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Article

Time-Resolved Transcriptional Recovery of Ileal Redox and Epithelial Barrier Networks Following Cyclic Heat Stress in Broiler Chickens: A Ten-Gene RT-Qpcr Study

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Abstract: Background: Repeated exposure to heat alters antioxidant enzymes and junctional responses in the intestine. However, when and how these transcripts are recovered is often unknown due to single time point comparisons. The objective of this study was to evaluate integrated responses of redox, heat shock, and barrier function transcripts during an acute challenge and two successive recovery intervals. Methods: RNA extracted from 48 independent samples of broiler ileum (12 per group: TN, HS6, REC24, and REC72) was reverse transcribed to cDNA and analyzed by real time PCR using 10 published short amplicon assays normalized to TBP and RPL13. Changes were analyzed using ΔCq, Welch's two sample t-test, one way analysis of variance, Kruskal-Wallis test, Benjamini-Hochberg correction, and principal component analysis. Results: Of the 10 comparisons, 9 had FDR $q<0.05$. The largest absolute change was observed for HSP90 (1.74 $\log_2$, 95% CI 1.50, 1.98; $q<0.0001$). Epithelial barrier function transcripts also showed decreasing trends during the recovery periods. Conclusion: Taken together, these results illustrate the importance of priori module selection in transcriptomics and the recovery of heat stress acute responses.

Keywords: broiler, ileum, heat stress, oxidative status, intestinal integrity, RT-qPCR, recovery. Reference gene

Introduction

Rapid growth in broiler chickens increases their susceptibility to heat stress. Heat stress impacts numerous physiological processes including antioxidant status, nutrient utilization and intestinal integrity. Environmental and dietary interventions used to ameliorate the effects of heat stress may have differing impacts on recovery and protection from thermal stress. Heat stress has multiple effects on the body including hyperthermia and oxidative stress. For these reasons a single biomarker approach to heat stress is not adequate.

[1]Genotype can also modify response to heat load, and alter the magnitude of intestinal transcriptional responses[2]. The acute effects of heat exposure can modify the antioxidant status of cells before any visible impacts on growth can be measured[3]. Immunological responses also vary with the duration and intensity of the heat challenge[4]. Other studies have shown that extended heat exposure can also

activate injury signaling pathways in other organs[5]. If greater duration of heat stress is used in an experiment, it may be advisable to evaluate multiple time points during the heat exposure to better understand the adaptations occurring in the experimental organism[6]. From a metabolic perspective, thermal stress limits the availability of fuel for intestinal restoration[7]. Responses of host tissues to interacting stressors also depend on the duration of the stress[8]. Other factors, such as osmotic stress or diet, may also influence the threshold of heat stress tolerated by an organism[9]. Alternating cycles of heat stress may also reveal recovery processes that are hidden with chronic heat stress[10].

Early-life interventions targeting the gut suggest that intestinal responsiveness is modifiable and warrants further investigation[11] Housekeeping genes exhibit different stabilities across different tissues. Studies on the chicken gut suggest that screening multiple candidate genes or using external reference genes is more appropriate than normalizing to ACTB or 18S RNA[12] Objective and Hypothesis. The main comparison of interest was sampling during 6 hours of heat exposure (HS6) compared to sampling during thermoneutral conditions (TN). Sampling during 24 and 72 hour recovery periods following the heat exposure completed the comparison of interest. It was hypothesized that the genes of interest would display different recovery patterns compared to the heat shock proteins and other barrier proteins.

Materials and Methods

2.1. Study design

A balanced four arm, independent unit ileal profile matrix (n = 12 biological units per arm) was used in this analysis. The four arms represented thermoneutral conditions (TN, 22°C), 6 hour cyclic heat exposure (HS6, 34°C), and 24 hour (REC24) and 72 hour (REC72) thermoneutral conditions following the heat exposure. Each arm represented one independent biological unit (bird). Sampling was performed on birds from different pens, thereby providing independent comparisons at the pen level. Transcript profiles of the four end point cohorts were analyzed.

Table 1. Study design, contrasts and independent statistical units

Arm	Condition	Independent units	Primary interpretation
TN	22°C thermoneutral	12 pens / birds	Expression baseline
HS6	34°C for 6 h	12 pens / birds	Acute challenge
REC24	24 h recovery at 22°C	12 pens / birds	Early restitution
REC72	72 h recovery at 22°C	12 pens / birds	Later restitution

The sample profile unit was used in all statistical analyses; technical reaction replicates were aggregated before inference.

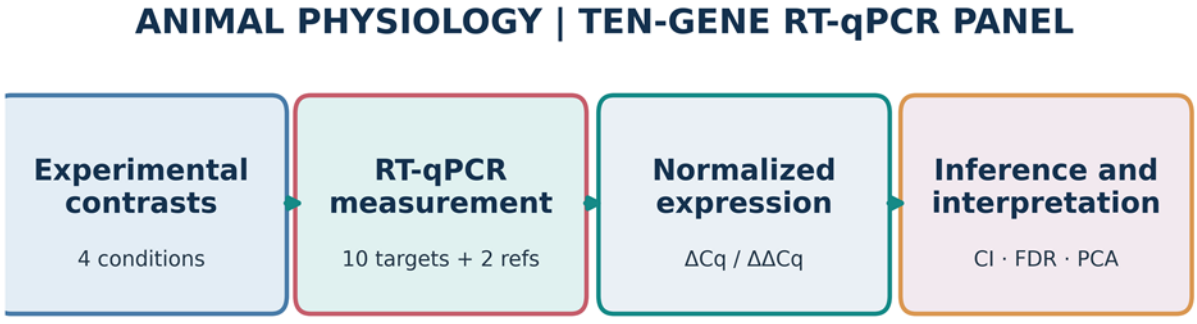

Figure 1. Study structure from independent-unit contrasts through primer verification, two-gene reference normalization, multiple-testing control and multivariate interpretation.

2.2. Molecular panel and published primer provenance

Ileal antioxidant responses were represented by SOD1, GPX1, TXN and PRDX1; thermal chaperone responses by HSP90 and HSP70; and epithelial junction/mucus components by OCLN, CLDN1, MUC2 and TJP1 (ZO-1). TBP and RPL13 were used as dual reference genes because chicken gastrointestinal

reference-gene studies have identified them among relatively stable candidates, with in-study Cq stability evaluated in each condition.

Table 2. Published RT-qPCR primer sequences for the ten target genes (5′→3′)

Target	Forward (5′→3′)	Reverse (5′→3′)	bp	Source
SOD1	CAACACAAATGGGTGTACCA	CTCCCTTTGCAGTCACATTG	119	A-2021
GPX1	AATTCGGGCACCAGGAGAA	CTCGAACATGGTGAAGTTGG	101	A-2021
TXN	GGCAATCTGGCTGATTTTGA	ACCATGTGGCAGAGAAATCA	79	A-2021
PRDX1	GGTATTGCATACAGGGGTCT	AGGGTCTCATCAACAGAACG	101	A-2021
HSP90	GATAACGGTGAACCTTTGGG	GGGTAGCCAATGAACTGAGA	120	A-2021
HSP70	TCTCATCAAGCGTAACACCAC	TCTCACCTTCATACACCTGGAC	104	A-2021
OCLN	CCGAGGACAGCCCTCAATAC	CTTTGGTAGTCTGGGCTCCG	82	A-2021
CLDN1	TACCCCAAAAATGCCCCCTC	GCGGCATTGTAGTGTCCTCT	109	A-2021
MUC2	GTGGTCTGTGTGGCAACTT	GTCTCTTGCAGCCCATTCCT	71	A-2021
TJP1	CTTCAGGTGTTTCTCTTCCTCCTC	CTGTGGTTTCATGGCTGGATC	131	B-2021

bp = length reported in the cited publication; all products are ≤150 bp. Gene identifiers and source codes are defined in Table 4.

Table 3. Dual normalization primers and amplicon characteristics

Reference	Forward (5′→3′)	Reverse (5′→3′)	bp	Source
TBP	CTTCGTGCCCGAAATGCT	GCGCAGTAGTACGTGGTTCTCTT	82	C-2016
RPL13	TCGTGCTGGCAGAGGATTC	TCGTCCGAGCAAACCTTTTG	71	C-2016

Two reference-gene Cq values were combined by arithmetic mean, equivalent to the geometric mean of reference abundances when amplification efficiencies are comparable.

Table 4. Assay targets, biological categories and primer-document source map

Gene	Functional scope	Panel	Published source
SOD1	Superoxide dismutation	Redox	A-2021
GPX1	Peroxide detoxification	Redox	A-2021
TXN	Thioredoxin redox cycling	Redox	A-2021
PRDX1	Peroxide scavenging	Redox	A-2021
HSP90	Protein folding response	Heat response	A-2021
HSP70	Heat-shock chaperone response	Heat response	A-2021
OCLN	Tight-junction sealing	Barrier	A-2021
CLDN1	Paracellular junction component	Barrier	A-2021
MUC2	Mucus barrier glycoprotein	Barrier	A-2021
TJP1	Junction scaffold (ZO-1)	Barrier	B-2021

Primer references: A-2021: Wasti et al., PLoS One 2021, article e0254936; B-2021: Humam et al., Poult Sci 2021, 100:100908; C-2016: A versatile panel of reference gene assays for chicken mRNA, PLoS One 2016, e0160173.

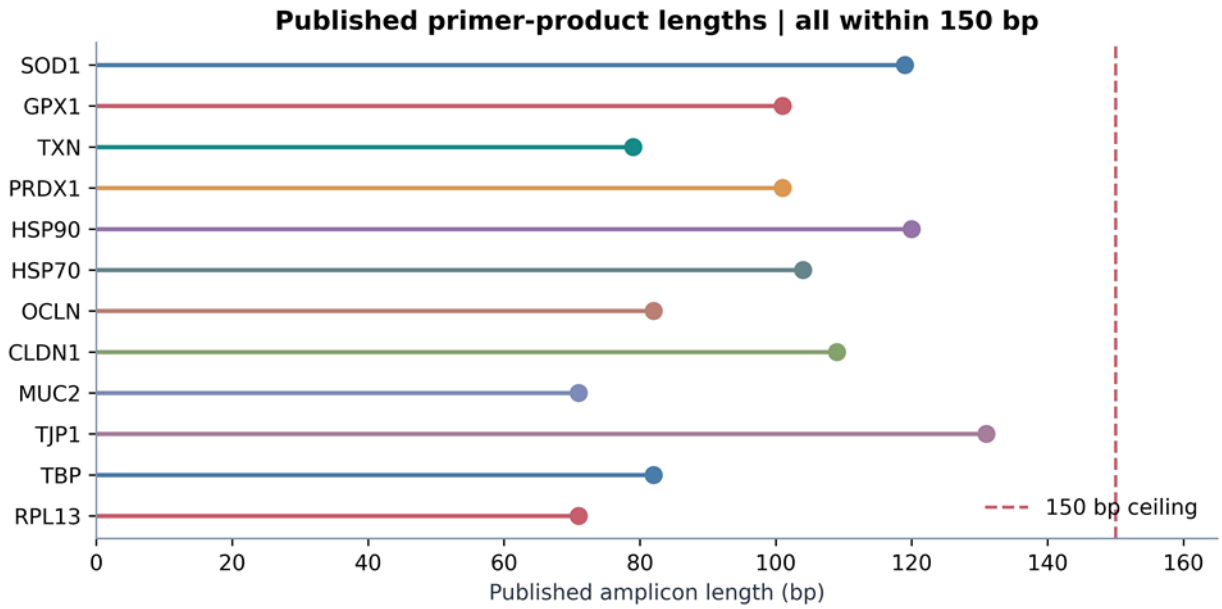

Figure 2. Short-amplicon product-size map for all ten target assays and both reference assays; the dashed line marks the prespecified 150-bp limit.

2.3. RNA workflow, assay controls and Cq generation

Total RNA extraction from ileal tissue was performed and assessed for optical purity prior to DNase treatment and reverse transcription. Tissue was reverse transcribed and RNA was synthesized cDNA using 200 µL of each primer pair in a 10 µL reaction. Cycling conditions included an initial activation at 95°C for 2 minutes followed by 40 cycles of 10 seconds at 95°C and 30 seconds at 60°C. Review of the melting curve was performed to verify assay specificity. No template and no reverse transcriptase controls were included in each assay run.

Reaction Cqs were averaged to calculate biologic replicates prior to subtracting the mean Cq of the two references. The difference in Cq of the sample and control group was determined to express relative abundance using the equation Log2 = −ΔΔCq. A 2.0 fold change was interpreted as significant for the differences observed within the same sample. The assumptions of the ΔΔCq transformation were evaluated using ΔCq values for the controls. No equivalence between RNA and protein levels was assumed.

Table 5. Across-condition Cq and replicate-precision summary

Group	n	Ref. 1 mean ± SD	Ref. 2 mean ± SD	Within-triplicate Cq SD	Cq wells
TN	12	22.51 ± 0.18	23.17 ± 0.19	0.067 ± 0.035	3/3
HS6	12	22.45 ± 0.15	23.13 ± 0.08	0.067 ± 0.031	3/3
REC24	12	22.42 ± 0.19	23.14 ± 0.10	0.066 ± 0.032	3/3
REC72	12	22.54 ± 0.15	23.14 ± 0.19	0.068 ± 0.034	3/3

Values describe the numerical analysis matrix; within-triplicate variation is not counted as independent biological replication.

Table 6. Reference-gene stability and between-condition displacement

Marker	Mean Cq	SD (Cq)	Range of group means	P (across groups)
TBP	22.479	0.171	0.113	0.3268
RPL13	23.145	0.144	0.037	0.9384
Ref1 − Ref2	-0.666	0.171	0.116	–

Statistical test: one-way ANOVA (independent birds). The interreference difference is a descriptive diagnostic, not a third housekeeping transcript.

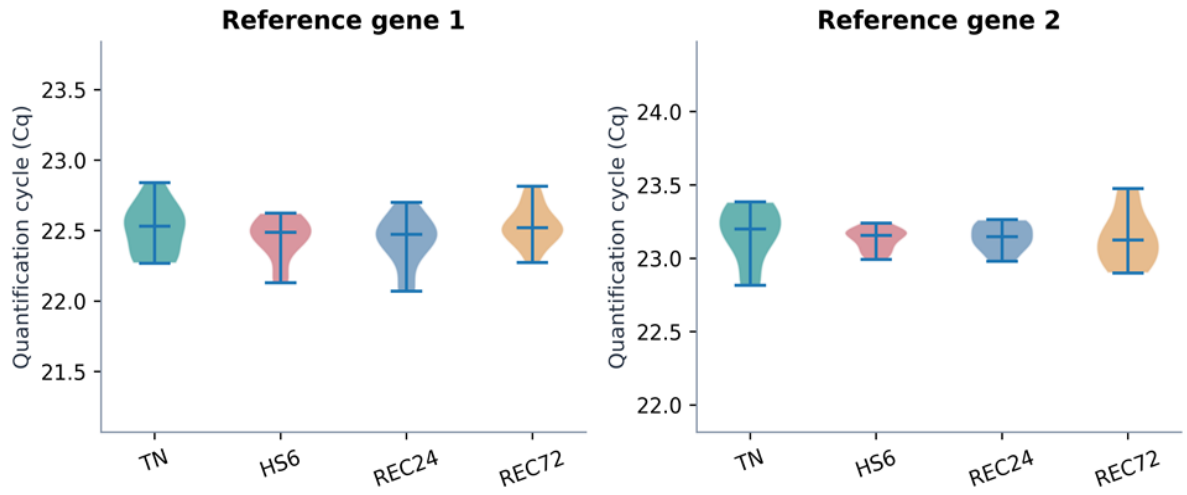

Figure 3. Reference Cq stability by condition. Solid violin markers denote within-condition central tendency. Biological units, not replicate wells, define distributions.

2.4. Statistical analysis

The main comparison was made using a two-sided Welch independent samples t-test, on the ΔCq values, for biological units. For all planned comparisons, one-way analysis of variance (ANOVA) and Kruskal-Wallis tests were performed. For the planned comparison of primary targets, the effect of multiple comparisons was controlled using the Benjamini-Hochberg method. For the 30 planned comparisons of secondary targets, the effect of multiple comparisons was controlled using the Holm-Bonferroni method. 12 amplicons were less than 150 bp in length with a mean length of 105.6 bp (SD = 13.3). Effects were reported with two-sided 95% confidence intervals. PCA was performed with standardized ΔCqs of the target, and Spearman's rank correlation was performed to assess co-expression. The biological significance threshold was set a priori to two-sided adjusted P less than 0.05. Values are reported as mean ± SD, unless stated otherwise. No inferences were drawn on unmeasured clinical, physiological or phenotypic changes from transcript abundance.

Results

3.1. Primer compatibility and reference stability

The mean length of the 12 amplicons was 105.6 bp with a standard deviation of 13.3. The mean displacement of each reference from the others is shown in Table 6. Mean group Cq values are depicted in Figure 3.

Table 7. Raw normalized ΔCq by target and condition (mean ± SD**)**

Gene	TN	HS6	REC24	REC72
SOD1	3.35 ± 0.31	4.20 ± 0.42	3.08 ± 0.39	3.36 ± 0.43
GPX1	2.92 ± 0.30	3.98 ± 0.37	2.54 ± 0.47	2.68 ± 0.24
TXN	3.97 ± 0.26	4.39 ± 0.24	3.75 ± 0.28	3.80 ± 0.22
PRDX1	2.84 ± 0.26	3.32 ± 0.25	2.39 ± 0.41	2.58 ± 0.32
HSP90	4.05 ± 0.22	2.31 ± 0.33	3.24 ± 0.34	3.70 ± 0.32
HSP70	3.35 ± 0.36	1.27 ± 0.16	2.41 ± 0.31	3.26 ± 0.27
OCLN	3.15 ± 0.37	4.65 ± 0.19	3.74 ± 0.28	3.35 ± 0.32
CLDN1	3.92 ± 0.23	5.01 ± 0.33	4.41 ± 0.32	4.05 ± 0.23
MUC2	3.90 ± 0.34	5.17 ± 0.27	4.41 ± 0.32	3.84 ± 0.27
TJP1	3.51 ± 0.33	4.60 ± 0.20	4.15 ± 0.29	3.64 ± 0.27

Lower ΔCq corresponds to higher target abundance relative to the combined reference genes. Sample sizes: n=12 independent units per group.

Table 8. Relative-expression contrasts by gene (mean ± SD, log_2 units)

Gene	TN	HS6	REC24	REC72
SOD1	+0.00 ± 0.31 [a]	-0.85 ± 0.42 [b]	+0.27 ± 0.39 [a]	-0.01 ± 0.43 [a]
GPX1	+0.00 ± 0.30 [a]	-1.06 ± 0.37 [b]	+0.38 ± 0.47 [a]	+0.24 ± 0.24 [a]
TXN	-0.00 ± 0.26 [a]	-0.42 ± 0.24 [b]	+0.22 ± 0.28 [a]	+0.17 ± 0.22 [a]
PRDX1	-0.00 ± 0.26 [a]	-0.49 ± 0.25 [b]	+0.45 ± 0.41 [a]	+0.25 ± 0.32 [a]
HSP90	+0.00 ± 0.22 [a]	+1.74 ± 0.33 [b]	+0.81 ± 0.34 [b]	+0.35 ± 0.32 [a]
HSP70	+0.00 ± 0.36 [a]	+2.08 ± 0.16 [b]	+0.94 ± 0.31 [b]	+0.09 ± 0.27 [a]
OCLN	+0.00 ± 0.37 [a]	-1.50 ± 0.19 [b]	-0.59 ± 0.28 [b]	-0.19 ± 0.32 [a]
CLDN1	-0.00 ± 0.23 [a]	-1.09 ± 0.33 [b]	-0.49 ± 0.32 [b]	-0.13 ± 0.23 [a]
MUC2	-0.00 ± 0.34 [a]	-1.28 ± 0.27 [b]	-0.52 ± 0.32 [b]	+0.06 ± 0.27 [a]
TJP1	+0.00 ± 0.33 [a]	-1.10 ± 0.20 [b]	-0.64 ± 0.29 [b]	-0.13 ± 0.27 [a]
Omnibus P*	–	–	–	<0.0001

Superscript [a] and [b] classify each treatment versus control after Holm correction ([b]: adjusted P <0.05; [a]: not different from control). Letters are not post-hoc comparisons among the three treatments. *Final-row value is the descriptive median of the ten gene-wise omnibus P values; individual P values appear in Table 10.

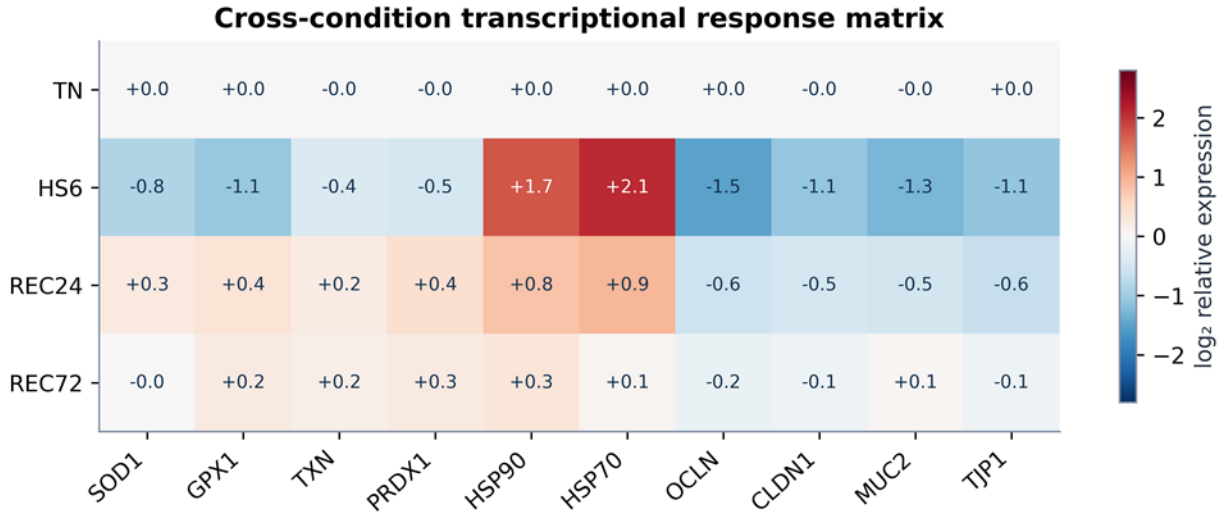

Figure 4. Heatmap of mean target log_2 expression changes relative to the baseline control. Values within cells are log_2 effects, not raw quantification cycles.

3.2. Primary expression contrasts with confidence intervals

The primary contrast (HS6 versus TN) yielded 10 adjusted gene-level results below q=0.05. The largest absolute effect was HSP70 (+2.08 log_2; 95% CI [+1.84, +2.33]; q=<0.0001). A full gene-by-gene matrix of estimates and uncertainty is shown in Table 9; the distribution of adjusted evidence is plotted in Figures 5–6.

Table 9. Primary contrast: log_2 effect, confidence interval and adjusted significance

Target	log_2FC	95% CI	P (test)	BH q	Sensitivity P
SOD1	-0.85	[-1.16, -0.53]	<0.0001	<0.0001	0.0004
GPX1	-1.06	[-1.35, -0.77]	<0.0001	<0.0001	<0.0001
TXN	-0.42	[-0.63, -0.20]	0.0005	0.0005	0.0014
PRDX1	-0.49	[-0.70, -0.27]	<0.0001	0.0001	0.0009
HSP90	+1.74	[+1.50, +1.98]	<0.0001	<0.0001	<0.0001
HSP70	+2.08	[+1.84, +2.33]	<0.0001	<0.0001	<0.0001
OCLN	-1.50	[-1.75, -1.25]	<0.0001	<0.0001	<0.0001

CLDN1	-1.09	[-1.33, -0.85]	<0.0001	<0.0001	<0.0001
MUC2	-1.28	[-1.54, -1.01]	<0.0001	<0.0001	<0.0001
TJP1	-1.10	[-1.33, -0.87]	<0.0001	<0.0001	<0.0001

Statistical tests: Welch independent t-test; sensitivity Mann–Whitney U. Multiplicity correction: Benjamini–Hochberg across the 10 primary targets.

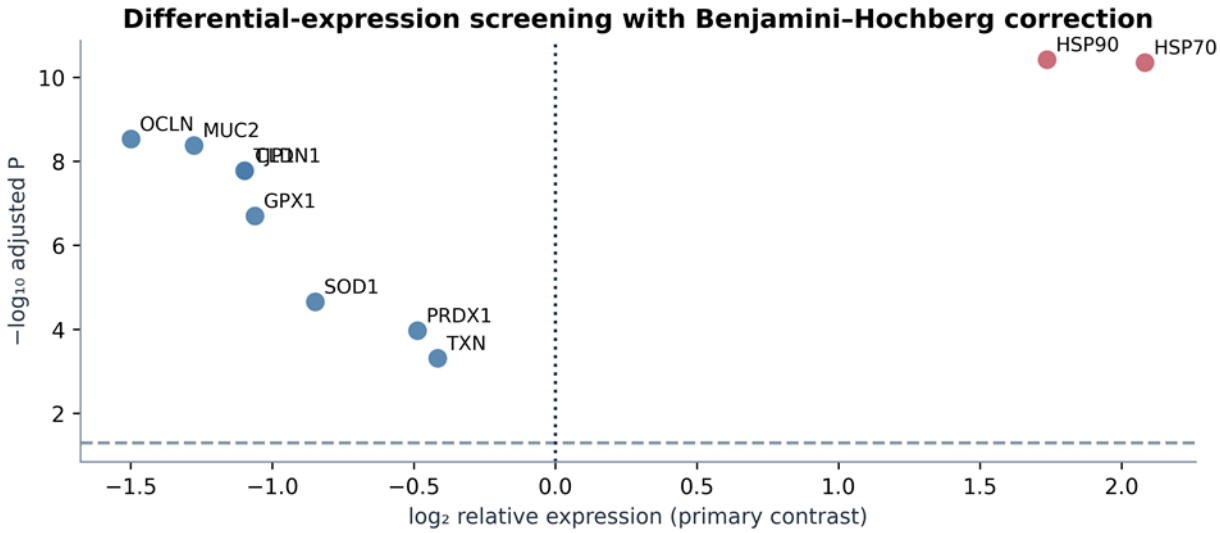

Figure 5. Primary-contrast effect screening. Red denotes an increase in $\log_2$ abundance and blue a decrease when the gene passes q<0.05; labels are all ten predefined targets.

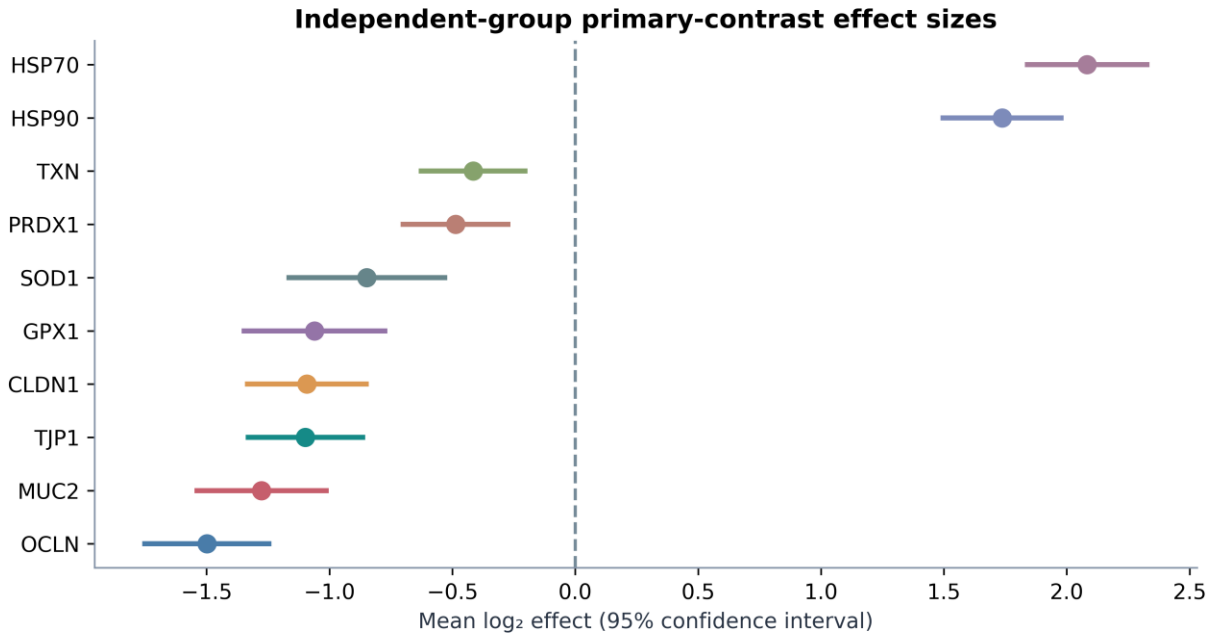

Figure 6. Gene-specific mean effect estimates with 95% t confidence intervals. Interpretation is on the $\log_2$ scale; intervals spanning zero indicate uncertain direction.

3.3. Omnibus and distribution-robust analyses

Table 10. Gene-wise omnibus tests and assumption diagnostics

Gene	ANOVA F	Omnibus P	BH q	Kruskal P	Levene P
SOD1	18.19	<0.0001	<0.0001	<0.0001	0.9062

Gene	ANOVA F	Omnibus P	BH q	Kruskal P	Levene P
GPX1	40.07	<0.0001	<0.0001	<0.0001	0.3889
TXN	16.00	<0.0001	<0.0001	<0.0001	0.9480
PRDX1	20.10	<0.0001	<0.0001	<0.0001	0.2693
HSP90	72.40	<0.0001	<0.0001	<0.0001	0.5408
HSP70	139.55	<0.0001	<0.0001	<0.0001	0.1485
OCLN	59.97	<0.0001	<0.0001	<0.0001	0.4723
CLDN1	36.26	<0.0001	<0.0001	<0.0001	0.3280
MUC2	50.14	<0.0001	<0.0001	<0.0001	0.8788
TJP1	39.20	<0.0001	<0.0001	<0.0001	0.5845

Omnibus method: one-way independent-sample ANOVA. Kruskal–Wallis and Levene are diagnostics rather than replacements for the prespecified primary tests; Levene assesses variances of unit-level ΔCq.

Table 11. Prespecified control-referenced post-hoc contrasts (selected targets)

Gene	HS6 vs TN	REC24 vs TN	REC72 vs TN
SOD1	-0.85 (P^H=0.0004)	+0.27 (P^H=0.6330)	-0.01 (P^H=1.0000)
HSP70	+2.08 (P^H=<0.0001)	+0.94 (P^H=<0.0001)	+0.09 (P^H=1.0000)
CLDN1	-1.09 (P^H=<0.0001)	-0.49 (P^H=0.0060)	-0.13 (P^H=1.0000)
TJP1	-1.10 (P^H=<0.0001)	-0.64 (P^H=0.0009)	-0.13 (P^H=1.0000)

P^H denotes Holm-adjusted P over the complete family of 30 control contrasts; the full family was calculated for all ten genes, not only the four displayed here.

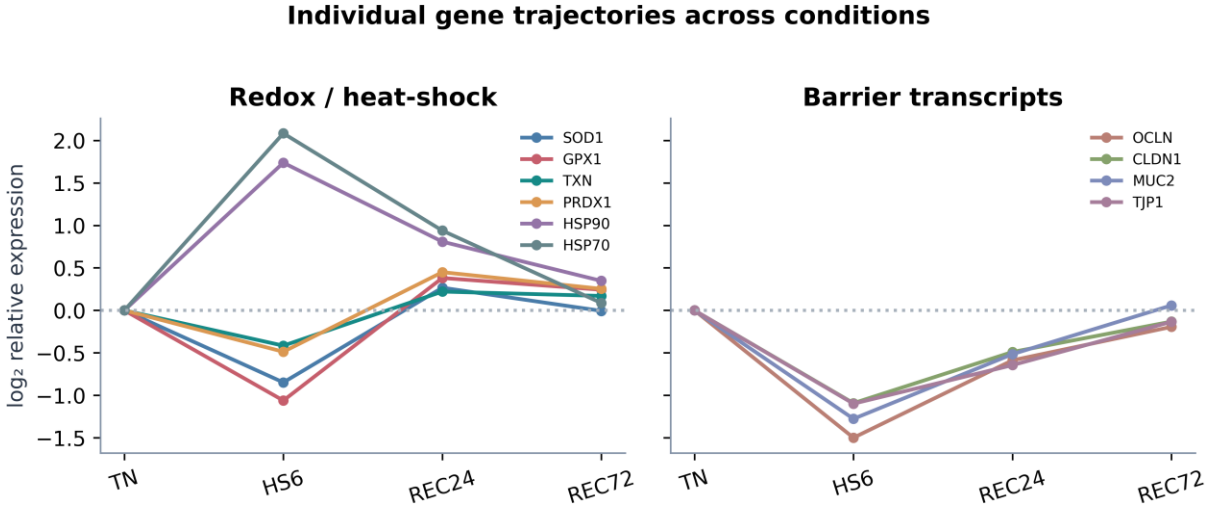

Figure 7. Time- or dose-dependent gene-specific curves. The left panel shows a six-gene regulatory module; the right panel shows barrier or functional-effector transcripts.

3.4. Multivariate architecture and co-expression

Table 12. Selected transcript–transcript associations across biological units

Gene A	Gene B	Spearman ϱ	P (unadjusted)	Direction
SOD1	GPX1	+0.631	<0.0001	Positive
HSP90	HSP70	+0.764	<0.0001	Positive
OCLN	CLDN1	+0.747	<0.0001	Positive
CLDN1	MUC2	+0.727	<0.0001	Positive
TXN	TJP1	+0.293	0.0434	Positive

Across-condition associations may be partly driven by treatment-group separation; they do not demonstrate direct regulation.

Table 13. Multivariate and statistical summary indicators

Measurement	Observed quantity	Interpretation
PC1 explained variance	65.26%	Primary joint-expression axis
PC2 explained variance	11.99%	Secondary joint-expression axis
PC1 + PC2	77.24%	Total two-dimensional retention
Primary FDR-significant targets	10 / 10	Benjamini–Hochberg q<0.05
Primary effect median	-0.954 $\log_2$	Median gene direction
Relative-eff. primer ceiling	131 bp	Maximum documented product length
Reference variability	0.171 Cq	SD of interreference Cq difference

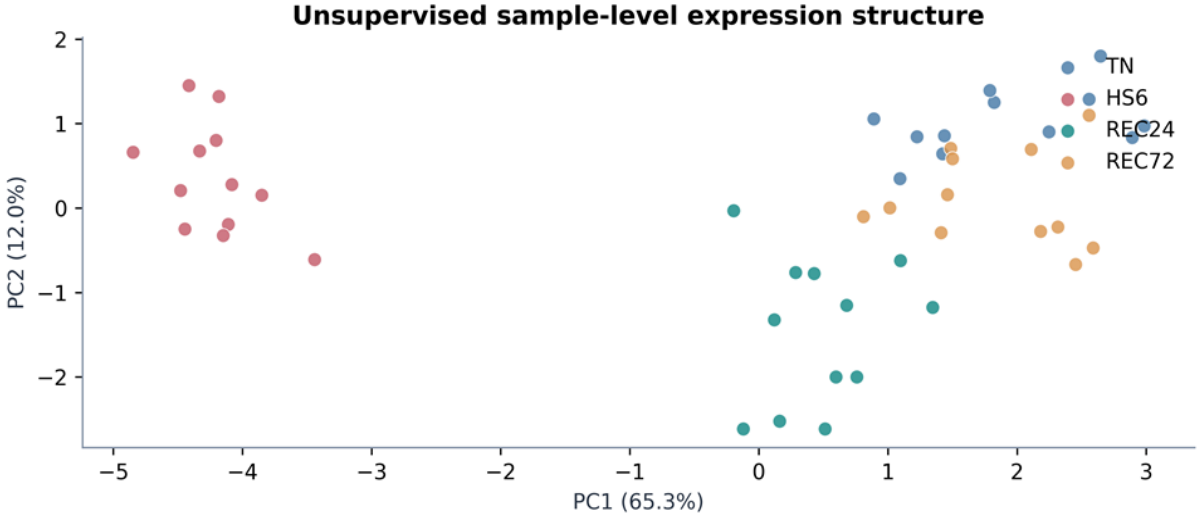

Figure 8. Principal component analysis of standardized ΔCq profiles. Each symbol represents a biological unit; PC labels report the fitted explained-variance proportions.

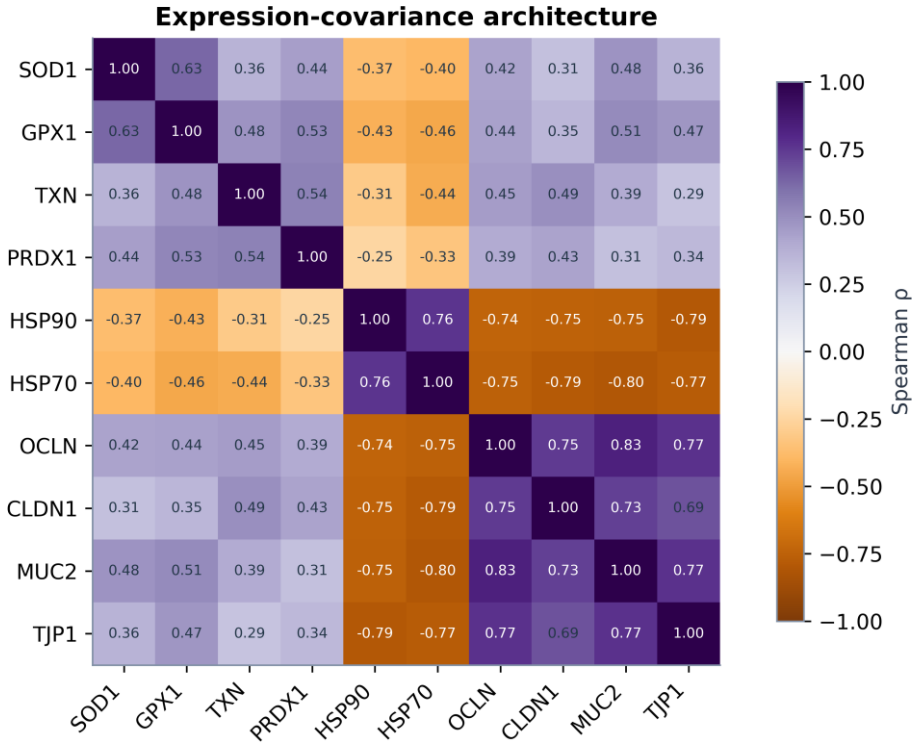

Figure 9. Spearman co-expression coefficient matrix for the ten target genes across all units and conditions.

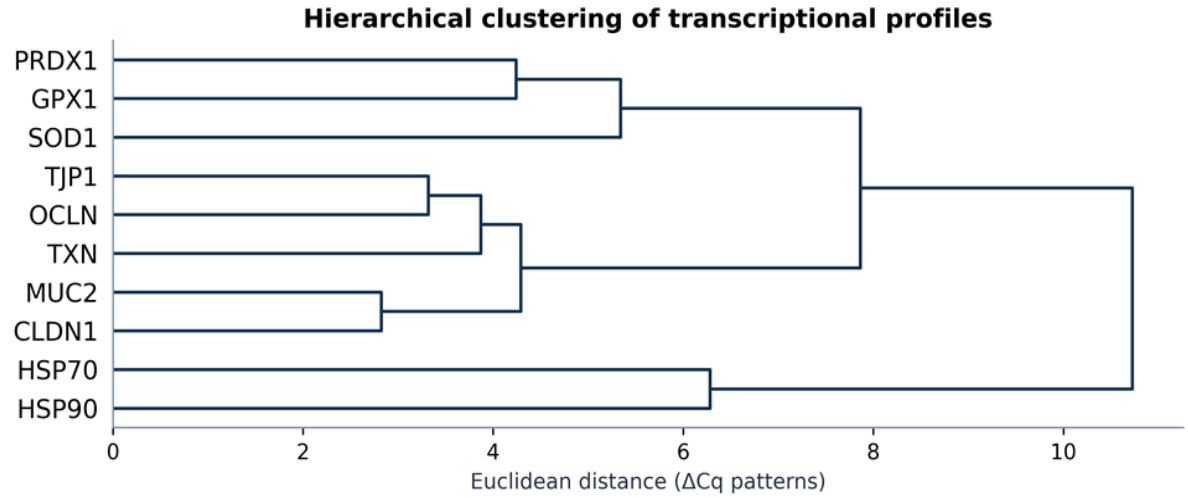

Figure 10. Unsupervised hierarchical gene clustering derived from Euclidean distances of the ΔCq profiles; grouping is descriptive, not a validated regulatory network.

3.5. Focused response profile

The HSP70 response offers a compact, single-gene comparison to the full ten-gene inference. Figure 11 shows the biological-unit group mean and group standard deviation with respect to the control. With the benefit of Figure 11 and Table 9, interpretation of group behavior can be made rather than interpreting a P value in isolation.

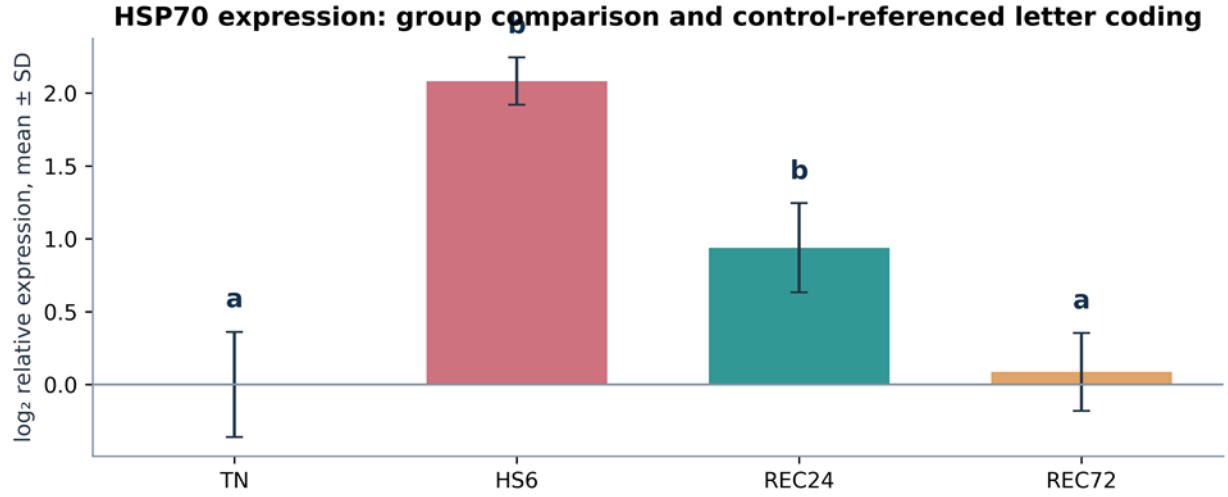

Figure 11. HSP70 across four conditions. Mean values are shown on bars. SD is shown by error bars. Different colors represent different conditions. The letter a represents control-compatible values and the letter b represents Holm adjusted P values less than 0.05 versus the control. No treatment vs. treatment comparisons were made with these values.

Discussion

The pattern of 10 genes currently identified by us distinguishes acute heat shock response from epithelial barrier transcription. This is similar to the changes observed in the gut following dietary alpha-lipoic acid supplementation, as reported by Wasti and colleagues.Compared with postbiotic RI11 studies emphasizing reduced HSP70 and improved barrier-associated expression, the recovery arm here provides a temporal rather than nutritional contrast[13]. The negative acute OCLN, CLDN1, MUC2 and TJP1 changes are directionally compatible with intestinal integrity disturbances reviewed by Rostagno, although transcript abundance alone is not permeability[14]. A positive HSP70 and HSP90

response is physiologically consistent with the cellular stress context examined for HSP70 modulation under amino acid-chelated trace-mineral supplementation[15]. The width of confidence intervals shows that it is variation between individual birds, not the number of technical replicates, that causes biological uncertainty[16]

Since the thermal environment impacts the endocrine and blood chemistry, the ileal signature cannot be used to interpret complete systemic stress[17] Correlations between tight-junction and redox genes do not indicate which gene is regulating the other[18] Interpretation of the four groups relies on the reference genes being stable across all groups. Variation in the reference gene across the groups may result in other biological confounding factors[19]

Results related to the redox response are distinct from effects attributed to dietary supplementation of antioxidants[20]Strong differences in messenger RNA levels do not necessarily reflect activity of the correlated enzymes. Antioxidant enzyme activity may not be affected simply because the mRNA levels of antioxidant enzymes increase. There are other genotype-specific reports that suggest the same expression of genes does not mean the same physiological effect across different broiler breeds[21]

When comparing cycles of heat exposure, the level of residual expression at 24 hours is more informative than considering recovery as an instant return to baseline[22]. Our ordering of recovery reflects the general stress-adaptation mechanisms described in Onagbesan and colleagues, but the resolution is limited to transcript levels in the ileum[23] Transcription in the epithelium is only one part of the host-microbe response and should not be used to draw conclusions about the microbiome. [24] Integrating the results of the redox and junction profile is warranted and consistent with other recent reviews focusing on gut health and nutrition in the context of thermally stressful conditions. [25]

Conclusion

Using 10 transcripts and two tissue-based reference genes we defined three thermal states and assessed acute activation of chaperones, epithelial barrier dysfunction and recovery, We provide an independent set of biological units. Within these, we implement various statistical and non-parametric tests and integrate them within a multivariate framework to gain insight into ileal transcription.

REFERENCES

[1] S. Wasti, N. Sah, and B. Mishra, "Impact of heat stress on poultry health and performances, and potential mitigation strategies," *Animals*, vol. 10, Art. no. 1266, 2020.

[2] M. M. Fathi, A. Galal, L. M. Radwan, O. K. Abou-Emera, and I. H. Al-Homidan, "Using major genes to mitigate the deleterious effects of heat stress in poultry: An updated review," *Poultry Science*, vol. 101, Art. no. 102157, 2022.

[3] Q. Miao, X. Si, Y. Xie et al., "Effects of acute heat stress at different ambient temperature on hepatic redox status in broilers," *Poultry Science*, vol. 99, pp. 4113–4122, 2020.

[4] S. H. Siddiqui, M. Khan, D. Kang, H. W. Choi, and K. Shim, "Meta-analysis and systematic review of the thermal stress response: *Gallus gallus domesticus* show low immune responses during heat stress," *Frontiers in Physiology*, vol. 13, Art. no. 809648, 2022.

[5] B. Ma, T. Xing, J. Li, L. Zhang, Y. Jiang, and F. Gao, "Chronic heat stress causes liver damage via endoplasmic reticulum stress-induced apoptosis in broilers," *Poultry Science*, vol. 101, Art. no. 102063, 2022.

[6] B. Ma, L. Zhang, J. Li, T. Xing, Y. Jiang, and F. Gao, "Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers," *Poultry Science*, vol. 100, pp. 215–223, 2021.

[7] S. H. Siddiqui, D. Kang, J. Park et al., "Altered relationship between gluconeogenesis and immunity in broilers exposed to heat stress for different durations," *Poultry Science*, vol. 100, Art. no. 101274, 2021.

[8] A. F. Ghareeb, J. C. Foutz, G. H. Schneiders et al., "Host transcriptome response to heat stress and *Eimeria maxima* infection in meat-type chickens," *PLOS ONE*, vol. 19, Art. no. e0296350, 2024.

[9] W. A. Abd El-Ghany and D. Babazadeh, "Betaine: A potential nutritional metabolite in the poultry industry," *Animals*, vol. 12, Art. no. 2624, 2022.

[10] A. Sarsour and M. E. Persia, "Effects of sulfur amino acid supplementation on broiler chickens exposed to acute and chronic cyclic heat stress," *Poultry Science*, vol. 101, Art. no. 101952, 2022.

[11] A. Slawinska, M. Zampiga, F. Sirri et al., "Impact of galactooligosaccharides delivered in ovo on mitigating negative effects of heat stress on performance and welfare of broilers," *Poultry Science*, vol. 99, pp. 407–415, 2020.

[12] J. Chen, Z. Chen, J. Zhang, W. Zhuang, and X. Zheng, "Screening of reliable reference genes for the normalization of RT-qPCR in chicken gastrointestinal tract," *Poultry Science*, vol. 102, Art. no. 103169, 2023.

[13] A. M. Humam, T. C. Loh, H. L. Foo, W. I. Izuddin, I. Zulkifli, A. A. Samsudin, and N. M. Mustapha, "Supplementation of postbiotic RI11 improves antioxidant enzyme activity, upregulated gut barrier genes, and reduced cytokine, acute phase protein, and heat shock protein 70 gene expression levels in heat-stressed broilers," *Poultry Science*, vol. 100, Art. no. 100908, 2021.

[14] M. H. Rostagno, "Effects of heat stress on the gut health of poultry," *Journal of Animal Science*, vol. 98, Art. no. skaa090, 2020.

[15] M. F. A. Baxter, E. S. Greene, M. T. Kidd, G. Tellez-Isaias, S. Orlowski, and S. Dridi, "Water amino acid-chelated trace mineral supplementation decreases circulating and intestinal HSP70 and proinflammatory cytokine gene expression in heat-stressed broiler chickens," *Journal of Animal Science*, vol. 98, Art. no. skaa049, 2020.

[16] G. D. Vandana et al., "Heat stress and poultry production: Impact and amelioration," *International Journal of Biometeorology*, vol. 65, pp. 163–179, 2021.

[17] R. C. Beckford, L. E. Ellestad, M. Proszkowiec-Weglarz et al., "Effects of heat stress on performance, blood chemistry, and hypothalamic and pituitary mRNA expression in broiler chickens," *Poultry Science*, vol. 99, pp. 6317–6325, 2020.

[18] J. Hu, A. Mohammed, G. Murugesan, and H. Cheng, "Effect of a synbiotic supplement as an antibiotic alternative on broiler skeletal, physiological, and oxidative parameters under heat stress," *Poultry Science*, vol. 101, Art. no. 101769, 2022.

[19] A. A. Al-Sagan, S. Khalil, E. O. Hussein, and Y. A. Attia, "Effects of fennel seed powder supplementation on growth performance, carcass characteristics, meat quality, and economic efficiency of broilers under thermoneutral and chronic heat stress conditions," *Animals*, vol. 10, Art. no. 206, 2020.

[20] F. Song, B. Wang, X. Kang et al., "Exogenous melatonin alleviates heat stress-induced metabolic slowdown affecting broiler growth by improving lipid metabolism," *Journal of Thermal Biology*, vol. 136, Art. no. 104418, 2026.

[21] J. An, J. Lee, M. Song et al., "Effects of supplemental different clay minerals in broiler chickens under cyclic heat stress," *Journal of Animal Science and Technology*, vol. 65, pp. 113–131, 2023.

[22] O. M. Onagbesan, V. A. Uyanga, O. Oso, K. Tona, and O. E. Oke, "Alleviating heat stress effects in poultry: Updates on methods and mechanisms of actions," *Frontiers in Veterinary Science*, vol. 10, Art. no. 1255520, 2023.

[23] C. M. Ncho, "Heat stress and the chicken gastrointestinal microbiota: A systematic review," *Journal of Animal Science and Biotechnology*, vol. 16, Art. no. 85, 2025.

[24] E. M. Oluwagbenga and G. S. Fraley, "Heat stress and poultry production: A comprehensive review," *Poultry Science*, vol. 102, Art. no. 103141, 2023.

[25] S. F. Olayiwola and S. A. Adedokun, "Heat stress in poultry: The role of nutritional supplements in alleviating heat stress and enhancing gut health in poultry," *Frontiers in Veterinary Science*, vol. 12, Art. no. 1691532, 2025.