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Stress levels of mangalita, large white, and pietrain pigs reared in different housing systems in south eastern Europe

[*Níveis de estresse de mangalita, grandes porcos brancos e pietrain criados em diferentes sistemas de alojamento no sudeste europeu*]

R.N. *Malancus*[1], R.O. *Rusu*[2], V. *Arsenoaia*[3], L.I. *Ailincai*[4]

[1] University of Life Sciences "Ion Ionescu de la Brad", Iasi, Faculty of Veterinary Medicine, 8, Mihail Sadoveanu Alley, 700489, Iasi, Department of Physiology and Pathophysiology, Romania
[2] University of Life Sciences "Ion Ionescu de la Brad", Iasi, Faculty of Veterinary Medicine, 8, Mihail Sadoveanu Alley, 700489, Iasi, Department of Inspection and control of products of animal origin, Romania
[3] University of Life Sciences "Ion Ionescu de la Brad", Iasi, Faculty of Agriculture, 3, Mihail Sadoveanu Alley, 700489, Iasi, Department of Pedotehniques, Romania
[4] University of Life Sciences "Ion Ionescu de la Brad", Iasi, Faculty of Veterinary Medicine, 8, Mihail Sadoveanu Alley, 700489, Iasi, Department of Etology and animal hygiene, Romania

ABSTRACT

Cortisol is a steroid hormone that regulates a wide range of vital processes throughout the body, including metabolism and the immune response. It also has a very important role in helping the body respond to stress. Numerous studies have been conducted around the world to compare the development rates of pigs raised indoors to those raised outdoors, but the results have been ambiguous and, in many cases, conflicting. The objective of this study was to determine the effects of various housing systems (indoors with no free access to outdoor runs or indoors with free access to outdoor runs) on stress levels and slaughter value in growing-finishing pigs.

Keywords: swine, stress, cortisol

RESUMO

O cortisol é um hormônio esteroide que regula uma ampla gama de processos vitais em todo o corpo, incluindo o metabolismo e a resposta imune. Ele também tem um papel muito importante em ajudar o corpo a responder ao estresse. Numerosos estudos foram realizados em todo o mundo para comparar as taxas de desenvolvimento de porcos criados em ambientes fechados com aqueles criados ao ar livre, mas os resultados foram ambíguos e, em muitos casos, conflitantes. O objetivo deste estudo foi determinar os efeitos de vários sistemas de alojamento (dentro de casa sem acesso livre a corridas ao ar livre ou dentro de casa com acesso livre a corridas ao ar livre) nos níveis de estresse e no valor de abate em suínos em crescimento e terminação.

Palavras-chave: suínos, estresse, cortisol

INTRODUCTION

Cortisol is a steroid hormone that regulates a wide range of vital processes throughout the body, including metabolism and the immune response. It also has a very important role in helping the body respond to stress.

Cortisol is a hormone that helps the body progress daily, in addition to its well-known activities. High quantities of cortisol, on the other hand, will overburden the adrenal glands, resulting in unfavorable consequences. Cortisol is a well-known stress marker, and when combined with catecholamine discharges, it can have a deleterious impact on the development of neoplastic lesions (Averos *et al*., 2003).

The release of cortisol is under control of the hypothalamus-pituitary-adrenal (HPA) axis. Corticotropin-releasing hormone (CRH) is released by the paraventricular nucleus (PVN) of

Corresponding author: razvanmalancus@gmail.com
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the hypothalamus. It then acts on the anterior pituitary to release adrenocorticotropic hormone (ACTH), which subsequently acts on the adrenal cortex. In a negative feedback loop, sufficient cortisol inhibits the release of both ACTH and CRH. The HPA axis follows a circadian rhythm. Thus, cortisol levels will be high in the morning and low at night (McKay and Cidlowski, 2003).

Numerous studies have been conducted around the world to compare the development rates of pigs raised indoors to those raised outdoors, but the results have been ambiguous and, in many cases, conflicting. In a study by Gentry *et al*. (2002), pigs reared outdoors were characterized by higher growth rates than pigs born and finished in an indoor environment. Similar trends were noted by Stern *et al*. (2003) and Millet *et al*. (2005). In another experiment (Gentry *et al*., 2004), pigs reared outdoors were heavier and had higher gain/feed ratios. Different results were reported by Hoffman *et al*. (2003) and Enfält *et al*. (1997), who noted higher daily gains in pigs kept indoors. According to other authors (Sather *et al*., 1997; Stern *et al*., 2003; Lebret, 2008; Lebret *et al*., 2014), free-range pigs needed a longer period to reach the desired market weight and used their feed less efficiently.

Considering these issues, it's worth noting that alternative pig housing arrangements have become increasingly popular in recent years. Environmental enrichment farming approaches are a way to improve animal welfare and disease resistance while also boosting the development of organic, high-nutrient-value products in response to the growing customer demand.

The objective of this study was to determine the effects of various housing systems (indoors or indoors with free access to outdoor runs) on stress levels and slaughter value in growing-finishing pigs.

MATERIAL AND METHODS

The study was conducted over a period of 6 months, between March-September 2021, in 3 swine farms, on 3 different breeds – Mangalita, Large white and Pietrain. Two farms were in Romania (Mangalita and Large white) and one in Republic of Moldova (Duroc). A total number of 156 pigs were investigated, at least 35 from each farm, 3 to 4 months old, weighting between 80 – 110kg, both males and females, to investigate stress levels and the quality of the carcass in terms of pH values. Pigs were housed in the finishing facility, indoor, on partially slatted floors (Large white and Pietrain) or reared with access to outdoor (Mangalita). Pigs were provided feed and water *ad libitum*.

Mangalita is one of the old type swine breeds, originating several centuries ago because of crossing between European and Asian primitive pigs. Mangalita was introduced in Romania from Serbia in the 19th century. Red Mangalita is one of the varieties, established by successive crossbreeding between the Blond Mangalita and the Salonta pig. The main features of these pigs are curly hair, thick backfat, palatable meat and stress and disease resistance (Ciobanu *et al*., 2001; Zăhan *et al*., 2009).

The Large White is one of the most extensively utilized pig breeds for crossbreeding in intensive pig farming around the world. It was created as an outdoor breed, but it is now one of the most popular among commercial pig breeders, providing consistency to pigs raised for meat production on a big scale.

Pietrain pigs occupy one of the top places in the world list of meat breeds. They are especially popular in European countries, where they are known for their relatively lean meat and high-quality bacon, with low-fat fiber. In households, Pietrain has not yet gained the popularity it deserves. This is because it is a sensitive breed to maintenance factors and certain diseases.

Samples have been collected pre-slaughter, twice, at interval of 4 weeks to determine serum cortisol levels and hematological parameters or post-slaughter, for determining the pH values. The inclusion criteria considered clinically healthy swine, without any expressed pathology.

Hematology was performed using Abaxis HM5 Vet Scan automated hematological analyzer. Blood samples were collected from the jugular vein. The vacutainers contained EDTA, as anticoagulant substance and the samples were analyzed immediately after collecting them. For each case have been determined the following parameters: red blood cells (RBCs), packed cell volume (PCV), hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin

(MCH) and mean corpuscular hemoglobin concentration (MCHC), WBC (white blood cells) and platelets. Also, a blood film was analyzed for each sample using Diff-Quick staining method.

Biochemistry was performed in an accredited laboratory, having been determined the cortisol levels.

Post-slaughter processing and carcass evaluation were carried out in accordance with meat industry regulations.

The pH was measured in the longissimus lumborum (LL) 45 min after bleeding (pH45) and after 24 hours of carcass chilling (pH24). The parameters, pH45 and pH24, were determined with the WTW 3310 pH meter and combination electrode (WTW-Wissenschaftlich-Technische Werkstaetten GmbH, Weilheim, Germany) and calibrated with the same standard solutions of pH 4.01 and 7.00 at 20ºC.

Two-tailed Fisher`s exact test was used to analyze contingency tables and examine the significance of two parameters association. The level of statistical significance was $P<0.05$.

RESULTS AND DISCUSSION

Domestic animals react to stress by an alteration of behavioral and physiological responses, which are the results of individual emotional reactivity (Selye, 1976).

The stimulation of the sympathetic-adrenergic system leads to the increase of adrenaline concentration as a fast, energetic, and short mechanism. However, the effects of adrenaline stimulation are strengthened and completed by the slow engagement into action of the hypothalamic-pituitary-adrenal system, with high secretions of glucocorticoid hormones. The growth, thyrotrophic, prolactin hormones also interfere in stress. Cortisol, along with epinephrine is best known for its involvement in the "fight-or-flight" response and temporary increase in energy production, at the expense of processes that are not required for immediate survival. The resulting biochemical and hormonal imbalances resolve due to a hormonally driven negative feedback loop (Rusu *et al*., 2021).

Pre-slaughter stress activates the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system, which are both stress-responsive neuroendocrine systems. The HPA axis regulates cortisol release in response to stress by activating it. Increased serum cortisol levels are frequently associated with higher blood glucose and lactate levels, leading in a rapid drop in muscle pH, greater drip loss, and lightness in pork. Previous research has investigated the link between cortisol levels and meat quality. However, we believe that cortisol levels are unrelated to meat quality, which is explained by the fact that cortisol plasma clearance is quick (Peres *et al*., 2014).

Table 1 reveals pre-slaughter cortisol values highlighting decreased cortisol amount in Mangalita pigs with elevated values for both Pietrain and Large white breeds.

Table 1. Average cortisol levels for Large White, Pietrain and Mangalita

Breed	Mean Cortisol levels (µg/dl)
Large White	7.76±0,5
Pietrain	7.25±0.7
Mangalita	5.86±0,5

Serum cortisol is widely used as an indicator of stress (Choe and Kim, 2014). In the present study, the levels of this hormone were very similar for Large white and Pietrain breeds with lower values for Mangalita pigs. One of the reasons for these results may be associated to very similar growth systems for these two breeds. There is a very significant statistical correlation between lower levels of cortisol serum in pigs reared with free access to outdoor runs compared with those reared indoors ($P<0,05$). It could be understood as a determinant of better welfare (Shaw *et al*., 1995).

As far as hematology results are concerned (Table 2) eosinophils count showed decreased values for Large white and Pietrain breeds, eosinopenia being regularly observed in stressed organisms. Also, there is a specific correlation between increased cortisol levels and decreased eosinophil counts for individuals within Pietrain and Large white breeds with $p<0.05$.

Table 2. Hematology results in Large white, Pietrain and Mangalita breeds

Parameter	Large white	SD	Pietrain	SD	Mangalita	SD
RBC ($\times 10^6$/mm3)	6.63	0.83	6.88	0.5	7.12	0.25
Hemoglobin (g/dL)	11.63	1.62	12.01	1.31	12.5	1.23
Haematocrit (%)	33.15	3.96	35.25	3.55	38.25	3.51
MCV (fL)	53.26	3.11	52.30	3.02	53.72	3.02
MCH (pg)	16.99	1.25	18.05	1.20	17.55	1.20
MCHC (g/dL)	33.25	1.25	33.60	1.35	32.67	1.35
Platelets ($\times 10^3$/mm3)	356.56	91.23	320.00	96.05	343.00	91.10
WBC ($\times 10^3$/mm3)	19.92	6.36	20.33	6.36	18.33	5.42
Neutrophils (%)	66.10	9.12	69.10	11.10	74.15	9.10
Eosinophils (%)	0.12	0.06	0.02	0.01	1.05	0.31
Lymphocytes (%)	29.30	8.98	28.20	8.60	23.40	6.30
Monocytes (%)	2.65	1.02	2.05	1.10	2.15	1.23
Neutrophil/lymphocyte ratio	2.25	0.71	2.44	0.95	3.18	0.63

Other changes that have been observed were consistent with modified neutrophil/lymphocyte ratio where lower values were observed for Large white (2.25) and Pietrain breeds (2.44), values which correspond to an inhibitory effect towards inflammatory processes exercised by increased cortisol values and therefore a stimulation of the HPA axis (Etim *et al*., 2013).

The value of the carcass might be also influenced by stress levels in slaughtered pigs so decreased pH levels may be an indicator of poorer quality. The values of pH at 45 minutes and 24 hours post-slaughter are presented in Table 3.

Table 3. Differences in pH levels at 45 minutes and 24 hours post-slaughter for Large White, Pietrain and Mangalita breeds

Breed	pH45	pH24
Large White	6.48±0,3	5.41±0,32
Pietrain	6.43±0.4	5.42±0.34
Mangalita	6.64±0,4	5.48±0,10

The samples of longissimus lumborum (LL) obtained from all the pigs in the current study could be regarded as normal and were defined as good quality, according to meat classification standards based on pH24 (5.44 - 5.52) with slightly lower values for Large white and Pietrain. However, Mangalita pigs showed better results as a reduced impact of stress on this breed given their genetical advantages in terms of resistance to diseases.

Long-term preslaughter stress, such as fighting, cold weather, fasting and transit, which occurs 12 to 48 hours prior to slaughter depletes muscle glycogen, resulting in meat which has a higher pH, darker color, and is drier (Hambrecht *et al*., 2004). Short-term acute stress, such as excitement or fighting immediately prior to slaughter, produces lactic acid from the breakdown of glycogen. This results in meat which has a lower pH, lighter color, reduced water binding capacity, and is possibly tougher (Grandin, 1980).

CONCLUSION

The study revealed higher levels of cortisol while eosinophil counts severely decreased in Large white and Pietrain breeds, changes which are associated with a strong reaction to stress for individuals that were housed in indoor finishing facilities without outdoor access. Another explanation for the results might be related to the housing total surface per capita, Mangalita breed thus benefiting from a larger housing area. These changes however have just a slightly negative impact on the carcass of the animals, the physical and chemical properties of the end products and by products found in the market ultimately not affecting the consumer. However, by lower serum cortisol levels and increased meat pH values, the Mangalita breed confirms its high resistance in the face of stress and disease.

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