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Research note: using rapid adjustments of mitochondrial function to dietary changes as an indicator? an experimental test in slower growing broiler hybrid

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ABSTRACT

This study investigated the effects of dietary energy and lysine levels on mitochondrial respiration and its association with growth performance in slower-growing broiler chickens. A 2 × 3 factorial arrangement of treatments varying in apparent metabolizable energy (AME; standard and -100 kcal/kg) and standardized ileal digestible lysine (SID Lys; 100 %, 90 %, and 80 %) was applied, resulting in 6 dietary treatments. Blood samples were collected on day 49 to assess mitochondrial respiration parameters, including routine respiration, LEAK respiration, oxidative phosphorylation (OXPHOS), combined Complex I and II (CI+CII) activity, OXPHOS coupling efficiency (OxCE), and the flux control ratio (FCR_Routine/CII). These parameters were further correlated with body weight and blood cell counts. Routine and OXPHOS respiration showed no statistically significant response to AME or Lys levels, although a numerical increase was observed with reduced Lys concentrations. LEAK respiration remained stable across all treatments, indicating preserved mitochondrial membrane integrity. Body weight, cell count and AME × Lys interaction had no significant effect on mitochondrial respiration parameters. While differences were not statistically significant, the trends suggest that nutrient availability may modulate mitochondrial activity, with potential implications for energy metabolism in slow-growing broilers.

Introduction

Mitochondrial respiration (especially cellular energy produced as ATP in oxidative phosphorylation) can play a pivotal role affecting nutrient balance, health, and optimal cellular function in broiler chickens, as it is intricately linked to energy metabolism and overall metabolic health (Ouyang et al., 2023). Understanding mitochondrial respiration is essential for evaluating how chickens convert dietary nutrients into energy, which can be influenced by various biochemical pathways and supplementation strategies that target mitochondrial efficiency (Bottje and Kong, 2017; Greene et al., 2025). Key indicators of mitochondrial function in nutrient metabolism include the activity of enzymes involved in cellular respiration and the electron transport chain (Peng et al., 2016).

Energy level and lysine (Lys) are key nutritional elements for broiler chicken health and performance. Previous studies indicate that various nutritional strategies, including the optimization of energy and amino acid levels, can enhance mitochondrial function; approaches to mitigate mitochondrial dysfunction include reducing mitochondrial membrane potential through mild uncoupling, improving electron transport chain efficiency, and strengthening the host's capacity to detoxify reactive oxygen species (Prates, 2025). Furthermore, because energy requirements change across life stages and conditions (e.g., reproduction, hypoxia, cold), mitochondrial function is also expected to vary (Stier et al., 2019). The correlation between red blood cells (RBCs) and tissue mitochondrial respiration is particularly significant under altered oxygen demand, such as during ischemia or chronic respiratory conditions (Johnston et al., 2023). Therefore, observing mitochondrial metabolism in RBCs is encouraging for testing how mitochondrial traits can rapidly respond to changes in energy constraints, supporting the hypothesis that

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Table 1
Analyzed nutritional composition of the experimental diets.

	Standard AME			Low AME		
SID Lys	**TR1**, 100 %	**TR2**, 90 %	**TR3**, 80 %	**TR4**, 100 %	**TR5**, 90 %	**TR6**, 80 %
Starter (0–10d)						
Dry matter (DM), g/kg	94.1	93.9	93.9	94.1	94.0	93.1
Crude protein, g/kg DM	251.2	251.3	220.4	260.9	248.4	237.8
Lysine, g/kg DM	18.6	14.6	12.7	18.8	15.0	15.0
Methionine, g/kg DM	7.65	6.18	5.54	7.27	6.36	4.7
Met + Cys, g/kg DM	11.6	10.0	9.6	11.7	10.3	8.8
Threonine, g/kg DM	12.1	10.0	8.8	12.1	9.9	10.4
Valine, g/kg DM	14.1	11.6	10.3	13.9	11.4	12.5
Grower I (11–21d)						
Dry matter (DM), g/kg	93.8	93.5	93.4	93.2	93.3	93.5
Crude protein, g/kg DM	243.8	231.3	222.1	247.9	234.6	219.2
Lysine, g/kg DM	14.9	13.2	11.5	15.9	12.8	11.7
Methionine, g/kg DM	5.76	4.4	4.55	5.7	5.85	4.78
Met + Cys, g/kg DM	10.0	7.73	8.33	10.0	9.49	8.69
Threonine, g/kg DM	9.76	8.91	7.69	10.1	8.51	7.97
Valine, g/kg DM	11.7	10.4	9.83	12.2	10.1	9.75
Grower II (22–30d)						
Dry matter (DM), g/kg	93.9	93.8	92.5	92.7	92.3	92.5
Crude protein, g/kg DM	246.4	219.9	210.5	241.0	235.0	194.2
Lysine, g/kg DM	14.4	12.3	11.1	13.8	12.1	11.4
Methionine, g/kg DM	4.92	5.18	4.12	6.27	5.35	3.63
Met + Cys, g/kg DM	8.80	9.10	8.07	10.4	9.27	7.48
Threonine, g/kg DM	9.86	8.42	7.37	8.18	7.50	7.10
Valine, g/kg DM	11.7	9.96	9.29	10.7	9.94	9.95
Finisher I (31–40d)						
Dry matter (DM), g/kg	92.4	92.1	92.2	94.4	92.2	92.5
Crude protein, g/kg DM	231.0	211.3	199.3	219.4	209.7	205.2
Lysine, g/kg DM	13.4	12.0	10.6	13.7	11.6	10.2
Methionine, g/kg DM	5.07	4.45	3.52	5.52	5.28	4.49
Met + Cys, g/kg DM	9.22	8.12	7.11	9.42	8.73	8.30
Threonine, g/kg DM	8.91	7.99	7.28	9.20	8.07	7.19
Valine, g/kg DM	10.6	10.3	9.78	11.0	9.34	9.01
Finisher II (41–49d)						
Dry matter (DM), g/kg	92.6	92.2	92.4	92.2	92.3	92.2
Crude protein, g/kg DM	213.9	207.7	189.8	219.1	207.2	193.0
Lysine, g/kg DM	12.0	11.0	10.2	12.0	11.0	9.8
Methionine, g/kg DM	5.27	3.6	3.38	5.82	4.91	3.37
Met + Cys, g/kg DM	8.95	7.24	6.96	9.44	8.55	6.93
Threonine, g/kg DM	8.17	7.43	7.23	8.51	7.47	6.63
Valine, g/kg DM	9.68	9.38	9.30	9.34	8.74	8.46

these traits are plastic.

Consumer demand for higher-welfare poultry products is rising, particularly in Europe, where production systems have increasingly shifted toward slower-growing broiler strains. These genotypes are associated with improved welfare outcomes, including better leg health, lower metabolic disorders, and greater suitability for alternative and free-range systems. Rustic Gold, part of Aviagen's Rowan Range, is a slow-growing genotype derived from exclusively slower-growing parental lines (Rustic female × Gold male) selected for robustness, welfare traits, and acceptable production efficiency. Despite its increasing use in welfare-friendly systems, there are no established feeding guidelines specific to Rustic Gold. Consequently, Ross 308 nutrient specifications were used as a reference baseline, which is a common practice when genotype-specific recommendations are unavailable. Most existing research on mitochondrial function and nutrient metabolism has focused on fast-growing commercial strains. However, studies have shown that different breeds exhibit variations in mitochondrial function and some commercial broiler breeds have enhanced expression of genes related to energy metabolism, correlating with a higher abundance of mitochondrial proteins that regulate processes such as lipolysis and fatty acid oxidation (Zheng et al., 2016). Therefore, slow-growing genotypes may differ in their physiological and metabolic responses to dietary interventions, but these characteristics remain poorly characterized.

Therefore, the objective of this study was to assess how mitochondrial respiration in blood is influenced by AME and Lys levels, along with correlating mitochondrial activity to body weight (BW) and blood cell count in slow-growing broilers. We hypothesized that the AME/Lys ratio in broiler diets may modulate mitochondrial proton leak, mitochondrial oxygen consumption, and the maintenance of energy homeostasis in RBCs of slow-growing broilers.

Material and methods

Birds and housing

A total of 864 one-d-old Rustic Gold slow growth broiler chicks, with equal numbers of males and females, were purchased from a local commercial hatchery (DanHatch Finland Ltd, Kokemäki, Finland) and housed in environmentally controlled facilities of Natural Resources Institute Finland (Luke) in Jokioinen, Finland according to Ross 308 broiler management instructions. Upon arrival, chicks were weighed and randomly distributed into 36 peat litter floor pens (1 width * 2 m length) with 24 chicks per pen. During the experiment, the animals were managed according to Finnish legislation. The flock was tested negative for salmonella on day 40. This study design was reviewed and approved by Animal Welfare body (Government decree 564/2013 22§) of Natural Resources Institute Finland.

Experimental treatments

The study employed a 2 × 3 factorial arrangement of treatments with

a random blocked design, with 6 dietary treatments replicated 6 times. The experimental diets varied in energy and Lys levels: diets were formulated with either standard or reduced AME (−100 kcal), and standardized ileal digestible lysine (SID Lys) at 100 %, 90 %, or 80 % of the Aviagen Ross 308 recommended level. The treatments were as follows: TR1: Standard AME, 100 % SID Lys; TR2: Standard energy, 90 % SID Lys; TR3: Standard energy, 80 % SID Lys; TR4: Low energy (−100 kcal), 100 % SID Lys; TR5: Low energy (−100 kcal), 90 % SID Lys; TR6: Low energy (−100 kcal), 80 % SID Lys. Wheat-soybean meal-based diets were formulated according to Ross 308 nutrition specifications for all plant-protein based feeds by Aviagen Ltd, 2022. The experimental diets were formulated using the dilution technique: a diet containing 100 % SID Lys diet was diluted with a 70 % SID Lys diet to achieve the desired lysine levels. The analyzed nutrient content of the diets is presented in Table 1.

Upon completion of the experimental period on day 49, after weighing and euthanizing the birds, the blood samples were collected, using EDTA-treated BD Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, USA), from the heart of 8 birds randomly selected from different pens within each treatment. The tubes were placed on ice, and the whole-blood samples were sent to laboratory at University of Jyväskylä for mitochondrial analysis. To reduce variation and obtain more consistent data, only males were sampled.

Mitochondria measurements

Mitochondrial respiration was analyzed using high-resolution respirometry (2 Oroboros Instruments, Innsbruck, Austria) at 41°C adapted from a protocol described in Stier et al. (2019) with: digitonin (20 μg ml−1), pyruvate (5 mmol $l-1$), malate (2 mmol $l-1$), ADP (1.25 mmol $l-1$), succinate (10 mmol $l-1$), oligomycin (2.5 μmol $l-1$), antimycin A (2.5 μmol $l-1$). We used 40 μl of fresh whole blood, suspended in Mir05 buffer. Four distinct respiration rates were analyzed: (1) the endogenous cellular respiration rate before permeabilization (ROUTINE), (2) the maximum respiration rate fueled with exogenous substrates of complexes I and II, as well as ADP (CI+CII), (3) the respiration rate contributing to the proton leak (LEAK), (4) the respiration rate supporting ATP synthesis through oxidative phosphorylation (OXPHOS). We also calculated two mitochondrial flux ratios (flux control ratio, FCR): (1) OXPHOS coupling efficiency (OxCE= (CI+CII−LEAK)/CI+II), (2) the proportion of maximal respiration capacity being used under the endogenous cellular condition (i.e. FCRROUTINE/CI+II). OxCE FCR provides an index of mitochondrial efficiency in producing ATP, whereas FCR_ROUTINE/CI+II reflects the cellular control of mitochondrial respiration by endogenous ADP/ATP turnover and substrate availability. Respiration rates were standardized by the blood volume. The number of cells in each sample, measured by a Bio-Rad TC20 automated cell counter, was used as a covariate in the statistical model. We noticed that in 11 samples, there was little or no response to oligomycin, and often this was with two samples that were processed at the same time, which suggests that it is likely a technical error in delivering the oligomycin to the cells. Therefore, the leak respiration values of these samples (distributed across treatments) were omitted from the analyses.

Statistical Analysis

Statistical analyses were conducted using RStudio (version 4.4.1), using linear mixed models (package lme4, lmer) where main factors were AME levels (standard or reduced AME), lysine level (100, 90 or 80 %) and their interaction, and BW and blood cell count as covariates. If there was no statistically significant interaction, the interaction was excluded to be able to interpret the main treatment effects. Degrees of freedom are shown in the tables as subscripted F-values. Pen was included as a random intercept to account for pseudoreplication of individuals from the same pens. Normality and homoscedasticity were visually inspected and deemed satisfactory.

Results and discussion

The study investigated the effect of different dietary treatments, BW and blood cell count on mitochondrial respiration parameters in slow-

Table 2
The effect of dietary treatments on different mitochondrial respiration parameters.[1,2].

Treatments	Routine	CI	CI+CII	Leak	OXPHOS	OxCE	FCR: Routine/CI+CII
AME							
Low AME	0.298	0.371	0.470	0.077	0.393	0.835	0.672
Standard AME	0.289	0.342	0.438	0.088	0.350	0.741	0.883
Pooled SEM	0.026	0.047	0.064	0.017	0.058	0.076	0.288
Lysine							
80 % SID	0.287	0.320	0.391	0.0.72	0.318	0.744	1.141
90 % SID	0.325	0371	0.510	0.085	0.424	0.841	0.685
100 % SID	0.257	0.361	0.426	0.087	0.339	0.771	0.633
Pooled SEM	0.033	0.065	0.088	0.024	0.078	0.100	0.371
AME × Lysine							
Low AME × 80 % SID	0.330	0.330	0.347	0.057	0.290	0.828	0.761
Low AME × 90 % SID	0.328	0.393	0.554	0.094	0.460	0.831	0.626
Low AME × 100 % SID	0.253	0.383	0.448	0.070	0.377	0.842	0.560
Standard AME × 80 % SID	0.275	0.337	0.435	0.088	0.347	0.660	1.074
Standard AME × 90 % SID	0.323	0.349	0.466	0.076	0.389	0.851	0.740
Standard AME × 100 % SID	0.421	0.340	0.404	0.103	0.300	0.700	0.642
Pooled SEM	0.051	0.102	0.135	0.038	0.123	0.158	0.327
AME	$F = 0.27_{(1,32.1)}$	$F = 0.66_{(1,31.6)}$	$F = 0.66_{(1,34)}$	$F = 0.07_{(1,22)}$	$F = 0.03_{(1,22)}$	$F = 0.18_{(1,22)}$	$F = 0.67_{(1,34)}$
	$P = 0.60$	$P = 0.42$	$P = 0.59$	$P = 0.78$	$P = 0.86$	$P = 0.67$	$P = 0.41$
Lysine	$F = 2.33_{(2,32.3)}$	$F = 0.28_{(2,32.0)}$	$F = 0.22_{(2,34)}$	$F = 0.46_{(2,\ 22)}$	$F = 0.59_{(2,\ 22)}$	$F = 0.54_{(2,\ 22)}$	$F = 0.09_{(2,\ 34)}$
	$P = 0.11$	$P = 0.75$	$P = 0.79$	$P = 0.63$	$P = 0.56$	$P = 0.58$	$P = 0.91$
AME × Lysine	$F = 0.28_{(2,30.6)}$	$F = 0.23_{(2,33.9)}$	$F = 0.44_{(2,32)}$	$F = 1.25_{(2,20)}$	$F = 0.37_{(2,20)}$	$F = 0.84_{(2,20)}$	$F = 0.10_{(2,32)}$
	$P = 0.75$	$P = 0.79$	$P = 0.64$	$P = 0.31$	$P = 0.69$	$P = 0.44$	$P = 0.90$
BW	$F = 0.12_{(1,33.3)}$	$F = 0.14_{(1,33.0)}$	$F = 0.11_{(1,34)}$	$F = 0.89_{(1,22)}$	$F = 0.45_{(1,22)}$	$F = 0.17_{(1,22)}$	$F = 0.13_{(1,34)}$
	$P = 0.73$	$P = 0.71$	$P = 0.73$	$P = 0.35$	$P = 0.50$	$P = 0.67$	$P = 0.72$
Cell count	$F = 0.03_{(1,33.9)}$	$F = 0.001_{(1,33.9)}$	$F = 0.22_{(1,34)}$	$F = 0.22_{(1,22)}$	$F = 1.85_{(1,22)}$	$F = 0.37_{(1,22)}$	$F = 0.006_{(1,34)}$
	$P = 0.85$	$P = 0.99$	$P = 0.64$	$P = 0.63$	$P = 0.22$	$P = 0.54$	$P = 0.94$

[1] Data are given as treatment means.
[2] F-values indicated the between-group-to-within-group variance ratio. Degrees of freedom (df) are shown in the table as subscripts of F-values.

growing broiler chickens. The parameters analyzed included Routine respiration, Complex I (CI) respiration, combined Complex I and II (CI+CII) respiration, Leak respiration, OXPHOS, OXPHOS coupling efficiency (OxCE), and Flux Control Ratio (FCR: Routine/CI+CII). Table 2 summarizes the results of the mitochondrial respiration parameters across the six experimental treatment groups.

Routine: In this study, the analysis of routine mitochondrial respiration (R), the endogenous (unstimulated) respiration of living cells necessary for meeting their aerobic energy demands, showed notable trends despite the lack of statistically significant differences across treatments. R levels did not significantly vary with different AME levels or Lys concentrations ($F = 0.27$, $P = 0.60$; $F = 2.33$, $P = 0.11$, respectively). However, a numerical tendency towards increased R was observed with reduced Lys levels, indicating possible physiological adaptations in the cells to meet bioenergetic demands under these constrained conditions. This responsiveness, while not statistically significant, may signal that there exists an intrinsic compensatory mechanism in mitochondrial function aimed at maintaining adequate ATP production despite dietary limitations. The potential influence of Lys, an essential amino acid, on mitochondrial respiration underscores the critical relationship between nutrient availability and metabolic activity (Bottje and Kong, 2017; Ouyang, et al., 2023). As previously discussed by Bottje and Kong (2017), nutrient fluctuations can lead to remarkable adjustments in mitochondrial bioenergetics, suggesting that the observed numerical increases in R could reflect an adaptive response to maintaining energy homeostasis under suboptimal Lys conditions. Notably, the method of measuring routine respiration in live cells, as opposed to permeabilized cells or isolated mitochondria, reinforces the validity of these trends by capturing the comprehensive metabolic responses of mitochondria to physiological and nutritional factors.

Leak: Leak respiration levels showed no significant differences across varying treatments concerning AME and Lys concentrations, ($F = 0.07$, $P = 0.78$; $F = 0.46$, $P = 0.63$, respectively). This stability suggests that dietary manipulations did not substantially affect mitochondrial proton leak, implying preserved membrane function. However, the specific contributions of basal and inducible leak in broiler chicken mitochondria need further investigation. Such findings imply that the dietary interventions used in this study did not induce mitochondrial dysfunction. Leak respiration, which corresponds to the mitochondrial oxygen consumption associated with proton leaks, releases energy as heat without contributing to ATP synthesis. This metric reflects the efficiency of the mitochondrial system, as higher leak respiration rates may indicate compromised mitochondrial function and impaired energy efficiency (Nord et al., 2021). While leak respiration can be indicative of mitochondrial dysfunction when elevated, understanding the context and biological significance of these values, especially in relation to dietary nutrients, is critical.

OXPHOS: The effect of AME on OXPHOS respiration was not significant ($F = 0.03$, $P = 0.86$). Similarly, Lys levels did not significantly affect OXPHOS respiration ($F = 0.59$, $P = 0.56$). However, following a pattern similar to routine respiration, decreasing Lys levels tended to numerically increase OXPHOS respiration at 90 % (not at 80 %) level compared to the 100 % Lys level when AME was provided at the standard level. This observation suggests that lower Lys availability might drive compensatory mechanisms within the mitochondria to enhance energy production, emphasizing the complex relationship between dietary Lys and mitochondrial function. OXPHOS is a vital metabolic process in which reduced fuel substrates are oxidized to transfer electrons (e^-) and protons (H^+) to oxygen. This electron transport is coupled with ATP formation from ADP, driven by the proton gradient across the mitochondrial membrane. In addition to ATP-coupled respiration, a portion of oxygen consumption is uncoupled from ATP production, primarily due to proton leak. The efficiency of OXPHOS is therefore often evaluated by accounting for residual oxygen consumption not linked to ATP synthesis.

Combined Complex I and II (CI+CII): Complex I transfer electrons to ubiquinone from NADH, while Complex II, or succinate: quinone oxidoreductase, is part of the electron transfer pathway and the TCA cycle. The analysis showed no significant differences in CI+CII levels for different AME and Lys levels ($F = 0.66$, $P = 0.59$; $F = 0.22$, $P = 0.79$, respectively).

OXPHOS Coupling Efficiency (OxCE): OxCE measures the ratio of OXPHOS of the maximum oxidative capacity, If the efficiency is at a maximum of 1, the leak respiration is zero. The analysis showed no significant differences in OxCE levels among the AME and Lys treatments ($F = 0.18$, $P = 0.67$; $F = 0.54$, $P = 0.58$, respectively). Similar to routine respiration, decreasing Lys level to 90 %, numerically increased OxCE compared to 100 % Lys level when AME was provided at the standard level.

Flux Control Ratio (FCR_Routine/CII): FCR can be interpreted as how much of their maximum oxidative capacity the individuals are using, here higher values referring to less flexibility to increase respiration (i.e. individuals already working close to their maxima). The analysis showed no significant differences in FCR_Routine/CII levels among the AME and Lys treatments ($F = 0.67$, $P = 0.41$; $F = 0.09$, $P = 0.91$, respectively).

Neither BW nor cell count significantly influenced mitochondrial respiration parameters (Table 2). For both parameters there is mixed evidence in previous studies; Thoral et al. (2024) demonstrated that analytical conditions, such as sample volume, can strongly influence the observed relationships between cell count and mitochondrial function, suggesting that methodological factors may mask real associations. Similarly, Stier et al. (2019) emphasized that variability in cell count can affect respiration metrics and complicate interpretation. Additionally, the interaction between AME and Lys levels had no significant effect on mitochondrial respiration parameters. Insights gained from this study aim to improve nutritional management and overall productivity in poultry studies by deepening our understanding of the relationship between mitochondrial function and nutrient status. In summary, mitochondrial respiration serves as a crucial indicator for the assessment of nutrient balance in broiler chickens, modulated by genetic factors, dietary supplementation, and environmental stressors. Recognizing the intricate balance of mitochondrial function and its direct correlation with energy metabolism emphasizes the need for targeted strategies to enhance nutritional efficiency and animal health in poultry production.

In this study, variations in dietary AME and lysine concentrations did not result in statistically significant alterations in any measured parameters of mitochondrial respiration. However, the numerical differences observed among treatments indicate that the assays were capable of detecting biological variation. Moreover, the low number of replicates reduced the statistical power of the analysis and likely caused the lack of statistical significance. Therefore, additional studies with greater replication are needed to determine whether the numerical differences seen here reflect true physiological effects. Such work would help clarify whether nutrient availability can influence mitochondrial respiration in blood cells and what this might mean for energy metabolism and nutritional management in broiler chickens.

CRediT authorship contribution statement

Ali Kiani: Writing – original draft, Visualization, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Heidi Högel:** Writing – original draft, Resources, Project administration, Methodology, Conceptualization. **Suvi Ruuskanen:** Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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