Hormonal panel of various breeds of cattle under the conditions of temperature stress

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Abstract. The article gives the data on the content of a number of steroid and thyroid hormones in the population of Tagil, Suksun and Holstein cattle under the conditions of increasing environmental temperature resulting in moderate stress reaction, as well as on the changes in endocrine system after temperature stress. Changes in function of vascular glands were found showing high resistance of Tagil cattle, moderate resistance of Suksun cattle and low resistance of Holstein cattle to temperature stress. The most significant changes were noted in thyroid panel of Tagil cattle that was shown by increasing synthesis of iodothyronine while environmental temperature changed from high to normal values (during post-stress period). The breeds with lower resistance had the signs of deterioration of adaption mechanism expressed in stronger unbalance reaction of hormonal axis.

1 Introduction

Nowadays the issue of preservation of aboriginal cattle in the Russian Federation is getting especially important. In 2021 the subprogramme “Improvement of genetic potential of dairy cattle» was included in the Federal science and technology programme of agricultural development during 2017-2025” (Project of Governmental Decree of the Russian Federation prepared by the Agriculture Ministry dated by 20.04.2021).

As a result of selection of dairy cattle in the last century, Holstein breed has become the most dominating and highly productive in the world, compared to other less numerous aboriginal breeds. Artificial selection was aimed at achieving high results in milk yield and body condition scoring. However, it causes the risk of breeding of less surviving animals with low adaptation abilities [1, 2, 3].

High milk productivity of Holstein cattle is connected with lower health quality and reproductive function and immune biological response, as well as with vulnerability to mastitis, and reduction of productive longevity [1, 4, 5]. Productive longevity of animals is the result of complex of physiologically favorable parameters specific for cattle, and search for their genetic determinants is still in process [2, 6]. One of the most important biological mechanisms affecting complete realization of genetic potential of cattle is adaptation to

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high-technology industry, as well as to climatic changes [6, 7, 8]. Climatic changes, like significant increase of environmental temperature in the summer time, cause the development of thermal stress of animals. The animals with bad adaptation to environmental factors (temperature and humidity) are noted to have lower milk yield, poorer milk quality and lower reproduction function [1, 9, 10]. One of the strategies aimed at reduction of negative influence of thermal stress is selection of animals, genetically resistant to climatic changes. However, genetic determinants and biological properties that form this adaptation have yet to be studied [1, 11].

As stress is hormonal body reaction, we have researched interconnection of iodothyronine and a number of hormones of steroid nature among Tagil, Suksun and Holstein cattle, in order to define adaptation potential under the conditions of influence of increased temperatures and during the period of temperature optimum.

2 Materials and methods

The object of research was cattle of Holstein and aboriginal breeds – Tagil and Suksun.
The first stage of research was done under environmental conditions – 26-29^{0}C (Point 1, Extreme Temperature Conditions – ETC), the second stage – 13-18^{0}C (Point 2, Standard Temperature Conditions – STC). For analysis of microclimate, we did temperature, humidity and airspeed measures in livestock buildings. The air temperature was measured with a no-contact infrared thermometer VKTECH GM320 (China), relative air humidity – with a digital hygrometer KKMOON TL-500 (China), airspeed by an anemometer HoldPeak HP-866B (China). Also, we calculated temperature-humidity index (THI) according to the rating scale of thermal stress prepared by Zimbelman, R.B., & Collier, R.J. [12].

By evaluation of conditions of animal welfare for all breeds, the parameters common for moderate thermal stress were registered (air temperature – 26-29 ^{0}C, relative humidity – 78-84%, THI index – 77-79). The parameters were higher than optimum ones stated by Order of Agriculture Ministry dated October, 21,2020, N 622 “About Veterinary Rules of cattle welfare aimed at is reproduction, breeding and realization”.

The research included defining the level of the following hormones: dehydroepiandrosterone (DHEA), cortisol, progesterone, total triiodothyronine (tT3), total thyroxine (tT4) and estradiol. Blood for analysis was collected in evacuated plastic blood tube with anticoagulant lithium-heparin. Samples were taken from *vena caudalis* in the morning time.

Hormonal profiling was done using immunoenzyme method on diagnostical sets «Khema Ltd” (Russian Federation). The evaluation of biochemical indicators was performed on the automatic biochemical and immunoenzyme analyzer ChemWell-2910 Combi (USA).

Mathematical analysis was done with calculations of average number and mean squared error (SD). Statistical analysis was performed by Statistics 10,0. For sample analysis normality of parameter distribution was assessed by the Shapiro-Wilk test, parametric methods by Gauss distribution and non-parametric methods by non-Gauss distribution. All the samples in this research showed non-Gauss distribution, so all the methods of statistical analysis were regarded as non-parametric – the Spearman's Rank Correlation Coefficient (R) and the Wilcoxon rank-sum test (W).

3 Results and discussion

For evaluation of the effect of thermal stress three cattle breeds were studied: Tagil, Suksun and Holstein, that suggested different (both in quality and level) responses to influence of unfavorable conditions of microclimate. Also, for evaluation of adaptation abilities of each breed, the state of their endocrine system during post-stress period (after normalization of temperature regime) was analyzed.

To evaluate population special aspects of influence of thermal stress on key regulation systems of animal body, we conducted hormonal profiling, firstly, of steroid nature, because they are very sensible to extreme conditions. According to literature reports, under extreme conditions the level of hormones of glucocorticoid function should increase, whereas synthesis of reproductive hormones can be supressed.

The main regulator of metabolism is thyroid gland, and its level of hormones is also changing under the conditions of thermal stress. Hormones of thyroid function were studied in order to evaluate total level of metabolism, including thermotaxic component, namely, iodothyronines: prohormone – total thyroxine (tT4) and all forms of triiodothyronines (tT3).

Endocrine system reacts to any changes inside, outside the body and it regulates homeostatic mechanisms, and get them adjusted for optimum existence, providing survival of both separate cells and body as a whole. It is worth noting that the more resistant some definite organism is for various effects, the more resistant is endocrine system, as there is no urgent need to stimulate some metabolic or physiological mechanisms, providing existence under changing conditions. At the same time, any adaptation mechanism is not unlimited and can deteriorate affected by long harmful influence.

For evaluation of glucocorticoid function under stress, we did the research on the main representative of these hormones for cattle – cortisol, which, by changing metabolism of glucose, amino acids, proteins and fats, provides metabolic effects for organism to react to stress.

Analysis of hormonal steroid system was done according to the data of main reproduction hormones and by-products of synthesis of steroid hormones – estradiol and some of its initials (and dehydroepiandrosterone (DHEA). It is worth noting that progesteron is an independent regulatory molecule, as well as a progenitor of cortisol and aldosterone.

Evaluation of thyroid hormones was done both for total state of metabolism and thermotaxic function. It is known that growing level of iodothyronines increase thermogenesis in mammals. Probably, there is feedback, whereas the increase of environmental temperature can reduce the synthesis of thyroid hormones, that can result in negative effect on stress-resistance and reduce level of main metabolic processes, and, consequently, influence reproduction function of cows.

The research on population of cows of Tagil breed, under two different temperature conditions, showed that by normalization of microclimatic parameters, there was a tendency to insignificant decrease of cortisol level by 4.8%, increase of progesterone level by 37%, estradiole – by 9.6%. At the same time there was verified growth of thyroxine by 13.7% (Table 1).

Table 1. Changes in endocrine status of cows of Tagil breed during the thermal stress

Tagil	DHEA, ug/ml	Cortisol, nmol/l	Progesterone, nmol/l	tT3, nmol/l	tT4, nmol/l	Estradiole, pg/ml
Point 1 (ETC) (n=55)	0.1	5.1	4.1	2.5	128.2	43.1
SD (1)	0.0	15.7	4.8	0.8	10.7	15.1
Point 2 (STC) (n=55)	0.1	4.9	5.6	2.5	145.8	47.2
SD (2)	0.1	15.2	5.8	0.8	11.4	30.1
STC / ETC, ±%	0.0	-4.8	**37.0**	-1.7	**13.7**	9.6
W	1.000	0.893	0.470	0.917	0.002	0.594

In general, among the cows of Tagil breed, no significant changes in their endocrine status by normalization of temperature regime were found, except of predicted increase of thyroxine level that means high stress-resistance of that breed, at least to thermal stress.

Population evaluation of changes in endocrine status of cows of Suksun breed by changes of temperature conditions from extreme to standard ones showed tendency of decrease of progesterone level by 14.9% and tT3 by 15.9%, whereas there are separate tendencies, maximum close to verified ones, to decrease of DHEA level by 22.2% and estradiole level by 28.8%. At the same time there was verified growth of tT4 level by 13.5% (Table 2).

Table 2. Population evaluation of changes in endocrine status of the cows of Suksun breed during the thermal stress

Suksun	DHEA, ug/ml	Cortisol, nmol/l	Progesterone, nmol/l	tT3, nmol/l	tT4, nmol/l	Estradiole, pg/ml
Point 1 (ETC) (n=50)	0.1	4.2	3.7	2.9	128.1	51.9
SD (1)	0.1	15.6	2.9	1.0	25.5	27.5
Point 2 (STC) (n=50)	0.1	4.3	3.1	2.5	145.5	37.0
SD (2)	0.0	16.0	2.6	0.6	9.1	13.9
STC / ETC, ±%	**-22.2**	2.9	**-14.9**	**-15.9**	**13.5**	**-28.8**
W	0.068	1.000	0.807	0.196	0.026	0.064

Among the cows of Suksun breed there was a tendency and verified changes in levels of steroid and thyroid hormones, with more significant non-balance of hormones of steroid nature, which might mean less expressed resistance to temperature effects. At the same time the level of changes in thyroid system was practically identical to the one of the cows of Tagil breed.

Changes in hormonal status in the population of cows of Holstein breed as response to changing temperature conditions were very notable, namely, there was a tendency to decrease of cortisole level by 17.5% and total triiodothyronine by 19.1%, by verified decrease of : DHEA level – by 88%, progesterone level – by 88.7%, estradiole level – by 73.8% and total thyroxine level – by 34.7% (Table 3).

Table 3. Population evaluation of changes in endocrine status of the cows of Holstein breed during the thermal stress

Holstein	**DHEA, ug/ml**	**Cortisol, nmol/l**	**Progesterone, nmol/l**	**tT3, nmol/l**	**tT4, nmol/l**	**Estradiole, pg/ml**
Point 1 (ETC) (n=40)	0.8	49.1	17.4	5.9	156.1	161.7
SD(1)	1.2	47.9	13.5	1.8	22.9	73.2
Point 2 (STC) (n=40)	0.1	40.6	2.0	4.8	101.9	42.4
SD (2)	0.1	24.4	0.6	1.3	15.3	29.1
STC / ETC, ±%	-88.0	-17.5	-88.7	-19.1	-34.7	-73.8
W	0.04	0.88	0.03	0.97	0.01	0.01

This hormonal reaction defines high sensibility of Holstein cattle to stress-factors influence. It is proved by increased secretion of hormones during the thermal stress and significant decrease during the post-stress period.

Evaluation of endocrine system of cattle populations of three breeds (Tagil, Suksun, Holstein) proved the fact that registered parameters of environment in Point 1 (t – +26+29 ^{0}C, humidity – 78-84%, index THI – 77-79), can be considered to be the conditions of thermal stress, that was also proved by the data of animals' clinical research (increased body temperature and acceleration).

For more detailed analysis of animals' resistance to thermal stress, we did intrapopulation research on reaction of hormonal systems depending on adaptation potential (high, low), based on the criteria – metabolic markers, which were stated in previous research.

By analysis of specific features of hormonal status of cows of Tagil breed with "high" adaptation potential during changing temperature regime from extreme to standard, there was a tendency to increased level of main gestagenic hormones, whereas the changes didn't affect other researched steroid hormones. At the same time increased level of total iodothyronines with verified increase of level of total thyroxine was registered. That increase is connected with decrease of harmful influence of high temperatures on thyroid system, that resulted in activation of steroidogenesis and, consequently, in growth of synthesis of gestagens (Table 4).

Also, correlation analysis under extreme temperature conditions showed positive correlation between cortisole and estradiole (0.8> R <0.9) and negative correlation between tT4 and estradiole (0.8> R <0.9). At the same time, after normalization of temperature regime correlation dependence of those animals changed, that was expressed in high positive correlation between tT3 and tT4 (0.8> R <0.9).

Table 4. Evaluation of hormonal status of the cows of Tagil breed with high adaptation potential under different temperature conditions

Tagil	DHEA, ug/ml	Cortisol, nmol/l	Progesterone, nmol/l	tT3, nmol/l	tT4, nmol/l	Estradiole, pg/ml
Point 1 (ETC) (n=35)	0.1	Less than 10.0	6.7	2.5	133.9	46.9
SD (1)	0.0	22.0	5.8	0.9	7.8	17.6
Point 2 (STC) (n=35)	0.1	Less than 10.0	7.1	2.6	150.8	58.9
SD (2)	0.1	21.2	7.5	0.9	8.2	36.9
STC / ETC ±%	12.5	-0.1	6.0	5.1	12.6	25.4
W	0.655	1.000	0.866	0.735	0.018	0.866

Among the cows of Tagil breed with "low" adaptation status by similar changes in temperature regime there was a tendency to decrease of estrogens and interjacent reproductive hormones with increase of progesterone; verified increase of prohormone – total thyroxine with decrease of level of total triiodothyronine (Table 5). These changes indicate disfunction of steroidogenesis, namely, the decrease of synthesis of estradiole from its progenitors, that is probably caused by decreased transformation of thyroxine prohormone into active form of triiodothyronine. The interconnection between tT3 level and estradiole is proved by correlation analysis with high positive interconnection by both temperature regimes (extreme temperature conditions (ETC): tT3 and estradiole (0.8> R <0.9), standard temperature conditions (STC) (0.7> R <0.8).

Table 5. Evaluation of hormonal status of the cows of Tagil breed with low adaptation potential under different temperature conditions

Tagil	DHEA, ug/ml	Cortisol, nmol/l	Progesterone, nmol/l	tT3, nmol/l	tT4, nmol/l	Estradiole, pg/ml
Point 1 (ETC) (n=20)	0.09	Less than 10.0	1.5	2.6	122.4	39.3
SD (1)	0.04	1.2	0.6	0.9	10.4	12.2
Point 2 (STC) (n=20)	0.07	Less than 10.0	4.1	2.3	140.7	35.6
SD (2)	0.05	0.0	3.4	0.6	12.5	17.1
STC / ETC, ±%	-16.67	-100.0	176.9	-8.4	15.0	-9.4
W	0.593	1.000	0.128	0.600	0.046	0.499

By analysis of specific features of hormonal status of the cows of Suksun breed with "high" adaptation status during changing temperature regime from extreme to standard

there was a tendency to decrease in formation of all steroid hormones that resulted in the decreased concentration of both gestagens and glucocorticoids. At the same time there was decreased transformation of thyroxine into triiodothyronine (Table 6). Also, high positive correlation between level of tT3, tT4 and DHEA was noted (0.8> R <0.9).

Table 6. Evaluation of hormonal status of the cows of Suksun breed with high adaptation potential under different temperature conditions

Suksun	DHEA, ug/ml	Cortisol, nmol/l	Progesterone, nmol/l	tT3, nmol/l	tT4, nmol/l	Estradiole, pg/ml
Point 1 (ETC) (n=31)	0.2	Less than 10.0	4.4	3.1	140.5	62.7
SD (1)	0.1	22.0	2.6	1.1	16.9	36.8
Point 2 (STC) (n=31)	0.1	Less than 10.0	3.1	2.3	149.0	39.2
SD (2)	0.1	22.0	2.6	1.1	16.9	36.8
STC / ETC, ±%	-36.4	-100.0	-29.5	-23.8	6.1	-37.5
W	0.068	1.000	0.612	0.173	0.237	0.128

Among the cows of Suksun breed with “low” adaptation potential by identical changes of temperature regime there was decrease of estradiole level and decrease of total thyroxine level by 22.6% (Table 7). At the same time in different temperature regimes there were positive correlation dependencies, different from each other, namely, by thermal stress there was dependency between progesterone and tT4 (0.8> R <0.9), by normalization of temperature conditions – between estradiole and tT3 (0.8> R <0.9).

Table 7. Evaluation of hormonal status of the cows of Suksun breed with low adaptation potential under different temperature conditions

Suksun	DHEA, ug/ml	Cortisol, nmol/l	Progesterone, nmol/l	tT3, nmol/l	tT4, nmol/l	Estradiole, pg/ml
Point 1 (ETC) (n=19)	0.1	Less than 10.0	2.9	2.8	115.8	41.2
SD (1)	0.0	0.0	3.2	0.9	27.6	4.2
Point 2 (STC) (n=19)	0.1	Less than 10.0	3.1	2.6	141.9	34.8
SD (2)	0.0	0.0	2.5	0.6	9.0	14.6
STC / ETC, ±%	0.0	0.0	7.4	-7.2	22.6	-15.4
W	1.000	1.000	0.753	0.735	0.063	0.499

In general, the above-mentioned data on realization of mechanisms of hormonal regulation under the conditions of thermal stress among the cows of Suksun breed indicates the deterioration of estrogenic steroid hormones, independently of adaptation group of animals. Other changes may indicate tension of endocrine function, but their specific features in connection with division into groups with “high” and “low” adaptation potential requires detailed research.

Table 8. Evaluation of hormonal status of the cows with high adaptation potential under different temperature condition (Holstein)

(Holstein)	**DHEA, ug/ml**	**Cortisol, nmol/l**	**Progesterone, nmol/l**	**tT3, nmol/l**	**tT4, nmol/l**	**Estradiole, pg/ml**
Point 1 (ETC) (n=25)	1.0	43.0	21.2	6.1	157.7	157.3
SD (1)	1.5	33.4	16.6	2.1	27.8	92.6
Point 2 (STC) (n=25)	0.1	31.5	2.2	3.9	99.6	50.9
SD (2)	0.2	21.0	0.8	0.7	19.9	39.2
STC / ETC, ±%	-88.0	-26.8	-89.7	-36.0	-36.9	-67.6
W	0.046	0.249	0.046	0.046	0.221	0.028

Among Holstein breed with both “high” and “low” adaptation status by changing temperature regime there was significant decrease of content of all hormones during shifting from thermal stress to standard temperature regime (Table 8, 9). The stated changes indicate longer disfunction of adaptation system during the post-stress period.

Table 9. Evaluation of hormonal status of the cows with low adaptation potential under different temperature condition (Holstein)

(Holstein)	**DHEA, ug/ml**	**Cortisol, nmol/l**	**Progesterone, nmol/l**	**tT3, nmol/l**	**tT4, nmol/l**	**Estradiole, pg/ml**
Point 1 (ETC) (n=15)	0.7	84.1	9.8	6.6	158.3	184.6
SD (1)	0.6	70.3	9.6	1.6	18.8	45.5
Point 2 (STC) (n=15)	0.1	49.6	1.8	5.7	104.2	33.9
SD (2)	0.1	26.0	0.4	1.2	10.3	12.2
STC / ETC, ±%	-90.8	-41.1	-82.1	-14.0	-34.2	-81.7
W	0.028	0.600	0.046	0.753	0.028	0.028

It appears that the representatives of this breed, independently of their adaptation potential, have low resistance to thermal stress.

4 Conclusion

According to population evaluation of resistance of various breeds to thermal stress, we can conclude that the Tagil breed has high resistance, the Suksun breed – average resistance, and the Holstein breed – low resistance.

The level of hormones of thyroid function had most significant and predicted changes among the cows of Tagil breed, that was expressed in increase of synthesis of iodothyronines by changing environmental temperature from high to normal. Also, there was reaction of reproductive hormones showing a tendency to growth, that, besides, showed the normalization of processes of steroidogenesis. Less resistant breeds showed signs of deterioration of adaptation mechanisms that was proved by stronger non-balance reaction of hormonal system.

At the same time, intrapopulation analysis, taking into account division of animals, according to metabolic criteria, into groups with low and high adaptation potential, showed specific changes of hormonal regulation among the cows of Tagil breed, controversial reactions of endocrine system among the cows of Suksun breed and significant non-balance in changing hormone level among the cows of Holstein breed, independently of the adaptation group.

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