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Requirement of Digestible Sulfur Amino Acids in Laying Hens Fed Sorghum- and Soybean Meal-Based Diets

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ABSTRACT

Two experiments were done to evaluate the effect of increasing levels of dietary digestible methionine (Met) and Met:cysteine (Met:Cys) ratio on the productivity of Hy-Line W-36 laying hens fed sorghum- and soybean meal-based diets. In Exp. 1, 160 hens from 68 to 75 weeks of age were assigned to four dietary levels of digestible Met (0.20 0.24, 0.28 and 0.32%). The digestible total sulfur amino acids:Lysine (TSAA:Lys) ratios were: 62, 68, 76 and 84%. In Exp. 2, 192 hens from 76-83 weeks of age were assigned to four dietary digestible Met:Cys ratios (160, 116.7, 85.7 and 62.5%). The digestible TSAA:Lys ratio was kept constant across diets (80%). Results were subjected to ANOVA and linear regression analyses. In Exp. 1, optimal egg production, egg mass, and feed efficiency responses were observed at 0.30 and 0.50% of dietary digestible Met and TSAA, respectively (quadratic effect, $p<0.05$). Live performance was maximized with digestible Met and TSAA in takes of 288 and 478 mg/hen/d, respectively. In Exp. 2, optimal egg production and feed efficiency responses were observed at 151 and 150% of dietary digestible Met:Cys ratios, respectively (quadratic effect, $p<0.05$). The digestible Met, Cys and TSAA intake to maximize egg production and feed efficiency were 313, 207 and 510 mg/hen/d, respectively. The requirements for sulfur AA in Hy-Line W-36 hens from 68 to 83 weeks of age fed sorghum- and soybean meal-based diets fell inside the range of the requirements previously estimated in hens fed corn-soybean meal based diets.

INTRODUCTION

Grain sorghum (*Sorghum bicolor* L. Moench) is the fifth largest cereal crop in the world after wheat, rice, corn, and barley, with an annual production in the order of 56 million metric tons (Taylor & Shewry, 2006; Wong *et al.,* 2009). Sorghum crops can be adapted to many different agro-ecological environments due to its rusticity, resistance to adverse weather conditions, and lower water requirements relative to corn crops. Sorghum tolerates heat and salinity better than corn, and can grow in a wide variety of soils with a limited supply of nutrients (Dendy & Dobraszczyk, 2001; Gómez *et al.,* 2009). Given the advantages of sorghum, its production is strategic in several parts of the world and is one of the best animal feeding alternatives in places where other crops have poorer yields or their production is not feasible. Furthermore, due to the declining global grain stocks due to global warming, it is expected that in the near future heat resistant crops such as sorghum are routinely utilized as food and feed resources for humans and animals (Selle *et al.,* 2010; García *et al.,* 2013).

Considering the above scenario, significant amounts of sorghum can be locally available at very competitive prices compared with other cereals, and its inclusion in animal diets may be economically attractive. In addition, the use of locally-produced sorghum could reduce the dependence on imported cereals in countries where the production of other cereal grains is limited. However, sorghum-based diets have been associated with inconsistent, and even sub-optimal growth performance of broiler chickens. Sorghum is unique in that it contains kafirin, phytate, and may contain condensed tannin; these factors can negatively influence the nutritive properties of sorghum (Selle *et al.,* 2010; García *et al.,* 2013).

In experiments with pigs fed different low- and high-tannin containing sorghum varieties (Mariscal-Landín *et al.,* 2004; Mariscal-Landín *et al.,* 2010), low or very low ileal standardized digestibility values of arginine (Arg), histidine (His), and proline (Pro), and the lowest lysine ileal standardized digestibility were determined in high-tannin sorghum compared to the remaining sorghum varieties. The use of improved sorghum varieties with low levels of anti-nutritional factors may partially alleviate the problems associated with the poor quality of sorghum. Ramírez *et al.* (2005) conducted a study in Mexico with 216 sorghum samples and found PC and tannin concentration ranges of 5.2 to 11.0 and 0.02 to 5.06%, respectively. Tannins were the most variable component with a coefficient of variation of 152.8%. It was also reported that 91.7% of the samples had tannin levels below 1.5% and only 1.86% of the samples contained tannin values higher than 3.9%. These results suggest that the probability of the inclusion of high-tannin sorghum varieties in poultry diets is low.

Furthermore, the application of the ideal AA profile concept to set the AA requirements in animals is expected to derive in better and more reliable estimates of the AA requirements (Rostagno *et al.* 2005), which can be applied to poultry reared under different production conditions (Angeles & Gómez, 2005; Gomez & Angeles, 2009). An advantage of using the ideal AA concept is the possibility to substitute conventional for non-conventional feed ingredients with different nutritional quality, such as the substitution of corn by sorghum as a source of energy and AA. For instance, it was shown that the digestible Lys, Met and Thr requirements of laying chickens fed sorghum- and soybean meal-based diets formulated according to the ideal AA profile were within the range of the requirements of the same AA established in laying hens fed corn-soybean meal based diets (Angeles & Gómez, 2005; Gomez & Angeles, 2009).

Methionine is the first limiting AA in hens fed corn- or sorghum- and soybean meal-based diets (Cortes *et al.,* 2001; Novak *et al.,* 2006; Saki *et al.,* 2012). An important aspect of the Met requirement is that this AA and Cys contain sulfur in their structure, and within the body, under conditions of deficiency of Cys, Met can be used to synthesize one molecule of Cys. This can lead to a deficiency of Met, which must be supplied in the diet because it cannot be synthesized by animal tissues. Therefore, in poultry diet formulation, in addition of supplying Met and Cys requirements, it is important to supply the exact amounts and ideal profile of total sulfur AA (TSAA) needed for optimal production. Such concepts should be considered, particularly when diets are based on sorghum, which contains levels of digestible sulfur amino acids compared to corn, which may cause marginal Met, Cys, or TSAA deficiencies. Therefore, the objective of this study was to evaluate the effect of increasing Met levels and Met:Cys ratios on the productivity of Hy-Line W-36 laying hens fed sorghum- and soybean meal-based diets.

MATERIALS AND METHODS

The experiments were carried out in the facilities of the Experimental Poultry Unit of the National Center of Disciplinary Research in Animal Physiology (CENIDFyMA-INIFAP), located in Querétaro, México. The experimental procedures were revised and approved by the Ethical Committee of Animal Use of the CENIDFyMA-INIFAP.

Experiment 1

One hundred and sixty Hy-Line W-36 hens were evaluated from 68 to 75 weeks of age and fed sorghum- and soybean meal-based diets with four levels of digestible Met (0.20, 0.24, 0.28, and 0.32%). The amino acid profiles of sorghum and soybean meal were calculated using prediction equations based on the analyzed protein composition and on the digestibility coefficients estimated from the table published by Mariscal *et al.* (1998). A basal diet was formulated to contain 12.4% CP, 2840 kcal ME/kg of feed, 0.20% digestible Met and 0.20% digestible Cys (Table 1). The limiting AA Lys, Thr and Ile were added from crystalline sources to meet or exceed the optimum ratio of essential AA.

Table 1 – Ingredients and nutritional composition of the experimental diets (as-fed basis).

Ingredients	%	Nutrient	Content
Ground sorghum	62.67	ME, kcal/kg	2840
Soybean meal	20.74	CP, % [b]	12.8
Calcium carbonate	10.0	Calcium, %	4.0
Calcium phosphate	1.45	Available P, %	0.32
Soybean oil	4.10		
Salt	0.39	Amino acids [c]	
Premix[a]	0.20	Lysine	0.65 (100)
Sodium bicarbonate	0.07	Methionine	0.20 (31)
Antioxidant	0.02	Cysteine	0.20 (31)
L-Lysine	0.13	Met + Cys	0.40 (62)
L-Threonine	0.07	Threonine	0.45 (69)
L-Isoleucine	0.16	Tryptophan	0.16 (25)
		Isoleucine	0.58 (89)
		Valine	0.65 (100)
		Arginine	0.84 (129)

[a] Each kg provided: vitamin A, 7,700 IU; vitamin D_3, 3,000 IU; vitamin E, 6.6 IU; vitamin K, 2.0 mg; riboflavin, 4.4 mg; niacin, 22 mg; pantothenic acid 5.5 mg; choline, 300 mg; folic acid, 0.11 g; vitamin B_{12}, 8.8 mg; manganese, 100 mg; zinc, 100 mg; iron, 33 mg; copper, 9 mg; iodine, 0.9 mg; selenium, 0.3 mg. [b] Analyzed. [c] Amino acids are given on digestible basis. The amino acid ratios to Lys are shown between parentheses.

The basal diet was mixed and divided into four equal portions, to which four increasing levels of DL-Met were added (0.0, 0.04, 0.08, and 0.12%) to achieve the desired dietary Met levels. The dietary digestible TSAA concentrations were 0.40, 0.44, 0.48, and 0.52% and the TSAA:Lys ratios were 62, 68, 76, and 84%, as the digestible Met levels increased in the basal diet.

Experiment 2

One hundred ninety two Hy-Line W-36 hens, between 76 and 83 weeks of age (from a different group of those used in the first experiment) were fed sorghum- and soybean meal-based diets with four digestible Met:Cys ratios (160, 116.7, 85.7, and 62.5%). The basal diet was similar to that used in Experiment 1, but in Experiment 2, dietary digestible TSAA level was kept constant across all diets (80%). To accomplish this, the basal diet was also mixed and then divided in four portions to which the crystalline Met and Cys were added to obtain the proper Met:Cys ratios. Met and Cys were adjusted in 0.04% units as follows: (0.32:0.20, 0.28:0.24, 0.24:0.28 and 0.20:0.32 %).

General procedures

There were 10 and 12 replicates per treatment in Experiment 1 and 2, respectively. Each replicate consisted of two adjacent cages with two hens per cage for a total of four hens in each replicate. In both experiments hens were kept on treatments for eight weeks; the first two weeks were considered as adaptation period, and for the statistical analysis only data from weeks 3-8 were included.

Hens were housed in battery cages equipped with trough feeders and bowl drinkers. Feed allowance was restricted to 95 g of feed/hen/d in both experiments to prevent AA over feeding. This was considered a minor feed restriction since the normal feed intake expected in hens from 65 to 80 weeks of age is on average 96 g of feed/hen/d (Hy-Line Management Guide, 2011). Body weight was recorded at the beginning and end of each experiment, egg production was daily recorded, and all eggs were weekly weighed. The change in body weight was estimated by the difference between the initial and final weight. Egg mass was estimated by multiplying egg production percentage by egg weight, and the feed efficiency was estimated by dividing the egg mass over feed intake.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the general linear models procedures of SAS software (1990) according to a randomized block design (Steel & Torrie, 1980). In all analyses, percentage data were transformed to arsine data before analysis. Least square means are shown in Tables 1 and 2. Variables that were affected by dietary Met level or Met:Cys ratios were submitted to linear and non-linear regression analyses. Results from both experiments were best fitted using polynomial (quadratic response) regression analysis of the data. Based on the quadratic equation developed, the dietary requirements of Met (Experiment 1) and Met:Cys ratios (Experiment 2) were estimated as the curve inflection point from the analyses of the first derivative. The Met levels and Met:Cys ratios required to achieve maximal responses were obtained by dividing the derivative of the responses by the derivative of Met and Met:Cys ratios.

RESULTS

Experiment 1

The means of the live performance responses observed in Experiment 1 are shown in Table 2. The results indicate a quadratic response ($p< 0.05$) of egg production ($y = - 14.975 + 658.13x -1109.4x^2$; $R^2 = 0.95$), egg mass ($y = - 45.87 + 584.5x -937.5x^2$; $R^2 = 0.98$), and feed efficiency ($y = - 0.4828 + 6.1526x -9.8684x^2$; $R^2 = 0.98$) to increasing dietary digestible Met levels. Using these equations, the curve inflection point from the analysis of the first derivative show maximum responses of 0.30, 0.31 and 0.30% dietary digestible Met levels for egg production, egg mass, and feed efficiency, respectively (Table 4). Egg weight showed a positive linear response ($p< 0.05$) to dietary digestible Met level ($y = 88.5x + 27.84$; $R^2 = 0.91$). Dietary Met level did not affect body weight.

Table 2 – Performance parameters of laying hens fed increasing levels of dietary methionine (Exp. 1)

Variable response	Level of dietary Met, %				SEM [a]
	0.20	0.24	0.28	0.32	
Feed intake, g	95.0	95.0	95.0	95.0	---
Digestible Met intake, mg/d	190	228	266	304	---
Digestible TSAA intake, mg/d	380	418	456	494	---
Egg production, % [b]	72.7	77.8	83.6	81.6	2.006
Egg weight, g [c]	44.2	50.6	53.6	55.0	1.068
Egg mass, g [b]	33.8	39.6	45.1	44.9	1.491
Feed efficiency [b]	0.36	0.42	0.47	0.47	0.016
Body weight change, g	-8.3	-28.9	-26.7	-17.4	0.030

[a] SEM = Standard error of the mean. [b] Quadratic effect, p<0.05. [c] Linear effect, p<0.05.

Experiment 2

The performance results obtained in Experiment 2 (Table 3) indicate that egg production ($y = 35.022 + 0.5451x - 0.0018x^2$; $R^2 = 0.99$), feed efficiency ($y = 0.3182 + 0.003x -0.00001x^2$; $R^2 = 0.99$), and body weight change ($y = - 86.812 + 1.1092x - 0.0045x^2$; $R^2 = 0.87$) presented a quadratic response ($p< 0.05$) when dietary digestible Met:Cys ratio was reduced from 160 to 62.5%. Using these equations, the curve inflection point from the analysis of the first derivative show maximum egg production and feed efficiency responses and minimum body weight loss with 151, 150, and 123% of dietary Met:Cys ratio, respectively (Table 4). Egg mass linearly decreased ($p<0.05$) with the reduction of Met:Cys ratios ($y = 36.952 + 0.0894x$; $R^2 = 0.94$), but egg weight was not affected by dietary Met:Cys ratios.

Table 3 – Performance parameters of laying hens fed increasing dietary methionine:cysteine ratios (Exp. 2)

Response variables	Methionine:Cysteine ratio, %				SEM [a]
	160.0	116.7	85.7	62.5	
Feed intake, g	95.0	95.0	95.0	95.0	---
Digestible Met intake, mg/d	304	266	228	190	---
Digestible Cys intake, mg/d	190	228	266	304	---
Egg production, % [b]	76.3	75.2	68.3	62.5	2.088
Egg weight, g	65.1	65.1	65.2	64.8	0.590
Egg mass, g [c]	49.5	48.8	44.5	42.0	1.635
Feed efficiency [b]	0.55	0.54	0.50	0.47	0.018
Body weight change, g [b]	-25.0	-21.7	-21.7	-36.7	0.009

[a] SEM = Standard error of the mean. [b] Quadratic effect, p<0.05. [c] Linear effect, p<0.05.

DISCUSSION

In the present study, it was assumed that AA requirements for egg production were the same, regardless of the age of the hens. The NRC (1994) recommends a single requirement for each AA during the first and the second cycles of egg production, given that the changes in AA requirements are regulated by changes in the level of feed intake of the hens. This argument is questionable since the level of feed intake may widely vary among flocks, and in many cases, laying hens can consume more feed than that required to support egg production. In the present study, in order to prevent significant differences in feed intake, and consequently in AA intake, the daily feed allowance was fixed in both experiments reported here.

In Experiment 1, based on the estimated optimal dietary digestible Met and TSAA requirements for egg production and feed efficiency (0.30 and 0.50%) and for egg weight (0.31 and 0.51%) and on feed intake (95 g/d), the digestible Met and TSAA intake for maximum egg production, egg mass, and feed efficiency were calculated as 282, 296, and 287 mg/hen/d, and 472, 486, and 477 mg/hen/d, respectively. On average, the digestible Met and TSAA intake to maximize the productive performance were 288 and 478 mg/hen/d, respectively.

According to the AA recommendations of the NRC (1994), white-egg layers consuming 100 g of feed/d during the first laying cycle have a requirement of 258 and 499 mg of digestible Met and TSAA/hen/d, respectively. In a recent study, Saki *et al.* (2012) reported that white commercial laying hens fed a corn-wheat-soybean meal diet from 22 to 36 weeks of age presented digestible Met and TSAA/hen/d requirements of 229 and 438 mg, respectively. The Met requirement estimated in the present study was higher than that reported by the NRC (1994) and by Saki *et al.* (2012). On the other hand, the TSAA requirement estimated in the present study was lower than the NRC (1994) and higher compared to that of Saki *et al.* (2012).

Our estimated Met intake is also in close agreement to the requirements reported by Coon & Zhang (1998) of 283 mg/hen/d, but the TSAA is lower than the value of 496 mg/hen/d observed in Hy-Line W36 hens fed corn-soybean meal based diets from 60-72 weeks of age. In the same report of Coon & Zhang (1998), but for Dekalb XL layer hens fed corn-soybean meal based diets from 29 to 41 weeks of age, the digestible Met and TSAA requirements were 352 and 596 mg/d, respectively. In a previous research by the same

Table 4 – Quadratic equations, inflection point and intake of digestible methionine, cysteine and total sulfur amino acids in Exp. 1 and Exp. 2

				Intake, mg/hen/d[c]		
Item	Regression equation	R^2	Inflection point	Met	Cys	TSAA
Experiment 1[a]						
Egg production, %	$y = -14.975 + 658.13x - 1109.4x^2$	0.95	0.30	282	200	472
Egg mass, g	$y = -45.87 + 584.5x - 937.5x^2$	0.98	0.31	296	200	486
Feed efficiency	$y = -0.4828 + 6.1526x - 9.8684x^2$	0.98	0.30	287	200	477
Experiment 2[b]						
Egg production, %	$y = 35.022 + 0.5451x - 0.0018x^2$	0.99	151	314	206	510
Feed efficiency	$y = 0.3182 + 0.003x - 0.00001x^2$	0.99	150	312	208	510
Body weight change, g	$y = -86.812 + 1.1092x - 0.0045x^2$	0.87	123	287	233	510

[a] Quadratic effect of dietary digestible Met level, $p<0.05$.

[b] Quadratic effect of dietary digestible Met:Cys ratios, $p<0.05$.

[c] The intake of Met and Cys was estimated by multiplying the dietary concentration of digestible Met and Cys at the inflection point in each experiment. The intake of digestible TSAA was obtained by summing the intake of digestible Met and Cys.

laboratory, requirements of 350 and 595 mg digestible Met and TSAA were estimated (Cao *et al.,* 1995). In these last two reports, the reported Met and TSAA requirement values were higher than those estimated in our research.

In other studies published during the last decade, reported Met and TSAA requirements were higher compared with the estimations of the present research. Bunchasak & Silapasorn (2005) reported a requirement of 420 mg digestible Met for maximum egg production in Isa-brown laying hens from 24-44 weeks of age. Polese *et al.* (2012) reported a requirement of 682 mg of digestible TSAA/hen/d in brown egg laying hens from 50 to 66 weeks of age. Filho *et al.* (2006) estimated a requirement of 697 mg digestible TSAA/hen/d for semi-heavy laying hens from the beginning to the peak of egg production. In the last three reports hens were fed corn-soybean meal based diets.

In laying hens fed sorghum- and soybean meal-based diets, digestible Met requirements of 313 and 343 mg/hen/d were reported for maximum egg production and egg weight for 100- to 108-week-old layers (Gomez & Angeles, 2009). Also, a digestible TSAA requirement of 553 mg was estimated fro Isa-Babcock B300 hens fed sorghum- and soybean meal-based diets from 24 to 34 weeks of age by Fuente *et al.* (2005).

The above observations indicate that in previous research, laying hens fed corn-based diets showed either lower (NRC, 1994; Saki *et al.,* 2012), similar (Coon & Zhang, 1998; Hy-Line W36 hens) or greater (Coon & Zhang, 1998 [using Dekalb XL laying hens]; Bunchasak & Silapasorn, 2005) digestible Met requirements compared with the layers in the present research, which were fed sorghum-based diets. Furthermore, Gomez & Angeles (2009) reported higher digestible Met requirement in layers fed sorghum-based diets compared with the present study. Regarding TSAA requirements, our estimates are higher only than that reported by Saki *et al.* (2012), but are lower compared to the remaining reports (NRC, 1994; Coon & Zhang, 1998; Filho *et al.,* 2006; Polese *et al.,* 2012), including one in which hens were also fed sorghum-based diets (Fuente *et al.,* 2005).

The findings of the present report were also compared with two recent meta-analyses based on literature reviews. In the review of Joly (2010), the results of 15 experiments published between 1990 and 2005 indicated that the digestible Met and TSAA requirements were 420 and 650 mg/hen/d, respectively. Furthermore, in the meta-analysis performed by Lemme (2009) of 19 experiments published between 1990 and 2006, digestible Met and TSAA requirements of 415 and 777 mg/hen/d, respectively, were estimated. It should be noted that the estimated values in both reviews are also higher than the values of the present research.

It may be argued that the estimated digestible Met and TSAA requirements reported here may have been lower because of the lower egg mass shown by the older hens used in the present study (Table 2 and 3). This argument could be clarified by expressing the daily digestible Met and TSAA requirements per gram of egg content. The estimated maximum egg mass (45.0 g) observed in the present report was multiplied by 0.911 (Coon & Zhang, 1998) and then divided by the average intake of digestible Met and TSAA (288 and 478 mg/hen/d, respectively). On this basis, the daily

Met and TSAA required per gram of egg content were 6.96 and 11.60 mg. Coon & Zhang (1998) reported requirements of 7.04 mg Met and 10.96 mg TSAA per gram of egg content in Hy-Line W36 hens with 60-72 weeks of age producing 50 g of egg mass d^{-1}. Also, in a previous study, Cao *et al.* (1995) reported digestible Met and TSAA requirements of 6.48 and 11.02 mg per gram of egg content in hens producing 54 g of egg mass d^{-1}. In laying hens in the second cycle of lay, Gomez & Angeles (2009) obtained a Met requirement of 7.02 mg per gram of egg content. In the literature review of Joly (2010), digestible Met requirement was estimated as 7.11 mg per gram of egg content. The results of Fuente *et al.* (2005) indicate a digestible TSAA requirement of 12.14 mg per gram of egg content. The estimated Met and TSAA requirements per gram of egg content in the present research were within the range of the values previously reported.

The estimated digestible Lys content of the basal diet was 0.65% and the estimated digestible Lys intake was 617.5 mg/hen/d. Therefore, the average Met:Lys and the TSAA:Lys ratio were 47 and 77%, respectively.

The estimated Met:Lys ratio is higher than the ratios of 43 and 44% recommended by the NRC (1994) and Jais *et al.* (1995), respectively, similar to the 47% ratio reported by Bregendhal *et al.* (2008), and slightly lower than the 49% ratio obtained by Coon & Zhang (1998) and the 50% ratio determined by the CVB (1996) and Rostagno *et al.* (2005).

Furthermore, the observed TSAA:Lys ratio is consistent with the 81% ratio recommended by Coon & Zhang (1998), but it is slightly lower than the 84% ratio of the NRC (1994) and far lower than other ratios, of 91-94% reported by other sources (CVB, 1996; Rostagno *et al.,* 2005; Bregendhal *et al.,* 2008).

Egg weight presented a positive linear response to increasing dietary Met levels. This response is supported by a previous report (Gomez & Angeles, 2009) and is consistent with earlier results indicating that the dietary Met level required to achieve maximum egg weight is higher than that required for maximum egg production (Shafer *et al.,* 1996; Shafer *et al.,* 1998; Narvaez-Solarte *et al.,* 2005). The same pattern was observed in other studies in which TSAA requirements were estimated (Filho *et al.*, 2006; Polese *et al.,* 2012).

In Experiment 2, the greater egg production and feed efficiency and lower weight loss obtained at the higher Met:Cys ratios emphasizes the essentiality of Met over Cys. Based on the estimated optimum Met:Cys ratios for egg production (152%), feed efficiency (150%), and body weight change (123%), as well on feed intake results (95 g/d), the digestible Met and Cys intake for maximum egg production and feed efficiency and to reduce body weight loss were calculated as 314 and 206, 312 and 208 and 287 and 233 mg/hen/d, respectively. In all cases, the digestible TSAA intake was 510 mg/hen/d.

On average, the digestible Met and Cys intake to maximize the egg production and feed efficiency were 313 and 207 mg/hen/d, respectively. These values are higher than the estimated digestible Met and Cys intake of 288 and 200 mg/hen/d observed in Experiment 1. The main reason for these differences may be that the higher average egg weight obtained in Experiment 2 compared to Experiment 1. However, the estimated digestible Met in Experiment 2 is considered normal because it is within the range of the values already presented in the discussion of Experiment 1. This comment also applies to the digestible TSAA intake (510 mg/hen/d) observed in Experiment 2.

The basal diet used in Experiment 1 and 2 provided a minimum of 200 mg digestible Cys. Results from Experiment 2, in which synthetic Cys was supplemented to the basal diet, indicate that only a small extra amount (7 mg/hen/d) of Cys may be needed for maximum egg production and feed efficiency. On the other hand, for maximum egg mass, no extra Cys was required since this response was linearly depressed as Cys addition to the basal diet increased. This result resembles a previous report of Harms & Russell (1996), who stated that a corn-soybean meal diet would always supply more Cys than required by the commercial laying hen. This seems to hold true also for sorghum- and soybean meal-based diets.

A wide variation in Met:Cys ratios (from 107 to 183%) and Cys requirements (from 197 to 362 mg/hen/d) can be found in the previously cited literature (Coon & Zhang, 1998; NRC, 1994; Joly, 2010; Lemme, 2009). For instance, the tables of requirements published by the NRC (1994) suggest a Met:Cys ratio and digestible Cys requirement of 107% and 241 mg/hen/d, respectively. From the analysis of published articles, Joly (2010) reported a Met:Cys ratio of 182% and a Cys requirement of 230 mg/hen/d, whereas Lemme (2009) estimated a Met:Cys ratio and Cys requirement of 115% and 362 mg/hen/d. In the experiment of Coon & Zhang (1998) with 33-week-old Hy-Line W36 hens, a Met:Cys ratio of 180% and a Cys requirement of 197 mg/hen/d were reported. These results suggest that there is not a defined relationship between the Met:Cys ratio and the Cys requirement. The same conclusion was drawn from the literature reviews reported by Joly (2010) and Lemme (2009).

In Experiment 2, differently from Experiment 1, egg weight was not affected by Met:Cys ratio changes, possibly because in Experiment 2 the dietary TSAA was kept constant across all treatments (80%), while in Experiment 1, this ratio was increased from 62% in the basal diet to 84% in the diet with the highest Met addition.

In a previous experiment (Gomez & Angeles, 2009), Hy-Line W-36 hens between 100 and 108 weeks of age (in the second egg-production cycle) fed sorghum-soybean meal diets showed similar Met and Thr requirements compared to hens on the first cycle of egg production fed corn-soybean meal diets. Also, Hy-Line W-36 hens between 60-70 weeks of age fed sorghum-soybean meal diets presented similar Lys requirement as younger hens fed corn-soybean meal diets (Angeles & Gómez, 2005). These previous reports, in addition to the results observed in the present research, suggest that laying hens fed sorghum-based diets have the same AA requirements as hens fed corn-based diets.

It is concluded that for Hy-Line W-36 hens between 68 and 75 weeks of age (Experiment 1), optimum egg production, egg mass, and feed efficiency were obtained with 0.30 and 0.50% of dietary digestible Met and TSAA, respectively. The productive performance was maximized at digestible Met and TSAA intakes of 288 and 478 mg/hen/d, respectively, and at Met:Lys and TSAA:Lys ratios of 47 and 77%, respectively. Also, in Hy-Line W-36 hens between 76-83 weeks of age (Experiment 2), optimum egg production and feed efficiency responses and the least body weight loss were observed at 151, 150, and 123% of dietary Met:Cys ratio, respectively. Digestible Met, Cys, and TSAA intake to maximize egg production and feed efficiency were 313, 207 and 510 mg/hen/d, respectively. These data suggest that Met, Cys and TSAA requirements of layers between 68 and 83 weeks of age fed sorghum- and soybean meal-based diets obtained in the present study are within the range of the values established in younger layers in the first egg production cycle and fed corn-soybean meal based diets.

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