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Effects of Drinking Water Temperature on Laying Hens Subjected to Warm Cyclic Environmental Conditions

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ABSTRACT

Different drinking water temperatures (T_w) were provided to 24 individually caged, initially 29 wk- (Expt 1) and 30 wk-old (Expt 2) laying hens subjected to warm diurnal cyclic air temperature (T_a) in two separate experiments. Two levels of T_w (27 and 18 °C) in Expt 1 and four (15, 19, 23 or 27 °C) in Expt 2 were tested. Each experiment consisted of a 1-wk acclimation period under thermoneutral conditions ($T_a = T_w = 21$ °C), a 4-wk exposure to the treatment conditions (T_a = 27 – 35 °C and 27 – 38 °C for Expt 1 and 2, respectively), and a 2-wk recovery period with conditions same as the acclimation period. Cooler T_w tended to be more conducive to feed and water intake of laying hens during the early stage of heat exposure. An optimal range of T_w seems to exist for hens subjected to heat stress. However, large variations among the individual hens may have tempered statistical significance of the treatment effects. Further investigation using more experimental hens is warranted to evaluate T_w effects on the hen production performance.

KEYWORDS: Laying hens, Feed intake, Water intake, Egg production, Heat stress relief.

INTRODUCTION

Factors influencing feed and water consumption and thereby, meat and egg production of poultry, are of economic importance. While the literature contains considerable information concerning environmental effects on feed and water intake of modern broilers (May et al., 1997; May and Lott, 1994; Xin et al., 1994; Xin et al., 1993; May and Lott, 1992a; May and Lott, 1992b), less information is available for modern breeds of laying hens. Feed use of white leghorn chickens has been reported to decrease from 13 to 7 kg/100 birds per day when the maximum house air temperature increased from 4.4 to 37.8 °C yielding a feed use reduction rate of 1.0 kg/100 birds per day per 5.6 °C increase in temperature (Poultry Times Supplement, 1999). At the same time, daily water use increased from 18.2 to 59.0 L per 100 birds for these temperatures. Decreased feed consumption during hot weather affects the intake of calcium and other nutrients essential for strong shells. High environmental temperature results in reduced shell quality and decreased shell thickness (Yamamoto, et al., 1997; North and Bell, 1990).

The benefit of providing cooled drinking water to birds, in terms of body heat loss used to warm the water, is insignificant (less than 0.2W assuming 10 °C cooler water and 300g daily water intake). Yet, if cool water can induce additional water intake, thereby ensuring adequate moisture supply for respiratory (panting) heat loss, then the benefit can be substantial (Brody, 1945). Further, we hypothesize that providing cooler drinking water temperature promotes increased feed consumption and thus eggshell quality. Largely unknown is the relationship between feed and water consumption over the course of diurnal heat stress. For example if birds were to drink more during the hottest portion of a day, they may alter their feeding behavior as well.

Puma et al. (2001), using a unique feeding and drinking monitoring system, reported that when provided cooler drinking water (27, 22, or 20 °C vs. 32 °C), broilers tended to maintain feed and water intake under a warm (35 °C) environment. No information is available for modern layers regarding the influence of water temperature during warm or hot environments. Water-cooling equipment is commercially available, but scientific data to justify investment on such equipment are lacking.

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The objectives of this research were: (1) to delineate dynamic feeding and drinking patterns of the birds as influenced by drinking water temperature, (2) to evaluate the effects of drinking water temperature on production performance of individual laying hens subjected to warm/hot cyclic air temperature, and (3) to determine whether an optimal drinking water temperature exists.

MATERIALS AND METHODS

Experimental Hens and Procedure

These studies were conducted at the Livestock Environment and Animal Physiology (LEAP) laboratory at Iowa State University, Ames, Iowa. Hy-line W-36 laying hens were procured from a commercial egg farm in north-central Iowa and transported to the LEAP laboratory. Initial age of the hens was 29 wk (Expt 1) and 30 wk (Expt 2), with corresponding body weights of 1.57 to 1.58 kg and 1.61 to 1.67 kg. Hens were housed in individual wire-mesh cages (25 cm W $\times$ 46 cm D $\times$ 46 cm H) that were located in two environmental chambers (2.4 m W $\times$ 2.4 m D $\times$ 3.0 m H), 12 hens per chamber. Hens were provided with a 16 h (5:00– 21:00 hr) fluorescent light and 8 h dark photoperiod as used on the commercial farm. They were fed *ad libitum* a commercial ration containing 19% crude protein, 4.2% Ca, and 0.8% P. Feed and water were replenished once daily.

Hens were held for one week, after which 24 hens with similar body weights were selected for testing. A one week acclimation period (week 0) was initiated with T_a and T_w at 21 ^oC. At the start of the following week (week 1), a diurnal T_a was applied to both chambers, and T_w was controlled to achieve respective target values. For Expt 1, six of the 12 hens in each chamber received a warm T_w of 27 ^oC and the other six received cool T_w of 18 ^oC; T_a varied diurnally from 27 to 35 ^oC. For Expt 2, four T_w were randomly assigned to the hens (six replications/treatment, three per chamber): T_w = 15, 19, 23 or 27 ^oC; T_a varied diurnally from 27 to 38 ^oC. In both experiments, the highest T_a was programmed to occur at 18:00 hr while the lowest at 06:00 hr. Hens were subjected to this environment for four weeks, followed by a 2-wk recovery period during which T_a and T_w were returned to the acclimation condition of 21 ^oC. T_a and T_w were maintained within 0.3 to 0.5 ^oC for T_a and 0.1 to 0.2 ^oC for T_w. Humidifiers were placed in each chamber to maintain relative humidity between 45 and 60%.

Measurement and Analysis of Performance Variables

Each cage was equipped with a feeder weighing station and a water-use measurement device whose signal outputs were transmitted to a central data acquisition PC. The specially designed watering devices featured control of T_w by controlling the temperature of a water jacket surrounding the water reservoir column. Detailed information on the design and operation of the Individual Bird Use (IBU) feed and water monitoring system has been described by Puma et al.(2001). Monitoring of feeding and drinking commenced at the start of the acclimation period and continued for seven weeks. Data for the transition days, from acclimation to treatment (days 13 and 14) and from treatment to recovery (day 43), were excluded in the data analysis of Expt 1, but not Expt 2.

Feeding and drinking events, including event duration and amount of ingestion, were quantified from time-series recordings (4 or 30 s intervals) of the feed scales and pressure transducers in each hen's waterer. Mean hourly feed and water use were determined for each bird from these data. Daily feed and water intake (DFI, DWI) were also directly measured from the feeder and waterer weight readings each 24 h. DFI, DWI, and water-to-feed use ratio (WFR), along with the egg production parameters, were used to evaluate the treatment effects. Daily values were averaged (summed for egg weight) into period intervals and analyzed statistically with an independent sample t-test (Expt 1) and analysis of variance (Expt 2) to evaluate the treatment effect (SAS, 1999).

Eggs were collected and recorded daily, cleaned, weighed and kept in cold (4 ^oC) storage, and analyzed weekly for the following parameters: yolk, albumen, shell weight, yolk to white ratio, and Haugh unit. In Expt 1, eggs were pooled by T_w treatment; in Expt 2, eggs were analyzed on a per hen basis. Yolk was weighed after separating albumen and chalaza from the yolk. Chalaza was removed using forceps. Shell weight was measured after removing any residual albumen from the inner eggshell surface with a vacuum. Albumen weight was calculated by subtracting yolk and shell weights from total egg weight. Albumen heights (to nearest 0.1 mm) were measured using a dial caliper device (Ames Co., Waltham, MA). Haugh unit was calculated according to Stadelman and Cotterill (1977). Expt 2 utilized similar procedures, except four eggs/hen weekly were used for yolk/white ratio determination and the remainder for Haugh unit determination; also eggshells were dried for 24 hr at 85 ^oC prior to weighing. For both experiments, feed conversion (FC) - the ratio of feed intake to egg production- was determined for each hen for various periods by summing DFI and dividing by mass of egg produced.

RESULTS AND DISCUSSION

Daily Feed and Water Intake (DFI, DWI), Water-to-Feed Intake Ratio (WFR)

For Expt 1, during the acclimation period, DFI of hens in both T_w groups was similar at 105 and 106 g/hen-d (Table 1). DFI dropped significantly during the treatment period. For the first two weeks of the treatment period, DFI for cool T_w (82 g/hen-d for week 1 and 86 g/hen-d for week 2) was significantly higher (P<0.10) than that for warm T_w (77 g/hen-d for week 1 and 81 g/hen-d for week 2). During the last two weeks of the treatment period and the recovery period, however, DFI was not significantly different between the two treatments (P>0.10). DFI for both treatments showed a similar compensatory increase during the recovery period, and stabilized at 114 g/hen-d at the end of the period.

Table 1. Daily feed and water intake (DFI, DWI), water to feed intake ratio (WFR), and body weight (BW) of laying hens for Experiment 1, at starting age of 29 weeks, during acclimation, treatment and recovery periods. Drinking water temperature (T_w) and air temperature (T_a) were 21 ^oC during the acclimation and recovery periods. During the treatment period, T_w was 18 ^oC (cool) or 27 ^oC (warm) and T_a varied from 27 to 35 ^oC.

Trial Week	DFI (SE) g/hen-d		DWI (SE) g/hen-d		WFR (SE)		BW (SE) kg/hen	
	Warm T_w	Cool T_w	Warm T_w	Cool T_w	Warm T_w	Cool T_w	Warm T_w	Cool T_w
Acclimation								
0	105 (4)	106 (3)	194 (7)	193 (7)	1.9 (0.1)	1.8 (0.1)	1.64 (0.02)	1.65 (0.02)
Treatment								
1	77 ^{a} (2)	82 ^{b} (3)[1]	262 (13)	278 (24)	3.4 (0.2)	3.4 (0.2)	1.56 (0.02)	1.58 (0.02)
2	81 ^{c} (1)	86 ^{d} (1)	260 (13)	277 (24)	3.2 (0.2)	3.2 (0.3)	1.53 (0.02)	1.56 (0.02)
3	90 (3)	91 (3)	257 (15)	274 (24)	2.9 (0.2)	3.0 (0.3)	1.54 (0.02)	1.56 (0.02)
4	89 (2)	91 (3)	264 (13)	287 (27)	3.0 (0.1)	3.2 (0.3)	1.54 (0.02)	1.56 (0.02)
1-4	84 (1)	87 (1)	261 (13)	279 (23)	3.1 (0.2)	3.2 (0.3)		
Recovery								
5	107 (2)	108 (2)	196 (4)	195 (8)	1.8 (0.04)	1.8 (0.08)	1.57 (0.02)	1.59 (0.02)
6	114 (2)	114 (2)	204 (6)	201 (6)	1.8 (0.04)	1.8 (0.05)	1.62 (0.02)	1.63 (0.02)

[1]Row means of response variables with different superscripts are significantly different (P<0.10).

DWI for both T_w regimens was similar (194 and 193 g/hen-d for warm and cool T_w, respectively) during the acclimation period, and increased to 262 and 278 g/hen-d, respectively, during the first week of the treatment period. Although cool T_w hens had a numerically higher DWI (up to 6%) than the warm T_w hens, the difference was not significant (P>0.10) throughout the 4-wk treatment period. Large variations among individual hens contributed to this non-significant outcome. During the recovery period, DWI returned to almost the same levels as during the acclimation period and there was no significant difference between the two regimens (P>0.10).

WFR were not significantly different (P>0.10) between the two treatments during the acclimation period (1.9 vs 1.8 for the warm and cool T_w, respectively). It increased during the treatment period, averaging 3.1 and 3.2, respectively, but no significant difference was detected (P>0.10) (Table 1). WFR returned to the acclimation levels during the recovery period.

For Expt 2, the same suppressing effect of the warm environment on DFI, as seen in Expt 1, was observed. Also, as in Expt 1, DFI recovered quickly upon return to thermoneutral conditions (Table 2). Treatment effect on DFI was not significant for any period of the test (Table 3). There was a trend for treatment 3 hens (T_w=23^{o}C) to experience a smaller reduction in DFI as verified by linear contrast (P<0.042). A linear regression of treatment means against week of heat stress suggested a recovery of approximately 4.3 g feed/day for each sequential week of heat stress (R^2 = 62.6%); incorporating the two weeks of recovery increased this to 8.5 g/day (R^2 = 70.8%).

Treatment effects were noted for DWI during the treatment period and the first week of recovery. Hens provided the warmest drinking water consistently reduced DWI, whereas mean values for the 15 and 23 ^{o}C T_w were greatest, and 21^{o}C intermediate. There was a significant difference for the first week of recovery, presumably as birds readjusted to 21^{o}C water, but DWI was less than the previous week. WFR varied by treatment, but were not statistically significant. Week 1-4 treatment means in increasing temperature order were: 3.3, 3.2, 3.2 and 2.9, SE=0.067.

Table 2. Daily feed and water intake (DFI, DWI) of laying hens for Experiment 2, at starting age of 30 weeks, during acclimation, treatment and recovery periods. Drinking water temperature (T_w) and air temperature (T_a) were 21 °C during the acclimation and recovery periods. During the treatment period, T_w was 15, 19, 23, or 27 °C, T_a varied from 27 to 38 °C.

	T_w °C					Test of:		T_w °C					Test of:	
Trial week	15	19	23	27	overall (MSE)[1]	Treatment Effect (P>F)[2]	Week Effect (P>F)[3]	15	19	23	27	overall (MSE)	Treatment Effect (P>F)[2]	Week Effect (P>F)[3]
Acclimation														
	103	101	101	102	102 (9)	P=0.98		205	181	188	185	190 (20)	P=0.21	
Treatment														
1	73	64	78	70	71 (10)	P=0.18		246	216	257	218	234 (35)	P=0.14	
2	77	74	81	74	77 (9)	P=0.43		244	223	249	209	231 (31)	P=0.13	
3	80	77	83	78	79 (7)	P=0.41		258	234	255	226	243 (28)	P=0.15	
4	84	84	89	81	84 (7)	P=0.35		264^{c}	239^{abc}	267^{bc}	224^{a}	248 (30)	P<0.067	
1-2	75	69	80	72	74 (5)	P=0.27	<0.0001	245	219	253	214	233 (15)	P=0.125	P=0.50
2-4	82	80	86	79	82 (3)	P=0.36	<0.0001	261^{c}	237^{b}	261^{c}	225^{a}	246 (11)	P<0.085	P=0.112
1-4	71	77	79	84	78 (5)	P=0.28	<0.0001	253^{c}	228^{b}	257^{c}	219^{a}	239 (14)	P<0.095	P<0.0001
Recovery														
5	109	106	113	104	108 (8)	P=0.26		220^{bc}	204^{ac}	223^{b}	197^{a}	211 (18)	P<0.058	
6	119	109	114	107	111 (8)	P=0.39		213	203	216	195	207 (19)	P=0.213	

[1]Mean and overall mean square error of mean for single weeks from ANOVA; for multiple weeks, values are from the repeated measures ANOVA

[2]Test of treatment effect uses SS(hen(trt))

[3]Test of week effect uses residual SS for model

Body Weight (BW) Change

For Expt 1, average BW at the end of the acclimation period was 1.64 and 1.65 kg for the warm T_w and cool T_w, respectively (Table 1). BW decreased by 6-7% (1.54 kg for both regimens) during the treatment period. It returned to nearly the acclimation period level during the recovery period (1.62 and 1.63 kg for the warm and cool T_w, respectively).

For Expt 2, average BW for acclimation, treatment, and recovery periods are shown in Table 3. There was no treatment effect noted. Mean reduction in BW was significant for each period tested (Table 3). Mean BW loss was 144g at the end of the treatment period. After two weeks of recovery, the mean BW was 42 g lower than that prior to heat stress exposure ($P < 0.0001$). This suggests that more than two weeks are necessary for full weight recovery from extended heat stress episodes.

Effects of T_w on Egg Production (EP) and Feed Conversion (FC)

EP (g/hen-d) for Expt 1 was not affected by T_w during the heat stress period (Table 4, $P>0.10$), although the cool T_w hens tended to have higher EP. For Expt 2, there was also no treatment effect on EP (Table 5). However, hens in treatment 3 (T_w = 23 ^oC) demonstrated somewhat higher (1.5 ~ 2.4 g/hen) EP than the other treatments. This result was consistent with the trend of less reduction in DFI for the same hens.

FC was not affected by the T_w treatments except during the first week in Expt 1. The effect of heat exposure on FC was evident in both experiments.

Effects of the Treatments on Egg Quality

For both Expt 1 and 2, yolk-to-white ratio and Haugh unit were unaffected by T_w (Tables 6 and 7). For Expt 1, eggshells for cool T_w were heavier than those for warm $T_{w,}$ for six sampling days. In Expt 2, there was not a significant treatment effect (with acclimation period used as a covariate).

Hourly Feeding and Drinking Patterns

Hourly feed and water intake data for both experiments are unavailable at the time of this writing. Discussion of results will be included in future reports.

CONCLUSIONS

Effects of drinking water temperature (T_w) on laying hens under diurnal warm cyclic air temperatures were investigated. Two levels of T_w (18, 27 ^oC) were used in Expt 1 and four (15, 19, 23, 27 ^oC) in Expt 2. The hens were subjected to 1-wk acclimation, 4-wk treatment, and 2-wk recovery. Cooler T_w tended to enhance feed and water intake of laying hens during the early stage of the heat exposure. There may exist an optimal range of T_w for hens exposed to heat stress. However, large variations among the individual hens tempered these findings. Further investigation using more experimental hens is warranted to evaluate T_w effects on production performance of the hen.

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Table 3. Suppression and recovery of daily mean feed intake (DFI) and body weight (BW) of laying hens for Experiment 2, at starting age of 30 weeks, during acclimation, treatment and recovery periods. Drinking water temperature (T_w) and air temperature (T_a) were 21 °C during the acclimation and recovery periods. During the treatment period, T_w was 15, 19, 23 or 27 °C and T_a varied from 27 to 38 °C.

Trial Week	DFI (SE) g/hen-d	DFI change from acclimation[1] (g/hen-d)	BW (SE) kg/hen	BW change from acclimation g/hen	SE change for BW[2] g/hen
Acclimation					
0	102 (2)	-	1.67 (0.01)	-	
Treatment					
1	72 (3)	-30			
2	77 (2)	-24	1.55 (0.01)	-0.11	0.01
3	80 (1)	-22			
4	85 (1)	-16	1.53 (0.01)	-0.14	0.01
Recovery					
5	109 (1)	7	1.62 (0.01)	-0.05	0.01
6	111 (1)	10	1.62 (0.01)	-0.04	0.01

[1] SE=1.2g, all means significantly different from zero (P<0.0001)
[2] all means significantly different from zero (P<0.0001).

Table 4. Egg production (EP), feed conversion (FC), and egg size (ES) of laying hens for Experiment 1, at starting age of 29 weeks, during acclimation, treatment and recovery periods. Drinking water temperature (T_w) and air temperature (T_a) were 21 °C during the acclimation and recovery periods. During the treatment period, T_w was 18 °C (cool) or 27 °C (warm) and T_a varied from 27 to 35 °C.

Trial Week	EP (SE) g/hen-d		FC (SE)		ES (SE) g	
	Warm T_w	Cool T_w	Warm T_w	Cool T_w	Warm T_w	Cool T_w
Acclimation						
0	52.7 (0.9)	51.3 (1.3)	1.91 (0.06)	1.90 (0.04)	54.7 (0.7)	54.6 (0.7)
Treatment						
1	51.7 (1.6)	52.1 (1.7)	1.41 (0.02)	1.49 (0.04)	54.2 (0.5)	55.3 (1.0)
2	47.8 (1.8)	49.5 (1.9)	1.46 [a] (0.02)	1.55 [b] (0.02)[1]	54.9 (0.6)	55.3 (1.0)
3	49.6 (1.8)	49.2 (1.8)	1.64 (0.03)	1.65 (0.05)	54.7 (0.6)	55.2 (0.8)
4	53.5 (1.8)	52.4 (1.9)	1.63 (0.02)	1.64 (0.06)	54.8 (0.7)	55.6 (0.8)
1-4	50.7 (1.2)	50.8 (0.8)	1.54 (0.02)	1.58 (0.04)	54.7 (0.8)	55.4 (0.8)
Recovery						
5	51.5 (1.7)	52.4 (1.5)	1.91 (0.03)	1.90 (0.04)	56.1 (0.5)	57.1 (0.7)
6	52.0 (2.7)	53.6 (1.6)	2.01 (0.87)	1.97 (0.03)	56.8 (0.5)	57.8 (0.9)

[1]Row means of response variables with different superscripts are significantly different (P<0.10).

Table 5. Egg production (EP), feed conversion (FC), and egg size (ES) of laying hens for Experiment 2, at starting age of 30 wk, during acclimation, treatment and recovery periods. Drinking water temperature (T_w) and air temperature (T_a) were 21 °C during acclimation and recovery periods. During the treatment period, T_w was 15, 19, 23 or 27 °C and T_a varied from 27 to 38 °C.

Trial week	EP, g/hen-d at T_w (°C) of				
	SE	15	19	23	27
Acclimation					
0	1.6	50.4	51.2	55.2	54.2
Treatment					
1	2.9	35.8	36.7	37.8	39.2
2	2.7	47.0	48.0	49.3	42.2
3	2.1	48.3	49.2	50.4	51.7
4	2.9	48.4	47.1	51.6	50.0
1-4	1.4	44.9	45.2	47.3	45.8
Recovery					
5	2.2	49.0	49.8	52.0	52.8
6	2.0	52.8	56.9	60.3	57.4

Trial week	FC at T_w (°C) of				
	SE	15	19	23	27
Acclimation					
0	0.09	2.06	2.00	1.83	1.87
Treatment					
1	0.20	1.45	1.22	1.67	1.31
2	0.08	1.64	1.54	1.67	1.83
3	0.08	1.66	1.58	1.69	1.51
4	0.05	1.74	1.79	1.72	1.68
1-4	0.03	1.63	1.54	1.63	1.58
Recovery					
5	0.12	2.27	2.14	2.19	2.05
6	0.08	2.13	1.94	1.90	1.93

Trial week	ES (g) at T_w (°C) of					
	SE	15	19	23	27	Treatment effect
Acclimation						
0	1.2	57.1	56.7	58.1	59.9	P=0.28
Treatment						
1	1.3	55.6 ab	55.1 a	58.5 bc	59.9 c	P<0.06*
2	1.2	54.9 ab	54.4 a	57.7 bc	59.3 c	P<0.03**
3	1.1	54.9 ab	54.4 a	57.5 bc	58.7 c	P<0.04**
4	1.4	55.0	55.0	57.1	58.4	P=0.28
1-4	1.2	55 ab	54.7 a	57.7 bc	59.0 c	P<0.07
Recovery						
5	1.4	55.6	56.5	59.2	60.0	P=0.15
6	1.2	56.9	58.3	60.4	61.1	P=0.10

* Significance level = 10%, ** Significance level = 5%.

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Table 6. Internal egg quality parameters of laying hens in Experiment 1, at starting age of 29 weeks, during acclimation, treatment and recovery periods. Drinking water temperature (T_w) and air temperature (T_a) were 21 ^oC during the acclimation and recovery periods. During the treatment period, T_w was 18 ^oC (cool) or 27 ^oC (warm) and T_a varied from 27 to 35 ^oC.

Trial week	Yolk/white ratio (SE), %		Haugh unit (SE)	
	Warm T_w	Cool T_w	Warm T_w	Cool T_w
Acclimation				
0	39.7 (0.3)	39.7 (0.4)	83.2 (1.3)	80.7 (0.5)
Treatment				
1	41.0 (0.2)	40.6 (0.3)	88.9 (1.6)	89.0 (1.4)
2	41.3 (0.4)	40.7 (0.7)	81.8 (1.9)	83.1 (1.1)
3	40.3 (0.7)	39.1 (0.8)	80.6 (1.4)	82.5 (1.1)
4	40.6 (0.3)	40.0 (0.5)	83.0 (1.4)	85.0 (1.3)
1-4	40.8 (0.5)	40.1 (0.6)	83.6 (1.0)	84.9 (0.8)
Recovery				
5	41.7 (0.2)	40.3 (0.4)	81.4 (0.8)	83.1 (1.0)
6	42.3 (0.2)	42.3 (0.2)	80.7 (0.5)	81.4 (0.9)

Table 7. Internal egg quality parameters of laying hens in Experiment 2, at starting age of 30 weeks, during acclimation, treatment and recovery periods. Drinking water temperature (T_w) and air temperature (T_a) were 21 ^oC during the acclimation and recovery periods. During the treatment period, T_w was 15, 19, 23 or 27 ^oC and T_a varied from 27 to 38 ^oC.

Trial week	SE	Yolk/white ratio (%) at T_w (^oC) of 15	19	23	27
Acclimation					
0	0.9	39.4	38.8	37.6	37.2
Treatment					
1	1.0	41.8	42.7	40.1	40.5
2	0.9	39.2	40.8	39.0	39.2
3	0.9	39.5	40.2	39.0	37.9
4	0.9	39.8	40.0	39.6	36.9
Recovery					
5	1.0	37.9	37.2	35.8	36.3
6	0.9	37.9	39.0	36.5	35.6

Trial week	SE	Haugh unit at T_w (^oC) of 15	19	23	27
Acclimation					
0	2.0	87.7	89.5	87.7	86.7
Treatment					
1	1.5	88.2	90.3	86.0	87.8
2	1.5	88.2	88.4	83.9	87.4
3	2.0	87.9	91.3	87.7	90.5
4	1.8	88.1	87.7	87.0	87.3
Recovery					
5	1.6	89.9	90.0	90.4	89.3
6	1.6	88.7	90.9	89.9	86.7

Trial week	SE	Shell dry weight (g) at T_w (^oC) of 15	19	23	27
Acclimation					
0	0.1	5.51	5.39	5.26	5.79
Treatment					
1	0.1	5.05	4.85	5.02	5.13
2	0.1	5.16	5.04	5.23	5.29
3	0.1	5.17	5.04	5.20	5.15
4	0.1	5.18	5.12	5.15	5.19
Recovery					
5	0.1	5.44	5.42	5.47	5.76
6	0.1	5.44	5.43	5.42	5.80

Note: test of treatment effect not significant during weeks 1-4 (repeated measures, week 0 as covariate).