

Changes in the Content of Major Proteins and Selected Hormones in the Blood Serum of Piglets During the Early Postnatal Period

Roman SZYMECZKO, Wojciech KAPELAŃSKI, Anna PIOTROWSKA, Jan DYBAŁA, Monika BOGUSŁAWSKA-TRYK, Katarzyna BURLIKOWSKA, Iwona HERTIG, Maciej SASSEK, Ewa PRUSZYŃSKA-OSZMAŁEK, and Paweł MAĆKOWIAK

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The aim of the study was to determine the changes in the content of major proteins, glucose and selected hormones in the blood of piglets during the first 7 days of neonatal life. The study involved an entire litter of eight newborn piglets of F1 hybrids (Polish White Large x Polish Landrace) delivered from one sow in the second gestation. In blood samples collected directly after parturition (before colostrum intake), in the 12th, 24th and 48th hour and in 7th day of life, the content of total protein and its fractions, glucose concentration and the level of insulin, T₃ (total and free), T₄ (total and free), leptin, resistin and ghrelin (total and active) was determined. In the blood serum of newborn piglets a low content of total protein, albumins, gamma globulins and a high share of alpha- and beta globulins was found. In the 12th hour of life, after colostrum intake, a significant (P<0.05) increase in the content of total protein, albumins, beta-globulins and a rapid increase of gamma globulins as well as decrease of alpha-globulins level were observed. In the consecutive periods of postnatal life a significant (P<0.05) decrease of total protein, beta- and gamma globulins as well as a steady increase in the content of albumins in the blood serum of piglets was observed. The content of glucose, insulin, leptin, resistin and ghrelin in the blood serum of neonates increased significantly (P<0.05) after colostrum intake. During the successive experimental periods a progressive increase (P<0.05) of glucose and T₃ as well as systematic decrease of insulin, T₄, ghrelin and resistin in the blood serum was observed as compared to the 12th hour of life.

Key words: Neonatal pigs, serum proteins, glucose, hormones.

Roman SZYMECZKO, Anna PIOTROWSKA, Monika BOGUSŁAWSKA-TRYK, Katarzyna BURLIKOWSKA, University of Technology and Life Sciences in Bydgoszcz, Faculty of Animal Breeding and Biology, Department of Animal Physiology, Mazowiecka 28, 85-084 Bydgoszcz, Poland.

E-mail: romansz@utp.edu.pl; piotrowska@utp.edu.pl; monikab@utp.edu.pl; kasiab@utp.edu.pl
Wojciech KAPELAŃSKI, Jan DYBAŁA, University of Technology and Life Sciences in Bydgoszcz, Faculty of Animal Breeding and Biology, Department of Pig Breeding, Mazowiecka 28, 85-084 Bydgoszcz, Poland.

E-mail: kapelanski@utp.edu.pl; dybala@utp.edu.pl

Iwona HERTIG, Maciej SASSEK, Ewa PRUSZYŃSKA-OSZMAŁEK, Paweł MAĆKOWIAK, University of Agriculture in Poznań, Faculty of Animal Breeding and Biology, Department of Animal Physiology and Biochemistry, Wołyńska 35, 60-637 Poznań, Poland.

E-mail: ibialik@jay.up.poznan.pl; macsass@up.poznan.pl; ewaprusz@up.poznan.pl; pawmacko@jay.up.poznan.pl

The postnatal period in piglets is characterised by rapid morphological-functional changes stimulated by feed intake. The survival of piglets, particularly those which are born with low body weight, because of lack hair and fat tissue, low glycogen reserves and low activity of oxidising enzymes, depends on the composition and quantity of the consumed colostrum. Nutrients contained in the colostrum and sows milk do not only provide components for metabolic requirements and enhance the immunological system of the growing

organism but, equally importantly, by mitogenic and trophic action, they also stimulate the growth of tissues and the development of organs (LEPINE *et al.* 1991; BURRIN *et al.* 1995, 1997a; DAVIS *et al.* 1998; JENSEN *et al.* 2001; WOLIŃSKI *et al.* 2003; KOTUNIA & ZABIELSKI 2006). It was found that the neonatal period in piglets, apart from a high rate degradation and regeneration of protein as well as protein accumulation in their organisms, is also characterised by intensive changes in carbohydrate and fat metabolism which take place during the

first 7 days after parturition (MERSMANN 1974; PÉGORIER *et al.* 1981; DAVIS *et al.* 1998; HERPIN *et al.* 2007). The protein level in blood serum and the hormone content in the peripheral blood affecting metabolism provide information on the intensity of changes taking place in the organism and also indicate its health.

The aim of the study was to determine the changes in the content of major proteins, glucose and selected hormones in the blood serum of piglets during the first 7 days of life.

Material and Methods

Experimental material

The experiment was carried out in a typical pig farm in the Kujawy-Pomerania region which rears pigs in a closed cycle system. The study involved an entire litter of eight newborn piglets (six females and two males) of F1 hybrids (Polish Large White x Polish Landrace). The piglets were derived from one sow (second gestation). Both maintenance and feeding of animals on the farm were in accordance with current standards (rearing without litter, Frydland pen type, sows fed on complete rations in accordance with Polish standards). The experimental animals remained under veterinary care for the duration of the study. The study was conducted under a research protocol approved by the Local Ethical Committee in Bydgoszcz, Poland.

Chemical analyses

Blood samples from piglets were collected from the axillary vein (*vena axillaris*) five times during the experiment: directly after parturition (before first colostrum intake) in the 12th, 24th and 48th hour and 7th day of life. Prior to each blood collection, the piglets were weighed individually and, after blood

sampling, immediately returned to the nursing sow. Following centrifugation, samples of the collected blood serum were stored at -20°C before being analysed. The following parameters were determined in the samples of blood serum: total protein and its fractions: albumins, alfa₁-, alfa₂-, beta-, gamma-globulins, glucose concentration and the level of insulin, T₃ (total and free), T₄ (total and free), leptin, resistin and ghrelin (total and active).

The total protein content in the blood serum was determined by the biuret method using an Advia 1650 haematological analyser and application notes of the Bayer Company. The level of protein fractions was estimated by the agar gel electrophoresis method using Beckman Company equipment and application notes of the Beckman Coulter Company. Serum glucose content was measured enzymatically using glucoseoxidase and peroxidase (Sigma). Hormones in the serum were analysed radio-immunologically using the insulin RIA kit, ghrelin (total and active) – RIA kits, multi-species leptin – RIA kit and mouse resistin – RIA kit obtained from Linco Res., (USA), and with RIA-gnost T₃, RIA-gnost FT₃, RIA-gnost T₄, RIA-gnost FT₄ obtained from CIS Bio International (France).

Statistical analysis

The content of total protein and its fractions were analysed by analysis of variance with repeated measures. Due to lack of orthogonality, the remaining parameters (hormones and glucose) were analysed by one way analysis of variance (ANOVA). The post hoc Bonferroni test was applied in both cases. The significance of differences was set at P<0.05.

Results and Discussion

The content of total protein and its fractions in the blood serum of the experimental piglets is presented in Table 1. The profile of the blood serum

Table 1

Blood serum proteins (g l⁻¹) of neonatal pigs ($\bar{x} \pm \text{SEM}$)

Parameter	Age of piglets				
	at birth ¹	12 h	24 h	48 h	7 days
Total protein	23.45 ^a ± 0.85	65.69 ^b ± 1.81	62.78 ^{bc} ± 1.14	60.61 ^c ± 0.81	52.78 ^d ± 0.96
Albumin	3.32 ^a ± 0.37	8.99 ^b ± 0.34	9.39 ^b ± 0.31	11.27 ^c ± 0.49	19.09 ^d ± 0.54
Globulin	20.13 ^a ± 0.73	56.70 ^b ± 1.58	53.39 ^c ± 1.01	49.34 ^d ± 0.53	33.69 ^e ± 0.78
– alpha ₁ -globulin	4.03 ^a ± 0.59	1.45 ^b ± 0.16	1.30 ^b ± 0.08	1.46 ^b ± 0.17	1.00 ^b ± 0.09
– alpha ₂ -globulin	11.85 ^a ± 0.67	8.69 ^b ± 0.53	7.81 ^b ± 0.29	8.65 ^b ± 0.15	7.85 ^b ± 0.25
– beta-globulin	3.96 ^a ± 0.18	16.25 ^b ± 0.83	14.02 ^c ± 0.36	13.77 ^c ± 0.50	10.86 ^d ± 0.10
– gamma-globulin	0.29 ^a ± 0.06	30.31 ^b ± 1.41	30.26 ^b ± 0.85	25.46 ^c ± 0.70	13.98 ^d ± 0.59
Albumin: Globulin	0.17 ^a ± 0.02	0.16 ^a ± 0.01	0.18 ^a ± 0.01	0.23 ^b ± 0.01	0.57 ^c ± 0.02

¹ – before suckling

^{a,b,c,d,e} – means in the rows with different letters differ significantly (P<0.05).

proteins of the animals before the first intake of the colostrum was characterised by a low content of total proteins (23.45 g l^{-1}) and albumins (3.32 g l^{-1}) which constituted only 14.2% of the total proteins and trace quantities of gamma-globulins (0.29 g l^{-1}). Alfa- and beta-globulins were the dominant fractions of the blood serum proteins (84.6% of total protein) in which alfa₂-globulins prevailed distinctly (50.5% of total protein). Similar results were obtained by LECCE and MATRONE (1960), BENGTTSSON (1974), WINNICKA (2004) and MARTIN *et al.* (2005), who found that the composition of blood serum proteins of newborn piglets before colostrum intake was distinctly different from that of older animals with fully developed physiological processes. In comparison with mature pigs, newborn piglets display a lower level of total protein (over 2-times), albumins (over 9-times), beta-globulins (2-3 times) and higher alfa-globulins concentration (about 50%). Earlier investigations revealed that, at birth, piglets are less mature physiologically in comparison with newborns of other species of domestic animals and the ability of the liver to synthesise and release blood proteins typical for mature animals appears in pigs at a later stage (MERSMANN 1974; STONE 1981). Furthermore, the epitheliochorial placenta of sows is an effective barrier between the mother and her foetus making the transfer of both large-size molecules such as gamma- and beta-globulins as well as alfa-globulins and albumins impossible. Therefore, the profile of the blood serum proteins during the perinatal period is more similar to the foetal profile than that of mature pigs (BENGTTSSON 1974; STONE 1981; BUTLER *et al.* 2001; LE DIVIDICH *et al.* 2005; MARTIN *et al.* 2005). The trace quantities of gamma-globulins in the blood of newborn piglets observed in our study may result from a low level of selective transport of these proteins from their mother's blood to the foetus or from *de novo* synthesis taking place in the thymus, spleen, liver and bone marrow, which constitute the main lymphopoietic tissue during the period of foetal life (WADDILL *et al.* 1962; BENGTTSSON 1974; FRENÝÓ *et al.* 1980; BUTLER *et al.* 2001; SINKORA *et al.* 2002; MARTIN *et al.* 2005).

In the 12th hour of life, a significant ($P < 0.05$) increase in the content of total protein, albumins and beta-globulins and a rapid (over 100-fold) increase of gamma-globulins were observed in piglet blood serum. This was accompanied by a significant ($P < 0.05$) decline in the levels of alfa₁- and alfa₂-globulins. In the consecutive periods of postnatal life (12, 24 and 48 hours and day 7), a statistically significant ($P < 0.05$) decrease of total protein, beta- and gamma-globulins as well as a steady increase of albumins in blood serum of piglets were recorded. The concentration of alfa₁- and alfa₂-

globulins remained at a similar level. The present study shows that in piglets reared with sows, rapid changes both in the level as well as in the mutual ratios of individual protein fractions in the blood serum occur already at an early period of postnatal life. These parameters reach a profile similar to that of mature animals on the 7th day of life (LECCE & MATRONE 1960; WINNICKA 2004). Similar trends in the content of the total proteins, albumins and globulin fractions in the blood serum of piglets from birth until the 7th day of life were reported by LECCE and MATRONE (1960), STONE (1989), and MARTIN *et al.* (2005). In newborn piglets there is a fundamental transition in the nutritional conditions at the time of birth. Sow's milk during the first 24 hours after parturition is a rich source of effectively utilised energy and proteins. The intake of the colostrum by piglets directly after birth is the main factor stimulating the development and functions of the gastrointestinal tract and determining the passive immunity of the organism by the intestinal absorption of maternal immunoglobulins (LECCE & MATRONE 1960; BENGTTSSON 1974; BURRIN *et al.* 1995; JENSEN *et al.* 2001; LE DIVIDICH *et al.* 2005). A rapid increase of the IgG concentration in the blood of the newly born piglets was determined already half an hour after the first intake of colostrum and its highest concentration was observed 24 hours after birth (FRENÝÓ *et al.* 1980). The absorption of immunoglobulins through the intestinal barrier takes place in piglets during the first 24 hours after birth and develops the immunity of the organism until it is capable to initiate its *de novo* synthesis. According to ROOKE *et al.* (2003) this occurs from the 7th day of life in piglets. The rapid increase in the content of protein and albumins in the blood of suckling piglets observed in the present study was caused by the absorption of "intact" colostrum proteins as well as by intensive liver synthesis of albumins strongly activated already in the first day of life (INGVARSSON *et al.* 1978; STONE 1989).

The neonatal period in piglets is characterised by a high rate of body weight increase. In the present investigation the mean body weight of experimental piglets directly after birth (prior to colostrum intake) amounted to $1.39 \text{ kg} (\pm 0.27 \text{ kg})$ and doubled in the first 7 days of life ($2.71 \pm 0.53 \text{ kg}$). This indicates a proper growth rate of animals. Earlier investigations carried out on piglets showed that high efficiency of tissue protein synthesis, including proteins of skeleton muscles, is strongly stimulated by feed ingestion, becoming apparent within the first 2-3 hours after birth and remaining at a high level during the first days of life. In this context the role of polypeptide growth factors present in the sow's colostrum (IGF-1, IGF-II, insulin) and the response of the endocrine system of piglets

Table 2

Blood serum glucose and hormones of neonatal pigs ($\bar{x} \pm \text{SEM}$)

Parameter	Age of piglets				
	at birth ¹	12 h	24 h	48 h	7 days
Glucose, mmol l ⁻¹	1.79 ^a ± 0.16	4.38 ^b ± 0.37	6.11 ^c ± 0.32	5.37 ^{bc} ± 0.38	7.55 ^d ± 0.19
Insulin, nmol l ⁻¹	2.55 ^a ± 0.86	15.62 ^b ± 0.86	13.53 ^b ± 2.54	6.70 ^{ab} ± 1.70	7.04 ^{ab} ± 0.69
T ₃ total, nmol l ⁻¹	0.89 ^a ± 0.14	1.11 ^{ab} ± 0.08	1.44 ^{ab} ± 0.09	1.70 ^{bc} ± 0.28	2.27 ^c ± 0.15
T ₃ free, pmol l ⁻¹	5.54	6.56 ± 0.48 ²	7.81 ± 0.33	7.30 ± 1.01	6.24 ± 0.20
T ₄ total, nmol l ⁻¹	47.39 ^{ab} ± 3.66	55.29 ^a ± 3.29	50.46 ^{ab} ± 3.51	43.00 ^{ab} ± 4.05	37.81 ^b ± 3.04
T ₄ free, pmol l ⁻¹	27.41	31.65 ^{a,b} ± 2.76 ²	34.47 ^a ± 1.97	22.91 ^b ± 2.92	11.52 ^c ± 0.91
Leptin, pmol l ⁻¹	49.06 ^a ± 4.53	116.55 ^{bc} ± 6.28	117.07 ^b ± 5.82	89.88 ^c ± 5.54	117.35 ^b ± 7.62
Resistin, pmol l ⁻¹	14.10 ^a ± 7.61	171.13 ^b ± 26.91	81.88 ^c ± 10.31	48.18 ^{ac} ± 9.68	42.84 ^{ac} ± 6.29
Ghrelin total, pmol l ⁻¹	112.65 ^a ± 5.18	172.90 ^b ± 7.55	157.33 ^b ± 2.40	121.60 ^a ± 5.67	132.25 ^a ± 6.23
Ghrelin active, pmol l ⁻¹	10.52 ± 1.50	15.45 ± 1.03	14.99 ± 1.43	13.67 ± 1.21	15.05 ± 1.20

¹ – before suckling² – statistical analysis were performed for piglet groups between 12h and 7 days of age^{a,b,c,d} – means in the rows with different letters differ significantly (P<0.05).

suckling colostrum were studied (BURRIN *et al.* 1995, 1997a,b; DAVIS *et al.* 1996, 1998).

The content of glucose and the levels of selected hormones in the blood serum of the newborn piglets are given in Table 2. The low glucose content in serum of experimental piglets at birth (1.79 mmol l⁻¹) increased rapidly after the intake of colostrum and it reached the value of 4.38 mmol l⁻¹ in the 12th hour of life. During the subsequent days a further increase in the level of blood glucose was observed. Our results correspond with the data of other investigators (MERSMANN 1974; CAMPION *et al.* 1986). In the 7th day the content of the blood glucose in experimental piglets reached 7.55 mmol l⁻¹ and was significantly (P<0.05) higher in comparison with the values obtained during the previous experimental periods. Numerous investigations confirmed that in newborn piglets with a very low fat content (1-2%), the main source of energy is glucose. It was found that the amount of glucose ingested by piglets with colostrum (high activity of intestinal lactase determined in piglets) covers only 35 to 55% of their daily requirements (MERSMANN 1974; PÉGORIER *et al.* 1981; LEPINE *et al.* 1991). The maintenance of normoglycemia is therefore conditioned by processes of glycogenolysis and gluconeogenesis. During the first 24-48 hours of life, the reserves of liver and muscular glycogen are depleted in piglets and the process of gluconeogenesis develops reaching its peak 2-3 days after birth (MERSMANN 1974; PÉGORIER *et al.* 1981; LEPINE *et al.* 1991). Changes in metabolic processes taking place directly after birth are correlated with the endogenous secretion of hormones which is strongly stimulated by feed ingestion by piglets (PÉGORIER *et al.* 1981; CAMPION *et al.* 1986; BURRIN *et al.* 1995; HERPIN *et al.* 1995; DAVIS *et al.* 1998). The content of blood insulin of

the experimental piglets prior to the colostrum intake amounted to 2.55 nmol l⁻¹ and increased 5 to 6-fold after its intake, remaining at a high level for the first 24 hours of life. According to SHEN and XU (2000), a significant increase in the insulin content in the serum of newborn piglets can be attributed both to the direct absorption of this hormone from the colostrum or to increased secretion of endogenous insulin. During the successive experimental periods (48th hour and 7th day), a decline in the insulin content was observed. Presumably, this is connected with the intensification of processes of gluconeogenesis and release of glucagon which stimulates this process in newborn piglets. This was confirmed by PÉGORIER *et al.* (1981) who found a decrease in the plasma insulin/glucagon molar ratio in piglets during the first 24 hours of life.

The absence of subcutaneous fat tissue as well as sparse hair cover force young piglets to activate processes of thermogenesis, controlled primarily by thyroid hormones. The content of total triiodothyronine and thyroxine in the experimental piglets prior to the intake of colostrum amounted to 0.89 and 47.39 nmol l⁻¹, respectively (Table 2). Similar values in the level of total T₃ in the blood of newborn piglets prior to suckling were reported by KASSER *et al.* (1981, 1982) and HERPIN *et al.* (1995). In our investigation, the content of thyroxine was over two times lower in comparison with the values of the above-mentioned researchers. The level of total T₃ in the blood serum of experimental piglets increased in consecutive experimental periods and the content of this hormone determined in blood on the 7th day of life (2.27 nmol l⁻¹) was by 2.5 times higher (P<0.05) in comparison with the period directly after birth. The concentration of the total T₄, following its slight

increase during the first 12 hours of life, declined gradually. The content of active T_3 in the blood of piglets remained at a similar level, while the concentration of free T_4 decreased significantly ($P < 0.05$) after the 48th hour of life. It should be mentioned here that the lack of statistically significant results concerning the content of active T_3 and T_4 in the experimental piglets serum directly after birth (Table 2) was caused by the insufficient quantity of biological material obtained from newborn piglets. It is commonly accepted that the catabolic and thermogenetic activity of thyroid hormones are important regulators of metabolic processes. In the case of newborn piglets, processes of gluconeogenesis, oxidation of fatty acids and temperature regulation are not developed fully and undergo improvement during the first three days of life (MERSMANN 1974). It was found that the low rate of fatty acid oxidation observed at birth increased four times before the end of the 7th day of life and the activity of mitochondrial oxidation was positively correlated with the content of thyroid hormones in blood (MERSMANN 1974; KASSER *et al.* 1981). Presumably, the gradual increase in the concentration of total T_3 demonstrated in the experiment on piglets is associated with the intensification of oxidation processes of fatty acids, whereas the decline in the thyroxine levels is a sign of the conversion of this hormone into triiodothyronine which is a metabolically active form of thyroid hormones.

The protein hormone leptin is one of the adipokines and its main source is fat tissue, but it is also secreted by tissues of other organs, including the placenta and mammary gland. Investigations revealed that this hormone regulates fat metabolism and energy administration of the organism and, in the case of newborn individuals, it stimulates the process of maturation of the small intestine and its functions (ESTIENNE *et al.* 2000; WOLIŃSKI *et al.* 2003; SKOWROŃSKA *et al.* 2005). The leptin content in the blood serum of the experimental piglets at birth amounted to 49.06 pmol l⁻¹ and increased rapidly after feed intake. During the successive experimental periods (12th, 24th, 48th hour and 7th day of life), the concentration of this hormone fluctuated from 89.88 to 117.35 pmol l⁻¹ and remained at a statistically higher level ($P < 0.05$) in comparison with the period before colostrum intake. The rapid increase in the leptin content in the blood of suckling piglets can probably be attributed to its high concentration in the colostrum and milk of the sow (ESTIENNE *et al.* 2000; WOLIŃSKI *et al.* 2003).

Another hormone released by adipocytes is resistin. A high level of expression of this protein was also found in leucocytes of peripheral blood, especially in pigs (ADEGHATE 2004; SKOWROŃSKA *et al.* 2005; DAI *et al.* 2006). The main physiological function of resistin is to maintain glycae-

mia during starvation by the activation of enzymes of liver gluconeogenesis and intensification of glycogenolysis (BENERJEE *et al.* 2004; SKOWROŃSKA *et al.* 2005; LIU *et al.* 2008). The content of resistin in the blood of experimental piglets directly after birth amounted to 124.10 pmol l⁻¹ and in the 12th hour of their life it increased 12 times after colostrum intake (171.13 pmol l⁻¹) ($P < 0.05$). The level of resistin decreased significantly ($P < 0.05$) and on day 7, its content was 42.84 pmol l⁻¹. The few studies on the subject published so far indicated that resistin present in the placenta and transported to the foetus as well as mother's milk may be the sources of this hormone in the newborn's blood. It was found that the concentration of resistin in the blood and milk of mothers is the highest directly after parturition and decreases in the course of lactation (ADEGHATE 2004; ILCOL *et al.* 2008). A significant decline in the level of resistin in the blood of newborn experimental piglets recorded from the 12th hour after birth is probably associated with the decreasing content of this hormone in sow's milk.

Ghrelin is synthesized, primarily, by the secretory cells of the mucous membrane of the stomach and intestines. Apart from its orexigenic action, it exerts a positive impact on the development of foetuses and the neonatal development of organisms. According to PIETRZAK *et al.* (2007), ghrelin may delay the closure of the intestinal barrier in newborns and, in this way, facilitate the transfer of the colostrum and milk proteins into the organism. In addition, by stimulating food intake, motor activity of the gastrointestinal tract and liberation of the growth hormone, ghrelin enhances body weight growth and plays an important role in the maintenance of energetic homeostasis (YOKOYAMA *et al.* 2005; KOTUNIA & ZABIELSKI 2006; PIETRZAK *et al.* 2007; ZABIELSKI 2007). In the present study, the content of total and active ghrelin in the blood of piglets directly after birth amounted to 112.65 and 10.52 pmol l⁻¹, respectively. The presence of this hormone in the blood of newborn piglets can be explained by endogenous secretion. Experiments carried out on rats revealed that the quantity and activity of the stomach secretory cells determined in foetuses at the end of pregnancy increased together with the development of this organ until somatic maturity (HAYASHIDA *et al.* 2002). The highest total ghrelin concentration in the blood of experimental piglets was determined on the 12th and 24th hour of life and after that its level declined significantly ($P < 0.05$). On day 7, the concentration of total and active ghrelin reached 132.25 and 15.05 pmol l⁻¹, respectively and was still higher in comparison with the period directly after birth. It was demonstrated that the concentration of this hormone in the blood of animals shows considerable variability; it peaks just

before meal times and then returns to the basal level. (YOKOYAMA *et al.* 2005; PIETRZAK *et al.* 2007). In our study, suckling piglets had unlimited access to feed, so the observed changes in the level of this hormone in blood did not result from feeding restrictions. Therefore, it can be presumed that they were caused by changes in secretion of endogenous ghrelin as well as its content in the colostrum and milk of the sow (PIETRZAK *et al.* 2007).

In conclusion, the performed investigations demonstrated that the profile of the blood serum proteins of newborn piglets was characterised by low contents of total proteins, albumins and gamma globulins as well as high proportions of alfa- and beta-globulins. In the 12th hour of life, a significant increase in the content of total protein, albumins and beta-globulins as well as a rapid increase of gamma-globulins and a decline in the level of alfa-globulins in the blood serum of piglets suckling colostrum were observed. During the consecutive periods of the neonatal life, the levels of total proteins, beta- and gamma-globulins in blood decreased significantly, while the concentration of albumins systematically increased. Following colostrum ingestion, a significant increase in the content of glucose, insulin, leptin, resistin and ghrelin in the blood of newborn piglets was recorded; the level of thyroid hormones also increased. Changes in the level of glucose and the examined hormones in the blood of newborn piglets occurring during the successive hours and days of life result from changes in the dynamics of metabolic processes in the organisms.

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