

# The Influence of Slaughter Stress on Cortisol and Glucose Levels in Fatteners' Blood Serum

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**Abstract** – The research aimed at an assessment of the changes in cortisol and glucose levels in the blood serum of fatteners of various races and of the meat-content as a result of slaughter stress. The material comprised the blood obtained from 120 fatteners of Polish Landrace, Polish White, Pietrain and Duroc breeds. Samples of blood to mark cortisol and glucose levels were taken twice. The first blood samples before stunning (sample I) were taken in the pre-slaughter room, immediately before the transfer of animals to the slaughter post. Fatteners underwent slaughter in a slaughterhouse located in Pig Performance Testing Station in Chorzelow, Poland. Alternating current of 400V and frequency of 50Hz and amperage of 2.5A was used as a stunning agent. The power was applied for 10-15s using Lotterschmidt-Weinberger tongs. The second blood samples (sample II) were taken during slaughter exsanguination. Blood was taken from the slaughter wound 20s after slaughter piercing. The level of glucose was marked by colorimetric method with HITACHI 912 automatic chemistry analyzer. The level of cortisol was marked by electrochemiluminescence method with COBAS E-410 Rosche apparatus.

The highest concentration of cortisol in the serum before slaughter was observed in Pietrain fatteners. It was observed that the concentration of cortisol in the serum of Pietrain fatteners was statistically significantly higher in comparison to Polish Landrace and Duroc breeds. As far as the concentration of glucose in the serum before slaughter is concerned, a significantly lower concentration of glucose in comparison to other breeds was observed in Polish Large White. The research proved that slaughter stress caused a significant increase in the concentration of cortisol and glucose in the serum in the blood of all the analysed breeds. The highest increase in the concentration of the examined hormone was observed in Pietrain fatteners. The greatest changes in the concentration of glucose between the blood samples were observed in Polish Large White and Polish Landrace fatteners. The research proved that slaughter stress caused a significant increase in the concentration of cortisol and glucose in the serum in all the analysed classes of meat-content. The researched proved a correlation between the changes in the concentration of cortisol and glucose under the influence of slaughter stress. An increase in the concentration of cortisol in all the analysed groups of animals was accompanied by an increase in the concentration of glucose. The most visible increase in the examined blood indices was observed in Pietrain fatteners and in fatteners with the highest meat content. The research showed that in the assessment of the intensity of the course of slaughter stress apart from the measurement of cortisol concentration, the measurement of the concentration of glucose may be used, with a simultaneous consideration of differences associated with breed and meat-content of the examined animals.

**Keywords** – Cortisol, Glucose, Fatteners, Slaughter Stress.

## I. INTRODUCTION

During the production cycle animals are exposed to numerous stress factors, both physical and emotional. According to many authors [1]–[3], a particularly intensified impact of stressors can be observed during slaughter. Although slaughter stress is of short duration, its course is particularly acute and leads to significant changes in the physiological and metabolic processes in the body, which are reflected in the level of biochemical indices.

From among the slaughter factors it is stunning that affects fatteners the most. Authors agree [3]–[4] that each of the stunning methods used nowadays is an intensive stimulant of the stress response, and stunning by means of electric current has been assessed as particularly negative [3]. It has been proved that porkers stunned with electric current show a two hundredfold increase in blood pressure and a multiple increase in stress hormones produced by adrenal glands. According to some authors [5], slaughter exsanguination is also considered a strong stress stimulant. Exsanguination, similarly to stunning, is accompanied by a significant increase in catechol amines and glucocorticoids. Exsanguination may also cause a significant increase in glucose levels [6].

The functional disorders in the body occurring during slaughter may lead to a decrease in the amount and quality of the meat obtained after slaughter due to a faster glycolysis. According to [7] and [8], slaughter factors have a significantly greater role in determining the quality parameters of the meat than genetic factors. A significant role of slaughter factors in determining the quality of the meat should be associated with the influence of many stress stimulants, which have a primary character or are characterized by a high intensity in the case of most animals [9]–[11]. This causes acute disorder of the haemostatic balance in the body mainly due to a disorder in the stress hormones cycle and metabolism in the muscles and subsequently post-slaughter changes in the meat.

The measurement of blood biochemical indices provides a lot of important information concerning the stress response. Studies [12], [9], [10], [13] show that a significant increase in the level of catechol amines, glucocorticoids, glucose and total protein can be observed in animals as a result of stress. The measurement of stress hormones, especially of cortisol, plays a particularly

significant diagnostic function in the evaluation of the adaptation phenomena and of the course of stress response [14], [15], [10]. This results from the stimulation of the nervous system and of the hypothalamic–pituitary–adrenal axis. The activation of these systems leads to the stimulation of the adrenal cortex to produce steroid hormones, mainly cortisol.

Studies [16], [2] show that the intensity of the secretion of cortisol to the blood due to a stress stimulus may be different depending on its type, intensity and individual experiences of the animal. The same factor, which in one animal may cause an increase in adrenal hormones, leading to general exhaustion, may cause body activation in another. An increase in the level of cortisol in response to stress also depends on sex, age, physiological condition as well as genetic factors [1]–[2], [13].

An increase in the blood cortisol level activates most of the energy reserves of the body. The activation of the gluconeogenesis process and an increase in the production of glucose is the most important result of the effect of cortisol. This process is the main mechanism of the body's response to stress stimuli. An increase in the cortisol level in the first stage of the stress response is accompanied by an increase in the level of glucose; therefore, apart from the measurement of the cortisol level, the evaluation of the level of glucose in the blood serum may be an indicator of the stress response, especially in the first stage.

The research aimed at an assessment of the changes in cortisol and glucose levels in the blood serum of fatteners of various races and of the meat-content as a result of slaughter stress.

## II. MATERIALS AND METHODS

The research was conducted at SKUTCh in Chorzelów. The material comprised the blood obtained from 120 fatteners of Polish Landrace, Polish Large White, Pietrain and Duroc breeds. Samples of blood to mark cortisol and glucose levels were taken twice. The first blood samples before stunning (sample I) were taken in the pre-slaughter room, immediately before the transfer of animals to the slaughter post. The time of the activity did not exceed one minute. Fatteners underwent slaughter in the slaughterhouse located in Pig Performance Testing Station in Chorzelow, Poland. Fatteners were stunned with the STZ-2 stunning apparatus powered by electric current of 220V and 50Hz. Alternating current of 400V and frequency of 50Hz and amperage of 2.5A was used as a stunning agent. The power was applied for 10-15s using Lotterschmidt-Weinberger tongs. After stunning fatteners were hung by their left rear leg and in this position a two-sided slaughter piercing was performed, which consisted in cutting the carotid vascular trunks. The time between the completion of stunning and slaughter piercing was around 20 seconds.

The second blood samples (sample II) were taken during slaughter exsanguination. Blood was taken from the slaughter wound 20s after slaughter piercing.

To mark cortisol and glucose levels blood was taken to dry samples with pellets to guarantee quick coagulation.

To obtain serum to mark biochemical indices the blood was spun at the speed of 3000 r/min for 10 minutes. Until measurements were completed the serum was stored at the temperature of  $-25^{\circ}\text{C}$  [17]. The level of glucose was marked by colorimetric method with HITACHI 912 automatic chemistry analyzer. The level of cortisol was marked by electrochemiluminescence method with COBAS E-410 Rosche apparatus. Measurements were performed in *Lawet* analytical laboratory which carries out animal blood tests located in Rzeszów.

Numerical material obtained in the research was submitted to statistical analysis. The method of single-factor variance analysis in a non-orthogonal system was used in the calculations considering the following factors: the influence of the breed (Polish Landrace, Polish Large White, Pietrain and Duroc), meat-content of the carcass (S, E, U classes of the EUROP system). While calculating the percentage changes in the levels of the analysed biochemical indices of the blood between samples, the values from the first blood samples before stunning were used as the 100% value. The significance of the difference between the analysed indices was calculated using Tukey's test. Two levels of significance  $p \leq 0.05$  and  $p \leq 0.01$  were applied to verify the obtained results. The results were worked out using Statistica 9.0 software.

## III. RESULTS

Table 1 presents average cortisol and glucose levels in the blood serum of the fatteners of the analysed breeds. The data show that before slaughter the highest cortisol level in the serum, amounting on average to 30.62nmol/l, was observed in Pietrain fatteners. It was shown that the concentration of cortisol in the serum of Pietrain fatteners was statistically significantly higher in comparison to the concentration of this hormone in the serum of Polish Landrace ( $\bar{x} = 22.00\text{nmol/l}$ ) and Duroc pigs ( $\bar{x} = 23.77\text{nmol/l}$ ). In the serum from the blood obtained during slaughter exsanguination a greater variation in the concentration of the analysed hormone among the examined breeds was observed. The highest concentration of cortisol in the serum, amounting to 47.92nmol/l was observed in Pietrain fatteners, analogically to the situation before slaughter. Statistical analysis showed that the concentration of cortisol in the blood of Pietrain fatteners was significantly higher in comparison to all the other breeds (Table 1). It was also observed that the serum obtained from Polish Large White fatteners was characterized by a significantly higher concentration of cortisol in comparison to Polish Landrace and Duroc breeds.

Examining the concentration of glucose in the serum, it was observed that Large White fatteners were characterized by its significantly lower amount (Table 1). The conducted studies did not show any differences in the level of glucose among the other breeds. Differences in the concentration of glucose among the examined breeds were observed in the samples of blood obtained during slaughter (Table 1). From among the four examined breeds, Pietrain fatteners were characterized by a

significantly higher concentration of glucose in comparison to other breeds. A significant difference in the concentration of glucose in the serum between Polish Landrace and Polish Large White fatteners was also observed (Table 1). It is worth noting that the observed statistical differences between the breeds in the concentration of glucose in the serum from the blood obtained during exsanguination were identical to those observed during the analysis of cortisol levels.

The studies show that slaughter stress caused a statistically highly significant increase in the cortisol level in the blood of all the analysed breeds. The highest increase in the concentration of the examined hormone, amounting on average to 56.49%, was observed in Pietrain fatteners (Fig. 1.). In fatteners of the white breeds (Polish Landrace and Polish Large White) the increase in the concentration of cortisol was lower. The smallest changes in the level of cortisol as a result of slaughter stress was observed in Duroc fatteners. The difference in the concentration of cortisol in the blood obtained before and after slaughter in the fatteners of this breed was the smallest and amounted on average to 5,19nmol/l, (21.83%).

The studies show that slaughter stress caused a statistically highly significant increase in the concentration of glucose in the blood of all the examined breeds. The greatest changes in the concentration of glucose between the blood samples were observed in Polish Large White and Polish Landrace fatteners (Fig. 1). Calculations show that changes in the concentration of glucose in the case of these breeds exhibited a nearly twice higher dynamics in contrast to Polish Landrace and Duroc breeds.

Results shown in Table 2 illustrate the influence of slaughter stress on the concentration of cortisol and glucose in fatteners with different meat-content in the carcass. Statistically significant influence of meat-content on the concentration of cortisol either in the samples of the serum obtained before stunning or during exsanguination was not observed. Meat-content also did not influence the level of glucose in the samples of blood obtained before slaughter. A significant influence of this factor on the concentration of glucose in the blood obtained during slaughter was observed. A higher concentration of glucose was observed in fatteners whose carcass was classified in the S class of the EUROP system (Table 2).

The research proved that slaughter stress caused a significant increase in the concentration of cortisol and glucose in the serum in all the analysed classes of meat-content. The greatest increase in the level of both examined biochemical indices (cortisol – 69.32%, glucose – 44.87%) was observed in fatteners characterized by the highest meat-content (Fig. 2). In the serum of the blood obtained from fatteners whose carcass was classified in the S class of the EUROP system an increase in the concentration of cortisol by 10.85nmol/l and glucose by 1.85mmol/l was observed, whereas in the fatteners whose carcass was classified in the E class, the increase in the concentration of cortisol amounted on average to 7.2 nmol/l, and of glucose to 1.43mmol/l (Table 2).

#### IV. DISCUSSION AND CONCLUSION

Significant changes in the physiological and metabolic processes take place in animals as a result of the influence of stressors [18]–[19].

According to many authors, the measurement of plasma components may provide information on the intensity of the stress response in animals. An increase in the level of cortisol in the blood serum is a commonly acknowledged indicator of the stress response [9], [18]–[19]. Previous studies [20], [7], [14], [15], [21] proved that stress connected with transport and pre-slaughter handling causes an increase in the concentration of cortisol in fatteners' blood serum. At the same time, authors emphasize that the extent of the changes in the concentration of cortisol depends on the time and intensity of the influence of stress stimuli. Animals' response to unfavourable stress stimuli may differ depending also on sex [22], meat-content [18], genotype of the animals [8], and their physiological condition [9]. In the case of pigs, genetic differences mainly associated with the presence of the RYR1 stress sensibility gene play an important role [8]. Significant individual differences in the body's response to the influence of stressors should also be underlined.

The results of the study and the studies by other authors [22]–[23] show that breed was a factor which significantly influenced the amount of cortisol in the serum both before slaughter and during slaughter exsanguination. However, under slaughter stress a similar mechanism of responding to stress stimuli connected with slaughter was observed. A comparison of the level of cortisol in the blood serum before and after slaughter showed that under slaughter stress a statistically highly significant increase in the concentration of the analysed hormone occurred in the blood serum (Table 1).

A significant increase in the level of cortisol in the serum obtained from the blood drawn during exsanguination, which was observed in this study, proves an intensive course of the stress response caused by factors associated with slaughter, which was proved by the results of the previous analyses as well [10]–[11]. At the same time it should be observed that significant differences between the analysed breeds in the extent of the changes in the concentration of cortisol occurred as a result of slaughter. The greatest differences were observed between Pietrain and Duroc breeds. As shown, the highest cortisol surge (an increase of 56.49%) was observed in Pietrain fatteners, whereas the lowest increase of 21.83% in Duroc fatteners. The observed differences between the breeds in the hormonal reaction may depend on genetically conditioned differences in the functioning of the limbic system in the brain. Such differences between Duroc and Pietrain breeds were observed before by [23]. The observed marked increase in the concentration of cortisol in the blood serum of Pietrain breed may indicate a high sensibility of pigs of this breed to stress associated with slaughter. The obtained results also prove a greater reactivity of the endocrine system in Pietrain pigs to environmental stimuli. A greater sensibility to the stress

associated with slaughter in Pietrain fatteners seems to be confirmed by the changes in the concentration of glucose (Fig. 1).

The changes in the concentration of glucose between the blood samples in Pietrain breed observed in the study exhibited a twice higher dynamics in comparison to the other breeds. As stated by [7], short-term but very intensive effort causes a significant surge of glucose to the blood stream as a mechanism of body adaptation. It can be expected that this process takes place also during slaughter stress. A less intensive response to stress in Duroc pigs in contrast to Landrace breeds, which this study showed, is confirmed by [18].

According to [9], an increase in the concentration of cortisol in the first stage of the stress response is accompanied by an increase in the concentration of glucose. As a result of stunning by means of electric current, a 10% increase in the concentration of glucose is observed in fatteners' blood [3]. This study proved a significantly higher increase in the concentration of glucose as a result of slaughter stress (Table 1, Table 2). According to [1], a highly intensive secretion of glucose to the cardiovascular system takes place during electric stunning. It should be observed that the change in the concentration of glucose depended on breed and meat-content, as was the case with cortisol. A particularly high dynamics of the increase in the concentration of glucose was observed in the fatteners of Polish Large White and Pietrain breeds (an increase of more than 56%) and fatteners whose carcass was classified in the S class of the EUROP system. The conducted study confirmed the results obtained by other authors [22] about a special reactivity to stress stimuli in Pietrain fatteners and in fatteners with a very high meat-content.

An interdependence of both analysed parameters was observed in the course of the changes in the concentration

of cortisol and glucose as a result of slaughter stress. An increase in the concentration of cortisol in all the analysed groups of animals was accompanied by an increase in the concentration of glucose. It was the most clearly visible in fatteners of Pietrain breed, and at the same time it exceeded the upper limit of the reference index provided by [17] by 0.79mmol/l. The observed increase in the concentration of glucose in the blood obtained during slaughter exsanguination was most probably connected with a higher concentration of cortisol.

The research shows that in the assessment of the intensity of the course of slaughter stress apart from the measurement of cortisol concentration, the measurement of the concentration of glucose may be used, with a simultaneous consideration of differences associated with breed and meat-content of the examined animals. The method and time of blood drawing is also significant for the obtained results and their interpretation, which was indicated by [18] and [24]. The interpretation of results should also involve the daily cycle of cortisol secretion and its fluctuation and a high individual variability in fatteners' responses to stress.

The consideration of the period of pre-slaughter fasting, the length of which may also influence the content of liver glycogen and the course of gluconeogenesis, is also significant for the assessment of the changes in the concentration of glucose as a result of slaughter stress. A good understanding of the correspondence between the two examined parameters may lead to a more exact measurement of the course of stress reaction and may significantly decrease the cost of such examination. The application of modern analytic methods allowing fast and direct measurement of the concentration of glucose may be the alternative to the examination of the course of stress reaction using the concentration of glucocorticoids.

Table 1: The influence of slaughter stress on the concentration of cortisol and glucose in the blood serum of the examined breeds

Traits	Breed							
	Polish Landrace		Polish Large White		Pietrain		Duroc	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Cortisol sample I (nmol/l)	22.00* <sup>ac</sup>	5.75	26.28*	8.47	30.62* <sup>b</sup>	8.20	23.77* <sup>c</sup>	3.09
Cortisol sample II (nmol/l)	31.36* <sup>a</sup>	8.97	37.41* <sup>b</sup>	7.92	47.92* <sup>c</sup>	8.64	28.96* <sup>ad</sup>	4.58
Changes in the concentration of cortisol (%)	142.54	-	142.35	-	156.49	-	121.83	-
Glucose sample I (mmol/l)	4.24* <sup>ac</sup>	0.65	3.56* <sup>b</sup>	0.75	4.04* <sup>c</sup>	0.72	4.03* <sup>ad</sup>	0.46
Glucose sample II (mmol/l)	5.37* <sup>a</sup>	0.59	5.65* <sup>b</sup>	0.63	6.39* <sup>c</sup>	1.03	5.36* <sup>ad</sup>	0.23
Changes in the concentration of glucose (%)	126.65	-	158.70	-	158.16	-	133.00	-

\* – statistically significant differences between samples have been marked with stars in the columns  
a, b, c, d – statistically significant differences between breeds have been marked with different letters

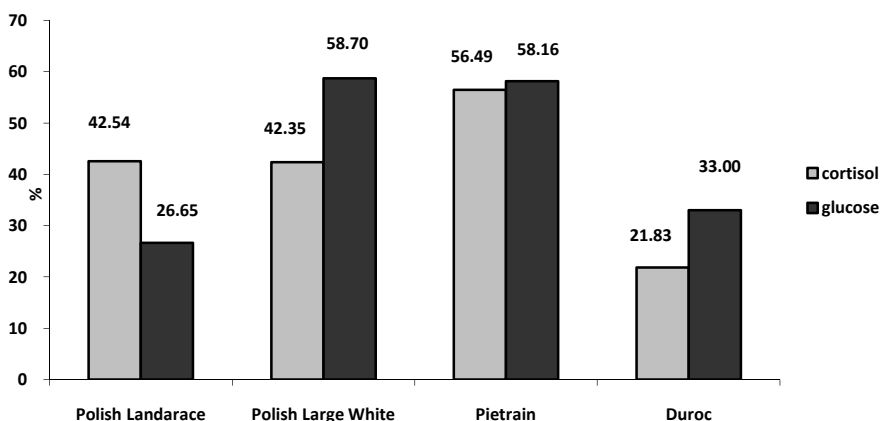


Fig.1. Increase in the concentration of cortisol and glucose in the blood serum as a result of slaughter stress in fatteners of the examined breeds

Table 2: The influence of slaughter stress on the concentration of cortisol and glucose in the blood serum of fatteners with different meat-content.

Traits	Meat-content of carcass					
	U		E		S	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Cortisol sample I (nmol/l)	15.70*	9.44	16.20*	6.95	15.65*	6.82
Cortisol sample II (nmol/l)	26.11*	10.16	23.40*	7.07	26.50*	11.01
Changes in the concentration of cortisol (%)	166.30	-	144.44	-	169.32	-
Glucose sample I	3.85*	0.74	3.99*	0.59	4.10*	0.70
Glucose sample II	5.53* <sup>ac</sup>	0.74	5.42* <sup>c</sup>	0.59	5.95* <sup>b</sup>	0.89
Changes in the concentration of glucose (%)	139.72	-	135.83	-	144.87	-

\* – statistically significant differences between samples have been marked with stars in the columns  
a, b, c – statistically significant differences between breeds have been marked with different letters

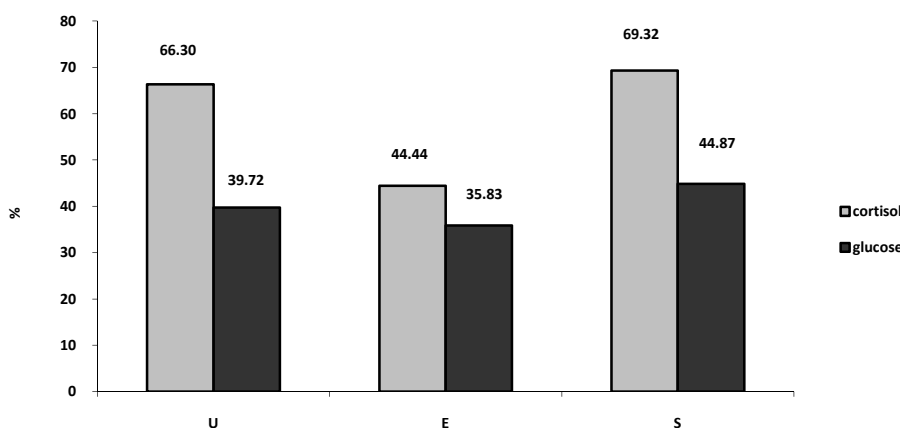


Fig.2. Increase in the concentration of cortisol and glucose in the blood serum as a result of slaughter stress in fatteners with different meat-content

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