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Effects of dietary total sulphur amino acids to lysine ratio on performance, nitrogen utilization of Ac layers (black-boned chicken)

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Abstract

A study was conducted to evaluate the effects of dietary total sulphur amino acids (TSAA) to lysine (Lys) ratio on performance of Ac layers (black-boned chicken). A total of 800 laying hens from 38 to 50 weeks old was allocated according to a completely randomized design with five treatments and 20 replicates of 8 birds each. The basal diet was formulated with 16% crude protein (CP), 2755 kcal/kg of metabolizable energy (ME), 0.482% methionine, 0.925% TSAA and 1.12% lysine. The TSAA/Lys ratio of the basal diet was 0.85. The basal diet plus four diets were formulated, two with TSSA levels at 10% and 20% below and two at 10% and 20% above that of the basal diet, giving 0.762%, 0.857%, 1.047% and 1.142% TSAA, respectively with the basal at 0.925% TSAA. The 10% decrease or increase in TSAA from 0.952% did not affect feed intake or egg production. However, further TSAA decreases from 0.857% to 0.762% led to reducing egg weight, which increased with a curvilinear trend as the level of TSAA in the diets was increased. TSAA influenced nitrogen retention in a quadratic trend, with hens fed diets containing 0.952% and 1.047 % TSAA retaining more nitrogen than the other treatments. Increasing dietary TSAA increased the white and yolk index and Haugh unit, whereas egg shell thickness, yolk colour and egg components were not influenced. The TSAA/Lys ratio of 0.85 improved egg weight and nitrogen retention of Ac layers.

Keywords: Egg weight, feed intake, performance, production, nitrogen retention, sulphur amino acid

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Introduction

Ac chicken is a native black-boned breed that is characterized by white feathers, skin, meat and bones, which is kept in the Mekong Delta of Vietnam. This breed has a small body size (0.9 kg), and is kept for meat and egg production. The Ac chicken has potential in meat and egg production because of its good flavour and relatively high price. Like other indigenous breeds, the growth performance and egg production of Ac chicken are low compared with those of commercial breeds. However, the consumption of Ac chicken meat and eggs has grown in Vietnam, particularly in Ho Chi Minh City. According to Phuong & Thien (2008), because they are kept in a scavenging system, Ac egg production is low with 90 - 95 eggs/year, though it could be improved to 180 - 190 eggs/year (Phuoc *et al*., 2016a) by providing good nutrition and disease control.

Non-ruminant animals have specific requirements for the amino acid, lysine (Han & Baker, 1991; Baker *et al*., 2002). The important role of lysine has been indicated by NRC (1984) and is considered a 'reference amino acid', and other essential amino acids are related to the percentage of lysine in diet formulation for pigs and poultry to maintain the balance among amino acids. Cereals such as maize and soybean are low in lysine (D'Mello, 1993) and methionine content (Waibel *et al*., 1995), but are used as main feed ingredient sources of poultry diets. Lysine is necessary for synthesis and improving protein deposition (Tesseraud *et al*., 1992) and changes metabolism in chicken (Tesseraud *et al*, 1996). Improved lysine intakes increased the final weight and carcass weights of broilers (Kidd & Flancher, 2001) and broiler chicks (Kidd *et al*., 1998), and enhanced the meat yield of Chinese local chicken (Yuan *et al*., 2015). High

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methionine intakes improved egg production (Harms & Russell, 2003) and increased egg weight (Sohail *et al*., 2002).

Many studies have been conducted on the nutrient requirements of poultry, and the need for essential amino acids such as lysine and methionine for laying hens has been pointed out (NRC, 1994; Leeson & Summers, 2005). However, information on protein, energy and amino acid requirements of Ac hens is limited.

Phuoc *et al*. (2016a, 2017) reported that a dietary protein level of 160 g/kg and 2700 kcal/kg of feed enhanced egg weight and egg mass of Ac hens in confinement. For commercial laying hens the requirements for lysine and methionine + lysine have been studied extensively, but few studies have been done using Ac hens. Judicious supplementation of amino acids for hens is one way to reduce dietary protein levels, decrease excreta nitrogen (N) and improve production performance.

Therefore, the aims of this study were to evaluate the effects of five ratios of TSAA/lysine (TSAA/Lys), at 10% and 20% lower and 10% and 20% higher than the NRC (1994) recommendations, on productivity, nutrient digestibility and N utilization of Ac hens from 38 to 50 weeks old.

Materials and Methods

This study was carried out for 10 weeks in Tien Giang Province. Eight hundred Ac layers (0.9 ± 0.05 kg) in an opened-side house were used in a completely randomized design. Diets were formulated on an available amino acid basis. The recommendation for TSAA/Lys (NRC 1994) in the diet is 0.85%, in which TSAA and lysine were 0.962% and 1.12%, respectively (Phuoc *et al*., 2018). The experiment consisted of five dietary treatments, which varied in TSAA/Lys ratios of 0.73%, 0.81%, 0.85%, 0.89% and 0.94%, with 20 replicates. One hundred cages with drinkers and troughs were randomly allocated to the five treatments with eight birds per each.

Table 1 Diet formulation and composition of basal diet of experimental Ac laying hens

Ingredients (%)	**(g/kg)**	**Nutrient composition (air dry basis)**	**%**
Maize	485.0	Dry matter	89.77
Wheat bran	133.3	Ash	11.35
Rice bran	65.0	Crude protein	16.04
Fish meal	28.0	Crude fat	4.37
Soybean meal	181.3	Crude fibre	3.00
Tra fish oil	10.0	Neutral detergent fibre	13.44
Coarse limestone	38.0	Calcium	4.07
Fine limestone	40.0	Phosphorus	0.67
Di-calcium phosphate	8.40	Lysine	1.12
Premix minerals[1]	2.50	Methionine	0.48
Premix vitamin[1]	2.50	Cystine	0.47
L-lysine HCl	2.60	TSAA[2]	0.96
DL-methionine	1.89	Tryptophan	0.18
L-cystine	1.52	AMEn (kcal/kg)[3]	2755
Total	1000		

[1] Supplied per kg diet: Fe: 200 mg, Cu: 40 mg, Zn: 60 mg, Mn: 60 mg, Co: 0.3 mg, iodine: 0.3 mg, Se: 0.3 mg, vitamin A: 8000 IU, vitamin B_6: 3 mg, vitamin D_3: 2500 IU, vitamin B_{12}: 15 mcg, vitamin E: 30 mg, pantothenic acid: 8 mg, vitamin B_1: 1.5 mg, folic acid: 0.5 mg, vitamin B_2: 4 mg, biotin: 100 mcg, vitamin K_3: 2 mg, niacin: 20 mg, vitamin C: 100 mg, choline chloride: 500 mg
[2] TSAA: total sulphur amino acids
[3] MEn: metabolizable energy, nitrogen balanced (Phuoc *et al*., 2016b)

Experimental diets:

Treatment 1: basal diet (BS) (TSAA/Lys = 0.85; TSAA = 0.952%) (BS100)

Treatment 2: 90% BS (TSAA/Lys = 0.81%; TSAA = 0.857%) (BS90)

Treatment 3: 80% BS (TSAA/Lys = 0.73%; TSAA = 0.762%) (BS80)
Treatment 4: 110% BS (TSAA/Lys = 0.89%; TSAA = 1.047%) (BS110)
Treatment 5: 120% BS (TSAA/Lys = 0.94&; TSAA= 1.142%) (BS120)

Before formulation, the amino acid contents of feed ingredients were determined (method 999.13, AOAC, 2012) and individual amino acids were added to the experimental diets to achieve the desired TSAA/Lys ratios (Table 1).

Hens were offered free access to the experimental diets from 38 to 50 weeks old, with the first two weeks as an adaptation period. The experimental data were taken from the last 10 weeks of the experiments. Feed consumption, egg production and egg weight were recorded daily on a cage basis. Egg mass was calculated as egg weight multiplied by egg production. Feed conversion efficiency was calculated by dividing the average feed intake (g/hen/day) by grams of egg mass.

For egg quality assessment, three eggs from 10 replicates were used. Egg shape index was assessed with Vernier callipers to measure the egg length (longitudinal axis) and width (equatorial axis) (Anderson *et al*., 2004). The shell, yolk and white were weighed to calculate the egg components (Romanoff & Romanoff, 1949; Abudabos, 2011). Haugh unit (HU) was calculated using Haugh's (1937) formula:

$$HU = 100 \log (h - 1.7w^{0.37} + 7.6)$$

where: HU = Haugh unit
h = albumen height (mm)
w = egg weight (g)

The thickness of the dried eggshell without membrane was measured with a micrometre to the nearest 0.01 mm and was calculated as the average of three positions taken at the broad end, equator and narrow end (Saleh *et al*., 2016).

Feed samples were collected three times during the experimental period, stored, sub-sampled and ground, using a 1–mm screen cyclotec grinder and analysed for dry matter (DM), ash, crude protein (CP), ether extract, crude fibre, calcium and phosphorus according to AOAC (1984). Amino acids of feed ingredients were analysed by Invivo Labs, Vietnam, using the method of AOAC (2012)

An N-balance experiment was carried out before the trial ended. Two hens were housed per metabolism cage with six replicates, each cage being equipped with a plastic self-feeder and a nipple drinker. All birds were fed pelleted diets. A 48-hour collection, excreta (faeces plus urine) was recorded twice daily at 7:00 and 16:00 from trays and packed in plastic bags. Each experimental unit was identified, and the excreta were frozen at -18 ^{o}C. Prior to analysis, the excreta were homogenized and sub-sampled, then determined for DM and N by a macro Kjeldahl method using Vapodest -20 (990.03 AOAC, 1984).

The calculations of apparent nutrient digestibility and N retention were done as follows (McDonald *et al*., 2011)

DM digestibility (%) = DM excreta (g)*100/DM feed consumed (g)
N excretion (g) = excreta (g)*N excreta (%)
N retention (%) = (N consumed - N excreta)*100/ N consumed (g)
Final N retention (%) = (N consumed (g) - N egg (g) - N excreta (g))*100/N consumed (g)
N egg (g) = N egg (%)*egg mass (g/hen/day)
N retained in egg = N egg (g)*100/N consumed

All calculations were done on a DM basis, and results were expressed per hen per day.

Data were analysed with the general linear model option of the ANOVA program in Minitab software. The model used was:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where: Y_{ij} is the individual observation,
μ the overall mean
$T_{i:}$ treatment effect (I = 1 - 5)
e_{ij} is the residual error

When F-test was significant at *P* <0.05, paired comparisons were performed with Tukey's procedure.

Results and Discussion

The TSAA/Lys ratio did not influence the intake of feed, protein, lysine or metabolizable energy (ME) (Table 2), but the intakes of methionine and TSAA increased as a result of increasing dietary methionine levels (P <0.01).

The TSAA/Lys ratio did not affect egg production (P = 0.78), while egg weight increased as the TSAA content of the diet increased from 0.762% to 0.857%. The results were similar to those of Novak *et al.* (2004) in that TSAA/Lys did not influence feed conversion and egg production. The authors' previous studies indicated that Ac hens fed diet of 160 CP*2750 kcal ME/kg of feed had similar egg production, egg weight and egg mass compared with those of 170*2850 and 180 g CP*2950 kcal/kg of feed (Phuoc *et al.*, 2017). According to Summers *et al*. (1991) and Penz & Jensen (1991), dietary CP of 16% was best for laying hens. Almeida *et al*. (2012) concluded that Hy-Line W-36 layers kept in hot temperature conditions and fed 2700 kcal ME/kg of feed had higher egg production and egg mass than those fed 3100 kcal/kg. The requirements of CP and energy of Ac hens are therefore not exceptional.

Phuoc *et al.* (2018) found that the ratio of lysine/ME influenced the feed intake of Ac hens. At the level of 0.32 (0.88/2750), birds consumed more feed than that of 0.43 (1.18/2750), while egg production, egg weight and egg mass were higher in hens fed a ratio of 0.41 (1.12/2750 (Lys/ME). Thus, the lysine level to optimize production performance for Ac hens was higher than that recommended for commercial laying hens in a hot climate (Rao *et al*., 2011). The inclusion of lysine at 0.7% in the diet of Lohman brown layers increased egg production and egg mass, while at 1%, hen day production was reduced and feed cost increased (Onimisi *et al*., 2012).

The NRC (1994) stated that commercial brown-egg layers with 100 g daily feed consumption need 690 mg lysine per day. Among chicken breeds, hens with 80 g and 120 g daily feed intake require lysine concentration of 0.86% and 0.58%, respectively. The two concentrations provided the same amount of 690 mg lysine per hen per day. According to NRC (1994) birds with low feed intakes required high concentrations of nutrients. However, many researchers (Scheideler *et al*., 1996; Novak & Scheideler, 1998) considered that amount was not enough to enhance laying hen performance, and thought that the daily lysine intake should be between 850 mg and 900 mg. Prochaska *et al.* (1996) found that egg production and egg weight were increased in hens (Hy-Line W-36) that consumed 828 mg to 1062 mg lysine/day. In Ac hens, which are small in size, have low feed intake, and are kept in a hot climate, Phuoc *et al*. (2017) found that the optimum dietary lysine concentration of 1.12% provided 700 to 750 mg per hen per day as the hens feed ad libitum. Thus, this figure was used as a reference to control TSAA levels in formulating the experimental diets.

The present results indicated that the amounts of protein, energy and amino acid, lysine, formulated in the basal diet seemed enough for Ac hens.

The TSAA levels when increased above 10% or 20% of the basal diet were probably more than the needs of the birds. Thus egg production, egg mass and feed efficiency were not affected (Table 2). These results were similar to those reported by Alagawany & Abou-Kassem (2014), who found that feed intake, feed efficiency, egg production and egg mass of Lohmann Brown hens were not affected by dietary methionine and lysine levels. However, Bateman *et al*. (2008) found that feed efficiency improved with increasing TSAA/Lys ratios.

There was a quadratic effect (P = 0.01) of increasing levels of TSAA on egg weight by second-order equation as follows:

$$\text{Egg weight} = 13.79 + 45.32\ \text{TSAA} - 23.43\ \text{TSAA}^2 \ (R^2 = 0.92;\ \text{Figure 1})$$

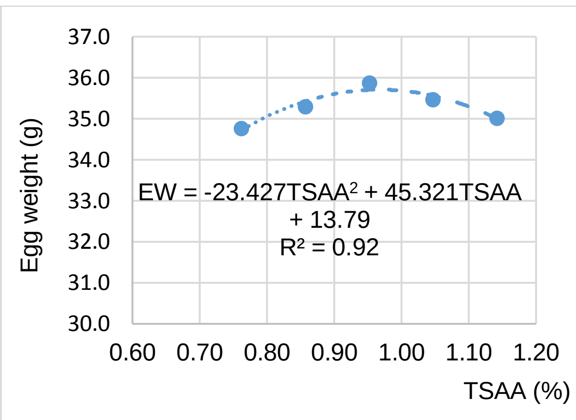

Figure 1 Effect of dietary total sulphur amino acids (TSAA)/lysine on egg weight (EW) of Ac hens

This observation was in agreement with the finding of Harms & Russell (2003) that increasing the methionine level above the requirements recommended by NRC (1994) produced a quadratic response in Hy-Line hens. Similarity, Fouad *et al*. (2016) said that increasing methionine levels did not affect egg production, but improved the egg weight and feed efficiency of ducks. Bertram *et al.* (1995) reported that dietary methionine improved the production performance of Leghorn laying hens. The best optimum responses of egg production, egg weight and egg mass were when TSAA levels were 0.58%, 0.64% and 0.61%, respectively. Narváez-Solarte (2005) said that enhancing the dietary TSSA ameliorated egg production and egg weight. In a study on local Sinai laying hens, Elkloub *et al.* (2015) reported that dietary methionine levels did not influence egg weight, but increased egg mass.

Egg production in this study was not affected by dietary methionine and TSAA levels and indicated that TSAA intake of the basal diet was enough to maintain the egg production, egg mass and feed efficiency of Ac hens.

Saki *et al.* (2012) used a broken line model to estimate methionine and TSAA requirements, which showed that dietary methionnine below 0.34% (0.62% TSAA) reduced the egg weight of commercial laying hens. Polese *et al*. (2012) reported that dietary TSAA levels displayed a quadratic effect on egg weight of Shaver Brown laying hens. This response was also mentioned by Ahmad & Roland (2003), who stated that the intake of various TSSA levels had no effect on egg production and feed conversion, but increasing TSAA levels improved egg weight of Dekalb Delta hens.

The maximum value obtained in the TSAA/Lys ratio of 0.85, corresponds to the consumption of 319 mg methionine and 361 mg TSAA/bird/day, respectively, with daily DM intake of 59.49 g.

Although feed efficiency was not influenced by the TSAA/Lys ratio (P = 0.22), there was a quadratic effect (P = 0.02) of decreased TSAA levels:

$$\text{Feed conversion efficiency} = 7.653 - 8.625\text{TSAA} + 4.329\text{TSAA}^2 \; (R^2 = 0.98) \; \text{(Figure 2)}.$$

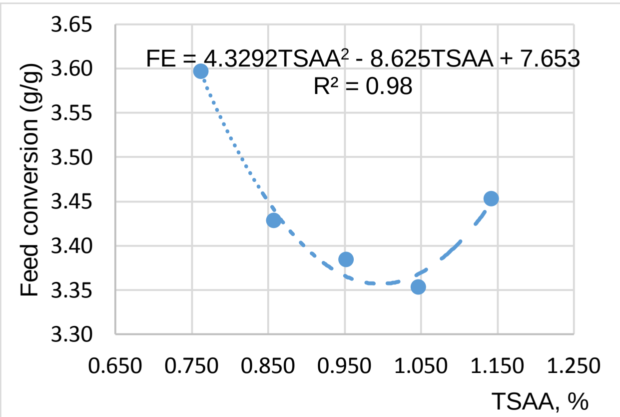

Figure 2 Effect of dietary total sulphur amino acids (TSAA) on feed conversion efficiency (g feed/g egg weight)

The best feed efficiency values received, were from 3.35 to 3.39 (g/g) with hens the basal and BS110 diets.

The requirements for methionine and TSAA of commercial laying hens vary widely (Wu *et al*., 2005; Novak *et al*., 2006; Domingues *et al*., 2012). According to Bregendahl *et al*. (2008), many studies on amino acid requirements had been conducted under various experimental conditions of dietary nutrient concentrations, lines or breeds and ages. These affected the amino acid requirement of hens. In 1984, the NRC recommended a daily intake of 350 mg methionine/day, which was reduced to 300 mg/day in 1994. This figure was considered low for commercial laying hens (Novak *et al*., 2004). Ahmad & Roland (2003) indicated that the maximum requirements for TSAA depended on environmental and market conditions.

The production of Ac chickens on the traditional small scale has low input in the form of low egg production, small-sized eggs (30 - 32 g) and a slow growth rate. Ac hens stay broody for almost 21 days. This causes a decrease in egg production. Owing to market demand, Ac hens are now kept commercially. Their egg production has improved substantially because the birds are provided with more nutrients and lighting is controlled. The sitting day is reduced and is quickly disappearing. Ac hens produce approximately 200 eggs per production year. This indicates that nutrition has improved the production performance of Ac hens.

The results in Table 2 indicate that the optimum egg weight and feed conversion responses for Ac hens were observed at 100% (BS) and 110% of dietary TSAA/Lys.

Table 2 Effect of total sulphur amino acids/lysine ratio on feed and nutrient intake and production performance of Ac hens

	BS	BS90	BS80	BS110	BS120	SEM	*P*-value
Intake (g/day)							
Feed consumption	66.27	64.23	66.91	65.78	65.65	1.12	0.53
Dry matter	59.49	57.66	60.07	59.05	58.94	1.00	0.53
Crude protein	10.63	10.30	10.73	10.55	10.53	0.18	0.53
Lysine	0.743	0.720	0.750	0.736	0.737	0.01	0.53
Methionine	0.319[c]	0.278[d]	0.258[d]	0.380[a]	0.348[b]	0.01	0.01
Methionine + cystine	0.631[c]	0.550[d]	0.510[d]	0.751[a]	0.687[b]	0.01	0.01
MEn, kcal/day	164	159	165	163	162	2.76	0.53
Production performance							
Egg production (%)	55.47	53.42	53.80	55.24	55.41	1.47	0.78
Egg weight (g)	35.87[a]	35.29[ab]	34.76[b]	35.01[ab]	35.47[ab]	0.22	0.01
Egg mass (g/hen/day)	19.91	18.85	18.71	19.36	19.66	0.55	0.49
Feed efficient (g/g)	3.39	3.43	3.60	3.45	3.35	0.08	0.22

[a,b,c,d] Row means with different superscripts differ significantly at P <0.05
BS: basal diet; BS90: TSAA/Lys = 0.81%, TSAA = 0.857%; BS80: TSAA/Lys = 0.73%, TSAA = 0.762%; BS110: TSAA/Lys = 0.89%, TSAA = 1.047% and BS120: TSAA/Lys = 0.94, TSAA = 1.142%
Lys: lysine; TSAA: total sulphur amino acids

The TSAA/Lys ratio slightly increased the DM digestibility of the basal diet (P = 0.09) but did not influence the digestibility of organic matter and ether extract (Table 3).

Table 3 Effect of dietary total sulphur amino acids/lysine ratio on nutrient digestibility and nitrogen (N) utilization

	BS diet	BS80	BS90	BS110	BS120	SEM	*P*-value
Digestibility (%)							
Dry matter	73.43	68.66	67.83	71.44	71.11	1.49	0.09
Organic matter	77.24	73.96	72.62	76.47	75.43	1.37	0.15
Ether extract	66.88	59.82	60.72	62.68	63.64	3.33	0.61
Nitrogen utilization (%)							
N-retention[1]	52.47[a]	46.87[ab]	44.83[b]	51.36[a]	48.85[ab]	1.53	0.01
Egg nitrogen (g/day)	0.327	0.318	0.275	0.263	0.281	0.02	0.12
N egg/N intake	19.60	18.76	16.77	16.08	17.05	1.05	0.13
Final N-retention[2]	32.87	28.11	28.06	35.27	31.80	1.86	0.05

[1] = N intake - N excreta,
[2] = N intake - N excreta – N egg,
[a,b]: Row means with different superscripts differ significantly at P <0.05.
BS: basal diet; BS90: TSAA/Lys = 0.81%, TSAA = 0.857%; BS80: TSAA/Lys = 0.73%, TSAA = 0.762%; BS110: TSAA/Lys = 0.89%, TSAA = 1.047% and BS120: TSAA/Lys = 0.94, TSAA = 1.142%.
Lys: lysine; TSAA: total sulphur amino acids

Nitrogen retention (Nr) was affected significantly by treatments (P =0.01) with the third-order equation as follows:

Nitrogen retention = 327.1 – 1045 x + 1252 $TSAA^2$ - 482 $TSAA^3$ (R^2 = 0.92; Figure 3).

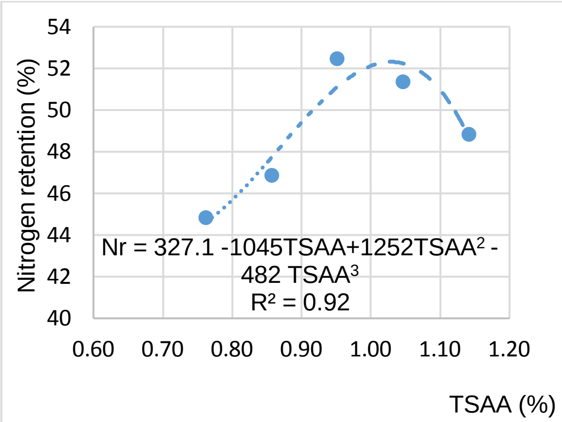

Figure 3 Effect of percentage dietary total sulphur amino acids (TSAA) on nitrogen retention (Nr)

The maximal value obtained in the TSAA/Lys ratio of 0.85 corresponds to the consumption 319 mg methionine and 361 mg TSAA/bird/day, with daily DM intake of 59.49 g.

Similarity, final N retention was increased with increasing dietary TSAA (P = 0.05) as shown:

Final N retention (Fi Nr) = 813.64 -2626 TSAA + 2882.5 $TSAA^2$ -1035.2 $TSAA^3$ (R^2 = 0.99; Figure 4).

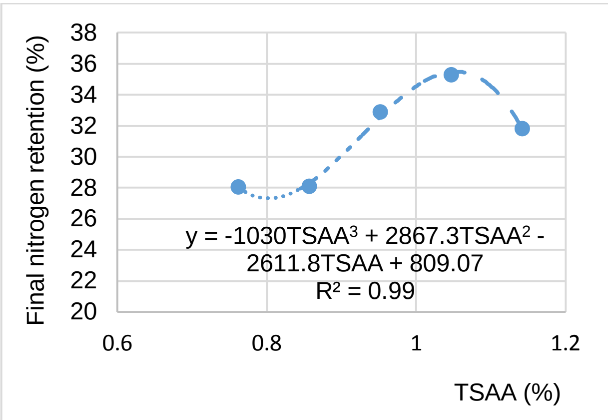

Figure 4 Effect of percentage dietary total sulphur amino acids (TSAA) on final nitrogen retention (y) of Ac hens

According to Alagawany (2014), N retention was affected by TSAA intake, meaning that reducing dietary TSAA led to decreasing N excretion. The dietary methionine addition reduced N retention (Nonis & Gous, 2006). In the present study, reducing dietary TSAA/Lys ratio did not cause negative effects on egg production, egg mass and feed conversion. However, increasing the TSAA/Lys ratio over 20% or reducing the BS diet by 10% to 20% led to reduced N retention. To achieve both, using the average level of 0.952% of TSAA, the BS diet was more effective in term of economy.

The TSAA/Lys ratio had no effect on shade index and egg components such as albumen, yolk and shell percentage, while albumen and yolk index increased with enhancing TSAA levels (Table 4). Amaefule *et al*. (2004) found that methionine addition did not affect the egg quality of black Bovan Nera laying hens in the hot season. Bunchasak *et al*. (2012) reported that DL-methionine supplementation decreased the albumen percentage of egg. Shafer *et al*. (1998) concluded that albumin protein was affected by dietary methionine. An increasing albumen index could be affected by raising TSAA intake. Albumen weight responded with increasing methionine in the diets (Fouad *et al*., 2016). This present study indicated that the Haugh unit was higher in diets containing more TSAA and eggshell thickness tended to be higher as dietary TSAA increased. Narváez-Solarte *et al*. (2005) found an improvement in the Haugh unit as the TSAA levels in the diet reduced, but Omara & Romeilah (2009) said that the Haugh unit was higher in hens fed diet that included 0.40% methionine compared with that of 0.35%. Novak *et al*. (2004) did not observe any effect of digestible TSAA levels on the Haugh unit. However, many factors such as storage time, temperature and hen age could influence the internal quality of eggs (Akyurek & Okur, 2009; Jin *et al*., 2011; Chung & Lee, 2014), thus varied results were commonly found among studies.

Table 4 Effect on egg quality of dietary total sulphur amino acids to lysine ratio

	BS diet	**BS90**	**BS80**	**BS110**	**BS120**	**SEM**	***P* value**
Shade index	79.56	77.90	78.54	78.73	78.52	0.52	0.28
Albumen index	0.065[ab]	0.054[b]	0.055[b]	0.063[ab]	0.07[a]	<0.01	0.01
York index	0.39[ab]	0.36[c]	0.37[bc]	0.38[bc]	0.41[a]	0.01	<0.01
Albumen rate (%)	54.88	54.54	55.04	55.31	55.29	0.52	0.82
Yolk rate (%)	34.14	34.33	33.76	33.64	33.53	0.49	0.74
Yolk/albumen (%)	62.41	63.1	61.49	61.05	61.89	0.01	0.79
Egg shell rate (%)	10.97	11.14	11.20	11.04	11.17	0.15	0.82
Haugh unit	75.99	71.02	72.13	74.76	76.95	1.49	0.03
Yolk color	6.63	6.13	6.37	6.80	6.53	0.18	0.10
Egg shell thickness (mm)	0.355	0.352	0.353	0.367	0.365	<0.01	0.08

[a,b] Row means with different superscripts differ significantly at $P < 0.05$
BS: basal diet; BS90: TSAA/Lys = 0.81%, TSAA = 0.857%; BS80: TSAA/Lys = 0.73%, TSAA = 0.762%; BS110: TSAA/Lys = 0.89%, TSAA = 1.047% and BS120: TSAA/Lys = 0.94, TSAA = 1.142%.
Lys: lysine; TSAA: total sulphur amino acids

Conclusion

It is concluded that for Ac laying hens between 38 to 50 weeks old, optimum egg weight, feed conversion and N retention were obtained at the level of 0.482% methionine and 0.952% TSAA, corresponding to methionine and TSAA intake of 319 and 613 mg/hen/day, respectively. The productive performance was maximized at a TSAA/Lys ratio of 0.85, as recommended by NRC (1994).

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Authors' Contributions

TVP conducted this experiment, performing laboratory and statistical analysis as part of a PhD degree in Animal Science under the supervision of NNXD and LHM for all processes through performing the experiment to writing the manuscript.

Conflict of Interest Declaration

There is no conflict of interest with regard to this work.

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