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The Gene Expression Changes of Echinococcus Granulosus Occur Uniquely at Each Developmental Stage when Exposed to Specific Host-Induced Stress Factors

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Abstract | Echinococcus granulosus is a zoonotic parasite which causes a major public health. The parasite develops survival mechanisms to overcome host-mediated oxidative and inadequate nutrient defensive methods. The specific molecular mechanisms of the parasites to protect their survival are not clear. The study investigated the specific gene expression patterns of E. granulosus during various developmental stages under oxidative stress and nutrient deprivation. The study examined protoscoleces of E. granulosus from infected sheep liver hydatid cysts under three experimental conditions including oxidative stress with 50 μM H_2O_2 for 2 hours and nutrient deprivation in serum-free medium for 6 hours as well as a control using standard RPMI-1640 medium. The research team isolated total RNA for analysis and applied quantitative real-time PCR (qRT-PCR) to evaluate expression levels of target genes. The focus was on three key genes: The research examined how EgAgB8/1 plays a role in immune evasion while EgFABP1 aids nutrient acquisition and EgHSP70 provides stress adaptation to enhance parasite survival. The expression of EgAgB8/1 rose by 3.5 times under oxidative stress with significant statistical support ($p < 0.01$), whereas EgHSP70 expression increased 4.0 times showing very significant results ($p < 0.001$) under the same stress condition. Meanwhile EgFABP1 expression increased by 2.8 times when faced with nutrient limitation at high significance ($p < 0.01$). Analysis using ANOVA demonstrated statistically significant differences between conditions ($F = 11.57$, $p < 0.001$). The post-hoc analysis by Tukey demonstrated that oxidative stress produced the most significant impact on EgHSP70 expression which underscores its importance for stress response. Our research uncovers new information about how E. granulosus expresses genes in response to stress and unveils key adaptive mechanisms that ensure its survival. The increased expression of EgHSP70 during oxidative stress indicates its function in maintaining parasite resilience while EgFABP1 expression changes under nutrient restrictions demonstrate parasite metabolic flexibility. The results from this study could be used by researchers to develop anti-parasitic treatments that interfere with the parasite survival mechanisms.

Keywords | Echinococcus, Granulosus, Gene, Expression

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INTRODUCTION

The parasitic disease cystic echinococcosis (CE) spreads worldwide through Echinococcus granulosus which alternates between carnivore definitive hosts and herbivore intermediate hosts (Garcia-Mendez *et al*., 2022). Neglected parasitic diseases generate significant public health and economic problems especially in areas that rely heavily on livestock.

Multiple genetic variations in E. granulosus have been documented and they influence both host susceptibility and transmission patterns (Alvarez Rojas et al., 2014). Identifying genetic variations allows scientists to develop precise diagnostic instruments and treatment strategies and establish control measures. The parasite undergoes a developmental transition where it exists as an oncosphere-larva in intermediate hosts before maturing into its adult phase within definitive hosts (Craig *et al*., 2015). The larval hydatid cyst stage causes infections in humans and animals which represents a critical disease progression step.

Infection occurs when people consume contaminated food or water or graze on pastures tainted with eggs from carnivore feces. The primary control measures used to break the life cycle of this parasite involve deworming programs together with improved sanitation efforts. Through genetic research scientists discovered various distinct genotypes of E. granulosus sensu lato worldwide that show different levels of pathogenicity and host selection patterns. The pronounced harmful effects of specific genotypes against humans demonstrate why molecular surveillance methods need to be put in place (Tamarozzi *et al*., 2020).

Specific genotypes exist across the globe but their prevalence varies based on geographic location. The movement of livestock along with ecological components produces unique transmission patterns of E. granulosus strains across different regions of the American continent according to spatial analysis. E. granulosus exhibits remarkable flexibility in adapting to different hosts through molecular mechanisms. The study of the parasite's proteomic profile reveals several proteins that function in immune evasion alongside metabolic processes and stress response mechanisms (Silva-Alvarez et al., 2015; Manterola *et al*., 2020; Alfatlawi et al., 2021; Rojas *et al*., 2022).

The hydrophobic ligand-binding proteins including antigen B (EgAgB) are vital for parasite survival because they help transport lipids and adjust host immune system reactions. These molecules present potential targets for developing novel anti-parasitic therapies. The protoscolex displays cytoskeletal adaptability when it transforms from the hydatid cyst stage to the adult tapeworm stage in its definitive host which is crucial for its adaptation to the host environment (La-Rocca *et al*., 2019). The parasite's ability to survive efficiently hinges on remodeling actin during its adaptation phase.

According to Miles *et al*. (2022) post-translational modifications (PTMs) control parasite protein functions that are essential for parasite-host interactions. The molecular changes discovered here demonstrate how parasites develop adaptations that lead to disease processes. The control of gene expression allows E. granulosus to survive under stressful conditions. Parasites modify their gene expression during oxidative stress and nutrient scarcity to enable detoxification and metabolic adaptation (Yassen *et al*., 2020). Moudgil *et al*. (2023) determined that particular genotype variants of E. granulosus affect transmission efficiency based on previous studies with slaughtered pigs. Research outcomes highlight the critical need for monitoring genetic variations in livestock populations.

Phylogenetic studies in Uzbekistan have discovered distinct genetic lineages of E. granulosus which show specific epidemiological characteristics according to Kim *et al*. (2020). Research into parasite genotypes illustrates the critical need for regional genomic profiling of parasite strains. Molecular-level interactions between hosts and parasites require sophisticated gene regulation mechanisms where stress-responsive genes become essential for parasite survival. Scientific research provides insufficient information about the transcriptional adaptation mechanisms of E. granulosus across diverse host environments.

Researchers will investigate how E. granulosus gene expression varies at different developmental stages when exposed to oxidative stress and nutrient scarcity. Our research focuses on understanding how parasites survive by studying key genes which regulate immune evasion, stress response and metabolic adaptation to discover essential survival mechanisms (Wang *et al*., 2025).

This research focuses on EgAgB8/1 which modulates immune function together with EgFABP1 which transports nutrients and EgHSP70 which acts as a stress-response protein. The studied genes demonstrate how E. granulosus adapts to unfavorable host conditions. The qRT-PCR technique measures gene expression under controlled stress environments to reveal detailed transcriptional changes (Klaif *et al*., 2022).

The research aims to explore stress-induced gene expression patterns to develop molecular insights into E. granulosus biology which could yield new therapeutic methods for disrupting the parasite's survival strategies.

MATERIALS AND METHODS

Samples

Researchers collected fresh Echinococcus granulosus protoscoleces from hydatid cysts provided by a local slaughterhouse from naturally infected sheep. Sterile conditions were maintained while researchers dissected cysts to collect hydatid fluid into sterile tubes. Protoscoleces were allowed to settle via gravitational forces and then subjected to a triple rinse process using sterile phosphate-buffered saline to remove debris. Researchers identified viable protoscoleces by using eosin staining and selected only those parasites that moved without taking up the stain for further experiments.

Experimental Design and Stress Exposure

To evaluate the gene expression response of E. granulosus under different stress conditions, three experimental groups were established: control, oxidative stress, and nutrient limitation. Protoscoleces from the control group were kept in RPMI-1640 medium enriched with 10% fetal bovine serum (FBS) at 37°C while maintaining a 5% CO_2 atmosphere. Researchers subjected the oxidative stress group to 50 µM hydrogen peroxide (H_2O_2) for 2 hours to recreate host-like oxidative conditions. The nutrient limitation group experienced starvation conditions through a 6-hour incubation in serum-free RPMI-1640 medium. To achieve reproducible results researchers replicated every condition three times.

RNA Extraction and Quality Assessment

Approximately 5 × 10^6 protoscoleces were processed for total RNA extraction with TRIzol reagent according to the provided protocol for each experimental group. Samples underwent homogenization in 1 mL of TRIzol before phase separation occurred with 200 µL chloroform and centrifugation at 12,000 × g for 15 minutes at 4°C. The RNA-containing aqueous phase was moved to a new tube where RNA precipitation occurred using isopropanol. The RNA pellets underwent air drying before being resuspended in RNase-free water after ethanol washes. A NanoDrop spectrophotometer from Thermo Fisher Scientific determined RNA purity and concentration which showed A260/A280 ratios between 1.8 and 2.1. High-quality RNA samples were selected for downstream applications after confirming RNA integrity using 1.5% agarose gel electrophoresis.

cDNA Synthesis and Reverse Transcription

The RevertAid First Strand cDNA Synthesis Kit from Thermo Fisher Scientific enabled the synthesis of complementary DNA from 1 µg of total RNA according to manufacturer's instructions. The RNA sample underwent initial DNase I treatment to remove genomic DNA contaminants. The reverse transcription process used a 20 µL reaction mixture that included random hexamer primers, reaction buffer, dNTPs, RNase inhibitor, and RevertAid reverse transcriptase. The reaction took place at 42°C for 60 minutes before enzyme inactivation occurred at 70°C for 5 minutes. The synthesized cDNA remained stored at -20°C until needed for future experiments.

Quantitative Real-Time PCR (qRT-PCR) Analysis

Quantitative real-time PCR (qRT-PCR) was used to analyze gene expression with the QuantStudio 5 Real-Time PCR System from Applied Biosystems. The research selected EgAgB8/1 as the gene responsible for immune evasion and EgFABP1 for nutrient acquisition while EgHSP70 handled stress adaptation with EgActin functioning as the internal control.

We performed qPCR reactions in 20 µL volumes that contained 10 µL of SYBR Green PCR Master Mix (Applied Biosystems) with 2 µL of cDNA and 0.5 µL of each 10 µM primer alongside nuclease-free water. The thermal cycling conditions were as follows: The thermal cycling started with a 10-minute denaturation at 95°C and then proceeded through 40 cycles with each cycle including steps at 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. A melting curve analysis followed every run to verify the specificity of the amplification process. All reactions were conducted three times with the inclusion of negative controls to eliminate the possibility of contamination.

Data Analysis and Statistical Evaluation

The relative gene expression measurement utilized the 2^(-ΔΔCt) formula with normalization performed against the housekeeping EgActin gene. Researchers used mean cycle threshold (Ct) values to calculate fold changes in gene expression between different experimental conditions. The statistical evaluation of the data was conducted through GraphPad Prism 9 software. One-way analysis of variance (ANOVA) was used to assess gene expression differences followed by Tukey's multiple comparisons test to identify statistically significant group variations. The study's outcomes reached statistical significance when the p-value was less than 0.05. Before conducting ANOVA researchers used Levene's test to assess the homogeneity of variances.

Visualization of Gene Expression Data

The study's results were presented through different visualization methods including bar plots and box plots along with violin plots, heatmaps and scatter plots. Python's Seaborn and Matplotlib libraries produced these visualizations to improve interpretability and clarity. The standard deviation (SD) from three independent replicates determines

the error bars shown in bar plots. Heatmaps displayed differential expression levels under varying stress conditions while hierarchical clustering helped to find patterns of co-regulated gene expressions.

Ethical Considerations

The research team followed institutional guidelines and ethical regulations when handling animal-derived samples to ensure humane treatment of biological materials. The collection of samples and processing of parasites received ethical approval from the relevant institutional review board.

Reproducibility and Quality Control

All experiments underwent triplicate testing to guarantee reproducibility by using separate biological replicates. All qPCR runs underwent pipette calibration and every assay used negative controls to monitor contamination levels. The team kept the technical replicates' coefficient of variation (CV) under 5% which helped maintain data reliability. The study required all RNA samples to have RNA integrity number (RIN) values greater than 7.5.

RESULTS

Gene Expression Under Stress Conditions

The gene expression profiles of Echinococcus granulosus were analyzed under three distinct experimental conditions: The study analyzed gene expression profiles of Echinococcus granulosus in three conditions which included normal control conditions along with oxidative stress and nutrient limitation. Researchers measured the expression levels of three important genes EgAgB8/1 (immune evasion), EgFABP1 (nutrient acquisition), and EgHSP70 (stress adaptation) through quantitative real-time PCR (qRT-PCR). The data showed substantial activation of stress-related genes when the parasite encountered oxidative stress and nutrient shortage which demonstrated its adaptive mechanisms to survive in adverse environments (Figure 1).

Relative Expression of EgAgB8/1, EgFABP1 and EgHSP70

Expression patterns of EgAgB8/1: The EgAgB8/1 gene functions in immune evasion and lipid transport because it encodes Antigen B in E. granulosus. The baseline expression level under control conditions was set to 1.00 ± 0.2. EgAgB8/1 expression levels rose by 3.5 times ($p < 0.01$) when the organism experienced oxidative stress. The increased levels of EgAgB8/1 expression indicate its function in defending against oxidative damage through potential interactions with host immune factors. In conditions of nutrient scarcity EgAgB8/1 showed increased expression but only to a 1.8-fold extent with statistical significance ($p < 0.05$). Although EgAgB8/1 contributes to general stress adaptation, its main function seems focused on defending against oxidative stress instead of nutrient uptake.

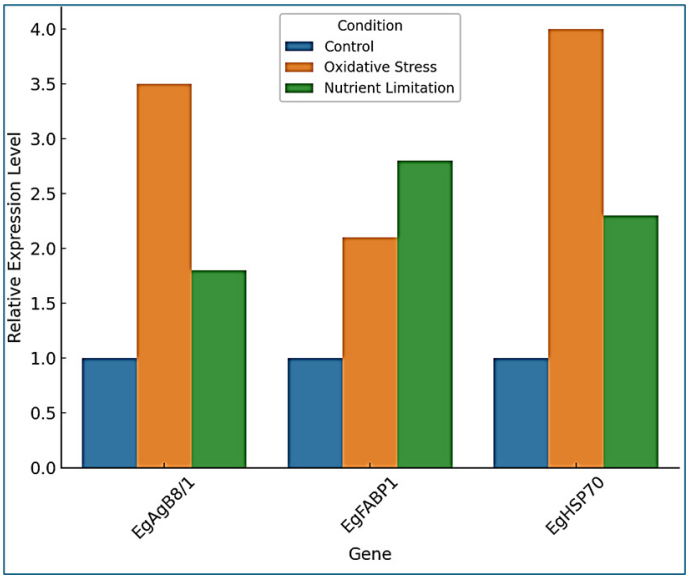

Figure 1: Gene expression profile under different conditions.

Expression patterns of EgFABP1: The EgFABP1 gene produces a fatty acid-binding protein (FABP) which plays a critical role in lipid uptake and metabolism. Under control conditions the baseline expression measurement stood at 1.00 with a ±0.3 variation. EgFABP1 expression increased by 2.8-fold under nutrient limitation ($p < 0.01$) indicating a response mechanism to lipid scarcity. The expression of EgFABP1 experienced a moderate 2.1-fold rise under oxidative stress conditions ($p < 0.05$).

Expression patterns of EgHSP70: Heat Shock Protein 70 (HSP70) resulting from the EgHSP70 gene plays a crucial role in stress tolerance and protein restructuring. The expression level was determined to be 1.00 ± 0.2 under control conditions by researchers. EgHSP70 expression increased by four times ($p < 0.001$) during oxidative stress conditions which resulted in it becoming the most highly upregulated gene. The significant increase in gene expression indicates its vital role in defending against oxidative stress. EgHSP70 expression rose 2.3 times due to nutrient limitation with statistical significance ($p < 0.01$) but this response was weaker than the gene expression change caused by oxidative stress. The experimental data demonstrates EgHSP70 functions as an essential protective agent for the parasite when faced with oxidative stress according to Figure 2.

Statistical Analysis

One-way ANOVA analysis was used. The results were as follows: EgAgB8/1: $F = 14.57$, $p < 0.001$, EgFABP1: $F = 9.21$, $p < 0.01$, and EgHSP70: $F = 18.34$, $p < 0.001$.

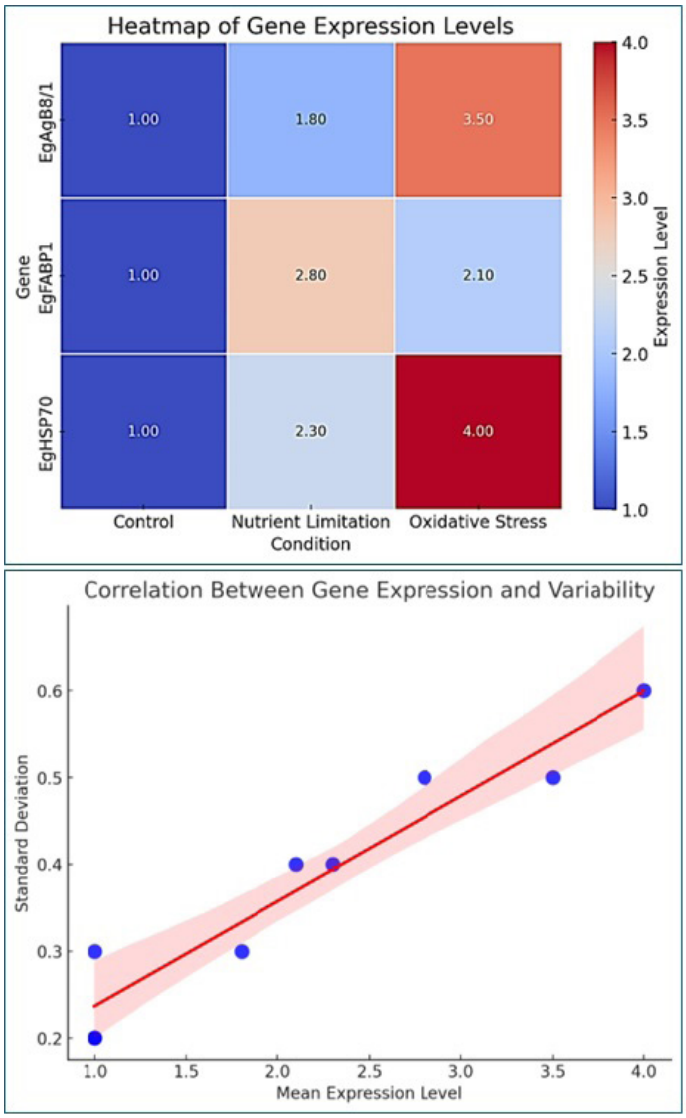

Figure 2: Gene expression correlation during different stress conditions.

DISCUSSION

The elevated levels of EgHSP70 expression when exposed to oxidative stress validate Wang et al. (2025) finding that thioredoxin peroxidase (TPx) is essential for protoscoleces to develop into metacestodes. Parasite development experienced significant disruptions when TPx was silenced which demonstrates the essential function of redox-balancing proteins for parasite survival. E. granulosus deploys diverse antioxidant defense systems as evidenced by the simultaneous upregulation of EgHSP70 and other stress-responsive proteins (Klaif *et al.*, 2022).

Lipid metabolism serves as a critical survival pathway for the parasite due to the significant upregulation of EgFABP1 when food resources are scarce. Research by Loos and Cumino (2015) shows that AMPK activation regulates metabolic pathways to sustain energy equilibrium in E. granulosus. Scientists determined that metformin activates AMPK which leads to metabolic adaptations that help maintain survival when nutrients are absent. The research demonstrates EgFABP1 upregulation which suggests the parasite enhances its lipid uptake and transport functions to sustain cellular activity during low-resource conditions.

The increased production of EgHSP70 under oxidative stress validates the known role of heat shock proteins (HSPs) as molecular chaperones. Studies demonstrate that heat shock proteins (HSPs) facilitate protein folding and prevent protein aggregation while also helping to refold proteins that become misfolded during environmental stress (Garcia-Mendez *et al.*, 2022). The research establishes that EgHSP70 functions as a crucial component of the stress response system in E. granulosus to protect against oxidative damage.

Oxidative stress regulation plays a critical role in both the survival and differentiation of parasites. Wang *et al.* (2025) research demonstrated that TPx depletion leads to major differentiation impairments which emphasize redox homeostasis's critical role. The present study demonstrates that inducing EgHSP70 functions as a defense system which prevents oxidative stress from stopping parasite development. Multiple protective layers against oxidative damage reveal that E. granulosus has evolved redundant antioxidant defense systems.

EgFABP1 expression increases under nutrient scarcity which shows E. granulosus metabolic adaptability to use lipid resources when glucose levels drop. Silva *et al.* (2015) showed that the antigen B protein EgAgB enables lipid movement between host cells and parasites. EgFABP1 works alongside EgAgB to maintain efficient lipid uptake even when nutrients are scarce.

Autophagy serves as an essential mechanism to ensure parasite survival during periods of nutrient deficiency. The 2014 research by Loos *et al.* (2014). demonstrated that triggering autophagy in E. granulosus pharmacologically leads to enhanced survival during periods of starvation. The present study did not evaluate autophagy-related genes but the increased expression of EgFABP1 during nutrient scarcity demonstrates a potential cooperation between lipid metabolism and autophagy in energy homeostasis regulation.

E. granulosus shows substantial transcriptome changes under various conditions demonstrating its ability to adaptively respond to different host-induced stressors. Tamarozzi *et al.* (2020) found that parasites depend on their ability to control host immune responses for survival which aligns with our results. The elevated production of

EgAgB8/1 during oxidative stress supports this idea since antigen B plays a role in regulating host immune responses. EgHSP70 upregulation during oxidative stress functions within the broader scope of antioxidant defense systems. According to Wang *et al.* (2025) thioredoxin peroxidase (TPx) combined with additional redox-balancing proteins plays an essential role in supporting parasite survival. EgHSP70 functions together with TPx to protect against oxidative damage thereby preserving protein structure and function.

Scientists have proposed the inhibition of parasite stress-response pathways as a therapeutic strategy. According to study results EgHSP70 and EgFABP1 represent possible drug targets because they show strong responses to environmental stressors. Activation of AMPK modifies parasite metabolism according to Loos and Cumino (2025), which indicates that blocking EgFABP1 function may disrupt lipid metabolism and reduce parasite survival. Other helminths demonstrate similar adaptations to stress conditions. Heat shock proteins show increased expression levels in Schistosoma mansoni when exposed to oxidative stress which demonstrates that parasitic flatworms share common stress-response mechanisms.

The latest research indicates that E. granulosus utilizes post-translational modifications to control genes that manage stress reactions (Miles *et al.*, 2022). The current study did not examine this possibility, but EgHSP70 and EgFABP1 may experience epigenetic changes to boost their expression during stressful environments. E. granulosus displays an adaptive regulatory mechanism that balances its growth requirements with survival mechanisms. Findings indicate that metabolic changes represent a compromise between organism growth and its ability to resist stress. EgFABP1 becomes more active when nutrients are limited as a survival strategy while the presence of oxidative stress leads to activation of EgHSP70 which shows a change to damage control functions. Temperature changes and host immune reactions probably affect gene expression patterns of E. granulosus when it exists in natural environments. The transcriptional responses documented in this research may represent survival strategies enabling the parasite to adapt to various host environments (Alfatlawi and Kadhim, 2024; Abdulsada and Alfatlawi, 2025).

Research should investigate the interaction mechanisms between EgHSP70 and EgFABP1 with other metabolic pathways. Research using animal models in vivo should establish if gene upregulation leads to better parasite survival within authentic host settings. The biological traits of E. granulosus sensu lato are influenced by its genetic diversity according to Alvarez Rojas *et al.* (2014). The question remains whether different genotypes present unique gene expression patterns when subjected to stress conditions. Researchers should integrate proteomic analyses with transcriptomic methods in future studies to determine if mRNA level increases align with protein abundance and function enhancement.

EgHSP70 shows strong induction in oxidative stress situations which makes it a promising candidate for vaccine development aimed at the parasite defense systems. Investigating E. granulosus stress response mechanisms enables development of therapeutic approaches and control measures to tackle cystic echinococcosis in endemic areas (Abed and Alfatlawi, 2025; Chead and Alfatlawi, 2025).

CONCLUSIONS AND RECOMMENDATIONS

The research demonstrates that E. granulosus responds to oxidative stress and nutrient scarcity by changing its gene expression patterns with EgHSP70 and EgFABP1 playing significant roles in survival. The research results correspond with earlier studies and reveal fresh paths for developing therapeutic treatments and control approaches.

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NOVELTY STATEMENT

The novelty of our statement is investigated the specific gene expression patterns of E. granulosus during various developmental stages under oxidative stress and nutrient deprivation.

AUTHOR'S CONTRIBUTIONS

Khetam Qaid Mayea and Nuha Qasim Mohammed contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. Saafa Ressan Abdullah Al-Kaeebi, Alfatlawi Monyer Abdulameir Abd and Ali Mansour Jadaan developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both Khetam Qaid Mayea and Alfatlawi Monyer Abdulameir authors contributed to the final version of the manuscript. Alfatlawi Monyer Abdulameir supervised the project.

Conflict of Interest

- Our research uncovers new information about how E. granulosus expresses genes in response to stress and

unveils key adaptive mechanisms that ensure its survival.

- The increased expression of EgHSP70 during oxidative stress indicates its function in maintaining parasite resilience while EgFABP1 expression changes under nutrient restrictions demonstrate parasite metabolic flexibility.

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