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Chapter

Perspective Chapter: Using Feed Additives to Eliminate Harmful Effects of Heat Stress in Broiler Nutrition

László Babinszky, Csaba Szabó and Márta Horváth

Abstract

Global warming is one of the major challenges for mankind, with animal breeding one of the most affected sectors in the agricultural industry. High ambient temperatures negatively affect all domestic animals. While it is true that pork and dairy production suffer the consequences of heat waves, it is actually the poultry industry which is hit the hardest by the heat stress poultry must endure due to hotter weather. Consequently, we have a fundamental interest in reducing and/or eliminating the negative effects of climate change, i.e. prolonged high ambient temperatures. The aim of this chapter is to present the adverse effects of heat stress on energy metabolism, anti- and pro-oxidant capacity and production in birds. A further goal is to show how various feed additives (e.g. vitamin A, C and E, selenium, zinc, betaine, plant extract, and probiotics) can reduce the negative effects of heat stress. Based on the large number of recent scientific findings, the following conclusions were drawn: Using fat in the diet (up to 5%) can reduce heat production in livestock. Vitamins (e.g. A, E and C) are capable of reacting with free radicals. Vitamin E and Vitamin C, Zn, and Se supplementation improved antioxidant parameters. Antioxidant potential of vitamins and micro minerals is more efficient in combination under heat stress in poultry nutrition. Plant extracts (e.g. oregano) could decrease the negative effects of heat stress on antioxidant enzyme activity due to its antioxidant constituents. Betaine reduces heat production in animals at high ambient temperatures. While acute heat stress induces a drop in feed intake, with the resulting increased nutrient demand leading to weight loss, if heat stress is prolonged, adaptation will occur. Probiotics and vitamins (C and E) seem to be the most effective means to reduce the negative effects of heat stress.

Keywords: Broiler, Feed additives, Heat stress, Antioxidant status, Performance

1. Introduction

Global warming is one of the major challenges for mankind, with animal breeding one of the most affected sectors in the agricultural industry. The impacts of increasing environmental temperatures on livestock will most likely differ from place to place, depending on latitude, geographical features and local farming systems [1–3].

High ambient temperatures negatively affect all domestic animals, but in addition to pork and dairy production, perhaps the poultry industry is hit the hardest. In 2020, the world's broiler meat production amounted to about 100.81 million metric tons, and is forecasted to increase to about 101.02 million metric tons by 2021 [4]. According to FAO data [5], total egg production in the world was 1.528 billion units in 2018. In 2019, this figure reached 1.577 billion.

These statistics clearly show that broiler meat and egg production play a crucial role in the global supply of animal origin foodstuffs.

Thus, we have a fundamental interest in reducing and/or eliminating the negative effects of climate change, i.e. prolonged high ambient temperature. The main question is, what tools do we have to reduce the harmful effects of high environmental temperatures-especially in the case of heat stress? A solution for prevention of heat stress in animals includes biological (e.g. genetics, thermal conditioning, nutrition) [6, 7] or keeping technology devices (e.g. air conditioning, intensive ventilation, humidification) [8]. However, housing methods are expensive and the service costs are high. Therefore, reducing the biochemical and physiological negative effects of heat stress with different nutritional tools is one of the primary interests for the economical production of food produced from animals.

According to Babinszky et al. [9], basically the following nutritional possibilities are available to eliminate the harmful effects of the heat stress:

- reduce animal's own heat production (e.g. feeding more dietary fat);
- compensate for the lower nutrient supply; (e.g. feeding more concentrated diets); and
- mitigate heat stress induced metabolic changes (e.g. using different feed additives: vitamins, micro minerals).

It should, however, be noted that during severe heat stress, these methods should be used in combination in order to maintain the production performance of the farm animals and the quality of their products [9]. While this chapter focuses on the third option, i.e. the use of feed additives, we would like to emphasize that whatever feeding method we use, we need to be aware of the changes in the intermediate metabolism of farm animals caused by heat stress, because without this knowledge, there is no effective defense against high ambient temperatures.

Therefore, the aim of this chapter is to summarize the adverse effects of heat stress on energy metabolism, anti- and pro-oxidant capacity, and production in birds. A further goal is to show how various feed additives (vitamin A, C and E, selenium, zinc, betaine, plant extract, and probiotics) can reduce the negative effects of heat stress.

2. Methodology of the literature review

The methodology of the literature review was basically the same as the internationally applied methodology used in animal science. Firstly relevant literature was searched. This follows by evaluation of sources. The third step was identifying the database and gaps in the published scientific findings, than setup the outline structure. Finally the literature review was written.

The literature searching was based on the keywords, using university database, own department data collection on the research field of heat stress, and different international scientific databases of life sciences, animal science and Google Scholar.

In each of the studied paper or book chapter, we asked the same questions as, for example:

What was the aim and methodology of the particular publication (in this case: what kind of heat stress was applied, how many animals were included in the experiment per treatment, whether there were repetitions, what dietary treatments (type of feed additives and their concentration in the diet) were used, what parameters were measured, what was the statistical analysis applied, etc.), furthermore, whether experimental data were correctly evaluated, what results were presented by the authors and what main conclusions were drawn from the data.

To have more clear information on effectiveness of various feed supplements in case of production parameters: daily gain (g/d), average daily gain (g/d) and feed conversion ratio (kg diet/kg gain), the so called mitigation capacity was calculated using the following formula:

$$\textbf{Mitigation capacity of a certain trait}\ (\%) = (\text{measured value in HS environment and fed with the experimental diet} - \text{measured value in HS environment and fed with the control diet})/(\text{measured value in TN environment and fed with the control diet–measured value in HS environment and fed with the control diet}) \times 100 \quad (1)$$

where: HS = heat stress; TN = thermoneutral.

All collected information (data) was placed in a large work database. This information formed the basis of the subchapter titles of our review chapter and of the chapter outline. Based on this information, the evaluation of research data from more than 90 publications started. The writing of the review chapter then began, including the drawing of main conclusions as well. The investigated and systematized research findings are summarized in tables.

3. Heat production of animals and heat stress

It is well known that heat production of animals is the sum total of non-productive energy utilized by the animal and of the energy lost in the course of transformation dietary nutrients [10]. Animals use this so called non-productive energy for maintenance (i.e. satisfy the energy requirement for the maintenance of body temperature, the functioning of the nervous system, the organs, for minimal activity, etc.) [10]. The extra heat produced in the course of digestion, excretion and metabolism of nutrients is called the heat increment. It is also well known that within a certain range of ambient temperature - with unvarying feed and nutrient intake - the total heat production of the animal remains constant. This temperature range is called the thermoneutral zone. The general scheme of the relationship between ambient temperature and heat production of livestock can be seen in **Figure 1** [10].

In a thermoneutral environment, the heat production of the animal is at the minimum, and thus the dietary energy can be used for production (growth, egg and milk production) efficiently [9, 10]. Therefore, whenever the daily amount of energy intake changes, the temperature range of the thermoneutral zone is changed, too. So, if for some reason the animal leaves the thermoneutral zone, this result in an increased heat production by the animal. This means that there is more loss of energy, and in consequence, less energy remains for production and moreover the efficiency of energy utilization deteriorates too. The upper and lower critical temperatures for poultry are summarized in **Table 1** [11].

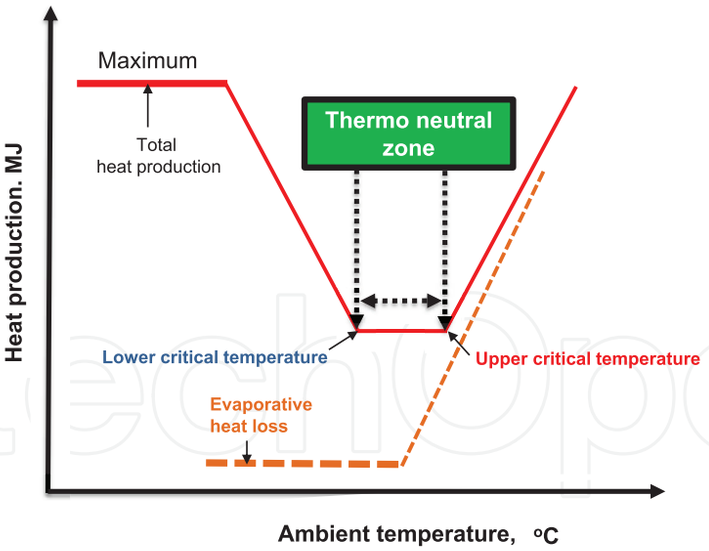


Figure 1.
Relationship between ambient temperature and heat production of livestock [10].

	Lower	Upper
	Critical temperatures (°C)	
1-day-old chicken	32	35
Finishing broiler	16	26
1-day-old turkey	35	38
Finishing turkey	16	26
Laying hen	16	27–29

Table 1.
Recommended thermal conditions for poultry [11].

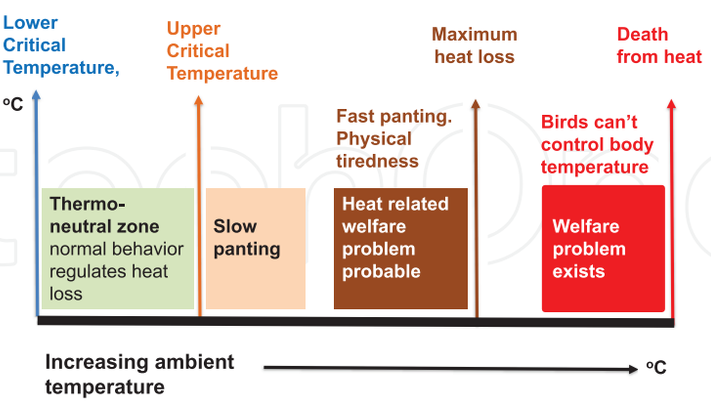


Figure 2.
The effect of increasing ambient temperature on birds [12].

The general scheme of the relationship between broiler behavior and the increasing ambient temperature is shown in **Figure 2** [12].

As can be seen in **Figure 2**, in the thermoneutral zone, birds can lose heat at a controlled rate using normal behavior [12]. Between the lower and upper temperatures, there is no heat stress and body temperature remains constant. If the environmental temperature exceeds the upper critical temperature, birds must lose heat actively by panting. However, it should be noted that panting is a normal response

to heat and is not initially considered a welfare problem [12]. However, as temperatures increase, the rate of panting increases. If heat production is greater than maximum heat loss, birds may die due to heat stress. In other words, heat stress occurs when the body cannot get rid of excess heat.

It is well established that heat stress increases the energy cost of maintenance and adversely affects productive and reproductive performance. In a hot environment, the respiration rate in birds can increase 10–20 times, causing increased CO_2 loss through the lungs [13]. This loss results in an increase in blood pH and this can upset the acid–base balance, which can impair the health and performance of birds [14–16].

There are usually two types of heat stress, acute and chronic heat stress. Acute heat stress refers to a short and rapid increase in environmental temperature (a few hours), whereas under chronic heat stress, high temperatures persist for more extended periods (several days) [17].

Heat stress exposed animals can use different ways to maintain thermoregulation and homeostasis. They can increase radiant, convective and evaporative heat loss by vasodilatation and perspiration [18]. However, birds have an extra mechanism which is promote heat exchange between their bodies and the environment. These are the air sacs. Air sacs are very useful especially during panting, as they promote air circulation on surfaces and consequently, the evaporative loss of heat [19, 20].

Unfortunately, there are only few scientific papers that report on the heat production and the heat loss of heat-exposed birds. Consequently, there is only a limited number of scientific publications that report on nutritional possibilities for reducing the heat production of birds under heat stress.

Syafwan et al. [21] concluded in their excellent review that the heat production of broilers is particularly high due to the high growth rate and the high daily feed intake. Developments in the genetic selection of meat-type birds has led to rapid growth and a high metabolic rate, which is accompanied by a higher heat production level due to increased feed intake [22]. Therefore, it can be stated that high genetic capacity hybrid broilers (so called "improved chicken") are much more sensitive to a hot environment than their unimproved counterparts.

Summarizing the relevant scientific findings, it can be stated that in practical animal agriculture, and especially in factory farming, it is particularly difficult to keep animals in a thermoneutral zone. Therefore, in order to reduce the negative effect of heat stress, it is important to use nutritional tools in addition to technical devices.

4. Reduction of heat production by nutritional tools

4.1 Using fat in the diets

It is well known that if more fat is used in pig diets in high ambient temperature, the total heat production of the animals reduces significantly. Babinszky et al. [23] concluded from their study that lactating sows fed a high level of dietary fat (125 g fat/kg diet) produce significantly less heat than those fed a carbohydrate rich (low-fat level) diet. Babinszky [10] is also concluded, that the energetic efficiency of milk production was improved, when sows received high dietary fat diet (125 g/kg diet). This phenomenon can be explained by the fact that synthesizing milk fat from dietary fat is more efficient than it's synthesizing from dietary carbohydrates.

In poultry nutrition, relative limited literature data are available on fat feeding against heat stress and its effect on heat production of birds. Das et al. [24], in an

excellent review, stated that heat stress may be combated by adding fat and reducing crude protein in poultry diets. Higher energy diets were effective in partially mitigating the effects of heat stress in poultry. This can be explained by the fact that during metabolism, fat produces a lower heat increment than protein and carbohydrates [25].

In other studies, it was concluded that supplementation of fat in the poultry diet increase the nutrient utilization in the gastrointestinal tract by lowering the rate of food passage [26] and also helps increase the energy value of the other feed constituents [27, 28]. Feeding a high fat diet (up to 5%) to heat-exposed broilers reduces heat production. This result occurs because the heat increment of fat is lower than that of either proteins or carbohydrates [21, 25, 29, 30].

4.2 Using vitamin C in chicken diet to change energy metabolism

Because the animal body derives all its energy from oxidation, the magnitude of energy metabolism can be determined from the amount of carbon-dioxide produced and oxygen consumed. The ratio of the volumes of carbon dioxide produced to oxygen consumed is called the respiratory quotient (RQ) [31]. The respiratory quotients are: for protein: 0.809; for fat: 0.711; for starch: 1.000; for sugar: 1.000; and for glucose: 1.000, respectively [32]. If the RQ value is equal to 1.00, this means that e.g. burning 1 g of starch produces as much carbon dioxide as oxygen is needed to burn it (0.829 liter CO_2/0.829 liter O_2).

However, it should also be noted that RQ values significantly higher than 1 can be achieved if the animals convert the carbohydrate to fat, since in this case oxygen-poor fat is formed from oxygen-rich glucose. During starvation, the RQ value is less than 0.7 [31].

As it can be seen above, the RQ may provide valuable information about the metabolic processes in the body. Therefore, RQ values are very often determined in respiratory studies.

McKee et al. [33] investigated the effect of vitamin C on different variables of energy metabolism of young heat exposed chickens in indirect calorimeters. The experiment started at day 9 and lasted until day 17 posthatch. In this study, CO_2 production and O_2 consumption were measured in the thermoneutral zone (27.7°C) and in a hot environment (34°C). On the basis of these values, RQ and heat production were calculated daily through day 17 of the experiment. The basal diet was supplemented by a 150 mg/kg diet of ascorbic acid (vitamin C). They found that heat exposure lowered ($P < 0.001$) the respiratory quotient. Heat-exposed birds consuming the ascorbic acid supplemented diet expressed lower respiratory quotients than their unsupplemented counterparts. The authors concluded that this effect resulted from a nonsignificant increase in O_2 consumption and decrease in CO_2 production. They also concluded that further investigations are needed to determine whether the ascorbic acid-induced change in the RQ value towards 0.70 reflects an increase in protein or lipid catabolism or both. In further study, the effect of ascorbic acid on the energy metabolism and heat production of domestic animals should also be elucidated.

Despite the many open questions, based on the findings made by McKee et al. in their study, it seems that supplemental ascorbic acid may influence the body energy stores during periods of reduced energy intake (during heat stress).

4.3 Using betaine as feed additive in the diet

Betaine chemical structure ($C_5H_{11}NO_2$) contains three methyl groups which play a role in transmethylation reactions [34]. Betaine (trimethylglycine) is an

intermediate metabolite in the catabolism of choline which can modify osmolarity, act as a methyl donor, and has potential lipotropic effects [9]. As a by-product of sugar beet processing, betaine is commercially available as a feed additive [35]. Currently, betaine is available in several purified forms (anhydrous, monophosphate and hydrochloride betaine) [36].

Betaine mainly functions as an osmolyte and a methyl-group donor [37]. Under heat stress, betaine plays an important role in cellular osmotic regulation, preventing dehydration by increasing the water-holding capacity of cells. It helps in maintaining the protective osmolytic activity in birds under heat stress. Betaine may promote various intestinal microbes against osmotic variations and this results in improve microbial fermentation activity [38]. Furthermore, betaine is also found to have anti-inflammatory properties and improves intestinal function [39].

Because betaine influences fat and protein deposition, it can also be used to improve carcass quality and reduce fatty livers. Schrama et al. [40] showed that energy retention in pigs improves over time following the supplementation of betaine to the diet. They also found that under thermoneutral conditions, dietary betaine supplementation (1.23g/kg diet) reduced the total heat production of pigs. They suggest the same concentration of betaine in poultry diet, as well.

These scientific findings suggest that betaine may be suitable for reducing heat production in livestock (e.g. in poultry) at high ambient temperatures. However, only few research results have been published in this area to date. Therefore, further studies are needed to determine the impact of betaine on heat production in animals under high ambient temperature.

Another problem is that many of published papers are not clear on the source of betaine used (natural, extracted betaine anhydrous or synthetic betaine hydrochloride). The source is important, as it is likely that the efficacies of the different betaine sources differ.

5. Effects of heat stress on anti- and prooxidant status in birds. Mitigation using different feed additives

5.1 Anti- and prooxidant status

5.1.1 Impacts of heat stress

Increased environmental temperature caused increased lipid peroxidation (as well as induced formation of malondialdehyde (MDA), which is an indicator for lipid peroxidation). Therefore, the antioxidant defense system is altered [41–43].

According to the latest research, the elimination of the free radicals activates three level antioxidant systems (**Figure 3**, based on Babinszky et al. [10]).

Elimination is done by the first level of the antioxidant system which functions at the same time as the detoxification and regeneration pathways of the second level. The third level starts working after damage has been done, to repair and eliminate damaged cells. This first level (direct enzymatic pathway) includes the neutralization of the oxygen and nitrogen centred free radicals by enzymes. The second level includes the detoxification and regeneration reactions of the small molecule antioxidants. The third level is activated when damaged systems (proteins, DNA) have to be repaired and/or removed from the cells by chaperones and DNA-repair enzymes.

In general, it can be concluded that a large amount of Reactive Oxygen Species (ROS) causes disruption of mitochondrial function, increased lipid peroxidation,

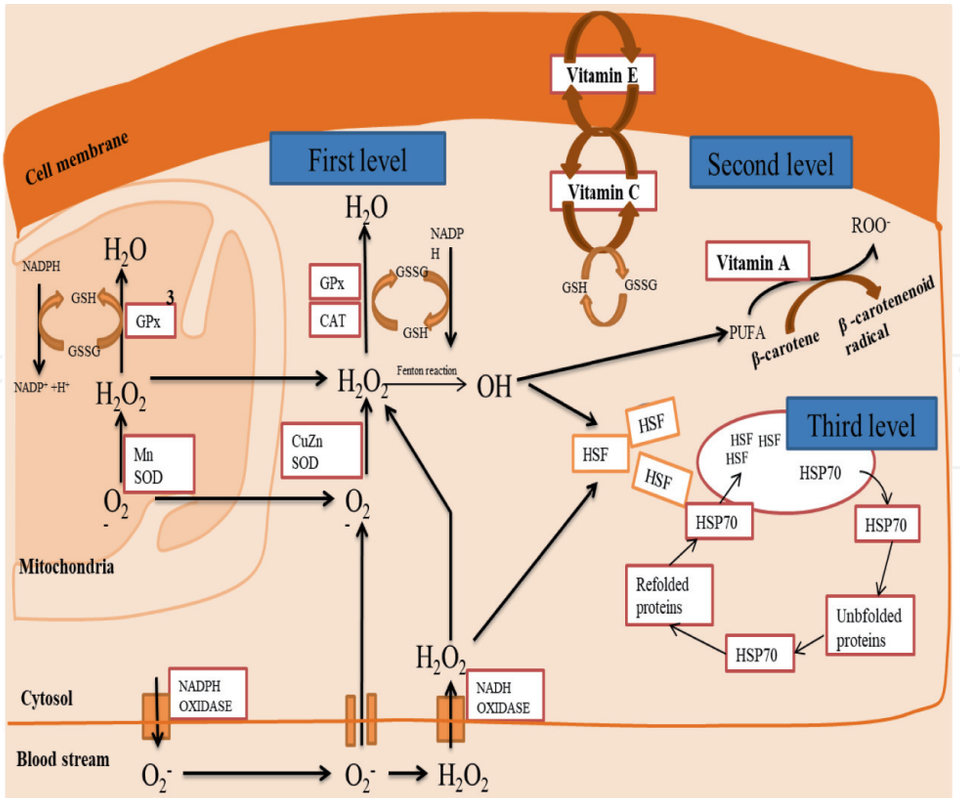


Figure 3.
Three level antioxidant system. Based on Babinszky et al. [10]. CAT = catalase; Cu SOD = copper superoxide dismutase; GPx = glutathione peroxidase; GR=glutathione reductase; GSH = glutathione; GSSG = glutathione disulphide; H_2O = water; H_2O_2 = hydrogen peroxide; HSF = heat shock factors; HSP70 = heat shock protein 70; Mn SOD = manganese superoxide dismutase; NADH=nicotinamide-adenine-dinucleotide; $NADP^+$ = oxidised nicotinamide-adenine-dinucleotide-phosphate, NADPH=nicotinamide-adenine-dinucleotide-phosphate, O_2^- = superoxide anion radical; OH = hydroxyl radical; PUFA = polyunsaturated fatty acids; ROO^- = peroxyl radical.

and decreased the concentration of so called antioxidant vitamins, furthermore induce stress gene expression, and finally it leads to dysfunction in antioxidant enzymes and also causes DNA damage.

According to Yang et al. [44] in heat stressed broilers (35°C for 3h/day), the activity of the mitochondrial respiratory chain is reduced, which led to over-production of ROS. This situation results in lipid peroxidation and oxidative stress in the birds.

In another study [7] lipid peroxidation and superoxide dismutase (SOD) activity was measured in broilers under heat stress (32°C for 6 h/day). The results showed that high temperature disturbed the equilibrium between the synthesis and catabolism of ROS production. Glutathione peroxidase (GPx) and SOD activity increased and catalyze (CAT) activity decreased under heat stress (34°C 5 h/day from d28 to d38) [45].

ROS production reduced Vitamin A and E levels. Vitamin C concentration decreased under heat stress in poultry [46]. It has been reported that heat stress increased zinc (Zn) mobilization from tissues, and thus may cause marginal Zn deficiency and increase requirements [47]. According to Zeng et al. [48, 49], SOD, MDA, CAT activity and the total antioxidant capacity (T-AOC) in Muscovy duck liver increased under short term heat stress (39°C for 1 hour then 3-hour recovery at20°C). The same results were found in broilers [50]. During heat stress in broilers, the serum concentrations of Vitamin C, E, A, iron (Fe), and Zn decreased, while the copper (Cu) concentration increased [51].

5.1.2 Using feed additives

5.1.2.1 Vitamin supplementation

High environmental temperature decreases the concentrations of vitamins and micro minerals in serum and increases excretion [52]; therefore, supplementation of direct or indirect antioxidant compounds (e.g. vitamins and micro nutrients) at higher levels is commonly recommended. These additives support mechanisms against lipid peroxidation, improve immune status and performance.

5.1.2.1.1 Vitamin E

Vitamin E functions as a fat-soluble antioxidant which protects cellular and membrane lipids from peroxidation-catalyzed free radicals due to heat stress. In cell membranes and lipoproteins, the essential antioxidant function of Vitamin E is to trap ROO^{-} and to break the chain reaction of lipid peroxidation. While it cannot prevent their formation, it can reduce the formation of secondary radicals [53]. Vitamin E is known as the first line of defense against lipid peroxidation caused by heat stress. It has free radical quenching activity and attacks free radicals in an early stage. When feed was supplemented with Vitamin E (200-250 mg/kg feed), the serum concentration of Vitamins E and A increased in serum and MDA concentration decreased under long term heat stress [51]. Maini et al. [54] reported that CAT, GR, GSH, MDA and SOD level decreased under heat stress due to Vitamin E supplementation. Short term heat stress increased the concentration of Zn in serum when the diet was supplemented with Vitamin E [55] (**Table 2**, [56]).

Author(s)	Spices	Duration of the study	Environmental temperature	Amount of vitamin supplementation	Effects on antioxidant status
Kucuk et al. [57]	broiler	1–42 day	34°C (24 h/day)	Vitamin A: 15 000 IU/kg diet	↓MDA
Mahmoud and Edens, [58]	broiler	1–42 day	30 °C (3.5 h/ for 3 days)	Vitamin C: 500 mg/ kg diet	↑plasma ascorbic acid concentration ↓Hsp70 expression
Sahin et al. [51]	Japanese quail	10–40day	34°C (24 h/day)	Vitamin E: 250 mg/ kg diet	↑Vitamin E, A concentration in serum ↓ MDA
Maini et al. [54]	broiler	1–49 day	38.6 ± 1.3 °C (24 h/day)	Vitamin E: 200 mg/ kg diet	↓CAT, GR, GSH, MDA, SOD
Harsini et al. [55]	broiler	1–49 day	37°C (8 h/day)	Vitamin E: 250 mg/ kg diet	↑Zn concentration in serum

CAT = catalase concentration in blood; GR = glutathione reductase concentration in blood; GSH = reduced glutathione concentration in blood; MDA = malondialdehyde concentration in blood; SOD = superoxide dismutase concentration in blood; Zn = zinc.
↑ Increase; ↓ decrease.

Table 2.
Effects of selected vitamin supplementation under heat stress based on different studies [56].

5.1.2.1.2 Vitamin C

Vitamin C protects against oxidative stress-induced cellular damage in the presence of scavenging ROS, and is capable itself of inhibiting lipid peroxidation in plasma. Ascorbic acid can directly scavenge radicals in the aqueous compartment. Ascorbate can scavenge O_2^-, H_2O_2, the OH, hypochlorous acid, aqueous ROO^-, and singlet oxygen. Under its antioxidant activity, ascorbate has a two-electron reduction [53]. Although chickens are known to synthesize ascorbic acid in the kidney, increased supplementation has proved beneficial effects in broilers reared under heat stress [59]. Ascorbic acid is actively absorbed. This active transport is supported by the sodium electrochemical gradient. However, the vitamin C requirements increase under heat stress. According to different studies, ascorbic acid supplementation (200 mg/kg feed) caused a significant increase in plasma ascorbic acid levels [59, 60] in broilers under heat stress. This indicates that the higher Vitamin C concentrations in the broiler diet could be used against heat stress successfully.

5.1.2.2 Micro-mineral supplementation

5.1.2.2.1 Zinc

Zn is a "member" of the antioxidant network because it is a cofactor of a very important antioxidant enzyme: Cu/Zn-SOD. Zinc plays a role in depressing the free radicals and inhibiting lipid peroxidation and GSH depletion. Zn can have direct antioxidant function and it is necessary for the prevention of free radical formation. However, it does not act directly against them [53]. Zinc supplementation has positive effects on antioxidant status of birds [61–63]. Zinc may play an important role in suppressing free radicals because it works as a cofactor (Cu/Zn-SOD) and inhibits NADPH-dependent lipid peroxidation [64], thus improving antioxidant status: increased serum Vitamin C and E concentrations [65] and decreased MDA levels [57, 66] (**Table 3**, [56]).

5.1.2.2.2 Selenium

Organoselenium compounds are essential micronutrients and are required for cellular defense against oxidative stress and optimal immune function. Selenium is necessary for cellular function and is a component of antioxidant enzymes: an important part (cofactor) of GPx, which works as an important antioxidant enzyme, protecting cells against free radical damage and oxidative stress [53]. Selenium supplementation improved antioxidant status in poultry under heat stress [51, 55, 58]. It is suggested that the metabolic role of Se is to protect cells against oxidation and tissue damage. Rapid oxidation of GSH to GSSH is necessary to compensate the heat stress caused ROS production. However, Se supplementation increases the level of available NADPH to promote the activation of GR, leading to increased GSSH reduction to GSH [67]. Therefore, Se supplementation affected GPx activity and the GPx/GSH ratio (**Table 3**).

Results of studies done with separated supplementation of Vitamin A (9000–15000 IU/kg diet), Vitamin E (150–500 mg/kg diet), Vitamin C (150–500 mg/kg diet), Zn (30 or 60 mg/kg diet) and Se (0,1-1 mg/kg diet), show that antioxidant status improved in poultry under heat stress. Antioxidant potential has been reported to be more efficient and important in combination than single antioxidant nutrients [68]. The latest research studies show that interactions between vitamin-vitamin and vitamin-minerals used in combination have more improved effects on

Author(s)	Spices	Duration of the study	Environmental temperature	Amount of supplementation (mg/kg diet)	Other effects
Sahin et al. [65]	Japanese quail	10–42 day	34°C (8 h/day)	Zn:30 or 60 mg/kg diet	↑Vitamin C concentration in serum ↓ MDA
Kucuk et al. [57]	broiler	1–42 day	34°C (24 h/day)	Zn: 30 mg/kg diet	↓MDA
Kucuk, [66]	Japanese quail	10–40day	35°C (8 h/day)	Zn: 30 mg/kg diet	↓ MDA
Harsini et al. [55]	broiler	1–49 day	37°C (8 h/day)	Se: 1 mg/kg diet	⌀Zn concentration ↑GPx
Mahmound and Edens, [58]	broiler	1–28 day	33°C (24 h/day for 4 weeks)	Se: 0.46 ppm/ kg diet	↑GSH, GSSG, GPx, GPx/GR ratio
Sahin et al. [51]	Japanese quail	10–40 day	34°C (24 h/day)	Se: 0.1 / 0.2 mg/kg diet	↑serum Vitamin E, A, Zn ↓ MDA

GPx = glutathione peroxidase concentration in blood; GR = glutathione reductase concentration in blood; GSH = reduced glutathione concentration in blood; GSSG = glutathione disulphide concentration in blood; GPx = glutathione peroxidase concentration in blood; MDA = malondialdehyde concentration in blood; Se = selenium; Zn = zinc.
↑ Increase; ↓ decrease; ⌀ no effect.

Table 3.
Effects of micro mineral supplementation under heat stress based on different studies [56].

Supplementation	Author(s)	Spices	Duration of the study	Environmental temperature	Amount of supplementation	Other effects
Vitamin C + Zn	Naila et al. 2014	broiler	22-42d	40 °C (12 h/day)	300 mg Vit. C + 60 mg Zn	↓mortality
Vitamin A+ Zn	Kucuk et al. [57]	broiler	1-42d	34°C (24 h/day)	15 000 IU Vit A + 30 mg	↓MDA
Vitamin E+ Se	Harsini et al. [55]	broiler	1-49d	37°C (8 h/day)	0.5 mg Se + 152 mg Vit.E	↓MDA ↑SOD

MDA = malondialdehyde concentration in blood; SOD = superoxide dismutase concentration in blood, Se = selenium, Zn = zinc.
↑ Increase; ↓ decrease.

Table 4.
Effects of vitamin and mineral interactions under heat stress based on different studies [56].

the antioxidant status and performance of poultry under heat stress than they do separately. Literature data on combinations of vitamin and mineral supplementation can be seen in **Table 4** [56].

5.1.2.3 Plant extracts

Dried oregano powder (0.5% and 1%) can be supplemented for ducks. Oregano (*Origanum vulgare* L.) is an herb extract used as an additive in poultry nutrition. It is an aromatic plant, containing more than 30 phenolic antioxidants constituents, including also anti-inflammatory and anti-microbial activity. It can also have

beneficial effects on production, mortality, microflora, and the immune system [69]. Antioxidant enzyme activity (SOD, GPx) was improved in poultry [69]. These results suggest that dried oregano powder addition could decrease the changes in antioxidant enzymes under heat stress.

5.1.2.4 Probiotics

Probiotics are non-digestive alternative growth promoters used in poultry nutrition. Probiotics can improve animal performance, and it can manipulate and maintain beneficial microflora in the gut. Several studies prove that probiotics supplementation to feed improves production parameters in poultry [70, 71]. Supplementation of probiotics (*Bacillus Subtilis*: 1x108 CFU/kg feed) decreased MDA activity and uric acid concentration, and also improved antioxidant response in ducks [72, 73].

6. The effect of heat stress on the performance of broilers reduced by using feed additives

6.1 The effect of heat stress on the performance of broilers

After reviewing the relevant research, we identified three different types of heat stress (HS) that have been applied in experiments: acute, cyclic and chronic. In the case of acute HS, the elevated temperature lasts from several hours up to 24 hours. After exposure to HS, sample and data collection occurs. This type of arrangement is suitable to study the immediate effect of heat stress. However, in temperate countries, even in the case of cooled stables, the actual barn temperature shows a daily cycle which can be mimicked by the cyclic HS environment (4–10 hours per day in the range of three days per week to daily up to 10 days long). In tropical countries, this kind of fluctuation is much less pronounced. Therefore, the environmental conditions can be best modeled with a chronic HS (continuous HS environment usually during the second half of fattening) model [74].

The most often claimed effect of heat stress is a reduction of feed intake. As an immediate effect, acute heat stress reduces feed intake by about 25% (**Table 5**).

Applying the heat stress repeatedly but allowing regeneration at thermoneutral (TN) temperature (cyclic HS-simulating the day-night temperature fluctuation) results in an adaptation, as during this period the lowest decline (in between 5 and 15%) in performance data (feed intake, daily gain and feed conversion ratio) can be observed (**Table 5**). Chronic heat stress will approximately double the negative effect compared to cyclic heat stress (7–11% point), but still some adaptation can be seen compared to acute heat stress. Acute HS has a dramatic effect on daily gain, as even negative values (weight loss) can occur, which makes it impracticable to calculate the feed conversion ratio. Therefore, researchers did not publish such data. The nutrient content of the unconsumed feed itself does not justify the negative energy balance; therefore, one can assume that the energy and nutrient needs of the HS response are high. When adaptation can occur; cyclic HS has a less adverse effect than chronic HS on both daily gain and the feed conversion ratio (**Table 5**).

6.2 Mitigation capacity of various feed additives on HS in broilers

One long term aim of researchers is to be able to mitigate the negative effects of heat stress. Supplementation of effective feed additives could be useful for improving intestinal absorption and minimizing the adverse effects of HS [92]. To have

Thermo-neutral temperature, °C	Heat stress temperature, °C	HS period, day of life	HS type	Feed intake, g/day			Average daily gain, g/day			Feed conversion ratio, kg/kg			Source
				Treatment		Change to TN %[c]	Treatment		Change to TN %[c]	Treatment		Change to TN %[c]	
				TN[a]	HS[b]		TN	HS		TN	HS		
18–19	38	20,41	acute[d]	115.2 ± 32.1	79.5 ± 16.2	−26.5 ± 24.7	78.5 ± 13.0	−1.3 ± 45.7	−102.9 ± 55.4				[75, 76]
20–28	31–36.2	15–49	cyclic[e]	125.8 ± 44.8	114.7 ± 33.6	−7.2 ± 9.0	69.4 ± 17.9	57.8 ± 13.7	−15.4 ± 12.8	1.95 ± 0.32	2.15 ± 0.54	9.1 ± 14.26	[77–84]
20–26	27.8–35	1–42	chronic[f]	132.8 ± 42.0	112.7 ± 33.5	−14.4 ± 10.0	70.5 ± 14.2	51.5 ± 11.5	−26.3 ± 14.0	1.85 ± 0.27	2.17 ± 0.33	18.1 ± 10.2	[81, 85–91]

[a]*Thermoneutral environment.*
[b]*Heat stress environment.*
[c]*(TN-HS)/TN*100, average values of the calculations from the research cited.*
[d]*HS environment from several hours to 24 hours.*
[e]*HS environment 4–10 hours per day in the range of three days per week to daily up to 10 days.*
[f]*Continuous HS environment (24 hours/day) usually during the second half of the fattening.*

Table 5.
Effect of heat stress on the growth performance of broilers (means ± s.d.).

TNt[a], °C	HSt[b], °C	HS period, days of life	HS type	Dietary treatment	Treatment type	Concentration of feed additives in the diet	Feed intake, g/day				Source
							TN[c]	HS[d]	HS + treatment[e]	Mitigation[f] %	
22	32	1–42	chronic[g]	betaine + vit E	combined[l]	1.2 g/kg + 500 mg/kg	111.1	78.5	83.3	14.7	[85]
22	32	1–42	chronic	Cr + vit C	combined	1.2 mg/kg + 500 mg/kg	111.1	78.5	83.1	14.1	[85]
22	32	1–42	chronic	cumin + tumeric	combined	1% + 1%	111.1	78.5	87.9	28.8	[85]
22	32	1–42	chronic	KCl + Na bicarbonate	combined	1.5 + 2 g/kg	111.1	78.5	83.1	14.1	[85]
22	32	1–42	chronic	propolis + vit A	combined	1 g/kg +15000 IU/kg	111.1	78.5	84.3	17.8	[85]
26	35	26–42	chronic	MOS + probiotic [i]	combined	0.5% + 0.1%	76.5	64.0	62.4	−12.8	[87]
	32	22–42	chronic	fumaric acid	single[m]	5 g/kg	160.7	142.4	143.4	5.7	[86]
23	32	22–42	chronic	fumaric acid	single	10 g/kg	160.7	142.4	138.7	−20.0	[86]
23	32	22–42	chronic	fumaric acid	single	15 g/kg	160.7	142.4	148.6	33.8	[86]
26	35	26–42	chronic	MOS	single	0.5%	76.5	64.0	63.3	−5.6	[87]
24	35	15–35	chronic	probiotic [j]	single	0.1%	86.1	82.0	85.6	87.8	[88]
26	35	26–42	chronic	probiotic [i]	single	0.1%	76.5	64.0	65.9	15.2	[87]
25	36	25–42	cyclic[h]	vit E + vit C	combined	100 mg/kg + 200 mg/kg	78.2	73.6	74.6	21.7	[78]
25	36	25–42	cyclic	vit E + vit C+ probiotic [k]	combined	100 mg/kg +200 mg/kg + 2 g/kg	78.2	73.6	74.4	17.4	[78]
25	36	25–42	cyclic	probiotic	single	2 g/kg	78.2	73.6	73.5	−2.2	[78]
25	36	25–42	cyclic	vit C	single	200 mg/kg	78.2	73.6	74.8	26.1	[78]
25	36	25–42	cyclic	vit E	single	100 mg/kg	78.2	73.6	73.5	−2.2	[78]
24	34	15–35	cyclic	vit E	single	150 mg/kg	99.5	91.9	89.2	−35.2	[79]
24	34	15–35	cyclic	vit E	single	150 mg/kg	104.1	87.1	89.3	13.0	[79]
			chronic		combined				Average ± sd	*12.8 ± 13.7*	

TNt[a], °C	HSt[b], °C	HS period, days of life	HS type	Dietary treatment	Treatment type	Concentration of feed additives in the diet	Feed intake, g/day				Source
							TN[c]	HS[d]	HS + treatment[e]	Mitigation[f] %	
			chronic		single				Average ± sd	*19.5 ± 38.1*	
			chronic		overall[n]				Average ± sd	16.1 ± 27.6	
			cyclic		combined				Average ± sd	*19.6 ± 3.1*	
			cyclic		single				Average ± sd	*−0.1 ± 22.9*	
			cyclic		overall				Average ± sd	5.5 ± 21.1	

[a]*Thermoneutral temperature.*
[b]*Heat stress temperature.*
[c]*Thermoneutral environment, control feed.*
[d]*Heat stress environment, control feed.*
[e]*Heat stress environment and specialty feed supplement fortified diet.*
[f]*% mitigation = (HS treatment - HS control)/(TN control – HS control) x 100.*
[g]*HS environment 4–10 hours per day in the range of three days per week to daily up to 10 days.*
[h]*Continuous HS environment (24 hours/day) usually during the second half of the fattening.*
[i]Lactobacillus plantarum, Lactobacillus delbrueckii *ssp. Bulgaricus*, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, *and* Streptococcus salivarius *ssp. Thermophilus and* Enterococcus faecium.
[j]Lactobacillus pentosus *ITA23 and* Lactobacillus acidophilus *ITA44.*
[k]Saccharomyces cerevisiae *and* Lactobacillus acidophilus.
[l]*Combination of two or more feed additives are used.*
[m]*Only one feed additive used.*
[n]*HS type averages regardless of single or combined supplementation.*

Table 6.
Mitigation capacity of various feed additives on HS in broilers' growth performance (daily feed intake, g/day).

TNt[a], °C	HSt[b], °C	HS period, days of life	HS type	Dietary treatment	Treatment type	Concentration of feed additives in the diet	Average daily gain, g/day				Source
							TN[c]	HS[d]	HS + treatment[e]	Mitigation[f] %	
22	32	1–42	chronic[g]	betaine + vit E	combined[l]	1.2 g/kg + 500 mg/kg	62.0	33.9	42.0	28.8	[85]
22	32	1–42	chronic	Cr + vit C	combined	1.2 mg/kg + 500 mg/kg	62.0	33.9	41.6	27.4	[85]
22	32	1–42	chronic	cumin+tumeric	combined	1% + 1%	62.0	33.9	41.0	25.3	[85]
22	32	1–42	chronic	KCl + Na bicarbonate	combined	1.5 g/kg +2 g/kg	62.0	33.9	40.9	24.9	[85]
22	32	1–42	chronic	propolis + vit A	combined	1 g/kg +15000 IU/kg	62.0	33.9	41.1	25.6	[85]
26	35	26–42	chronic	MOS + probiotic [i]	combined	0,5% + 0,1%	57.4	38.7	41.5	15.0	[87]
23	32	22–42	chronic	fumaric acid	single[m]	5 g/kg	83.3	61.4	68.6	32.6	[86]
23	32	22–42	chronic	fumaric acid	single	10 g/kg	86.3	61.4	69.1	30.6	[86]
23	32	22–42	chronic	fumaric acid	single	15 g/kg	83.3	61.4	76.2	67.5	[86]
26	35	26–42	chronic	MOS	single	0,50%	57.4	38.7	45.4	35.8	[87]
24	35	15–35	chronic	probiotic [j]	single	0,10%	53.8	43.3	48.0	44.8	[88]
26	35	26–42	chronic	probiotic [i]	single	0,10%	57.4	38.7	41.1	12.8	[87]
25	36	25–42	cyclic[h]	vit E + vit C	combined	100 mg/kg + 200 mg/kg	43.6	38.6	41.2	52.0	[78]
25	36	25–42	cyclic	vit E + vit C + probiotic [g]	combined	100 mg/kg +200 mg/kg + 2 g/kg	43.6	38.6	42.7	82.0	[78]
25	36	25–42	cyclic	probiotic [k]	single	2 g/kg	43.6	38.6	41.3	54.0	[78]
25	36	25–42	cyclic	vit C	single	200 mg/kg	43.6	38.6	40.1	30.0	[78]
25	36	25–42	cyclic	vit E	single	100 mg/kg	43.6	38.6	39.8	24.0	[78]
24	34	15–35	cyclic	vit E	single	150 mg/kg	55.7	46.4	48.5	23.1	[79]
24	34	15–35	cyclic	vit E	single	150 mg/kg	54.0	48.7	48.4	−4.7	[79]
			chronic		combined				Average ± sd	*24.5 ± 4.9*	

TNt[a], °C	HSt[b], °C	HS period, days of life	HS type	Dietary treatment	Treatment type	Concentration of feed additives in the diet	Average daily gain, g/day				Source
							TN[c]	HS[d]	HS + treatment[e]	Mitigation[f] %	
			chronic		single				Average ± sd	*37.4 ± 18.1*	
			chronic		overall[n]				Average ± sd	30.9 ± 14.3	
			cyclic		combined				Average ± sd	*67.0 ± 21.2*	
			cyclic		single				Average ± sd	*25.3 ± 20.9*	
			cyclic		overall				Average ± sd	37.2 ± 27.9	

[a]*Thermoneutral temperature.*
[b]*Heat stress temperature.*
[c]*Thermoneutral environment, control feed.*
[d]*Heat stress environment, control feed.*
[e]*Heat stress environment and specialty feed supplement fortified diet.*
[f]*% mitigation = (HS treatment - HS control)/(TN control – HS control) x 100.*
[g]*HS environment 4–10 hours per day in the range of three days per week to daily up to 10 days.*
[h]*Continuous HS environment (24 hours/day) usually during the second half of the fattening.*
[i]Lactobacillus plantarum, Lactobacillus delbrueckii *ssp. Bulgaricus*, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, *and* Streptococcus salivarius *ssp. Thermophilus and* Enterococcus faecium.
[j]Lactobacillus pentosus *ITA23 and* Lactobacillus acidophilus *ITA44.*
[k]Saccharomyces cerevisiae *and* Lactobacillus acidophilus.
[l]*Combination of two or more feed additives are used.*
[m]*Only one feed additive used.*
[n]*HS type averages regardless of single or combined supplementation.*

Table 7.
Mitigation capacity of various feed additives on HS in broilers' growth performance (average daily gain, g/day).

TNt[a], °C	HSt[b], °C	HS period, days of life	HS type	Dietary treatment	Treatment type	Concentration of feed additives in the diet	Feed conversion ratio, kg/kg				Source
							TN[c]	HS[d]	HS + treatment[e]	Mitigation[f]%	
22	32	1–42	chronic[g]	betaine + vit E	combined[l]	1.2 g/kg + 500 mg/kg	1.8	2.3	2.0	64.2	[85]
22	32	1–42	chronic	Cr + vit C	combined	1.2 mg/kg + 500 mg/kg	1.8	2.3	2.0	62.3	[85]
22	32	1–42	chronic	cumin + tumeric	combined	1% + 1%	1.8	2.3	2.1	34.0	[85]
22	32	1–42	chronic	KCl + Na bicarbonate	combined	1.5 g/kg + 2 g/kg	1.8	2.3	2.0	54.7	[85]
22	32	1–42	chronic	propolis + vit A	combined	1 g/kg + 15000 IU/kg	1.8	2.3	2.0	52.8	[85]
26	35	26–42	chronic	MOS + probiotic [i]	combined	0.5% + 0.1%	1.3	1.7	1.5	50.0	[87]
23	32	22–42	chronic	fumaric acid	single[m]	5 g/kg	1.9	2.3	2.1	59.0	[86]
23	32	22–42	chronic	fumaric acid	single	10 g/kg	1.9	2.3	2.0	82.1	[86]
23	32	22–42	chronic	fumaric acid	single	15 g/kg	1.9	2.3	2.0	94.9	[86]
26	35	26–42	chronic	MOS	single	0.5%	1.3	1.7	1.4	82.4	[87]
24	35	15–35	chronic	probiotic [j]	single	0.1%	1.6	1.9	1.8	34.5	[88]
26	35	26–42	chronic	probiotic [i]	single	0.1%	1.3	1.7	1.6	20.6	[87]
25	36	25–42	cyclic[h]	vit E + vit C	combined	100 mg/kg + 200 mg/kg	1.8	1.9	1.8	81.8	[78]
25	36	25–42	cyclic	vit E + vit C+ probiotic[k]	combined	100 mg/kg +200 mg/kg + 2 g/kg	1.8	1.9	1.7	145.5	[78]
25	36	25–42	cyclic	probiotic	single	2 g/kg	1.8	1.9	1.8	109.1	[78]
25	36	25–42	cyclic	vit C	single	200 mg/kg	1.8	1.9	1.9	27.3	[78]
25	36	25–42	cyclic	vit E	single	100 mg/kg	1.8	1.9	1.9	45.5	[78]
24	34	15–35	cyclic	vit E	single	150 mg/kg	1.8	2.0	1.8	73.5	[79]
24	34	15–35	cyclic	vit E	single	150 mg/kg	1.9	1.8	1.8	40.1	[79]
			chronic		combined				Average ± sd	*53.0 ± 10.8*	
			chronic		single				Average ± sd	*62.2 ± 29.6*	

TNt[a], °C	HSt[b], °C	HS period, days of life	HS type	Dietary treatment	Treatment type	Concentration of feed additives in the diet	Feed conversion ratio, kg/kg				Source
							TN[c]	HS[d]	HS + treatment[e]	Mitigation[f]%	
			chronic		overall[n]				Average ± sd	57.6 ± 21.8	
			cyclic		combined				Average ± sd	*113.6 ± 45.0*	
			cyclic		single				Average ± sd	*59.1 ± 32.7*	
			cyclic		overall				Average ± sd	74.7 ± 41.9	

[a]*Thermoneutral temperature.*
[b]*Heat stress temperature.*
[c]*Thermoneutral environment, control feed.*
[d]*Heat stress environment, control feed.*
[e]*Heat stress environment and specialty feed supplement fortified diet.*
[f]*% mitigation = (HS treatment - HS control)/(TN control – HS control) x 100.*
[g]*HS environment 4–10 hours per day in the range of three days per week to daily up to 10 days.*
[h]*Continuous HS environment (24 hours/day) usually during the second half of the fattening.*
[i]Lactobacillus plantarum, Lactobacillus delbrueckii *ssp. Bulgaricus*, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, *and* Streptococcus salivarius *ssp. Thermophilus and* Enterococcus faecium.
[j]Lactobacillus pentosus *ITA23 and* Lactobacillus acidophilus *ITA44.*
[k]Saccharomyces cerevisiae *and* Lactobacillus acidophilus.
[l]*Combination of two or more feed additives are used.*
[m]*Only one feed additive used.*
[n]*HS type averages regardless of single or combined supplementation.*

Table 8.
Mitigation capacity of various feed additives on HS in broilers' growth performance (feed conversion ratio, kg/kg).

more clear information on effectiveness of various feed supplements Ortega and Szabó [93] suggested the calculation of mitigation capacity (see subsection 2 in the present chapter). However, researchers [93] also point out that contrary to the numerous publications in the field, only a limited number of studies are suitable for using this calculation, as it requires at least three treatment groups. HS effect mitigating supplements are usually vitamins expressing antioxidant capacity, probiotics and plant extracts (**Tables 6–8**).

It can be seen that - overall - the most difficult parameter to improve is that of feed intake (**Table 7**), as the feed additives studied have quite variable mitigation capacities. The overall mitigation percentage is only about 5–15%.Bettervalueswere obtained in the case of chronic heat stress compared to cyclic heat stress, but different feed additives were used. The highest improvement (above 85%) was achieved with a probiotic [88]. However, other probiotics were much less effective.

The adversely affected daily gain can be improved by about 30–35%, and the feed conversion ratio increased up to 60–70%. Observing the mitigation capacity of different feed additives in this regard, it seems that probiotics and vitamins can be the most effective mitigators, especially when they are applied in combination [84]. However, further research is needed to determine the most effective microbe combination(s), and the most effective levels of vitamins, as well as their interactive effects. Only one study reported results with fumaric acid supplementation which also seems promising, but more research is still needed [86]. Quite a few authors have tested the feed supplements in combination, which is in line with feed industry trends. Therefore, we calculated the average % mitigation value for combined and single applications. Data shows that combined applications are more effective in cyclic heat stress conditions, while that benefit cannot be observed in chronic heat stress.

7. Conclusions

Based on the scientific findings presented in this chapter, the following important conclusions can be drawn:

- Using fat in the diet (up to 5%) can reduce heat production in livestock.
- Vitamins (e.g. A, E and C) are capable of reacting with free radicals, thereby reducing their amounts and lipid peroxidation in the poultry. However, micro minerals (e.g. Zn, Se) are not directly capable of preventing or reducing ROS-formation, but they are essential cofactors for those enzymes which are reacting with free radicals.
- Vitamin E and Vitamin C supplementation improved antioxidant parameters (CAT, GR, GSH, MDA, SOD) due to their essential antioxidant function. Both Zn and Se are also improving antioxidant parameters (GR, GSH, GPx, and MDA).
- Antioxidant potential of vitamins and micro minerals is more efficient in combination under heat stress in poultry nutrition.
- Plant extracts (e.g. oregano) could decrease the negative effects of heat stress on antioxidant enzyme activity due its antioxidant constituents.
- Betaine reduces heat production in animals at high ambient temperatures.

- Acute heat stress induced a drop in feed intake and increased nutrient demand will result even in weight loss. However, if heat stress is prolonged, adaptation will occur.

- Probiotics and vitamins (C and E) seem to be the most effective means of reducing the negative effects of heat stress.

- Main conclusion for the practice: Different feed additives and supplementation strategies (single vs. combined) can be more effective in temperate and tropical countries. Therefore, in order to decide which feed additive to use and in what form (single or combined) the most effectively, it is recommended that farmers carry out a pre-study in the given climatic and feeding conditions.

Author details

László Babinszky*, Csaba Szabó and Márta Horváth
Department of Animal Nutrition and Physiology, University of Debrecen, Debrecen, Hungary

*Address all correspondence to: babinszky@agr.unideb.hu

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