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Original article

Influences of total sulfur amino acids and photoperiod on growth, carcass traits, blood parameters, meat quality and cecal microbial load of broilers

Diaa E. Abou-Kassem [a], Mohamed M. El-Abasy [b], Muhammad S. Al-Harbi [c], Salah Abol-Ela [b], Heba M. Salem [d], Amira M. El-Tahan [e], Mohamed T. El-Saadony [f], Mohamed E. Abd El-Hack [b,]*, Elwy A. Ashour [b]

[a] Animal and Poultry Production Technology Department, Faculty of Technology and Development, Zagazig University, Zagazig 44519, Egypt
[b] Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
[c] Department of chemistry, College of Science, Taif University, P.O. Box 11099, Taif 21944. Saudi Arabia
[d] Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, 12211, Egypt
[e] Plant Production Department, Arid Lands Cultivation Research Institute, The City of Scientific Research and Technological Applications, SRTA-City. Borg El Arab, Alexandria, Egypt
[f] Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, 44511 Zagazig, Egypt

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ABSTRACT

The current study aimed to discuss the impact of total sulfur amino acids (TSAA) %, photoperiod, and their interaction on growth performance, carcass and blood indices of broiler chicks. A total of 300 unsexed IR broiler chicks one-week old were used in a factorial arrangement (2 × 3), including two photoperiod systems (22 L: 2 D and 16 L: 8 D) and three experimental rations having three grades of Met + Cyst (TSAA) (70%, 85% and 100% of digestible lysine in starter and finisher diets). Results revealed that the higher LBW and BWG were noticed in birds given TSAA at grades of 1.1 or 0.90 % under 22L: 2D photoperiod at five weeks of age and the whole experimental period (1–5 weeks of age), respectively. The highest live body weight (LBW (and body weight gain (BWG) were recorded in birds received 1.1% TSAA under the long photoperiod compared to the control and the other groups. Birds fed 1.3% TSAA consumed more feed than the other groups. The opposite was found in birds fed 1.1% TSAA under the short photoperiod (16L: 8D). The best feed conversion (FCR) was detected by birds fed 1.1% and 0.90% TSAA diets during the whole experimental period. All carcass traits studied were significantly influenced by TSAA levels, except for the relative weights of abdominal fat and spleen. The interaction effect on was significant on all carcass traits except spleen %. In conclusion, the addition of TSAA at level 1.1 and 0.9 % to starter and finisher diets under a long photoperiod regime improved broiler's performance, carcass traits, and blood parameters studied.

1. Introduction

Effective broiler production relies on fast growth rates and an effectual feed conversion rate. To achieve these goals, it is usual for feeds to be available as desired and stocks are kept under continuous or semi-continuous lighting (23 or 23.5 h). The impact of light on broiler production has shown inconsistent results in several research reports (El-Sayiad et al. (2005)). The photoperiod is defined as the relative amount of light per day to which an organism is exposed (Lee et al., 2017). Previous researchers showed that hours of darkness are important like lighting for the growth and health of broilers (Classen et al., 1991). Many companies implement continuous or semi-continuous lighting systems to improve growth rate and feed intake (Olanrewaju et al., 2013). Increased photoperiods, too, have favorable impacts on broiler performance and feed conversion (Lien et al., 2007).

The European Union (EU) has mandated rearing birds with lighting that follows a 24-hour rhythm and includes periods of darkness lasting at least six hours in total, with at least one period of continuous darkness lasting at least four hours (EU, 2007). El-

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* Corresponding authors.
E-mail address: m.ezzat@zu.edu.eg (M.E. Abd El-Hack).

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Sayiad et al. (2005) found that live body weight of chicks improved when chicks were exposed to 16 or 24 hrs photoperiods as compared to 8 hrs during all the experimental periods, while no significant effect was found on feed conversion ratio. Physical activity and walking ability are markedly improved when darkness is included in the lighting program (Lardner et al., 2016). It is hypothesized that short photoperiods early in life reduces feed intake and limit growth.

Yang et al. (2015) showed that four hours of darkness had a good impact on the growth rate of broilers and their carcass characteristics. Also, Baykalir et al. (2020) concluded that broiler chicks subjected to 16 L: 8d had less slaughter, carcasses, and lower weights, while an 8 L: 4 D treatment gave a positive effect on broiler chicks in terms of carcass characteristics. A pathogenic period of semi-continuous photophobia is being applied in modern broiler breeding. Recently, Abo-Al-Ela et al. (2021) confirmed that the exposure time and light level modulated the bird's health and increased performance under normal or stress conditions.

Adding high levels of artificial amino acids like methionine and lysine to corn and soybean meals can enhance insulin secretion (Murray et al., 1988). This raises the uptake of amino acids and protein synthesis in multiple tissues (Sigolo et al., 2019). Methionine (Met) is the top amino acid in classical diets applied for young birds. It plays a serious role in protein formation and metabolism (Baker, 2006). Methionine is a major amino acid for livestock, utilized to synthesize protein and many amino acids. Optimum methionine addition can improve growth performance (Yang et al., 2016; Abd El-Hack et al., 2017). The use of synthetic amino acids as a nutritional supplement has a large impact on growth and production rates, as stated by Alagawany and Mahrose (2014) and Alagawany et al. (2016). The best performance can be gained with an adequate level of needful amino acids, mostly Met (Yuan et al., 2012). The study by Abou-Kassem (2006) showed that the improvements in body weight and body weight gain in quail chicks were detected by birds given 0.95% total sulpher amino acids (TSAA) followed with 0.85% compared to the control (0.75%). Recently, in other species, Ashour et al. (2020a, 2020b) reported that high dietary TSAA level gave a significant ($p < 0.05$) increase in feed consumption and improved FCR through 12–18 weeks of age. The present study aimed to investigate the impact of photoperiod, dietary TSAA levels and their interactions on broiler's growth performance, carcass characteristics, and blood parameters during 1–5 weeks of age.

2. Materials and Methods

2.1. Birds, design, and diets

This study was conducted in Poultry Research Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. All procedures were carried out according to the guidelines of the local committee for the care of experimental animals and confirmed by the ethics of the Institutional Council of the Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. A total of 300 un-sexed one-week old IR broilers with an initial body weight of 104.90 ± 0.12 g was used in a factorial arrangement involving six groups: 60 birds in five replicates ($6 \times 5 \times 10$). Chicks were purchased from a commercial hatchery. Birds were exposed to semi-continuous photoperiod length a 23L:1D with rotation at the first week. At the begging of the seventh day, empirical light was used. A 2×3 factorial arrangement was performed including two photoperiods (22 L: 2 D and 16 L: 8 D) with rotation and three experimental diets were formulated by utilizing three levels of Met + Cyst (TSAA) (70%, 85% and 100% of digestible lysine in starter and finisher periods). The birds were kept in proper pens under the same managerial, health and environmental conditions. All birds were given the basal diets (in pellet form) according to NRC (1994) as shown in Table 1. The experimental rations were fed in two stages: starter (1–3) and finisher (4–5) weeks of age.

2.2. Performance, carcass, and blood biochemical parameters

The parameters were measured one time a week. Average daily feed intake (FI), live body weight (LBW), body weight gain (BWG), and feed conversion ratio (FCR) were determined. Thirty chicks were randomly selected from all groups and slaughtered at the age of 35 days. The carcasses were weighed, and the edible parts (liver, gizzards, and hearts) and spleen, bursa, and intestinal fat were weighted as g/ kg of the slaughter weight (SW). Carcass and dressed weights were expressed as (dressed weight = carcass weight + edible weight)/ live body weight.

Blood samples were taken from five birds per group. Samples were promptly centrifuged (Janetzki, T32c, 5000 rpm, Germany) at 2146.56 xg for 15 min. The obtained serum was then frozen at -25 °C till the biochemical tests (Sitohy et al., 2013). The levels of total protein, albumin, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea-N were determined following Salvaggio et al. (1991). Triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were measured using kits according to the protocol provided by the manufacturer (Spinreact Co., Spain) (Abdel-Hamid et al., 2020).

2.3. Microbial analysis in broiler cecum

The microbial analysis in broiler cecum was estimated as follows: In a screw bottle, broiler cecal samples were collected and transferred quickly to the microbiological laboratory. 10 g from broiler cecal samples were homogenized in 90 ml sterilized saline peptone water (1 g peptone: 8.5 g NaCl) to obtain 10^{-1} dilution. Serial dilutions from the previous (10^{-1}) were prepared up to 10^{-7} (El-Saadony et al., 2021a; El-Saadony et al., 2021b; El-Saadony et al., 2021c). The total bacterial count was enumerated on Plate count Agar (PCA) following El-Saadony et al. (2021d); El-Saadony et al. (2021e). According to Ashour et al. (2020a, 2020b), Sheiha et al. (2020) and Saad et al. (2021a), total coliforms were counted on the MacConkey agar medium. *Salmonella* spp., were counted on S.S. agar with black colonies, according to Abdelnour et al. (2020) and Abd El-Hack et al. (2021). Molds were also estimated (Saad et al., 2021b; Abou-Kassem et al., 2021). The count of lactic acid bacteria was calculated on MRS-medium following (Alagawany et al., 2021a; Alagawany et al., 2021b). *Enterococcus* spp., with red colonies, was counted at chromocult enterococci agar (Reda et al., 2020; Reda et al., 2021a; Reda et al., 2021b).

2.4. Breast meat quality and sensory evaluation

The color parameters [L^* (lightness), a^* (redness), and b^* (yellowness)] of raw and cooked meat samples (cubes, 2 cm) were measured by Hunter Lab colorimeter (Color Flex EZ, USA) following the procedure described in (Wattanachant et al., 2005). The Lipid peroxidation was measured by a 2-thiobarbituric acid test (TBA) (Fernández-López et al., 2005). Total volatile bases nitrogen (TVBN) was estimated according to Botta et al. (1984). The pH value of minced meat samples was assessed using a pH meter (pH 211 HANNA instruments Inc. Woonsocket, USA). The chemical composition of meat was also estimated by Wattanachant et al., 2005. Moisture content was determined by oven method (AOAC, 2005); protein was determined by Kjeldahl method (AOAC, 2005); fat

Table 1
Composition and chemical analysis of the starter and finisher basal diets as fed.

CP%	Starter			Finisher		
Methionine %	0.9	1.1	1.3	0.7	0.9	1.1
Ingredients (g/kg diet)						
Yellow Corn	55.88	55.88	55.88	57.00	57.00	57.00
Soybean meal	31.50	31.50	31.50	29.50	29.50	29.30
Gluten meal	6.50	6.30	6.10	4.83	4.63	4.63
Di Calcium phosphate	1.70	1.70	1.70	1.70	1.73	1.77
Limestone	1.24	1.24	1.24	1.15	1.15	1.15
Vit-min Premix[**]	0.30	0.30	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.14	0.34	0.53	0.07	0.24	0.40
L-Lysine HCl	0.24	0.24	0.24	0.11	0.11	0.11
Choline 50%	0.20	0.20	0.20	0.20	0.20	0.20
Soybean oil	2.00	2.00	2.00	4.84	4.84	4.84
Total	100	100	100	100	100	100
Calculated analysis[**]						
Dry matter %	91.72	91.73	91.76	90.43	90.51	90.66
Crude protein %	23	22.9	22.9	20.94	20.96	20.96
Metabolizable energy kcal./kg	2996	2996	2996	3150	3150	3153
Calcium %	1.00	1.00	1.00	0.96	0.96	0.96
Phosphorous, Available) %	0.44	0.44	0.44	0.44	0.44	0.44
Lysine %	1.30	1.30	1.30	1.10	1.10	1.10
Methionine + Cysteine %[*]	0.91	1.11	1.30	0.77	0.94	1.10
Crude fibre %	3.52	3.52	3.51	3.38	3.37	3.37

[***] Calculated according to NRC (1994).

[*] Met + Cyst. at the average of 70, 85 & 100% of digestible lysine, respectively.

[**] Vitamins and minerals mix provide per kilogram of ration Trace mineral (milligrams per kilogram of diet) Mn, 66; Zn, 50; Fe, 30; Cu, 4; Se, 0.1 and Ethoxyquin 3 mg, and vitamin A (as alltransretinyl acetate); 1200 IU; Vitamin E (all racatocopheryl acetate); 10 IU; k3 3 mg; Niacin, 20 mg; Vitamin B12, 10 mg; Vitamin B6, 1.5 mg; Thiamine (as thiamine mononitrate); 2.2 mg; Folic acid, 1 mg; D biotin, 50 mg Vit.D3, 2200 ICU; Riboflavin, 10 mg; Ca Pantothenate, 10 mg.

was estimated by the Soxhlet apparatus method (AOAC, 2005); and a muffin assessed ash at 600 °C (AOAC, 2005).

2.5. Sensory evaluation

The cooked meat samples were cut into cubes (2 cm) (Zhao et al., 2019). Eight experienced panelists have received meat samples in foam plate coded with random 3-digits. The sensory panel followed the descriptive sensory assessment carried out using a variation of the Sow and Grongnet (2010), Zhuang, and Savage (2011) process. The panelists have evaluated the following attributes (color, flavor, appearance, and juiciness) using a 9-point Hedonic scale, where 1 = strongly dislike and 9 = strongly like. Tap water was provided between sessions to alter the mouth-feel.

2.6. Statistical analysis

Data were statistically analyzed using (SPSS, 2014) according to Snedecor and Cochran (1982), as shown in following the model:

$$Y_{ijk} = M + A_i + S_j + AS_{ij} + e_{ijk}$$

where: Y_{ijk} = an observation, M = overall mean, A_i = influence of photoperiods (i = 1 to 2), S_j = influence of total sulfur amino acids levels (j = 1 to 3), AS_{ij} = the interaction effect between photoperiods and total sulfur amino acids grades and e_{ijk} = random error. Significant differences among means were tested according to Duncan's multiple tests (Duncan, 1955).

3. Results

3.1. Growth performance

Data in Table 2 suggested a considerable ($p < 0.01$) decrease in live body weight of broilers with reducing photoperiod regime during all stages studied (2, 3, 4 and 5 wks of age). On the contrary, the TSAA addition had significant impacts ($p < 0.01$) on the body weight of chicks through various experimental periods. For TSAA effect, in the starter period (1–3 wks), the highest LBW value was associated with the level of 1.3% TSAA (100 % of digestible lysine). For the interaction impact, there was a significant influence due to the main factors studied. The highest body weight was obtained by long photoperiod followed by a short one, respectively. The highest body weight was obtained under long photoperiod by 1.1% (85% of digestible lysine) level of addition of TSAA (689.91 gm/bird) during the starter period. For the finisher period (4–5 wks) the highest value was gained at a level of TSAA 1.1% (85 % from digestible lysine), (1935.40 gm/bird) at the end of the finisher period (5 wks). The short photoperiod (16L: 8D) recorded the lowest body weights with the different levels of TSAA tested in all studied periods (Table 2).

Body weight gain significantly decreased ($p < 0.01$) with decreasing the length of the photoperiod, as shown in Table 3. The interaction effect was the best during all interval periods. The highest values of LBW and BWG were observed in birds fed TSAA at levels of 1.1 or 1.3 % (85 or 100% of digestible lysine) and 1.1 or 0.9% (85 or 70 % from digestible lysine) under 22L: 2D photoperiod at five weeks of age and during the whole experimental period (1–5 weeks of age).

Data in Table 2 reported that mortality rate was not influenced by the photoperiod, TSAA levels, and their interactions during the different experimental periods.

3.2. Feed intake and feed conversion ratio

Results in Table 4 showed a significant ($p < 0.001$) impact on feed consumption of chicks due to photoperiod's regime (22 or 16 L) during all ages studied except the whole period. In contrast, TSAA significantly influenced feed intake of broilers. At the same time, the interaction effect was significant on the main factors studied. The highest FI was noticed in birds given 1.3% TSAA. In contrast, the worst FI was recorded by birds fed 1.1% TSAA under the short photoperiod (16 L: 8 D) (Table 4).

Table 2
Body weight as affected by photoperiod, TSAA and their interaction.

Items	Body weight					MR
	1w	2w	3w	4w	5w	1-5w
Photoperiod (h/day)						
22	104.53	309.66a	664.92a	1097.10a	1988.20a	2.30
16	104.70	295.32b	628.38b	1052.30b	1726.00b	2.72
SEM	0.10	1.47	2.50	10.44	21.13	0.54
p-value	0.282	<0.001	<0.001	0.011	<0.001	0.204
TSAA (%)						
1.30	104.25	293.48c	656.62a	1101.20	1798.15c	1.82
1.10	105.15	311.27a	646.04b	1064.35	1935.40a	0.00
0.90	104.45	302.70b	637.29c	1059.40	1838.75b	1.82
SEM	0.13	1.80	3.06	12.79	17.54	0.69
p-value	0.110	<0.001	0.003	0.075	<0.001	0.327
Interaction						
22/1.30	104.20	302.69c	667.41b	1113.80	1843.30c	1.82
22/1.10	104.30	322.64b	689.91a	1112.50	2161.00a	0.00
22/0.90	104.10	303.64c	637.44c	1064.25	1958.65b	1.82
16/1.30	104.30	384.27a	645.82c	1088.35	1752.70d	0.00
16/1.10	105.00	299.91c	602.18d	1016.10	1707.95e	2.72
16/0.90	104.80	301.77c	637.15c	1053.00	1717.05e	0.00
SEM	0.53	12.06	4.03	39.33	24.80	0.83
p-value	0.151	0.004	0.038	0.078	<0.001	0.416

Table 3
Body weights gain as affected by photoperiod, TSAA and their interaction.

Items	Body weight gain g bird/week				
	1-2w	2-3w	3-4w	4-5w	1-5w
Photoperiod (h/day)					
22	205.13a	355.26a	432.18a	891.10a	1883.67a
16	190.62b	333.06b	423.92b	673.70b	1621.30b
SEM	8.47	9.54	13.81	11.04	19.38
p-value	<0.001	0.002	0.023	<0.001	<0.001
TSAA (%)					
1.30	189.23b	363.14a	444.58a	696.95c	1693.75c
1.10	206.12a	334.77b	418.31b	871.05a	1830.40a
0.90	198.24ab	334.59b	422.11b	779.35b	1734.30b
SEM	8.75	9.70	12.93	15.81	21.05
p-value	<0.001	<0.001	0.035	0.002	0.003
Interaction					
22/1.30	198.49c	364.72a	446.39a	729.50c	1739.10c
22/1.10	218.34b	367.27a	422.59b	1048.50a	2056.70a
22/0.90	199.54c	333.80b	426.81b	894.40b	1854.55b
16/1.30	279.77a	261.55d	442.53a	664.35e	1648.20d
16/1.10	194.91c	302.27c	413.92c	691.85d	1602.95e
16/0.90	196.97c	335.38b	415.85c	664.05e	1612.25e
SEM	11.83	26.52	37.64	39.58	32.08
p-value	0.005	0.020	0.003	<0.001	<0.001

Feed conversion ratio was not influenced ($p < 0.01$) by photoperiod's regime (22 or 16 h L) as reported in Table 5. Also, the dietary addition of TSAA significantly ($p < 0.01$) improved the FCR of birds. The best FCR was given by birds fed 1.1% and 0.90% TSAA which recorded 1.47 and 1.52 through 1–5 wks of age, respectively. The interactions among photoperiods and dietary TSAA levels significantly ($p < 0.05$) affected FCR values at only at 2–3 and 3-4wks of age (Table 5).

3.3. Carcass traits

Results in Table 6 show insignificant ($p < 0.01$ and 0.05) impacts of photoperiod systems on all carcass properties except for giblets and abdominal fat , M, by using the short-term photoperiods outcomes given the best carcass traits studied compared to another long photoperiod used. On the other hand, TSAA addition significantly influenced all carcass traits studied, except the relative weights of abdominal fat and spleen (Table 6). Also, the interaction effect on all carcass traits studied was significant except for spleen. The highest value of dressing % was recorded with 1.1% TSAA under short photoperiod regime (16 L: 8 D). The lowest abdominal fat % was found in birds fed 0.90% TSAA under the short photoperiod program as reported in Table 6.

3.4. Some blood parameters

Results in Table 7 revealed an important ($p < 0.001$) influence due to photoperiod on each of AST and ALT activities. The highest levels were recorded with 16 h photoperiod. Due to TSAA% addition, each of total protein, AST and ALT were statistically ($p < 0.01$) impacted. The highest levels of total protein, AST and ALT were accompanied with 0.90% TSAA compared to the other treatment groups. For the interaction effect, only total protein, AST and ALT were significantly ($p < 0.05$ and 0.01) influenced. The highest levels were recorded with 0.90% TSAAbelow 16 h photoperiod compared with the other groups.

Results in Table 8 showed a significant ($p < 0.01$) influence due to photoperiod on only LDL level. The highest level of LDL was

Table 4
Feed intake as affected by photoperiod, TSAA and their interaction.

Items	Feed intake g bird/day				
	1-2w	2-3w	3-4w	4-5w	1-5w
Photoperiod (h/day)					
22	37.96a	60.19a	128.19a	126.35b	88.17
16	36.19b	54.35b	119.62b	146.22a	89.09
SEM	0.38	1.06	2.32	2.69	1.16
p-value	0.016	0.002	0.023	<0.001	0.586
TSAA (%)					
1.30	37.39	65.07a	129.41a	139.17	92.76a
1.10	36.83	54.82b	117.47b	134.57	85.92b
0.90	37.00	51.90b	124.83a	135.11	87.21b
SEM	0.46	1.30	2.84	3.91	1.42
p-value	0.799	<0.001	0.035	0.574	0.012
Interaction					
22/1.30	39.31a	70.74a	124.23b	116.48e	87.69b
22/1.10	38.42a	58.27b	123.35b	140.08bc	90.03b
22/0.90	36.16b	51.55c	136.99a	122.50d	86.80b
16/1.30	35.48b	59.40b	134.59a	161.86a	97.83a
16/1.10	35.25b	51.37c	111.59c	129.07c	81.82c
16/0.90	37.85a	52.26c	112.67c	147.73b	87.63b
SEM	1.84	4.28	9.94	11.58	5.74
p-value	0.005	0.020	0.003	<0.001	0.002

Table 5
Feed conversion ratio as affected by photoperiod, TSAA and their combination.

Items	Feed conversion ratio g gain/ g feed				
	1-2w	2-3w	3-4w	4-5w	1-5w
Photoperiod (h/day)					
22	1.30	1.52	1.79	1.29b	1.47b
16	1.33	1.47	1.69	1.95a	1.61a
SEM	0.01	0.02	0.03	0.03	0.02
p-value	0.148	0.139	0.051	<0.001	<0.001
					TSAA (%)
1.30	1.38a	1.61a	1.75	1.82a	1.64a
1.10	1.25c	1.48b	1.69	1.44c	1.47b
0.90	1.31b	1.39c	1.77	1.61b	1.52b
SEM	0.02	0.03	0.04	0.04	0.02
p-value	0.001	0.001	0.358	<0.001	0.001
Interaction					
22/1.30	1.39	1.75a	1.67d	1.44	1.56
22/1.10	1.24	1.43d	1.77c	1.21	1.41
22/0.90	1.27	1.39d	1.92a	1.23	1.45
16/1.30	1.38	1.48c	1.83b	2.19	1.72
16/1.10	1.27	1.53b	1.62e	1.68	1.53
16/0.90	1.35	1.40d	1.62e	1.66	1.59
SEM	0.07	0.14	0.14	0.39	0.11
p-value	0.274	0.002	0.004	0.054	0.797

recorded with 16 h photoperiod as compared to 22 h/ day. On the contrarily, concentrations of glucose, urea, triglycerides, cholesterol and VLDL were not significantly affected. On the other hand, levels of triglycerides, LDL and VLDL were statistically ($p < 0.01$ & 0.05) raised with decreasing dietary TSAA% level. Only triglyceride and LDL levels were significantly ($p < 0.001$) affected by the interaction among photoperiod systems and dietary TSAA levels. The highest levels of triglycerides and LDL were obtained by birds received 0.90% TSAA under a photoperiod of 22 h and 1.30% TSAA under a photoperiod of 16 h.

3.5. Microbial analysis in broiler cecum

All microbial counts (total bacterial count, total yeasts and molds, *E.* coli, coliform, Enterococcus spp., and Salmonella spp.) were significantly $p \leq 0.05$ decreased with methionine concentration in two photoperiod systems (16 and 23 h) (Table 9). However, the lactic acid bacterial count was significantly increased. The total bacterial count decreased by 11%. E. coli and coliform count decreased by 13%. Yeast and molds count depressed by 18%. Salmonella count decreased by 65% in 1.1% TSAA addition and did not appear in 1.3% methionine in 23 h photoperiod and 1.1, 1.3 % in 16 h photoperiod. Lactic acid bacterial count increased by 16% in (23 h photoperiod) and 25% in (16 hrs photoperiod). Generally, broilers exposed to 16hrs photoperiod had better microbial load than that of 23 hrs.

3.6. Breast meat quality and sensory evaluation

Meat moisture, protein content, and pH increased with increasing methionine levels (Table 10). However, lipid content was significantly lower than that of the control. Additionally, TBVN and TBA values were significantly lower than the control after methionine supplementation. Furthermore, methionine addition (1.3%) dramatically enhanced the meat's appearance, juiciness, and flavor. In addition, the whiteness and yellowness of meat were enhanced; however, redness was declined compared to 0.9 and 1.1% levels. On the other hand, broilers exposed to photoperiod

Table 6
Carcass traits as affected by photoperiod, TSAA and their interaction.

Items	Carcass traits								
	Carcass %	Giblets %	Liver %	Gizzard %	Heart %	Dressing %	Abdominal fat %	Spleen %	Bursa %
Photoperiod (h/day)									
22	74.03	3.65b	2.06	1.10b	0.49	77.68	1.11a	0.11	0.11
16	73.80	4.04a	2.06	1.53a	0.44	77.83	0.89b	0.13	0.12
SEM	0.21	0.06	0.03	0.02	0.02	0.62	0.04	0.01	0.01
p-value	0.220	0.002	0.726	<0.001	0.140	0.428	0.019	0.159	0.635
TSAA (%)									
1.30	73.37b	3.63b	1.89c	1.31a	0.44	77.00b	0.95	0.12	0.10b
1.10	74.38a	3.71b	1.99b	1.24b	0.49	78.09a	0.94	0.11	0.09b
0.90	74.35a	4.12a	2.33a	1.32a	0.46	78.47a	1.08	0.12	0.14a
SEM	0.27	0.07	0.03	0.04	0.02	0.28	0.06	0.01	0.01
p-value	0.005	0.001	<0.001	0.039	0.409	0.002	0.259	0.566	0.001
Interaction									
22/1.30	73.96bc	3.25d	1.72d	1.06e	0.46	77.21d	1.22a	0.13	0.09bc
22/1.10	73.57c	3.58c	1.97c	1.09e	0.54	77.15d	1.09bc	0.10	0.13b
22/0.90	74.56b	4.13a	2.49a	1.16d	0.48	78.68b	1.03c	0.11	0.11b
16/1.30	72.06d	4.14a	2.00c	1.71a	0.44	76.20e	0.73e	0.12	0.12b
16/1.10	75.19a	3.85b	2.01c	1.39c	0.44	79.04a	0.80d	0.13	0.05c
16/0.90	74.14bc	4.12a	2.18	1.49b	0.45	78.25c	1.13b	0.13	0.18a
SEM	1.46	0.35	0.24	0.19	0.05	1.13	0.20	0.02	0.04
p-value	0.002	0.003	0.001	0.011	0.360	0.006	0.013	0.219	<0.001

Table 7
Some blood feature as influenced by photoperiod, TSAA and their interaction.

Items	Blood parameters				
	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AST (U/l)	ALT (U/l)
Photoperiod (h/day)					
22	5.56	3.76	2.17	26.13b	19.61b
16	5.83	4.07	2.53	31.44a	24.00a
SEM	0.12	0.09	0.13	0.51	0.49
p-value	0.270	0.105	0.094	<0.001	<0.001
TSAA (%)					
1.30	5.52b	3.80	2.27	26.44b	19.50c
1.10	5.43b	4.10	2.30	26.37b	21.33b
0.90	6.12a	3.93	2.29	32.83a	24.33a
SEM	0.14	0.12	0.16	0.62	0.60
p-value	0.010	0.120	0.737	<0.001	0.001
Interaction					
22/1.30	5.00c	3.40	2.35	21.00e	15.50d
22/1.10	5.43b	4.10	2.17	25.73d	20.00c
22/0.90	6.23a	3.77	1.98	31.67b	23.33b
16/1.30	6.07a	4.00	2.57	33.33a	24.00b
16/1.10	5.43b	4.10	2.43	27.00c	22.67bc
16/0.90	6.00a	4.10	2.60	34.00a	25.33a
SEM	0.49	0.32	0.39	4.63	3.25
p-value	0.020	0.269	0.636	<0.001	0.007

of 16L were better than that of 23L regarding meat quality parameters.

4. Discussion

Our results for the photoperiod studied agree with Olanrewaju et al. (2018) who found a major impact of photo-time on body weight at the age of 14 d ($p \leq 0.006$), 28 d ($p \leq 0.034$) and 42 d ($p \leq 0.026$. Also, Pal et al. (2019) reported that providing broiler chicks with 20 h photoperiod for a day gave the highest growth rate at all ages of fattening. On the other hand, our findings agree with that of Boon et al. (2000), who noticed that longer photoperiods were associated with larger weight gains in 18 L: 6 D growing quail. On the contrarily, the current results disagree with Fidan that of et al. (2017) who reported that final body weight of male broiler chicks (Ross 308) was not affected by photoperiod length.

Body weight and weight gain of broilers were significantly improved ($p < 0.05$) in the starter and finisher stages as a result to dietary TSAA addition. Likely, Ahmed and Abbas (2011) studied the effect of dietary methionine supplementation on the nutritional needs of broilers (NRC, 1994) on growth and carcass traits. Dietary levels of methionine, 0, 100, 120 and 130% of the NRC recommendation were utilized. The weight gain was higher by 110 & 130% of NRC methionine than that of the main ratio. This improvement may be because methionine has the main part in the production of energy via the synthesis of protein. Also, it could activate the livability of broilers, efficiency of diet and growth performance (Binder, 2003). Moreover, the methyl group given by sulfur-adenosyl methionine is needed for several metabolic reactions like the synthesis of epinephrine, carnitine, choline and creatine (Binder, 2003). Also, our results are in line with that of Ashour et al. (2020a,b) who noticed an increase ($p < 0.01$) in LBW of geese due to dietary TSAA supplementation. In contrast, our results disagree with Si et al. (2000) who found no significant influences due to methionine supplementation to corn-soybean diet on broiler chicks greater than that needed to meet the minimum NRC recommendation.

The current results agree with that Abbas et al., (2008), who noticed that non-intermittent restricted lighting program had no

Table 8
Several blood features as influenced by photoperiod, TSAA and their interaction.

Items	Blood parameters					
	Glucose (g/dl)	Urea (g/dl)	Triglycerides mmol/L))	Cholesterol mmol/L))	LDL mmol/L))	VLDL mmol/L))
Photoperiod (h/day)						
22	6.33	5.38	1.26	4.60	1.76b	0.16
16	6.30	5.46	1.34	4.47	2.21a	0.15
SEM	0.11	0.13	0.23	0.10	0.06	0.03
p-value	0.818	0.886	0.308	0.506	0.002	0.989
TSAA (%)						
1.30	6.42	5.33	1.16c	4.59	2.32b	0.19b
1.10	6.34	5.33	1.28b	4.35	1.03c	0.08c
0.90	6.12	5.63	1.44a	4.57	2.65a	0.22a
SEM	0.13	0.15	0.05	0.12	0.08	0.04
p-value	0.208	0.243	0.004	0.226	<0.001	0.043
Interaction						
22/1.30	6.40	5.15	0.93e	4.60	1.65d	0.15
22/1.10	6.23	5.23	1.33c	4.50	1.07e	0.07
22/0.90	6.35	5.77	1.53a	4.70	2.57c	0.27
16/1.30	6.43	5.43	1.43b	4.77	2.90a	0.23
16/1.10	6.00	5.43	1.23d	4.20	1.00e	0.07
16/0.90	6.47	5.50	1.35c	4.43	2.73b	0.17
SEM	0.30	0.34	0.19	0.31	0.21	0.09
p-value	0.618	0.394	<0.001	0.418	<0.001	0.319

Table 9
The impact of different supplementation levels of methionine and illumination of 16 and 23 h on cecal bacterial count.

Microbial count	Treatments (Methionine + illumination)							
	23 h			Mean	16 h			Mean
	0.9	1.1	1.3		0.9	1.1	1.3	
TBC	9.41ab	9.21b	8.99b	9.41a	8.78b	8.58bc	8.36c	8.78a
TYMC	4.33d	4.09de	3.87e	4.29d	3.7 cd	3.46d	3.24d	3.66d
E. coli	5.65 cd	5.43 cd	5.21d	5.58c	5.02c	4.8c	4.58c	4.95c
Salmonella spp.	1.7f	0.8 g	ND	1.56f	0.9e	ND	ND	0.96e
Enterococcus spp.	6.25c	6.08c	5.87c	6.35b	5.62bc	5.45c	5.24c	5.72b
coliform	6.31b	6.11bc	5.92c	6.34b	5.68bc	5.48c	5.29c	5.71b
Lactic acid bacteria	3.99e	4.12d	4.33d	3.97e	4.62bc	4.75bc	4.96b	4.38c
Mean	*5.38a*	*5.16b*	*4.88c*		*4.88a*	*4.63a*	*4.51b*	
ANOVA				*p*-value				*p*-value
Treatments (T)				<0.001				<0.001
Bacterial count (BC)				<0.001				<0.001
T × BC				<0.001				<0.001

*TSAA 0.9, 1.1 and 1.3 in begging and TSAA 0.7, 0.9 and 1.1 in ending terms indicate inclusion of methionine + cystine at the average of 70, 85 and 100% of digestible lysine, respectively. Bold and non-italic lowercase letters indicate significant differences between methionine levels. Blod and italic lowercase letters indicate significant differences between microbial counts. Total bacterial count (TBC), Total yeasts and molds count (TYMC).

influence on mortality rate and suppressed LBW by about 10% compared with the continuous photoperiod (control). On the contrarily, Julian (2005) reported that the fast growth rate of new broiler chickens is linked with a chain of physiological disorders meaning to high death rate through develop-out. Also, Brickett et al. (2007) visualized that shortened lighting days (12L: 12D) decreases gross death rate compared to longer light days (20L: 4D). For another main factor studied, Abou El-Wafa et al. (2003) reported no variation between dietary methionine levels used on mortality rate for Arbor Acres broiler chicks. In line, Abou-Kassem, (2006) found that mortality rate during the whole growing period (1–6 weeks of age) was not influenced by dietary methionine level in growing quails.

Continuous lighting schedule (24L: 0D) or semi-continuous (23L: 1D, 16L: 8D) lighting program causes highly feed consumption (Mahmood et al., 2014). Also, Brickett et al. (2007) illustrated that below the schedule of light by 20L: 4D chicks ate more feed than birds exposed to 12L: 12D. Whereas, Rahimi et al. (2005) showed no variation in feed intake in broilers kept below 23L: 1D and sporadic lighting program 1L: 3D. El-Sayiad et al. (2005) found that feed consumed through the whole experimental periods (1–3, 4–6 and 1–6 weeks of age) was significantly ($p < 0.01$) influenced by various photoperiods.

Li et al. (2010) noticed that FCR of chickens raised below 12 L:12 D was superior ($p < 0.05$) to those under another lighting schedule at 1 wk and 4 wks and inferior ($p < 0.05$) at 6 wks. The 16L: 8D schedule presented higher FCR ($p < 0.001$) than others from 8 to 14 d and was superior ($p < 0.05$) for the 12 L:12 D schedule for days 15 to 21 d. Broilers grew under nearly continuous lighting programs showed the best FCR (Maria et al., 2011). Longer day lengths (20L: 4D) significantly improved FCR in broilers compared to those exposed to 12L: 12D (Brickett et al., 2007). Also, Wen-bin et al. (2010) found that FCR ratio was higher in broiler birds when raised with 12L: 12D compared to 23L: 1D and 16L: 8D lighting patterns. On the other hand, Scott (2002) concluded that after 35 days of age, FCR reduced in broilers reared below 16L: 8D lighting patterns compared to the other photoperiods (23L: 1D, 20L: 4D). On the contrarily, Downs et al. (2006) found no impact of photoperiod on feed conversion ratio. Our findings are in line with those of Elamin Ahm and Abbas (2011), who reported that dietary levels of methionine, influenced ($p < 0.05$) feed consumption and FCR. However, BWG was influenced ($p < 0.01$) by methionine level. Feed

Table 10
Chemical, color parameters and meat quality of broiler fed diet supplemented with methionine under illumination levels.

Chemical composition	Met. Levels (%) + 16L			Mean	Met. Levels (%) + 23L			**Mean**
	0.9	1.1	1.3		0.9	1.1	1.3	
Moisture	66.88ab	69.71ab	70.85a	**69.15a**	65.54ab	67.22ab	68.70a	**67.15a**
Protein	20.78bc	21.40b	22.98b	**21.72b**	20.68bc	21.00b	21.99b	**21.22b**
Lipids	11.00c	8.45c	6.11d	**8.52c**	10.89c	10.22c	9.55 cd	**10.22c**
Ash	1.10de	0.89de	0.29e	**0.76e**	0.90e	0.77e	0.21ef	**0.63e**
pH	5.60d	6.00d	6.30 cd	**5.97d**	6.83d	7.30 cd	7.46 cd	**7.20 cd**
TBA	0.39e	0.32ef	0.25f	**0.32e**	0.66e	0.59e	0.52e	**0.59e**
TBVN	5.61d	5.35d	5.10de	**5.35d**	6.06d	5.81d	5.56de	**5.81d**
Mean	***15.91***	***16.02***	***15.98***		***15.94***	***16.13***	***16.28***	
ANOVA				*p*-value				*p*-value
Treatments (T)				<0.001				<0.001
Chemical composition (CC)				<0.001				<0.001
T × CC				<0.001				<0.001
Color parameters								
L^*	60.00a	59.03ab	58.77ab	**59.27a**	59.35a	59.25a	58.39ab	**59.00a**
a^*	6.50 cd	7.07c	6.17 cd	**6.58c**	6.16c	6.23c	6.29c	**6.23c**
b^*	14.30b	15.00b	14.50b	**14.60b**	13.14b	13.44b	13.61b	**13.40b**
Mean	***26.93ab***	***27.03a***	***26.48b***		***26.22***	***26.31***	***26.10***	
ANOVA				*p*-value				*p*-value
Treatments (T)				<0.001				<0.001
Color (C)				0.043				0.039
T × C				0.049				0.033
Sensorial traits								
Tenderness	8.47b	9a	8.99a	**8.82**	7.98b	8.31a	8.44a	**8.24a**
Juiciness	8.53b	8.93a	9.00a	**8.82**	7.98b	8.11b	8.45a	**8.18b**
Aroma	8.60b	8.60b	8.74a	**8.65**	8.05b	8.18b	8.24a	**8.16b**
Taste	9.00a	8.81a	8.65b	**8.82**	8.28a	8.39a	8.17b	**8.28a**
Mean	***8.71b***	***8.78b***	***8.80a***		***8.10b***	***8.23a***	***8.29a***	
ANOVA				*p*-value				*p*-value
Treatments (T)				0.168				<0.001
Sensorial traits (S)				0.015				<0.001
T × S				0.026				<0.001

Bold and non-italic lowercase letters indicate significant differences between methionine levels. Blod and italic lowercase letters indicate significant differences between microbial counts.

intake was numerically improved with 110 and 130% NRC methionine but not with 120% NRC methionine. These results agree with the findings of Pillai et al. (2006), who noticed that feed intake, weight gain and feed efficiency increased with the addition of methionine. On the other hand, results obtained for feed intake disagree with those of Saki et al. (2007), who found no effect of methionine addition on either feed intake or FCR. Supplementation of 120 and 130% of NRC methionine significantly ($p < 0.05$) improved FCR and PER. Ashour et al. (2020a, 2020b) illustrated that high TSAA level improved ($p < 0.05$) feed intake and FCR through the whole and 12–18 wks of age, respectively.

The present results for the photoperiod agree with the study of Olanrewaju et al. (2012), who indicated that lengthy/continued and uniform/sporadic photoperiod evenly improved carcass feature compared to shortened/non-sporadic photoperiod (Li et al. (2010) reported that breast muscle ratio of birds raised below 12L: 12 D reduced ($p < 0.05$) than below 23L: 1D and 16L: 8D schedules. The low-intensity diet reduced ($p < 0.001$) the ratio of abdominal fat and improved ($p < 0.05$) the ratio of wings and legs. On contrarily, the current results disagree with Fidan et al. (2017), who noticed that all carcass traits of male broiler chicks (Ross 308) were not affected due to photoperiod length either semi-continued photoperiod (23L: 1D of 1 to 42 d of age) or rising photoperiods of (23L: 1D, 14L: 10D, 16L: 18D, 18L: 6D, 20L: 4D, followed by 23L: 1D for six weeks of age, respectively. El-Sayiad et al. (2005) found improvements in carcass and dressing ratio in birds fed methionine as recommended by the NRC or higher for the interaction effect, our findings are in line with that of El-Sayiad et al. (2005) who found that the highest values of carcass and dressing percentages were recorded by birds under the photoperiod of 24 h along with 115% methionine of the NRC recommended level. Our results are in line with that of Ur Rehman et al. (2019), who clarified that the level of methionine had an impact ($p < 0.05$) on carcass, breast and thigh weights. Methionine has a beneficial impact on traits because it is the first essential amino acid in broiler nutrition and plays a role in building the body tissues, which reflects on body conformation and finally some carcass traits (Rehman et al., 2019).

Our findings agree with that of Onbasilar et al. (2008), who studied the effect of photoperiods of 16L: 8D and 24L: 0D and found that light period didn't affect serum glucose, cholesterol, triglyceride levels of broiler chicks. Li et al. (2010) observed that the photoperiod of 12L: 12D lowered MDA activity by 54.35% in breast meat of broiler chicks compared to 23L: 1D treatment. Olanrewaju et al., (2013) reported that the shortened lighting term influenced most blood physiological variables in broiler chickens. It may be agreed with Pandey (2019), who explained that broiler chickens need to be given four hours for sleep, but it may demand higher hours at certain points in the growth. Our results agree with that of Ashour et al. (2020a,b), who noticed that glucose, total protein, and albumin levels were insignificantly ($p > 0.05$) influenced, while results for AST, ALT and cholesterol disagree with the same researchers. Also, current findings disagree with Yang et al. (2017), who reported that dietary methionine addition led to a linear increase in serum levels of total protein and its fractions.

Ravangard et al., (2017) found that dietary inclusion of feed additives (prebiotic and probiotic) had no significant effect on cecal *Lactobacillus* and *Escherichia coli* counts at 21 days of age. While, at 42 days of age, feed additives increased *Lactobacillus* and decreased the counts of *Escherichia coli* ($p < 0.05$). On the other hand, photoperiod has a positive correlation with microbial counts. The bacterial colonies increased with light intensity (Schmidt et al., 2018) following the obtained results. In addition, light exposure led to lower abundances of viable bacteria and communities that were compositionally distinct from dark rooms, suggesting preferential inactivation of some microbes over others under daylight conditions. Day lighting was associated with the loss of a few numerically dominant groups of related microorganisms and apparent increases in the abundances of some rare groups, suggesting that a small number of microorganisms may have exhibited modest population growth under lighting conditions (Fahimipour et al., 2018).

Our results are in line with wen et al. (2017) who found that broilers fed the high methionine enriched diets had higher pH but lower L^*, cooking loss and ether extract content in breast muscle than those fed the lower methionine diets. Additionally, Albrecht et al. (2019) studied the effect of dietary DL-methionine supplementation on broiler (ROSS 308) meat quality. The pH values were between 6.1 and 6.4. The study revealed a significant influence of methionine supplementation on the quality of broiler breast meat compared to the control group. Methionine supplementation led to higher pH values and higher water binding. Higher concentrations of methionine had a positive influence on the water-holding capacity by lowering the cooking loss. The L^* -value showed a significant negative correlation to the methionine concentration supplemented. On the other hand, Tuell et al. (2020) studied the impact of photoperiod 20L:4D, 18L:6D, 16L:8D, and 12L:12D on broiler meat quality and found no considerable impact of photoperiods on general carcass and meat quality attributes. However, color and oxidative stability were influenced by the photoperiod. Color of muscles from 20L:4D appeared lighter and more discolored, coupled with higher lipid oxidation ($p < 0.05$) and protein denaturation ($p = 0.058$) compared to 12L:12D.

5. Conclusion

The addition of TSAA at levels of 1.1 and 0.9% TSAA (122 and 128% of NRC recommendation) in the starter and finisher diets, respectively under a long photoperiod regime (22 L: 2 D) improved the broiler's growth, carcass traits and blood parameters. It also decreased pathogenic microbial counts and increased lactic acid bacterial counts. TBVN and TBA values were significantly lower than the control as a response to dietary methionine supplementation. Furthermore, dietary methionine addition (1.3%) dramatically enhanced meat's appearance, juiciness, flavor as well as whiteness and yellowness.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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