



Impacts of Restricting Ribonucleotide Reductase on Performance, Meat Quality, and Intestinal Health in Broiler Chickens Evaluated by Evans Blue Dye

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ABSTRACT

Ribonucleotide reductase (RNR) enzyme is essential for DNA synthesis and overall cellular health. Its inhibition interferes with metabolic pathways and mitochondrial function, leading to increased oxidative damage. Evans blue dye evaluates oxidative damage in tissues by penetrating through ruptured or destabilized membranes, thereby serving as an indicator of cell viability. The present study aimed to investigate the effects of restricting RNR on growth performance, meat quality, and intestinal health in broiler chickens after intestinal development was mainly completed. Twenty male broiler chickens, aged 14 days and weighing 445 ± 5 grams, were randomly divided into two equal groups: a control group and an experimental group, which received an RNR inhibitor (RR) at 20 mg/kg body weight/day for two weeks. Half of the chickens in both groups (5 broiler/group) were injected with Evans blue dye (EB) on the last day of the study. The oxidative damage was measured at the end of the study. The present results indicated that growth performance and feed intake were unchanged during the study. The RNR enzyme increased meat drip loss and lightness while reducing ultimate pH (pHu) compared to the control group. Evans blue concentration increased in the muscle, duodenum, jejunum, and ileum of the experimental group compared to the control group. Although RNR inhibition did not affect growth performance, it led to reduced meat quality and increased oxidative damage. These findings were evidenced by elevated drip loss and increased EB concentration in muscle and in all segments of the small intestine. The present results highlighted the essential role of RNR in maintaining meat quality and tissue integrity of broiler chickens.

Keywords: Evans blue dye, Intestine, Meat quality, Ribonucleotide reductase



INTRODUCTION

Ribonucleotide reductase (RNR) plays an essential role in DNA synthesis, a vital process for cell proliferation and the overall growth (Liew et al., 2016). As the enzyme responsible for converting ribonucleotides into deoxyribonucleotides, RNR ensures a steady supply for DNA synthesis (Liew et al., 2016). Disruption or inhibition of RNR activity can impair cellular metabolism and replication, resulting in developmental delays and reduced physiological function (Liew et al., 2016). In poultry, reduced RNR expression has been associated with

the incidence of muscle-related disorders (Shakeri et al., 2024; 2025).

Quantifying tissue damage is challenging, but this issue can be partially addressed by using markers such as Evans blue dye (EB; Shakeri et al., 2019). Evans blue dye binds tightly to plasma albumin and is used as an exogenous marker of plasma volume (Crooke and Morris, 1942). Following tissue injury, EB extravasates into the surrounding tissue, where it may be quantified as a marker of tissue damage (Radu and Chernoff, 2013).

Previous studies indicated that broiler chickens with woody breast muscle had reduced RNR activity and impaired meat quality parameters (Shakeri et al., 2024; 2025). Nevertheless, factors other than RNR may also influence the occurrence of woody breast (Hisasaga and Makagon, 2024). To investigate RNR's specific role in tissue health, its activity was artificially reduced using a targeted inhibitor during the present study. Therefore, the present study aimed to investigate the adverse effects of RNR inhibition on growth performance and meat quality, with an additional focus on measuring tissue damage using the EB marker.

MATERIALS AND METHODS

Ethical approval

All procedures used in the present study followed the guidelines of the Institutional Animal Care and Use Committee of the University of Melbourne, Melbourne, Australia.

Study design

In total, twenty 14-day-old male broiler Ross-308 chickens with an average body weight of 445 ± 5 g were randomly assigned to 10 equally sized pens. Body weight, feed intake, and feed conversion ratio (FCR) were recorded weekly. Feed for the grower phase (days 14-28), with a CP of 27.69% and ME of 3020 kcal/kg, was provided *ad libitum*, along with water, throughout the trial. The temperature was maintained at 21°C throughout the experiment. Humidity was maintained at $60 \pm 5\%$. Light was provided 24 hours/day throughout the study. The treatment groups included a control group and an experimental group receiving the RNR inhibitor (RR, Sigma-Aldrich USA, Hydroxyurea) at 20 mg/kg body weight/day administered orally for two weeks. The inhibitor dose was determined based on *in vitro* experiments and a study in other species (Geetha, 2018; Shakeri et al., 2025). Each treatment consisted of 10 broiler chickens, with two per pen. On day 28, all broiler chickens were slaughtered using the cervical dislocation or cutting the jugular vein for EB dye analysis. Samples from the duodenum, jejunum, and ileum, and breast muscle were collected for meat quality measurements. The experiment was limited to the growing phase, as this period focuses on supporting rapid muscle growth and skeletal development. The growing phase is crucial for setting a healthy growth and efficient feed conversion during the finishing phase of broiler chickens.

Meat quality assessment

A total of 10 broiler chickens from each group were used to measure the parameters. Approximately 10 g of breast muscle was suspended on a wire surface in a sealed box at 4°C to measure drip loss. To measure drip loss, initial weight (< 15 minutes) and 24-hour weights were recorded to calculate the drip loss percentage. The pH was measured at < 15 minutes using a pH meter (HannaInst, Australia). Then, the muscles were kept at 4°C in sealed plastic bags for measuring color (Minolta, USA) and pHu at 24 hours post-mortem.

Evans blue dye injection, extraction, and qualification

Five chicks per group (total 10 chicks) were injected with 10 mg/kg body weight of EB in 0.9% saline (Sigma Aldrich, USA) into the brachial vein at the end of the experiment. After 1 hour, broiler chickens were bled, and samples were collected from the ileum, jejunum, duodenum, and muscle tissue. All tissues were dried in an oven at 70°C for 48 hours, then EB was extracted from 100 mg of dried tissues with 500 µL formamide (Sigma Aldrich, USA) and incubated at 55°C for 24 hours. Samples were then centrifuged for 15 minutes at 14,000× g and 4°C. Finally, 200 µL of supernatant was loaded in duplicate against standards at 610 nm. The results were expressed as ng EB per mg of dry tissue weight.

Statistical analysis

Data was analyzed using a t-test (GraphPad Prism, version 10.2.2). The present results were considered significant at a p-value less than 5% ($p < 0.05$) and trending towards significance at $p = 0.05-0.1$. Mean values are given as standard error of the mean (SEM).

RESULTS

The current findings on performance and meat quality are presented in Table 1. There were no significant differences between the two groups in growth ($P = 0.37$), feed intake ($P = 0.57$), and FCR ($P = 0.82$) during the two-week experiment. Inhibition of ribonucleotide reductase lowered pHu ($P = 0.005$), increased drip loss, and decreased meat lightness (both $P = 0.03$) compared to the control group. Evans blue concentration was increased in the duodenum, ileum, and muscle of the experimental group ($P < 0.0001$, $P = 0.01$, and $P = 0.01$, respectively) compared to the control group, indicating possible greater damage to these tissues when RNR activity was restricted (Figure 1). Evans blue concentration tended to be higher in the jejunum of the experimental group than in the control group ($P = 0.11$).

Table 1. Growth, meat quality, and distribution of Evans blue in tissues of broiler chickens aged 42 days for two weeks with administration of ribonucleotide reductase inhibitor

Parameters	Control	SEM	RNR ¹ inhibitor	SEM	P-value [*]
Weight gain(g)²					
Day 14-21	343.4	2.03	341.1	3.99	0.61
Day 22-28	584.9	4.02	582.0	5.10	0.66
Day 14-28	928.3	4.09	923.1	4.07	0.37
Final body weight	1374.2	3.95	1367.1	4.18	0.24
Feed intake (g)					
Day 14-21	579.9	2.66	581.7	2.34	0.61
Day 22-28	1028.0	6.64	1020.1	5.86	0.40
Day 14-28	1608.1	6.92	1602.2	7.41	0.57
Feed conversion ratio					
Day 14-21	1.68	0.01	1.70	0.02	0.46
Day 22-28	1.75	0.01	1.75	0.02	0.90
Day 14-28	1.73	0.01	1.74	0.02	0.82
Meat quality measurements					
Drip loss (%)	1.76	0.16	4.22	0.97	0.03
pH (< 15 minutes)	6.64	0.04	6.61	0.16	0.87
pHu (24 hours)	5.60	0.06	5.22	0.07	0.005
Color (24 hours) ³					
Redness	0.69	0.28	1.01	0.34	0.52
Yellowness	11.15	0.98	12.75	1.05	0.29
Lightness	55.07	1.26	59.95	1.50	0.03
Evans blue (ng/mg)					
Muscle	47.8	10.78	187.0	41.9	0.01
Duodenum	145.1	25.60	499.9	16.50	< 0.0001
Jejunum	334.2	28.53	458.2	64.46	0.11
Ileum	315.2	37.62	492.2	44.01	0.01

¹RNR: Ribonucleotide reductase, SEM: Standard error of mean, ²Initial body weight for each broiler: 445 ± 5g, ³Skin side, ^{*}Data are compared within a row.

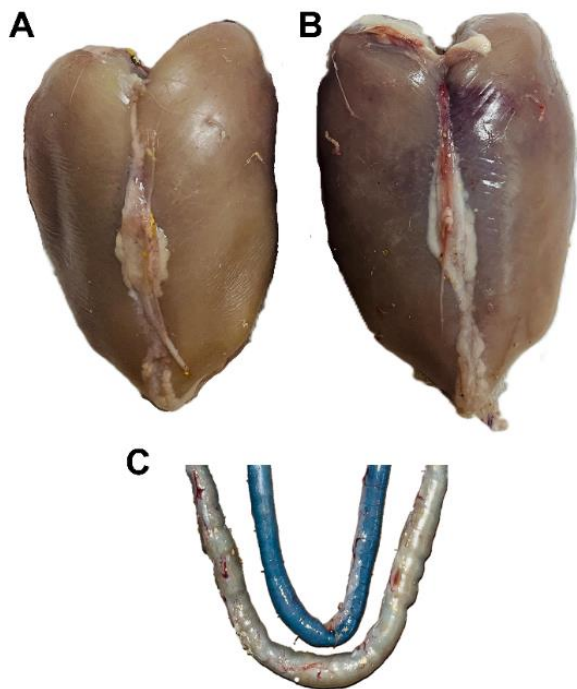


Figure 1. Breast muscle and duodenum samples from untreated broiler chickens or treated with Hydroxyurea at 20 mg/kg body weight for 14 days. Evans blue dye was administered to evaluate tissue damage in muscle from untreated (A), treated with Hydroxyurea (B), and duodenum from treated (inner loop) and untreated (outer loop) broiler chickens (C).

DISCUSSION

The current findings indicated that although growth performance remained unaffected by RNR restriction, meat quality parameters were compromised in the experimental group. Furthermore, the experimental group exhibited elevated EB concentration in muscle and intestinal tissues, indicating more severe tissue damage. This increase in EB aligns partially with previous findings of Shakeri et al. (2025a;b) in broiler chickens with woody breasts, in which reduced RNR activity was linked to poorer meat quality.

The RNR is the key enzyme that synthesizes deoxyribonucleotides, the major factor for DNA synthesis and cell proliferation (Liew et al., 2016). Additionally, RNR impacts the production of reactive oxygen species (ROS) and is itself influenced by the oxidative stress process. The RNR does not directly produce ROS, but its activity affects the cell's redox balance, and inhibiting RNR can increase ROS levels and cause oxidative stress (Andrs et al., 2023). Although the present findings demonstrated negligible changes in body weight gain, feed intake, and FCR in both groups, adding the RNR inhibitor negatively affected meat quality parameters, increasing

drip loss and meat lightness. The minimal effects on growth performance after treatment with RNR inhibitors might have resulted from compensatory metabolic changes, such as shifting metabolism to promote fat storage or altering the energy-use balance. The negative impacts on meat quality were associated with more severe oxidative tissue damage as EB concentration increased in broiler chickens treated with the RNR inhibitor. It has been shown that serious oxidative damage negatively impacts meat pHu and drip loss in broiler chickens (Chen et al., 2022).

CONCLUSION

Inhibiting RNR activity negatively affected meat quality parameters and intestinal health during the growing stage of broiler chickens, as evidenced by increased drip loss and elevated EB concentrations in tissues. The current findings might be beneficial in confirming RNR's direct role in the development of woody breast myopathy and related abnormalities. Further studies are warranted to explore different dosages of RNR inhibitors and assess their influence on growth performance, meat quality parameters, and the regulation of alternative growth-related pathways.

DECLARATIONS

Ethical considerations

The authors affirmed that this manuscript is original and has not been submitted for publication elsewhere. The authors assured that the data included in this manuscript are truthful and have not been manipulated. No AI-assisted technologies were used in the generation of this manuscript.

Availability of data and materials

All available data within the manuscript can be provided upon reasonable request from the corresponding author.

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The authors conducted the study independently, with no external contributions received.

Authors' contributions

Majid Shakeri contributed to the conceptualization of the study, performed the formal data analysis and statistical evaluations, drafted the original manuscript, and managed the overall project. Hamid Reza Rafieian-Naeini supported the development of the methodology and contributed to the review and editing of the manuscript. Hassan Khanaki conducted the execution of the

experiment and the collection of experimental data and contributed to the review and editing of the manuscript. All authors have read and approved the final version of the manuscript before publication in the present journal.

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Competing interests

The authors declared no conflict of interest.

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