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Abstract
Market-size (61-68 day-old) AA broiler chickens were exposed to simulated high-cyclic summer temperatures of North, Central and South China for 5 continuous days. Blood samples were collected at 0AM, 4AM, 8AM, 0PM, 4PM and 8PM each day, and concentrations of triiodothyronine (T3) and thyroxine (T4) were determined by double-antibody radioimmunoassay (RIA). T3, T4 concentration and T3/T4 ratio had two peaks, but the daily variation patterns of thyroid hormones were different between each other. T3 peaked at 12 AM and 12 PM, while T4 peaked at 8 AM and 12 PM, with the two peaks of T3/T4 ratio showing at 4 AM and 12 AM. The lowest concentrations of both T3 and T4 occurred at 4 PM. According to above results, the blood samples should be collected around the time corresponding to the peak of temperature sinusoid, when thyroid hormones (both T3 and T4 concentrations) are used to evaluate the heat stress status of broilers.

Keywords
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DAILY VARIATION OF THYROID HORMONES IN BROILER UNDER HIGH-TEMPERATURE CONDITIONS

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ABSTRACT

Market-size (61-68 day-old) AA broiler chickens were exposed to simulated high-cyclic summer temperatures of North, Central and South China for 5 continuous days. Blood samples were collected at 0AM, 4AM, 8AM, 0PM, 4PM and 8PM each day, and concentrations of triiodothyronine (T_3) and thyroxine (T_4) were determined by double-antibody radioimmunoassay (RIA). T_3, T_4 concentration and T_3/T_4 ratio had two peaks, but the daily variation patterns of thyroid hormones were different between each other. T_3 peaked at 12 AM and 12 PM, while T_4 peaked at 8 AM and 12 PM, with the two peaks of T_3/T_4 ratio showing at 4 AM and 12 AM. The lowest concentrations of both T_3 and T_4 occurred at 4 PM. According to above results, the blood samples should be collected around the time corresponding to the peak of temperature sinusoid, when thyroid hormones (both T_3 and T_4 concentrations) are used to evaluate the heat stress status of broilers.

KEYWORDS. Heat stress, broiler, thyroid hormone, diurnal rhythm

INTRODUCTION

The major hormone products of thyroid gland, 3,5,3´-triiodothyronine (T_3) and thyroxine (T_4), play important roles in the control of metabolic rate and thermogenesis of poultry. In birds, T_4 and T_3 have a half-life span of a few hours, the diurnal rhythms of thyroid hormones in chickens have been detected and the results have been variable. Du et al. (1995) found that roosters exhibited three diurnal peaks of T_3, occurring at 8 AM, 4 PM and 12 PM, under natural environmental condition with the average outside temperature in between 7.9-11.1^{o}C. Sadovsky and Bensadoun (1971) observed similar diurnal variations for T_3 and T_4 with two peaks at 8 AM and 4 PM when roosters were exposed to a 14L:10D photoperiod (darkness onset at 8 PM). Newcomer (1974) reported one peak of T_3 and T_4 occurring at 4 PM and 7 AM, respectively, for birds exposed to a 16L: 8D photoperiod (darkness onset at 9 PM). But Smoak and Birrenkott (1986) demonstrated no circadian variations for either T_3 and T_4 in cockerels exposed to 23L:1D. There is therefore a lack of information on daily variations in thyroid hormones under hot environment, which chickens inevitably undergo in the summer. The objectives of the present study were to delineate the circadian rhythms of thyroid hormones in market-size broiler chickens under high temperatures, and to provide guideline information for selection of blood sample collection timing when using thyroid hormones as a heat stress indicator of the bird.

MATERIALS AND METHODS

EXPERIMENTAL BIRDS

Day-old Arbor Acres AA chicks (400 total) were procured from a commercial hatchery and were

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reared at the Key Lab of Animal Nutrition, Ministry of Agriculture, Beijing, China. At 58 d of age (mean mass of 2631 ± 64 g), 270 lively and uniform broilers were selected and randomly assigned to three groups, 45 birds of both sex per group. The selected experimental birds were transported to three environmental chambers, where the birds were acclimated for 3 d under the thermoneutral (TN) condition of 23 ± 1.2^{o}C dry-bulb temperature (t_{db}) and 39%±6.9 RH. The birds were fed two times at 8 AM and 8 PM with free access to water under a 24:0 light – dark photoperiod. Trials lasted 5 d.

ENVIRONMENTAL CONDITIONS

After acclimation, the three groups of birds were treated with different high cyclic temperatures, simulating the average of the top 10 hot summer days during 1991-2001 in Harbin (northern China), Wuhan (central China) and Guangzhou (southern China), which were designated as treatment 1, treatment 2, and treatment 3, respectively.

The original temperature data were provided by China Meteorological Administration, the temperature ranges were 23.1-35.5^{o}C for Harbin, 30.1-37.9^{o}C for Wuhan, and 27.9-36.5^{o}C for Guangzhou. The set-point temperature profile of each treatment corresponding to blood sample time was showed in Figure 1. To highlight the effect of temperature on serum thyroid hormones in broilers, the RH in the three chambers were maintained at constant averaging 40%, in which the absolute humidity varied over the day. A programmable, battery-powered RH/Temp data logger (HOBO Pro H8 series, Onset Computer Corporation, bourne, MA, USA) was used to record temperature and RH in each room at 15-min intervals, and the recording data were used in the following analysis.

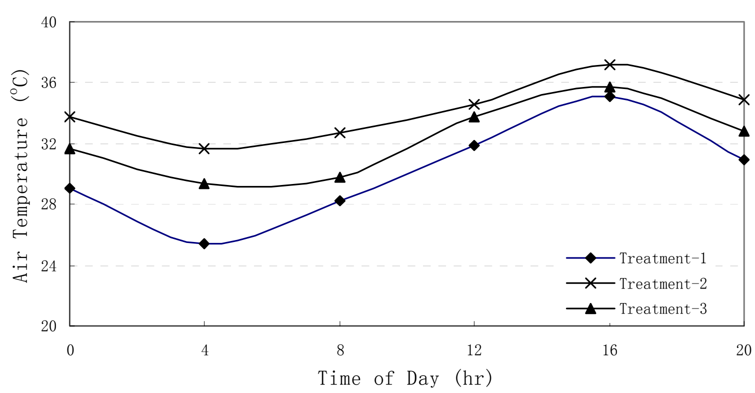


Figure 1. The setpoint temperature profile of each treatment

COLLECTION AND ANALYSIS OF BLOOD SAMPLES

Blood samples were collected by a skilled avian physiologist 6 times a day during the 7-d trial period, at 0AM, 4AM, 8AM, 12PM, 4PM and 8PM. Five birds (3 males and 2 females or 2 males and 3 females) from each treatment chamber were randomly selected. Four ml of blood from each bird was drawn from a wing vein with one-time-use syringes, and was placed in polycarbonate tubes. The birds were handled with care and the blood drawn within 2 minutes to minimize artifact on hormone responses due to handling. Serum was collected after 10-minute centrifugation at a constant temperature of 4^{o}C with Hitachi Refrigerated Centrifuge (Himac CR20B, Hitachi Ltd, Japan), and stored at -20^{o}C until hormone analysis was performed. Some birds were sampled more than once with intervals no less than 3 days. T_3/T_4 concentrations were determined by double-antibody radioimmunoassay (RIA), using commercially available RIA kits (China Institute of Atomic Energy, Beijing, China), in which the correlation between the curve of standard concentrations and that of the predicted values was 0.9976.

DATA ANALYSIS

All statistical analyses, including analysis of variance (ANOVA), regression and two-tail t-tests, were performed using the general linear model procedure of Statistical Package for the Social Sciences (SPSS Inc. Release 10.0).

RESULTS

Thyroid Hormones Concentration in Broilers

Both male and female broilers were used in the experiments, and the number of birds bled in each sex at each sampling time was approximately equal. The T_3 andT_4 concentrations and T_3/T_4 ratio were analyzed for significant differences between the male and female broilers using two-tailed t-tests. No significant difference was found.

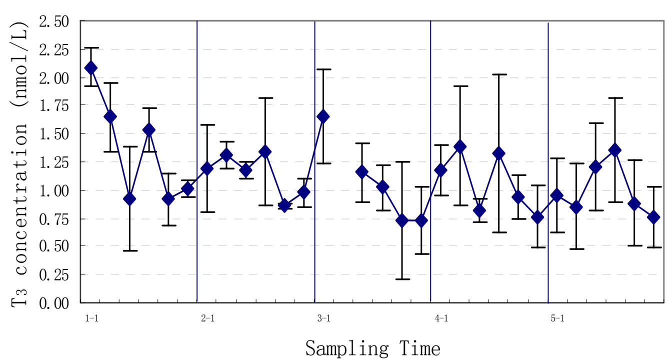
Some broilers were bled more than once during the trials, with the sampling interval being no less than 3 d, the secondary bleeding occurred at the fourth day and lasted to the end of the experiment. A few broilers were bled three times, with the last bleeding taken on the last (7th) day. T_3 and T_4 concentrations and T_3/T_4 ratio were analyzed for significant differences between sampling times on the same day using two-tailed t-tests. The results showed no significant differences ($P>0.05$, Table 1).

Table 1. Effects of sampling times on thyroid hormone of 61-68 day-old AA broilers（Mean±SD）

Day	Sampling times	Thyroid Hormone（nmol/L）				T_3/ T_4 ratio	P
		T_3	P	T_4	P		
4	First	1.31±0.88	0.510	16.72±9.38	0.719	0.09±0.08	0.794
	Second	1.19±0.86		16.02±8.75		0.09±0.07	
7	Second	1.07±0.73	0.655	14.42±11.42	0.119	0.08±0.05	0.454
	Third	0.99±0.79		11.01±8.38		0.09±0.07	

On each of the heat exposure day, no significant difference in thyroid hormone was found between treatments, blood samples were pooled over sampling time of each day, and the values of pooled T_3, T_4 concentrations, and T_3/T_4 ratio of broilers in the five heat-challenged days were shown in Table 2.

T_3 concentrations ranged from 3.975nmol/L to 0.014nmol/L, the range of T_4 concentrations were 47.69nmol/L to 0.21nmol/L, and the T_3/ T_4 ratio varied between 0.34 to 0.002. The daily mean of both T_3 and T_4 concentrations kept decreasing as heat stress extended, however, the daily average of T_3/T_4 ratio changed differently, it fluctuated. Within each day, T_3, T_4 concentrations, and T_3/T_4 ratio varied with the sampling time. The profiles of the pooled T_3, T_4 concentrations, and T_3/T_4 ratio during the entire testing periods are shown in Figure 2.



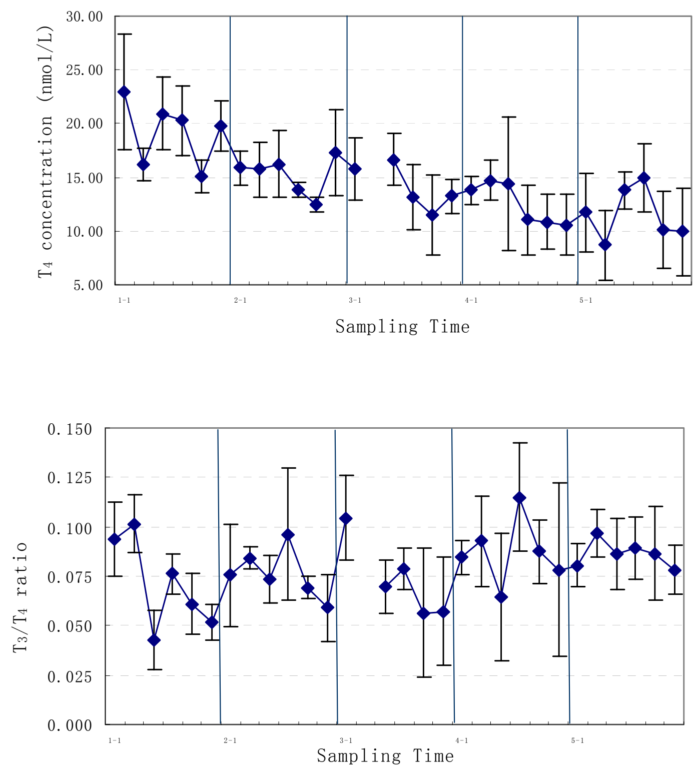
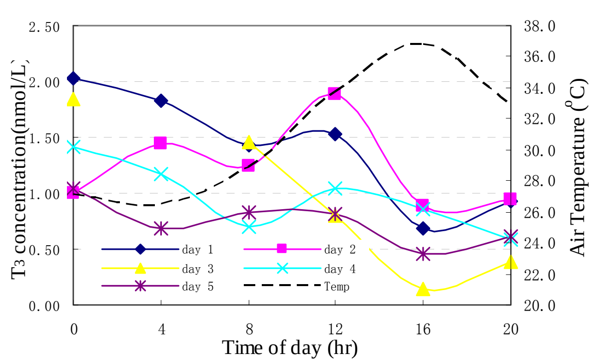


Figure 2. Profile of pooled serum T3 and T4 concentrations, and T3/T4 ratio of market-sized AA broilers subjected to heat challenge at different sampling time in 5 days. Data are means ± standard deviation. 1-1 indicate the first sampling time at 0 hr of the first day and so on.

Diurnal Rhythms of Thyroid Hormones

As indicated above, the pooled T_3, T_4 concentrations, and T_3/T_4 ratio at one sampling time were different from their counterparts at the adjacent sampling time although no significant differences existed. To give a closer view of the daily fluctuation of thyroid hormone, the diurnal variations of thyroid hormones in the three treatments were analyzed separately. Results showed that the values of thyroid hormones changed with both time of day and heat exposure length, however the daily patterns of each thyroid hormone presented similarity, the profiles of T_3, T_4 concentrations and T_3/T_4 ratio in treatment 3 were plotted day by day, as shown in Figure 3, T_3, T_4 concentrations, and T_3/T_4 ratio exhibited different variation patterns.



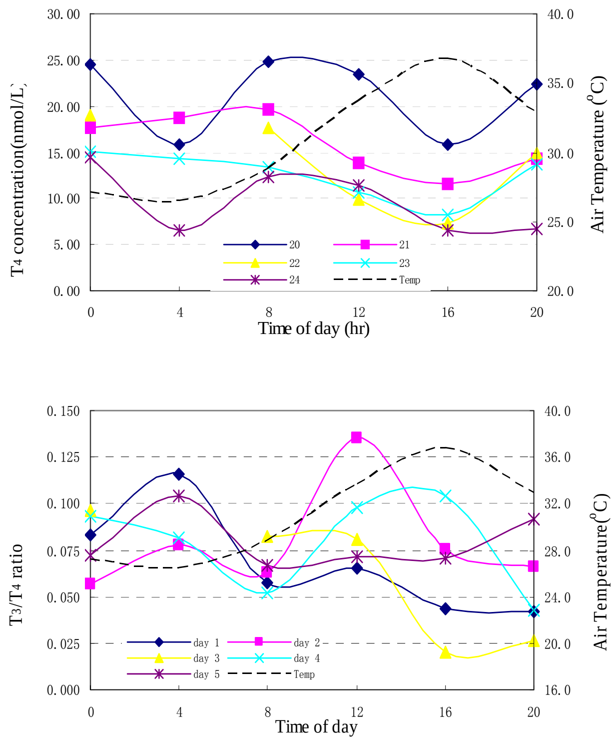
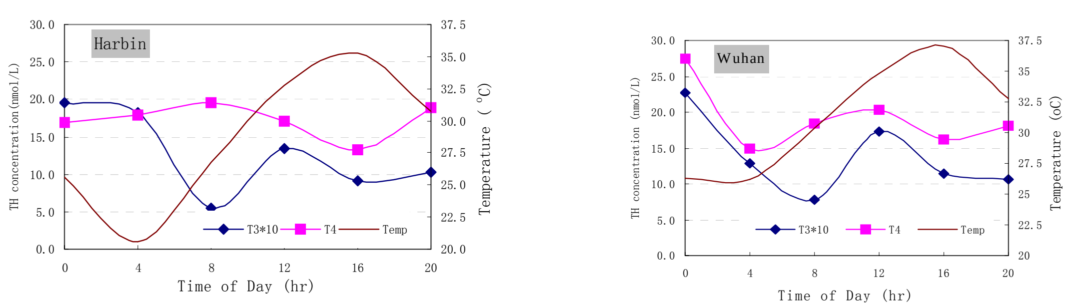


Figure 3. Daily Variations of T_3, T_4 concentrations and T_3/T_4 ratio in treatment 3, simulating high cyclic temperature of Guangzhou (Southern China)

Although daily variations of T_3, T_4 concentrations, and T_3/T_4 ratio were different between each other, the circadian rhythms of each thyroid hormone under different hot conditions were quite similar, so T_3 and T_4 concentrations in each treatment varying with the corresponding environmental temperatures in the first heat exposure day as shown in Figure 4, with T_3 concentration T_3/T_4 ratio amplified by a factor of 10. The results indicated that T_3, T_4 concentration, and T_3/T_4 ratio had consistent circadian rhythms under different high temperature environments. T_3 concentration had two peaks, one at 12 AM and the other and the higher at 12 PM, The highest T_3 level at 0 AM averaged 2.09±0.17nmol/L, was 2.3 times higher than the lowest level (0.92±0.23nmol/L) occurring at 4 PM. T_4 concentration also had two peaks, showing at 8 AM and 12 PM, respectively. The lowest T_4 concentration also presented at 4 PM. T_3/T_4 ratio peaked at 4 AM and 12 AM, respectively. Thyroid hormones had a peak at midnight, and a valley of both T_3 and T_4 concentrations appeared at 4 PM, when environmental temperature reached the highest.

When thyroid hormones (T_3 and T_4 concentrations) were used as heat stress indicators of chickens, the optimum sampling time should be around 4 PM.



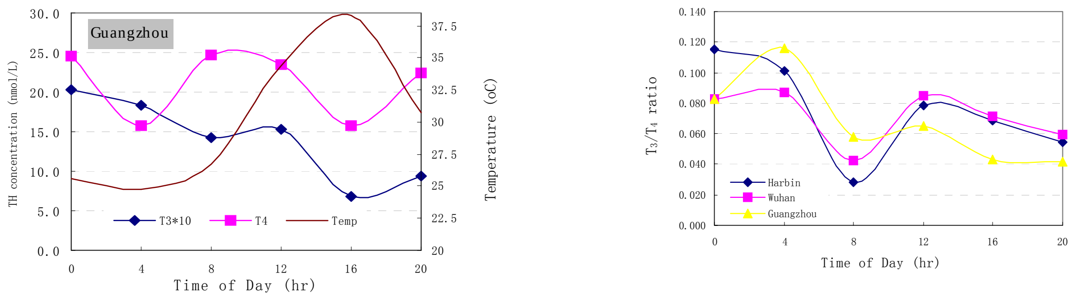


Figure 4. Thyroid hormones (T_3, T_4 and T_3/T_4 ratio) changing with environmental temperatures in the first heat challenge day under different conditions

DISCUSSION

Sadovsky and Bendadoun (1971) found that the hen plasma iodohormone concentration was significantly higher than that of roosters. However, the results of the present study showed no significant difference in the thyroid hormones (T_3, T_4 concentration and T_3/T_4 ratio) between male and female broiler chickens. This disparity probably resulted from the differences in genetics (White Leghorn *vs.* AA), bird age (10 months *vs.* 61-68 d), and rearing conditions.

Our observations were likely consistent with the reports by Sinurat et al (1987), who found that broilers in high ambient temperature had T_3, T_4 concentration and T_3/T_4 ratio range of 0.24-2.02μg/L (0.37-3.10 nmol/L), 4.20-20.9μg/L (5.41-26.90 nmol/L), and 0.02-0.51, respectively, but could partially agreed with the literature in which T_3, T_4 concentration and T_3/T_4 ratio for 60 d old chickens ranged 3.42-3.80ng/ml (5.25-5.84nmol/L), 14.4-18.4ng/ml (18.53-23.68nmol/L), and 0.23-0.20, respectively (Astier, 1980), T_3/T_4 ratios were 2/3 (Sturkie, 1976) and 1.33-2.12 (Sadovsky and Bensadoun, 1971), because their ranges of thyroid hormones were much narrower than the our records of their counterparts, T_3, and T_3/T_4 ratio were beyond our results, it was probably that heat challenge incurred these differences.

Sadovsky and Bendadoun (1971) indicated that T_3, T_4 concentration and T_3/T_4 ratio of 10-month old White Leghorn roosters presented very similar circadian rhythms, with two peaks showing at 8 AM and 4 PM, our research also found that T_3, T_4 concentration and T_3/T_4 ratio exhibited two peaks, but the daily variations of thyroid hormones were different between each other, and both T_3 and T_4 concentration showed lowest value at 4 PM, which was contrary to the reports of Sadovsky and Bendadoun (1971). However, Du et al (1995) observed three peaks for T_3, occurring at 8 AM, 4 PM, and 12 PM for adult White Leghorn roosters under natural conditions in Beijing during 27 March to 1 April, with the average outside temperature in between 7.9-11.1^{o}C, our observation were in agreement with highest peak at midnight, but only one peak was detected in the daytime instead of two. Sadovsky and Bendadoun (1971) suggested that diurnal rhythms of thyroid hormones had some relation to feeding activities, increased food intake may cause an increase in the production of thyroid hormone. But in our research, the patterns of temperatures exerted an influence on the daily variations of thyroid hormones. When ambient temperature reached the highest point at 4 PM, both T_3 and T_4 concentration appeared the lowest values, which were contrary to the pattern of thyroid hormones in natural conditions. Geraert et al (1996) observed specific effects of heat on thyroid hormones, which was independent of feed intake. As potent metabolic regulators, thyroid hormones play a major role in controlling body temperature and metabolism. A decrease in basal metabolic rate and plasma thyroid hormones were reported in chickens exposed to heat stress (Brigmon et al, 1992; Bowen and Washburn, 1985; Yahav et al, 1996). Decreased production of thyroid hormones was one of the pathways for the control of thermogenesis and maintenance of homeostasis. In view of the specific circadian rhythms of thyroid hormones in high temperature, the blood samples should be collected around the time corresponding to the peak of temperature sinusoid, when thyroid hormone concentrations are used to evaluate the heat stress status of broilers in both production practices and laboratory experiments.

CONCLUSIONS

Daily variations of thyroid hormones of 61-68 d old AA chickens challenged with three high cyclic temperatures were studied. Blood samples of broilers were collected at 0AM, 4AM, 8AM, 0PM, 4PM and 8PM on five consecutive days. Each thyroid hormone showed similar patterns in different environmental conditions and on different days. T_3, T_4 concentration and T_3/T_4 ratio had two peaks, but the daily variation patterns of thyroid hormones were different between each other. T_3 peaked at 12 AM and 12 PM, while T_4 peaked at 8 AM and 12 PM, with the two peaks of T_3/T_4 ratio showing at 4 AM and 12 AM. The lowest concentrations of both T_3 and T_4 occurred at 4 PM. The circadian rhythms of the thyroid hormones of the broilers under hot conditions had specific patterns. Blood samples should be taken around the time of daily temperature peak to assess the heat stress status of broiler chickens more accurately when thyroid hormones are used as physiological indicator under high temperature conditions.

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Table 2. Serum T_3 and T_4 concentrations, and T_3/T_4 ratio values of market-sized AA broilers subjected to heat challenge at different sampling time in 5 days

Heat Exposure	Time of day	T_3 concentration (nmol/L)			T_4 concentration (nmol/L)			T_3/ T_4 ratio		
		Mean±SD	Max	Min	Mean±SD	Max	Min	Mean±SD	Max	Min
Day 1	0	2.09±0.17	3.44	0.61	22.96±5.45	40.43	4.75	0.094±0.019	0.27	0.03
	4	1.65±0.31	3.67	0.16	16.21±1.56	42.84	8.99	0.101±0.015	0.13	0.01
	8	0.92±0.46	2.78	0.18	20.90±3.39	35.59	1.89	0.043±0.015	0.13	0.01
	12	1.53±0.20	2.14	0.17	20.30±3.24	31.72	8.58	0.076±0.010	0.17	0.02
	16	0.92±0.23	2.40	0.17	15.10±1.55	25.18	1.52	0.061±0.015	0.21	0.01
	20	1.01±0.07	1.87	0.15	19.79±2.30	37.67	10.15	0.052±0.009	0.11	0.01
Day 2	0	1.19±0.39	2.97	0.43	15.86±1.61	44.88	4.69	0.075±0.026	0.18	0.02
	4	1.31±0.12	3.91	0.43	15.72±2.58	31.30	8.21	0.084±0.006	0.14	0.03
	8	1.17±0.07	2.56	0.05	16.22±3.12	25.85	5.77	0.073±0.012	0.14	0.00
	12	1.33±0.48	2.76	0.10	13.82±0.68	27.73	3.34	0.096±0.034	0.17	0.01
	16	0.86±0.02	3.36	0.09	12.42±0.69	37.08	1.92	0.069±0.006	0.26	0.01
	20	0.98±0.13	1.96	0.20	17.29±3.98	34.94	7.13	0.059±0.017	0.15	0.01
Day 3	0	1.65±0.42	2.93	0.06	15.78±2.93	39.18	1.69	0.105±0.021	0.27	0.03
	4									
	8	1.16±0.26	3.39	0.50	16.66±2.39	41.64	4.38	0.070±0.014	0.20	0.02
	12	1.02±0.20	2.06	0.02	13.13±3.03	38.70	3.45	0.079±0.011	0.34	0.01
	16	0.73±0.53	2.37	0.03	11.45±3.75	29.72	1.07	0.057±0.032	0.32	0.00
	20	0.73±0.30	1.82	0.08	13.27±1.60	47.69	3.38	0.057±0.027	0.14	0.01
Day 4	0	1.18±0.23	3.47	0.03	13.81±1.30	36.66	7.74	0.085±0.008	0.22	0.00
	4	1.39±0.52	3.26	0.20	14.70±1.86	43.44	8.25	0.093±0.023	0.26	0.02
	8	0.81±0.10	3.15	0.10	14.41±6.19	37.22	2.52	0.065±0.032	0.13	0.02
	12	1.32±0.70	3.29	0.06	11.04±3.22	23.59	2.68	0.115±0.027	0.33	0.01
	16	0.94±0.19	2.47	0.05	10.87±2.55	29.94	0.72	0.088±0.016	0.22	0.01
	20	0.76±0.28	2.14	0.11	10.58±2.81	31.54	4.20	0.078±0.044	0.20	0.01
Day 5	0	0.95±0.33	2.24	0.01	11.76±3.66	33.03	3.34	0.081±0.011	0.20	0.00
	4	0.85±0.38	2.43	0.03	8.70±3.24.	21.47	2.74	0.097±0.012	0.21	0.01
	8	1.20±0.39	2.18	0.02	13.79±1.68	32.50	0.25	0.086±0.018	0.32	0.00
	12	1.35±0.49	3.24	0.30	14.88±3.18	37.05	0.21	0.089±0.016	0.30	0.05
	16	0.88±0.37	2.01	0.08	10.13±3.57	23.25	1.12	0.087±0.023	0.30	0.02
	20	0.76±0.27	2.79	0.10	9.95±4.06	34.57	4.12	0.078±0.012	0.15	0.02