

Effect of dietary methionine levels on growth, production performance, and egg quality in Hy-Line Brown laying hens

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ABSTRACT. Determining the dietary requirements of sulphur containing amino acids (SAA), particularly methionine (Met), is critical for formulating nutritionally balanced poultry diets. The objective of this study was to evaluate the effects of different dietary Met levels on growth, production and egg quality of laying hens from 65 to 72 weeks of age. A total of 480 Hy-Line Brown laying hens were randomly assigned to six dietary groups for eight weeks. The treatments consisted of diets formulated to provide 90, 95, 100, 105, 110, and 120% of the SAA levels recommended by the National Research Council (NRC), with 100% SAA serving as the control (CON). Growth was recorded at the beginning and at weeks 2, 4, 6, and 8, while egg production and feed intake data were measured weekly. Egg quality parameters were recorded at weeks 2, 4, 6, and 8. Supplementation with 90, 95, and 120% SAA significantly reduced egg production, egg weight, and feed conversion ratio (FCR), while 105 and 110% SAA levels were comparable to the CON. Egg quality traits, including Haugh unit, eggshell strength, and eggshell thickness were unaffected by the dietary treatments. These findings demonstrate that decreasing Met to levels below NRC recommendations, or increasing it by more than 10%, negatively affects production performance. The NRC-recommended dose is therefore appropriate for Hy-Line Brown laying hens at 64 weeks of age.

Introduction

Feed costs in poultry production are largely determined by energy and protein, which account for over 85% of total expenses (Gunawardana et al., 2008). Protein and amino acids play a pivotal role in laying hen diets, not only by improving egg production and bird health but also by reducing environmental nitrogen pollution (Ji et al., 2014). The effective utilisation of dietary protein, which depends on the quantity, composition, digestibility, and availability of amino acids, is essential for maintaining bird health and productivity (Dozier et al., 2008;

Dersjant-Li and Peisker, 2011). Feeds formulated with balanced digestible amino acids help to lower feed costs, meet the precise nutritional requirements, and decrease nitrogen excretion. Among the amino acids necessary for optimal laying hen productivity, methionine (Met) is of particular importance.

Met is the first limiting amino acid in laying hen diets due to its low concentration in plant protein sources and the high dietary requirements of birds (Kakhki et al., 2016). It is essential for protein synthesis and the maintenance of egg production (Brede et al., 2018; Liu et al., 2024). Met also functions as a methyl donor and a precursor for compounds such

as cystine, thereby supporting metabolic and physiological processes. To address deficiencies and meet nutritional requirements, Met is commonly supplemented in poultry diets (Harms and Russell, 1996; Bunchasak and Silapsorn, 2005; Rao et al., 2011).

Dietary Met levels play a significant role in regulating egg production, egg weight, egg quality, and feed efficiency in laying hens (Carvalho et al. 2018, Novak et al., 2004; Liu et al., 2005; Kakhki et al., 2016; Carvalho et al., 2018; Santana et al., 2021). Consequently, accurate determination of Met demand is therefore essential for optimising laying hen nutrition. Safaa et al. (2008) reported that reducing dietary Met from 0.36 to 0.31% in 60-week-old Lohman Brown hens decreased egg weight. However, recommendations for Met vary considerably between the National Research Council (NRC, 1994), breed-specific guidelines, and published studies, creating challenges for poultry nutritionists. Furthermore, advances in genetics, management practices, and health protocols have enabled modern laying hens to produce larger eggs and sustain longer peak production periods (Narvaez-Solarte et al., 2005). Despite extensive research, estimates of Met requirements remain inconsistent, largely due to variations in experimental conditions, including genetic strain, bird age, dietary composition, feed intake levels, environmental factors, housing systems, and methodologies for estimating requirements (Leeson et al., 2001; Bregendahl et al., 2008; Kakhki et al., 2016; Carvalho et al., 2018; Santana et al., 2021).

Given that Met requirements vary significantly with the age and laying phase of hens, these factors must be considered when formulating diets and balancing amino acids. The present study evaluated the effects of varying dietary Met doses on production performance and egg quality of 64-week-old Hy-Line Brown laying hens.

Material and methods

Ethical approval

The experimental protocol for this study was reviewed and approved by the Animal Care and Use Committee and the Ethics Committee of the College of Animal Resources and Sciences, Dankook University (Approval No. DK-1-2419, Cheonan, South Korea).

Experimental design, animals, diets, and housing

A total of 480 Hy-Line Brown laying hens (64-week-old) of approximately uniform body weight (BW) were randomly allocated to six dietary

treatment groups for an eight-week trial. The experiment followed a randomised complete design with five replicates per treatment and 16 hens per replicate. The birds were housed in a windowless, environmentally controlled room maintained at 23 °C with a 16-h light schedule (05:00 to 21:00). Hens were individually housed in cages (50 cm length × 38 cm width × 40 cm height) arranged in large double-sided, four-tier battery systems. Feed and water were provided *ad libitum*. The experimental treatments consisted of mash diets formulated to provide six different sulphur containing amino acids (SAA) concentrations: 90, 95, 100 (control, CON), 105, 110, and 120% of the requirement. A basal diet with the lowest methionine content was prepared, divided into six equal portions, and supplemented with methionine to achieve the target SAA levels. The composition of the experimental diets is provided in Table 1. BW was recorded individually for all hens at the start of the experiment, and at the end of weeks 2, 4, 6, and 8 to calculate body weight gain (BWG).

Laying performance

Egg production was recorded daily for each replicate and averaged weekly. Eggs were categorised as normal or damaged to calculate the percentage of downgraded eggs (dirty, broken, soft, or small eggs). Average egg weight was obtained by dividing the total egg mass by the number of eggs laid per replicate. Feed intake (FI) was measured weekly by recording the difference between feed offered and residual feed, with results averaged per replicate to determine average daily feed intake (ADFI). Feed conversion ratio (FCR) was calculated weekly as feed intake divided by total egg mass produced ($FCR = FI / \text{no. of eggs} \times \text{avg. egg weight}$).

Egg quality traits

At the end of weeks 2, 4, 6, and 8, 30 eggs were randomly collected from each treatment group at 17:00 for same-day evaluation of egg quality traits. The traits assessed included Haugh unit (HU), egg weight, eggshell strength, and eggshell thickness. Each egg was individually weighed with a digital precision scale (accuracy: 0.1 g) before quality analysis. HU, eggshell strength, and eggshell thickness were determined using a fully automatic DET 6000 egg tester (Kyoto, Japan).

Statistical analysis

The data were analysed using a complete randomised design with the General Linear Model procedure implemented in SAS software (SAS Institute, Cary, NC, USA). Replicates ($n = 5$) served

Table 1. Composition of laying hen diets (as fed basis)

Item	Experimental diets					
	SAA (90%)	SAA (95%)	SAA (100%) (CON)	SAA (105%)	SAA (110%)	SAA (120%)
Ingredients, %						
maize	67.01	66.88	66.73	66.54	66.44	66.11
soybean meal (CP 44%)	15.42	15.45	15.47	15.50	15.51	15.57
maize gluten meal (CP 60%)	5.00	5.00	5.00	5.00	5.00	5.00
tallow	0.60	0.64	0.70	0.77	0.81	0.93
MDCP	1.07	1.07	1.07	1.07	1.07	1.07
limestone	9.80	9.80	9.80	9.80	9.80	9.80
salt	0.25	0.25	0.25	0.25	0.25	0.25
methionine (50%)	0.09	0.15	0.22	0.31	0.36	0.51
threonine (99%)	0.03	0.03	0.03	0.03	0.03	0.03
tryptophan (99%)	0.03	0.03	0.03	0.03	0.03	0.03
lysine (78%)	0.20	0.20	0.20	0.20	0.20	0.20
vitamin mix ¹	0.20	0.20	0.20	0.20	0.20	0.20
mineral mix ²	0.20	0.20	0.20	0.20	0.20	0.20
choline (50%)	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated value, %						
metabolizable energy, kcal/kg	2850	2850	2850	2850	2850	2850
crude protein	15.00	15.00	15.00	15.00	15.00	15.00
crude fat	3.11	3.15	3.20	3.26	3.30	3.40
crude fibre	2.48	2.48	2.48	2.47	2.47	2.47
crude ash	11.66	11.66	11.66	11.66	11.66	11.66
calcium	4.00	4.00	4.00	4.00	4.00	4.00
available phosphorus	0.31	0.31	0.31	0.31	0.31	0.31
lysine	0.78	0.78	0.78	0.78	0.78	0.78
methionine	0.31	0.34	0.38	0.42	0.45	0.52
methionine + cysteine	0.65	0.68	0.72	0.76	0.79	0.86
threonine	0.58	0.58	0.58	0.58	0.58	0.58
tryptophan	0.18	0.18	0.18	0.18	0.18	0.18

SAA – sulphur containing amino acids, CON – control group, CP – crude protein, MDCP – monocalcium phosphate; ¹ provided per kg of diet: IU: vit. A 8 000, vit. D₃ 3 300; g: vit. E 20, vit. K₃ 2.5, vit.;B₁ 2.5, vit. B₂ 5.5, vit. B₆ 4, folic acid 0.9, niacin 30, D-calcium pantothenate 8; mg: vit. B₁₂ 23, biotin 75; ² provided per kg of diet: g: Fe 40 as ferrous sulphate, Cu 8 as copper sulphate, Mn 90 as manganese oxide, Zn 80 as zinc oxide, K 1.2 as potassium iodide, Se 0.22 as sodium selenite

as the experimental unit. Treatment differences were assessed by Tukey's multiple range test, with results presented as means and pooled standard error of the mean (SEM). Differences were considered statistically significant at $P < 0.05$.

Results

Growth and production performance

Table 2 summarises the effects of varying dietary methionine levels on growth and production performance of laying hens. BW, downgraded egg percentage, and ADFI did not differ between the treatments ($P > 0.05$). Dietary Met levels influenced egg production, egg weight, and FCR over the experimental period. During the first two weeks, no differences were observed in egg production, egg weight, or FCR ($P > 0.05$). Egg production was

significantly reduced ($P < 0.05$) in hens receiving 90, 95, and 120% SAA diets during weeks 3, 4, and 8 compared to CON (SAA 100%). Egg weight decreased significantly ($P < 0.05$) in the 90 and 120% SAA groups from weeks 3 to 8, except in week 4, when only 120% SAA reduced egg weight relative to CON. The response of FCR mirrored egg production, increasing significantly ($P < 0.05$) in the 90 and 95% SAA groups from weeks 3 to 8, and in 120% SAA during weeks 3 and 6 compared with CON (Table 2).

Egg quality traits

Table 3 shows the effects of different levels of Met supplementation on egg quality traits. HU, egg weight, eggshell strength, and eggshell thickness did not differ significantly ($P > 0.05$) between dietary groups with SAA levels ranging from 90 to 120% of NRC recommendations (NRC, 1994).

Table 2. Effects of dietary methionine + cysteine (Met + Cys) levels on growth and production performance of Hy-Line Brown laying hens

Parameter	SAA (90%)	SAA (95%)	SAA (100%) (CON)	SAA (105%)	SAA (110%)	SAA (120%)	SEM	P-value
Body weight, g								
initial	1981	1980	1980	1981	1980	1981	0.414	0.913
week 2	1986	1985	1986	1988	1989	1990	1.073	0.705
week 4	1990	1991	1992	1994	1996	1999	2.277	0.880
week 6	1994	1996	1998	1999	2001	2004	2.919	0.943
week 8	1997	1999	2002	2004	2006	2009	3.24	0.912
Week 1								
downgraded egg, %	1.42	1.36	1.46	1.46	1.44	1.38	0.201	0.991
egg production, %	85.56	85.72	88.22	87.86	87.68	86.26	0.538	0.588
egg weight, g	63.87	63.50	63.73	63.55	63.25	63.21	0.126	0.654
FCR	2.03	2.04	1.97	1.98	1.99	2.03	0.014	0.627
ADFI, g	110.8	110.8	110.6	110.5	110.6	110.6	0.210	0.997
Week 2								
downgraded egg, %	1.40	1.38	1.42	1.40	1.46	1.40	0.238	0.925
egg production, %	85.20	85.36	88.58	89.30	90.02	85.74	0.680	0.116
egg weight, g	63.07	62.87	63.48	63.45	63.13	62.42	0.202	0.704
FCR	2.07	2.07	1.97	1.95	1.95	2.07	0.019	0.100
ADFI, g	110.9	110.5	110.6	110.3	110.5	110.1	0.215	0.984
Week 3								
downgraded egg, %	1.48	1.36	1.46	1.44	1.42	1.36	0.224	0.911
egg production, %	84.84 ^b	85.00 ^b	90.38 ^a	90.20 ^a	90.20 ^a	85.54 ^b	0.718	0.011
egg weight, g	62.08 ^b	62.87 ^{ab}	63.91 ^a	63.78 ^a	63.77 ^a	62.32 ^b	0.177	0.011
FCR	2.08 ^a	2.08 ^a	1.91 ^b	1.92 ^b	1.92 ^b	2.07 ^a	0.021	0.003
ADFI, g	110.8	110.6	110.4	110.3	110.4	110.2	0.215	0.978
Week 4								
downgraded egg, %	1.50	1.48	1.36	1.40	1.40	1.38	0.160	0.925
egg production, %	84.30 ^b	84.48 ^b	89.30 ^a	89.12 ^a	89.66 ^a	85.34 ^b	0.758	0.045
egg weight, g	62.88 ^{ab}	63.12 ^a	63.64 ^a	63.74 ^a	63.68 ^a	62.33 ^b	0.132	0.039
FCR	2.08 ^a	2.09 ^a	1.94 ^b	1.94 ^b	1.93 ^b	2.06 ^{ab}	0.020	0.023
ADFI, g	110.9	110.6	110.3	110.0	110.3	109.8	0.217	0.782
Week 5								
downgraded egg, %	1.74	1.44	1.48	1.42	1.42	1.44	0.184	0.935
egg production, %	83.56 ^b	83.74 ^b	87.50 ^{ab}	88.22 ^a	88.76 ^a	85.00 ^{ab}	0.650	0.043
egg weight, g	62.21 ^c	62.54 ^{bc}	63.32 ^{ab}	63.32 ^{ab}	63.52 ^a	62.30 ^c	0.144	0.016
FCR	2.14 ^a	2.11 ^a	1.98 ^{bc}	1.98 ^{bc}	1.95 ^c	2.08 ^{ab}	0.020	0.006
ADFI, g	110.9	110.6	110.2	109.7	110.0	109.8	0.229	0.682
Week 6								
downgraded egg, %	1.72	1.58	1.54	1.46	1.44	1.44	0.174	0.908
egg production, %	82.84 ^d	83.76 ^{cd}	88.04 ^{ab}	87.50 ^{bcd}	89.66 ^a	84.48 ^{abc}	0.661	0.015
egg weight, g	62.22 ^b	62.51 ^{ab}	63.50 ^a	63.50 ^a	63.70 ^a	62.18 ^b	0.189	0.024
FCR	2.15 ^a	2.11 ^a	1.97 ^b	1.98 ^b	1.93 ^b	2.09 ^a	0.020	0.001
ADFI, g	110.8	110.6	110.3	109.8	110.0	109.7	0.205	0.687
Week 7								
downgraded egg, %	1.82	1.56	1.60	1.50	1.52	1.50	0.171	0.996
egg production, %	82.16 ^b	83.50 ^b	85.12 ^{ab}	87.02 ^a	88.74 ^a	85.94 ^{ab}	0.887	0.019
egg weight, g	62.28 ^c	62.69 ^{bc}	63.46 ^{ab}	63.76 ^a	63.72 ^a	62.30 ^c	0.165	0.013
FCR	2.17 ^a	2.14 ^a	1.95 ^{bc}	1.92 ^c	1.90 ^c	2.10 ^{ab}	0.025	0.001
ADFI, g	110.7	110.4	110.0	110.1	109.7	109.6	0.240	0.776
Week 8								
downgraded egg, %	1.92	1.68	1.62	1.60	1.48	1.54	0.211	0.994
egg production, %	81.26 ^c	81.78 ^c	88.76 ^a	88.04 ^{ab}	88.94 ^a	84.12 ^{bc}	0.819	0.010
egg weight, g	61.86 ^b	62.49 ^{ab}	63.48 ^a	63.44 ^a	63.48 ^a	62.14 ^b	0.171	0.026
FCR	2.19 ^a	2.16 ^a	1.95 ^c	1.98 ^{bc}	1.94 ^c	2.10 ^{ab}	0.022	0.011
ADFI, g	110.7	110.3	109.9	110.2	109.6	109.3	0.220	0.483

SAA – sulphurcontaining amino acids, CON – control group; FCR – feed conversion ratio, ADFI – average daily feed intake; SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 3. Effects of dietary methionine + cysteine (Met + Cys) levels on egg quality of Hy-Line Brown laying hens

Parameter	SAA (90%)	SAA (95%)	SAA (100%)(CON)	SAA (105%)	SAA (110%)	SAA (120%)	SEM	P-value
Week 2								
HU	81.9	81.8	81.8	81.6	81.5	81.3	0.176	0.943
egg weight, g	63.7	63.7	63.9	64.1	64.2	64.3	0.088	0.259
eggshell strength, kg/cm ²	3.91	3.92	3.95	3.96	3.97	3.97	0.02	0.904
eggshell thickness, mm ²	39.5	39.6	39.9	39.9	39.9	40.1	0.24	0.98
Week 4								
HU	81.7	81.7	81.5	81.2	81.0	80.7	0.221	0.697
egg weight, g	63.7	63.8	64.1	64.3	64.4	64.6	0.262	0.839
eggshell strength, kg/cm ²	3.89	3.91	3.94	3.96	3.96	3.97	0.018	0.76
eggshell thickness, mm ²	39.3	39.3	39.7	39.9	40.0	40.1	0.257	0.909
Week 6								
HU	81.6	81.5	81.3	81.0	80.6	80.2	0.440	0.932
egg weight, g	63.8	64.0	64.3	64.5	64.6	64.9	0.461	0.887
eggshell strength, kg/cm ²	3.89	3.89	3.91	3.94	3.96	3.97	0.051	0.991
eggshell thickness, mm ²	39.1	39.0	39.5	39.8	39.8	39.8	0.554	0.991
Week 8								
HU	81.5	81.3	81.1	80.7	80.2	79.8	0.367	0.733
egg weight, g	63.8	64.1	64.4	64.6	64.9	64.1	0.282	0.777
eggshell strength, kg/cm ²	3.86	3.87	3.89	3.92	3.94	3.95	0.048	0.992
eggshell thickness, mm ²	38.7	38.9	39.2	39.3	39.6	39.6	0.562	0.997

SAA – sulphurcontaining amino acids, CON – control group; HU – Haugh unit; SEM – standard error of the mean; $P > 0.05$ – not statistically significant

Discussion

The nutritional effects of Met supplementation on layer performance vary considerably depending on genetic strains, bird age, dietary formulations, production phases, and environmental conditions (Kakhki et al., 2016; Carvalho et al., 2018; Castro et al., 2019; Santana et al., 2021). This study evaluated six graded levels of SAA, based on variations in Met supplementation on growth, production performance, egg weight, and egg quality in Hy-Line Brown laying hens aged 65 to 72 weeks.

In the present study, no significant differences were observed in BW and ADFI between treatments with varying SAA levels based on Met supplementation. These findings are consistent with previous reports by Novak et al. (2004), Safaa et al. (2008), and Kakhki et al. (2016), who documented stable feed intake patterns in layers receiving different levels of Met and total SAA. However, contrasting results have also been reported in literature, with some studies showing increased ADFI (Sohail et al., 2002; Brumano et al., 2010; Carvalho et al., 2018) and others decreased ADFI (Shafer et al., 1996; Shafer et al., 1998) in response to graded dietary Met supplementations. While Austic (1986) and Harper et al. (1970) speculated that plasma amino acid fluctuations might influence appetite regulation, our findings indicate that the relatively small incremental differences (5–10%) between treat-

ments may have been insufficient to trigger detectable changes in the FI of laying hens.

Laying hens showed reduced egg production, egg weight, and FCR after two weeks of feeding diets containing either lower (90 and 95%) or higher (120%) Met levels compared to CON (100%). These results are consistent with the study of Sohail et al. (2002), who reported decreased egg production and egg weight in response to reduced SAA supplementation. The adverse effects of both deficient (90, 95%) and excessive (120%) Met supply became statistically significant after 2 weeks of dietary intervention. However, previous studies show inconsistent evidence regarding the impact of Met and total SAA supplementation on egg production, indicating that the effects may vary depending on factors such as breed, bird age, feed composition, and environmental conditions.

A particularly noteworthy finding of the current study was the non-linear response of egg production, egg weight, and FCR to varying Met supplementation levels. Performance metrics remained statistically unchanged when Met supplementation was increased up to 10% above NRC recommendations (NRC, 1994) but declined significantly in the 120% treatment group. Hens receiving suboptimal (90 and 95%) or excessive (120%) Met concentrations showed overall poorer performance compared to intermediate dietary treatments (100, 105, and 110%) during weeks 3 to 8 of the experiment.

This clearly demonstrates that both insufficient and excessive SAA provision can disrupt amino acid homeostasis, leading to measurable production losses. These findings are in line with the results of Santana et al. (2021) and Jordao et al. (2006), who similarly recorded reduced productivity at extreme Met and total SAA concentrations.

Numerous studies have reported diverse findings on the production performance of laying hens using varying levels of Met and SAA in experimental diets (Schutte and Van Weerden, 1978; Schutte et al., 1983; Schutte et al., 1994; Shafer et al., 1996; Waldroup and Hellwig, 1995; Harms and Russell, 1996; Shafer et al., 1998; Bunchasak and Silapsorn, 2005; Narvaez-Solarte et al., 2005). Among essential amino acids, Met is particularly important due to its sulphur content and potential toxicity in poultry. Here, the reduced production performance observed at 120% Met supplementation may reflect metabolic stress caused by excessive SAA intake, as suggested by Santana et al. (2021). Supporting this interpretation, previous research has documented increased hepatic lipid accumulation in layers receiving high Met diets (Bunchasak and Silapsorn, 2005; Santana et al., 2021), indicating potential liver dysfunction associated with SAA overload.

Egg quality parameters, including HU, eggshell thickness, and eggshell strength, were not affected by different levels of Met supplementation in the present study. These observations align with findings of Kakhki et al. (2016) and Santana et al. (2021), but differ from reports of improved HU in younger hens supplemented with higher SAA levels (Solarte et al., 2005; Carvalho et al., 2018). Interestingly, Carvalho et al. (2018) observed that the effects of different Met levels on egg quality diminished in older hens (34 vs. 50 weeks), suggesting that the production phase of laying hens significantly influence nutrient utilisation efficiency, including Met and other amino acids. These age-dependent responses emphasise the importance of stage-specific nutritional strategies in layer production.

Conclusions

In conclusion, this study demonstrates that both deficient and excessive dietary methionine (Met) levels relative to National Research Council (NRC) (1994) recommendations negatively affect production performance in 64-week-old Hy-Line Brown laying hens. These findings confirm that NRC-recommended levels provide an optimal balance for maintaining egg production, egg weight, and feed conversion ratio during the late laying period,

supporting their continued application in commercial layer nutrition. Further studies should evaluate potential toxic effects of Met over-supplementation in hens during the later stages of production.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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