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A systematic review on the modulation of heat shock protein 70 in broilers at different stages of growth under thermal stress

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Abstract: This systematic review synthesises evidence from published articles investigating nutritional strategies to modulate heat shock protein 70 (HSP70). A total of 1 616 records were identified from four databases and snowballing. After screening and eligibility assessment, 25 studies comprising 29 reports published between 2004 and 2025 were included. The analysis shows that targeted interventions particularly antioxidants (vitamins C, E, and selenium), amino acids and their derivatives (methionine, betaine), and phytogenic compounds effectively downregulate HSP70 expression in a dose or tissue-dependent manner. This modulation is associated with improved growth performance, enhanced redox balance, and normalised stress hormone profiles. Key findings indicate that synergistic combinations outperform single additives in suppressing HSP70 and improving carcass yield under chronic heat stress (32–38 °C); the early-life nutrient delivery modulates HSP70 expression and influences post-hatch thermotolerance; and organic mineral or methionine hydroxy analogue are more effective than inorganic forms. However, efficacy varies by life stage, with most studies focused on Ross and Cobb genotypes, and is influenced by gut microbiota interactions. Major research gaps remain, including defining stage-specific optimal HSP70 thresholds, clarifying links between HSP70 modulation, immunity or gut health, understanding long-term effects of early-life nutritional programming, and determining the influence of administration routes on nutrient efficacy.

Keywords: antioxidant; gene expression; intervention; nutrient; thermotolerance

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INTRODUCTION

Poultry farming is a cornerstone of global agriculture, critically underpinning food security, economic development, and rural livelihoods (Tallentire et al. 2016). Driven by rising demand for animal protein, technological advancements, and the pursuit of production efficiency, the industry has evolved significantly from traditional backyard systems to intensive commercial operations. This transformation is particularly evident in broiler production, where genetic selection and optimised nutrition have yielded remarkable improvements in growth rates, feed conversion ratios (FCR), and disease resistance. However, these gains come with vulnerabilities: the high metabolic rates of fast-growing genotypes increase metabolic heat output, rendering them highly susceptible to heat stress (HS), a challenge amplified by global warming (Nawaz et al. 2021; Gouda et al. 2024).

The poultry sector faces persistent challenges, including disease outbreaks, environmental sustainability, and the need for cost-effective alternative feed sources (Wongtangtintharn et al. 2025). Among these, heat stress has emerged as a critical constraint, especially in the tropical regions where poultry farming is economically vital (Wongtangtintharn et al. 2025). Heat stress disrupts physiological homeostasis, leading to reduced feed intake, impaired growth performance, increased mortality, and heightened disease susceptibility (Zulkifli et al. 2018). It also induces significant oxidative stress, damaging cellular structures (e.g. through lipid peroxidation) and compromising the immune function (Shehata et al. 2020; Abdel-Moneim et al. 2021; Qin et al. 2023).

At the molecular level, a key adaptive response to HS is the induction of heat shock protein 70 (HSP70). This cytoprotective chaperone prevents protein denaturation and aggregation, maintains cellular integrity, mitigates HS-induced inflammation (e.g. by reducing IL-6 and TNF-α), and reduces oxidative damage (Goel et al. 2021; Balakrishnan et al. 2023). Chronic HS (typically 32–35 °C) upregulates HSP70 in critical tissues like the liver, jejunum, and *pectoralis major* muscle. However, this protective response is energetically costly and can divert resources away from growth (Teyssier et al. 2022; Balakrishnan et al. 2023). Broilers exhibiting higher basal HSP70 levels often demonstrate better survivability, highlighting its dual role as both a biomarker of stress severity and a potential therapeutic target (Zulkifli et al. 2018). Despite its importance, the regulation of HSP70 and its interactions with other physiological pathways, particularly under nutritional interventions, remain incompletely understood (Abdel-Moneim et al. 2021; Nawaz et al. 2021).

Nutritional strategies offer a promising avenue for mitigating HS impacts. Supplementation of antioxidants (e.g. ascorbic acid, selenium) and probiotics has shown a potential to enhance antioxidant defences, modulate immune responses, and improve thermotolerance, partly by modulating HSP70 expression (Kumbhar et al. 2018; Shakeri et al. 2020; Wasti et al. 2020). However, the efficacy of these interventions is highly variable, influenced by factors such as dosage, broiler life stage, supplement form (organic vs inorganic), and interactions with the gut microbiota (Deng et al. 2022; Apalowo et al. 2024). Critically, broilers exhibit distinct metabolic and physiological responses to HS across different growth stages, necessitating the development of stage-specific dietary approaches.

Despite growing research, a significant gap exists in comprehensive reviews synthesising recent findings on the application of these nutritional strategies in the modulation of heat stress in broilers of different stages. This systematic review, therefore, aims to critically analyse current knowledge on HS impacts in broilers, focusing on the roles of HSPs, oxidative stress pathways, and nutritional interventions. Specific objectives are to (*i*) assess the efficacy of amino acids and other nutrients in mitigating HS; (*ii*) analyse the relationship between HSP70 regulation and key performance, health, and stress parameters; and (*iii*) identify research gaps and future directions for developing sustainable HS management strategies. By integrating findings from recent studies, this review will provide actionable insights for researchers, nutritionists, and poultry producers striving to enhance broiler welfare and productivity in heat-challenged environments.

MATERIAL AND METHODS

Inclusion and exclusion criteria

This review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. It included only published

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peer-reviewed journal articles in English, without applying any restrictions on publication dates. Studies that focused on other non-nutritional interventions, were not on broiler birds and did not measure HSP70 were excluded. This systematic review focuses on broiler chickens (population) subjected to heat stress conditions, evaluating the impact of nutritional supplementation (intervention), including vitamins, minerals, amino acids, phytogenics, and probiotics, on the modulation of heat shock protein 70 (HSP70). Interventions have been compared against non-supplemented control groups or baseline pre-treatment measurements (Comparison), with all groups exposed to standardised heat stress protocols. Primary outcomes include changes in HSP70 expression (measured via mRNA levels or protein concentration), while secondary outcomes encompass performance metrics such as growth rate, feed efficiency, and mortality (outcome). Finally, only articles indexed in the Web of Science (WOS) database were included to ensure a high level of credibility.

Literature search, screening and selection

The search process was performed twice using a consistent strategy across all selected databases. The initial round of searches was carried out between June 15th and June 22nd, 2025, and a follow-up search took place from June 27th to July 5th, 2025, to validate and update the results. All records retrieved during both phases underwent a rigorous screening process, including full-text and abstract reviews, to determine their eligibility based on predefined criteria.

Literature searches were conducted using Google Scholar (https://scholar.google.com/), Scopus (https://www.scopus.com), ScienceDirect (https://www.sciencedirect.com/), and PubMed (https://pubmed.ncbi.nlm.nih.gov). The comprehensiveness of searched articles was enhanced via a snowballing approach by examining the reference lists of relevant articles identified through the databases (Saba et al. 2024; Ishaq et al. 2025; Saba et al. 2025). Details of keywords used in carrying out a comprehensive search based on PICO headings in the various databases can be found in Table 1.

Table 1. Search keywords from different databases based on PICO headings

Component	Description
Google Scholar	("heat shock protein 70" OR HSP70) AND ("broilers" OR "broiler chicken") AND ("nutrients" OR "dietary supplements" OR "feed additives" OR "vitamins" OR "minerals" OR "amino acids" OR "ascorbic acid" OR "betaine" OR "selenium") AND ("modulate" OR "regulate" OR "expression" OR "suppressions") AND ("developmental stage" OR "embryonic" OR "in ovo" OR "starter" OR "grower" OR "finisher" OR "age-dependent") AND ("heat stress" OR "thermal stress") –layers –breeders –ruminants –swine
PubMed	("heat-shock proteins" OR "HSP70 heat-shock proteins" OR "heat shock protein 70" OR "HSP70") AND ("Chickens" OR "broilers" OR "Gallus gallus") AND ("dietary supplements" OR "nutrient" OR "feed additives" OR "amino acids" OR "vitamins" OR "minerals" OR "ascorbic acid" OR "betaine" OR "polyphenols") AND ("modulate" OR "regulate" OR "expression") AND ("growth and development" OR "embryonic structures" OR "in ovo" OR "starter" OR "grower" OR "finisher") AND ("heat stress sisorders" OR "heat stress" OR "thermal stress") NOT ("layer" OR "turkey" OR "duck")
Scopus	("heat shock protein 70" OR "HSP70") AND ("broilers" OR "broiler chicken") AND ("nutrients" OR "dietary supplements" OR "functional feed" OR "antioxidants" OR "ascorbic acid" OR "betaine" OR "chromium" OR "selenium" OR "zinc") AND ("modulate" OR "regulate" OR "gene expression" OR "protein synthesis") AND ("developmental stage" OR "life stage" OR "embryonic" OR "early posthatch" OR "starter" OR "grower" OR "finisher") AND ("heat stress" OR "thermal stress" OR "high temperature stress") AND NOT ("layer" OR "breeder" OR "swine" OR "fish")
ScienceDirect	("HSP70" OR "heat shock protein 70") AND ("broilers" OR "meat chickens") AND ("nutritional intervention" OR "dietary strategy" OR "nutraceutical" OR "phytogenic" OR "vitamin" OR "trace minerals" OR "methionine" OR "lysine") AND ("modulate" OR "downregulate" OR "suppression") AND ("growth phase" OR "embryonic development" OR "hatching stage" OR "starter phase" OR "grower phase" OR "finisher phase") AND ("heat stress" OR "heat challenge" OR "hyperthermia") AND NOT ("layer" OR "turkey")

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At the end of the literature search, a total of 1 616 records were identified. After initial title screening, 747 duplicates were removed, and 689 records were excluded for not meeting the inclusion criteria, leaving 180 records for abstract screening (Figure 1). Of these, 120 records were excluded based on abstract relevance, and 60 records were selected for full-text retrieval. All 60 full texts were successfully retrieved and assessed for eligibility. During full-text screening, 33 records were excluded for the following reasons: not focusing on broilers (n = 6), not addressing HSP70 (n = 11), not being related to heat stress (n = 10), being duplicates (n = 3), or not being published in English (n = 1),

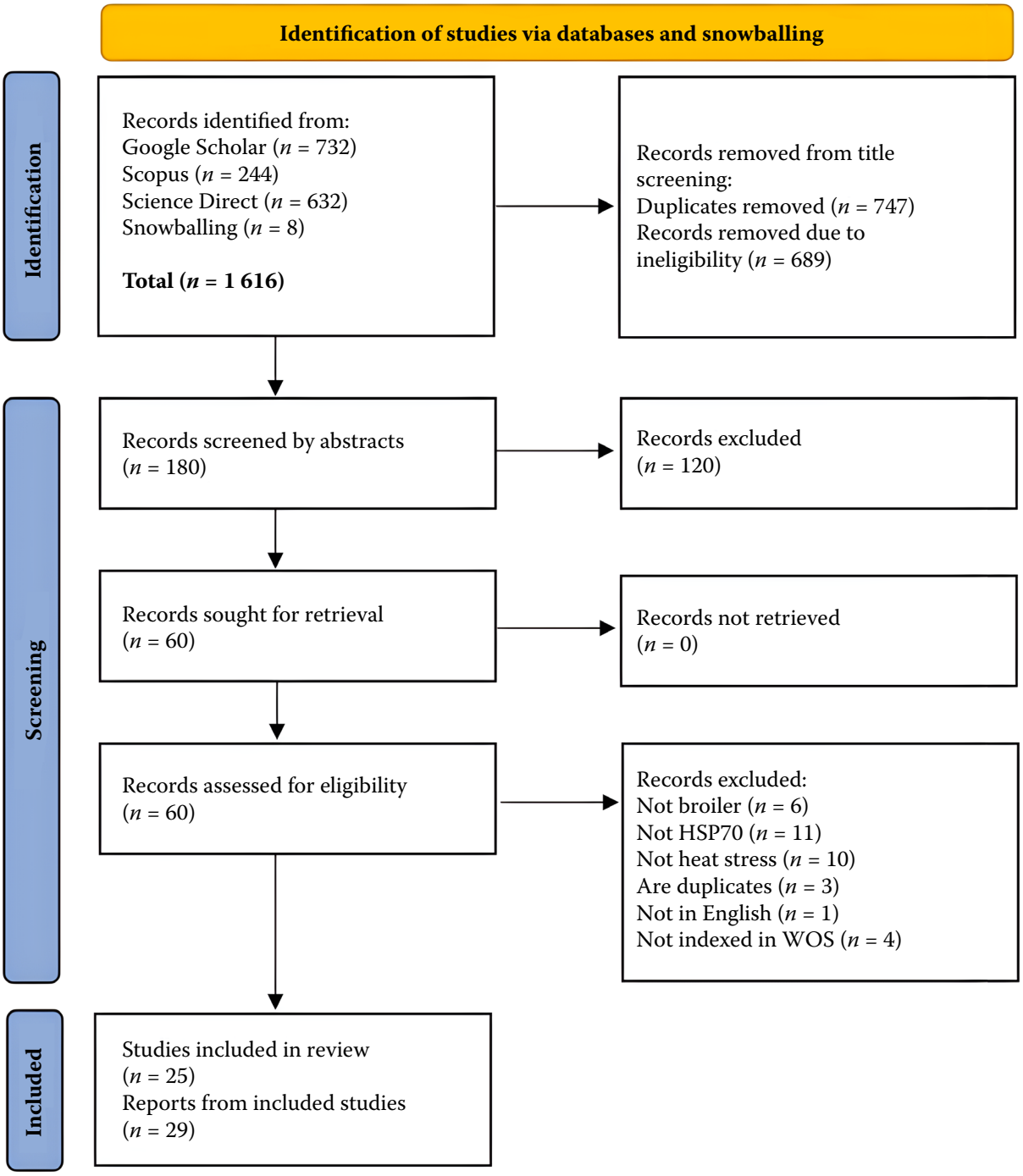

Figure 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart of literature search, screening and selection process

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or not indexed in the Web of Science database (4). Ultimately, 25 studies were included in the review, comprising a total of 29 relevant reports.

Identification, screening, and selection of article

Most of the search results from each database were exported as CSV files into the Rayyan QCRI online platform (https://www.rayyan.ai/), apart from results obtained through snowballing. To remove duplicates, two key steps were followed: verifying the presence of relevant studies and discarding those not aligning with the inclusion criteria. Thereafter, titles and abstracts were screened using Rayyan. After completing the initial screening on Rayyan, the full texts of selected studies were retrieved for in-depth evaluation (Ouzzani et al. 2016; Saba et al. 2023). These articles were carefully reviewed, and relevant data were systematically extracted using Microsoft Excel 365 (Redmond, WA, USA). Articles not previously screened in Rayyan had their abstracts independently reviewed prior to the full-text analysis. As the comprehensive review of the full texts progressed, several studies were excluded due to failure to meet the predefined eligibility criteria.

Data extraction and synthesis

The full-text versions of all identified articles were downloaded as PDFs for further review. Relevant information was tabulated and analysed descriptively using Microsoft Office Excel 365. General information about the included studies was extracted, including treatments used, broiler stage, dependent variables, study design, sample size, broiler genotype, age of birds (days), heat-stress conditions (temperature/duration), nutrient type, nutrient dose, duration of supplementation, control group details, HSP70 measurement method and tissue used.

RESULTS AND DISCUSSION

Experimental design and intervention details

For most of the studies, randomised control trials (RCT, 72%) were used, with the randomised factorial design (RFD, 16%) (Figure 2). Other designs include randomised block design (RBD), *in vitro*, and *in vivo* testing. Antioxidants constituted 48% of interventions, primarily ascorbic acid (200–600 mg/kg diet or 100–300 mg/bird/day), vitamins (100–250 mg/kg), and selenium (0.2–0.3 mg/kg). Amino acids/derivatives (31% of studies) included methionine, betaine, and leucine. Minerals featured chromium (0.1–1.2 mg/kg CrMet or 1 200 µg/kg picolinate) and zinc/manganese. Other agents included melatonin (0.5 mg/kg) and probiotics (Table 2). Furthermore, about 80% of studies used long-term supplementation (≥35 days). *In ovo* interventions involved single embryonic injections, e.g. leucine (Han et al. 2019). All controls received basal diets without additives under identical heat stress (32–38 °C). Three studies included thermoneutral controls (24–25 °C) (Mahmoud et al. 2004b; Toplu et al. 2014; Abo-Samaha et al. 2021).

For HSP70 measurement, most studies (65%) quantified mRNA via qRT-PCR (SYBR® Green/TAQMan), often normalised to GAPDH or β-actin (Table 2). The tissue-specific analysis dominated (liver: 40%; muscle: 30%; intestine: 15%). For protein detection, studies mostly (35%) used ELISA (commercial chicken HSP70 kits) or Western blot (e.g. recombinant HSP70 standards: Mahmoud et al. 2004a). Only two studies combined mRNA/protein assessment (Zhu et al. 2015; Belal et al. 2018).

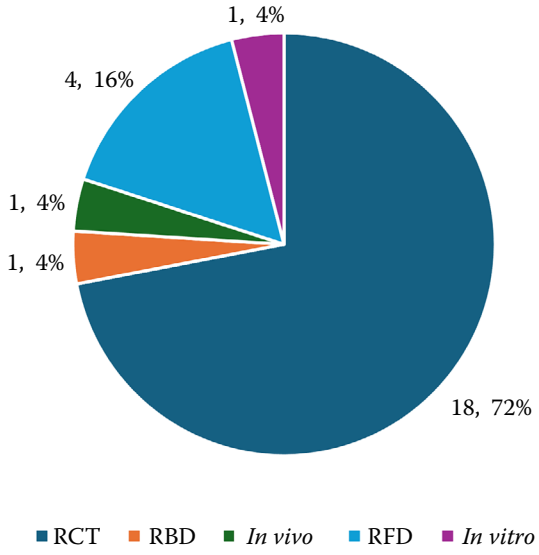

Figure 2. Experimental design of included studies relating to the modulation of heat stress in broilers at different stages

RBD = randomised block design; RCT = randomised control trials; RFD = randomised factorial design

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Table 2. Nutrient used, duration, control and HSP70 measurement method in the selected studies

Nutrient type	Nutrient dose	Supplementation duration	Control group details	HSP70 measurement method/ tissue used	Reference
Ascorbic acid (AA)	200 mg/kg diet	days 1–33	basal diet	qRT-PCR (liver)	Abo-Samaha et al. (2021)
Ascorbic acid (AA)	500 mg/kg diet	days 1–28	basal diet	Western blot (heart tissue)	Mahmoud et al. (2004a)
Ascorbic acid (AA)	100–300 mg/bird	42-day trial	basal diet	ELISA kit (plasma)	Mishra et al. (2015)
Chromium-methionine	0–1.20 mg/kg diet	days 22–43	basal diet	RT-qPCR (*Pectoralis major*)	Dalolio et al. (2024)
Inorganic & organic Mn	120 mg Mn/kg diet	32–45 weeks	basal diet	RT-qPCR & Western blot: liver, heart, and breast	Zhu et al. (2015)
Vitamin E and selenium	100–150 mg/kg Se 0.2–0.3 mg/kg Vit E	days 42/ trial period	basal diet	chicken HSP70 ELISA kit from serum	Bora et al. (2024)
L-Ascorbic acid (vitamin C)	500 mg/kg of diet	days 22–42	basal diet	immunohistochemistry assay: liver, kidneys and brain	Toplu et al. (2014)
L-Methionine and L-cysteine dissolved in saline	5.90 mg/L-methionine 3.40 mg/L-cysteine	days 17.5 of incubation	negative and positive controls	ELISA & qPCR: mRNA expression in tissues and serum	Elnesr et al. (2019)
L-Leucine (L-leu)	34.5 µmol L-leu per egg in 500 µl sterile water	injection at day 7 of incubation	sterile water injection	qPCR: mRNA expression in diencephalon, liver, muscle	Han et al. (2019)
Probiotic (*Saccharomyces cerevisiae*); ascorbic acid	1 g/kg of feed; ascorbic acid: 200 mg/kg of feed	days 1–35	basal diet	RT-qPCR from serum	Sumanu et al. (2023)
Selenium-enriched probiotics (SP)	0.30 mg Se/kg feed (total Se: 0.41 mg/kg)	days 1–42	basal diet	qRT-PCR from heart tissue	Khan et al. (2016)
Serotonin	20 µg/egg	single injection before incubation	normal saline (0.9% NaCl) injection	qPCR from brain tissues	KHasti et al. (2025)
Vitamin C (L-ascorbic acid)	1 g/kg basal diet	days 1–42	basal diet	qPCR from liver, heart and kidney	Albokhadaim et al. (2019)
Sodium selenite, vitamin E	Se: 0.2 mg/kg; VE: 250 mg/kg	days 1–42	basal diet	real-time PCR of the breast muscles	Kumbhar et al. (2018)
Selenium yeast (Sel-Plex®)	0.2 mg/kg feed	days 1–42	basal diet	ELISA (liver tissue)	Mahmoud and Edens (2005)

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Table 2 to be continued

Nutrient type	Nutrient dose	Supplementation duration	Control group details	HSP70 measurement method/ tissue used	Reference
DL-Methionine + L-carnitine	200 mg/kg diet	days 1–42	basal diet	qRT-PCR (mRNA level) in liver and hypothalamus	Ghasemi and Nari (2023)
MHA + L-carnitine	200 mg/kg diet + MHA	days 1–42	basal diet	qRT-PCR (mRNA level) in liver and hypothalamus	Ghasemi and Nari (2023)
Recombinant chicken leptin	8 µg/kg per hour	6 h	saline solution	RT-PCR/Southern blot analysis	Figueiredo et al. (2007)
Recombinant chicken leptin	10–1 000 ng/ml	24 h	untreated cells	RT-PCR/Southern blot analysis	Figueiredo et al. (2007)
Ascorbic acid	200, 400, 600 mg/kg diet	days 1–42	basal diet	RT-qPCR in liver tissue	Biswas et al. (2024)
Taurine	0.1% in water	5 weeks	no taurine	qRT-PCR (mRNA) and Western blot (protein) from liver and breast	Belal et al. (2018)
Inorganic phosphorus (P)	0.50% available P	3 weeks	no P	Northern blot (mRNA)	Mahmoud et al. (2004b)
Vitamin C	300 mg/kg diet	days 1–42	basal diet	qRT-PCR (mRNA) from liver, brain, heart and spleen	Hajati et al. (2015)
Vitamin C	0.2% in all periods	days 1–42	basal diet	real-time PCR (qPCR) with β-actin as a control from the liver	Erfani et al. (2021)
Methionine hydroxy analogue (MHA)	0.46% (starter), 0.36% (grower), 0.32% (finisher)	days 1–42	basal diet	real-time PCR (qPCR) with β-actin as a control from the liver	Erfani et al. (2021)
MHA + Vitamin C	MHA: 0.36–0.46% Vit C: 0.2–0.32%	days 1–42	basal diet	real-time PCR (qPCR) with β-actin as a control form liver	Erfani et al. (2021)
Amino acid-chelated trace minerals	not specified	42 days	basal diet	qPCR (RNA isolation + SYBR Green) from blood	Baxter et al. (2020)
Inorganic Zn sulphate (iZn), organic Zn lysine chelates (oZn)	50 µmol/l	8 h preincubation + 4 or 6 h incubation	basal diet	real-time quantitative PCR, Western blot	Li et al. (2021)
Amino acids (lysine, methionine, etc.)	80–110% of Aviagen AA specifications	36 days	basal diet	qRT-PCR (Power SYBR Green) on pectoralis muscles	Alhotan et al. (2021)

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Sample characteristics

Commercial Ross strains (predominantly Ross 308) were used in 44% of studies (11/25), followed by Cobb variants (Cobb 500: 16%; Cobb 400: 8%) and Arbor Acres (16%). Only two studies used unspecified commercial broilers. This reflects the industry alignment with high-yield genetic lines. About 84% of studies (21/25) tested aged birds, while 16% (4/25) included embryonic stages, for example, *in ovo* injections at ED7-18 (Elnesr et al. 2019; Han et al. 2019) or starter phases, 0–10 days (Alhotan et al. 2021). Zhu et al. (2015) studied laying broiler breeders (32–45 weeks), while Li et al. (2021) used embryonic hepatocytes (*in vitro*). Bird numbers ranged widely, with small-scale testing involving 40–96 birds (Figueiredo et al. 2007; Han et al. 2019) and large-scale testing involving 240–720 birds (e.g. Ghasemi and Nari 2023; Biswas et al. 2024). As per replication, most studies used pen replicates (4–8 birds/pen; 3–6 replicates/treatment), while some studies reported unclear replication. Moreover, about 70% of the studies specified male-only cohorts to eliminate sex-based variability. All birds were reared under standard commercial pre-trial conditions, with heat stress applied at defined ages (32–38 °C from days 21–42) (Table 3). Most studies reported that water was provided *ad libitum* to all experimental birds throughout the heat stress exposure period, consistent with standard poultry research protocols and essential for supporting evaporative cooling, thermoregulatory balance, and animal welfare while avoiding confounding dehydration stress.

Other parameters

Research assessing HSP70 (heat shock protein 70) in broilers commonly evaluates it alongside several key categories of dependent variables, reflecting the multifaceted impact of heat stress. These include physiological and hormonal stress markers, growth and production performance, antioxidant status, oxidative stress, haematological and biochemical blood parameters, immune and inflammatory markers, molecular markers (beyond HSP70), tissue integrity, and morphology. A primary focus is on indicators of the stress response itself. This consistently includes measuring the plasma or serum levels of corticosterone (CORT or CS), the major avian stress hormone (Mahmoud et al. 2004a,b; Mishra et al. 2015; Toplu et al. 2014; Elnesr et al. 2019). Body temperature is a direct physiological indicator that is also monitored (Toplu et al. 2014; Dalolio et al. 2024).

Hormonal assessments sometimes extend to thyroid hormones (Erfani et al. 2021), linking stress to metabolic regulation. Organ weights, particularly lymphoid organs (thymus, spleen, bursa) (Hassan and Assim 2020; Bora et al. 2024; Dalolio et al. 2024) and sometimes the heart or liver, serve as indicators of immune status and metabolic strain. Fear-related behaviour (tonic immobility) is also assessed as a stress indicator (Toplu et al. 2014). Direct measures of economic impact are frequently correlated with HSP70. These include body weight (BW), body weight gain (BWG or weight gain), feed intake (FI), feed efficiency ratio (FCR), performance index (PI), body performance efficiency index (BPEI), and mortality (Mahmoud and Edens 2005; Mishra et al. 2015; Baxter et al. 2020; Abo-Samaha et al. 2021; Alhotan et al. 2021; Erfani et al. 2021; Ghasemi and Nari 2023; Biswas et al. 2024; Bora et al. 2024; Dalolio et al. 2024).

Carcass characteristics such as dressing percentage, prime cuts yield, and giblet weights are also evaluated as end-point production traits (Baxter et al. 2020; Bora et al. 2024). Given the strong link between heat stress and oxidative damage, numerous studies measure markers of antioxidant defence and lipid peroxidation. Key variables include malondialdehyde (MDA) levels (a marker of lipid peroxidation), activities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and levels of glutathione (GSH, TGSH, GSH/GSSG ratio) (Mahmoud and Edens 2005; Zhu et al. 2015; Kumbhar et al. 2018; Albokhadaim et al. 2019; Elnesr et al. 2019; Li et al. 2021; Ghasemi and Nari 2023; Sumanu et al. 2023). The oxidative DNA damage biomarker 8-OHdG is also measured (Sumanu et al. 2023).

In studies where they were reported, corticosterone levels were typically measured using validated radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) kits on plasma or serum samples collected via wing vein, jugular, or cardiac puncture, centrifuged at 1 500–3 000 × *g* for 10–15 min at 4 °C, stored at –20 °C or –80 °C, and assayed in duplicate with coefficients of variation below 5–6%.

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Table 3. Broiler production stages, sample size used and genotype in the selected studies under heat stress conditions

Stage of production	Sample size	Genotype	Heat stress condition	Reference
Grower (33 days)	96 (4 × 3 reps of 8 birds)	Cobb 500	34 °C from day 21 through day 24	Abo-Samaha et al. (2021)
Grower phase (28 days)	160 (2 × 2 factorial)	Arbor Acres	41 °C (over 3.5 h/3 days)	Mahmoud et al. (2004a)
Grower phase (42 days)	96 (4 × 2 reps, 12 birds)	Cobb 400	40 ± 5.0 °C heat stress	Mishra et al. (2015)
Grower to finisher (22–43 days)	experiment 1: 336 broilers	Cobb 500	33 °C for 12 h/day for 21 days	Dalolio et al. (2024)
Broiler breeders (18–45 weeks)	144 (6 × 6 reps, 4 birds)	Arbor Acres	32 ± 1 °C from 32 to 45 weeks	Zhu et al. (2015)
Grower phase (0 to 42 days)	240 (4 × 3 reps, 20 birds)	Cobb 400	36.5 °C with 92.5% RH	Bora et al. (2024)
Grower phase (0 to 42 days)	320 (4 × 4 reps of 20 birds)	Ross 308	35 °C for 6 h/day	Toplu et al. (2014)
Embryonic stage (1–18)	150 eggs (50 per group)	Ross broiler	39.6 °C for 6 h daily	Elnesr et al. (2019)
Grower (0 to 35 days)	56 (14 groups of 4 birds)	Ross 308	not explicitly stated	Sumanu et al. (2023)
Grower phase (0 to 42 days)	200 (4 × 5 reps of 10 birds)	Ross 308	34 ± 1 °C with 60% to 80% RH	Khan et al. (2016)
Embryogenesis (ED 13–18)	120 eggs	Ross 308	39.5 °C for 2 h, 55–65% RH	KHasti et al. (2025)
Grower phase (0 to 42 days)	200 (50/group of 4 birds)	Ross 308	34 °C for 8 hours/day	Albokhadaim et al. (2019)
Grower phase (0 to 42 days)	200 (50/group in 4 reps)	Ross 308	34 °C to 36 °C for the first 14 days	Kumbhar et al. (2018)
Grower phase (0 to 42 days)	128 (64/group in 2 reps)	Arbor Acres	40 °C for 1 h	Mahmoud and Edens (2005)
Grower phase (0 to 42 days)	720 (5 × 6 reps, 24 birds)	Ross 308	34 °C for the first 3 days	Ghasemi and Nari (2023)
Grower phase (0 to 42 days)	192 (4 × 6 reps × 8 birds)	not specified	35.4 °C, 79% RH	Biswas et al. (2024)
Grower (0 to 35 days)	40 (10 × 4)	Ross broilers	34 °C for 3 weeks at 60% RH	Belal et al. (2018)
Grower phase (0 to 21 days)	60 (10 × 6)	not stated	41 °C for 60 min	Mahmoud et al. (2004b)
Grower phase (0 to 42 days)	60 (12 × 5)	Cobb-500	34 ± 1 °C with 65% to 70%, 5 h	Hajati et al. (2015)
Grower phase (0 to 42 days)	400 (5 reps of 20 birds)	Ross 308	32–34 °C with 60 and 70% RH	Erfani et al. (2021)
Grower phase (0 to 42 days)	480 (12 × 24 reps, 20 birds)	Cobb 500	35 °C and 20% to 30% RH	Baxter et al. (2020)
Embryo hepatocytes in vitro	6 reps per treatment	Arbor Acres	44 °C for 1, 2, 4, 6, or 8 h	Li et al. (2021)
Starter phase (0 to 10 days)	576 (24 reps, 6 chicks)	Ross 308	32 °C for 8 h daily	Alhotan et al. (2021)
Grower phase (0 to 21 days)	5 per group	Ross strain	37 °C at 5% humidity	Figueiredo et al. (2007)
Grower phase (0 to 42 days)	3 per group	Cobb strain	37 °C at 5% humidity	Figueiredo et al. (2007)
Embryonic stage	120 eggs (60/group)	Ross strain	37.6 °C with 58–68% RH	Han et al. (2019)

ED = embryonic days; reps = replicates; RH = relative humidity

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The blood analysis provides a snapshot of the overall physiological state. Commonly assessed parameters include haemoglobin (Hb), red blood cell count (RBCs), packed cell volume (PCV), white blood cell count (WBC), heterophil/lymphocyte (H/L) ratio (a stress leukogram), glucose, total protein, liver enzymes (SGOT/AST, SGPT/ALT), lipid profile (triglycerides, cholesterol, HDL, LDL, VLDL), and electrolytes (Toplu et al. 2014; Hajati et al. 2015; Hassan and Assim 2020; Erfani et al. 2021). Immune function assessment includes measuring cytokine gene expression (Abo-Samaha et al. 2021), antibody titres (e.g. against Newcastle Disease Virus – NDV) (Toplu et al. 2014), interleukin (IL-10) levels (Sumanu et al. 2023), general assessments of immunity (Ghasemi and Nari 2023; Biswas et al. 2024), and inflammation markers (Baxter et al. 2020). Lymphoid organ weights also indirectly reflect the immune status.

Molecular markers (beyond HSP70)

Research often explores the broader molecular response by measuring the gene or protein expression of other heat shock proteins (HSP60, HSP90) and heat shock factors (HSF1, HSF3) (Figueiredo et al. 2007; Zhu et al. 2015; Khan et al. 2016; Albokhadaim et al. 2019; KHasti et al. 2025). Expression of genes related to antioxidant defence (MnSOD, GPx1, GPx4, selenoproteins, GSH-Px), metabolism (mTOR pathway genes: mTOR, S6K1, 4EBP1; IGF-1; amino acid transporters; aquaporins – AQPs), and selenoprotein P (SelP) are also evaluated (Zhu et al. 2015; Khan et al. 2016; Kumbhar et al. 2018; Elnesr et al. 2019; Han et al. 2019; Alhotan et al. 2021; Dalolio et al. 2024). Heart histopathology provides a tissue-level insight (Khan et al. 2016).

Tissue integrity and morphology

Some studies delve into structural changes, examining intestine morphology (villus height, crypt depth) (Erfani et al. 2021) and intestinal integrity markers (Baxter et al. 2020). In summary, HSP70 serves as a central biomarker for cellular stress response in poultry research, consistently evaluated alongside core indicators of physiological stress (corticosterone, body temperature), growth performance (BW, BWG, FI, FCR), and oxidative stress/antioxidant status (MDA, SOD, GSH). It is further contextualised by measures of immune function, haematology, blood biochemistry, carcass traits, and the expression of related molecular pathways (other HSPs/HSFs, antioxidant genes, and metabolic genes).

Heat shock protein 70 modulation

This systematic review comprehensively assessed the impact of nutrient supplements on heat stress modulation in broilers of different growth stages, specifically with respect to HSP70, and the relationship with other parameters such as physiological and hormonal stress markers, growth and production performance, antioxidant status and oxidative stress, haematological and biochemical blood parameters, immune and inflammatory markers, molecular markers (beyond HSP70), tissue integrity and morphology. Various research gaps were identified with recommendations for future research.

The induction of heat shock protein 70 (HSP70) is a hallmark response to thermal stress in broilers, with most studies in this review identifying it as a critical biomarker for evaluating the heat stress resilience. Numerous interventions, from dietary antioxidants to amino acid supplements, have demonstrated the potential to attenuate HSP 70 expression. For instance, supplementation of ascorbic acid (AA) significantly downregulated HSP 70 and pro-inflammatory cytokines, though the weight gain was not significantly affected in the short post-stress recovery period (Abo-Samaha et al. 2021).

Similarly, organic selenium (Se) and vitamin E (VE) combinations consistently reduced HSP 70 mRNA or protein levels across multiple studies (Kumbhar et al. 2018; Bora et al. 2024), underscoring their synergistic antioxidant roles. Importantly, tissue specificity emerged as a recurring theme. HSP 70 expression was often assessed in breast muscle (Dalolio et al. 2024), or hypothalamus (Figueiredo et al. 2007), reflecting differential stress responses. However, the lack of protein-level validation in many mRNA-based studies (e.g. Han et al. 2019; KHasti et al. 2025) limits interpretability, highlighting the need for multi-level molecular assessments at different stages of broiler chicken production (starter and finisher phases), which needs to be investigated.

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Antioxidant and micronutrient interventions

Antioxidants such as vitamins C and E, selenium, chromium (Cr), and zinc (Zn) have shown promise in enhancing thermotolerance by reducing oxidative damage and HSP70 expression. Dalolio et al. (2024) demonstrated that chromium methionine (CrMet) enhanced metabolic efficiency and downregulated HSP70, while Zhu et al. (2015) showed that organic manganese improved antioxidant enzyme activity and reduced HSP70 expression in muscle tissues. Organic mineral sources, particularly selenium and zinc, appear superior to their inorganic counterparts in terms of bioavailability and stress mitigation (Mahmoud and Edens 2005; Li et al. 2021). Nevertheless, Bora et al. (2024) cautioned that the form of supplementation (organic vs inorganic) was often unspecified, and controlled comparisons were scarce.

Role of amino acids and *in ovo* interventions

Specific amino acids, notably methionine, leucine, and tryptophan, have been investigated for their regulatory effects on HSP70. Han et al. (2019) found that the *in ovo* L-leucine injection modulated arginine and lysine metabolism, attenuating HSP70/90 expression via mTOR signalling, albeit in a tissue-specific manner. Methionine analogues such as MHA [2-hydroxy-4-(methylthio)butanoic acid] showed superior effects under heat stress compared to DL-methionine, with improved antioxidant profiles and lowered HSP70 (Erfani et al. 2021). Despite these promising results, many *in ovo* studies lacked the post-hatch performance data (e.g. Elnesr et al. 2019; Han et al. 2019), which limits practical translation.

Phytogenic and hormonal modulation of HSP 70

Phytogenic-like compounds including taurine (Belal et al. 2018) and citrulline (amino acid derivatives; Ghasemi and Nari 2023) have demonstrated efficacy in modulating HSP70 expression, often accompanied by improved growth or antioxidant status. Likewise, serotonin and leptin have been investigated for their central roles in stress adaptation. KHasti et al. (2025) reported that serotonin increased HSP70/90 mRNA expression, indicating a potential neuromodulatory effect, while leptin was shown to act indirectly via the central nervous system to regulate HSP70 (Figueiredo et al. 2007). Although these findings open novel avenues, most hormonal studies were constrained by small sample sizes and limited mechanistic validation at the protein level, necessitating further work.

Synergistic supplementation strategies

A growing body of evidence supports the use of combined supplementation strategies to achieve maximal mitigation of heat-induced stress. For example, vitamin E + selenium (Bora et al. 2024) showed superior HSP70 suppression versus single agents, while the *in ovo* injection of sulphur amino acids (methionine and cysteine) in heat-stressed embryonated eggs enhanced antioxidant markers and GSH-Px expression while reducing HSP70 expression, corticosterone levels, and plasma lipids in newly hatched broilers (Elnesr et al. 2019). This suggests it may be an effective strategy to mitigate heat and oxidative stress effects; moreover, combining MHA and citrulline restored the performance and minimised the oxidative stress markers better than either supplement alone (Ghasemi and Nari 2023). Similarly, Khan et al. (2016) found that combining selenium and probiotics suppressed HSP70 expression and improved cardiac resilience, suggesting synergistic effects at both molecular and physiological levels. Despite this, few studies explored interaction effects formally, and dose-response data were often missing or limited (Baxter et al. 2020; Kadim and Alhamadani 2023).

Early life conditioning and developmental plasticity

Early thermal conditioning has emerged as a non-nutritional strategy to build the heat resilience. Toplu et al. (2014) demonstrated that early exposure to 36 °C for 24 h at 5 days of age was more effective than vitamin C in reducing HSP70 and improving the tissue protection. This finding supports the theory of developmental plasticity, wherein controlled early-life stress induces adaptive responses that persist later in life. Nevertheless,

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long-term implications and performance metrics were not reported in most such studies, leaving critical gaps regarding practical utility.

Inverse association with performance and growth

Elevated HSP70 (mRNA or protein) is often associated with impaired growth performance under heat stress (HS). Studies report significant increases in HSP70 alongside reduced body weight (BW), body weight gain (BWG), and feed efficiency (FCR) in HS groups compared to thermoneutral controls (Abo-Samaha et al. 2021). Conversely, interventions that successfully reduce HSP 70 expression or concentration [e.g. ascorbic acid (AA), chromium methionine (CrMet), selenium, betaine, vitamins, and melatonin] consistently demonstrate concomitant improvements in BW, BWG, FCR, carcass yield, and reduced mortality (Mahmoud and Edens 2005; Mishra et al. 2015; Elnesr et al. 2019; Erfani et al. 2021; Ghasemi and Nari 2023; Biswas et al. 2024; Bora et al. 2024; Dalolio et al. 2024). This inverse relationship positions HSP 70 as a reliable biomarker of HS-induced performance loss.

Strong link to physiological stress markers

The HSP70 elevation is frequently associated with key stress hormones, particularly corticosterone (CORT). Higher HSP70 consistently coincides with higher plasma CORT levels (Mahmoud et al. 2004a,b; Toplu et al. 2014; Mishra et al. 2015), and interventions reducing HSP70 also lower CORT (Elnesr et al. 2019; Baxter et al. 2020; Ghasemi and Nari 2023; Dalolio et al. 2024). HSP70 also correlates positively with the stress leukogram marker heterophil/lymphocyte (H/L) ratio (Toplu et al. 2014) and often with hyperglycaemia and dyslipidaemia (Toplu et al. 2014; Elnesr et al. 2019; Erfani et al. 2021). This underscores the role of HSP70 as part of the integrated physiological stress response.

Association with oxidative stress and antioxidant response

Elevated HSP70 is frequently associated with increased oxidative damage (higher malondialdehyde – MDA) and often with reduced antioxidant enzyme activity (SOD, CAT, GPx, GSH) (Zhu et al. 2015; Albokhadaim et al. 2019; Sumanu et al. 2023). Interventions that mitigate oxidative stress (e.g. Mn, Zn, vitamins, selenium, melatonin, and betaine) typically reduce both MDA and HSP70 while enhancing the antioxidant enzyme activity (Mahmoud and Edens 2005; Zhu et al. 2015; Kumbhar et al. 2018; Elnesr et al. 2019; Li et al. 2021; Ghasemi and Nari 2023). More importantly, injecting sulphur amino acids (methionine and cysteine) into embryonated eggs exposed to heat stress improved the antioxidant status and upregulated GSH-Px gene expression, while decreasing HSP70 expression, corticosterone levels, and lipid parameters in newly hatched broiler chicks. This approach shows a potential for reducing the negative impacts of heat and oxidative stress. This highlights the interconnectedness of heat shock response and other oxidative stress pathways.

Complex relationship with immunity

The link between HSP70 and immune outcomes is less consistent. While elevated HSP70 often associates with immune suppression, e.g. reduced antibody titres (Toplu et al. 2014), HSP70 reduction via supplements sometimes correlates with improved immune organ weights like spleen (Bora et al. 2024) or humoral/cell-mediated immunity (Ghasemi and Nari 2023; Biswas et al. 2024) and reduced inflammation (Baxter et al. 2020). However, some studies found no significant effect on lymphoid organs despite HSP70 reduction (Zhu et al. 2015; Dalolio et al. 2024). The pro-inflammatory cytokine elevation alongside HSP70 (Abo-Samaha et al. 2021) suggests context-dependent roles, potentially related to the severity/duration of stress or tissue location. Anti-inflammatory IL-10 showed a variable response (Sumanu et al. 2023).

The magnitude and direction of HSP70 change, and its relationship with outcomes, can be tissue specific. For example, leucine supplementation reduced HSP70 in the diencephalon and muscle but increased it in the liver under HS (Han et al. 2019). Similarly, the efficacy of interventions in modulating HSP70 and associated outcomes varies. Optimal doses are often identified, for example, CrMet at 0.80 mg/kg (Dalolio et al. 2024), betaine at 600 mg/kg (Biswas et al. 2024), and some supple-

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ments show gender-dependent effects (Figueiredo et al. 2007). Serotonin increased HSP70 mRNA expression while reducing neuronal damage (KHasti et al. 2025), contrasting with the general inverse performance relationship.

Critical considerations

While there exist strong associations, most data demonstrate correlation. The HSP70 induction is a protective mechanism; its reduction often indicates successful stress mitigation rather than being the direct cause of improved outcomes. Outcomes depend on whether HSP70 is measured at the mRNA or protein level, in specific tissues (liver, muscle, brain, blood) or systemically (serum), and the timing relative to stress exposure. HSP70 is one component of a complex network, e.g. interacting with other HSPs, HSFs, antioxidant systems, and hormones like IGF-1 and melatonin (Dalolio et al. 2024). Its relationship with outcomes reflects this integration. Genotype, age, diet composition, and specific HS conditions significantly influence results, making direct comparisons between studies challenging. Some studies lack direct performance measures alongside molecular data (Zhu et al. 2015; Han et al. 2019).

LIMITATIONS AND RESEARCH GAPS

A major limitation across studies is the inconsistent reporting of performance metrics (e.g. weight gain, feed conversion ratio), despite their centrality to poultry production. Furthermore, many studies relied solely on the mRNA analysis of HSP70 without corresponding protein validation, weakening the physiological inference. Additionally, small sample sizes, single genotypes, and short trial durations undermine the generalisability of several findings (e.g. Sumanu et al. 2023; Bora et al. 2024; KHasti et al. 2025). Environmental variability, such as natural vs controlled heat stress, also complicates cross-study comparison. Moreover, the form of nutrient supplements (e.g. organic vs inorganic Se) was inconsistently described, calling for standardisation.

Significant knowledge gaps persist regarding HSP70 dynamics and heat stress responses across broiler development stages. While most research focuses on acute heat stress during finisher phases (21–42 days), critical deficiencies exist in understanding early life and chronic exposure scenarios. The role of HSP70 in immune development, thermoregulation, and growth plasticity during the vulnerable post-hatch and chick stages remains poorly defined, despite hints of early impacts (Erfani et al. 2021). Similarly, the potential for the *in ovo* modulation of HSP70 (via nutrients or temperature) to program lasting stress resilience and performance outcomes requires investigation, as embryonic studies like Han et al. (2019) often lack performance linkages. Furthermore, the effects of prolonged, subclinical heat exposure on HSP70 adaptation and tolerance mechanisms throughout the growth cycle are inadequately characterised.

Beyond stage-specific gaps, the causal relationships between HSP70 modulation and physiological outcomes remain elusive. While there exist correlations, it is unclear whether reduced HSP70 expression directly enhances growth or merely serves as a biomarker of successful stress mitigation through other pathways, such as enhanced antioxidant capacity or hormone regulation. The tissue-specific roles of HSP70 (e.g. in liver metabolism versus muscle growth versus neural stress integration) and their varying importance across development stages are also underexplored, despite evidence of tissue divergence (Han et al. 2019). The mechanistic dissection of HSP70 interactions with other heat shock proteins (HSPs), heat shock factors (HSFs), and key signalling pathways (e.g. mTOR, IGF-1, selenoproteins) is needed throughout growth.

The relationship between HSP70 and immune function presents unresolved complexities. Conflicting findings exist on whether HSP70 elevation suppresses or activates specific immune functions (e.g. innate vs adaptive) at different ages (Abo-Samaha et al. 2021 vs Toplu et al. 2014), and its impact on gut integrity and microbiota composition, critically linked to immunity, across development stages is largely unknown, with Baxter et al. (2020) being a notable exception. Translating mechanistic understanding into practical strategies faces hurdles. Standardised, stage-optimised protocols (e.g. for starter vs grower phases) for dosing key nutrients and compounds (e.g. betaine, CrMet, vitamins) are lacking, despite the clear evidence of dose-dependency (Biswas et al. 2024; Dalolio et al. 2024). Synergistic approaches combining anti-

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oxidants, amino acids, probiotics, and management practices require rigorous, stage-specific efficacy testing. Furthermore, the long-term consequences of early-life HSP70 modulation on productivity and welfare remain unknown.

Addressing these gaps necessitates specific methodological improvements: standardising HSP70 reporting (units, tissues), conducting dose-optimisation studies for emerging agents (e.g. melatonin), and directly comparing embryonic versus grower-phase intervention efficacy. The current research focus on Ross 308/Cobb strains in finisher stages underscores the limited understanding of HSP70 modulation in early development and non-industrial genotypes. Future studies must prioritise expanding embryonic and starter-phase cohorts, including both sexes and diverse genotypes, and ensuring adequate sample sizes and replication.

FUTURE DIRECTIONS

Figure 3 gives a summary of the future directions in understanding the modulation of heat stress via nutritional strategies based on HSP 70. To address the critical knowledge gaps, future research must adopt a comprehensive developmental perspective, moving beyond isolated studies in finisher broilers. Longitudinal investigations profiling HSP70 expression (mRNA/protein), stress hormones (e.g. corticosterone – CORT), redox status, and immune markers from embryo to market age under controlled thermal challenges are essential. These studies should establish stage-specific HSP70 thresholds that differentiate adaptive responses from detrimental stress levels, providing crucial benchmarks for intervention timing and efficacy evaluation.

Understanding the mechanistic approaches requires leveraging multi-omics studies (transcriptomics, proteomics, and metabolomics) to map HSP70-interacting networks and identify key resilience drivers across tissues and developmental stages. Concurrently, exploring the epigenetic regulation (e.g. DNA methylation) of HSP70 and associated genes will illuminate mechanisms underlying the lifelong stress tolerance programming. This mechanistic foundation is vital for designing targeted nutritional interventions optimised for specific developmental windows: *in ovo* delivery for embryonic programming, neonatal inoculation

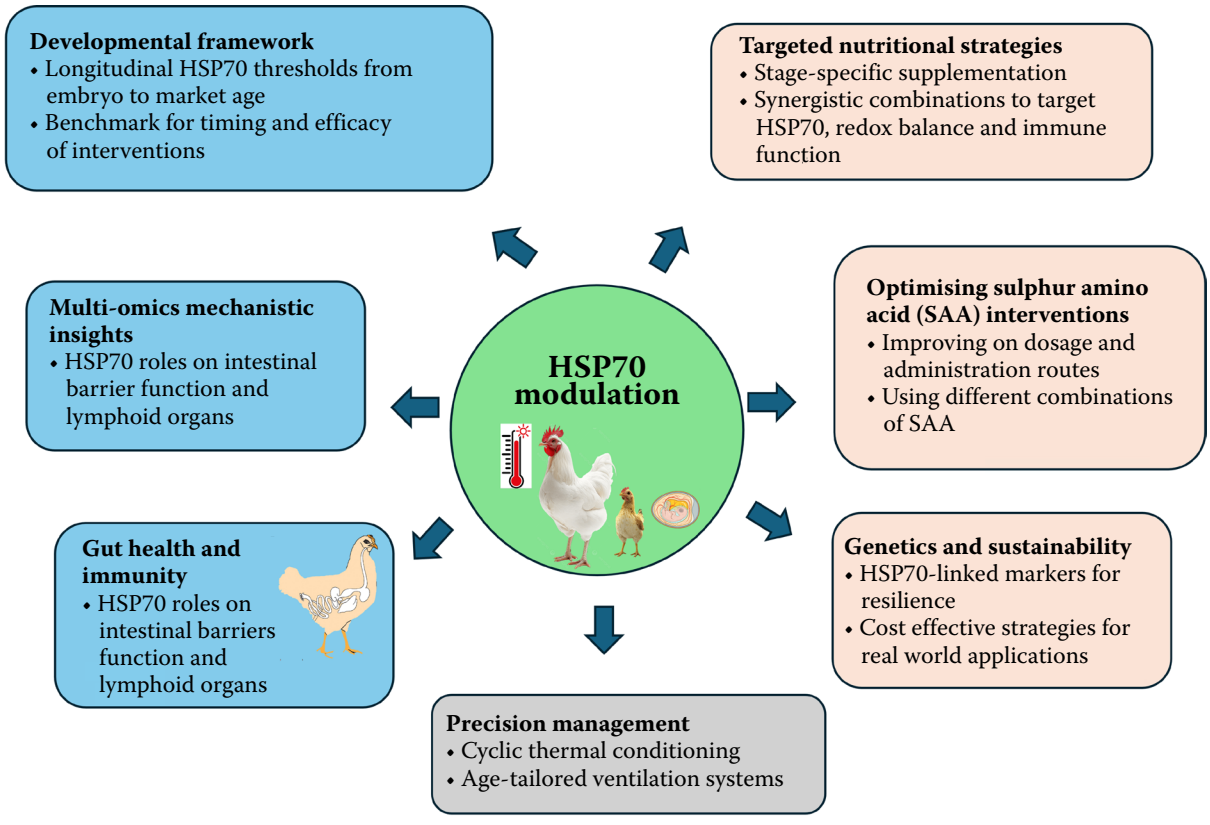

Figure 3. Future directions in research for the modulation of heat shock protein 70 at different stages of broilers

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or starter diets enhancing early thermotolerance, and finisher strategies preserving carcass yield under heat stress.

Advanced mitigation strategies should focus on developing and testing synergistic combinations of compounds (e.g. methionine + cysteine or betaine + vitamin C + selenium) at optimised, stage-specific doses to holistically address performance, HSP70 dynamics, redox balance, and immunity. The potential of candidate modulators – such as serotonin precursors (KHasti et al. 2025), and phytogenics to influence HSP70 across ages warrants thorough exploration. In addition, the *in ovo* inoculation of SAA requires further investigation due to its potential in heat stress modulation (Elnesr et al. 2019). Furthermore, integrating these nutritional approaches with precision management techniques (e.g. cyclic heat conditioning, age-tailored ventilation control) offers a promising avenue for enhanced resilience.

A dedicated focus on immunity and gut health is paramount. Research must define how the HSP70 modulation influences gut barrier integrity, microbiome composition, and mucosal immunity at different stages, building upon initial findings like Baxter et al. (2020). Clarifying the dual role of HSP70 in pro- and anti-inflammatory signalling within key immune organs (e.g. thymus, spleen) during development is also critical to resolve existing inconsistencies. Finally, breeding and sustainability efforts should aim to identify genetic markers linked to favourable HSP70 expression patterns and associated stress resilience traits for selective breeding programmes. Concurrently, rigorous assessment of the long-term sustainability and cost-efficacy of HSP70-modulating strategies across the entire production cycle is necessary for practical implementation.

CONCLUSION

This systematic review establishes heat shock protein 70 (HSP70) as a critical biomarker and therapeutic target for mitigating heat stress (HS) in broilers. Collectively, the evidence demonstrates that targeted nutritional interventions, particularly those rich in antioxidants (e.g. vitamins C, E, and selenium) and balanced in key amino acids (sulphur-containing, branched-chain ones) effectively modulate HSP70 expression and enhance thermotolerance, thereby preserving productivity under thermal challenge. However, the interactions between nutrients and HSP70 are complex, exhibiting significant tissue specificity and dependence on stress severity and duration.

While these strategies offer substantial promise, a deeper understanding of long-term physiological effects, tissue-specific molecular interactions, inoculation route and timing, and the metabolic trade-offs associated with HSP70 induction is essential. Moving forward, translating this knowledge into practical, scalable solutions requires future research focused on (*i*) refining stage-specific nutritional protocols to optimise the protective benefits of HSP70 while minimising its energetic costs; (*ii*) establishing standardised methodologies for HSP70 assessment; and (*iii*) validating approaches across diverse genotypes and commercial settings. Implementing these evidence-based, nutritionally modulated HSP70 strategies is paramount for the poultry industry to enhance broiler resilience, welfare, and economic sustainability in the face of escalating global temperatures.

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Conflict of interest

The authors declare no conflict of interest.

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