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Impacts of Nicotinamide Adenine Dinucleotide Supplementation on Longevity, Egg Quality, and Immunity in Laying Hens

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ABSTRACT

The productivity and health of laying hens are major challenges for the global poultry industry, especially as flocks age and egg production declines. The present study aimed to evaluate the effects of nicotinamide riboside chloride (NRCl), a bioavailable precursor of nicotinamide adenine dinucleotide (NAD^+), on longevity, egg quality, and immune performance in laying hens. A total of 180 Hy-Line Brown hens, 24-week-old, were randomly assigned to three dietary groups for 24 weeks, including a control group receiving standard layer feed, a low-dose NRCl group receiving 25 mg NR/kg feed, and a high-dose NRCl group receiving 50 mg NR/kg feed. The present study assessed survival rate, daily egg production, egg weight, shell strength, yolk nutrient composition, and immune parameters, including white blood cell (WBC) count, immunoglobulins (IgA and IgG), and cytokines (IL-6 and IFN-γ). The present findings indicated that supplementation with NRCl significantly increased the survival rate, resulting in 100% survival in the high-dose group compared to 93.3% in the control group. Egg production and feed conversion ratio were also enhanced in NRCl groups, indicating improved physiological resilience. The NRCl groups demonstrated significant improvements in egg quality, including stronger, thicker shells and enhanced nutritional content such as higher yolk protein, vitamin D3, and omega-3 fatty acid levels. Furthermore, immune markers were markedly elevated, reflecting enhanced immune modulation and potential disease resistance. The NRCl supplementation effectively supported metabolic efficiency, immune competence, and egg quality in laying hens. The current findings highlighted the potential of NAD^+ precursor supplementation as a nutritional strategy to prolong the productive lifespan of commercial layers while promoting sustainable poultry production.

Keywords: Egg quality, Immune response, Laying hens, Longevity, Nicotinamide adenine dinucleotide supplementation, Poultry feed

INTRODUCTION

Egg production plays an essential role in global food security, providing an affordable and high-quality source of protein worldwide (Supriya et al., 2023). Poultry farming is vital to the rural livelihoods in many developing countries, providing direct household consumption and income through poultry-based activities. However, the poultry industry faces several challenges, particularly the ageing of laying hens, which leads to a gradual decrease in egg production after approximately 72 weeks of age (Li et al., 2021).

In many regions, including the United States and Australia, increasing feed and transportation costs, along with recurrent disease outbreaks such as avian influenza, have intensified these challenges (Rehman et al., 2023). The progressive decline in productivity associated with hen ageing is closely linked to metabolic and cellular changes, including reductions in nicotinamide adenine dinucleotide (NAD^+) levels, which are known to influence energy metabolism, DNA repair, and mitochondrial function (Covarrubias et al., 2021). Therefore, strategies that restore or maintain NAD^+ balance may help mitigate the effects of ageing and improve the longevity and productivity of laying hens.

In recent years, advances in molecular biology have highlighted NAD^+ as a crucial regulator in cellular energy metabolism, aging, and immune function (Zhang et al., 2025). The NAD^+ plays an essential role in redox reactions, mitochondrial activity, and DNA repair, all of which depend on adequate intracellular NAD^+ levels (Braidy et al., 2019). Supplementing the diet with NAD^+ precursors such as nicotinamide riboside chloride (NRCl) in mammals has been demonstrated to extend lifespan, enhance metabolic efficiency, and improve immune performance (Elhassan et al., 2019). These findings suggested exploring the potential use of NAD^+ supplementation in non-mammalian species, particularly in agricultural settings such as poultry production (Wang et al., 2022). However, there are a limited number of studies that investigated the avian use of NAD^+ and its physiological effects in laying hens (Xie et al., 2020).

The present study aimed to evaluate the effects of NRCl on the longevity, egg quality, immune function, and overall productivity in ageing laying hens.

METHODS AND MATERIALS

Ethical approval

All experimental procedures were conducted in accordance with national and institutional ethical standards for animal welfare. Approval for the present study was obtained from the Institutional Animal Care and Use Committee (IACUC) of PWI Research and Technology Pty Ltd, Somersby, New South Wales, Australia. Humane euthanasia, performed according to the American Veterinary Medical Association (AVMA) guidelines, was conducted in cases of severe mortality.

Nicotinamide adenine dinucleotide formulation

During the present study, a stable and bioavailable NAD^+ precursor formulation was developed in collaboration with a certified poultry feed manufacturer. The NAD^+ precursor was NRCl, which was purchased from Sigma-Aldrich, USA, with a stated purity of ≥ 99%. The NRCl was selected because it is highly bioavailable and has been shown to increase NAD^+ levels in mammalian models, *in vivo* (Yang et al., 2019). The NRCl powder was mixed into the basal feed by simple physical mixing, rather than encapsulation, using 0.05% w/w of maltodextrin and microcrystalline cellulose as carrier materials. This method was selected to ensure a homogeneous distribution of the supplement while maintaining its stability during feed manufacturing. The NRCl remains stable at moderate temperatures (25-30°C) and humidity levels typical in poultry feed processing environments (Elhassan et al., 2019).

The toxicity and efficacy of NAD^+ precursor supplementation in poultry were initially assessed in a small-scale pilot trial involving 20 Hy-Line Brown hens, allocated to two NRCl dosages of 25 mg/kg and 50 mg/kg feed, for four weeks, as recommended by Elsherif et al. (2019). No adverse clinical signs, mortality, or reductions in feed intake were observed at either dose. However, hens receiving 50 mg/kg of NRCl exhibited improved activity, normal droppings, and a slight increase in egg production compared to the supplemented controls. Based on these preliminary observations, 50 mg NRCl/kg feed was selected as a safe and effective concentration for the experiment (Maynard et al., 2024). A carrier ratio of 1:10 (NRCl: carrier) was used to ensure even dispersion in the feed, and the mixture was processed with a horizontal feed mixer for 10 minutes to achieve uniform blending. The prepared feed was then stored in opaque, airtight containers at approximately 25°C to minimize light- and oxygen-induced degradation. The NAD^+ stability in the feed was verified through periodic sampling. The NAD^+ concentrations were quantified using high-performance liquid chromatography (HPLC) according to the method described by Jing et al. (2022).

Study design

The present experiment was conducted at a certified poultry research facility located in Punjab Province, Pakistan, under ambient temperatures of 24-27°C and relative humidity of 55-65%. Each hen was individually housed in a wire-floored cage (40 × 45 × 45 cm; 2 poultry /m^2 stocking density) within an environmentally controlled house. Hens were maintained on a 16-hour light: 8-hour dark photoperiod and had *ad libitum* access to feed and fresh water. The experimental period lasted 24 weeks.

Animals

A total of 180 Hy-Line Brown laying hens, 24 weeks of age and with an average initial body weight of 1.75 ± 0.05 kg, were selected based on the uniform health status and laying performance, according to the Hy-Line brown hen conventional system (2022) and Zhao et al. (2022). Baseline data on body weight and egg production were collected before treatment allocation and confirmed to have no significant differences across groups. Unpublished pre-test data showed that the average body weight (Control: X kg; NR-5: Y kg; NR-10: Z kg) and hen day egg production (Control: A; NR-5: B; NR-10: C) were similar across all groups. The hens were divided into three dietary groups. The control group was fed only the standard commercial layer diet (Group A). The second group received a low dose of nicotinamide riboside (NR), which was included in the basal diet containing 25 mg of NR per kilogram of feed (Group B), and the third group received a high dose of NR, fed with a basal diet containing 50 mg of NR per kilogram of feed (Group C).

Diet compositions

A lower NR inclusion rate (25 mg/kg) was expected to support metabolic balance, whereas a higher rate (50 mg/kg) was anticipated to exhibit a stronger physiological response without adverse effects. The expected physiological effects included biological responses at each NR inclusion level, supported by previous mammalian and initial avian studies on NRs. The basal diet was formulated according to NRC (1994) nutrient recommendations for layers and contained approximately 17.5% of crude protein, 11.3 MJ/kg of metabolizable energy, 3.5% of calcium, 0.45 % of available phosphorus, 0.35% of methionine, and 0.75% lysine, with vitamin-mineral premixes meeting or exceeding standard requirements. The metabolizable energy of the basal diet was 11.3 MJ/kg, meaning it provided 11.3 megajoules of usable energy per kilogram.

Premix composition of vitamin-mineral

The premix contained vitamins A, D3, E, K3, B-complex, and essential trace minerals such as zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), iodine (I), and selenium (Se), and required electrolytes. The vitamin-mineral premix was added at levels that meet or exceed the nutrient requirements of the laying hen as specified by the NRC (1994; Table 1).

Table 1. Inclusion levels of nicotinamide riboside and anticipated physiological consequences, and the basal diet composition used in the experiment

NR Inclusion Level	Justification	Expected physiological effect
25 mg/kg feed (Low dose)	Supported by prior mammalian and preliminary avian findings showing dose-dependent mitochondrial and antioxidant benefits	Supports metabolic balance without excessive stimulation
50 mg/kg feed (High dose)	Higher doses have been shown to enhance mitochondrial activity and antioxidant capacity	Stronger physiological response with no observed adverse effects
Basal diet composition (as-fed basis)		
Nutrient	**Level**	
Crude protein	17.5%	
Metabolizable energy	11.3 MJ/kg	
Calcium	3.5%	
Available phosphorus	0.45%	
Methionine	0.35%	
Lysine	0.75%	
Vitamin–mineral premix	Met or exceeded NRC (1994) requirements (Dale, 1994)	

NR: Nicotinamide riboside.

Data and sample collection

Throughout the experiment, trained personnel closely monitored the hens to ensure their well-being and health status. Veterinary staff inspected the poultry daily for any signs of disorder and administered appropriate interventions when required. Several performance parameters evaluated included hen longevity, daily egg production, egg weight, shell strength and thickness, egg nutrient composition, and immune parameters. Digital egg counters and radio frequency identification tracking systems were used to record individual egg production daily. Each week, egg quality traits, including shell strength and shell thickness, were measured using a breaking force tester (Model EFR-01, Imada Co., Japan) and micrometer calipers (Mitutoyo Corp., Japan). Calibrated digital weighing scales were used to accurately measure egg weight.

Egg nutrient composition was analyzed for protein, lipid profile, vitamins, and mineral content. Protein and lipid contents were determined using the Kjeldahl and Soxhlet extraction methods, respectively, while vitamins were quantified using HPLC (Agilent Technologies, USA). Mineral concentrations were assessed through atomic absorption spectroscopy (AAS; PerkinElmer, USA) following the method described by Jiang et al. (2023).

Sampling procedures were kept to a minimum to reduce stress and minimize blood collection. A subgroup of ten hens per group was sampled monthly via wing vein puncture, with approximately 3 mL of blood collected from each hen. White blood cell (WBC) counts, immunoglobulin levels, such as IgA and IgG, cytokine concentrations, including interleukin-6 (IL-6) and interferon-gamma (IFN-γ), were measured. Samples were analyzed using an automated hematology analyzer (Mindray BC-2800Vet, China) and enzyme-linked immunosorbent assay (ELISA) kits (Cusabio Biotech, Wuhan, China) according to the manufacturer's instructions. The ELISA method ensured consistent and repeatable data collection for both production and immune performance parameters.

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., USA). A one-way analysis of variance (ANOVA) was performed to determine significant differences among the three treatment groups, followed by Tukey's post hoc test for multiple comparisons. For repeated measures, such as egg production over time, a mixed-model ANOVA was applied. A significance level of $p < 0.05$ was used for all statistical analyses. Data visualization was conducted using GraphPad Prism software (version 9.0; GraphPad Software, San Diego, CA, USA) to generate line and bar graphs representing mean values and variations. Results are expressed as mean ± standard deviation (SD).

RESULTS

Hen longevity and egg production metrics

Hen survival and egg production were consistently monitored over the 24-week experimental period in all treatment groups. The present results indicated a significant difference in survival rates among the control group and both NR-supplemented groups ($p < 0.05$; Table 2. Group A demonstrated a survival rate of 93.3%, whereas Group B recorded 96.7%, and Group C exhibited 100% survival. The few deaths observed in the control group occurred between weeks 18 and 20, most likely due to age-related physiological decline. No necropsy was performed, so the precise cause of death could not be pathologically confirmed. These findings suggested that NR supplementation, particularly at the higher level, enhanced longevity and reduced mortality during the late laying period. These improvements might be associated with greater general health and physiological resilience in the supplemented hens.

The average daily egg production rate differed significantly among the treatment groups ($p < 0.05$). Group C indicated the highest production at 0.93 ± 0.01 eggs/hen/day, which was significantly greater than that of the control group (0.84 ± 0.02). Group B produced at an intermediate rate of 0.89 ± 0.01. The higher egg production in Group C indicated that NR supplementation helped maintain productivity as hens aged. By week 20, the control group exhibited a statistically significant 12% reduction in egg production, compared to 6% in Group B and 3% in Group C ($p < 0.05$). No significant differences were observed in body weight and egg production at the start of the study among groups ($p > 0.05$). Therefore, the enhanced performance observed in the NR-supplemented groups likely resulted from treatment rather than pre-existing health or physiological differences.

Furthermore, the slightly higher average egg weight in the supplemented groups might have contributed to the apparent improvement in shell strength, as larger eggs generally require stronger shells to maintain integrity. The present results demonstrated that NR supplementation improved survival rate and egg production in ageing hens during the experiment (Figure 1).

Table 2. Hy-Line Brown laying hens' survival and egg production among treatment groups

Group	Treatment (mg NR/kg feed)	Survival (%)	Avg. Egg Production (Eggs per hen per day ± SD)
A	0 (Control)	93.3 ± 1.0	0.84 ± 0.02^{a}
B	25	96.7 ± 0.6	0.89 ± 0.01^{b}
C	50	100 ± 0.0	0.93 ± 0.01^{b}

Avg: Average, SD: Standard deviation, $^{a, b}$ Different superscript letters in a column indicate significant differences ($p < 0.05$).

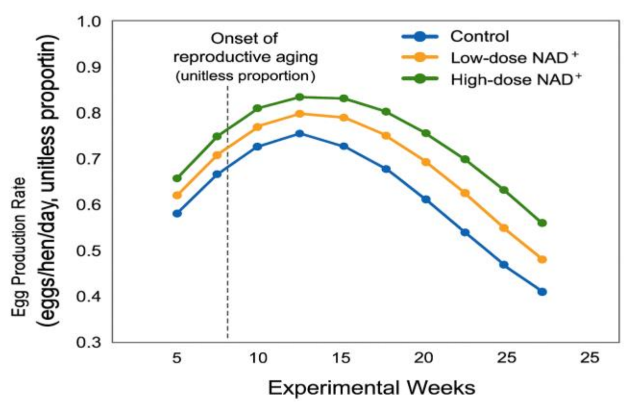

Figure 1. The y-axis is the egg production rate (eggs/hen /day, unitless proportion), whereas the x-axis is experimental duration (weeks). The study showed that high-dose NR supplementation (50 mg/kg feed) resulted in a high laying rate and retarded the onset of reproductive aging in high-dose supplementation as opposed to the control and low-dose groups. The dotted line represents the estimated age of reproductive aging towards Week 9.

Egg quality and nutrient analysis

The average egg weight across all groups increased gradually over 24 weeks. In week 24, the mean egg weights were 60.2 ± 1.5 g for Group A, 62.4 ± 1.2 g for Group B, and 63.8 ± 1.0 g for Group C. Group C exhibited the highest increase in egg weight among all groups, with statistically significant differences observed between Group A and Group C ($p < 0.05$; Table 3). Regarding shell quality, Group C produced significantly thicker shells (0.38 mm) compared to Group A (0.32 mm; $p < 0.05$). Likewise, shell strength was higher in Group C (3.5 kg) than in Group A (2.9 kg), indicating that NR supplementation improved shell thickness and strength. These findings suggested that NR supplementation enhanced the structural integrity and durability of eggshells, which are desirable characteristics in commercial egg production. The protein and vitamin levels in eggs differed significantly among treatment groups ($p < 0.05$). Eggs from Group C contained a higher level of protein (13.4%) and vitamin D_3 (3.8 µg/egg), compared to Group A (12.2%; 2.6 µg/egg, respectively). Similarly, the omega-3 fatty acid level was higher in Group C (230 mg/egg) than in Group A (180 mg/egg). These enhancements in egg nutrient content could benefit consumer health and the egg market value (Table 4).

Immune response and antiviral testing

The WBC count and immunoglobulin levels in the NR-supplemented groups were significantly higher than those in the control group ($p < 0.05$). The WBC count in Group A was 17,200 WBC/µL, in Group B was 19,400 WBC/µL, and in Group C was 20,100 WBC/µL; therefore, increasing NR supplementation was associated with a greater immune response in the treated groups. Similarly, IgA concentrations were significantly higher in Group C (4.1 mg/mL) compared to Group A (2.8 mg/mL; $p < 0.05$), suggesting that NR supplementation might enhance mucosal immunity by stimulating antibody production. The IFN-γ and IL-6 levels were significantly elevated in groups B and C ($p < 0.05$), particularly in Group C, indicating a stronger activation of immune regulatory pathways (Table 5). These findings demonstrated that NR supplementation improved immune competence, supporting humoral and cellular immune responses in laying hens during the experiment.

Observational data (behavioral health, feeding habits)

Improved overall health and well-being were observed in the NR-supplemented groups. The chickens in the control group demonstrated reduced activity and inconsistent feed intake compared to Group C, which exhibited consistent feeding behavior and remained more alert throughout the study period. Compared to the control group, hens in the NR-supplemented groups exhibited fewer signs of stress or nutrient deficiency, such as feather pecking and feather loss, thereby indicating improved welfare and physiological stability. In the NR-supplemented groups, the feed conversion ratio (FCR) improved, indicating greater feed efficiency. Group C had the most efficient FCR at 1.90, followed by Group B (1.98) and Group A (2.10; Table 6). These observations demonstrated that NR supplementation not only enhanced overall health and behavior but also enhanced feed utilization efficiency, thereby supporting improved performance in laying hens throughout the study.

Table 3. Effect of dietary supplementation of Nicotinamide Riboside on egg weight and shell quality of laying hens

Group	Egg weight (g ± SD)	Shell thickness (mm ± SD)	Shell strength (kg ± SD)
A (Control)	60.2 ± 1.5^{a}	0.32 ± 0.01^{a}	2.9 ± 0.1^{a}
B (25 mg/kg)	62.4 ± 1.2^{b}	0.35 ± 0.01^{b}	3.2 ± 0.1^{b}
C (50 mg/kg)	63.8 ± 1.0^{b}	0.38 ± 0.01^{b}	3.5 ± 0.1^{b}

SD: Standard deviation, $^{a, b}$ Different superscript letters in a column indicate significant differences ($p < 0.05$).

Table 4. Yolk nutrient composition of eggs from different dietary treatments in laying hens

Group	Protein (%)	Vitamin D_3 (µg/egg)	Omega-3 fatty acids (mg/egg)
A (Control)	12.2 ± 0.3^{a}	2.6 ± 0.1^{a}	180 ± 5^{a}
B (25 mg/kg)	12.8 ± 0.2^{ab}	3.2 ± 0.2^{b}	205 ± 4^{b}
C (50 mg/kg)	13.4 ± 0.2^{b}	3.8 ± 0.2^{b}	230 ± 6^{b}

$^{a, b}$ Different superscript letters in a column indicate significant differences ($p < 0.05$).

Table 5. Immune indicators of laying hens fed Nicotinamide Riboside-supplemented diets

Group	WBC (×10³ cells / µL)	IgA (mg/mL)	IgG (mg/mL)	IL-6 (pg/mL)	IFN-γ (pg/mL)
A (Control)	17.2 ± 0.3^a	2.8 ± 0.2^a	3.6 ± 0.2^a	21.4 ± 0.8^a	19.8 ± 0.7^a
B (25 mg/kg)	19.4 ± 0.4^b	3.6 ± 0.3^b	4.0 ± 0.3^b	25.1 ± 0.9^b	23.2 ± 0.6^b
C (50 mg/kg)	20.1 ± 0.3^b	4.1 ± 0.2^b	4.5 ± 0.3^b	28.3 ± 1.0^b	25.7 ± 0.7^b

$^{a, b}$ Different superscript letters in a column indicate significant differences ($p < 0.05$).

Table 6. Feed conversion ratio of laying hens during the experiment

Group	FCR (feed intake/egg mass)
A (Control)	2.10 ± 0.04^a
B (25 mg/kg)	1.98 ± 0.03^b
C (50 mg/kg)	1.90 ± 0.03^b

FCR: Feed conversion ratio, $^{a, b}$ Different superscript letters indicate significant differences ($p < 0.05$).

DISCUSSION

The current findings supported the hypothesis that NR improved physiological strength in laying hens and prolonged their productive lifespan, consistent with previous studies on other avian and mammalian species (Braidy et al., 2019; Elhassan et al., 2019). The improvement in egg production, particularly in group C, suggested that NR supplementation might delay reproductive ageing and promote sustained metabolic activity. This was likely due to the key role of NAD^+ in maintaining mitochondrial energy metabolism and cellular repair mechanisms.

The observed increase in eggshell thickness might be linked to enhanced calcium metabolism driven by improved mitochondrial efficiency in shell gland cells, resulting from elevated intracellular NAD^+ levels. Similarly, the improved egg nutrient composition, including higher levels of protein, vitamin D_3, and omega-3 fatty acids, indicated increased metabolic turnover and more efficient nutrient deposition due to enhanced redox status balance. Furthermore, the higher FCR efficiency observed in NR-supplemented groups could be associated with improved energy utilization and immune status. Enhanced NAD^+ metabolism is known to support avian immunometabolism by activating sirtuin pathways and improving cytokine regulation, thereby strengthening immune resilience and reducing physiological stress (Fang et al., 2023). These findings indicated that NR supplementation supported metabolic and immune homeostasis in laying hens, thereby enhancing productivity and product quality. This result is particularly relevant to commercial poultry production, where ageing hens and disease remain persistent challenges (Rehman et al., 2023).

Implications of increased egg weight, shell strength, and nutrient enrichment in eggs from NR-supplemented groups generally resulted in enhanced egg quality, which is essential for the poultry industry and consumer health. Therefore, eggs from supplemented groups with higher levels of omega-3 fatty acids and vitamin D_3 are marketed as premium eggs, meeting the growing consumer demand for value-added functional foods (Papanikolaou and Fulgoni, 2021; Palmieri et al., 2022). Moreover, elevated WBC and IgA levels indicated improved immune function following NR supplementation, which can serve as a natural strategy to enhance disease resistance, reduce antibiotic use, and strengthen biosecurity measures on poultry farms (Elhassan et al., 2019; Maynard et al., 2024). During the present study, NR supplementation resulted not only in behavioral improvements but also in enhanced hen activity and greater feed conversion efficiency. NR supplementation represented a strategy to improve the sustainability of poultry farming. Enhancing hen health and metabolic efficiency can potentially lower feed costs and increase the economic viability.

CONCLUSION

Supplementation with NR, particularly at higher inclusion levels, effectively enhanced egg production, eggshell quality, and immune function, thereby extending hens' lifespan. These findings suggested that NR can be considered a promising feed additive to support long-term poultry performance and welfare. To strengthen these findings, future investigations should focus on optimizing

NR dosage and evaluating its physiological impacts under different production and environmental conditions. This would help assess NR's full potential within the commercial poultry sector.

DECLARATIONS

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Authors' contributions

Bamboo Xiu Zhu Yan conceived and designed the study, monitored experimental implementation, and drafted the manuscript. Xiaoxuan Zhang performed data collection and laboratory analyses and assisted with the interpretation of results. Ramsha Shahbaz contributed to writing the manuscript, statistical analysis, and data interpretation. All authors read and approved the final edition of the manuscript prior to submission.

Competing interests

The authors declare that there are no financial or personal conflicts of interest.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical considerations

All the authors confirmed that ethical concerns, such as plagiarism, permission to publish, research misconduct, data fabrication or falsification, duplicate submissions, and redundant publication, have been thoroughly reviewed. The authors disclose that a generative artificial intelligence tool application was involved in the language editing and removing grammatical errors. Apart from that, no AI tool was utilized in data generation, statistical analysis, interpretation of results, or conclusion. The authors checked and approved the originality of the scientific content, data analyses, and interpretations very carefully before publication.

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