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Dietary Methionine and Broiler Chicken Growth: Evaluating Performance and the GH/IGF-I Axis

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Abstract
Methionine is an essential amino acid crucial for various physiological functions in broiler chickens, including growth, metabolism, and immune response. Understanding the optimal levels of methionine supplementation is vital for maximizing poultry production efficiency and bird health. This study aimed to evaluate the effects of dietary methionine supplementation on growth performance and gene expression in broiler chickens. The study was conducted at the College of Veterinary Medicine, Al-Qadisiyah University, from 26 September 2024 to 6 November 2024. The study utilized broiler chickens divided into experimental groups based on varying levels of dietary methionine supplementation. Broiler chickens were assigned to different dietary treatment groups with varying levels of methionine supplementation. Growth performance and gene expression were assessed at regular intervals throughout the study period. Statistical analyses were performed to determine significant differences between treatment groups. The results show in the dietary methionine supplementation improved body weight gain and feed conversion ratio. Optimal feed conversion ratios were observed in the T3 group, with mean FCR over the 6-week study ranging from 2.01 ± 0.42 (C) to 1.55 ± 0.17 (T3). It also significantly increased GH and IGF-1 concentrations (LSD = 1.81 and 2.059, respectively). Dietary methionine supplementation at varying levels influences growth performance and gene expression in broiler chickens, demonstrating its significant role in broiler physiology.
Keywords: *Broiler Chickens, Methionine Supplementation, Growth, Performance, Feed Conversion Ratio.*

INTRODUCTION

Over the past few decades, the poultry sector has seen tremendous progress, with notable enhancements in production standards and growth performance. The success of genetic selection, which is responsible for 85–90% of these developments (Ravindran *et al.*, 2017).Within the realm of poultry nutrition, methionine emerges as an essential amino acid that plays a critical role in various metabolic processes. It serves as a precursor for the synthesis of other amino acids, proteins, and a range of biomolecules (Liu & Kim, 2023). Previous research has indicated that dietary supplementation with methionine can influence the expression of growth-related genes, such as insulin-like growth factor (IGF) and growth hormone (GH), which are key regulators of growth and development in broiler chickens (Wang *et al.*, 2021).The pace of daily weight increase and the makeup of that weight gain (fat and protein) are two important aspects of poultry production. A complex interaction of biological processes, including growth hormone (GH) and the animal's nutrition, affects these traits. Through the GH receptor (GHR) pathway, GH promotes the production and release of IGF-I (insulin-like growth factor 1), acting as a crucial regulator of development and body composition (Abdelli et al., 2021). Previous studies have confirmed that methionine supplementation affects gene expression, including IGF-I and GHR, through cellular and molecular mechanisms. Research has also shown that different methionine sources exhibit different absorption pathways and biological efficacy, which influence broiler productivity.

These findings emphasize the importance of investigating the effects of methionine on growth performance and gene expression to improve poultry production (Del Vesco et al., 2013).This study aims to investigate the effects of dietary methionine supplementation on growth performance, with a specific focus on the underlying mechanisms involving IGF-I and GHR gene expression. A comprehensive understanding of how methionine influences these key growth-related factors can lead to the development of more effective nutritional strategies for broiler production. This research seeks to determine the optimal levels of methionine supplementation to maximize growth and efficiency in broiler chickens.

METHODS

This study was conducted at the College of Veterinary Medicine, Al-Qadisiyah University, from 26 September 2024 to 6 November 2024. Three hundred broiler chicks were used in this study. The experiment lasted six weeks (42 days). The experimental groups were as follows: Group C (control group): basal diet (n = 75) Group T1: basal diet + methionine 1 g/kg (n = 75) Group T2: basal diet + methionine 2 g/kg (n = 75) Group T3: basal diet + methionine 3 g/kg (n = 75) This group division allowed us to investigate the effects of different levels of methionine supplementation on broiler chickens.

2.1. Data Collection:

Body weight gain and feed consumption were recorded weekly. Feed conversion ratio (FCR) was calculated. Feed consumption was measured by recording the amount of feed offered to each group and subtracting the amount of feed remaining at the end of each week. Feed conversion ratio (FCR) was calculated using the following formula: FCR = Total feed consumed (g) / Total body weight gain (g) This calculation provides a measure of the efficiency with which the birds converted feed into body mass.

2.2. Gene expression analysis:

Blood samples were collected from the jugular vein, and gelatin and EDTA tubes were used for blood samples. Liver samples were taken to analyze insulin-like growth factor 1 (IGF1) gene expression, and breast tissue samples were taken to analyze growth hormone (GH) gene expression. Deoxyribonucleic acid (RNA) was extracted, complementary DNA (cDNA) was synthesized, and quantitative real-time polymerase chain reaction (qRT-PCR) was performed. 12 samples (total24) were used for each gene (IGF1, GH) expression analysis, Divided into three samples from each group for each gene (3/75), (total 6/75). This method allows for the determination of mRNA levels, allowing for a deeper understanding of the effects of dietary manipulations on gene transcription (Bustin *et al.,* 2009).

2.3. Statistical Analysis:

All data were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS). Analysis of variance (ANOVA) was used to determine the effects of dietary treatments on the measured parameters. When significant differences were detected, differences among means were compared using the Least Significant Difference (LSD) test. Differences were considered significant at a probability level of $P < 0.05$.

RESULTS

3.1. Body Weight

This section presents the results of the current study, which aimed to investigate the effects of dietary methionine supplementation on the growth performance, expression of insulin-like growth factor (IGF) and growth hormone (GH) genes in broiler chickens. The experimental groups consisted of a control group (C) that did not receive any additional methionine, and treatment groups that received additional methionine at doses of 1 g/kg (T1), 2 g/kg (T2), and 3 g/kg (T3), respectively, over a period of 42 days.

Table 1 displays the mean weekly body weight (g) of broiler chickens under different dietary treatments. Initial weights on Day 1 were similar across groups, ranging from 41 ± 0.57 g (C) to 43 ± 1.52 g (T2). By

Week 6, mean body weights were recorded as 2308 ± 22.3 g for the control (C), 2520 ± 11.5 g for T1, 2661.6 ± 30.8 g for T2, and 2816.6 ± 72.6 g for T3. Statistical analysis indicated significant differences between group means at Week 3 (LSD = 23.67) and Week 4 (LSD = 17.89).

Table 1: Effect of Dietary Methionine Supplementation on Weekly Body Weight (g) of Broiler Chickens

Group	Day 1	W1	W2	W3	W4	W5	W6	M± SE
C	41 ± 0.57	112.6 ± 1.45	262.3 ± 1.85	494.6 ± 2.60	910.6 ± 2.33	1366.6 ± 8.81	2308 ± 22.3	785.14 ± 169.8
T1	42 ± 1.15	113.6 ± 1.66	268.6 ± 0.33	553.3 ± 14.5	1056.3 ± 1.85	1583.3 ± 10.4	2520 ± 11.5	870.33 ± 187.5
T2	43 ± 1.52	117.6 ± 2.02	275 ± 2.88	581.3 ± 1.85	1110.6 ± 5.81	1648.3 ± 20.8	2661.6 ± 30.8	919.66 ± 199.1
T3	43 ± 1.15	120.3 ± 2.60	288.3 ± 1.66	592.3 ± 1.45	1152.3 ± 3.92	1690.3 ± 2.60	2816.6 ± 72.6	957.61 ± 209.7
M± SE	42.25 ± 0.55	116.08 ± 1.25	273.5 ± 3.01	555.4 ± 11.84	1057.5 ± 27.59	1560.9 ± 38.11	2576.5 ± 59.14	
LSD	23.67							17.89
Intermedian	47.34							

3.2. Feed Conversion Ratio (FCR)

Evaluation of feed conversion efficiency across the experimental period revealed variations in the Feed Conversion Ratio (FCR) among the groups. Weekly FCR values generally trended higher in the control group (C). In contrast, groups receiving supplemental methionine consistently demonstrated more favourable FCRs, with the lowest ratios observed in the T3 group throughout most of the trial. For instance, Week 4 FCR values spanned from 1.98 ± 0.04 (C) to 1.36 ± 0.01 (T3). The mean FCR over the 6-week study showed a clear gradient, from 2.01 ± 0.42 (C) to 1.55 ± 0.17 (T3). Significant differences between group means were statistically supported at Week 1 (LSD = 0.048) and Week 2 (LSD = 0.058). Detailed FCR data is presented in Table 2.

Table 2: Effect of Dietary Methionine Supplementation on Weekly Feed Conversion Ratio (FCR) of Broiler Chickens

Group	W1	W2	W3	W4	W5	W6	MEANS ± SD
C	1.38 ± 0.02	1.77 ± 0.05	2.16 ± 0.02	1.98 ± 0.04	2.75 ± 0.13	2.01 ± 0.07	2.01 ± 0.42
T1	1.36 ± 0.03	1.68 ± 0.02	1.77 ± 0.15	1.59 ± 0.07	2.16 ± 0.06	1.74 ± 0.03	1.72 ± 0.25
T2	1.37 ± 0.10	1.64 ± 0.04	1.61 ± 0.01	1.47 ± 0.02	1.94 ± 0.02	1.64 ± 0.07	1.61 ± 0.18
T3	1.43 ± 0.06	1.55 ± 0.05	1.60 ± 0.05	1.36 ± 0.01	1.87 ± 0.01	1.49 ± 0.18	1.55 ± 0.17
MEANS ± SD	1.39 ± 0.02	1.66 ± 0.08	1.78 ± 0.26	1.60 ± 0.26	2.18 ± 0.39	1.72 ± 0.22	
LSD	0.048	0.058					
Intermedian		0.117					

3.3. Gene Expression

Relative gene expression levels for Growth Hormone Receptor (GHR) and Insulin-like Growth Factor I (IGF-I) were determined for the dietary treatment groups. Mean GHR expression varied, showing values such as 1 ± 0 for the control, peaking at 5.12±0.59 units for T3, and as 4.07 ±1.02 units for T2. Statistical analysis (LSD = 1.81) placed T2 in statistical group A, T3 in A, and the control and T1 in B. For IGF-I expression, the control group mean was 1 ± 0 units, while supplemented groups exhibited higher values (T1: 1.21 ±0.8, T2: 2.07±0.23, T3: 4.33±1.09 units). Statistical comparison (LSD = 2.059) placed T2 in statistical group B, T3 in A, and the control and T1 in B. These expression data are summarized in table 3.

Table 3: Effect of Dietary Methionine Supplementation on Relative Gene Expression of GH/GHR and IGF-I.

Group	GHR	IGF
C (Control)	1±0 B	1±0 B

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T1 (1 g/kg)	2.11±0.29 B	1.21 ±0.82 B
T2 (2 g/kg)	4.07 ±1.02 A	2.07±0.23 B
T3 (3 g/kg)	5.12±0.59 A	4.33±1.09A
LSD	1.81	2.059

Relative gene expression levels of Insulin-like Growth Factor I (IGF-I) in broiler chickens, shown as fold change relative to the control group, varied across the dietary treatments. The control group (C) displays a relative expression level of 1-fold. Supplementation with dietary methionine resulted in different expression levels: the group receiving 1 g/kg (labelled T1) in the figure1 shows 1.21-fold expression, the 2 g/kg group (labelled T2) exhibits 2.07-fold expression and the 3 g/kg group (labelled T3) demonstrates 4.33-fold relative expression. Statistical analysis confirming the significance of these differences among groups is provided in Table 3. The visual representation of these IGF-I expression levels is presented in Figure 1.

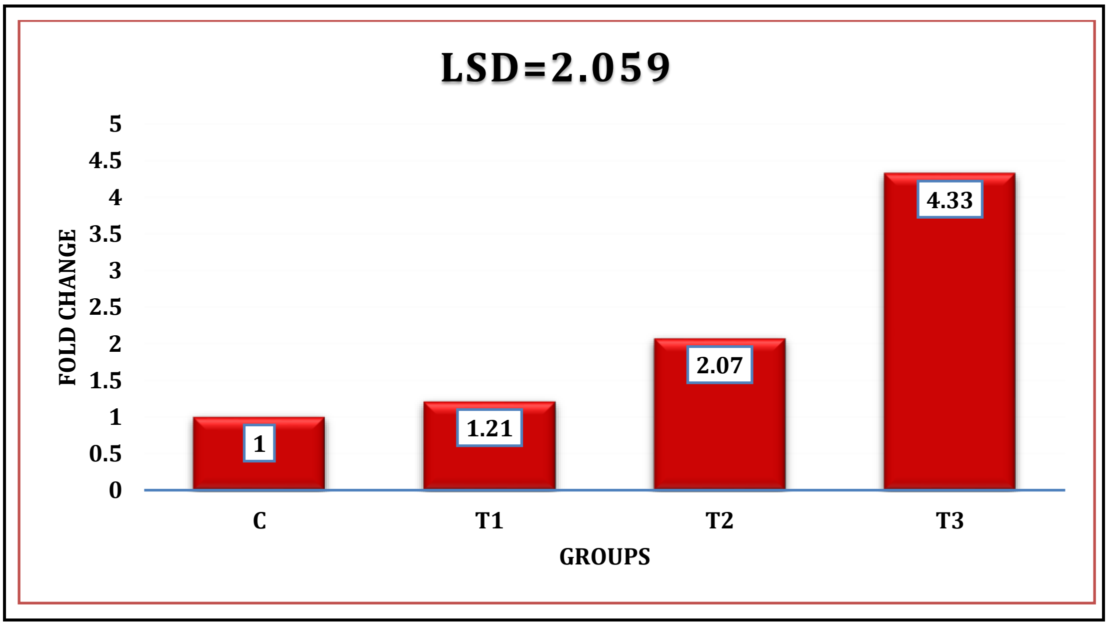

Figure 1 Effect of Dietary Methionine Supplementation on Relative Gene Expression of IGF-I.

The relative gene expression levels of the Growth Hormone Receptor (GHR) in broiler chickens are depicted as fold change relative to the control group across the dietary treatments. The control group (C) shows a relative expression level fixed at 1-fold. Supplementation with 1 g/kg methionine (labelled T1 in the figure2) corresponded to 2.11-fold expression. A notable observation is the high expression level in the group receiving 3 g/kg supplementation (labelled T3), reaching 5.12-fold. The group with 2 g/kg supplementation (labelled T2) corresponded expression of 4.07-fold relative to the control. Statistical analysis detailing the differences between these groups is presented in Table 3. Figure 2 visualizes these GHR expression levels.

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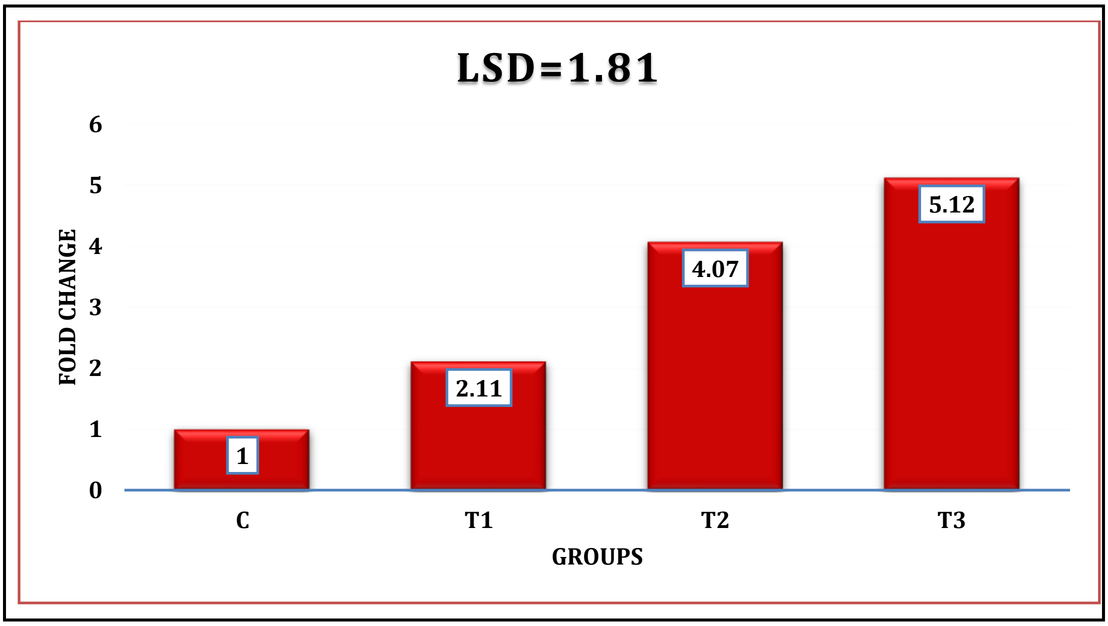
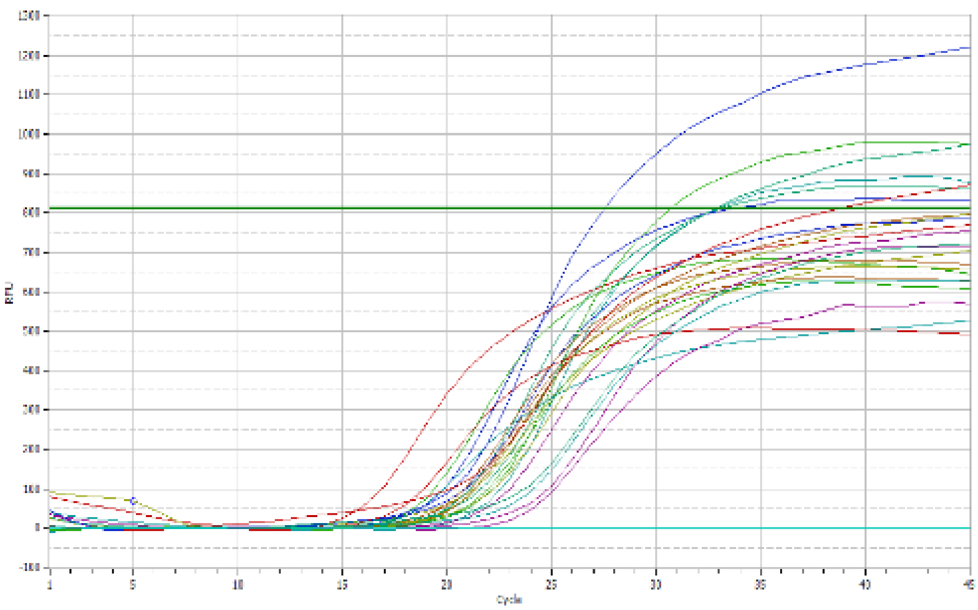
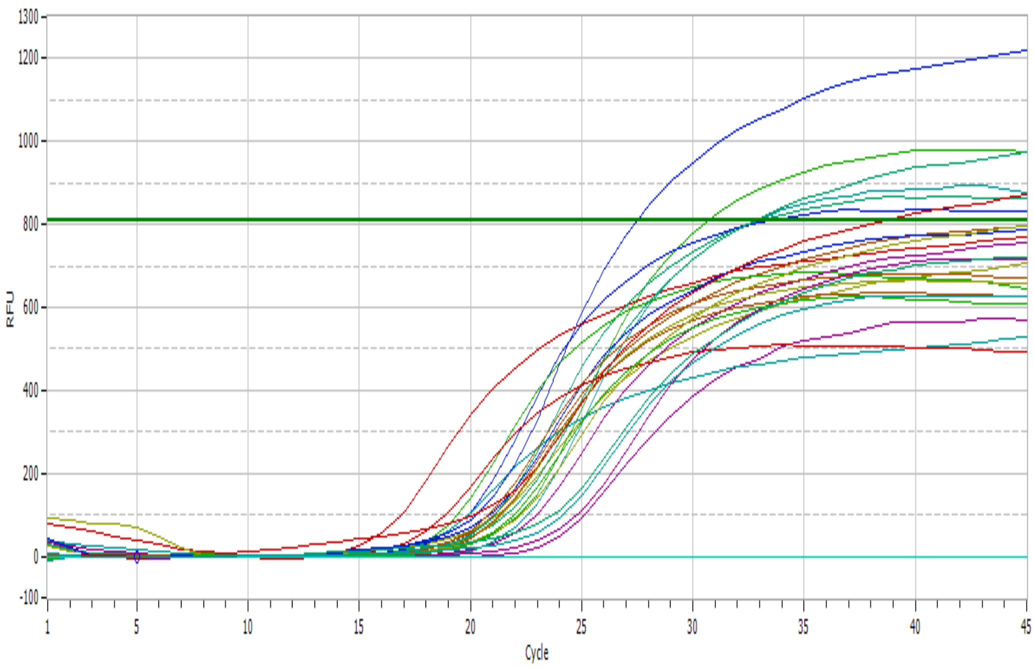


Figure 2 Effect of Dietary Methionine Supplementation on Relative Gene Expression of GHR.

Real-Time PCR amplification curves illustrating the cycle threshold (Ct) determination for A. IGF-I and B. GHR gene expression are displayed. Panel A presents the fluorescence signal amplification across PCR cycles for individual samples analyzed for IGF-I. Similarly, Panel B depicts the amplification curves for GHR gene expression. Within each panel, multiple colored lines represent different samples from the experimental groups. A horizontal threshold line is indicated; the point where an amplification curve intersects this line defines the Ct value for that specific sample. These curves represent the raw data from which relative gene expression levels were calculated for summarized results, as presented in Table 3 and Figures 1 & 2. Figure 3 shows the Real Time PCR curve of CT for A.IGF-I and B. GHR.



A



B

Figure 3 Real Time PCR curve of CT for A.IGF-I and B. GHR

DISCUSSION

The results in Table 1 show the mean weekly body weight (g) of broiler chickens across different dietary methionine supplementation groups (C, T1, T2, and T3) over a 42-day period. The results indicate that dietary methionine supplementation has a positive effect on the growth performance of broiler chickens, particularly in the later stages of growth. Perhaps the reason is that methionine plays a crucial role in protein synthesis, and higher availability of methionine enhances muscle tissue development, leading to increased body weight.A previous study, whose goal was to evaluate the effects of methionine supplementation on growth performance and carcass characteristics in beef steers, was conducted in 2021 in the USA by (Baggerman *et al.,* 2021). This study reached a conclusion that methionine supplementation increased longissimus muscle area in beef steers. This indicates that methionine's positive influence on growth is not limited to poultry and can be observed in other livestock as well. The aforementioned study agrees with the current study, where increased methionine led to increased body weight, which is directly related to muscle growth (Baggerman *et al.,* 2021).While (Baggerman *et al.,* 2021) observed increased muscle growth with methionine supplementation, another study, which aimed to determine the effect of dietary methionine concentrations on muscle protein turnover in rainbow trout, found results that differed from the current study, as it found that methionine deficiency led to decreased body weight and feed efficiency (Belghit et al., 2014). Perhaps this indicates that the effect of methionine on growth performance is more pronounced when methionine is deficient, and that the optimal level of methionine varies across species (poultry vs. fish). It also highlights the importance of methionine in protein metabolism.Table 2 presents the effects of dietary methionine supplementation on the weekly feed conversion ratio (FCR) of broiler chickens. The data reveal that the control group (C) generally exhibited higher (less favorable) FCR values across the experimental period, indicating less efficient feed utilization. Conversely, broiler chickens receiving supplemental methionine consistently demonstrated improved FCRs, with the most efficient feed conversion observed in the T3 group throughout much of the study. These results indicate a strong positive influence of dietary methionine supplementation on feed conversion efficiency in broiler chickens. The improved FCR in the supplemented groups, particularly T3, suggests that methionine enhances the birds' ability to convert feed into body mass. Perhaps the reason is that methionine plays a crucial role in protein synthesis and other metabolic processes that optimize nutrient utilization, leading to better feed efficiency. (Wilfred et al., 2024) conducted a study in South Africa to determine the effect of dietary methionine to crude protein (CP) ratio on broiler chicken performance. Their study reported that dietary methionine to CP ratio had no effect on feed efficiency. This result contrasts with the findings of the current study, which clearly demonstrate that methionine supplementation improves FCR. However, the (Wilfred et al., 2024) study focused on the ratio of methionine to crude protein, while the current study examined the effect of absolute methionine supplementation. This difference in approach may explain the discrepancy in results. It's possible that while the ratio of methionine to CP may not always affect feed efficiency, increasing the overall availability of methionine, as done in the current study, does. In contrast, (Zarghi & Ghavi, 2024) investigated the effects of dietary methionine hydroxy analogue-free acid (MHA-FA) supplementation levels on growth performance, blood metabolites, and immune responses in broiler chickens in Iran. Their research found that increasing dietary MHA-FA levels improved feed efficiency. This finding aligns with the results of the current study, supporting the conclusion that methionine supplementation enhances FCR in broiler chickens. This perhaps indicates that different forms of methionine supplementation (MHA-FA vs. direct methionine) can both positively influence feed conversion.Table 3 illustrates the effect of dietary methionine supplementation on the relative gene expression of GHR and IGF-I in broiler chickens. The results show that methionine supplementation influenced the expression of both genes, but to varying degrees. These results indicate that dietary methionine supplementation can modulate the expression of key growth-related genes in broiler chickens. The pronounced increase in IGF-I expression across all supplemented groups suggests that methionine promotes the activity of this important growth factor. The effect on GHR expression was more complex, with the highest expression at the 3 g/kg level, perhaps indicating an optimal level of methionine for GHR activation. A study by (Del Vesco et al., 2013) aimed to evaluate liver and breast muscle IGF-I and GHR gene expression in broilers fed different methionine levels and sources. Conducted in Brazil, the study reached a conclusion that methionine supplementation increased IGF-I gene expression in the liver. This aligns with the findings of the current study, where methionine supplementation led to higher IGF-I expression. This indicates that methionine's positive influence on IGF-I gene expression is consistent across different studies. In contrast, (Rolland et al., 2015) investigated the effect of dietary methionine level on the hepatic expression of genes related to the somatotropic axis, including GHR-I and IGF-I, in rainbow trout in Norway. The study found that the transcript levels of GHR-I and IGF-I increased linearly with increasing dietary methionine content. While this also shows a positive relationship between methionine and gene expression, the linear increase differs from the pattern observed in the current study, particularly for GHR. This perhaps indicates that the relationship between methionine and GHR expression may differ between species (broiler chickens vs. rainbow trout) or that there are other factors influencing GHR expression in broiler chickens.

CONCLUSION

Increasing levels of methionine supplementation led to significant improvements in body weight gain. Furthermore, methionine supplementation enhanced feed conversion efficiency, as evidenced by lower FCR values in the supplemented groups, indicating more efficient utilization of feed. Methionine also influenced the expression of IGF-I and GHR genes, with a general upregulation of IGF-I expression and a more complex effect observed for GHR. These findings suggest that dietary methionine plays a crucial role in optimizing growth and feed efficiency in broiler chickens, likely through its effects on protein synthesis and the GH/IGF-I signaling pathway. The results of this study have implications for optimizing broiler chicken nutrition and production practices.

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