 97-115.

Quinteiro-Filho, W. M., Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M., Sakai, M., Sá, L. R. M., Ferreira, A. J. P., & Palermo-Neto, J. (2010). Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult. Sci. 89, 1905–1914.

Rad, A. H., Maleki, L. A., Kafil, H. S., & Abbasi, A. (2020). “Postbiotics: A Novel Strategy in Food Allergy Treatment.” Critical Reviews in Food Science and Nutrition 12: 1–8

Ricke, S. C. (2017). “Insights and Challenges of Salmonella Infections in Laying Hens.” Current Opinion in Food Science 18: 43–49.

Rine, C. R., Shovon, L. S., Pravas, C. R., Azraf, A., Anwar H. M. & Iqbal K. J. (2021) Prebiotics, probiotics and postbiotics for sustainable poultry production, World's Poultry Science Journal, 77:4, 825-882.

Sahin, K., Sahin, N., Onderci, M., Gursu F., & Cikim, G. (2002). Optimal dietary concentration of chromium for alleviating the effect of heat stress on growth, carcass qualities, and some serum metabolites of broiler chickens. Biological Trace Element Research, 89: 53-64.

Sahin, K., Sahin, N., Kucuk, O., Hayirli, A. & Prasad, A. S. (2009). Role of dietary zinc in heat stressed poultry: A review. Poult. Sci., 88: 2176-2183

Salaj, R., Štofilová, J., Šoltesová, A., Hertelyová, Z., Hijová, E., Bertková, I., Strojný, L., Kružliak, P., & Bomba, A. (2013). The effects of two Lactobacillus plantarum strains on rat lipid metabolism receiving a high fat diet. Sci. World J.

Saleh, A. A., Hayashi, K., & Ohtsuka, A. (2013). Synergistic Effect of Feeding Aspergillus awamori and Saccharomyces cerevisiae on Growth Performance in Broiler Chickens; Promotion of Protein Metabolism and Modification of Fatty acid Profile in the Muscle. J. Poult. Sci., 50, 242–250.

Salim, H. M.; Huque, K. S., Kamaruddin, K. M., Beg, M. D. A. H. (2018). Global Restriction of Using Antibiotic Growth Promoters and Alternative Strategies in Poultry Production. Sci. Prog. 101, 52–75

Sanders, M. E. (1984). Benefits of feeding probiotics. Food Technology, 53(11): 67-77.

Shazali, N., Foo, H. L., Loh, T. C., Choe, D. W., Rahim, R. A (2014). Prevalence of antibiotic resistance in lactic acid bacteria isolated from the faeces of broiler chicken in Malaysia. Gut Pathog. 6, 1.

Shenderov, B. A. (2013). “Metabiotics: Novel Idea or Natural Development of Probiotic Conception”. Microbial Ecology in Health and Disease 24: 1–8.

Sinha, R., Lone, S. A., Ranjan, A., Rahim, A., Devi, I. & Tiwari, S. (2017). The impact of climate change on livestock production and reproduction: ameliorative management. International Journal of Livestock Research., 7(6): 1- 8.

Sohail, M. U., Ijaz, A., Yousaf, M. S., Ashraf, K., Zaneb, H., Aleem, & M., Rehman, H. (2010). Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic:

Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poult. Sci., 89, 1934–1938

Soltan, M. A. (2008). Effect of dietary organic acid supplementation on egg production, egg quality and some blood serum parameters in laying hens. Int J Poult Sci. ;7(6):613–621.

Song, J., Xiao, K., Ke, Y. L., Jiao, L. F., Hu, C. H., Diao, Q. Y., Shi, B., & Zou, X. T. (2014). Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult. Sci., 93, 581–588

Star, L., Juul-Madsen, H. R., Decuypere, E., Nieuwland, M. G., de Vries Reilingh, G., van den Brand, H., Kemp, B., & Parmentier, H. K. (2009). Effect of early life thermal conditioning and immune challenge on thermotolerance and humoral immune competence in adult laying hens. Poult. Sci., 88, 2253–2261.

Thanh, N. T., Loh, T. C., Foo, H. L., Bejo, M. H., & Azhar, K. (2010). Inhibitory activity of metabolites produced by strains of Lactobacillus plantarum isolated form Malaysian fermented food. International Journal of Probiotics and Prebiotics, 5:37–44

Thanh, N.T., Loh, T. C., Foo, H. L., Hair-Bejo, M., & Azhar, B. K. (2009). Effects of feeding metabolite combinations produced by Lactobacillus plantarum on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. Br. Poult. Sci., 50, 298–306.

Thu, T. V., Loh, T.C., Foo, H.L., Halimantun, Y., & Bejo, M. H. (2011). Effects of liquid metabolites combinations by Lactobacillus plantarum on growth performance, faeces characteristics, intestinal morphology and diarrhea incidence in postweaning piglets. Trop Anim Health Prod, 43:69–75.

Tomar, S. K., Anand, S., Sharma, P., Sangwan, V., & Mandal S. (2015). “Role of Probiotic, Prebiotics, Synbiotics and Postbiotics in Inhibition of Pathogens.” In The Battle against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs, edited by A. Méndez-Vilas, 717–732.

Traub-Dargatz, J. L., Ladely, S. R., Dargatz, D. A., & Fedorka-Cray, P. J. (2006). Impact of heat stress on the fecal shedding patterns of Salmonella enterica Typhimurium DT104 and Salmonella enterica Infantis by 5-week-old male broilers. Foodborne Pathog. Dis., 3, 178–183.

Tsilingiri, K., Barbosa, T., Penna, G., Caprioli, F, Sonzogni, A., Viale, G., & Rescigno, M. (2012). “Probiotic and Postbiotic Activity in Health and Disease: Comparison on a Novel polarised Ex-vivo Organ Culture Model.” Gut 61 (7): 1007–1015.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. USA,112,5649–5654.

Verbrugghe, E., Boyen, F., Gaastra, W., Bekhuis, L., Leyman, B., Van Paryz, A., Haesebrouck, F., Pasmans, F. (2012). The complex interplay between stress and bacterial infections in animals. Vet. Microbiol., 155, 115–127

Wang X., Farnell, Y. Z., Peebles, E. D., Kiess, A. S., Wamsley, K. G. S., & Zhai, W. (2016). Effects of prebiotics, probiotics, and their combination on growth performance, small intestine morphology, and resident Lactobacillus of male broilers. Poultry Science Volume 95, Issue 6, Pages 1332-1340

Wang, Y., Dong, Z., Song, D., Zhou, H., Wang, W., Miao, H., Wang, L., & Li, A. (2018). Effects of microencapsulated probiotics and prebiotics on growth performance, antioxidative abilities, immune functions, and caecal microflora in broiler chickens. Food Agric. Immunol. 29, 859–869