ISOPRENOIDS, A NATURAL ALTERNATIVE TO MITIGATE HEAT STRESS IN BROILER CHICKENS

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Abstract

Heat stress (HS) is a major problem in poultry farming, causing decreases in productive performance, deterioration of intestinal health, liver dysfunction, systemic inflammation, and impaired welfare in broilers. Nutritional treatments based on medicinal plants and, in particular, isoprenoid compounds have attracted increased interest as natural alternatives to synthetic additives due to their antioxidant, anti-inflammatory, antimicrobial, and stress response-modulating properties. Recent studies on broilers subjected to cyclic heat stress demonstrate the beneficial effects of administering isoprenoids (carotenoids, tocopherols/tocotrienols-vitamin E, coenzyme Q_{10}, phytosterols, and a variety of terpenoids/monoterpenes from essential oils) through biochemical mechanisms that can mitigate the effects of hyperthermia: they reduce oxidative stress, stabilize cell membranes, support mitochondrial function, modulate the inflammatory response, and influence the intestinal microbiota. However, there are limitations to the use of isoprenoids, such as: high variability of response depending on dose, exposure time, interactions with other nutritional components, maintenance conditions, bioavailability, compound stability, combinations, risk of unpleasant taste, or antagonism with the intestinal flora. This review summarizes how isoprenoids can improve the effects of HS, presents recent experimental evidence (powder, extracts, essential oils, and isolated molecules: carvacrol, thymol, carnosic acid, etc.), as well as current forms of administration and limitations.

***Key words**: heat stress, oxidative stress, broiler, isoprenoids*

INTRODUCTION

The increase in global demand for animal protein, as well as climate change, is leading to heat stress, which is becoming a major concern in the poultry industry [1]. Due to genetic improvements, broiler chickens, in addition to the advantages obtained, such as optimal feed efficiency, also have some disadvantages, such as low disease resistance and high sensitivity to temperature variations. In this regard, research shows that, in addition to adopting production systems that mitigate the adverse effects of heat stress caused by temperature variations, special attention is paid to feed and nutrition [2].

Heat stress occurs when birds, although homeothermic organisms, are unable to maintain their thermal balance by regulating heat production and loss. When exposed to factors that induce heat stress, behavioral and physiological changes are triggered to restore homeostasis [2]. Under high temperature conditions, thermoregulatory mechanisms are compromised when there is an imbalance between metabolic heat production and the ability to dissipate it efficiently, resulting in abnormal increases in body temperature [3].

Due to their feather cover, lack of sweat glands, and high metabolism, birds are more susceptible to temperature variations [4]. Exposure to heat stress causes a series of physiological changes in birds, such as acid-base imbalance, intestinal morphology, oxidative damage, and immune system

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dysfunction, which directly translate into decreased feed intake, inefficient feed conversion, reduced body weight, diminished meat quality, increased susceptibility to disease, and increased mortality [5]. Morphological changes due to heat stress promote the generation of reactive oxygen species (ROS) that compromise the antioxidant defense system, leading to oxidative stress. Research shows that, compared to other animals, birds are more vulnerable to temperature variations and oxidative stress [6]. Reactive oxygen species, generated physiologically during cellular metabolism, are radicals and chemically reactive molecules that play an essential role in redox signaling, ion transport, transcription, and cytokine immunomodulation [6].

Under conditions of environmental stress, intracellular ROS production increases significantly, leading to a marked activation of the physiological mechanisms involved in the regulation of thermal homeostasis [7], an additional stress that results in poor nutrient absorption and disruption of the redox status of the intestinal mucosa, causing an imbalance between pro-oxidative and antioxidant processes, thus reducing the efficiency of the immune response. In the long term, these changes compromise the integrity of the digestive tract and diminish both productivity and growth performance [8].

In poultry farms, exposure to heat stress is practically inevitable, thus leading to the adoption of various nutritional and technological strategies to mitigate oxidative stress, such as supplementing rations with minerals, antioxidants, vitamins, as well as the use of plant extracts and phytoadditives [9]. The introduction of medicinal plants into the diet can support health by stimulating the antioxidant and immune systems [10], modulating ROS production associated with heat stress, inhibiting enzymes involved in cell damage, resulting in improved mitochondrial function [11].

The purpose of this research is to provide an updated synthesis of the use of isoprenoids in the diet of broilers to mitigate heat stress by characterizing the types of compounds used, the inclusion levels (doses), and the effects on performance and physiology, as well as by highlighting the limitations and potential risks associated with their application.

MATERIAL AND METHOD

To conduct this analysis, scientific articles published between 2014 and 2025 were consulted, identified by querying the Google Scholar, Elsevier, MDPI, and PubMed Central® (PMC) databases. The keywords used included: "isoprenoids," "terpenes," "heat stress," and "oxidative stress." The selection of papers was based on their relevance to the proposed topic, including both original experimental studies and systematic or narrative review articles.

CLASSIFICATION OF ISOPRENOIDS

Exposure of broilers to high temperatures triggers a series of physiological and molecular responses, oxidative stress, inflammation, and endocrine dysfunction, which can be mitigated by administering plant-derived isoprenoids through antioxidant, anti-inflammatory, and endocrine-metabolic homeostasis modulation mechanisms [12].

Isoprenoids, or terpenes, are hydrocarbons composed of two or more isoprene units, each unit containing 5 carbon units, arranged in characteristic patterns. They are classified according to the number of isoprene units: hemiterpenoids (1 unit), monoterpenoids (2 units), sesquiterpenoids (3 units), diterpenoids (4 units), sesterterpenoids (5 units). triterpenoids (6 units), tetraterpenoids (8 units) and polyterpenoids (> 8 units) [12]. The use of isoprenoids extends to various fields, such as:

pharmaceuticals (artemisinins, taxol, cannabinoids), perfumes (geraniol, limonene, linalool), pigments (lycopene and β-carotene), cosmetics (esclarenol, patchoulol, santalol) [13].

Terpenes, such as pinene, myrcene, limonene, and p-cymene, are hydrocarbons with a relatively simple structure, while terpenoids are modified forms determined by the introduction of functional groups, resulting from the inclusion of oxygen atoms and rearrangements or oxidations of methyl groups. As secondary metabolites of medicinal and aromatic plants, terpenoids act as antimicrobial agents, providing protection against pathogens [14]. Terpenes and terpenoids are synthesized to form the precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) through two main metabolic pathways, namely the mevalonic acid (MVA) pathway in the cytosol and the 2C-methyl-d-erythritol-4-phosphate (MEP) pathway in the plastid [14].

ISOPRENOIDES USED IN POULTRY NUTRITION

Limonene, classified as a monoterpene, is a colorless liquid commonly found in essential oils extracted from citrus peels. D-limonene is the form with the highest biological activity (tab. 1). In addition to its aromatic role, limonene is recognized for its anti-inflammatory, immunomodulatory, and anticarcinogenic properties [15].

Carvacrol is a liquid monoterpenoid alcohol, insoluble in water but highly soluble in ethanol, acetone, and diethyl ether. It is found in essential oils obtained from aromatic plants such as black cumin (*Nigella sativa),* wild bergamot (*Citrus aurantium* var. *bergamia Loisel*), thyme (*Thymus glandulosus*), garlic mustard (*Alliaria petiolate*), savory (*Satureja hortensis*) and various species of oregano (*Origanum compactum, O. dictamnus, O. microphyllum, O. vulgare, O. scabrum*). Carvacrol is known for its antimicrobial properties and biological activities with neuro-, reno-, cardio-, hepato-protective, anticonvulsant, antidiabetic, immune-modulatory, antioxidant, and anti-inflammatory effects [16].

Thymol, a naturally occurring volatile monoterpenoid phenol, is the main active compound in essential oil extracted from thyme (Thymus vulgaris L.). This molecule is notable for its versatility, being used in a wide range of fields such as medicine, the food industry, veterinary medicine, and the chemical industry. From a pharmacological point of view, thymol is intensively studied due to its antioxidant, antimicrobial, anti-inflammatory, and healing properties [17]

Menthol is a cyclic monoterpene alcohol, a natural compound with a cooling effect, non-reactive, used as a flavoring agent in the food industry as well as in oral hygiene products, medicines, cosmetics, and pesticides. In nature, the predominant form is (-)-menthol (l-menthol), recognized for its superior cooling properties compared to other isomers [18]. Menthol is the dominant component of the essential oil of the genus *Mentha*, one of the most aromatic members of the subfamily *Nepetoideae*, including between 18 and 30 species and approximately 100 varieties [19].

Triterpenes and their saponins are a group of secondary metabolites in nature, numbering over 20,000 compounds, present in higher plants, monocotyledons, dicotyledons, fungi, and marine organisms. Triterpenes are a class of compounds with numerous valuable pharmacological effects and biological activities, with immunomodulatory and hypoglycemic actions, as well as anti-inflammatory, antioxidant, antiviral, cardioprotective, and neuroprotective properties [20].

Lutein is a natural antioxidant belonging to the carotenoid class, synthesized exclusively by plants such as spinach and carrots, contributing, according to studies, both to the protection and improvement of visual function [21] and to the optimization of productive parameters by improving feed conversion rates, feed consumption, and immunity in birds subjected to heat stress [22].

Table 1. Effects of major isoprenoids in heat-stressed broiler chickens

Compound	Effect	Reference
Thymol + Carvacrol	↑meat quality; ↑antioxidant capacity; ↑growth performance; ↓feed intake; ↓body temperature; ↑immunity; ↑enzymatic activity.	[29][30][31] [32]
Linalool	↑body weight; ↑slaughter yield; ↓water consumption; ↓body temperature; ↑crude protein digestibility; ↑antioxidant capacity; ↑increase in production parameters; - exhibits antimicrobial activity.	[34] [35] [36]
Limonene	↓MDA, NO; ↑SOD, CAT, GPx; ↑body weight.	[15]
Carnosic acid + Carnosol	↑levels of HSP70 and CRYAB; ↑growth performance; ↑average daily gain; - improves gut health, meat quality.	[37][38] [39]
Menthol	- activates innate immunity and anti-inflammatory response; ↑increased feed intake.	[33][40]
Vitamin E	↑performance; ↑skeletal characteristics and bone integrity; ↓mortality; ↓decreased SO impact.	[41]
Coenzyme Q10	- protective effect on cells by stimulating HSP70 expression.	[42]
β-carotene	- immunomodulatory and antioxidant effects; - improvement of performance.	[43]
Lycopene	↑SOD; ↑GPx; ↑meat quality; - enhancement of intestinal health.	[27][44]
Lutein	↑ antioxidant levels; ↑ improved oxidative balance; ↑ feed intake; ↑ improved feed conversion ratio; ↑ immunity.	[45] [46] [22]
Astaxanthina	↑meat quality; ↑ immune status; - improves growth performance	[47]
Saponins	↓ body temperature; ↑ feed intake.	[48]
Phytosterols	↑ antioxidant capacity; ↑ molecular biogenesis; ↑ growth performance.	[49]

1. Nf-κb- Nuclear Factor kappa-light-chain-enhancer of activated B cells; 2. AP-1-Activator Protein-1; 3. IRF3- Interferon Regulatory Factor 3; 4. MDA- Malondialdehyde; 5. NO- Nitric Oxide; 6. SOD- Superoxide Dismutase, 7. CAT- Catalase, 8. GPx- Glutathione Peroxidase; 9. HSP70- Heat Shock Protein 70; 10. CRYAB- Crystallin Alpha B.

Astaxanthin, a carotenoid without vitamin A activity, is a potent fat-soluble antioxidant that demonstrates exceptional free radical neutralizing properties and significant anti-inflammatory effects [23].

Naturally present in aquatic organisms and some birds, astaxanthin cannot be synthesized endogenously by animals, which is why it must be added exogenously to the basic diet of broiler chickens from sources such as *Haematococcus pluvialis*, red yeast, and crustacean by-products. [24].

β-carotene is a carotenoid with activity similar to vitamin A, being also a precursor of it. Although poultry has the most efficient conversion of β-carotene to vitamin A, exposure to stress factors can lead to a reduction in the ability to convert carotenoids to vitamin A [25].

One of the main functions of vitamin A is the ability of retinol to neutralize free radicals, thus contributing to maintaining optimal cell function and protecting the integrity of cell structures [26].

Lycopene is a typical isoprenoid molecule, a red carotenoid soluble in lipids, with promising antioxidant and nutritional properties, used as a natural feed supplement under heat stress conditions in the diet of broiler chickens [27].

Lycopene, among all the approximately 600 natural carotenoids identified, is the pigment with the highest protective capacity against reactive oxygen species, responsible for cell damage, playing an important role in protecting DNA against oxidative damage, contributing significantly to maintaining cell integrity [28].

MODE OF ADMINISTRATION OF ISOPRENOID COMPOUNDS IN BROILER DIETS

To mitigate heat stress in broiler chickens, the use of phytogenic feed additives in poultry diets has been widely adopted. Various sources and forms of administration have been explored to alleviate the effects of heat stress, including essential oils, nanoemulsions [50, 51], or direct supplementation through drinking water [52] and/or feed [53] (tab. 2).

Table 2. Dosage levels of major isoprenoid compounds reported in heat-stressed broiler chickens

Compound	Dosage	Reference
Tymol	250 mg/kg	[59]
Carvacrol	300–500 mg/kg oregano EO 50–100 mg/kg.	[60]
Licopene	20–30 mg/kg feed 200–400 mg/kg feed	[61]
Astaxanthina	10–80 mg/kg feed	[62]
Luteine	20–40 mg/kg feed;	[63]
β-carotene	0–60 mg/kg feed; Up to 120 mg/kg	[65]
Carnosic acid/ Carnosol	3% rosemary extract nanoemulsion in water; 20–80 mg/kg feed of carnosic acid.	[37]
Menthol	peppermint leaves 5 g/kg feed; essential oil blends 200–400 mg/kg	[33]
Vitamin E	200–500 mg/kg feed.	[41]
Coenzyme Q10	20–40 mg/kg feed.	[65]
Limonene	200 mg/kg feed, lemon EO.	[66]

1. Mg- miligram, 2. kg-kilogram, 3. EO- esential oil

Numerous studies have investigated the effects of essential oils on broiler chickens under heat stress conditions [54, 55], demonstrating that they can improve productive performance while simultaneously reducing the negative impacts of heat stress, such as oxidative stress and inflammation.

Some essential oils also exert immunomodulatory effects, strengthening the immune response and contributing to a reduced incidence of diseases under high-temperature conditions [14].

In addition, antimicrobial [56] and antioxidant [57] activities have been reported.

Owing to their multifunctional biological activities, essential oils represent a "green" and sustainable alternative in the nutritional and pharmaceutical fields [58].

CONCLUSIONS

Heat stress represents one of the most significant challenges in modern broiler production, exerting major negative effects on productive performance, health status, and bird welfare. In this context, the use of natural isoprenoids has proven to be a promising nutritional strategy for mitigating the adverse effects of heat stress.

Through their antioxidant, anti-inflammatory, and immunomodulatory properties, these compounds help neutralize reactive oxygen species, protect cellular membrane integrity, and maintain redox balance. They also support immune function, intestinal epithelial integrity, and metabolic adaptation, contributing to improved productive performance, enhanced feed conversion efficiency, and reduced mortality associated with hyperthermia.

However, the efficacy of these compounds depends on dosage, route of administration, and bioavailability.

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