Experimental Results (2021), 2, e24, 1–12
doi:10.1017/exp.2021.15

LIFE SCIENCE AND BIOMEDICINE

NEGATIVE-RESULT

Cloacal temperature responses of broiler chickens administered with fisetin and probiotic (*Saccharomyces cerevisiae*) and exposed to heat stress

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(Received 25 June 2021; Revised 07 July 2021; Accepted 08 July 2021)

Abstract

There is dearth information on the role of fisetin as an antistress agent in ameliorating heat stress in broiler chickens. Here, we experimentally compared probiotic, an antioxidant and antistress agent, with fisetin, an antioxidant agent with little or no report on its antistress effect. Sixty-day-old broiler chickens (Arbo Acre breed) were allotted into 4 groups of 15 birds each as follows; control, fisetin, probiotic, and fisetin + probiotic groups, respectively. All administrations were performed orally through gavage for the treatment groups. The environmental and cloacal temperature (CT) parameters were measured bi-hourly at Days 21, 28, and 35 from 7:00 to 7:00 hr, during the period of study. The environmental parameters exceeded the thermoneutral zone for broiler chickens. The probiotic-supplemented group had the least overall mean CT values all through the experimental period. Based on our findings, fisetin was not a potent antistress agent in mitigating heat stress in birds.

Key words: ambient temperature; antioxidants; broiler chickens; oxidative stress

1. Introduction

The major factor that negatively affects broiler chickens production in the subtropics and tropics is heat stress (Aluwong et al., 2017; Goel et al., 2021; Sugiharto et al., 2017). The combined effects of high relative humidity (RH) and high ambient temperature (AT) have been reported to induce thermal stress in chickens (Egbuniwe et al., 2015; Jiang et al., 2020; Kim et al., 2021; Qaid et al., 2021), therefore, lipid peroxidation resulting from hyperthermia may damage some of their vital organs (Rhoads et al., 2013; Al-Zghoul & Saleh, 2020; Aksoy et al., 2021; Gogoi et al., 2021). Antioxidants are used universally in dietary supplementation, and these supplements are beneficial in ameliorating tissue damages induced by stress (Gouda et al., 2020; Sun et al., 2015). Fisetin is a flavonoid found in vegetables and fruits, such as grapes, onion, strawberries, and cucumbers (Khan et al., 2013; Chen et al., 2015; Kikusato et al., 2021). Fisetin has several pharmacological and physiological activities that are extensive (Qaid et al., 2021). Probiotics are live microorganisms that are beneficial when adequately administered to the host (Aluwong et al., 2013). They function as both antioxidant and antistress agents, and are also gut effective (Sumanu et al., 2021).

2. Objectives

Our objective was to determine the cloacal temperature (CT) responses of broiler chickens administered with fisetin and probiotic and exposed to heat stress. We hypothesized that supplementation of fisetin either alone or in combination with probiotic would be beneficial to ameliorate the negative effects of thermal stress in broiler chickens. To test this hypothesis, we assessed broiler chickens CT responses in separate groups of chicks given these supplements alone and in combination.

3. Methods

3.1. Ethics statement

This research was approved by the Ahmadu Bello University, Zaria's Ethical Committee on Animal Use and Care, with the following reference number, ABUCAUC/2018/021.

3.2. Site of the experiment and experimental animal management

We conducted this experiment at the Department of Veterinary Physiology, Ahmadu Bello University, Zaria (11°10′ N, 07°38′ E). The chickens were exposed naturally to the fluctuating RH and AT conditions (Dzenda et al., 2013). Sixty newly hatched broiler chicks that were apparently healthy served as the subjects. They were raised in an intensive management system, feed and water were provided to the broiler chicks *ad libitum*. On Days 1–28, broiler starter were fed to the chicks, whereas broiler finisher was given from Days 29 to 42. The poultry pen was made of concrete floor littered with wood shavings, cement block with aluminum roofing and cardboard ceiling. The dimension of the pen was $8.4 \times 5.6 \times 1.91$ m and the birds were stocked at the density of 15 birds/m^2. Protective clothing was made available to ensure optimum biosecurity measures.

3.3. The study area meteorological data and experimental design

The dry-bulb temperature (DBT) and wet-bulb temperature (WBT) (Brannan® Sapphire Instruments, New Delhi, India) were recorded every 2 hr for 3 days, 1 week apart, on Days 21, 28, and 35 of the experiment. RH was calculated using Osmon's hygrometric table (Narinda Scientific Industries, Haryana, India). The temperature-humidity index (THI) is a measure of the degree of discomfort that the chickens experienced during the stressful season. The index is importantly an efficient temperature based on air temperature and humidity. THI was determined using the formula of Tao and Xin (2003): $\mathrm{THI} = 0.85(\mathrm{Tdb}) + 0.15\mathrm{Twb}$, where THI = THI for broiler chickens, Tdb = DBT, and Twb = WBT. The parameters were recorded inside the poultry house on each day of the experiment.

Sixty chicks were divided into four groups of 15 each by simple randomization. Group I control; Group II, fisetin (Sigma Inc., New Orleans, LA) at a dose of 5 mg/kg; Group III, probiotic (Montajat Pharmaceuticals, Bioscience Division, Dammam, Saudi Arabia) at a dose of 4.125×10^6 cfu/100 ml; and Group IV, fisetin and probiotic (same doses as stated above). All administrations were performed orally for the first 7 days of life via gavage.

3.4. CT and AT measurement

CT values, which is an index of the core body temperature of the broiler chickens, were recorded (Sinkalu et al., 2015), using a digital clinical thermometer (Krusser Thermometer®, Amazon). CT measurements were taken using procedures of good standard (Minka & Ayo, 2013) over a period of 24 hr, from 07:00 to 07:00 hr of the next day, on Days 21, 28, and 35 of the study. The AT was recorded simultaneously with CT values using a WBT and DBT (BrannanSapphire Instruments, New Delhi, India). RH and THI were calculated as described by Tao and Xin (2003).

For data analysis, one-way analysis of variance (ANOVA) was utilized, Tukey's multiple comparison *post-hoc* test was used to compare disparateness between the treatments and control groups means. Data obtained were expressed as mean ± standard error of the mean (SEM). GraphPad Prism 5.03 for windows was used (GraphPad Software, San Diego, CA). Significant values were considered to be $p < .05$.

https://doi.org/10.1017/exp.2021.15 Published online by Cambridge University Press

4. Results

The mean DBT at Day 28 (28.85 ± 0.45°C) of the study was higher compared to those of Days 21 (26.85 ± 0.25°C) and 35 (28.54 ± 0.39°C). Day 21 (77.92 ± 2.56%) had the least RH compared to Days 28 (81.46 ± 3.11%) and 35 (79.00 ± 2.62%). The THI was highest on Day 28 (28.47 ± 0.38) as compared to Days 21 (26.40 ± 0.23) and 35 (28.05 ± 0.33; Table 1). At Day 21 of the study period, overall mean values of the CT in the fisetin group (41.20 ± 0.03°C) showed no difference significantly ($p > .05$) when compared with that of the control group (41.39 ± 0.03°C). Values recorded in the probiotic (40.32 ± 1.90°C) and fisetin + probiotic groups (40.49 ± 0.03°C) were lower significantly ($p < .05$) when compared with the control group (41.39 ± 0.03°C; Table 2). The overall mean CT values recorded at Day 28 of the study was evident that fisetin group showed no difference (41.36 ± 0.04°C), whereas the co-administered group had a significant (41.23 ± 0.03°C, $p < .05$) decrease when compared with the values obtained from the control group. Also, the CT values were different significantly ($p < .05$) in the chickens supplemented with probiotic (41.10 ± 0.06°C), when compared with the corresponding values of the controls (41.58 ± 0.03°C; Table 3). At Day 35 of the study, the overall mean CT values in the fisetin group (41.68 ± 0.03°C) did not differ when compared with that of the control group (41.78 ± 0.03°C). The overall CT values of fisetin + probiotic-administered group differed significantly ($p < .05$) from the control group. Nevertheless, probiotic-administered group had the least CT values which differed significantly (40.10 ± 0.11°C; $p < .05$) as compared to the controls (41.78 ± 0.03°C; Table 4).

5. Discussions

From the present study, it was evident that the values of DBT (26.00–36.00°C), RH (49.00–93.00%), and the THI (28.47 ± 0.38) obtained exceeded the thermoneutral zone stipulated for broiler chickens above 3 weeks of age, which are 18–24°C, 65–70%, and 20.8, respectively in the tropics. This further support the fact that the experimental period was thermally stressful to the birds. The values of THI higher than 20.8 which elicited heat stress in broiler chickens agrees with previous findings (Sinkalu et al., 2015). Broiler chickens' energy balance and fitness might be affected negatively during the prevailing thermally stressful season as recorded by previous research (Tao & Xin, 2003; Yin et al., 2021). This might subsequently lead to low immunity, decrease performance, suppression, high morbidity, and death; hence, antioxidants, such as fisetin and probiotic could be helpful during the season when administered adequately. Oxidative stress is a resultant of heat stress which consequently, enhances reactive oxygen species (ROS) production (Ogbuagu et al., 2018; Shakeri et al., 2020; Sumanu et al., 2019), therefore, the findings of this study serves as the basis for modulating the detrimental effects of heat stress in animals *via* fisetin and/or probiotic administration.

Little or no information have been published to the best of our knowledge, on the use of fisetin or its combination with probiotic in modulating the detrimental effects of thermal stress in poultry, using CT as a potent biomarker, during the cause of this study. It is very difficult for the chickens to maintain core

Table 1. Variations in thermal environmental parameters on selected days of the study period

Parameters	Day 21	Day 28	Day 35
DBT (°C)	26.85 ± 0.25	28.85 ± 0.45	28.54 ± 0.39
	(26.00–28.00)	(27.00–32.00)	(27.00–31.00)
RH (%)	77.92 ± 2.56	81.46 ± 3.11	79.00 ± 2.62
	(58.00–92.00)	(63.00–93.00)	(62.0–93.00)
THI	26.40 ± 0.23	28.47 ± 0.38	28.05 ± 0.33
	(25.10–27.55)	(26.85–31.10)	(26.55–30.10)

Values in parenthesis are minimum–maximum. $n = 15$.
Abbreviations: DBT, dry-bulb temperature; RH, relative humidity; THI, temperature-humidity index.

https://doi.org/10.1017/exp.2021.15 Published online by Cambridge University Press

Table 2. Circadian variation in cloacal temperature of broiler chickens during Day 21 of study

Hours	Control (n = 15)	Fisetin (n = 15)	Probiotic (n = 15)	F + P (n = 15)
7:00	41.48 ± 0.06^{a}	41.43 ± 0.06^{a}	40.37 ± 0.08^{b}	40.54 ± 0.10^{a}
	(41.10–41.90)	(41.10–41.80)	(40.20–41.90)	(40.30–42.20)
9:00	41.53 ± 0.05^{a}	41.46 ± 0.06^{a}	40.37 ± 0.08^{b}	40.38 ± 0.10^{b}
	(41.20–41.90)	(41.10–41.80)	(40.10–41.90)	(40.20–42.00)
11:00	41.62 ± 0.11	41.23 ± 0.10	41.43 ± 0.06	41.07 ± 0.13
	(40.00–42.10)	(40.90–41.70)	(41.10–41.80)	(40.20–42.10)
13:00	41.55 ± 0.09	41.76 ± 0.11	41.02 ± 0.09	41.39 ± 0.08
	(41.40–41.90)	(41.30–42.20)	(40.60–42.00)	(40.50–41.90)
15:00	41.57 ± 0.10^{a}	41.49 ± 0.07^{a}	40.99 ± 0.10^{c}	41.07 ± 0.05^{b}
	(41.10–42.40)	(41.00–41.90)	(40.50–41.40)	(40.60–41.30)
17:00	41.83 ± 0.11^{a}	41.33 ± 0.11^{a}	40.93 ± 0.08^{b}	40.88 ± 0.14^{b}
	(41.00–41.90)	(40.00–41.60)	(40.10–41.30)	(39.80–41.80)
19:00	41.32 ± 0.11^{a}	41.27 ± 0.08^{a}	40.30 ± 0.06^{b}	41.00 ± 0.06^{b}
	(41.20–42.10)	(40.70–41.80)	(40.10–41.80)	(40.80–41.60)
21:00	41.04 ± 0.07^{a}	41.09 ± 0.09^{a}	40.95 ± 24.67^{b}	41.32 ± 0.08^{a}
	(41.00–41.60)	(41.00–41.50)	(40.80–41.30)	(40.60–41.80)
23:00	41.18 ± 0.09^{a}	41.15 ± 0.06^{a}	41.10 ± 0.06^{a}	41.34 ± 0.08^{b}
	(40.70–42.20)	(40.90–41.40)	(40.10–42.00)	(40.80–41.70)
1:00	41.29 ± 0.10	41.08 ± 0.08	41.07 ± 0.10	41.48 ± 0.10
	(41.00–42.20)	(40.98–41.70)	(40.80–42.10)	(40.90–42.60)
3:00	41.19 ± 0.11	41.15 ± 0.08	41.35 ± 0.10	41.48 ± 0.10
	(41.10–41.90)	(41.00–41.70)	(40.80–42.10)	(40.90–42.60)
5:00	41.33 ± 0.06^{a}	41.27 ± 0.08^{a}	40.15 ± 0.06^{b}	41.30 ± 0.14^{a}
	(41.00–41.90)	(41.20–42.00)	(40.00–41.90)	(40.80–42.50)
7:00	41.33 ± 0.10	41.28 ± 0.10	41.06 ± 0.08	41.45 ± 0.09
	(40.70–41.80)	(40.93–42.00)	(40.60–41.70)	(40.70–42.00)
Overall mean ± SEM	41.39 ± 0.03^{a}	41.20 ± 0.03^{a}	40.32 ± 1.90^{b}	40.49 ± 0.03^{b}
	(41.30–42.40)	(41.00–42.00)	(40.10–41.30)	(40.25–42.60)

Values in parenthesis are minimum–maximum.
Abbreviations: F + P, fisetin + probiotic; SEM, standard error of the mean.
^{a,b,c}Means with different superscript letters across rows are significantly different (p < .05).

body temperature amidst high AT, as they are void of sweat glands and rely more on evaporative cooling (panting) to keep their body cool. Several workers have reported CT increase in older chickens during heat stress (Robinson et al., 2016; Sahebi-Ala et al., 2021). Fluctuations in body temperature depicts the stressful nature of RH and AT, the mechanism of thermoregulation is furthermore required for the maintenance of homeothermy (Makeri et al., 2017; Yin et al., 2021). The greater the fluctuation of environmental parameters, the more prominent the thermal stress, and the higher the negative effects upon health and optimal growth (Lee et al., 2021).

At Days 21, 28, and 35 of the study, the CT values recorded in the control group were higher than the values recorded within the treatment groups. This may be attributed to the fact that the birds were

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Table 3. Circadian variation in cloacal temperature of broiler chickens during Day 28 of study

Hours	Control ($n = 15$)	Fisetin ($n = 15$)	Probiotic ($n = 15$)	F + P ($n = 15$)
7:00	41.35 ± 0.14	41.29 ± 0.16	41.76 ± 0.19	41.25 ± 0.21
	(40.00–42.20)	(41.10–42.00)	(39.00–41.90)	(39.60–42.70)
9:00	41.33 ± 0.14	41.34 ± 0.14	41.60 ± 0.13	41.17 ± 0.18
	(41.00–42.20)	(40.10–42.00)	(40.00–41.70)	(39.60–42.70)
11:00	41.68 ± 0.12	41.61 ± 0.13	41.48 ± 0.06	41.50 ± 0.09
	(40.10–42.00)	(40.70–42.50)	(40.10–41.90)	(41.10–42.20)
13:00	41.83 ± 0.16	41.56 ± 0.15	41.38 ± 0.11	41.65 ± 0.10
	(41.00–41.90)	(41.50–42.80)	(40.50–42.40)	(41.00–42.40)
15:00	41.72 ± 0.11[a]	41.77 ± 0.12[a]	41.00 ± 0.08[b]	41.44 ± 0.16[a]
	(41.70–41.90)	(40.90–42.70)	(40.30–42.30)	(40.20–42.60)
17:00	41.83 ± 0.12[a]	41.49 ± 0.14[a]	41.17 ± 0.06[b]	41.13 ± 0.10[b]
	(41.00–42.00)	(40.90–42.60)	(41.00–41.90)	(40.80–42.30)
19:00	41.79 ± 0.16	41.06 ± 0.08	41.13 ± 0.10	41.28 ± 0.08
	(41.50–42.00)	(41.00–42.90)	(40.10–41.80)	(40.80–42.00)
21:00	41.29 ± 0.11[a]	41.43 ± 0.14[a]	41.07 ± 0.06[b]	41.19 ± 0.08[b]
	(40.50–42.00)	(40.60–42.40)	(40.80–41.60)	(40.80–42.00)
23:00	41.69 ± 0.08[a]	41.65 ± 0.14[a]	40.90 ± 0.11[c]	41.14 ± 0.12[b]
	(40.80–42.00)	(41.00–42.60)	(39.00–41.90)	(41.00–42.00)
1:00	41.59 ± 0.15[a]	41.55 ± 0.16[a]	40.50 ± 0.14[c]	41.09 ± 0.11[b]
	(41.30–42.60)	(40.70–42.60)	(40.30–42.50)	(40.40–41.70)
3:00	41.48 ± 0.13[a]	41.35 ± 0.21[a]	40.30 ± 0.11[b]	41.27 ± 0.09[a]
	(41.00–42.00)	(40.20–42.70)	(40.10–42.70)	(40.70–41.80)
5:00	41.75 ± 0.09[a]	41.69 ± 0.16[a]	40.11 ± 0.67[c]	41.25 ± 0.08[b]
	(40.90–42.00)	(41.00–42.80)	(39.90–41.70)	(40.80–41.80)
7:00	41.50 ± 0.06[a]	41.45 ± 0.06[a]	40.07 ± 0.08[b]	41.54 ± 0.10[a]
	(41.10–41.90)	(41.10–41.80)	(40.00–41.90)	(40.90–42.20)
Overall mean ± SEM	41.58 ± 0.03[a]	41.36 ± 0.04[a]	41.00 ± 0.06[b]	41.23 ± 0.03[b]
	(41.30–42.60)	(41.10–42.90)	(40.00–42.70)	(40.60–42.70)

Values in parenthesis are minimum–maximum.
Abbreviations: F + P, fisetin + probiotic; SEM, standard error of the mean.
[a,b]Means with different superscript letters across rows are significantly different ($p < .05$).

naturally exposed to heat stress and their endogenous antioxidants were not potent enough to mitigate its adverse effects. These findings are in agreement with previous studies in the tropic (Egbuniwe et al., 2015). The CT values recorded in the fisetin-administered group were higher than the values recorded in the probiotic and the co-administered groups, respectively. It may be deduced that fisetin is devoid of antistress effect in mitigating heat stress in broiler chickens as evident in this study. This further rejects the hypothesis which was earlier stated that fisetin administration would be advantageous in ameliorating the detrimental effect of heat stress in broiler chickens. Values of CT recorded in the probiotic group during the period of study were lower as compared to all other groups. It could be speculated that probiotic was able to function not only as an antioxidant, but also as an antistress agent in mitigating heat

https://doi.org/10.1017/exp.2021.15 Published online by Cambridge University Press

Table 4. Circadian variation in cloacal temperature of broiler chickens during Day 35 of study

Hours	Control (n = 15)	Fisetin (n = 15)	Probiotic (n = 15)	F + P (n = 15)
7:00	41.67 ± 0.17	41.51 ± 0.08	41.48 ± 0.05	41.53 ± 0.07
	(41.10–42.70)	(41.00–42.10)	(41.20–42.00)	(41.20–42.20)
9:00	41.53 ± 0.05[a]	41.51 ± 0.05[a]	40.37 ± 0.11[b]	41.24 ± 0.07[b]
	(41.20–41.90)	(41.10–41.80)	(40.20–41.90)	(41.20–42.20)
11:00	41.60 ± 0.15	41.47 ± 0.09	41.36 ± 0.15	41.49 ± 0.20
	(41.20–42.00)	(41.00–42.00)	(40.00–42.40)	(40.40–42.60)
13:00	41.63 ± 0.09[a]	41.58 ± 0.11[a]	41.13 ± 0.08[b]	41.57 ± 0.10[a]
	(40.70–42.00)	(40.80–42.60)	(40.30–42.50)	(41.10–42.30)
15:00	41.83 ± 0.12[a]	41.72 ± 0.10[a]	41.13 ± 0.11[b]	41.34 ± 0.18[b]
	(41.80–42.30)	(41.00–42.50)	(40.80–42.50)	(41.20–42.40)
17:00	41.82 ± 0.15[a]	41.66 ± 0.08[a]	40.62 ± 0.12[c]	41.00 ± 0.10[b]
	(41.30–42.40)	(41.20–42.30)	(40.50–42.10)	(40.80–42.30)
19:00	41.74 ± 0.11[a]	41.60 ± 0.12[a]	41.24 ± 0.09[b]	41.41 ± 0.10[a]
	(41.50–42.10)	(41.30–42.30)	(40.70–42.10)	(40.09–42.00)
21:00	41.66 ± 0.15	41.60 ± 0.10	41.26 ± 0.08	41.35 ± 0.12
	(40.10–42.20)	(40.80–42.00)	(41.00–42.00)	(40.80–42.70)
23:00	41.74 ± 0.99[a]	41.52 ± 0.07[a]	40.31 ± 0.06[b]	41.39 ± 0.07[a]
	(41.70–42.10)	(41.30–42.10)	(40.10–41.90)	(41.00–42.00)
1:00	41.63 ± 0.09	41.65 ± 0.08	41.43 ± 0.08	41.53 ± 0.11
	(41.00–42.00)	(41.00–42.00)	(41.00–41.90)	(40.90–42.20)
3:00	41.54 ± 0.12	41.55 ± 0.08	41.43 ± 0.08	41.45 ± 0.06
	(40.60–42.00)	(41.00–42.30)	(40.90–42.00)	(41.10–41.80)
5:00	41.65 ± 0.10	41.62 ± 0.06	41.56 ± 0.09	41.41 ± 0.08
	(41.10–42.60)	(41.00–41.90)	(41.10–42.30)	(41.00–42.00)
7:00	41.48 ± 0.06[a]	41.46 ± 0.06[a]	40.37 ± 0.08[b]	41.54 ± 0.10[a]
	(41.10–41.90)	(41.10–41.80)	(40.00–41.90)	(40.90–42.20)
Overall mean ± SEM	41.78 ± 0.03[a]	41.68 ± 0.03[a]	40.10 ± 0.11[c]	41.11 ± 0.03[b]
	(41.10–42.70)	(41.30–42.60)	(40.00–42.50)	(40.40–42.70)

Values in parenthesis are minimum–maximum.
Abbreviations: F + P, fisetin + probiotic; SEM, standard error of the mean.
[a,b]Means with different superscript letters across rows are significantly different ($p < .05$).

stress, hence it could be potent in enhancing thermoregulation which reduces the metabolic heat production of the chickens, vis a vis increasing their productivity. Previous studies on broiler chickens demonstrated similar findings (Aluwong et al., 2017; Sugiharto et al., 2017). The co-administered group had CT values that were lower than that of the control and fisetin groups, respectively during the study period. This may be attributed to the thermoregulatory effect of the probiotic and not the fisetin. Nevertheless, this is the first time that fisetin, a phytonutrient, would be tried on food animals to the best of our knowledge. Therefore, fisetin alone was not potent in alleviating heat stress in broiler chickens.

The molecular mechanism by which probiotic was able to elicit thermoregulation should be further investigated.

https://doi.org/10.1017/exp.2021.15 Published online by Cambridge University Press

6. Conclusion

CT was significantly increased by the thermal environmental parameters, indicative that the study period was thermally stressful. Therefore, the administration of fisetin singly was not potent in eliciting antistress effect in broiler chickens exposed to thermal stress. Although, probiotic was potent in alleviating heat stress, hence its use singly is advocated.

Acknowledgments. Profs J. O. Ayo and T. Aluwong are highly appreciated for designing this research and for their mentorship all through the study. Their constructive criticism and meticulous corrections in bringing out the best in me and this research can never be underestimated.

Authorship Contributions. J.O.A. and T.A. conceived and designed the study. V.O.S., T.A., J.O.A., and N.E.O. conducted data gathering, V.O.S. performed statistical analyses, V.O.S., J.O.A., and T.A. wrote the article.

Data Availability Statement. The authors confirm that the data supporting the findings of this study are available within the article.

Conflict of Interest. The authors declare no conflict of interest.

Funding Statement. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

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Cite this article: Sumanu VO, Aluwong T, Ayo JO, Ogbuagu NE (2021). Cloacal temperature responses of broiler chickens administered with fisetin and probiotic (*Saccharomyces cerevisiae*) and exposed to heat stress *Experimental Results*, 2, e24, 1–12. https://doi.org/10.1017/exp.2021.15

Peer Reviews

Reviewing editor: Dr. Michael Nevels
University of St Andrews, Biomolecular Sciences Building, Fife, United Kingdom of Great Britain and Northern Ireland, KY16 9ST

This article has been accepted because it is deemed to be scientifically sound, has the correct controls, has appropriate methodology and is statistically valid, and has been sent for additional statistical evaluation and met required revisions.

doi:10.1017/exp.2021.15.pr1

Review 1: Cloacal temperature responses of broiler chickens administered with fisetin and probiotic (Saccharomyces cerevisiae) and exposed to heat stress

Reviewer: Dr. Mohammed Ibrahim[1,2]

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Date of review: 03 July 2021

Conflict of interest statement. Reviewer declares none

Comments to the Author: Dear Editor,

Thank you for considering me as a reviewer for this publication in your esteemed journal Experimental Results. I have provided my comments as follows.

The authors have achieved their objective and the manuscript is, generally speaking, well-written.

1- There are some grammar issues in L11 (There is a death), L19 (The probiotic-supplemented), L90 (CT values were), L127 (These findings are), L135 (also as an anti-stress).

2- In L79 and 80 (add the full name of the DBT and RH). In addition, please delete all the means values throughout the result in the text since you have mentioned them in the tables, this will be a repetition of the result.

3- In L114 (change the sentence to "fisetin or its combination with probiotics").

4- Could the authors explain what are these numbers between brackets in all tables, which is very confusing? since the authors have provided the mean values ± SEM.

Thank you

Mohammed Ibrahim

Score Card

Presentation

3.6 /5

Is the article written in clear and proper English? (30%)	4/5
Is the data presented in the most useful manner? (40%)	3/5
Does the paper cite relevant and related articles appropriately? (30%)	4/5

Context

3.8 /5

Does the title suitably represent the article? (25%)	4/5
Does the abstract correctly embody the content of the article? (25%)	3/5
Does the introduction give appropriate context? (25%)	4/5
Is the objective of the experiment clearly defined? (25%)	4/5

Analysis

4.0 /5

Does the discussion adequately interpret the results presented? (40%)	4/5
Is the conclusion consistent with the results and discussion? (40%)	4/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	4/5

https://doi.org/10.1017/exp.2021.15 Published online by Cambridge University Press

doi:10.1017/exp.2021.15.pr2

Review 2: Cloacal temperature responses of broiler chickens administered with fisetin and probiotic (Saccharomyces cerevisiae) and exposed to heat stress

Reviewer: Dr. Tadayuki Yanagi Junior

Universidade Federal de Lavras, Engenharia Agrícola, Escola de Engenharia, Caixa Postal 3037, Lavras, MInas Gerais, Brazil, 37200900

Date of review: 06 July 2021

Conflict of interest statement. Reviewer declares none.

Comments to the Author: Sumaru and co-authors compared the effect of fisetin, probiotic and combination of both on CT of broiler chickens subject to natural thermal challenges. The mitigation of CT, that is a physiological response used to indicate heat stress, is widely desirable, especially in the sub-tropics and tropics. Hot conditions are a worldwide problem that negatively affect poultry performance, causing economic loss. Therefore, this is an interesting study.

The authors reported that the experiment was conducted in an intensive management system. However, constructive details of the facility (dimensions and materials description) should be informed.

The bird`s thermal sensation is affected by wind and thermal radiation. For this study, can air velocity and thermal radiation be neglected? If so, a justification must be addressed in the text for using the THI. Otherwise, another thermal index should be used, such as the black globe-humidity index (BGHI).

Inform the accuracy of each sensor used.

In the discussion related to the overall mean CT values recorded at day 28, the probability ($P>0.05$) must be ($P<0.05$), as a significant difference was found.

Statistical interpretation on day 35 should be verified. The CT value for (F+P) is different in the text (41.41 ± 0.03) when compared to the value listed in Table 4 (41.11 ± 0.03). Which value is correct? Based on the letters attributed for the means of CT by the Tukey's test (Table 4), overall CT values in the (F+P) differ statically ($P<0.05$) from the control group.

Score Card

Presentation

4.3 /5

Is the article written in clear and proper English? (30%)	5/5
Is the data presented in the most useful manner? (40%)	4/5
Does the paper cite relevant and related articles appropriately? (30%)	4/5

Context

5.0 /5

Does the title suitably represent the article? (25%)	5/5
Does the abstract correctly embody the content of the article? (25%)	5/5
Does the introduction give appropriate context? (25%)	5/5
Is the objective of the experiment clearly defined? (25%)	5/5

Analysis

4.6 /5

Does the discussion adequately interpret the results presented? (40%)	4/5
Is the conclusion consistent with the results and discussion? (40%)	5/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	5/5

https://doi.org/10.1017/exp.2021.15 Published online by Cambridge University Press