Journal of the Hellenic Veterinary Medical Society

Vol 74, No 1 (2023)

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doi: 10.12681/jhvms.29349

To cite this article:

Kaya, M., & Dereli Fidan, E. (2023). The Effect of Drinking Water Temperature and Stocking Density on Broiler Performance, Meat Quality and Some Behavioral Traits at High Ambient Temperature. *Journal of the Hellenic Veterinary Medical Society*, *74*(1), 5315–5324. https://doi.org/10.12681/jhvms.29349 (Original work published April 11, 2023)

J HELLENIC VET MED SOC 2023, 74 (1): 5315-5324
ΠΕΚΕ 2023, 74 (1): 5315-5324

Research article
Ερευνητικό άρθρο

The Effect of Drinking Water Temperature and Stocking Density on Broiler Performance, Meat Quality and Some Behavioral Traits at High Ambient Temperature

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ABSTRACT: The present study examined the effect of drinking water temperature and stocking density on performance (cumulative body weight gain, feed intake, and feed conversion ratio), meat quality, and behaviour traits (eating, drinking, lying, standing, and dust bathing) in broilers. A total of 360 broiler chicks (Ross 308) were randomly divided into 6 experimental groups, in a 2x3 factorial arrangement of drinking water temperature (10°C or 31°C), with stocking density (12, 15 or 18 birds/m^2), each consisting of 4 replicate pens. Meat quality traits (pH_{15}, L*, CL, and WHC), lying and standing behaviours were affected by water temperature. The stocking density significantly affected the cumulative body weight gain, feed intake, feed conversion ratio, color of breast meat [lightness (L*), redness (a*), yellowness (b*)] of broiler chickens. In conclusion, management practices such as stocking density and drinking water temperature in broilers could reduce the detrimental effects of high ambient temperature on growth performance, meat quality traits, and behaviour.

***Keywords*: Broiler, Drinking water temperature, High ambient temperature, Performance, Stocking density.

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Date of initial submission: 27-01-2022
Date of acceptance: 24-07-2022

INTRODUCTION

The feed intake and performance change with water temperature in hot ambient temperature. Cold water helps to increase appetite for water and feed and reduces the metabolic temperature. Similarly, high water intake improves performance; decreases body temperature, heat production, respiratory rate and mortality at high ambient temperatures in poultry (Saeed et al., 2019).

Stocking density is defined as the number of birds or the birds live weight reared in an exact space. The European Union Council Directive and reported that the stocking density should be 33-39 kg/m^2 in broiler houses (EU, 2007). A high stocking density is beneficial and economical if it remains within an optimum range (Dozier et al., 2006). It reduces the fixed cost of production and produces more kilograms of chicken per area up to a certain extent, with an increasing profitability (Puron et al., 1995). However, a high stocking density exerts stress on broilers that negatively affects their growth performance and carcass quality (Feddes et al., 2002; Sohail et al., 2010). Stocking density in intensive poultry production is a crucial stressor and has negative correlation to poultry efficiency, health and welfare issues (Astaneh et al., 2018; Bailie et al., 2018). It causes lower production, higher incidence of disorders and cannibalism in poultry (Karcher and Mench, 2018). High stocking density causes lower meat production, decreased growth rate, higher incidence of disorder like poor walking ability and final body weight (Tahamtani et al., 2020; Costa et al., 2021).

It is well known that heat stress and stocking density negatively affects carcass characteristics and meat quality in poultry (Sandercock et al., 2001). Meat-quality characteristics such as water loss, muscle haemorrhages and tenderness were negatively affected by chronic heat stress (Attia et al., 2011). High stocking density has been shown to cause oxidative stress in broilers (Najafi et al., 2015), and oxidative stress is associated with high drip loss and lowered pH of broiler breast muscle (Chen et al., 2010; Wang et al., 2017). Moreover, high stocking density also affects meat quality in terms of reduced tenderness (Patria et al., 2016; Zhang et al., 2018).

High ambient temperature and high stocking density negatively impact the behavioral traits of animals while simultaneously increasing health problems and mortality (Daramola et al., 2012; Slimen et al., 2016). High stocking density change hormone release, physiological stress, center on aggression, feather pecking and behavioral disturbances (Ravindran et al., 2006; Estevez, 2007; Simsek et al., 2009). Andrews et al. (1997) investigated that the broilers spent more time walking and sitting and less time dozing and sleeping under the lower stocking density. The broilers under the higher stocking density performed more resting and standing and less locomotion (Buijs et al., 2009).

We hypothesized in this study that broiler welfare and meat quality traits can be improved with management tools at high ambient temperature. This study evaluated the effect of drinking water temperature, and stocking density on performance (cumulative body weight gain, feed intake, and feed conversion ratio), meat quality, and behaviour traits (eating, drinking, lying, standing, and dust bathing) in broilers exposed to high ambient temperature.

MATERIALS AND METHODS

This study was conducted at full controlled poultry house of Aydin Adnan Menderes University, Aydın, Turkey. The climate during the period of study was characterized by dry to hot season. Aydin district is situated at 37° 51' 27" N latitude and 27° 51' 14" E longitude. Aydin has the Mediterranean climate (Kirmaci and Ağcagil, 2009).

Ethical statement

All the experimental procedures involved in this study were peformed after an ethical approval was taken from Animal Care and Use Committee of Aydin Adnan Menderes University (64583101/2020/040).

Experimental design and treatments

The total of 360 male broiler chicks (Ross 308) were procoured from a local commercial hatchery (Egetav Tavukçuluk, İzmir, Turkey). Chicks were divided randomly into 6 treatments, in a 2x3 factorial arrangement of drinking water temperature (10°C or 31°C), stocking density (12, 15 or 18 birds/m^2), each consisting of 4 replicate pens. Day old chicks were individually weighed and grouped based on their average body weights. For cooling treatment, the water cooler (Cihso 2000) cooled the water to 10°C and circulate 5 L min^{-1} inside the pipes. Water temperature in drinkers was kept between 9.8-10.2°C by thermosensors and controlled twice daily in the system. The experiment was performed during the summer, during which the ambient temperature ranged from 27.4°C at night to 34.2°C at midday, and humidity 50.1-61.4%. Depending on the ambient temperature, the water temperature was measured as 31°C on average.

The broiler chickens were housed in floor pens (1.1 m wide x 1.5 m deep x 0.75 m high) for 42 d. Each pen had a drinker lines (three nipples) and a round feeders (0.4 m diameter). The stocking density was modified at three levels as per The European Union Council Directive describing the standards for stocking density of broilers as per final rearing body weight [2]. The adjustable partitions of pen setted to the stocking density area, when animals die. The broilers were kept in floor pens on wood shavings litter of 6 cm deep layer.

Photoperiod lengths were applied 24 h light (24L) from 1 to 7 days, whereas restricted lighting replicates were provided 18 h light: 6 h dark (18L:6D) from 8 to 39 days, followed by 24 h light (24L) onwards until the end of the experiment (EU, 2007). The brooding temperature for first 3 d was 34°C and was gradually reduced to around 31°C from 4 to 42 days. Afterwards, all the broilers were provided ambient temperature and recorded daily. During 42 days experiment humidity varied from 50 to 60%.

General management

The chickens were fed a starter diet (0-10 days; 3000 kcal ME kg^{-1}, 23.0% crude protein), grower diet (11-24 days; 3100 kcal ME kg^{-1}, 21.5% crude protein), and finisher diet (25-42 days; 3200 kcal ME kg^{-1}, 19.5% crude protein). Feed and water were ensured *ad libitum* throughout the study.

Performance parameters

In this study all broilers weight was recorded individually at hatch and every week till 6 the age of weeks. Feed intake (FI) was recorded every week. Feed conversion ratio (FCR) was calculated as the ratio of FI to body weight gain (BWG). A periodic estimation of cumulative body weight gain, feed intake and feed conversion rate was conducted for the periods from 0 to 7-14-21-28-35-42 days.

Carcass and meat quality measurements

On the 42nd d of age, 5 broilers were randomly chosen with average body weight from each pen, and were slaughtered to determine carcass and meat quality measurements.

Meat quality characteristics were determined using breast meat. The pH was recorded using a digital pH meter (Hanna Instrument HI 9124) equipped with a penetration electrode (Hanna FC-200), 15 min postmortem (pH_{15}), and 24 h postmortem (pH_{24}). Cooking loss (CL) was determined by heating the samples in a bag to internal temperature of 75°C in a water bath and were cooled under running tap water for 15 min. Afterwards the samples were dried with filter paper and weighed. Cooking loss was expressed as percent decrease in weight compared to the initial weight (Honikel, 1998). After 24 h of slaughter water holding capacity (WHC) was evaluated by using the methodology described (Barton-Gade et al., 1993). The postmortem samples of cranial side of the breast fillets were cut into 5-g cubes. Later, the samples were kept between two filter papers and a weight (2250 g) was applied for 5 min. The samples were weighed and WHC was determined by the exudated water weight by the formula: 100 - [(initial weight - final weight) / (initial weight)]. After 24 h slaugther muscle color was measured from the surface of left breast muscles. Breast color measurements were performed using a Minolta CR 400 chroma-meter in the CIELAB color space using a D65 illuminant [relative lightness (L*), relative redness (a*), relative yellowness (b*)].

Behavioral traits

The behaviors of all broilers was evaluated using a video recording program. Daily behaviors of chickens (eating, drinking, lying, standing and dust bathing) were monitored by software program equipped with cameras (ICU, B400) between 22-42 days. Cameras were placed on top of each pen. For each group, 24-hour camera recording was observed using the instantaneous scanning technique (Estevez et al., 2002) at 04:00, 08:00, 12:00, 16:00, 20:00 and 00:00, respectively. A total recording time of 15 minutes (3 minutes for each behavior) was evaluated for each day (Zhao et al., 2013).

Statistical analysis

The data were statistically analysed using the SPSS software package (version 22.0 SPSS Inc., Chicago, IL, USA). Using Levene's test, the assumption of homogeneity of variances was verified. The BW, BWG, FI, FCR, meat quality and behavior traits (eating, drinking, lying, standing and dust bathing) were analysed using a general linear model procedure and means were compared using least square difference (LSD) (Özdamar, 2004).

RESULTS

Performance findings

In a previous study, we found that there was no significant difference between the cold drinking water group (1912 g) and the normal drinking water group (1916 g) on slaughter weight. In terms of stocking density, the slaughter weight (2027 g) in 12 birds/m^2 stocking density was found to be significantly higher (P<0.001) compared to the 15 and 18 birds/m^2 stocking density (1891 and 1824 g) (Kaya and Dereli Fidan, 2021)

Stocking density affected cumulative BWG of broilers from 0 to 14, 21, 28, 35, and 42 days of age. The cumulative BWG was found the highest in 12 birds/m^2 stocking density (P<0.001) at 0-42 d. There was no significant between drinking water temperature groups on BWG (Table 1).

The drinking water temperature had no significant effect on cumulative FI and FCR. The cumulative FI was found highest in 12 birds/m^2 stocking density (P<0.01), and the cumulative FCR was found highest in 18 birds/m^2 stocking density (P<0.01) at 0-42 d (Table 2).

Meat quality findings

The effect of drinking water temperature and stocking density on color and pH traits of breast meat are given in (Table 3). The differences between cold water (CW) and normal water (NW) groups for pH_{24}, a*, and b*; the differences between 12, 15, 18 birds/m^2 stocking density groups for pH_{15}, pH_{24}, CL, and WHC were not significant. The stocking density have significant effect on muscle color parameters (L*, a*, b*).

Behavioral findings

The drinking water temperature and stocking density significantly generally effected drinking, lying, standing, and dust bathing behaviours; but no significant effect on eating at 0, 4, 8, 12, 16, and 20 hours was found. At 20 hour, the highest drinking incidence (1.16 and 1.18 respectively) was obtained for broilers in normal water and 18 birds/m^2 stocking density groups. The highest lying and dust bathing incidence was found at 12 hour (0.86, 0.83 and 1.48, 1.66 respectively). The cold water group had higher standing incidence, compared with the broilers in normal water group at 0, 4, 8, 12, 16, and 20 hours (Table 4).

Table 1. Effect of drinking water temperature and stocking density on cumulative body weight gain of broilers

Factors	Cumulative Body Weight Gain (g)					
	d 0-7	**d 0-14**	**d 0-21**	**d 0-28**	**d 0-35**	**d 0-42**
Water temperature						
Normal	126.87	356.72	669.33	955.47	1403.69	1865.91
Cold	125.54	351.68	675.79	992.65	1429.28	1870.28
Stocking density						
12 birds/m^2	125.89	365.67^a	701.74^a	1017.95^a	1503.52^a	1980.79^a
15 birds/m^2	126.31	355.01^{ab}	670.47^{ab}	972.06^{ab}	1421.87^b	1844.97^b
18 birds/m^2	126.41	341.92^b	644.46^b	932.17^b	1324.05^c	1778.53^b
SEM[1]	0.63	2.44	5.97	9.49	13.20	14.97
Water temp. x *Stocking dens.*						
Normal-12 birds/m^2	125.37	367.01	700.96	1001.18	1494.60	1971.36
Normal-15 birds/m^2	127.26	360.49	666.97	953.78	1395.11	1858.74
Normal-18 birds/m^2	127.97	342.67	640.04	911.43	1321.35	1767.62
Cold-12 birds/m^2	126.41	364.33	702.51	1034.71	1512.45	1990.21
Cold-15 birds/m^2	125.34	349.52	673.97	990.34	1448.62	1831.19
Cold-18 birds/m^2	124.87	341.18	650.89	952.91	1326.77	1789.46
SEM[2]	1.63	5.98	14.52	22.59	31.10	36.18
Effect (P-value)						
Water temperature	0.147	0.303	0.552	0.056	0.292	0.891
Stocking density	0.916	0.000	0.001	0.001	0.000	0.000
Water temp. X Stocking dens.	0.591	0.807	0.922	0.955	0.757	0.749

$^{a, b, c}$: Means with different superscript letters in the same column differ (P<0.05)

[1]: Standard error of the mean

[2]: Standard error of the mean for interaction effect

Table 2. Effect of drinking water temperature and stocking density on cumulative feed intake and cumulative feed conversion ratio of broilers

Factors	Cumulative Feed Intake (g)						Cumulative Feed Conversion Ratio (g feed/g gain)					
	d 0-7	d 0-14	d 0-21	d 0-28	d 0-35	d 0-42	d 0-7	d 0-14	d 0-21	d 0-28	d 0-35	d 0-42
Water temperature												
Normal	203.85	570.57	1032.36	1623.38	2512.02	3499.22	1.60	1.60	1.54	1.70	1.79	1.88
Cold	202.53	560.30	1067.13	1700.19	2576.51	3532.30	1.61	1.59	1.58	1.71	1.80	1.89
Stocking density												
12 birds/m^2	200.17	577.74	1070.15	1710.31^{a}	2683.84^{a}	3644.74^{a}	1.59	1.58	1.52	1.68^{b}	1.79^{b}	1.84^{b}
15 birds/m^2	203.98	568.06	1052.70	1662.40^{ab}	2552.38^{b}	3505.43^{ab}	1.61	1.60	1.57	1.71^{ab}	1.80^{b}	1.90^{a}
18 birds/m^2	205.42	550.50	1026.39	1612.64^{b}	2396.57^{c}	3397.11^{b}	1.62	1.61	1.59	1.73^{a}	1.81^{a}	1.91^{a}
SEM[1]	1.19	4.36	10.27	16.93	17.96	29.18	0.008	0.006	0.011	0.007	0.003	0.009
Water temp. x *Stocking dens.*												
Normal-12 birds/m^2	198.07	576.20	1058.44	1671.97	2660.39	3607.59	1.58	1.57	1.51	1.67	1.78	1.83
Normal-15 birds/m^2	206.16	580.39	1033.79	1621.42	2497.25	3531.60	1.62	1.61	1.55	1.70	1.79	1.90
Normal-18 birds/m^2	207.31	555.12	1004.85	1576.76	2378.43	3358.47	1.62	1.62	1.57	1.73	1.80	1.90
Cold-12 birds/m^2	202.26	579.27	1081.86	1748.66	2707.29	3681.89	1.60	1.59	1.54	1.69	1.79	1.85
Cold-15 birds/m^2	201.80	555.74	1071.61	1703.38	2607.52	3479.25	1.61	1.59	1.59	1.72	1.80	1.90
Cold-18 birds/m^2	203.54	545.88	1047.93	1648.53	2414.72	3435.75	1.63	1.60	1.61	1.73	1.82	1.92
SEM[2]	2.93	10.68	25.17	41.48	43.99	71.48	0.019	0.014	0.026	0.018	0.008	0.023
Effect (P-value)												
Water temperature	0.590	0.255	0.108	0.066	0.089	0.578	0.697	0.258	0.586	0.183	0.070	0.974
Stocking density	0.209	0.059	0.243	0.049	0.000	0.009	0.404	0.911	0.677	0.047	0.003	0.003
Water temp. X Stocking dens.	0.291	0.446	0.922	0.992	0.668	0.594	0.715	0.910	0.782	0.978	0.447	0.898

$^{a, b, c}$: Means with different superscript letters in the same column differ ($P<0.05$)

[1]: Standard error of the mean

[2]: Standard error of the mean for interaction effect

Table 3. Effect of drinking water temperature and stocking density on breast meat quality traits of broilers

Factors	Meat Quality Traits						
	pH_{15}	pH_{24}	L*	a*	b*	CL (%)	WHC (%)
Water temperature							
Normal	5.87	5.49	59.76	2.14	12.45	36.94	12.49
Cold	5.65	5.47	61.24	2.32	12.00	28.38	11.35
Stocking density							
12 birds/m^2	5.78	5.51	60.00^{b}	2.35^{a}	12.20^{ab}	31.52	11.79
15 birds/m^2	5.78	5.47	59.83^{b}	2.51^{a}	13.28^{a}	34.61	12.08
18 birds/m^2	5.71	5.47	61.68^{a}	1.82^{b}	11.19^{b}	31.86	11.89
SEM[1]	0.01	0.01	0.30	0.10	0.25	0.86	0.25
Water temp. x *Stocking dens.*							
Normal-12 birds/m^2	5.81^{bcd}	5.50	57.88^{c}	2.96^{a}	12.86	33.95^{abc}	12.11
Normal-15 birds/m^2	5.93^{a}	5.49	60.05^{abc}	1.73^{b}	13.01	37.36^{ab}	12.66
Normal-18 birds/m^2	5.87^{ab}	5.48	61.35^{ab}	1.73^{b}	11.48	39.51^{a}	12.71
Cold-12 birds/m^2	5.75^{c}	5.51	62.11^{a}	1.74^{b}	11.54	29.08^{cd}	11.48
Cold-15 birds/m^2	5.63^{d}	5.45	59.61^{bc}	3.29^{a}	13.56	31.86^{bc}	11.49
Cold-18 birds/m^2	5.56^{d}	5.45	62.01^{a}	1.91^{b}	10.91	24.21^{d}	11.08
SEM[2]	0.03	0.02	0.74	0.25	0.61	2.12	0.62
Effect (P-value)							
Water temperature	0.000	0.304	0.017	0.408	0.374	0.000	0.026
Stocking density	0.094	0.116	0.027	0.021	0.004	0.282	0.899
Water temp. X Stocking dens.	0.001	0.554	0.006	0.000	0.314	0.025	0.721

$^{a, b, c, d}$: Means with different superscript letters in the same column differ ($P<0.05$)

pH_{15}: Initial pH value measured 15 min postmortem; pH_{24}: Value measured 24 h postmortem

CL: Cooking loss; WHC: Water holding capacity

L*: Lightness; a*: Redness; b*: Yellowness.

[1]: Standard error of the mean

[2]: Standard error of the mean for interaction effect

Table 4. Effect of drinking water temperature and stocking density on the incidence of some behaviours in broilers at 6 weeks of age.

Factors	Eating						Drinking						Lying						Standing						Dust bathing					
	h0	h4	h8	h12	h16	h20	h0	h4	h8	h12	h16	h20	h0	h4	h8	h12	h16	h20	h0	h4	h8	h12	h16	h20	h0	h4	h8	h12	h16	h20
WT																														
NWT	0.31	0.33	1.14	1.03	1.08	1.72	0.13	0.13	1.11	1.33	1.10	1.08	0.31	0.40	1.94	1.89	2.21	1.54	0.41	0.33	0.62	0.77	0.98	1.16	0.30	0.32	0.43	0.86	0.67	0.44
CWT	0.36	0.40	1.31	1.15	1.14	1.30	0.22	0.19	1.24	1.48	1.33	1.19	0.87	0.71	2.89	3.01	3.50	3.10	0.51	0.37	0.80	0.98	0.95	0.93	0.18	0.14	0.22	0.54	0.41	0.30
SD																														
12 b/m^2	0.30	0.30	1.05^{b}	0.99	0.96	1.07^{b}	0.15	0.09	1.04	1.12^{b}	0.98^{b}	1.05	0.58	0.35^{b}	2.11^{b}	2.28	2.35^{b}	2.07	0.43	0.23^{b}	0.57^{b}	0.69^{b}	0.88	0.91^{b}	0.14^{b}	0.17	0.27	0.55^{b}	0.38^{b}	0.24^{b}
15 b/m^2	0.38	0.37	1.19^{b}	1.05	1.16	1.28^{ab}	0.19	0.16	1.24	1.44^{a}	1.15^{ab}	1.24	0.63	0.68^{a}	2.59^{a}	2.46	2.98^{a}	2.42	0.46	0.39^{a}	0.73^{ab}	0.89^{a}	0.97	1.05^{b}	0.23^{ab}	0.24	0.37	0.72^{a}	0.64^{a}	0.43^{a}
18 b/m^2	0.32	0.42	1.44^{a}	1.22	1.22	1.37^{a}	0.19	0.22	1.23	1.66^{a}	1.37^{a}	1.13	0.57	0.63^{a}	2.53^{ab}	2.61	3.24^{a}	2.48	0.49	0.41^{a}	0.83^{a}	1.05^{a}	1.05	1.18^{a}	0.36^{a}	0.28	0.34	0.83^{a}	0.61^{a}	0.44^{a}
SEM[1]	0.03	0.04	0.05	0.05	0.05	0.05	0.02	0.02	0.05	0.05	0.05	0.05	0.06	0.06	0.08	0.08	0.09	0.08	0.04	0.03	0.04	0.04	0.04	0.04	0.03	0.02	0.03	0.03	0.03	0.03
WT x *SD*																														
NWT-12 b/m^2	0.24	0.24	0.95	0.92	0.92	0.99	0.03^{b}	0.07	0.98	1.14	0.85	0.91	0.23	0.21	1.66	1.64	1.75	1.27	0.35	0.22	0.47	0.57	0.83	0.88^{b}	0.19$^{b.c}$	0.26	0.28^{b}	0.71	0.48	0.27
NWT-15 b/m^2	0.33	0.39	1.02	0.91	1.08	1.20	0.20^{a}	0.10	1.23	1.38	1.05	1.13	0.34	0.40	1.94	1.84	2.27	1.57	0.38	0.40	0.67	0.83	0.97	1.16^{b}	0.38^{a}	0.39	0.58^{a}	0.93	0.84	0.56
NWT-18 b/m^2	0.35	0.37	1.46	1.24	1.24	1.33	0.15$^{a.b}$	0.22	1.11	1.48	1.13	1.20	0.37	0.58	2.21	2.19	2.62	1.80	0.48	0.36	0.73	0.91	1.14	1.45^{a}	0.34$^{a.b}$	0.31	0.45^{a}	0.94	0.71	0.47
CWT-12 b/m^2	0.36	0.37	1.14	1.05	0.99	1.15	0.27^{a}	0.12	1.10	1.09	1.11	1.18	0.93	0.49	2.58	2.93	2.95	2.86	0.50	0.24	0.68	0.81	0.93	0.93^{b}	0.08^{c}	0.09	0.26^{b}	0.38	0.29	0.20
CWT-15 b/m^2	0.43	0.36	1.34	1.19	1.24	1.35	0.17$^{a.b}$	0.22	1.26	1.50	1.25	1.35	0.92	0.96	3.23	3.08	3.69	3.26	0.53	0.39	0.80	0.95	0.98	0.95^{b}	0.08^{c}	0.09	0.17^{b}	0.51	0.44	0.30
CWT-18 b/m^2	0.28	0.47	1.42	1.20	1.20	1.41	0.22^{a}	0.22	1.35	1.84	1.61	1.05	0.77	0.68	2.85	3.03	3.86	3.18	0.50	0.47	0.93	1.19	0.95	0.91^{b}	0.38^{a}	0.25	0.23^{b}	0.72	0.50	0.41
SEM[1]	0.08	0.09	0.13	0.11	0.12	0.11	0.06	0.05	0.12	0.12	0.11	0.11	0.14	0.14	0.20	0.18	0.23	0.19	0.07	0.08	0.09	0.10	0.11	0.11	0.05	0.06	0.06	0.08	0.07	0.07
P																														
WT	0.474	0.388	0.118	0.188	0.525	0.169	0.061	0.188	0.184	0.150	0.001	0.238	0.000	0.005	0.000	0.000	0.000	0.000	0.131	0.521	0.023	0.010	0.783	0.007	0.013	0.000	0.000	0.000	0.000	0.017
SD	0.556	0.433	0.014	0.115	0.073	0.032	0.720	0.060	0.158	0.000	0.005	0.215	0.899	0.028	0.033	0.226	0.000	0.056	0.751	0.033	0.035	0.003	0.278	0.040	0.002	0.190	0.195	0.004	0.002	0.003
WT x SD	0.496	0.608	0.342	0.400	0.724	0.932	0.040	0.485	0.686	0.262	0.458	0.158	0.576	0.257	0.280	0.449	0.873	0.708	0.691	0.772	0.900	0.706	0.392	0.026	0.019	0.129	0.006	0.509	0.319	0.250

WT: Water Temperature, NWT: Normal Water Temperature, CWT: Cold Water Temperature, SD: Stocking Density

[a, b, c]: Means with different superscript letters in the same column differ ($P<0.05$)

[1]: Standard error of the mean

DISCUSSION

In the present study, while cold water group showed higher values compared to normal water group on cumulative BWG from 0 to 21, 28, 35, and 42 days of age, drinking water temperature was no statistically significant effect on the body weight gain of broiler chickens. Similarly, weight gain on week basis and total weight of broilers in CW group were heavier than NW group at ambient temperature (Park et al., 2015). This finding could be attributed to high ambient temperature and thereby restricted growth of broilers due to lower feed consumption and panting of birds (May et al., 2000). High ambient temperature increases physiological response of releasing corticosteroids, which limits BWG in poultry (Gross and Siegel, 1981). Drinking cold water decreases the negative effects of high temperature in animals, and allows to gain more weight. The 12 birds/m^2 stocking group clearly increased the other stocking groups in terms of cumulative body weight gain during to all periods, except between 0 and 7 days of age, ($P<0.01$ or $P<0.001$). Similar studies have reported adverse effects of stocking density on performance (Zuowei et al., 2011; Gholami et al., 2020). It might be speculated that broilers face difficulty in accessing feeders or drinkers due to negative effects of high stocking density. It is possible that lowering the stocking density reduces the body temperature and increases the airflow at the surface of the bird and this phenomenon causes increaed meat yield.

Although CW group increased FI and improved FCR when compared to NW group, no significant effects were found for FC or FCR due to drinking water temperature from 0 to 21, 28, 35, and 42 days of age. Similarly Okelo et al. (2003) reported no significant effect of CW on FC or FCR of broilers reared under heat stress conditions. In the present study, the broilers reared at the lowest density had a significantly higher FI and lower FCR from 0 to 28, 35, and 42 days of age. In the current experiment FI by reduced by increasing the stocking density. These results are in agreement with previous studies reporting that high stocking density decreases the growth performance of broiler chickens (Shakeri et al., 2014; Cengiz et al., 2015). Such observations might be due to inbility of birds to move freely and acess the feeder or drinker in high stocking density groups. Furthermore, heat dessipation is hinderedin crowded pens causing more heat stress and lower feed intake and body weight gain cause to poor FCR (Vargas-Rodriguez et al., 2013). It may be because of the stocking density of 15 and 18 birds/m^2 groups may pose a considerable stress on the broilers as compared to 12 birds/m^2 group.

Breast meat quality parameters were within the normal range and it was found that cold water group has significant lower (5.65) on pH_{15} values from meat quality traits ($P<0.001$). It has been documented that pH is associated with lightness, cooking losses and water holding capacity in chicken breast meat (Hao and Gu, 2014).

Bianchi et al. (2007) showed that dark broiler breast meat has significantly lower L*, higher a*, and lower b* values than light broiler breat meat. Our findings suggest that cold water group significanlty increased the L* compared with normal water group ($P<0.05$). Similarly Froning et al. (1978) reported that lower L* value of broiler breast meat, as a result of heat stress.

Drinking water temperature significantly influenced CL and WHC of breast meat ($P<0.001$; $P<0.05$). In CW group cooking loss and water holding capacity of breast meats was lower than NW group. In line with this finding, Güler (2011) found that higher CL ($P<0.001$) was obtained in breast meat from stress group (%15.24) compare to those from control group (%13.78). McKee and Sams (1997) reported that increase in CL of the breast meat as a result of heat stress. It is thought that there was a decrease in stress levels related to the beneficial effects of the drink of cold water in broilers.

The quality of breast meat were significant main effects of stocking density on some measurements. Breast meats of the broilers in 18 birds/m^2 group had significantly higher L* value, compared with those in 12 and 15 birds/m^2 groups ($P<0.05$). Previous studies (Zhang et al., 2018; Wu et al., 2020) add more studies reported that there was negative effect of high SD on meat quality which is in agreement of this experimental study.

High meat L* value is typically associated with muscle diseases such as PSE (pale, soft, and exudative meat) and woody meat (Petracci et al., 2004; Cai et al., 2018). High stocking density has been shown to cause oxidative stress in broilers (Najafi et al., 2015). Stress conditions such as high CO_2 concentrations and heat stress have been shown to increase the L* value of breast meat in broilers (Lu et al., 2017; Xu et al., 2018), and high L* value in the 18 birds/m^2 group of present study may be due to stress induced by high stocking density. In the study, 18 birds/m^2 group determined the lowest a* value at 24 h after slaughter. Stressors such as heat stress can reduce the a* value of breast meat (Wen et al., 2019). Wu et al. (2020) reported that the low L* and increased a* values are desired as this indicates high myoglobin concentrations and an improvement in meat color. Gentry et al. (2004) indicated that the higher a* value also indicate higher oxidative capacity of muscles.

The mean standing duration increased significantly in CW group compared to NW group at all hours. However, lying behaviour decreased significantly in CW group. Cold water group increased on drinking behaviour at 8 and 12 hours. It can be explained that increase in eating behaviors due to increased activation. The standing behaviour of broilers increased with stocking density, and these results are in line with findings of Thomas et al. (2011) and Son (2013). It is possible due to less use of space, increased fearfulness and adverse envrionmental conditions (Stadig et al., 2017). The incidence of lying behaviour increased significantly high density groups compared to normal density group. Several studies have reported increased incidence of leg deformations and weakness due to less space and movement in high stocking density groups (Buijs et al., 2011; Abudabos et al., 2013). In the present study, eating and drinking behaviours increased with high stocking density.

CONCLUSION

The present study found that cold water temperature and low stocking density increased the cumulative body weight gain and feed intake. Cold drinking water temperature has a positive effect on meat quality at high ambient temperatures in broilers. Meat quality traits (pH_{15}, L*, CL, and WHC) and behaviours (lying, standing) were mainly effected by drinking water temperature. In addition, the stocking density significantly affected color of breast meat (lightness, redness, yellowness) of the broilers. Furthermore, cold water and high stocking density groups decreased the initial pH value (pH_{15}). The mean standing behaviour increased, but lying behaviour decreased significantly in cold water group compared to normal water group. Additionally, our results may contribute to the ongoing search for the drinking water temperature and stocking density.

CONFLICT OF INTEREST

None of the authors of this article has any conflict of interest.

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