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Mob: +92 300 3008585, Fax: +92 41 8815544
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Research Article
The Digestible Methionine and Cystine Requirements for Commercial Layers

[1]T. Cao, [2]J.T. Weil, [2]P. Maharjan, [3]J. Lu and [2]C.N. Coon

[1]Noveltech, LLC., 16600 43rd Ave N., Minneapolis, MN 55446, United States
[2]Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701, United States
[3]Syngenta, 410 S. Swing Rd., Greensboro, NC 27409, United States

Abstract

Background and Objective: Two experiments were conducted to determine the total sulfur amino acid requirements in laying hens. The objective of Experiment 1 was to determine the digestible methionine and cystine requirements for laying hens. An additional experiment (Experiment 2) was conducted to determine the cystine requirement for laying hens and determine the utilization efficiencies of supplemental methionine and cystine to meet the cystine requirement. **Materials and Methods:** In Experiment 1, one hundred and seventy-six laying hens were randomly assigned into 11 dietary treatments for a six-week period. One group of hens received a corn-soybean meal control diet containing 2,899 kcal ME kg^{-1} and 19.5% CP, while the remaining ten groups of hens received 10 test diets containing 2,850 kcal ME kg^{-1} and 15% CP. Five diets were deficient in cystine (0.148% digestible cystine), containing digestible methionine levels of 0.143, 0.240, 0.337, 0.434 and 0.531% and another five diets were excessive in cystine (0.450% digestible cystine), containing digestible methionine levels of 0.143, 0.231, 0.317, 0.407 and 0.495%. An additional experiment (Experiment 2) was conducted by assigning one hundred sixty laying hens to one of two series of diets, which were formulated to contain 0.319% digestible methionine and 0.148% digestible cystine, same as that in Experiment 1, with exception of the methionine level. Four levels of equimolar amounts of methionine or cysteine (½ cystine) were added to the basal diet. The added levels were 0.05, 0.10, 0.15 and 0.20% for methionine and 0.04, 0.08, 0.12 and 0.16% for cystine since the molecular weight of cysteine (½ cystine) is 80% of that of methionine. Data generated from each experiment was analyzed using the general linear models (GLM) and analysis of variance procedures with the help of statistical analysis software (SAS). A second-order polynomial regression analysis was conducted in order to determine the methionine requirements for laying hens. **Results:** The results showed that the requirement of digestible methionine and digestible cysteine for laying hens were 354 and 184 mg hen^{-1} day^{-1} for egg mass (EM), 349 and 193 mg hen^{-1} day^{-1} for feed conversion, 437 and 325 mg hen^{-1} day^{-1} for body weight change (BWC) and 367 and 189 mg hen^{-1} day^{-1} for EM+BWC, respectively. Deficient or excessive dietary methionine produced an increase of methionine degradation due to the increased body weight loss or the excessive dietary methionine, correspondingly. Optimum dietary methionine levels resulted in increased liver SAM/SAH concentration ratios (s-adenosylmethionine/s-adenosylhomocysteine) and decreased homocysteine (Hcy) levels. **Conclusion:** The results demonstrated that the utilization efficiencies of methionine and cysteine (½ cystine) were 100% on an equimolar basis for egg mass and 90% on an equimolar basis to prevent loss of body weight. When methionine was used to meet the cystine requirement, an utilization efficiency of 80% was adequate on a weight and concentration basis for egg mass and 72% for body weight maintenance. The practice of feeding ingredients with a substantial digestible cystine level for supporting body weight may be beneficial for laying hens.

Key words: Methionine, cystine, egg production, laying hens, poultry diet

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Corresponding Author: C.N. Coon, Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701, United States
Tel: 479-575-4134 Fax: 479-575-8755

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The sulfur amino acid requirement of laying hens has been traditionally expressed as a methionine requirement and total sulfur amino acid (TSAA) requirement[1]. The concept of the requirement implies that the demand for sulfur amino acids of laying hens can be satisfied with either methionine alone or through the combination of methionine and cystine. This is supported by the fact that methionine can be endogenously converted to cysteine whereas the opposite is not possible because of a lack of enzymes[2,3]. However, due to the limited or lack of information that exists about the efficiency of methionine conversion to cysteine, the quantitative relationship of cystine for sparing methionine and the utilization efficiency of supplemental methionine and cystine, there is confusion about the reported requirement.

In recent years, the indicator amino acid oxidation method has been used to determine the methionine requirement in broiler chicks, humans and farm animals such as lambs[4-6]. However, a frequently used method to determine the methionine requirement in laying hen is still a traditional one, which involves adding graded levels of methionine to a basal diet deficient in the amino acid[7]. The methionine requirement for laying hens has been reviewed at several occasions over time and reported to be in a range of 250, 320, 400, 440 and 451 mg hen^{-1} day^{-1} and at least 356 mg hen^{-1} day^{-1} with variable levels of dietary cystine[8-13]. Noticeably, Coon and Zhang[14] recommended a cystine inclusion of 216 mg hen^{-1} day^{-1}, while Bregendahl *et al*.[15] reported a higher requirement of 253 mg hen^{-1} day^{-1}. The National Research Council has reported different methionine requirements for laying hens over time: 280 mg hen^{-1} day^{-1} in 1977 and 300 mg hen^{-1} day^{-1} in 1994[1,16]. Although, many factors such as protein levels, choline and energy level could affect the methionine requirement of laying hens, the primary reason for the differences among reports can be related to dietary cystine levels. In order to eliminate the confusion on methionine requirement caused by dietary cystine levels, the methionine requirement should be, by definition, the amount of methionine needed only to satisfy the need for biological functions of methionine whereas the cystine function is satisfied by dietary cystine alone[12,17,18]. Therefore, the methionine requirement of laying hens should be determined with diets sufficient in cystine so that no extra methionine is needed for the biological function of cystine. Moreover, the key metabolites and enzyme activities involved in methionine metabolism (Fig. 1) should be determined in this type of study[2]. However, no such work has been reported in previous studies on the methionine requirement in laying hens.

A typical experiment for determining the TSAA requirement involves the addition of graded levels of methionine to a basal diet deficient in both methionine and cystine. The deficiency of cystine in test diets is crucial for the determination of the TSAA requirement since extra cystine in test diets can inflate the TSAA requirement[19-22]. Additionally, the amino acids other than that of interest, should be included in the diet at a rate of at least 90% of the amino acid recommendation[23]. Currently, there is no information on the efficiency of transsulfuration reactions in laying hens in the literature. Also, cysteine is not the only metabolic outlet for dietary methionine[2,3,24-26]. The efficiency of converting methionine to cysteine has presented another problem to use the TSAA requirement in practice. Due to the molecular weight difference between methionine and cysteine, the efficiency of utilizing methionine for cysteine on a weight or concentration basis is in the order of 80%, whereas an assumption of 100% molar efficiency is frequently used[19-22]. Moreover, utilization efficiencies of supplemental methionine and cystine for meeting the TSAA requirement were not equal, especially when the basal diet was limited in methionine[27]. In order to eliminate the confusion, separation of methionine and cystine requirements is needed. Kalinowski *et al*.[28,29] separately determined the methionine and cystine requirement of slow- and fast-feathering broiler males by using a corn-soybean basal diet. However, no enzymes activities and metabolites involved in methionine and cystine metabolism were determined in the aforementioned study.

The reported experiments herein were conducted to determine digestible methionine and digestible cystine requirements of laying hens for optimum production and to determine the utilization efficiency of supplemental methionine and cystine for the cystine requirement of laying hens. At same time, levels of key metabolites and enzymes activities involved in methionine metabolism were also obtained.

MATERIALS AND METHODS

All procedures regarding the use of live animals in this study were conducted in accordance with the University of Arkansas Institutional Animal Care and Animal Use Protocol #03008.

Experiment 1

Experimental birds, bird care and diets: One hundred and seventy-six 29-week old Dekalb-XL laying hens were randomly assigned to 44 cages (49.9 cm deep and 48.9 cm wide), four

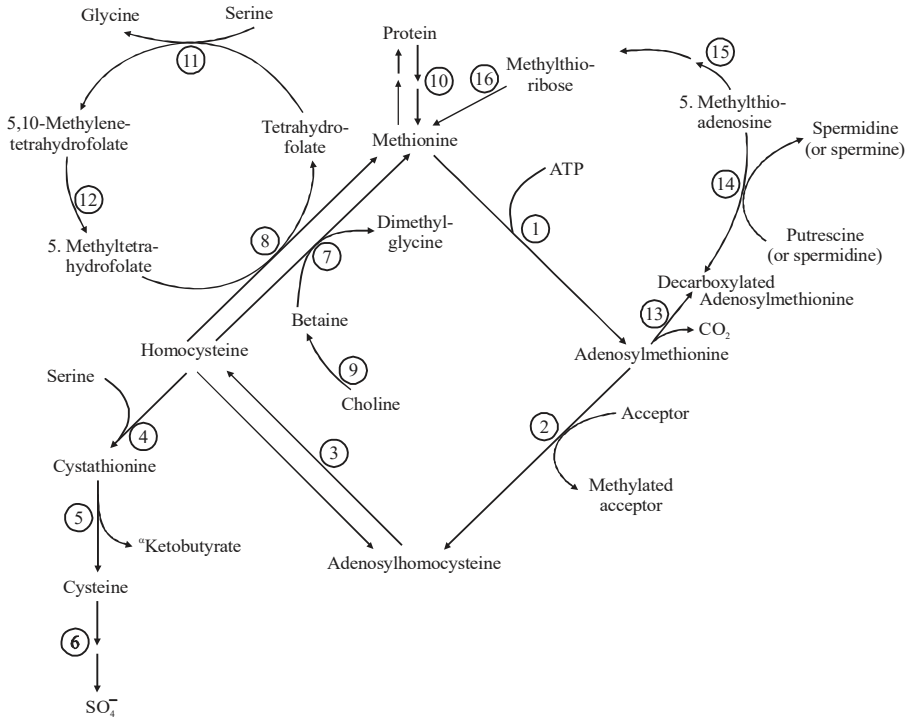

Fig. 1: Methionine metabolism in mammals

The numbers represent the following enzymes or sequences, 1: Methionine adenosyltransferase (EC 2.5.1.6), 2: AdoMet-dependent transmethylations, 3: Adenosylhomocysteinase (EC 3.3.1.1), *4* cystathionine-b-synthase (EC 4.2.1.22), 5: g-cystathionase (EC 4.4.1.1), 6: Further metabolism of cysteine, 7: Betaine-homocysteine methyltransferase (EC 2.1.1.5), 8: Methylfolate-homocysteine methyltransferase (EC 2.1.1.13), 9: Choline+betaine aldehyde dehydrogenases (EC1.1.99.1 and EC1.2.1.8), 10: Equilibrium between free and protein methionine, 11: Serine hydroxymethylase (EC 2.1.2.1), 12: Methylenetetrahydrofolate reductase (EC 1.7.99.5), 13: AdoMet decarboxylase (EC 4.1.1.50), 14: Spermidine (spermine) synthase (EC 2.5.1.16 and EC 2.5.1.22), 15-16: Methylthioadenosine phosphorylase (EC 2.4.2.28)+methionine formation via methylthioribose-1-phosphate. Pathway drawing of methionine and cysteine metabolism is from the publication of Finkelstein[2]

hens per cage. Hens from four cages received one of the 11 dietary treatments for a six-week period. Feed and water were provided *ad libitum*. The housing temperature was maintained at 21°C. The hens were provided 15 h of incandescent light (1 foot candle, 6:00 am to 9:00 pm) and a 9-hour dark period (9:00 pm to 6:00 am) each day for the first week of the experiment. The light period of a day was increased 15 min weekly until 16 h light a day was reached.

Two series of diets were mixed with either low cystine (0.148% digestible) or high cystine (0.416% digestible) levels in a basal diet containing 2,850 kcal ME kg^{-1}, 15% CP, 0.143% digestible methionine and 0.148% digestible cystine (Table 1). The basal diet was formulated to be equal to or in excess of NRC[1] requirements for all nutrients except methionine and cystine. Additions of methionine and cystine were made to prepare a 0.416% digestible cystine diet series consisting of five levels of digestible methionine (0.143, 0.231, 0.317, 0.407 and 0.495%, respectively) and a 0.148% digestible cystine diet series consisting five levels of digestible methionine (0.143, 0.240, 0.337, 0.434 and 0.531%, respectively). A control diet containing 2,899 kcal ME kg^{-1} and 19.5% CP (Table 1) was fed to one group of hens. Analysis for percent digestible amino acids for methionine and cysteine in diets was performed as discussed in Ekmay *et al*.[30].

Experiment 2

Experimental birds, bird care and diets: One hundred and sixty 35-week old Dekalb-XL laying hens were randomly assigned to 40 cages (49.9 cm deep and 48.9 cm wide), four hens per cage. Hens from four cages received one of the 10 dietary treatments for a six-week period. Feed and water were provided *ad libitum*. The housing temperature was maintained at 21°C. The hens were provided 16 h of incandescent light (1 foot candle) (6:00 am to 10:00 pm) and a 8 h dark (10:00 pm to 6:00 am) period each day during the experiment.

Two series of diets were formulated by either adding methionine or cystine to the basal diet containing 0.319% digestible methionine and 0.148% digestible cystine, same as that in Experiment 1 (Table 1) with exception of methionine level. Four levels of equimolar amounts of methionine or cysteine (½ cystine) were added to the basal diet. The added

Table 1: Diet composition of nutrients and ingredients

Ingredients and analysis	Control diet (%)	Basal diet (%)
Corn, ground	49.342	66.438
Soybean meal (47% protein)	32.320	5.701
Limestone, shell and bone builder[a]	4.955	5.000
Limestone, unical F[b]	4.955	4.506
Dicalcium phosphate (18.5%)	1.720	1.729
Sodium bicarbonate	0.272	0.196
Salt	0.189	0.200
Vitamin premix[c]	0.050	0.050
Mineral premix[d]	0.065	0.065
Choline chloride (50%)	0.085	0.085
Animal fat	5.842	3.000
Solk-a-floc[e]	---	3.983
Builder sand	---	1.992
Ethoxyquin	0.017	0.017
DL-Methionine	0.188	---
L-Lysine (78.4%)	---	0.559
Tryptophan (98.5%)	---	0.115
Threonine (98%)	---	0.374
Leucine	---	0.476
Isoleucine	---	0.478
Valine	---	0.420
Phenylalanine	---	0.415
Histidine	---	0.137
Arginine monohydrochloride	---	0.608
Glycine	---	0.074
Glutamic acid	---	1.348
Aspartic acid	---	1.220
Alanine	---	0.816
Total	100.000	100.000
Nutrient analysis		
ME (kcal kg^{-1})	2.899	2.850
CP	19.500	15.000
Calcium	4.205	4.000
Available P	0.451	0.400
Sodium	0.180	0.160
Digestible methionine	0.500	0.143
Digestible cystine	0.310	0.148

[a]Limestone, Shell and Bone Builder, had mean particle size of 3260.61 microns and consisted of 39.63% Ca (Shell and Bone Builder, ILC Resources, Des Moines, Iowa). [b]Limestone, Unical F, had mean particle size of 2166.85 microns and consisted of 39.53 % Ca (Unical F, ILC Resources, Des Moines, Iowa). [c]The vitamin premix provided the following per kilogram of diet; Vitamin A: 3,300 IU, Vitamin D3: 3,307 IU, Vitamin E: 40.6 IU, Vitamin K: 1.46 mg, Pantothenic acid: 23.06 mg and Vitamin B12: 0.017 mg. [d]The mineral premix provided the following in milligram per kilogram of diet; Mn: 91.88, Fe: 0.027, Zn: 82.65, Cu: 9.48 and I: 1.04. [e]Nonnutritive filler (cellulose), Brown Co., Berlin, NH

levels were 0.05, 0.10, 0.15 and 0.20% for methionine and 0.04, 0.08, 0.12 and 0.16% for cystine since the molecular weight of cysteine (½ cystine) is 80% of that of methionine. The control diet (Table 1) was also fed to two groups of hens. The objective of the experiment was to study the utilization efficiencies of supplemental methionine and cystine.

Production parameters and sampling: In both Experiments 1 and 2, feed intake and body weight gain were determined every two weeks. Egg production was determined daily. Egg weight was determined with one day's egg collection each week. At the end of each experiment, six hens from each dietary treatment, excluding the control treatment, were sacrificed at 3:00 pm by carotid exsanguination. Immediately after removal, liver samples were frozen and stored in liquid nitrogen for further processing.

Enzyme and metabolite assays: The enzyme extracts were prepared following the procedure introduced by Finkelstein and Martin[31]. A Tissuemizer® was used at 85% maximum speed for 1 min to homogenize liver samples in 4 volumes of ice-cold 1 mM potassium phosphate, pH 7.5. After the homogenate was centrifuged at 27,000 g for 20 min at 4°C, the supernatant was decanted into Sephadex G-25 columns for the removal of small molecular weight solutes through filtration. The protein-containing fraction was then lyophilized and stored at -40°C. All enzyme assays for each experiment were conducted in less than 10 days, which is the maximum time that enzyme activity has been shown to remain unchanged[31,32]. Immediately before conducting the enzyme assay, the enzyme extract was reconstituted with water and the final volume was such that 0.5 mL was the equivalent of 1 g of wet tissue. The enzyme activities were determined with the published methods described by Mudd *et al.*[32] for methionine s-adenosyltransferase (EC 2.5.1.6; MAT) and cystathionine ß-synthase (EC 4.2.1.22; CS); by Mudd *et al.*[33] and Finkelstein *et al.*[34] for N5-methyltetrahydrofolate-homocysteine methyltransferase (EC 2.1.1.13; MFMT) and by Finkelstein and Mudd[35] with a modification in determining the product formed in the assay[36] for betaine-homocysteine methyltransferase (EC 2.1.1.5; BHMT).

The metabolite extract was prepared following the procedure described by Cao and Coon[36]. Three volumes of 0.2 M $HClO_4$ were added to liver tissue and homogenized using a Tissuemizer® at 85% maximum speed for 1 minute at 4°C. After the homogenate was centrifuged at 27,000 g for 10 min, the supernatant was filtered through a 0.22 µm Millex-GS Millipore filter. The filtrate was treated for blocking thio-group of metabolites[37]. Fifty µL of 250 mM 2-mercaptoethanol in 700 mM $HClO_4$ and 67 µL of 6 N NaOH were added to 767 µL of filtrate sample. After mixing, 50 µL of 200 mM iodoacetic acid was added to each sample. After 30 min, 66 µL of 6 N HCl was added to each sample. Concentrations of methionine (Met), SAM, SAH, homocysteine and cysteine (Cys) were then analyzed for each treated sample by HPLC[37]. Due to the reaction of blocking thio-groups of metabolites that occurred due to the presence of 2-mercaptoethanol, the metabolites of oxidized forms could

proceed to their corresponding reduced forms. Therefore, the determined concentration was the total amount of metabolite including both oxidized and reduced forms.

Statistical analysis: Data were analyzed using the General Linear Models and one-way analysis of variance (ANOVA) procedures with the help of Statistical Analysis System (SAS[38]). Differences of $p<0.05$ were considered statistically significant. Differences between treatment means were compared by Duncan's Multiple range test. Methionine requirements with high or low cystine levels for laying hens were determined as 95% of the asymptote from the second-order polynomial regression analysis. The cystine requirement was obtained by the difference between two methionine requirements with high or low cystine level.

RESULTS AND DISCUSSION

Experiment 1: The effects of dietary digestible methionine and digestible cystine levels on laying performance determined in Experiment 1 are presented in Table 2. The egg production, egg weight, egg mass, feed intake, feed conversion and body weight gain were significantly influenced by dietary methionine levels. Hens on the high cystine (0.416% digestible) diets, containing 0.317% or greater digestible methionine and hens on the low cystine (0.148% digestible) diets, containing 0.337% or greater digestible methionine, demonstrated an equal amount of egg production to that of hens on the corn-soybean meal control diet. The high cystine (0.416% digestible) diets with variable methionine levels were used to determine the methionine requirement of laying hens since 0.416% digestible cystine is thought to be adequate or in excess for laying hens. Harms and Russell[39] reported the commercial layer had a cystine requirement about 175 mg hen^{-1} day^{-1} or 3.19 mg for 1 g of egg mass. Schutte *et al.*[40] reported that the cystine requirement of laying hens was 372.2 mg hen^{-1} day^{-1} when producing 54.7 g egg mass per hen per day.

Hens fed the high cystine (0.416% digestible) diets showed significant increases of egg production, egg weight, egg mass, feed intake and body weight gain and improved feed conversion when the dietary digestible methionine level was increased from 0.143-0.317% (Table 2). Further increases of dietary methionine levels resulted in non-significant changes of laying performance, as observed in other studies[41-43]. Polynomial regressions showed the digestible methionine requirement of laying hens fed 0.416% digestible cystine was 354 mg hen^{-1} day^{-1} for egg mass (Fig. 2a), 349 mg hen^{-1} day^{-1} for feed conversion (Fig. 3a), 437 mg hen^{-1} day^{-1} for body weight change (Fig. 4a) and 367 mg hen^{-1} day^{-1} for EM+ BWC (Fig. 5a). Results of egg mass, feed efficiency, body weight change and egg mass plus body weight change for hens fed 0.145% digestible cystine are shown in Fig. 2b-5b, respectively. In a meta-analysis study of methionine and cysteine requirements, the predicted values for combined methionine and cysteine requirements were 670 mg day^{-1} for egg mass and 675 mg day^{-1} for feed conversion for 95% of maximal hen response[44]. The digestible methionine requirement, as found in this study, was slightly higher than the previously reported requirements of 0.29-0.32% because of the higher egg mass produced[8-10,40,43,45-48]. Additionally, the estimated requirement is closer to the reported requirement of 0.36% when 16% of protein is provided in the diet and 0.34% when 17.5% of protein is provided in the diet[12,49].

The cystine requirement was obtained by the difference between two methionine requirements with high or low cystine levels. Since the digestible cystine level of 0.148% was approximately 55% of the reported cystine requirement of

Table 2: Effects of dietary digestible methionine and cystine levels on laying performance in experiment 1

Treatments	HDP (%)	Egg weight (g)	Egg mass (g hen^{-1} day^{-1})	Feed intake (g hen^{-1} day^{-1})	Feed conversion (g feed g^{-1} egg)	Body weight gain (g hen^{-1} day^{-1})
Control	93.45±1.37^{a}	59.79±0.39^{abc}	55.88±0.91^{a}	112.38±1.92^{a}	2.02±0.04^{c}	-0.28±0.87^{a}
Dig. Met (%)[1]						
0.143	45.31±4.44^{c}	51.14±0.90^{e}	23.44±2.22^{d}	75.96±2.37^{b}	3.88±0.52^{a}	-5.60±1.05^{c}
0.240	84.46±2.09^{b}	56.33±0.91^{d}	47.57±0.76^{c}	106.75±2.68^{a}	2.25±0.05^{c}	-3.24±0.93^{b}
0.337	90.33±0.52^{ab}	60.03±0.64^{abc}	54.25±0.54^{ab}	111.65±3.19^{a}	2.06±0.05^{c}	-0.92±0.42^{a}
0.434	90.10±3.58^{ab}	57.66±0.73^{cd}	52.02±2.24^{abc}	107.48±4.51^{a}	2.08±0.02^{c}	-0.50±0.72^{a}
0.531	94.05±1.35^{a}	58.85±0.73^{bc}	55.37±0.63^{a}	113.21±2.27^{a}	2.05±0.05^{c}	0.00±0.72^{a}
Dig. Met (%)[2]						
0.143	45.80±1.56^{c}	49.48±0.46^{e}	22.91±0.78^{d}	68.64±1.51^{b}	3.32±0.14^{b}	-7.45±0.64^{c}
0.231	89.47±2.27^{ab}	56.00±0.90^{d}	50.18±2.04^{bc}	110.89±1.82^{a}	2.22±0.08^{c}	-1.70±1.22^{ab}
0.317	91.70±1.29^{ab}	58.92±0.77^{abc}	54.07±1.46^{ab}	110.97±2.56^{a}	2.06±0.08^{c}	0.35±0.53^{a}
0.407	93.64±2.57^{a}	60.35±0.64^{ab}	56.46±1.15^{a}	112.61±3.01^{a}	2.00±0.09^{c}	0.50±0.53^{a}
0.495	91.96±4.40^{ab}	61.31±4.58^{a}	56.56±3.75^{a}	114.13±2.53^{a}	2.06±0.15^{c}	0.14±0.44^{a}

[1]Diets containing 0.148% digestible cystine. [2]Diets containing 0.416% digestible cystine. Each value represents Means±SEM (n = 4 cages). Each cage contained 4 laying hens. [a-e]Means within a column with no common superscript differ significantly

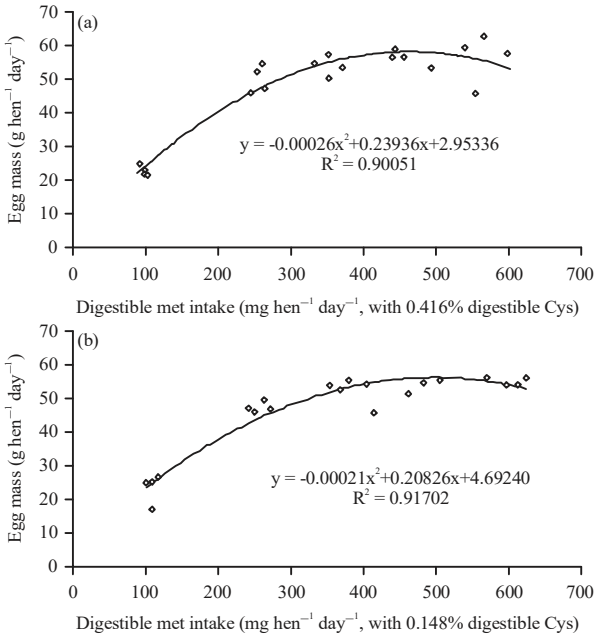

Fig. 2(a-b): Polynomial regressions depicting impact of digestible Met intake on egg mass (g hen^{-1} day^{-1}) in laying hens fed (a) 0.416% and (b) 0.148% digestible Cys in experiment 1

0.33%, extra cysteine (½ cystine) should be synthesized from methionine through the transsulfuration reactions[40]. Hens fed the low digestible cystine (0.148%) diets showed significant increments in egg production, egg weight, egg mass, feed intake and body weight gain and improved feed efficiency when the dietary digestible methionine level was increased from 0.143-0.337% (Table 2). Further increases of dietary methionine levels resulted in non-significant changes of laying performance in hens. Polynomial regressions showed the digestible methionine requirement of laying hens fed 0.148% digestible cystine for egg mass production was 380 mg hen^{-1} day^{-1} with 110 g feed intake day^{-1} (Fig. 2b). This estimation was 26 mg higher than that of the determined methionine requirement of 354 mg with high cystine diets. It is possible that the extra 26 mg of methionine was used for the synthesis of cysteine (½ cystine) in order to meet the cystine requirement of laying hens. Since the utilization efficiency of methionine for cysteine on a weight or concentration basis is in the order of 80%, the cysteine synthesized from methionine had provided an additional 21 mg cystine to the 0.148% dietary digestible cystine (163 mg cystine intake)[19-22]. Ultimately, hens required a dietary cystine level of 184 mg hen^{-1} day^{-1} for 55.2 g egg mass production. This dietary cystine intake was higher than 0.195% (or 175 mg hen^{-1} day^{-1} or 3.19 mg for 1 g of egg mass) as reported by Harms and Russell[39]. This could be due to the

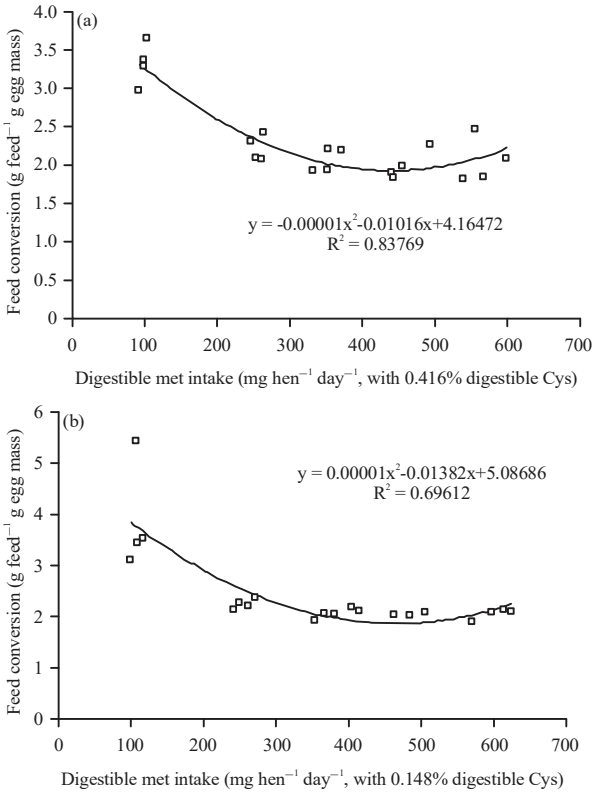

Fig. 3(a-b): Polynomial regressions depicting impact of digestible Met intake on feed efficiency in laying hens fed (a) 0.416% and (b) 0.148% digestible Cys in experiment 1

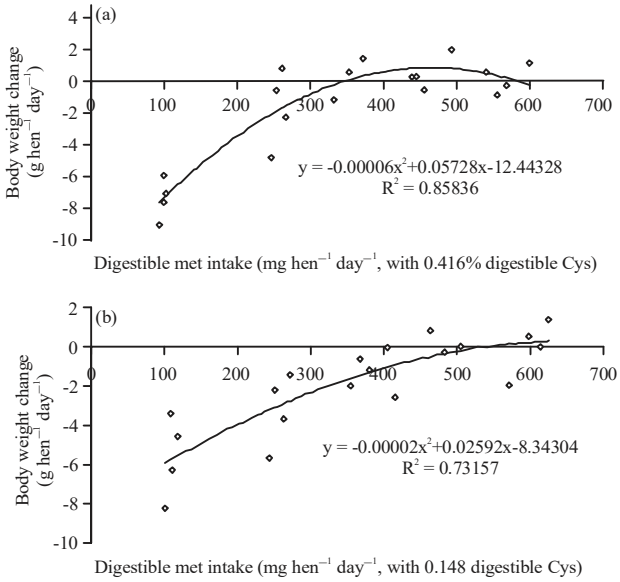

Fig. 4(a-b): Polynomial regressions depicting impact of digestible Met intake on body weight change (g hen^{-1} day^{-1}) in laying hens with (a) 0.416% and (b) 0.148% digestible Cys in experiment 1

higher feed intake by hens (110 g versus 90 g hen^{-1} day^{-1}) than in the previously reported study[39]. Based on same calculation method, the digestible cystine requirement was 193 mg hen^{-1} day^{-1} for feed conversion, 325 mg hen^{-1} for body weight change, 189 mg hen^{-1} day^{-1} for EM+BWC. These results show that the body weight change of laying hens is more sensitive to differences in digestible cystine intake.

The hepatic methionine, cysteine (½ cystine), SAM, SAH and homocysteine concentrations of laying hens were significantly influenced by dietary methionine levels with 0.416 or 0.148% digestible cystine (Table 3). The hepatic Met/Cys concentration ratio was relatively lower in hens fed the 0.143% digestible methionine diet containing either 0.148 or 0.416% cystine (Fig. 6a and b). The ratio was increased to the highest level when dietary digestible methionine level was increased to 0.240 or 0.231% for the low and high cystine diets, respectively. Further increases of methionine level in diets containing either one of the two cystine levels gradually

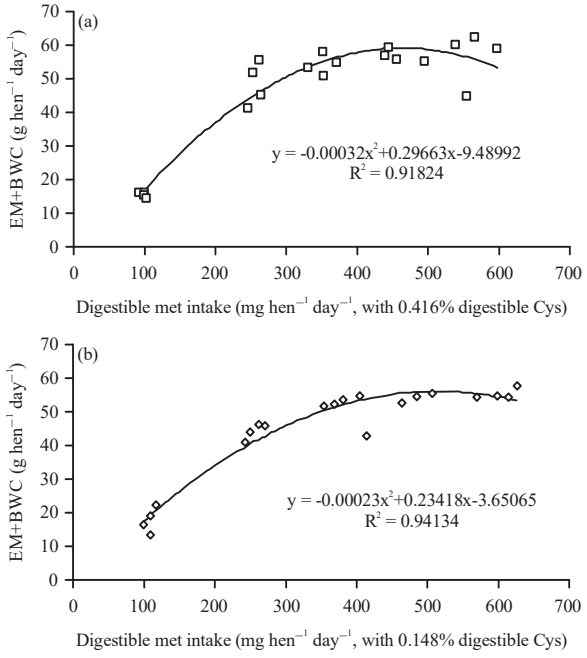

Fig. 5(a-b): Polynomial regressions depicting impact of digestible Met intake on egg mass (EM) plus body weight change (BWC) (g hen^{-1} day^{-1}) in laying hens with (a) 0.416% and (b) 0.148% digestible Cys in experiment 1

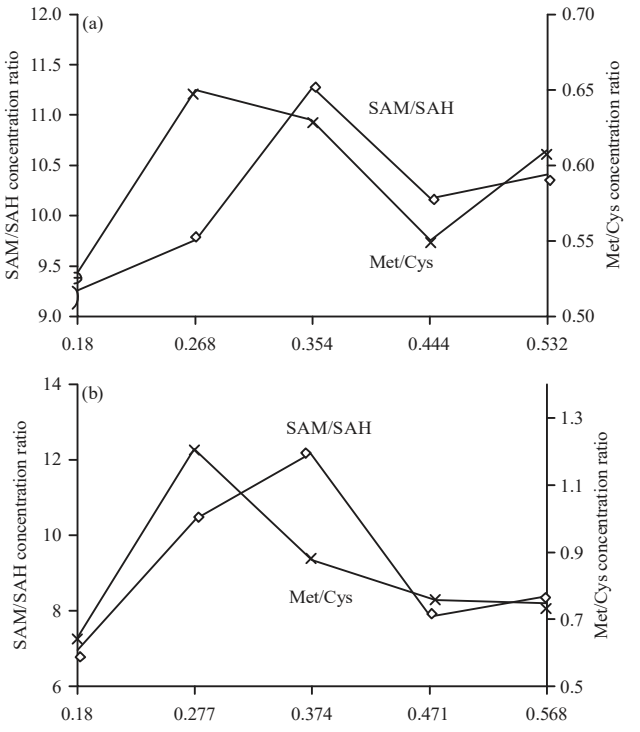

Fig. 6(a-b): Effects of dietary methionine level (X-axis, in percent) on methylation and the ratio conversion of methionine to cysteine in laying hen livers fed (a) Cystine sufficient diets (0.450%), (b) Cystine deficient diets (0.182%)

Table 3: Laying hen liver metabolite concentrations (nmoles g^{-1} wet liver) as affected by dietary digestible methionine and cystine levels in experiment 1

Treatments	Met	SAM	SAH	Cys
Dig. Met levels (%)[1]				
0.143	142.32±12.26^{e}	205.96±38.61^{c}	29.614±4.06^{ab}	220.20±13.92^{ef}
0.240	215.62±13.75^{bcd}	298.29±56.20^{ab}	28.647±5.44^{ab}	177.86±26.62^{f}
0.337	239.11±13.02^{abc}	353.4219.21^{a}	29.008±1.45^{ab}	272.71±11.46^{d}
0.434	260.11±2.78^{ab}	242.08±21.04^{bc}	30.630±0.95^{a}	342.29±9.21^{abc}
0.531	282.15±25.06^{a}	272.74±22.97^{bc}	32.520±1.02^{a}	374.05±17.39^{ab}
Dig. Met levels (%)[2]				
0.143	206.41±44^{cd}	211.51±36.13^{c}	22.832±1.17^{c}	391.50±21.33^{a}
0.231	170.96±58.41^{de}	248.38±44.68^{bc}	25.445±3.99^{bc}	263.29±25.80^{de}
0.317	193.83±34.69^{cd}	317.74±48.67^{ab}	28.157±3.20^{ab}	306.21±12.95^{cd}
0.407	215.47±29.88^{bcd}	300.31±23.87^{ab}	29.529±1.67^{ab}	391.38±35.49^{a}
0.495	205.19±11.80^{cd}	300.77±17.48^{ab}	28.889±1.15^{ab}	335.28±12.59^{bc}

[1]Diets containing 0.148% digestible cystine. [2]Diets containing 0.416% digestible cystine. Each value is Means±SEM (n = 6). $^{a-f}$Means within a column with no common superscript differ significantly. Met: Methionine, SAM: s-adenosylmethionine, SAH: s-adenosylhomocysteine, Cys: Cysteine

Table 4: Laying hen liver enzyme activities (nmoles min^{-1} g^{-1} wet liver) as affected by dietary digestible methionine and cystine levels in experiment 1

Treatments	MAT	BHMT	MFMT	CS
Dig. Met levels (%)[1]				
0.143	0.0607±0.04	42.998±4.33^{ab}	0.5092±0.03^{c}	62.206±6.33^{abc}
0.240	0.0325±0.02	13.376±4.08^{d}	0 .3416±0.07^{d}	44.717±1.86^{d}
0.337	0.0586±0.01	28.112±4.88^{c}	0.5400±0.06^{bc}	57.319±3.62^{bcd}
0.434	0.0328±0.01	33.149±3.92^{bc}	0.7112±0.03^{a}	66.474±2.67^{ab}
0.531	0.0157±0.00	37.637±5.55^{abc}	0.6889±0.06^{ab}	68.415±4.03^{ab}
Dig. Met levels (%)[2]				
0.143	0.0647±0.02	49.017±5.33^{a}	0.7051±0.06^{a}	75.359±10.19^{a}
0.231	0.0495±0.01	49.189±9.73^{a}	0.6343±0.10^{abc}	51.132±5.41^{cd}
0.317	0 .0433±0.01	32.665±3.10^{bc}	0.6570±0.04^{abc}	60.915±3.19^{bc}
0.407	0.0151±0.00	39.615±5.79^{abc}	0.6497±0.04^{abc}	61.457±7.27^{abc}
0.495	0.0396±0.02	32.216±3.90^{bc}	0.6919±0.01^{ab}	65.273±2.43^{abc}

[1]Diets containing 0.148% digestible cystine. [2]Diets containing 0.416% digestible cystine. Each value is Means±SEM (n = 6). [a-d]Means within a column with no common superscript differ significantly. MAT: Methionine s-adenosyltransferase, BHMT: Betaine-homocysteine methyltransferase, MFMT: N5-methyltetrahydrofolate-homocysteine methyltransferase, CS: Cystathionine ß-synthase

decreased the Met/Cys ratios. The changes of the Met/Cys ratio were largely caused by the liver cysteine concentrations (Table 3) due to the significant changes of liver CS activities (Table 4). Although the function of the enzyme BHMT and MFMT is opposite to that of enzyme CS in the methionine metabolism, the higher CS activities observed with layer hens in present experiment on the diets with either extremely deficient or excessive methionine levels was an indication of cysteine/GSH/taurine demand or methionine degradation, respectively. Finkelstein and Martin[31,50] reported that at higher levels of CS activity, the methionine metabolism was in favor of transsulfuration reactions than transmethylation reactions even though the BHMT activity was high.

The lower Met/Cys ratio observed with hens fed the diet containing 0.143% digestible methionine with the high or low cystine level was an indication of increased degradation of methionine for cysteine because of the increased CS activity. The elevated degradation of methionine might be stimulated by the increased body protein mobilization as evidenced by the significant body weight loss of hens. Hens fed the 0.143% digestible methionine diets also showed significant reductions of feed intake and laying performance (Table 2), indicating the inadequacy of dietary methionine level. The lower Met/Cys ratios observed with hens fed the diets containing 0.317% or greater digestible methionine were possibly caused by the increased CS activities that degraded the extra dietary methionine, as the hens did not show any significant improvement in laying performance with increased methionine intake. This is consistent with the finding that significantly increased CS activity with age in hatched broiler chicks[51].

Table 3 and 4 demonstrate the highest hepatic SAM/SAH concentration ratios for hens fed the diet containing the optimum digestible methionine levels (0.337 and 0.317%, respectively, for the 0.148 and 0.416% digestible cystine diets). Deficient or excessive dietary methionine levels caused reduced liver SAM/SAH concentration ratios. The reduced liver SAM/SAH concentration ratios of hens fed the diets containing deficient levels of methionine (<0.317% digestible) were possibly caused by the decreased available dietary methionine for SAM formation even though the MAT activities were slightly increased (Table 3 and 4). The decreased SAM/SAH ratios of hens fed the diets containing excessive methionine (>0.337% digestible) could be related to the reduced MAT activities although the liver methionine levels were slightly increased. The optimum methionine level in either the low or high cystine diets also resulted in a lack of detectable liver homocysteine concentrations. The cause of the decrease of liver homocysteine might be related to the enhanced methionine utilization for egg protein synthesis.

Experiment 2: This experiment was conducted to determine cystine requirement for laying hen and the utilization efficiencies of supplemental methionine and cystine to meet the cystine requirement. The basal diet contained sufficient methionine (0.319% digestible) and deficient cystine (0.148% digestible). The results of the experiment showed significant body weight gain of hens on diets with either methionine or cystine addition level up to 0.12% cystine equivalent. The addition of 0.12% cystine equivalent to the basal diet produced 3.68 g and 4.20 g egg mass per hen per day for hens that received the methionine addition diet and for hens that received the cystine addition diet, respectively. The hens fed the basal diet also showed a feed intake reduction of 10.72 g hen day^{-1} and a body weight loss of 4.25 g hen^{-1} day^{-1} than those of hens on the test diets, indicating the digestible cystine level of 0.148% was deficient. Polynomial regressions showed the digestible cystine requirement of laying hen with 0.319% digestible methionine (methionine

Table 5: Effects of dietary digestible methionine and cystine levels on laying performance in experiment 2

Treatments	HDP (%)	Egg weight (g)	Egg mass (g hen^{-1} day^{-1})	Feed intake (g hen^{-1} day^{-1})	Feed efficiency (g feed g^{-1} egg)	Body weight gain (g hen^{-1} day^{-1})
Control	89.44±2.68	61.34±0.71	54.83±1.53	105.58±1.85	1.93±0.03	0.41±0.35^{a}
Basal (B)	86.01±2.75	58.70±0.41	50.47±1.28	98.66±1.29	1.96±0.07	-3.24±0.47^{c}
B+0.05% Met	88.84±1.19	59.88±0.68	53.20±1.11	103.65±3.16	1.95±0.05	-1.35±0.55^{b}
B+0.10% Met	89.58±1.10	60.17±0.93	53.89±0.92	108.85±1.49	2.02±0.04	0.54±0.29^{a}
B+0.15% Met	88.99±1.11	60.82±0.55	54.15±1.51	109.38±2.88	2.02±0.05	0.54±0.57^{a}
B+0.20% Met	90.77±2.39	59.33±0.42	53.88±1.71	109.08±3.63	2.03±0.04	0.41±0.85^{a}
B+0.04% Cys	88.24±2.53	59.74±0.25	52.71±1.45	109.38±3.78	2.08±0.05	-0.41±0.82^{ab}
B+0.08% Cys	86.31±3.97	60.55±0.65	52.32±2.82	106.18±4.16	2.04±0.05	-0.20±0.45^{ab}
B+0.12% Cys	90.18±1.56	60.65±0.81	54.67±0.73	108.04±3.12	1.98±0.06	1.01±0.33^{a}
B+0.16% Cys	82.59±4.92	60.57±1.19	49.92±2.53	102.16±3.04	2.05±0.05	0.07±0.51^{ab}

Each value is Means±SEM (n = 4 cages). Each cage contained 4 laying hens. $^{a-e}$Means within a column with no common superscript differ significantly

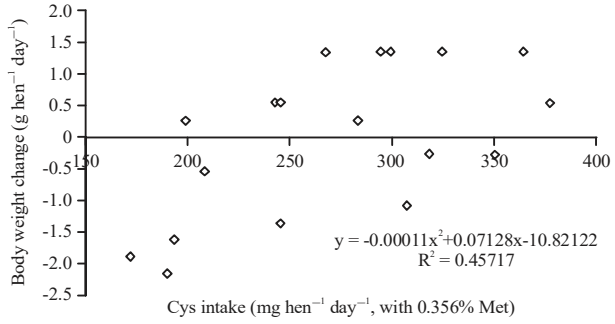

Fig. 7: Polynomial regressions depicting impact of Cys intake on body weight change (g hen^{-1} day^{-1}) in laying hens fed 0.356% Met in experiment 2

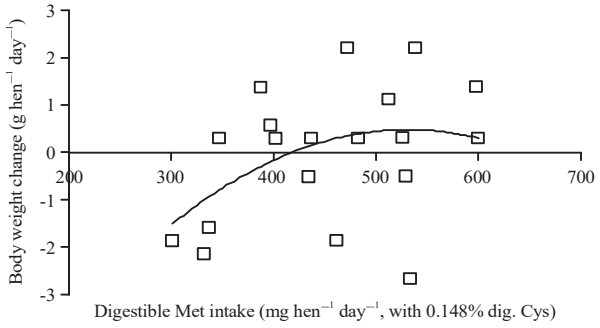

Fig. 8: Polynomial regressions depicting impact of Met intake on body weight change (g hen^{-1} day^{-1}) in laying hens fed 0.148% digestible Cys in experiment 2

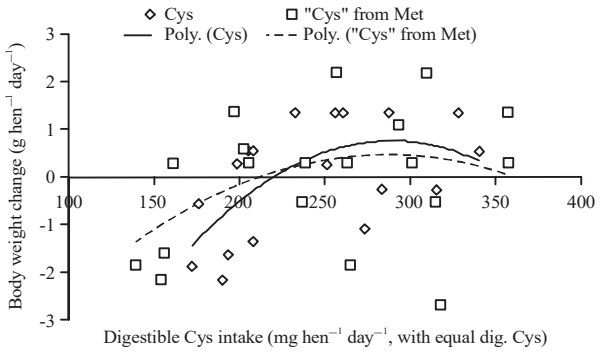

Fig. 9: Polynomial regressions depicting impact of Cys intake on body weight change (g hen^{-1} day^{-1}) in laying hens fed 0.182% Cys in experiment 2

requirement level from Experiment 1) is 276 mg hen^{-1} day^{-1} for body weight change in Experiment 2 (Fig. 7). Also, polynomial regressions showed that 157 mg hen^{-1} day^{-1} methionine were needed to provide for 113 mg plus 163 mg in basal diet to meet 276 mg hen^{-1} day^{-1} cystine requirement for prevent body loss (Fig. 8). Considering so, in this case, conversion efficiency from methionine to cysteine is about 90% (113/156/0.8*100%) (Fig. 9) based on weight or concentration basis.

The results also showed that there was a no significant difference in egg mass production between hens receiving the methionine addition treatment and the hens receiving the cystine addition treatment at each of the four addition levels (Table 5). The lack of change in egg mass also suggests that the cystine requirement of laying hens for optimal production of egg mass could be satisfied with either methionine or cysteine (½ cystine) on an equimolar basis. Since the molecular weight of cysteine is 80% of that of methionine, the utilization efficiency of methionine for cystine is in the order of 80% on a weight and concentration basis. In another study done in monogastric species (pig), the inferior performance of growing pigs on diets with cystine addition to that of pigs on diets with methionine addition was most likely caused by the limited methionine levels in the diets (0.20 and 0.16% for the 30-60 and 60-90 kg body weight ranges, respectively) since the methionine addition could provide pigs both additional methionine and cystine whereas the cystine addition could only provide the pigs with additional cystine[27]. This is explained with the irreversible conversion of methionine to cystine in the methionine metabolism[2,3].

The hepatic methionine, cysteine, SAM and SAH concentrations of laying hens were significantly influenced by

Table 6: Laying hen liver metabolite concentrations (nmoles g^{-1} wet liver) as affected by dietary digestible methionine and cystine levels in experiment 2

Treatment	Met	SAM	SAH	Cys
Basal (B)	240.11±25.69^{bcd}	234.25±18.40^{d}	32.296±2.57^{ab}	244.64±21.92^{de}
B+0.05% Met	236.02±7.29^{cde}	286.35±13.31^{c}	27.734±1.54^{c}	248.07±10.38^{de}
B+0.10% Met	214.50±9.83^{de}	300.04±2.44^{c}	28.57±21.80^{bc}	266.90±11.22^{de}
B+0.15% Met	179.93±8.35^{e}	286.83±4.13^{c}	27.703±1.36^{c}	232.11±4.89^{e}
B+0.20% Met	192.22±11.41^{de}	290.22±6.25^{c}	27.085±0.81^{c}	276.03±10.61^{d}
B+0.04% Cys	185.46±4.62^{de}	349.02±16.78^{b}	25.948±1.68^{c}	232.49±4.56^{e}
B+0.08% Cys	283.48±37.96^{abc}	411.62±36.99^{a}	25.829±0.61^{c}	316.81±1061^{c}
B+0.12% Cys	297.14±21.03^{ab}	305.55±5.63^{bc}	29.423±0.40^{abc}	364.75±16.68^{b}
B+0.16% Cys	332.69±26.36^{a}	308.58±16.87^{bc}	32.426±0.77^{a}	443.26±17.64^{a}

Each value is Means±SEM (n = 6). $^{a-d}$Means within a column with no common superscript differ significantly. Met: Methionine, SAM: s-adenosylmethionine, SAH: s-adenosylhomocysteine, Cys: Cysteine

Table 7: Laying hen liver enzyme activities (nmoles min^{-1} g^{-1} wet liver) as affected by dietary digestible methionine and cystine levels in experiment 2

Treatments	MAT	BHMT	MFMT	CS
Basal (B)	0.0643±0.01	5.254±1.31^{e}	0.1797±0.02^{b}	48.617±2.65^{c}
B+0.05% Met	0.0853±0.04	41.791±1.53^{a}	0 .3141±0.01^{a}	56.031±1.92^{b}
B+0.10% Met	0.0674±0.03	31.419±3.44^{abc}	0.3678±0.02^{a}	60.274±2.27^{b}
B+0.15% Met	0.0326±0.02	39.791±3.91^{ab}	0.3635±0.01^{a}	58.646±1.45^{b}
B+0.20% Met	0.0804±0.04	28.225±4.64^{bcd}	0.3155±0.02^{a}	67.586±2.79^{a}
B+0.04% Cys	0.0676±0.02	43.638±8.14^{a}	0 .3135±0.02^{a}	60.023±1.71^{b}
B+0.08% Cys	0.0310±0.03	24.142±1.10^{cd}	0.3171±0.04^{a}	58.977±3.14^{b}
B+0.12% Cys	0.0369±0.01	31.806±6.46^{abc}	0.3665±0.02^{a}	57.623±1.75^{b}
B+0.16% Cys	0.0755±0.02	16.599±3.83^{de}	0.3142±0.03^{a}	60.485±1.91^{b}

Each value is Means±SEM (n = 6). $^{a-d}$Means within a column with no common superscript differ significantly. MAT: Methionine s-adenosyltransferase, BHMT: Betaine-homocysteine methyltransferase, MFMT: N5-methyltetrahydrofolate-homocysteine methyltransferase, CS: Cystathionine ß-synthase

dietary methionine and cystine levels (Table 6). Hens fed the diets with methionine additions from 0.05-0.20% compared to the hens fed the basal diet containing 0.319% digestible methionine and 0.148% digestible cystine demonstrated gradual decreases of liver Met/Cys ratios from 0.98-0.70. The decreased Met/Cys ratios were caused by the increased liver cysteine concentrations due to the elevated CS activities (Table 7). The hens fed the diets with the methionine addition also showed increase in liver enzyme activities of BHMT and MFMT greater than those of hens fed the basal diet (Table 7). Methionine additions of more than 0.05% also showed a non-significant change in liver methionine level and MAT activity. The hens fed the diet with 0.05% methionine also showed an increase in liver SAM/SAH concentration ratio by 3.07 units from that of the value of 7.25 of hens fed the basal diet. Further increase of methionine addition level resulted in a non-significant change in SAM/SAH ratio. These observations generally agreed with the results from Experiment 1.

The cystine addition compared to the methionine addition to the basal diet produced similar changes of liver Met/Cys and SAM/SAH ratios in laying hens. The liver metabolite concentrations and enzyme activities of hens showed the same changes for the cystine and methionine addition treatments, with an exception that there was a significant increase of liver methionine concentration of hens fed the diets with an increased level of cystine. Additionally, there was a significant decrease of liver methionine concentration of hens fed the diets with an increased level of methionine in diets. The increased liver methionine concentration of hens fed the diets with an increased level of dietary methionine most likely resulted from the increased need for methionine degradation.

Although, the current findings are crucial to better understanding the utilization efficiencies of methionine and cystine in laying hens, further research should be conducted in order to create a more representative database. The digestible methionine and cystine requirements are reflective of genetics, gender, age and performance. Moreover, the hepatic enzymes and metabolites of total sulfur amino acids experience daily variations throughout light and dark periods[52]. Each of the aforementioned variables represents the need for additional experimentation.

CONCLUSION

Overall, the study demonstrates that the utilization efficiencies of methionine and cysteine (½ cystine) were 100% on an equimolar basis for egg mass and 90% on an equimolar basis to prevent body weight loss. When methionine is used to meet the cystine requirement, the utilization efficiency of 80% should be adequate on a weight

and concentration basis for egg mass and 72% for body weight maintenance. The practice of feeding ingredients with a substantial digestible cystine level for supporting body weight may be beneficial for laying hens.

SIGNIFICANCE STATEMENT

This study discovers the utilization efficiencies of methionine and cysteine for maintaining egg mass and preventing body weight loss in laying hens. This study will help the researcher to understand the digestible methionine and cystine requirements for optimum production and to determine the utilization efficiency of supplemental methionine and cystine for the cystine requirement of laying hens.

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