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ANIMAL SCIENCE
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Differential Incubation Temperature Effects on Growth of Hisex Brown Chick Embryos and Development of Their Visceral Organs

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Abstract—Surveys were carried out in order to study the effects of differential incubation temperatures (37.8, 39.5, 37.5, and 37.0°C on days 1–14, 15–17 for 2 h daily, 18, and 19–20, respectively) on growth of chick embryos of the Hisex Brown cross, development of their visceral organs, and early postnatal development of chicks. Increased (39.5°C on days 15–17 for 2 h) and decreased (37.5°C and 37.0°C on days 18 and 19–20, respectively) incubating temperatures during the test period of chick embryogenesis contributed to increased body mass of embryos and their visceral organs when compared to a constant temperature (37.6°C). Significant changes in the muscular-stomach relative growth rate, represented by an increase at 37.5°C on day 18 and decreases at 39.5 and 37.0°C on days 15–17 and days 19–29, respectively, were found at the differential incubation temperatures compared with the other temperature regime in the previous period. A comparative statistical analysis of the relative growth rates of body length and mass and visceral organ mass of chick embryos at the differential incubation temperatures compared to the constant temperature did not reveal any significant differences ($p > 0.05$). At the differential temperatures compared to the constant temperature regime, pronounced intensive allometric increases in the chick embryo heart and liver masses and their body length and stomach mass were recorded on days 15–17 to day 18 and on days 19–20, respectively. The effects of differential incubation temperatures manifested in increasing the chick embryo's body, heart, muscular stomach, and liver mass, early chick hatching on the 19th day, and increasing the size of body measured from gain in body weight and linear body measurements in early postnatal ontogenesis.

Keywords: chicken embryos, embryogenesis, differential incubation temperature, allometry, heart, liver, muscular stomach

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INTRODUCTION

Embryonic and early postnatal development is considered the most vulnerable phase in the life cycle of domestic poultry Gallus gallus in relation to the effects of artificial incubation temperatures [1]. It is the most important factor of artificial-incubation external environments, having an effect on the hatching eggs and the postnatal development. Taking into consideration that the temperature may vary in a wide range of 30 to 40°C [2] at natural hatching eggs of the domestic poultry Gallus gallus, special attention should be drawn to a probable effect combination of low and high temperatures at artificial incubation. However, the surveys dedicated to studying the effects of differential temperatures on growth and development of chick embryos and their visceral organs with the artificial incubation are insufficiently published [3, 4]. It was previously ascertained that a temperature maintained at 37.8°C on days 1–14, subsequently increased to 39.5°C on days 15–17 for 3 h daily, and decreased to 37.5°C and 37.0°C on day 18 and days 19–20, respectively, can rise the hatch rate and reduce the embryo death rate in chicks [5]. It was proven compared to a constant temperature in artificial incubation.

The objective of the survey is to assess the effects of a differential temperature regime on chick embryo growth, development of viscera, and early postnatal development of chicks of the Hisex Brown cross.

EXPERIMENTAL

The experiments were performed at the Velikie Luki State Agricultural Academia. The incubated eggs of the Hisex Brown laying hens, which were purchased at the *Yuzhny* subsidiary farm, Smolensk oblast, served as the object of the surveys. The age of the parent stock, whose eggs were used for incubation, was 30 weeks. The fertilized eggs were preliminary weighed and sampled by mass in the range of 58 to 60 g before incubation and then put into an ILB-0.5 incubator. The differential temperature regime for egg incubation ($n = 200$; 58.45 ± 4.14 g average egg mass) could maintain 37.8 ± 0.10°C temperature on days 1–14, increas-

ing it to 39.5 ± 0.10°C on days 15–17 for 2 h daily and decreasing to 37.5 ± 0.10°C on day 18 and 37.0 ± 010°C on days 19–20. The relative air humidity comprised 57.0%. In order to perform a comparative analysis, the fertilized-egg artificial incubation of ($n = 200$; 37.0 ± 0.10 g average egg mass) was carried out at 37.6 ± 0.10°C of constant temperature and 55.0% of relative air humidity (control).

The incubated eggs were opened daily from day 4 in compliance with ethical standards during practices with live biological objects. The extracted embryos and their viscera were dried on the filter paper. Morphometric assessment of the embryo's body growth and mass and their viscera (heart, muscular stomach, and liver) were performed from day 4 to day 20 and from day 9 to day 20, respectively. The embryo body length was measured from the scull top to the end of a tail with a digital caliper, Finch Industrial Tools 19856 (Canada Inc.). The chick's body length was measured from the comb to a middle toe. The mass of the embryo's body, certain visceral organs, and chicks were determined with the Sartogosm LV 210-A analytical balances (Russia).

The relative rates of increases in length, body mass, and viscera of chick embryos were estimated with the Brody's formula [6]:

$$K = \frac{(W_t - W_0) \times 100}{(W_t + W_0) \times 0.5}, \quad (1)$$

where W_t is the parameter value at age (t) and W_0 is the initial value for a parameter.

In order to estimate the relative rates of increases in the body length and the heart, muscular stomach, and liver masses proportionate to the embryo's body mass, the formula of simple allometry was used:

$$y = ax^b, \quad (2)$$

where x indicates embryo body mass, g; y indicates body length, cm (mass of an organ, g); a is a constant development for the embryo initial rise; b is allometric and degree coefficient for regression, showing faster ($b > 1$ for positive allometry) or slower ($b < 1$ for negative allometry) growth rates in the embryo length or its organ mass relative to the entire organism (growth is isometric at $b = 1$).

Statistical assessment of data was performed with the Statistica 10.0 software (Statsoft Inc, United States, 2010). In order to compare the analyzed parameters, the one-way ANOVA and the Newman-Keuls a posteriori test were used. A regression coefficient (b), an absolute term (a) in allometric equations, deterministic coefficient (R^2), and significance of relationship between the test traits (PF test) were estimated with the Multiple Regression Analysis.

RESULTS AND DISCUSSION

Despite the type of an incubation regime, significant increases in the body length, body mass, and masses of heart, muscular stomach, and liver in the period from day 15 to day 20 relative to day 4...14 (Table 1; $p = 0.000$) were recorded. It was previously ascertained that high growth rates in the Hisex Brown embryo-body length along with intensive pelvic-limb bones [7] and heart [8] development can be observed at the incubation temperature increased to 38.0°C on days 1 to 3 and the temperature subsequently decreased to 37.6°C on days 4 to 30 of incubation. In addition, positive effects of differential incubation temperatures on increasing the body mass of embryos of the cross Ross 308 broiler chickens were recorded by the other authors [3, 4].

The results of multiple comparison analysis indicate that the increased and decreased temperatures within the differential incubation regime can cause reliable increases in the embryo body mass and length and masses of heart, muscular stomach, and liver at the end of incubation (See Table 1). At the constant temperature, no variation in the body length on days 15...17 and day 18 of incubation ($p = 0.065$) was revealed.

Under the differential temperature effects in the first 20 days of embryogenesis, the chick embryo masses of body, muscular stomach, and liver were significantly higher on days 15–17, 18, and 19–20 and the heart mass was higher on days 18 and 19–20 when compared to the constant temperature (see Table 1). Under the temperature of 37.8°C on days 4(9)–14, the analyzed parameter values were insignificantly higher ($p > 0.05$) than that in the variant with the constant temperature. Under the differential incubation temperature regime on days 15–17, significant increases in the body mass, the muscular stomach mass, and the liver mass in the chick embryos were observed when compared to the control. Thus, the increases comprised 2.72 g ($p = 0.006$), 0.73 g ($p = 0.014$) and 0.071 g ($p = 0.006$), respectively. Under the temperature decrease to 37.5°C on day 18 and to 37.0°C on days 19–20, increases in the masses of body, heart, muscular stomach, and liver were recorded. Thus, the increases comprised 5.58 and 4.53 g ($p = 0.000$), 0.034 and 0.032 g ($p = 0.001$; $p = 0.002$), 0.180 and 0.145 g ($p = 0.000$), and 0.125 and 0.119 g ($p = 0.000$), respectively. In addition, the other authors [9] have proven that the differential incubation temperature regime (37.5°C on days 0–14, 39.5 or 40.7°C on days 15–17 for 3 h daily, 37.5°C on day 18, and 37.0°C on days 19–20) causes the increases in the embryo mass on the 18th day of embryogenesis and the chick mass on the first day of early postnatal onthogenesis.

During embryogenesis under the differential incubation temperatures, significant decreases in the relative growth rates in the chick embryo's body mass and length, muscular stomach mass, and liver mass were

Table 1. Effects of differential and constant temperatures of incubation on absolute values for sizes of chick body and visceral organs measured from gain in body weight and linear body measurements

Temperature	Incubation day	BM, g*	BL, cm	HM, g	MSM, g	LM, g
Differential temperatures						
37.8°C	4...14 (9...14**)	3.099 ± 0.273	3.793 ± 0.155	0.052 ± 0.004	0.106 ± 0.012	0.103 ± 0.011
at 39.5°C for 2 h	15...17	17.288 ± 0.661	7.367 ± 0.091	0.146 ± 0.007	0.491 ± 0.021	0.390 ± 0.018
37.5°C	18	28.638 ± 0.469	8.533 ± 0.037	0.241 ± 0.002	0.970 ± 0.004	0.678 ± 0.006
37.0°C	19...20	34.390 ± 0.446	9.717 ± 0.120	0.297 ± 0.008	1.157 ± 0.023	0.894 ± 0.014
		P value				
$37.8°C_{4(9)...14\ day} \times 39.5°C_{15...17\ day}$		0.000	0.000	0.000	0.000	0.000
$37.8°C_{4(9)...14\ day} \times 37.5°C_{18\ day}$		0.000	0.000	0.000	0.000	0.000
$37.8°C_{4(9)...14\ day} \times 37.0°C_{19...20\ day}$		0.000	0.000	0.000	0.000	0.000
$39.5°C_{15...17\ day} \times 37.5°C_{18\ day}$		0.000	0.013	0.000	0.000	0.000
$39.5°C_{15...17\ day} \times 37.0\ °C_{19...20\ day}$		0.000	0.000	0.000	0.000	0.000
$37.5°C_{18\ day} \times 37.0°C_{\ 19...20\ day}$		0.000	0.011	0.000	0.000	0.000
Constant temperature						
37.6°C	4...14 (9...14**)	2.497 ± 0.220	3.509 ± 0.143	0.042 ± 0.003	0.086 ± 0.010	0.084 ± 0.009
	15...17	14.569 ± 0.403	7.144 ± 0.093	0.127 ± 0.004	0.418 ± 0.014	0.319 ± 0.010
	18	23.057 ± 0.057	7.967 ± 0.237	0.207 ± 0.003	0.790 ± 0.002	0.553 ± 0.001
	19...20	29.857 ± 0.905	9.500 ± 0.108	0.265 ± 0.011	1.012 ± 0.029	0.775 ± 0.025
		P value				
$37.6°C_{4(9)...14\ day} \times 37.6°C_{15...17\ day}$		0.000	0.000	0.000	0.000	0.000
$37.6°C_{4(9)...14\ day} \times 37.6°C_{18\ day}$		0.000	0.000	0.000	0.000	0.000
$37.6°C_{4(9)...14\ day} \times 37.6°C_{19...20\ day}$		0.000	0.000	0.000	0.000	0.000
$37.6°C_{15...17\ day} \times 37.6°C_{18\ day}$		0.000	0.065	0.000	0.000	0.000
$37.6°C_{15...17\ day} \times 37.6°C_{19...20\ day}$		0.000	0.000	0.000	0.000	0.000
$37.6°C_{18\ day} \times 37.6°C_{19...20\ day}$		0.000	0.000	0.000	0.000	0.000
		P value				
Differential temperatures on days $_{4(9)...14\ day}$ × Constant temperature on days$_{4(9)...14\ day}$		0.543	0.535	0.346	0.504	0.456
Differential temperatures on days$_{15...17\ day}$ × Constant temperature on days$_{15...17\ day}$		0.006	0.627	0.068	0.014	0.006
Differential temperatures on days$_{18\ day}$ × Constant temperature on days$_{18\ day}$		0.000	0.216	0.001	0.000	0.000
Differential temperatures on days$_{19...20\ day}$ × Constant temperature on days$_{19...20\ day}$		0.000	0.636	0.002	0.000	0.000

* Here and in the other tables: body mass (BM), body length (BL), heart mass (HM), muscular stomach mass (MSM), and liver mass (LM); ** body mass and length were measured from the fourth day, while muscular stomach and liver masses were measured from the ninth day.

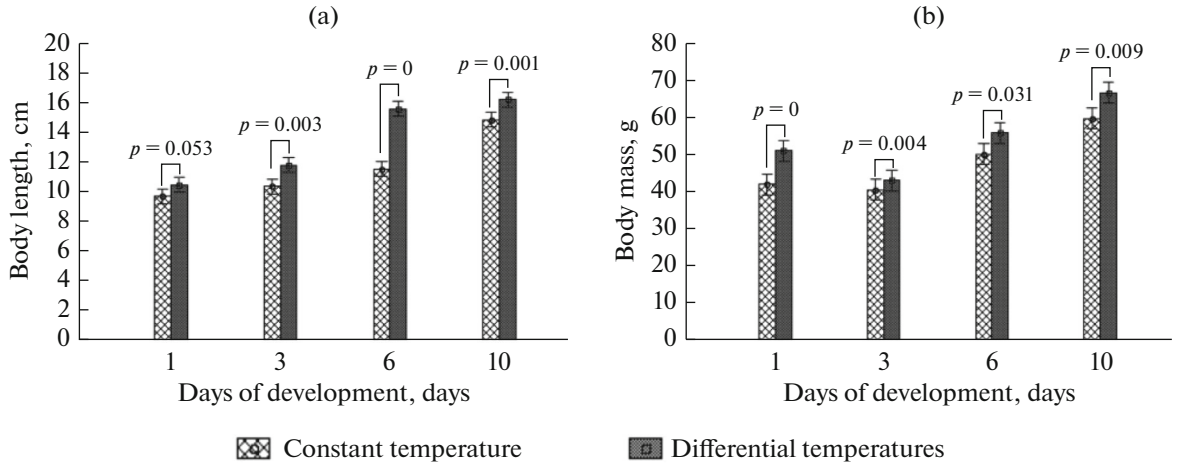

Fig. 1. Morphometric indicators for (a) length and (b) body mass of chicks (n = 10) produced under constant and differential incubation temperatures.

observed. The biggest decline in the relative growth rates in embryo body length and the masses of body, muscular stomach, and liver was recorded on day 18 (Table 2; $p = 0.003$) and on days 19–20 ($p = 0.000$). N.A.M. Elsayed et al. record some reduction in the relative growth rate in chick embryo liver mass and glycogen concentration in it with the temperature increased to 39.5 or 40.7°C on days 15–17 for 3 h daily [9]. The temperature increase from the 15th to 17th day can contribute to elevating the blood serum glucose level and decreasing the death rate at the later stages of embryo development. The authors report that increasing the temperature on days 15–17 contributes to increasing the rates of metabolism and glycogen degradation and releasing a large amount of energy for hatching chickens [9]. N. Leksrisompong et al. indicate that the temperature increase to 39.5°C after 14 days of embryo development can speed up chick hatching, decreasing the relative growth rates in the embryo body mass, heart mass, muscular stomach mass, glandular stomach, and small intestine, when compared to the temperature of 38.2°C [10]. According to the data reported by the other researchers, a temperature decrease to 36.7°C on day 19 of incubation results in increases in the relative growth rates in the chick embryo's liver mass and spleen mass when compared to the high temperature (38.9°C) [3].

The surveys proved that, under the differential temperatures, a significant increase in the muscular stomach relative growth rate at 37.5°C ($p = 0.004$) on day 18 compared to days 15–17 and its decreases at the temperatures of 39.5°C ($p = 0.000$) on days 15–17 compared to days 10...14 and 37.0°C ($p = 0.000$) on days 19–20 compared to day 18 were recorded.

Under the constant temperature of 37.0°C, significant decreases in the relative growth rates of body mass, body length, and liver mass from days 10 to 20 compared to days 5(10)–14 and heart and muscular stomach masses on days 15–17 and 19–20, respectively, when compared to days 10–14 were observed (see Table 2). During days 19–20, significant reduction in the relative growth rates in the chick embryo stomach mass ($p = 0.013$), when compared to the previous day of incubation, was revealed.

No significant variation in the relative growth rates in chick embryo body length, body mass, and visceral organs in relation to the incubation regime was found (see Table 2; $p > 0.05$).

Under both incubation regimes, negative allometry was observed (Table 3). Under the differential temperatures, evident growth rates in the chick embryo heart and liver masses and their body length and muscular stomach mass were recorded on days 15–17 to 18 and on days 19–20, respectively. With respect to the control variant, high allometric coefficients for body length, liver and heart masses, and muscular stomach mass on days 4 to 18, days 19–20, and on days 15 to 18, respectively, were identified. In addition, no data on the effects of incubation temperature regimes on the allometric growth of chick embryos and their organs were found in the observed scientific literature, which indicates the topicality of surveys of this kind.

Temperature modulation during incubation had positive effects on growth and development of chicks in the early postnatal onthogenesis (see Figs. 1a, 1b). Significant increases in their body length on the third, sixth, and tenth days by 1.42 cm ($p = 0.000$), 4.04 cm ($p = 0.000$), and 1.35 cm ($p = 0.000$), respectively, were recorded. In the postnatal period of their development, the body mass increased on the first, third, sixth, and tenth days by 9.06 g ($p = 0.000$), 2.50 g ($p = 0.004$), 5.66 g ($p = 0.031$), and 6.88 g ($p = 0.009$), respectively.

In addition, early hatching of chicks and their higher growth rates under the effects of incubation

Table 2. Effects of differential incubation temperatures on relative rates of increase in size of body components measured from body weight gain and linear body measurements of chick embryos and their visceral organs

Temperature	Incubation day	BM, g*	BL, cm	HM, g	MSM, g	LM, g
Differential temperatures						
37.8°C	5…14 (10…14**)	56.17 ± 2.90	15.74 ± 0.93	35.66 ± 1.63	53.71 ± 2.62	43.46 ± 2.47
at 39.5°C for 2 h	15…17	26.19 ± 2.32	9.22 ± 0.81	20.50 ± 2.41	28.90 ± 1.22	22.55 ± 3.92
37.5°C	18	28.05 ± 1.56	5.64 ± 0.65	26.08 ± 1.44	43.00 ± 1.34	31.31 ± 1.15
37.0°C	19…20	11.55 ± 1.15	9.09 ± 1.29	15.35 ± 1.69	12.66 ± 1.94	16.12 ± 1.79
		P value				
$37.8°C_{5(10)…14\ day} \times 39.5°C_{15…17\ day}$		0.002	0.026	0.001	0.000	0.003
$37.8°C_{5(10)…14\ day} \times 37.5°C_{18\ day}$		0.001	0.003	0.020	0.030	0.054
$37.8°C_{5(10)…14\ day} \times 37.0°C_{19…20\ day}$		0.000	0.061	0.000	0.000	0.000
$39.5°C_{15…17\ day} \times 37.5°C_{18\ day}$		0.837	0.444	0.173	0.004	0.164
$39.5°C_{15…17\ day} \times 37.0\ °C_{19…20\ day}$		0.105	0.964	0.208	0.001	0.306
$37.5°C_{18\ day} \times 37.0°C_{\ 19…20\ day}$		0.310	0.242	0.026	0.000	0.044
Constant temperature						
37.6°C	5…14 (10…14**)	57.48 ± 3.61	16.73 ± 1.14	33.83 ± 2.68	54.80 ± 4.08	45.36 ± 3.06
	15…17	26.52 ± 2.99	9.78 ± 0.83	22.81 ± 1.58	31.48 ± 3.13	24.12 ± 0.36
	18	26.30 ± 0.96	8.96 ± 0.73	28.10 ± 1.63	40.21 ± 0.27	32.11 ± 0.42
	19…20	18.63 ± 1.62	11.06 ± 1.68	19.74 ± 3.25	17.41 ± 1.49	22.92 ± 1.19
		P value				
$37.6°C_{5(10)…14\ day} \times 37.6°C_{15…17\ day}$		0.013	0.038	0.005	0.010	0.000
$37.6°C_{5(10)…14\ day} \times 37.6°C_{18\ day}$		0.034	0.040	0.330	0.067	0.011
$37.6°C_{5(10)…14\ day} \times 37.6°C_{19…20\ day}$		0.010	0.043	0.002	0.000	0.000
$37.6°C_{15…17\ day} \times 37.6°C_{18\ day}$		0.985	0.842	0.394	0.267	0.123
$37.6°C_{15…17\ day} \times 37.6°C_{19…20\ day}$		0.803	0.697	0.531	0.078	0.816
$37.6°C_{18\ day} \times 37.6°C_{19…20\ day}$		0.540	0.637	0.205	0.013	0.179
		P value				
Differential temperatures on days $_{5(10)…14\ day}$ × Constant temperature on days$_{5(10)…14\ day}$		0.796	0.759	0.702	0.867	0.739
Differential temperatures on days$_{15…17\ day}$ × Constant temperature on days$_{15…17\ day}$		0.971	0.862	0.628	0.700	0.959
Differential temperatures on days$_{18\ day}$ × Constant temperature on days$_{18\ day}$		0.911	0.307	0.672	0.670	0.889
Differential temperatures on days$_{19…20\ day}$ × Constant temperature on days$_{19…20\ day}$		0.525	0.929	358	0.468	0.460

* Relative rates of increase in mass of heart, muscular stomach, and liver are estimated from the tenth day, while the relative rates of increase in body are estimated from the fifth day.

Table 3. Allometry of relative rates of increase in length and mass of body and visceral organs at the differential and constant incubation temperatures

Item	Incubation day	Differential temperatures				Constant temperature			
		BL, cm	HM, g	MSM, g	LM, g	BL, cm	HM, g	MSM, g	LM, g
		37.8°C				37.6°C			
b		0.91 ± 0.04	0.98 ± 0.02	0.98 ± 0.03	0.98 ± 0.03	*0.92 ± 0.04**	0.99 ± 0.02	0.98 ± 0.02	0.99 ± 0.02
R^2	4...14	0.829	0.969	0.962	0.964	0.856	0.974	0.869	0.974
$P_{F\ test}$		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		At 39.5°C for 2 h				37.6°C			
b		0.82 ± 0.12	*0.89 ± 0.09*	0.95 ± 0.06	*0.96 ± 0.06*	*0.92 ± 0.08*	0.63 ± 0.16	*0.97 ± 0.05*	0.88 ± 0.09
R^2	15...17	0.664	0.800	0.900	0.914	0.847	0.391	0.934	0.787
$P_{F\ test}$		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		37.5°C				37.6°C			
b		0.57 ± 0.31	*0.99 ± 0.05*	0.26 ± 0.36	*0.27 ± 0.36*	*0.78 ± 0.12*	0.45 ± 0.33	*0.52 ± 0.33*	0.06 ± 0.33
R^2	18	0.327	0.980	0.066	0.071	0.616	0.207	0.270	0.004
$P_{F\ test}$		0.110	0.000	0.503	0.487	0.000	0.217	0.151	0.887
		37.0°C				37.6°C			
b		*0.84 ± 0.13*	0.88 ± 0.12	*0.88 ± 0.11*	0.91 ± 0.11	0.79 ± 0.12	*0.98 ± 0.05*	0.86 ± 0.12	*0.94 ± 0.08*
R^2	19...20	0.705	0.778	0.788	0.821	0.624	0.964	0.749	0.879
$P_{F\ test}$		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

* Values in italics for allometric coefficient of regression (*b*) indicate the intensive growth.

temperature varying at the embryo stage are recorded by other authors [8, 9, 4].

There is a range of arguments for positive effects of differential temperatures on chick embryo development in the current literature. Thus, the effect of temperature of 39.5°C for 12 h from the seventh to 16th days stimulates the development of blood vessels in the chorioallantoic membrane and the hypothalamic-pituitary-thyroid axis, which are involved in development of thermoregulatory mechanisms in the chick embryos [11]. They improve the thermal stability of chicks in the postembryonic period. The effects of high temperatures at 39.5°C for 2 h from the fifth to 12th day increase the expression of angiogenesis related genes and improve embryo thermotolerance during incubation [12]. In the opinion of G.K. Otryganieva, A.F. Otryganieva, and some foreign authors, the temperature increase in the second half of incubation suppresses the embryo development. Therefore, it should be decreased at the end of incubation, especially in the withdrawal period [3, 4, 13]. Low temperatures used on days 18–20 improve chick tolerance to low temperatures in the postembyonic period and decrease the risk factors for ascites [12]. A temporary increase or decrease in the egg incubation temperatures at regular intervals can first contribute to mobilization of the chick embryo's immune system, which plays an important role in developing the mechanisms of adaptation to changing external environments [14]. An adaptation response to variable (differential) temperatures, which is developed through embryogenesis, can improve the hen youngstock's health and their quality when compared to that under the constant incubation temperature regime [5].

CONCLUSIONS

Therefore, the analysis of the survey results allows us to ascertain that differential temperatures through the artificial incubation had significant effects, which were expressed in the higher rates of the chick embryo's development. At the end of incubation, their heart mass, muscular stomach mass, and liver mass were 0.032, 0.145, and 0.119 g higher, respectively. In addition, the differential temperature regime in the period of incubation has a positive effect on growth and development of chicks in the early postnatal onthogenesis. Thus, their body length and mass were 1.35 cm and 6.88 g greater on the tenth day.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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