



Studies of endocrine changes and morphological blood composition in gilts affecting puberty

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Abstract

Background: The puberty period in pigs depends on the functional activity of the endocrine system, which has an effect on the blood through the connective tissue. It has been established that during puberty period, the steroidal and thyroid hormones regulate the processes of metabolism and proliferation, differentiation and apoptosis of cells. The aim of the research was to find out the effects of the morphological composition of blood under the influence of steroidal and thyroid hormones during the process of puberty in different directions of productivity in the pigs.

Methods: Experiments were performed on two groups of clinically healthy piglets from Pietrain and Large White (LW) breed. To assess the steroidal and thyroid hormone levels, the morphological blood was collected from the anterior hollow vein at the puberty ages of 120, 150, 180 and 210 days, when their live weights achieved to 100 kg.

Results: The predominance of hemoglobin content and the amount of erythrocytes in the blood were observed higher ($p < 0,05$) in Pietrain piglets than LW piglets on the 120 and 150 days of puberty development, in contrast to the general decrease in leukocytes in piglets of both breeds at the age of 150 days with the next gradual increase. The age-related changes in the leukocyte formula were directly related to the direction of animal productivity. In the LW breed, from the beginning of the experiment, there was an increase in the number of rodenuclear neutrophils at 210 days of puberty, followed by a sharp decreasing on 36.4%. In Pietrain breed, the level of the rodenuclear neutrophils decreased on 28.6% ($p < 0.01$) at the age of 150 days, followed by a sharp increasing over the coming months. From the puberty ages of 150 days to the 210 days, the concentration of estradiol decreased in the first genotype: two times ($p < 0.05$); in the second genotype: 1,9 times ($p < 0,01$). The content of thyroxine and triiodothyronine increased in the blood serum of Pietrain ($p < 0.01$) and LW ($p < 0.001$) piglets at the puberty ages of 120 to 180 days. Study found that the amount of progesterone in LW piglets was higher relatively to Pietrain piglets in the all investigated periods. The content of estradiol in piglets of both breeds from the 120 and 150 days of puberty tended to decrease by 2.8 times ($p < 0.001$) in LW piglets, and 1.4 times ($p < 0.05$) in Pietrain piglets, and the testosterone level increases, 2.1 ($p < 0,05$) and 1,9 ($p < 0,05$) times respectively. The significant influence of progesterone on the number of lymphocytes and common leukocytes in Pietrain piglets was established. The level of estradiol significantly influenced the number of granulocytes in Pietrain piglets at the age of 150 days ($r = 0.92$ and 0.99), and in LW piglets, the maximum correlation level was observed at the puberty age of 150 days ($r = 0,61$) and 180 days ($r = 0.94$).

Keywords: pigs; progesterone; estradiol; testosterone; hematopoiesis.

introduction

The puberty period in pigs depends on the functional activity of the endocrine system, which has an effect on the blood serum through the connective tissue [1–2]. It was established that steroid and thyroid hormones regulate the anabolic and catabolic mechanisms of metabolism, the processes of proliferation, differentiation and apoptosis of cells. However, the role of these substances in regulation of hemopoiesis in the postnatal ani-

mals development was not fully discovered. Thyroxine and triiodothyronine are also functioning in cells of the hemopoiesis system, however, the age-related peculiarities of the conversion process and functional features of these substances in the bone marrow cells and animals blood are not fully investigated. The mechanisms of progesterone and estrogen, androgens influence on erythro- and leukopoiesis processes still remain under-researched and challenge to researchers [3–5].

The main role in regulation of erythropoiesis and leukopoiesis belongs to hormones, which take part in the paracrine and autocrine mechanisms of metabolism regulation in cells, and identify its ability to poliferation, differentiation, apoptosis, adhesion, migration [6]. Changes in the hemopoietic system which pigs have during the perinatal development and the reconstruction of regulatory mechanisms of hemopoiesis in the transition to the postnatal period of life, plays a leading role in determination of the functional activity of blood cells and the immune system, not only right after the birth but during the subsequent age-related periods of ontogenesis. Research studies [7–8] showed certain modifications of the hematopoietic and lymphoid organs in the process of embryonic and post-embryonic development. Meanwhile, the functioning peculiarities of hematopoietic organs in the process of puberty in different directions of productivity in the pigs remain uninvestigated.

Materials and methods

An experiment was performed on clinically healthy piglets from LW and Pietrain breed. For each breed five piglets were investigated. The experimental design was comprised of two groups: first group represented the piglets from Pietrain breed (n=5), second group represented the piglets from LW breed (n=5). Feeding of piglets were performed accordingly to the feed regulations of the Institute of Pig Breeding and Agro-industrial production of National Academy of Agrarian Sciences, Poltava, Ukraine. To assess the hormonal and morphological status, blood samples for research were taken from the piglets anterior hollow vein at the puberty ages

of 120, 150, 180 and 210 days (when their live weight reaches 100 kg). The levels of testosterone, progesterone, estradiol, thyroxine and triiodothyronine in the blood serum were measured by electro-chemiluminescence immunoassay method «ECLIA» on the analytic analyzer Elecsys 2010 system (RocheDiagnosticsGMBH, Germany). The amount of erythrocytes and leukocytes were measured with the help of microscope in Goryaev's camera with grid by Pyatnitskii method, leukogram and smears, by Romanowsky–Giemsa staining. The level of hemoglobin was measured by a hemoglobin cyanide method in a photoelectric colorimetric way. Erythrocyte sedimentation rate was measured by Panchenkov's method [9–14]. The received digital data were statistically processed with the help of STATISTIKA program for WindowsXP. Student's T-criterion was used for the comparison of researched indicators and their inter-group variances, and the result was considered to be significant at the levels of $p < 0,05$, $p < 0,01$, and $p < 0,001$, respectively.

results and discussions

Experimental data showed that the amount of erythrocytes and hemoglobin in piglets were decreasing at the puberty ages of 120, 150, 180 and 210 days. Meanwhile, the content of hemoglobin in LWbreed was lower relatively to Pietrain breed, where the maximum difference between breeds was ascertained at the 120th day – 16% ($p < 0,05$) (Table 1).

Table 1. Measurements of morphological and hormonal blood parameters in Pietrain piglets during different stages of puberty

Hematological value	Ages of puberty (in days)			
	120	150	180	210
Hemoglobin, g/dL	115,64±1,87	110,71±1,35*	95,92±1,08	87,63±2,07
Erythrocytes ,c/l	8,82±0,17	8,67±0,35*	6,82±0,11	6,12±0,34
Leukocytes, g/l	12,74±1,99	10,16±1,15	10,24±1,54	11,96±2,18

Hematological value	Ages of puberty (in days)			
	120	150	180	210
Eosinophils,%	2,4±0,89	2,6±0,89	2,2±0,83	2,8±0,84
Bands,%	0,8	0,8	0,6	1,2
Stab ,%	2,2±0,44	2,6±0,89	3,0±1,0	2,2±0,84
Segmented,%	44,0±2,24	43,2±3,03	45,6±3,21	45,0±4,30
Lymphocytes,%	47,8±4,6	46,4±1,82	43,0±4,64	45,4±4,72
Monocytes,%	2,6±1,14	3,8±1,79	5,0±0,71	2,6±1,52
ESR,mm	3,6±1,51	4,6±2,70	3,8±1,92	3,2±1,30
progesterone, nmol/l	14,96±2,53	10,89±2,44	12,63±2,01	15,92±1,56
Estradiol, nmol/l	19,12±1,56	13,51±1,74	8,11±1,11	9,56±1,15
Testosterone ,nmol/l	0,047±0,004	0,092±0,0063*	0,070±0,006	0,048±0,002*
Thyroxine, nmol/l	45,97±3,61	75,21±5,45	90,12±9,11***	66,18±4,48
Triiodothyronine, pmol/l	2,65±0,11	2,45±0,62	3,03±0,07	1,39±0,12

Significant at: *-p <0.05, **-p <0.01, ***- p <0.001 compared to the 120th day of development.

Table 2. Measurements of morphological and hormonal blood parameters in Large White piglets during different stages of puberty.

Hematological value	Ages of puberty (in days)			
	120	150	180	210
Hemoglobin, g/dL	99,7±5,29	94,8±3,13	84,5±3,92	78,7±5,12
Erythrocytes, c/l	7,81±0,32	6,35±0,37	6,81±0,56	6,23±0,17
Leukocytes, g/l	11,16±1,66	9,56±0,63	11,96±0,88	13,91±2,40
Eosinophils,%	2,4±1,14	2,0±0,70	1,8±1,30	3,0±1,0
Bands,%	0,6	1,2	0,4	0,6
Stab ,%	2,8±0,84	2,0±0,70	2,4±1,14	2,6±0,89
Segmented,%	44,0±2,65	41,2±1,92	44,6±3,44	46,4±4,67
Lymphocytes,%	46,6±4,51	49,8±3,34	45,8±3,70	42,6±7,83

Hematological value	Ages of puberty (in days)			
	120	150	180	210
Monocytes, %	3,2±1,0	3,2±1,79	4,2±1,48	4,2±1,92
ESR, mm	4,4±2,30	5,0±1,58	5,2±1,48	4,4±2,32
Progesterone, nmol/l	12,21±1,30	16,37±1,85	15,12±3,24	27,26±2,01***
Estradiol, nmol/l	26,63±1,58	9,47±1,28***	24,66±3,17	11,33±3,03
Testosterone, nmol/l	0,036±0,006	0,077±0,012*	0,037±0,005	0,039±0,003**
Thyroxine, nmol/l	56,04±4,10	93,56±7,88	131,15±13,24**	103,35±9,48
Triiodothyronine, pmol/l	3,01±0,13	3,35±0,30	3,08±0,27	2,35±0,11

Significant at: *-p < 0.05, **-p < 0.01, ***- p < 0.001 compared to the 120th day of development.

Comparing the hematological blood indicators of both animals groups it was noted, that erythrocytes number in blood of Pietrain breed of pigs at the 120 days age was higher to 17% ($p < 0,05$) over LW breed, relatively [15]. The research results of leukocytes amount in piglets blood during the 150 days of puberty, testify of their significant decrease in Pietrain's breed from the 120th day to 20,3% and in LW breed on 14,3%. It was found that from the 180th to the 210th day the content of leukocytes in this tissue of the first genotype pigs was almost constant. The concentration of leukocytes for LW breed peers had a similar dynamics, increasing the related indicators of Pietrain breed on the 180th day on 14,4%, the 210th day on 14,0%.

The leukocyte formula had its separate specificities [1]. Accordingly, in Pietrain breed, during the 150 and 180 days of puberty, there was an increase in the number of stab neutrophils, followed by a sharp decrease to the 210th day on 36,4%, segmented neutrophils didn't have any significant changes at the same time. In LW breed of pigs on the 150th day the level of stab and segmented neutrophils decreased on 28,6% ($p < 0,01$) and 6,4%, with the next increase to the 210th day on 30,0 and 12,6%. Breeding factor had a significant impact on the lymphocytes amount dynamics of the investigated breeds. Therefore, in Pietrains breed pigs

their content was gradually decreasing from the 120 days to the 180 days of a postnatal development, with the low increase on the 210th day. At the same time, maximum lymphocytes amount for animals of LW breed was at the 150 days of puberty with the following sharp decrease on 14,5% upon attaining the 210 days of puberty. The sharp increase of monocytes amount in blood of Pietrain breed animals almost by two times at the age of 180 days, whereas, for the LW breed only by 1,3 times at the age of 120 days was established.

Indicators of the erythrocyte sedimentation rate at the first genotype had maximum value in the 120 days of puberty, and at second genotype at the age of 150 days of puberty, with the excess among LW breed representatives, where the maximum difference between breeds was identified on the 180 days of puberty and was 26,9%. The obtained findings of the morphological blood composition of different breeds of pigs during the research period were dynamically changing, that was obviously caused by physiological changes at different periods of their puberty [16]. Such hematological profile changes in intensively growing animals, are obviously caused by variations of other biologically active substances, especially hormones. Variations of the hormones level can be accompanied by changes of erythrocytes properties and leukocytes activity [3–4, 6].

During our research reports it was established that the level of hormones in pigs blood serum in their puberty period for different breeds had distinctive numerical ranges. The progesterone content in Pietrain breed pigs had minor fluctuations from 120, 150, 180 and 210 days of puberty, reaching its maximum at the 210th day. However, animals of LW breed had an identical pattern to the 180 days of development, followed by next sharp increase of concentration on almost 80% ($p < 0,001$). The level of estradiol in researched breeds of animals in the 120-days-old age was the biggest. Sharp following decrease of this metabolite by almost 3 times ($p < 0,001$), was noted in LW breed on the 150th day, and when animals live weight reached 100kg (180th day) its level was promptly increasing. Similar variations, but of a minor range, was noted in pigs of Pietrain breed.

The concentration of testosterone in blood serum for both researched groups of animals from the 120th to 150th days promptly increased by two times ($p < 0,05$). Subsequently, its level in LW breed promptly decreased to the indicators of the initial research period. The amount of this hormone in pigs of Pietrain breed was gradually decreasing at the same period. Maximum difference in the indicators between breeds of this hormone was established on the 180th day – 52% ($p < 0,05$).

Determination of thyroxine content in blood serum of researched animals showed a significant difference in kinetics of these indicators in animals of different breeds. LW breed pigs had an increase of this hormone in a time period from 120 days to 180 days of puberty by 2,3 times ($p < 0,01$) with the following decrease. A relevant tendency in increasing of thyroxine concentration during 120–180 days of puberty was in Pietrain piglets by almost 2 times ($p < 0,001$) with the following decrease. It should be noted, that the level of thyroxine in blood of animals of both breeds was increasing with age, however, pigs of LW breed had much higher indicators. Maximum difference in breeds between levels of this hormone was established on the 180th day – 45% ($p < 0,05$) in favor of LW breed of pigs. Maximum concentration of thyroxine in blood serum in LW breed of pigs was established in the 180 days of puberty, with the following decrease on 21% till the end of the 210 days of puberty of a postnatal development.

It was established, that a triiodothyronine concentration in blood serum in both groups of researched pigs was significantly lower than thyroxine. In animals of LW breed its content was 2,35–3,01 nmol/L, in pigs of Pietrain breed 1,39–2,65 nmol/L. In the physiological process of animals development the triiodothyronine dynamic was close to established to thyroxine level. Maximum triiodothyronine concentration in pigs of LW breed was noted on the 150-day-old age. In pigs of Pietrain breed, maximum amount was noted at the 180th day of development. Moreover, it may be concluded, that thyroxine has a deposited function, since it exists in the organism in a substantially greater amount [17]. Although the T_4 concentration was ten times higher than T_3 , the content dynamics of the first is similar to the second, it demonstrates that triiodothyronine is an active form, and thyroxine is spare, therefore, the fluctuations of estrogens

level in pigs blood serum during the puberty period primarily affect T_3 level [17–18].

Research results of the hormones influence on the blood cells condition that were obtained on different species of animals, demonstrate the dependence of erythrocytes activity and separate groups of leukocytes on the amount of steroids during the constant estrus cycle and pregnancy [19–20]. To determine the influence of the hormonal background changes on the morphological blood composition in pigs during the establishment of sexual function the correlation linkages were calculated. Therefore, the content of progesterone in animals of Pietrain breed at the 120-days-old age significantly influenced the number of lymphocytes ($r = 0,78$), and in a period from the 150th to 180th day on the total number of leukocytes ($r = 0,60$). This hormone in pigs of LW breed more closely correlated with the content of leukocytes ($r = 0,73$) in the relevant age period. And in the age of 150-days-old an increase of progesterone significantly influenced a decrease of stab neutrophils ($r = 0,51$), which is confirmed by the research studies Shilpa Nandi and Reshma Rani [21]. Progesterone in representatives of Pietrain breed at the 210-age-old was closely correlated with the content of hemoglobin ($r = 0,76$) and the erythrocyte sedimentation rate ($r = 0,62$), strength of these linkages was higher in pigs of LW breed $r = 0,95$ and $r = 0,68$.

Our research shows the improving of a linkage of the estrogen concentration with the increase of granulocytes subpopulations (eosinophils, stab) in pigs of Pietrain breed at the age of 150 days and is $r = 0,99$, relatively. During the further development of animals, significant linkages are being established by segmented neutrophils ($r = 0,92$). Meanwhile, the sharp increase of estradiol level in blood serum in LW breed animals in a period from the 150 and 180 days of puberty, significantly influenced an immature stab neutrophils increase ($r = 0,94$), which confirms the influence of the female sex hormones on the level of a nonspecific resistance of females bodies [22].

It was established, that a linkage between testosterone content and morphological blood composition in females bodies, under the influence of progesterone and estradiol during the puberty process, was dependent

on the productivity direction of researched breeds. Therefore, an increase of a testosterone concentration in LW breed up to the 210 days of puberty of a postnatal development, significantly influenced the number of hemoglobin ($r = 0,91$ and $0,74$), and in Pietrain breed similar strong linkages between testosterone and hemoglobin were established during the maximum weight gain, caused by a genotype factor, at the age of 150-180 days of puberty and was $r = 0,53$ and $0,90$. The obtained results shows that the components which form a hematological homeostasis are connected to each other, and the level of their connection significantly depends on steroid and thyroid hormones during the puberty period and productivity direction. The defined dependence of values of coefficients of the correlation on age and physiological condition demonstrates an important role of researched hematological components in homeostasis formation in pigs at certain stages of a postnatal development.

conclusions

1. The predominance of a hemoglobin content and the number of erythrocytes in piglets blood of Pietrain breed were 16% on the 120th day and 17% on the 150th day of development ($p < 0,05$). The leukocytes number in pigs of both breeds decreases on the 150th day with the next gradual increasing. The values of this indicator in LW breed were higher on the 180th day – 14,4%, and on the 210th day – 14%.

2. The age-related changes in the leukocyte formula were related to the breed affiliation of pigs. In LW breed from the 120th day there was an increase in the number of stab neutrophils, followed by a sharp decreasing from 210-day-olds on 36,4%. In Pietrain breed, the level of stab neutrophils decreased on 28,6% ($p = 0,01$) on the 150th day, followed by next increasing to the 210th day on 30,0 and 12,6%. Maximum number of leukocytes in Pietrain breed was established on the 120th day, in LW breed on the 150th day of a postnatal development.

3. Study found that the amount of progesterone in pigs of LW breed was higher relatively to Pietrain breed in all investigated periods.

Maximum difference between them was observed on the 150th day 1,5 times ($p < 0,05$) and the 210th day 1,7 times ($p < 0,01$). The content of estradiol in pigs of LW breed from the 120th till the 150th day of development is decreasing by 2,8 times ($p < 0,001$), by 1,4 times ($p < 0,05$) – in Pietrain breed, and testosterone increases, respectively, 2,1 ($p < 0,05$) and 1,9 ($p < 0,05$) times. During the 180 and 210 days of puberty, the concentration of testosterone was decreasing in the first genotype by two times ($p < 0,05$); in the second genotype – by 1,9 times ($p < 0,01$).

4. The content of thyroxine and triiodothyronine in the blood serum of Pietrain breed pigs and LW breed from the 120th till the 180th day of development is significantly increasing by 2 times ($p < 0,01$) and 2,3 times ($p < 0,001$), relatively. The concentration of thyroxine in pigs of LW breed over Pietrain breed on the 150th day was higher on 24%, the 180th day – 45% and the 210th day on 56% ($p < 0,005$).

5. The significant influence of the progesterone content in Pietrain breed pigs at the 120-day-old age on the number of lymphocytes ($r = 0,78$), and in the period from the 150th till the 180th day on the total number of leukocytes ($r = 0,60$), was established. In pigs of LW breed this hormone was in closer correlation with the leukocytes content ($r = 0,73$) only on the 120th day of development.

6. The concentration of estradiol was significantly interrelated with granulocytes in Pietrain breed pigs at the age of 150 days ($r = 0,92 \dots 0,99$), and in animals of LW breed the maximum level of correlation was observed at the achievement of the 150th day – $r = 0,61$ and the 180th day of development – $r = 0,94$.

7. An increase of the testosterone level in LW breed up to the 210 days of puberty of a postnatal development, significantly influenced on the hemoglobin level ($r = 0,91, 0,74$), and in Pietrain breed similar linkages between testosterone and hemoglobin were noted at 150–180 days of puberty and were $r = 0,53$ and $0,90$.

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