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Methionine Supplementation of Maternal Diet Improves Hatching Traits, Initial Development, and Performance in Japanese Quail Fed Different Levels of Methionine During Growth

Thaís Pacheco Santana[1] | Alany Cristyane Felix da Silva[1] | Marisa Silva Bastos[1] | Juliana dos Santos Conceição[1] | Angélica de Souza Khatlab[2] | Eliane Gasparino[2] | Leandro Teixeira Barbosa[3] | Claudson Oliveira Brito[1,3] | Ana Paula Del Vesco[1,3]

[1]Integrated Graduate Program in Animal Science, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil | [2]Department of Animal Science, State University of Maringá, Maringá, Paraná, Brazil | [3]Department of Animal Science, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil

Correspondence: Thaís Pacheco Santana (thaisp100@gmail.com)

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ABSTRACT

This study examined the effects of dietary levels of methionine on lipid and intestinal metabolism in Japanese quail hens and their progeny. The experiment was conducted according to a 3 × 3 factorial design, with three maternal and three progeny diets, as follows: low-methionine (LMET), recommended methionine (MET), and high-methionine (HMET). Methionine supplementation improved reproductive performance during laying ($p < 0.05$). Intestinal morphometry revealed that MET and HMET diets increased duodenal villus width and crypt depth in hens ($p < 0.05$). Hens fed the HMET diet showed higher expression of amino acid transport and barrier function genes. Hens fed LMET produced offspring with lower body weight at 15 days of age and lower weight gain (1–15 days of age) than hens fed MET and HMET ($p = 0.0002$). During the grower phase, chicks fed LMET diet had lower body weight at 15 ($p < 0.0001$) and 35 ($p < 0.0001$) days and worse feed conversion ratio ($p = 0.0006$) than chicks fed MET and HMET. Progeny from MET or HMET hens had improved intestinal histomorphometry. Overall, methionine supplementation of quail diets enhances intestinal function and reproductive performance in hens, improving chick performance in the starter and grower phases.

1 | Introduction

Quail farming is gaining prominence in the field of animal production. Data show that the number of quail birds produced in Brazil grew by 89% from 2016 to 2021 (IBGE 2021). These excellent results are explained by numerous advantages of quail production, such as easy handling, rapid growth, early egg production, high yield (300 eggs year^{-1}), extended duration of high-production phases, low breeding cost, and rapid return on investment. Japanese quail (*Coturnix japonica*) are the most used for egg production because they have a higher laying rate than other species (Pastore, Oliveira, and Muniz 2012). Several technologies have been developed to enhance the efficiency of quail egg production and harness the genetic potential of these birds (Zuidhof et al. 2014).

In birds, egg production starts with follicle maturation, which increases yolk content, prompting the release of the oocyte. This complex process is hormonally controlled and largely dependent on the availability of nutrients, such as amino acids, vitamins,

and lipids. Yang et al. (2022) showed that supplementation of bird diets can increase serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and modulate the expression of FSH and LH receptor genes, promoting follicle development and maturation. Research has also shown that nutrition is one of the main factors influencing the reproductive performance of birds and fertile egg production, representing a critical factor for egg number, egg quality, and chick quality. Numerous studies have been conducted to determine the best diets for improved growth rate, bird production, and reproduction and increased tolerance to environmental stress (Santana et al. 2021, 2023).

A key aspect of poultry nutrition is formulating diets based on optimal amino acid ratios (Emmert and Baker 1997). Methionine, the first-limiting amino acid in birds, is a sulfur donor with essential functions in protein synthesis and cell metabolism. This amino acid participates in the synthesis of polyamines, is a precursor of carnitine and cystine, and acts as a methyl donor for the formation of the coenzyme *S*-adenosyl methionine. Additionally, methionine plays an important role in physiological functions, has antioxidant action, preventing the formation of reactive oxygen species (ROS), and is linked to immune function, participating in immune organ development and defense cell production (Pan et al. 2016; Kalvandi, Sadeghi, and Karimi 2019; Machado et al. 2020; Santana et al. 2021). Diets containing adequate methionine levels result in better feed efficiency, improving reproductive performance, egg quality, and egg production via increased protein deposition. Egg quality parameters, such as resistance, weight, and shell thickness, are improved by the antioxidant action of methionine. Its antioxidant properties also exert beneficial effects on lipid metabolism. During the laying phase, lipids are directed to the synthesis of yolk precursors, such as vitellogenin and very low-density lipoprotein (VLDL) cholesterol. Methionine supplementation of poultry diets increases plasma levels of high-density lipoprotein (HDL) cholesterol and decreases low-density lipoprotein (LDL) cholesterol, total cholesterol, and triglycerides (Reda et al. 2019).

From embryo to post-hatch development, the chick depends on nutrients from the maternal diet that are deposited in the egg (Emamverdi et al. 2019). In recent years, efforts have been made to better understand how the maternal diet influences progeny performance (Santana et al. 2021, 2023). Incompatibility of the maternal/offspring environment can cause positive or negative effects on offspring development and performance, modifying the physiological functioning of birds and causing changes in the digestive, reproductive, oxidative, and immune systems (Li et al. 2020; Liu et al. 2020). Waaij et al. (2011), for instance, demonstrated that hens reared under food restriction conditions produce offspring conditioned to low food availability; when these chicks are fed *ad libitum*, they reach adult weight early, attributed to compensatory gain, and nutrients are diverted to fat deposition instead of muscle mass deposition. According to Jiang et al. (2022), the maternal diet can improve the intestinal immune function of progeny by modulating the intestinal microbiota, accelerating intestinal function and maturation, and activating intestinal inflammation when progeny are challenged by stressors. Santana et al. (2023) found that dietary supplementation of laying hens with methionine contributes to progeny development and performance during growth, particularly for chicks subjected to challenges during growth.

As previously mentioned, dietary methionine supplementation has been widely investigated in poultry production. Nevertheless, it is still necessary to better understand the effect of methionine on the various metabolic pathways associated with hen and progeny phenotype. Thus, this study raised the following questions: (1) What is the effect of methionine supplementation on the intestinal environment of hens? (2) What is the effect of methionine supplementation on hen lipid metabolism and egg production? (3) How does the maternal diet influence the early progeny environment? (4) How does the maternal diet interact with progeny diet during the grower phase? To answer these questions, this study investigated the histomorphometry of the small intestine and the expression of genes related to glucose and amino acid transport and genes related to lipid metabolism in quail layers and their progeny supplemented with different levels of methionine.

2 | Material and Methods

2.1 | Animal Welfare Statement

This study was conducted at the experimental farm of the State University of Maringá, Paraná, Brazil, and was approved by the Animal Research Ethics Committee (CEUA, protocol no. 2402310719).

2.2 | Quail Layers

A total of 200 1-day-old female Japanese quail (*C. japonica*) were reared in cages up to 98 days of age. Bird development and laying rate were assessed daily during this period. At 98 days of age, 30 quail females with a mean weight of 154.6 g and a laying rate of 85% were transferred to individual cages and distributed in three dietary treatments: basal diet without methionine supplementation (low-methionine diet, LMET), basal diet supplemented with methionine at the recommended level, according to Rostagno et al. (2017) (MET); and basal diet supplemented with methionine at a level higher than the recommended (high-methionine diet, HMET) (Table 1). The experiment was conducted from 98 to 136 days of age, totaling 38 days; of these, 28 days corresponded to mating without egg collection, to ensure that all eggs were fertilized during the experimental period, and 10 days corresponded to egg collection. During the experimental period, birds had *ad libitum* access to feed and water.

For egg fertilization, 30 males (mean weight of 161.2 g) were placed in the same cage as females for 1 h daily. The parental effect was minimized by rotating males. Males were used for mating only and had *ad libitum* access to water and feed formulated to meet quail requirements. Eggs were collected daily, identified, weighed, and stored individually at room temperature. At the end of the collection period, eggs were transferred to an incubator (Chocomaster Luna 240) set at 37°C and 60% relative humidity. At the end of the 19 days of incubation, unhatched eggs were opened and classified as unfertilized eggs or dead embryos.

TABLE 1 | Ingredient and nutrient composition of experimental diets for Japanese quail layers in the laying phase (98 to 136 days).

Item	Experimental diet[a]		
	LMET	MET	HMET
Ingredient composition (%)			
Ground corn	56.65	56.32	56.10
Soybean meal	32.20	32.30	32.30
Salt	0.37	0.37	0.37
Vegetable oil	1.70	1.60	1.50
Calcitic limestone	7.52	7.52	7.52
Dicalcium phosphate	0.99	0.99	0.99
L-Lysine HCL	0.13	0.13	0.13
DL-Methionine	—	0.33	0.65
L-Threonine	0.04	0.04	0.04
Vitamin–mineral premix[b]	0.40	0.40	0.40
Total	100	100	100
Analyzed energy and nutrient composition			
Metabolizable energy (kcal kg^{-1})	2796	2795	2795
Crude protein (%)	19	19	19
Calcium (%)	3.15	3.15	3.15
Digestible phosphorus (%)	0.33	0.33	0.33
Sodium (%)	0.17	0.17	0.17
Available amino acids (%)			
Digestible methionine + cystine	0.53	0.86	1.17
Digestible lysine	1.04	1.05	1.05
Digestible threonine	0.71	0.71	0.71
Digestible tryptophan	0.21	0.21	0.21

[a]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[b]Provided (per kg of feed): vitamin A, 2,250,000 IU; vitamin D3, 500.00 IU; vitamin E, 7000 IU; vitamin B1, 450 mg; vitamin B2, 1000 mg; vitamin B6, 450 mg; vitamin B12, 3500 mg; vitamin K3, 420 mg; calcium pantothenate, 2500 mg; niacin, 7000 mg; folic acid, 180 mg; biotin, 15 mg; choline, 55 g; zinc, 12 g; iron, 12 g; manganese, 15 g; copper, 3000 mg; iodine, 250 mg; cobalt, 50 mg; selenium, 72 mg; ethoxyquin, 40 mg; and butylated hydroxyanisole (BHA), 40 mg.

TABLE 2 | Ingredient and nutrient composition of experimental diets for Japanese quail layers in the starter (1 to 14 days) and grower (15 to 35 days) phases.

Item	Starter diet	Grower diet[a]		
		LMET	MET	HMET
Ingredient composition (%)				
Ground corn	61.90	63.86	63.7	63.59
Soybean meal	34.00	30.50	30.60	30.60
Salt	0.44	0.60	0.60	0.60
Vegetable oil	0.30	1.70	1.60	1.50
Calcitic limestone	1.12	1.40	1.40	1.40
Dicalcium phosphate	1.53	1.40	1.40	1.40
L-Lysine HCL	0.12	0.13	0.13	0.13
DL-Methionine	0.18	—	0.16	0.37
L-Threonine	0.01	0.01	0.01	0.01
Vitamin–mineral premix[b]	0.40	0.40	0.40	0.40
Total	100	100	100	100
Analyzed energy and nutrient composition				
Metabolizable energy (kcal kg^{-1})	2898	2908	2910	2013
Crude protein (%)	20	19	19	19
Calcium (%)	0.88	0.92	0.92	0.92
Digestible phosphorus (%)	0.45	0.42	0.42	0.42
Sodium (%)	0.19	0.21	0.21	0.21
Available amino acids (%)				
Digestible methionine + cystine	0.75	0.53	0.70	0.90
Digestible lysine	1.10	1.02	1.03	1.02
Digestible threonine	0.72	0.68	0.68	0.68
Digestible tryptophan	0.22	0.21	0.21	0.21

[a]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[b]Provided (per kg of feed): vitamin A, 2,250,000 IU; vitamin D3, 500.00 IU; vitamin E, 7000 IU; vitamin B1, 450 mg; vitamin B2, 1000 mg; vitamin B6, 450 mg; vitamin B12, 3500 mg; vitamin K3, 420 mg; calcium pantothenate, 2500 mg; niacin, 7000 mg; folic acid, 180 mg; biotin, 15 mg; choline, 55 g; zinc, 12 g; iron, 12 g; manganese, 15 g; copper, 3000 mg; iodine, 250 mg; cobalt, 50 mg; selenium, 72 mg; ethoxyquin, 40 mg; and butylated hydroxyanisole (BHA), 40 mg.

2.3 | Progeny

After hatching, a total of 234 chicks were ringed, weighed, and housed in a circular pen heated with a heating lamp. Chicks had *ad libitum* access to starter feed (Table 2) and water. Chicks were housed according to maternal diet and reared under conventional conditions until 15 days of age.

At 15 days of age, progeny were weighed and distributed into three dietary treatments, namely LMET, MET, and HMET (Table 2). Birds of the same treatment were allocated in collective cages ($n = 13$), with two birds per cage. Quail had *ad libitum* access to feed and water.

2.4 | Assessments

During the 38 days of the experimental period, female breeders were assessed for feed intake, feed conversion per dozen eggs, feed conversion per egg mass, number of eggs, egg weight, egg mass,

and laying rate, as described by Bastos et al. (2017). Hatching rate (%) was calculated according to Koppenol et al. (2015).

Progeny performance was assessed from 1 to 14 days of age (starter phase) and from 15 to 35 days of age (grower phase). At the end of egg collection and at the end of the progeny experimental period, hens and progeny were sacrificed by cervical displacement and subjected to bleeding. Phase F1 eggs (ready to be released) were extracted from the ovary of hens and weighed (Figure 1). Relative weights of the ovary, liver, heart, and intestine of female breeders were calculated using the following equation: Relative weight = Organ weight / Bird weight × 100.

2.5 | Morphometric Analysis

Villus height, villus width, crypt depth, villus surface area, and villus/crypt ratio were determined on duodenum, jejunum, and ileum samples. Segments measuring 1.0 cm in diameter were cut longitudinally, washed in phosphate buffer (0.1 M, pH 7.4), and fixed in Bouin's solution for 36 h. Samples were prepared according to the technique described by Junqueira and Junqueira (1983). One slide was mounted per segment containing two cuts, and the length (μm, in a straight line) of 10 villi and 10 well-oriented crypts of the duodenum and jejunum was measured using an optical microscope.

2.6 | Gene Expression

For gene expression analysis, duodenal samples were collected in RNAlater™ (Life Technologies do Brasil, Brazil) and stored at −20°C until RNA extraction. Total RNA was extracted using Trizol® reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's recommendations, at a ratio of 1 mL of reagent to 80 mg of tissue. RNA integrity was evaluated by 1% agarose gel electrophoresis with ethidium bromide (10 mg mL^{-1}) staining and visualization under ultraviolet light.

All samples were treated with DNAse I (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, to eliminate any DNA contamination. For complementary DNA (cDNA) synthesis, the High-Capacity cDNA Reverse Transcription Kit (Life Technologies Corporation, Carlsbad, CA, USA) and 10 μL of DNA-treated RNA were used, according to the manufacturer's instructions.

For real-time PCR reactions, the SYBR™ Green PCR Master Mix (Applied Biosystems, USA) was used. The amplification reaction consisted of 5 μL of cDNA diluted to 40 ng, 0.5 μL of each primer diluted to 10 μM (the final concentration of the reaction was 200 μM), 12.5 μL of SYBR™ Green PCR Master Mix, and 6.5 μL of ultrapure water, in a final volume of 25 μL. The efficiency of each primer was assessed using a series of 25 μL reactions, performed similarly to the previous one, using 5 μL of a serial dilution (80, 40, 20, and 10 ng μL^{-1}) of cDNA. The thermocycler program for all genes was as follows: 95°C for 15 s and 60°C for 1 min. Melting curves were constructed to assess specificity.

Primers for apolipoprotein AI (*APOA1*), apolipoprotein B (*APOB*), acetyl-CoA carboxylase (*ACC*), and fatty acid synthase (*FAS*) genes were obtained according to Lei and Lixian (2014) and Jiang et al. (2014). Primers for glucose transporter (*SLC2A2*), sodium-dependent glucose cotransporter (*SLC5A1*), neutral amino acid transporter (*SLC6A19*), y + L amino acid transporter 1 (*SLC7A7*), and occludin (*OCLN*) genes were designed based on gene deposited in the NCBI database (www.ncbi.nlm.nih.gov). The β-actin gene was used as endogenous control (Table 3). All analyses were performed in a volume of 25 μL, in duplicate. Amplification efficiencies were similar between genes of interest, ranging from 90% to 110%.

2.7 | Statistical Analysis

The $2^{-\Delta CT}$ method was used for relative quantification of gene expression. The effect of maternal diet on hens and progeny during

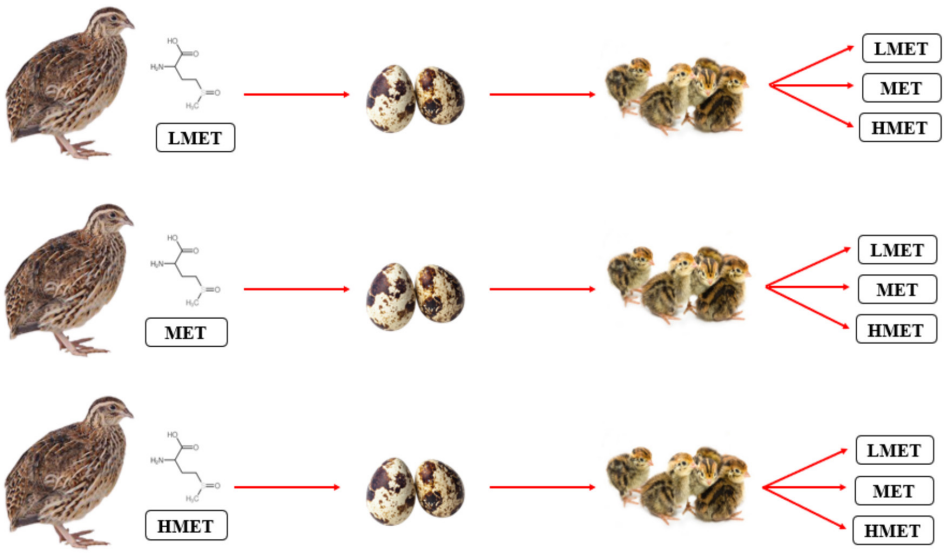

FIGURE 1 | Hierarchical follicles of Japanese quail in the laying phase (98 to 136 days of age). (A) Follicles of hens fed a low-methionine diet (LMET). (B) Follicles of hens fed a diet containing the recommended methionine level (MET). (C) Follicles of hens fed a high-methionine diet (HMET).

TABLE 3 | Primer sequences used for RT-qPCR.

Gene[a]	Accession number	Primer sequence (5′ → 3′)
APOA1	NM_205525	F: GTGACCCTCGCTGTGCTCTT R: CACTCAGCGTGTCCAGGTTGT
APOB	NM_001044633.2	F: GACTTGGTTACACGCCTCA R: TAACTTGCCTGTTATGCTC
ACC	NM_205505	F: AATGGCAGCTTTGGAGGTGT R: TCTGTTTGGGTGGGAGGTG
FAS	J03860	F: CTATCGACACAGCCTGCTCCT R: CAGAATGTTGACCCCTCCTACC
SLC2A2	XM_015878220.2	F: CGCAGAAGGTGATAGAAGC R: ACACAGTGGGGTCCTCAAAG
SLC5A1	XM_015878220.2	F: GCCATGGCCAGGGCTTA R: CAATAACCTGATCTGTGCACCAGTA
SLC6A19	XM_419056.6	F: TCTATTGAAGATTCGGGCAC R: AATGGTAAGCACAAGGTATGG
SLC7A7	XM_418326.6	F: TGTTGGAGCCAGAGAAGGA R: CACAAGGAGATAAAGCAAAGTC
OCLN	D21837.1	F: ACGGCAGCACCTACCTCAA R: GGGCGAAGAAGCAGATGAG
β-actin	L08165.1	F: ACCCCAAAGCCAACAGA R: CCAGAGTCCATCACAATACC

[a]*APOA1*, apolipoprotein AI gene; *APOB*, apolipoprotein B gene; *ACC*, acetyl-CoA carboxylase gene; *FAS*, fatty acid synthase gene; *SLC2A2*, glucose transporter 2 gene; *SLC5A1*, sodium/glucose cotransporter 1 gene; *SLC6A19*, sodium-dependent neutral amino acid transporter gene; *SLC7A7*, y + L amino acid transporter 1 gene; *OCLN*, occludin gene.

the starter phase was assessed using one-way analysis of variance (ANOVA). During the grower phase it was assessed the maternal diet × progeny diet interaction effect using two-way ANOVA.

When treatments exerted a significant effect, means were compared by Tukey's test at $p < 0.05$. Analyses were conducted using SAS software (SAS Institute Inc., NC, USA). All results are presented as mean and standard error.

3 | Results

3.1 | Quail Layers

Female breeders fed MET and HMET had higher egg weight, number of eggs, egg mass, laying rate, and hatching rate, as well as better feed conversion per egg mass, than hens fed LMET ($p < 0.05$). There was no effect of methionine supplementation on the other performance variables (Table 4).

There was no effect of diet on follicle number but there was an effect on follicle weight ($p = 0.0475$). Hens fed MET had higher follicle weight than birds fed LMET (1.28 vs 1.03 g). No effect of methionine supplementation was observed on relative weights of the ovary, liver, intestine, or heart (Table 5).

Regarding morphometry, there was a significant effect of diet on villus width and crypt depth in the duodenum of quail layers

TABLE 4 | Effects of dietary methionine level during the laying phase (98–136 days of age) on the laying performance and hatchability of Japanese quail.

	Experimental diet[†]				
Item[‡]	LMET	MET	HMET	SEM[§]	*p*
IW (g)	164.16	158.33	162.50	15.36	0.79
FW (g)	173.33	167.50	172.50	12.67	0.69
FI (g)	850.83	794.00	822.33	250.38	0.92
FCR_{dz}	2.94	2.24	2.67	0.74	0.17
FCR_{em}	3.73[a]	2.48[b]	2.87[ab]	2.46	0.03
EN	26.16[a]	29.00[a]	29.00[a]	1.72	0.01
EW (g)	9.43[b]	10.99[a]	11.12[a]	0.61	<0.01
EM (g)	234.43[b]	302,30[a]	320.87[a]	49.69	0.02
LR (%)	87.22[b]	96.66[a]	96.66[a]	5.76	0.01
HR (%)	62.40[b]	96.29[a]	69.39[b]	16.03	<0.01

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]IW, initial weight; FW, final weight; FI, feed intake; FCR_{dz}, feed conversion ratio per dozen eggs; FCR_{em}, feed conversion ratio per egg mass; EN, egg number; EW, egg weight; EM, egg mass; LR, laying rate; HR, hatching rate.
[§]Results are presented as mean and standard error of the mean (SEM).

TABLE 5 | Effects of dietary methionine level during the laying phase (98–136 days of age) on follicle number and relative organ weights of Japanese quail layers.

	Experimental diet[†]				
Item[‡]	LMET	MET	HMET	SEM[§]	*p*
FN	4.33	4.50	4.83	0.49	0.23
FW (g)	1.03[b]	1.28[a]	1.12[ab]	0.15	0.04
Ovary (%)	3.26	4.02	3.76	0.59	0.07
Live (%)	2.88	2.64	2.73	0.44	0.64
Intestine (%)	5.19	4.64	4.41	0.73	0.20
Heart (%)	0.86	0.93	0.94	0.15	0.59

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]FN, large follicle number; FW, large follicle weight.
[§]Results are presented as mean and standard error of the mean (SEM).

($p < 0.05$). Birds that received methionine supplementation (MET and HMET diets) had greater villus width (87.79 and 81.51 μm, respectively) than birds that received the LMET diet (74.03 μm). Greater crypt depth was observed in layers fed the MET diet than in birds subjected to the other treatments (Table 6).

Layers fed MET and HMET diets had greater jejunal crypt depth (67.76 μm and 66.63 μm, respectively) than those fed the LMET diet (61.50 μm) ($p < 0.05$) (Table 6). There was no treatment effect on ileal morphometry parameters.

In view of the high energy and nutrient demand of egg production and the importance of body composition for egg fertility, this study evaluated the expression of genes related to lipid metabolism in the liver of female breeders. The following genes were assessed: *ACC*, *FAS*, *APOA1*, and *APOB*. Birds fed diets with any level of methionine supplementation (MET and HMET) showed higher expression of *APOA1* and lower expression of *FAS* than birds fed LMET. Hens fed MET had lower *ACC* mRNA levels than birds fed MET or HMET (Figure 2).

The expression of genes related to nutrient transport and junction proteins was assessed in the duodenum. There was an effect of diet on the expression of all genes, namely *SLC2A2*, *SLC7A7*, *OCLN*, *SLC5A1*, and *SLC6A19* ($p < 0.05$). *SLC7A7* and *OCLN* expression was higher in birds fed HMET, not differing between MET and LMET groups. *SLC5A1* expression was higher among hens fed LMET. *SLC2A2* and *SLC6A19* expression was lower in birds fed MET than in birds fed HMET (Figure 3).

3.2 | Progeny Performance During Starter (1–14 Days) and Grower (15–35 Days) Phases

The effect of maternal diet on progeny development was evaluated in two phases: early development (1–14 days) and growth (15–35 days). The early phase was tested to demonstrate the effect of maternal diet on chick development. Hens fed LMET produced offspring with lower body weight at 15 days of age

TABLE 6 | Effect of dietary methionine level on the intestinal morphometry of Japanese quail layers.

	Experimental diet[†]				
Parameter[‡]	LMET	MET	HMET	SEM[§]	*p*
Duodenum					
VH (μm)	568.04	625.05	589.86	185.01	0.23
VW (μm)	74.03[b]	87.79[a]	81.51[ab]	28.48	0.03
CD (μm)	63.20[b]	71.80[a]	63.54[b]	13.77	<0.01
VSA (mm^2)	0.14	0.17	0.16	0.07	0.05
V/C	9.17	8.91	9.24	3.01	0.82
Jejunum					
VH (μm)	494.48	465.29	470.89	93.97	0.19
VW (μm)	72.07	78.52	77.31	16.19	0.07
CD (μm)	61.50[b]	67.76[a]	66.63[a]	11.24	<0.01
VSA (mm^2)	0.11	0.11	0.11	0.03	0.94
V/C	8.11	8.04	7.17	4.73	0.48
Ileum					
VH (μm)	369.45	364.07	364.08	64.74	0.87
VW (μm)	67.31	67.11	62.85	16.07	0.23
CD (μm)	60.11	60.34	57.20	12.09	0.28
VSA (mm^2)	0.08	0.07	0.07	0.02	0.38
V/C	6.30	6.27	6.51	1.39	0.57

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]VH, villus height; VW, villus width; CD, crypt depth; VSA, villus surface area; V/C, villus/crypt ratio.
[§]Results are presented as mean and standard error of the mean (SEM).

(33.28 g) and lower weight gain (25.48 g, 1–15 days of age) than hens fed MET and HMET ($p = 0.0002$) (Table 7).

The growth phase was monitored to evaluate the possible interaction effects between the maternal and chick diets.

There was no significant effect of interaction on chicks' performance during grower phase (Table 8). However, significant effect was observed of progeny diet on body weight at 15 and 35 days of age, on weight gain and feed conversion ratio. Chicks fed LMET diet had lower body weight at 15 ($p < 0.0001$) and 35 ($p < 0.0001$) days and worse feed conversion ratio ($p = 0.0006$) than chicks fed MET and HMET. Weight gain was higher for chicks fed MET than for those fed LMET.

In duodenum, there was significant interaction effect on villus height, villus width, villus area, and villus/crypt ratio. Among the progeny of hens fed LMET, chick fed MET or HMET showed higher duodenal villus height ($p < 0.0001$) and villus/crypt ratio ($p = 0.0086$) than chicks fed LMET. Chicks fed MET also had higher villus area ($p < 0.0001$) than chicks fed LMET (Table 9). Among progeny of hens fed MET, chicks also fed MET showed

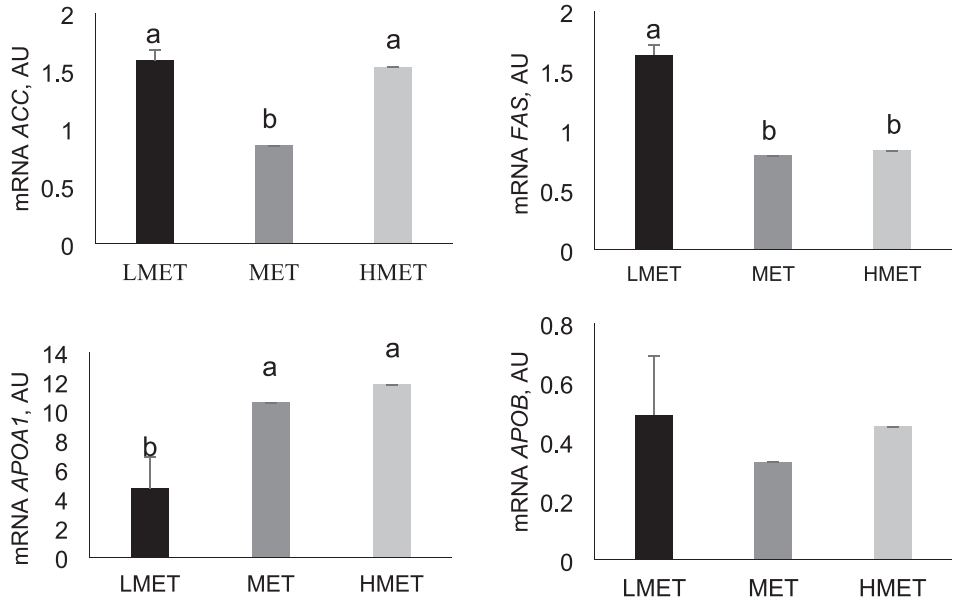

FIGURE 2 | Relative expression (arbitrary units, AU) of *ACC*, *FAS*, *APOA1*, and *APOB* genes in the liver of Japanese quail layers fed a low-methionine diet (LMET), a diet containing the recommended level of methionine (MET), or a high-methionine diet (HMET). *ACC*, acetyl-CoA carboxylase gene; *FAS*, fatty acid synthase gene; *APOA1*, apolipoprotein AI gene; *APOB*, apolipoprotein B gene. Results are presented as mean and standard error.[a,b]Different letters indicate significant differences by Tukey's test ($p < 0.05$).

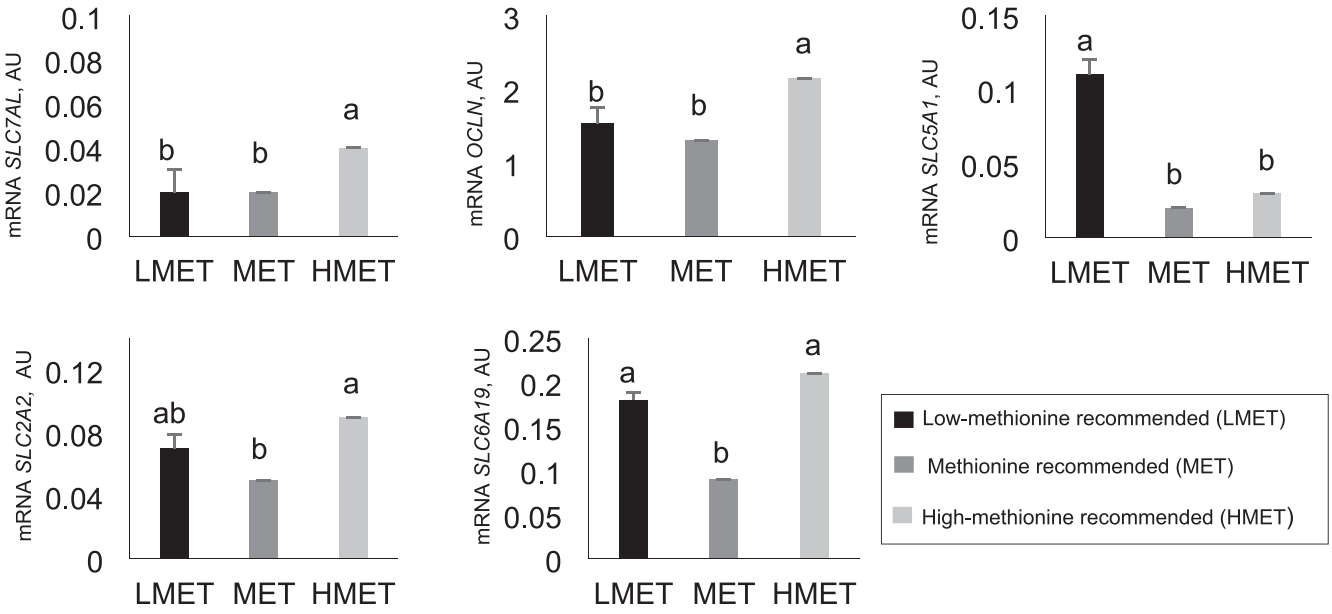

FIGURE 3 | Relative expression (arbitrary units, AU) of *SLC7A7*, *OCLN*, *SLC5A1*, *SLC2A2*, and *SLC6A19* genes in the duodenum of Japanese quail layers fed a low-methionine diet (LMET), a diet containing the recommended level of methionine (MET), or a high-methionine diet (HMET). *SLC7A7*, y + L amino acid transporter 1 gene; *OCLN*, occludin gene; *SLC5A1*, sodium/glucose cotransporter 1 gene; *SLC2A2*, glucose transporter 2 gene; *SLC6A19*, sodium-dependent neutral amino acid transporter gene. Results are presented as mean and standard error. [a,b]Different letters indicate significant differences by Tukey's test ($p < 0.05$).

the highest duodenal villus height, villus width, villus area and villus/crypt ratio (Table 9). Among chicks from hens fed HMET, increased duodenal villus width was observed in chicks fed MET or HMET. Villus area was larger in chicks fed HMET. Chicks fed LMET, on the other hand, had higher duodenal villus height and villus/crypt ratio (Table 9).

There was a significant interaction effect on villus height, villus width, crypt depth, villus area and villus/crypt ratio in jejunum. Among chicks from hens fed LMET, methionine supplementation caused an increase in jejunal villus width. Chicks fed MET ($p < 0.0001$) had higher villus area. Chicks fed HMET had a lower jejunal villus/crypt ratio ($p = 0.086$) than chicks fed MET or LMET (Table 10). Among chicks from hens fed MET, HMET-fed chicks had lower villus height ($p < 0.0001$), villus surface area ($p < 0.0001$), and villus/crypt ratio ($p = 0.0086$), whereas LMET-fed chicks had greater crypt depth (Table 10). Among the progeny of HMET-fed hens, chicks fed HMET showed higher

villus height, villus width, crypt depth, and villus surface area (Table 10).

There was a significant interaction effect on villus height, villus width, crypt depth, villus area, and villus/crypt ratio in ileum.

TABLE 7 | Effect of dietary methionine level of maternal diet on progeny performance in the starter phase (1–14 days of age).

	Experimental diet[†]				
Item[‡]	**LMET**	**MET**	**HMET**	**SEM[§]**	***p***
WH (g)	7.79	7.65	7.91	0.77	0.83
W15 (g)	33.28[b]	41.92[a]	45.69[a]	4.02	<0.01
WG (g)	25.48[b]	34.26[a]	37.96[a]	3.25	<0.01

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]WH, weight at hatch; W15, weight at 15 days; WG, weight gain.
[§]Results are presented as mean and standard error of the mean (SEM).

Among the progeny of hens fed LMET, chocks fed HMET showed higher ileal villus width, crypt depth and villus area and lower villus/crypt ratio than chicks fed LMET (Table 11). In the group of chicks from hens fed MET, those fed also MET showed higher villus width (Table 11). Among progeny of birds fed HMET, supplementation of methionine at any level led to an increase in villus height, villus width, crypt depth, villus surface area, and villus/crypt ratio compared with non-supplementation (Table 11).

The expression of genes related to glucose and amino acid transport and junction proteins in the duodenum is shown in Table 12. There was significant interaction effect on *OCLN* gene expression. For the progeny of hens fed LMET, chicks fed HMET ($p = 0.0362$) had higher *OCLN* expression than chicks fed LMET. For the progeny of hens fed MET and HMET, there was no effect of progeny diets.

There was a significant effect of maternal diet on *SLC2A2* and *SLC6A19* genes expression. *SLC2A2* expression was higher in LMET diet (p 0.0003). *SLC6A19* expression was lower in HMET ($p = 0.0277$) diet than LMET or MET diet.

TABLE 8 | Effects of dietary methionine level on progeny performance (15–35 days of age) in Japanese quail.

Hens fed	**Progeny diet**	**W15 (g)[‡]**	**W35 (g)**	**BW (g)**	**FCR**
LMET[†]	LMET	30.25	89.00	58.75	5.89
	MET	39.75	122.25	82.50	6.05
	HMET	50.20	119.40	69.20	6.12
MET	LMET	33.50	103.41	69.91	4.56
	MET	42.28	117.00	64.00	4.56
	HMET	44.33	114.97	70.72	4.18
HMET	LMET	34.90	98.65	63.75	4.40
	MET	43.37	124.37	81.00	4.98
	HMET	45.33	116.60	71.26	4.93
Mains effects					
Hens fed	LMET	40.84	110.21	70.07	4.94
	MET	40.13	111.79	71.94	5.16
	HMET	40.88	113.20	71.36	5.15
Progeny diet	LMET	33.53[b]	97.02[b]	64.00[b]	6.05[a]
	MET	42.23[a]	121.20[a]	79.00[a]	4.44[b]
	HMET	45.76[a]	116.99[a]	70.40[ab]	4.87[b]
p-value					
Hens fed		0.08	0.88	0.92	0.87
Progeny diet		<0.01	<0.01	<0.01	<0.01
Interation		0.53	0.41	0.36	0.96
SEM[§]		8.00	15.36	12.91	1.47

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]W15, weight at 15 days of age; W35, weight at 35 days of age; WG, weight gain; FCR, feed conversion ratio.
[§]Results are presented as mean and standard error of the mean (SEM).

TABLE 9 | Effects of dietary methionine level on the duodenal morphometry and absorption area of the progeny of Japanese quail layers.

		Duodenum				
Hens fed	**Progeny diet**	**VH[‡] (μm)**	**VW (μm)**	**CD (μm)**	**VSA (mm^2)**	**V/C**
LMET[†]	LMET	683.16^{c}	110.40^{ab}	75.96	0.23^{bc}	9.18^{d}
	MET	815.51^{b}	114.92^{a}	77.73	0.29^{a}	10.76^{b}
	HMET	820.66^{b}	107.08^{ab}	77.11	0.27^{ab}	10.98^{b}
MET	LMET	676.64^{c}	93.33^{c}	87.31	0.20^{c}	9.10^{d}
	MET	851.20^{b}	110.82^{ab}	82.56	0.30^{a}	10.64^{bc}
	HMET	635.66^{d}	100.42^{b}	69.59	0.20^{c}	9.28^{d}
HMET	LMET	915.47^{a}	85.75^{c}	76.66	0.24^{bc}	12.46^{a}
	MET	713.23^{c}	103.34^{ab}	76.63	0.23^{bc}	9.69^{cd}
	HMET	835.02^{b}	114.71^{a}	76.52	0.31^{a}	11.02^{b}
Mains effects						
Hens fed	LMET	777.35	111.21	77.02	0.27	10.35
	MET	721.17	101.53	79.82	0.23	9.68
	HMET	806.74	103.65	76.59	0.26	10.84
Progeny diet	LMET	743.55	95.92	81.31	0.22	10.04
	MET	791.73	109.32	79.06	0.27	10.33
	HMET	755.03	107.45	73.99	0.26	10.34
p-value						
Hens fed		<0.01	0.02	0.70	<0.01	<0.01
Progeny diet		0.03	<0.01	0.26	<0.01	0.78
Interation		<0.01	<0.01	0.38	<0.01	<0.01
SEM[‡]		137.87	28.65	35.21	0.09	2.49

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]VH, villus height; VW, villus width; CD, crypt depth; VSA, villus surface area; V/C, villus/crypt ratio.
[§]Results are presented as mean and standard error of the mean (SEM).

There was also a significant effect of progeny diet on *SLC2A2* gene expression. The highest value was observed in MET diet ($p = 0.0084$). There was difference between LMET and HMET diet.

The expression of genes related to lipid metabolism in the liver of progeny is shown in Table 13. There was interaction effect on *APOB* gene expression. For the progeny of hens fed HMET, chicks fed MET ($p = 0.0046$) had higher *APOB* expression than chicks fed LMET and HMET.

There was a significant effect of maternal diet on *APOA1* gene expression: higher expression was observed in MET diet ($p = 0.0188$).

There was significant effect of progeny diet on *ACC* ($p = 0.0111$), *FAS* ($p = 0.0024$), and *APOA1* ($p < 0.0001$) genes expression. Chicks fed LMET had higher expression of these genes than chicks fed HMET diet.

4 | Discussion

In this study, hens fed diets containing methionine at the recommended level (MET) or higher (HMET) showed better performance during the egg-laying phase. Previous studies revealed that methionine supplementation has a positive effect on hen performance, increasing laying rate (Alagawany et al. 2016), egg weight, nutrient conversion efficiency, and egg quality (Xiao et al. 2017). These findings show that supplementation of laying hens with methionine improves egg production and quality (Sumiati and Wiryawan 2016). The beneficial effect of methionine supplementation is partially due to the fact that the amino acid increases protein deposition, thereby enhancing egg production, egg weight, and egg mass (Reda et al. 2019).

The positive effect of methionine supplementation on reproductive performance may be related to the increased feed conversion efficiency. Animal performance is dependent on several

TABLE 10 | Effects of dietary methionine level on the jejunal morphometry and absorption area of the progeny of Japanese quail layers.

		Jejunum				
Hens fed	**Progeny diet**	**VH[‡] (μm)**	**VW (μm)**	**CD (μm)**	**VSA (mm^2)**	**V/C**
LMET[†]	LMET	563.27^{ab}	75.41^{c}	61.78^{c}	0.13^{c}	9.34^{a}
	MET	530.39^{b}	92.17^{a}	65.15^{c}	0.15^{ab}	8.41^{a}
	HMET	497.02^{b}	86.40^{ab}	67.63^{bc}	0.13^{bc}	7.51^{cd}
MET	LMET	528.49^{b}	83.79^{b}	70.31^{b}	0.14^{bc}	7.65^{cd}
	MET	517.65^{b}	79.80^{b}	64.09^{c}	0.13^{c}	8.23^{bc}
	HMET	439.48^{b}	79.40^{c}	60.18^{c}	0.11^{d}	7.48^{d}
HMET	LMET	511.59^{b}	91.77^{ab}	65.67^{bc}	0.14^{bc}	7.95^{bcd}
	MET	507.49^{b}	83.47^{bc}	67.59^{bc}	0.13^{c}	7.78^{bcd}
	HMET	577.51^{a}	93.00^{a}	76.53^{a}	0.17^{a}	7.71^{bcd}
Mains effects						
Hens fed	LMET	530.24	85.41	64.88	0.14	8.42
	MET	495.18	80.10	64.86	0.12	7.79
	HMET	535.37	89.05	70.58	0.15	7.79
Progeny diet	LMET	533.37	83.69	66.72	0.14	8.19
	MET	517.63	84.64	65.64	0.14	9.12
	HMET	505.85	86.24	68.19	0.14	7.58
p-value						
Hens fed		<0.01	<0.01	<0.01	<0.01	<0.01
Progeny diet		0.01	0.72	0.25	0.94	0.01
Interation		<0.01	<0.01	<0.01	<0.01	<0.01
SEM[§]		83.31	20.07	12.02	0.04	1.71

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[§]VH, villus height; VW, villus width; CD, crypt depth; VSA, villus surface area; V/C, villus/crypt ratio.
Results are presented as mean and standard error of the mean (SEM).

factors, including nutrient absorption and utilization. Dietary nutrients are digested and absorbed in the intestine, contributing to intestinal flora regulation, epithelium maintenance, and bird development, performance, and health. For adequate feed digestion and nutrient absorption, the integrity, morphology, functionality, and structure of the intestinal mucosa must be maintained (Khatlab et al. 2019).

Here, it was observed that birds fed MET had better reproductive performance, feed conversion per egg mass, and duodenal and jejunal crypt depth. Crypt depth is associated with cell regeneration, such as that of enterocytes that constitute villi. The greater the number of cells produced, the greater the size of villi, and, consequently, the greater the nutrient absorption area (Moraes et al. 2021). In addition to intestinal morphology, nutrient absorption depends on the action of various transporters present in the apical and basolateral membrane of enterocytes. Transporters are responsible for the entry of nutrients into intestinal cells and, subsequently, from within cells to the bloodstream (Garber et al. 2003; Osmanyan et al. 2017). Studies have shown that nutrient deficiencies, such as methionine deficiency, can alter the expression of transporters, affecting cell function and metabolism (Zhang et al. 2017). In the current study, *SLC7A7* and *OCLN* expression was increased in the duodenum of HMET-fed hens. *SLC7A7* encodes y+LAT1 transporter, an amino acid transporter belonging to the y+L system, a member of the SLC7 family of transporters. The transporter is present in the basolateral membrane of enterocytes and has high affinity for cationic and neutral amino acids (Bröer 2008; Bröer and Palacín 2011). y+LAT1 mediates the efflux of cationic amino acids from the intracellular medium into the bloodstream, in exchange for the influx of neutral amino acids from the bloodstream into the intracellular medium (Krehbiel and Matthews 2003). *OCLN* encodes the junction protein occludin. Occludin is part of the occluding junctions, which form a seal between adjacent enterocytes near the apical surface. The protein is crucial for regulation of paracellular permeability and inter-membrane diffusion (Awad, Hess, and Hess 2017). Studies have shown that challenged birds have low levels of junction proteins (Su

TABLE 11 | Effects of dietary methionine level on the ileal morphometry and absorption area of the progeny of Japanese quail layers.

		Ileum				
Hens fed	**Progeny diet**	**VH[‡] (μm)**	**VW (μm)**	**CD (μm)**	**VSA (mm^2)**	**V/C**
DM[†]	DM	345.82^{c}	67.14^{c}	55.75^{c}	0.07^{ef}	6.33^{ab}
	DL-Met1	351.13^{c}	72.30^{bc}	56.02^{c}	0.08^{cde}	6.42^{ab}
	DL-Met2	355.45^{c}	81.95^{a}	65.61^{ab}	0.09^{bcd}	5.58^{cd}
DL-Met1	DM	397.79^{b}	65.19^{c}	64.81^{ab}	0.09^{bcde}	6.32^{ab}
	DL-Met1	372.73^{bc}	81.52^{a}	63.45^{ab}	0.09^{bc}	6.09^{ab}
	DL-Met2	364.35^{c}	70.00^{c}	61.68^{b}	0.08^{de}	6.02^{ab}
DL-Met2	DM	297.89^{d}	67.96^{c}	58.26^{c}	0.06^{f}	5.23^{d}
	DL-Met1	367.61^{bc}	82.17^{a}	63.52^{ab}	0.09^{b}	5.87^{bc}
	DL-Met2	442.01^{a}	80.23^{ab}	67.89^{a}	0.11^{a}	6.47^{a}
Mains effects						
Hens fed	DM	350.83	73.65	58.82	0.08	6.14
	DL-Met1	378.29	72.24	63.32	0.08	6.15
	DL-Met2	380.14	78.15	63.99	0.09	5.96
Progeny diet	DM	356.37	66.48	60.56	0.07	6.03
	DL-Met1	364.73	79.12	61.35	0.09	6.11
	DL-Met2	392.17	76.69	64.97	0.09	6.10
p-value						
Hens fed		<0.01	0.03	0.02	<0.01	0.43
Progeny diet		<0.01	<0.01	<0.01	<0.01	0.83
Interation		<0.01	0.01	<0.01	<0.01	<0.01
SEM[§]		78.65	19.40	12.61	0.03	1.33

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]VH, villus height; VW, villus width; CD, crypt depth; VSA, villus surface area; V/C, villus/crypt ratio.
[§]Results are presented as mean and standard error of the mean (SEM).

et al. 2014). However, methionine supplementation can raise *OCLN* expression, thereby improving intestinal barrier function and preventing pathogen entry (Del Vesco et al. 2020; Barekatain et al. 2019).

Laying hens fed the methionine-deficient diet showed higher *SLC5A1* expression. *SLC5A1* encodes sodium glucose transporter 1, which is responsible for the uptake of glucose into the apical membrane of enterocytes. According to Harmad et al. (2010), the high *SLC5A1* expression and increased glucose absorption capacity observed in animals fed amino acid-deficient diets might be related to an increase in energy demand in intestinal cells, peripheral tissues, or both. Hens fed LMET and HMET showed higher *SLC2A2* expression. The protein is located at the basolateral membrane of the gut epithelium and is involved in glucose efflux (Shibata et al. 2019). *SLC6A19* expression was also higher. The gene encodes B^0AT, responsible for apical resorption of neutral amino acids (Fagundes et al. 2020). Interestingly, hens fed LMET and HMET showed the same expression patterns for *SLC2A2* and *SLC6A19* and also the same pattern of feed conversion per egg mass and follicle weight. These results indicate that not only deficiency but also excess of methionine can alter nutrient absorption, affecting the availability of nutrients and energy in intestinal cells and peripheral tissues.

The findings demonstrated that methionine supplementation increased the hatchability of fertile eggs. As shown in previous research (Xiao et al. 2017), methionine plays a key role in increasing reproductive performance, fertility rate, and hatchability. According to Bunchasak, Ratchadapornvanitch, and Thiengtham (2012), such effects are promoted by an increase in the antioxidant capacity and nutrient availability in embryos via the transfer of methionine from the maternal metabolism to eggs, benefiting embryo development.

Lipid metabolism in the liver is increased with the onset of sexual maturity and laying via the action of estrogen (Ren et al. 2021). Lipids are then transported to the ovary by very low-density lipoproteins (VLDLs) to ensure efficient fertile

TABLE 12 | Effects of dietary methionine level on the relative expression (arbitrary units) of *SLC2A2*, *SLC5A1*, *SLC7A7*, *SLC6A19*, and *OCLN* genes in the duodenum of the progeny of Japanese quail layers.

Hens fed	Progeny diet	*SLC2A2*[‡]	*SCL6A19*	*SLC7A7*	*SLC5A1*	*OCLN*
LMET[†]	LMET	0.13	0.07	0.03	0.23	0.84[b]
	MET	0.29	0.07	0.04	0.31	1.00[ab]
	HMET	0.22	0.07	0.05	0.22	1.12[a]
MET	LMET	0.09	0.06	0.02	0.21	0.49[c]
	MET	0.13	0.08	0.03	0.29	0.69[bc]
	HMET	0.15	0.07	0.02	0.27	0.60[c]
HMET	LMET	0.06	0.04	0.01	0.19	0.41[cd]
	MET	0.17	0.07	0.01	0.23	0.60[c]
	HMET	0.07	0.02	0.03	0.21	0.23[d]
Mains effects						
Hens fed	LMET	0.21[a]	0.07[a]	0.04	0.25	0.98
	MET	0.12[b]	0.07[a]	0.02	0.26	0.59
	HMET	0.10[b]	0.04[b]	0.02	0.21	0.41
Progeny diet	LMET	0.09[b]	0.05	0.02	0.21	0.58
	MET	0.20[a]	0.07	0.03	0.27	0.76
	HMET	0.14[ab]	0.05	0.03	0.23	0.64
p-value						
Hens fed		<0.01	0.02	0.07	0.52	<0.01
Progeny diet		<0.01	0.21	0.36	0.35	0.02
Interation		0.37	0.53	0.69	0.90	0.03
SEM[§]		0.07	0.02	0.02	0.10	0.15

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]*SLC2A2*, glucose transporter 2 gene; *SLC5A1*, sodium/glucose cotransporter 1 gene; *SLC7A7*, y + L amino acid transporter 1 gene; *SLC6A19*, sodium-dependent neutral amino acid transporter gene; *OCLN*, occludin gene.
[§]Results are presented as mean and standard error of the mean (SEM).

egg production and embryo development (Zhu et al. 2020). This is a finely controlled mechanism, given that egg fat is the major energy source of embryos (Speake, Noble, and Murray 1998). Here, methionine supplementation increased *APOA1* expression. The gene encodes the main protein component of high-density lipoprotein (HDL) and VLDL. Thus, APOA1 is associated with HDL, promoting the exit of cholesterol from peripheral extrahepatic cells to the liver, where it is metabolized (Xiao et al. 2023). The protein, along with VLDL, is also associated with the transport of liver lipids to peripheral tissues. The high *APOA1* expression in methionine-supplemented quail may suggest greater lipid availability for egg yolk deposition, contributing to increased follicle weight and egg hatchability. Because of its participation in LDL synthesis in birds, increased expression of *APOA1* suggests lower abdominal fat deposition (Yadav and Jha 2019). Here, supplemented birds also had lower expression of genes encoding acetyl-CoA carboxylase and fatty acid synthase, responsible for lipid synthesis.

The positive effect of methionine supplementation was also observed during the grower phase of progeny since methionine supplementation in progeny diet resulted into higher body weight at 35 days and weigh gain, better feed conversion ratio, and higher *SLC2A2* gene expression.

All nutrients necessary for bird development and growth must be provided by the maternal metabolism. Thus, any initial change in nutrient availability can have important effects on progeny growth and health (Cherian 2015). Maternal supplementation with methionine had positive effects on progeny performance in the starter phase: chicks from hens fed methionine-supplemented diets had higher weight gain (1–15 days of age) and body weight at 15 days of age than chicks from birds fed LMET. The effect of maternal diet on early progeny development might have been due to the better embryonic environment. Increased nutrient deposition in eggs is associated with increased dietary intake of nutrients (Dixon, Sparks, and Rutherford 2015). The results suggest that maternal diets

TABLE 13 | Effects of dietary methionine level on the relative expression (arbitrary units) of lipid metabolism genes in the liver of the progeny of Japanese quail layers.

Hens fed	Progeny diet	*ACC*[‡]	*FAS*	*APOA1*	*APOB*
LMET[†]	LMET	1.81	7.59	0.71	0.12[c]
	MET	1.67	3.98	0.13	0.31[b]
	HMET	1.51	2.09	0.42	0.21[bc]
MET	LMET	2.85	6.25	0.98	0.13[c]
	MET	2.80	3.98	0.38	0.27[bc]
	HMET	1.14	1.91	0.46	0.12[c]
HMET	LMET	1.85	5.50	0.53	0.16[bc]
	MET	1.81	4.16	0.27	0.67[a]
	HMET	1.53	1.93	0.47	0.12[c]
Mains effects					
Hens fed	LMET	1.66	4.67	0.48[b]	0.19
	MET	2.15	6.71	0.65[a]	0.15
	HMET	1.71	3.81	0.46[b]	0.25
Progeny diet	LMET	2.17[a]	8.66[a]	0.74[a]	0.13
	MET	2.09[ab]	4.04[ab]	0.26[b]	0.41
	HMET	1.39[b]	1.98[b]	0.45[b]	0.15
p-value					
Hens fed		0.15	0.46	0.01	0.08
Progeny diet		0.01	<0.01	<0.01	<0.01
Interation		0.10	0.40	0.06	<0.01
SEM[§]		0.60	4.11	0.15	0.09

Note: [a,b]Means within rows followed by different letters are significantly different by Tukey's test ($p < 0.05$).
[†]LMET, low-methionine diet; MET, diet containing the recommended level of methionine; HMET, high-methionine diet.
[‡]*ACC*, acetyl-CoA carboxylase gene; *FAS*, fatty acid synthase gene; *APOA1*, apolipoprotein A1 gene; *APOB*, apolipoprotein B gene.
[§]Results are presented as mean and standard error of the mean (SEM).

are essential to promote adequate embryo development and, consequently, enhance chick performance during the starter phase (Surai, Fisinin, and Karadas 2016). The nutritional status of hens is an important factor in the regulation of progeny development (Surai 2000), given that the maternal environment influences offspring growth. Therefore, hatchling weight and progeny growth may be negatively affected by maternal stress (Lordi et al. 2000; Tao et al. 2012).

Maternal supplementation, particularly at the high level (HMET), was able to improve the histomorphometric parameters of the progeny at grower phase. The best results were observed in HMET-fed chicks from HMET-fed hens. The effect of maternal methionine supplementation on the histomorphological parameters of offspring is still poorly understood. According to Azad et al. (2018), methionine supplementation can improve progeny intestinal morphology by increasing villus height, crypt depth, and villus/crypt ratio. Thus, it can be said that modulation of maternal diets can influence the intestinal functionality, barrier, and health of progeny (Xiao et al. 2023).

There was a a significant effect of maternal diet on *SLC2A2* and *SLC6A19* genes expression. *SLC2A2* expression was higher in LMET diet. *SLC6A19* expression was lower in HMET diet than in LMET or MET diet. The effect of progeny diet on the expression of gene related to lipid metabolism (*APOB*) and membrane transporters (*OCLN*) was also dependent on maternal diet. Studies have shown that maternal nutrition is one of the main factors influencing progeny phenotype via modifications in transcriptional agents (Lv et al. 2019). Hens provide the first environment experienced by offspring before and after hatching, having the ability to create and regulate prenatal environment and thereby influencing progeny development (Afrouziyeh, Zukiwisky, and Zuidhof 2021). Overall, it was found that, during the growth phase, the negative effect of methionine restriction in progeny diet was mitigated by maternal methionine supplementation, probably because supplementation of hens during laying was sufficient to ensure proper nutrient transfer to eggs.

Our results indicate that methionine supplementation of laying hens improves the intestinal environment and ensures better reproductive performance, in addition to enhancing progeny performance in the starter phase. During the growth phase, the effect of progeny diet seems to be dependent on maternal diet, in that the progeny of methionine-supplemented hens are able to respond better to their own environment.

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Conflicts of Interest

The authors declare no conflicts of interest.

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