EFFECT ON THE CORTISOL SERUM LEVEL OF GROWING PIGS SUPPLEMENTED WITH ESSENTIAL OIL OF *Lippia alba*

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ABSTRACT

Animal welfare has become more important as the consumer market is increasingly demanding good conditions of handling production animals, especially in swine farming. There is also scientific evidence of economic losses due to animal stress during growth and before slaughter, reducing body weight, carcass yield and meat quality. Thus, natural and economically viable therapeutic alternatives were researched, such as the use of herbal medicines, aiming to minimize these negative effects on animals. With emphasis on *Lippia alba*, a plant commonly used in Brazilian medicine with proven efficacy as a relaxant and antiparasitic, anti-inflammatory, anesthetic and anti-stress for most animal species. The aim of this study was to evaluate the effect of *L. alba* essential oil supplementation on the reduction of serum cortisol levels in growing pigs and as a welfare parameter to evaluate these animals. Twelve F1 castrated pigs with an average weight of 12.3 kg were divided into two groups of six animals. Control group (A1) received saline solution at a dose of 1 $mL.kg^{-1}$ and treatment group (A2) 0.5% *L. alba* essential oil at a dose of 1 $mL.kg^{-1}$ on days 0, 30, 60 and 90 by gavage. During these time points, blood was collected from the auricular vessel to measure cortisol levels using the radioimmunoassay technique (Kit Cort-CT2). The administration of *L. alba* essential oil showed a significant anti-stress effect in growing pigs, significantly decreasing blood cortisol levels, an indicator of animal welfare.

Keywords: Cortisol. *L. alba*. Phytotherapy. Pig farming. Welfare.

INTRODUCTION

Pig farming has an excellent position on the international scene owing to genetic improvements and high-quality nutrition. In this field, productivity indices and economic return are possible through rigorous sanitary and nutritional management control in all stages of the production cycle (DALLA COSTA et al., 2006).

The implementation of new technologies in production systems has increased pork consumption, both processed and unprocessed, and boosted the export market, which is evidenced by the annual Brazilian production of approximately 4.4 million tons of pork products. Of which, 1.0 million tons are exported to more than 70 countries, making Brazil the fourth largest pork producer in the world (ABPA, 2021).

However, despite consolidation in the market, pork production still faces some obstacles, mainly in the process of transporting animals to slaughterhouses. The lack of adequate management during housing, transport, and slaughter can produce inferior quality meat, known as dark, firm, and dry (DFD) or pale, soft, and exudative (PSE), resulting in great economic loss (DALLA COSTA et al., 2006). According to Simer (2015), slaughterhouses lose approximately US$ 5.00 per carcass with PSE defects, and up to 40% of the pork production can be considered non-tradable (GOETTEMS, 2011).

In addition, there is an increasing concern of the consumer about animal welfare, humane slaughtering, and proper stunning, since many production problems are caused by stress due to intensive production conditions that require physiological and behavioral adaptations that warrant investigation as animal welfare parameters (GRANDIN, 2014). In this context, the analysis of serum levels is an important evaluation tool; cortisol, particularly, is the most important neuroendocrine hormone indicative of stress response during production housing and pre-slaughter management. Increased levels of this hormone decrease carcass weight, growth rate, and feed efficiency (MORMÈDE et al., 2011).

New natural alternatives to minimize animal stress throughout the production chain willing to obtain better production results are gaining prominence, which include herbal products administered via drinking water or mixed with food (SANTANA et al., 2015). One of these

alternatives is the essential oil of *Lippia alba*, which is championed because of its pharmacological properties as a sedative, muscle relaxant, antioxidant, and tranquilizing drug, as well as its fast absorption and effect when administered to animals. Additionally, it leaves no residue in meat and byproducts and is easily produced on a large scale (NICULAU et al., 2013).

The objective of this study was to evaluate the effect of *L. alba* essential oil on reducing cortisol levels in growing pigs as a welfare parameter.

MATERIAL AND METHODS

Plant material was obtained from the *L. alba* cultivation in the Universidade Paranaense (UNIPAR) medicinal garden. The essential oil was extracted from the fresh leaves using the hydrodistillation technique in a Clevenger apparatus, according to the method previously described by Simões and Schenkel (2002). The project was approved by the Committee on Animal Research and Ethics (CEPEEA) of the UNIPAR on July 27, 2016, under protocol number 31.594.

The study included a total of twelve pigs F1 hybrids (Pietrain boar and Landrace sow), 1.2 month old with weight average of 12.3 kg, which were divided into two groups of six animals, being A1 control group and A2 treatment group. All the animals came from the experimental farm of Paranaense University, Umuarama, Campus. The animals were housed in 4 m^2 barns with cement floor oriented East-West, without lantern, with 3 meter ceilings throughout with long eaves of 3.4 meters, with tiles painted externally with reflective paint. There were open sides on the walls measuring 0.75 centimeters covered by polyethylene curtains with side opening control devices, with movements from bottom to top and vice versa, being generally opened at 7 am and closed around 6 pm, according to the temperature and the internal air humidity. These conditions were observed through two thermo-hygrometers placed inside the installation and between the barns and were maintained between 16 ^oC and 26 ^oC and relative humidity of 60% to 80%, remaining within the comfort range of the animals. The experiment was carried out in the winter period providing mild temperatures and the high humidity in the region. In addition, the installation also had 4 ventilators of 1,730 rpm (model

NBR - 7094) internally on the sides of the room at a height of 2 meters from the floor, however it was not necessary to activate them. The environment was quiet, away from all facilities, with no passage of machinery or any noise that could stress the animals. All handling was carried out exclusively by three people throughout the experimental period, with water and feed being supplied *ad libitum*, using semi-automatic collective feeder and automatic nipple drinkers, in the proportion of 1 every two animals. No fights or disputes between the animals were observed throughout the experimental period.

The essential oil of *L. alba* was diluted in water at a concentration of 0.5% and administered to the animals of A2 treatment group, 1 $mL.kg^{-1}$, on days 0, 30, 60 and 90 by gavage, with a 50 mL oral dosing gun (Höppner Aparelhos Veterinários Ltda®). The A1 control group received physiological solution 1 $mL.kg^{-1}$ like placebo in the same time points. Restraint was performed manually with the animals always standing, with their heads raised about 30 degrees and restrained by an assistant. Ropes or containment smoking pipe were not used as they made gavage difficult. After 3 hours of administration, blood was collected from an auricular vessel in heparinized Vacutainer® tubes for serum cortisol measurement and immediately sent to the Clinical Analysis Laboratory of Universidade Paranaense in a thermal box under refrigeration at 6 ^{o}C.

Plasma cortisol levels were measured using the radioimmunoassay technique with commercial double-cortisol antibody (Cort-CT2) kits, according to the method described by the National Committee for Clinical Laboratory Standards (NCCLS, 1993). The results were analyzed using the Fisher-Snedecor test to evaluate significant differences between groups ($p<0.05$) using SAS software version 9.2 (SAS, 2000).

RESULTS AND DISCUSSION

There was no difference between groups at day 0, while at days 30, 60, and 90, a significant difference was observed (Figure 1) between the groups. The A2 treatment group had lower cortisol levels than the control group, suggesting that *L. alba* essential oil used during the experimental period had an anti-stress effect, according to data presented in Table 1.

Table 1 – Mean of blood cortisol (µg/dL) concentration in pigs supplemented with *Lippia alba* essential oil at 0, 30, 60 and 90 days.

	Day 0	Day 30	Day 60	Day 90
Control (A1)	5.32 ± 0.32	5.70 ± 0.10	5.42 ± 0.18	4.85 ± 0.16
Treated (A2)	5.32 ± 0.16	5.12 ± 0.07	3.24 ± 0.10	1.63 ± 0.10
CV (%)	4.83	1.64	3.39	4.12
p-value	0.9825	<0.0001	<0.0001	<0.0001

CV: coefficient of variation. ( ) standard deviation.

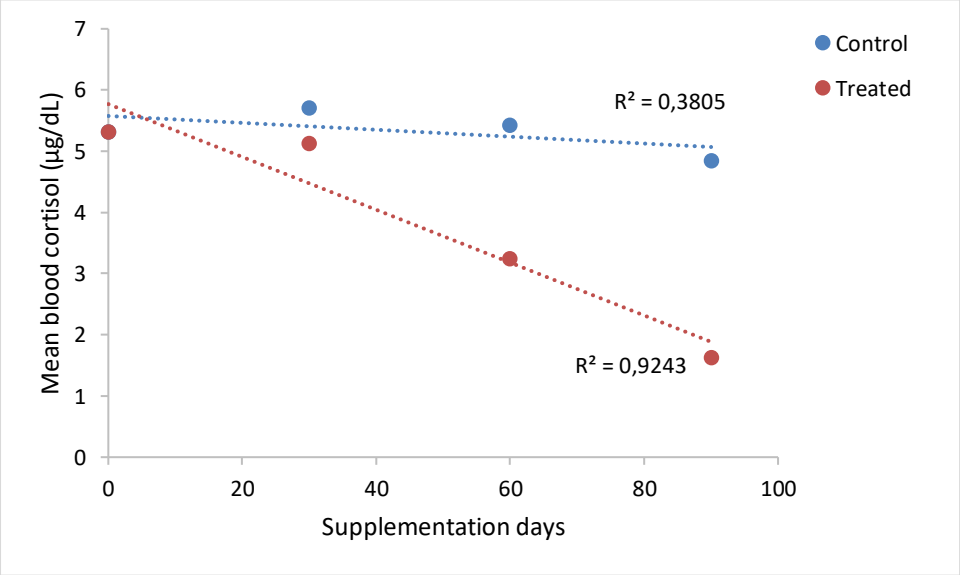

Figure 1 - Mean of blood cortisol concentration (µg/dL) in pigs orally supplemented with *Lippia alba* essential oil at 0, 30, 60 and 90 days.

Cortisol is a hypothalamic-pituitary-adrenocortical (HPA) axis glucocorticoid involved in calcium absorption regulation, blood pressure maintenance, gluconeogenesis, and pepsin and gastric acid secretion; in addition, also has anti-inflammatory and immunological effects. Its concentration varies according to response to stressors and environmental challenges (DALLA COSTA et al., 2006; KOEPPEN et al., 2009). Stress is defined as an environmental effect on an individual that overloads their control systems and results in adverse consequences (BROOM; FRASER, 2010).

Cortisol has been the hormone of choice in stress research because its high plasma concentration leads to homeostasis breakdown, stress induction, or absence of welfare. The objective of this response is to maintain and restore homeostasis, preserve oxygen supply to

important tissues, mobilize caloric substrate (glucose), reduce painful effects, and regulate body temperature (GOYMANN et al., 2003; STOCCHE et al., 2001).

As a result, animal stress related to welfare can range from very good to very poor. Whenever there is stress, welfare becomes poor (BROOM; MOLENTO, 2004). The only acceptable measurement of stress presence or absence is the adrenal corticosteroid blood level (RADOSTITS et al., 2007). Vining et al. (1983) and Cunningham (2004), reported that salivary and serum cortisol levels are the most appropriate clinical measurements of the adrenocortical function of serum cortisol level because its increase is reflected in salivary cortisol in less than 5 min; therefore, the analysis of salivary cortisol has been widely used to verify the stress level to which a growing animal is exposed due to management and during the pre-slaughter period.

Intense stress can lead to oxidative stress, muscle and cell injury, intense muscle glycogen breakdown, and consequently to release of large amounts of lactic acid into the bloodstream. Thus, serum cortisol concentration shows the effect of short-term stress events while salivary cortisol concentration shows the effect of long-term phenomena (BUSHONG et al., 2000; SCHÖNREITER et al., 1999).

Acute stress modulates systemic changes in the animal's body by stimulating it to adapt to the aggressive agent. As in acute stress, during chronic stress there is an elevation of serum cortisol concentration that may or may not remain elevated depending on the degree of stress. Plasma cortisol increases within a few minutes after exposure to acute stressor stimulus and returns to baseline levels in one hour or more. Lower stress levels result in lower cortisol production when compared to high stress levels (CUNNINGHAM, 2004).

In cases of chronic stress, cortisol levels can remain high, although much lower than the peak levels of acute cases. The glucocorticoid response is immediate and proportional to stress severity. Cortisol concentrations increase rapidly to reach values that are many times higher than normal (WENDELAAR BONGA, 1997).

In this context, it can be verified in Table 1 that the treated animals presented a lower stress level compared to the control group, showing a beneficial and active effect of the use of *L.*

alba essential oil for growing pigs. It also demonstrates its possible use in the diet of these animals as a beneficial method of reducing stress, consequently increasing carcass yield, since high cortisol levels may predispose to pre-pathological conditions (CHOI et al., 2006). The pre-pathological conditions are characterized by reduced number of eosinophils, neutrophils, and lymphocytes and lymphoid tissue atrophy, consequently resulting in a larger number of sick animals, deaths, and lower live and carcass weight for the industry (BEERDA et al., 1999; GUYTON; HALL, 2006).

In his study, Grandin (1994), reported that in situations of extreme stress, cortisol values can double or quadruple. In another study, Shaw et al. (1995), suggested that the group producing lower mean of cortisol levels should be considered the least stressed. Gispert et al. (2000), described a positive correlation between high cortisol plasma concentrations and degree of skin lesions caused by fights between growing pigs, concluding that the higher the stress, the higher the hormone plasma levels, corroborating this present study, in which the treated animals presented lower blood cortisol levels and less agitation during the whole period of experiment and management, inferring a lower level of stress.

Cortisol levels are also used to measure other welfare parameters. Fagundes et al. (2008) evaluated the cortisol levels of 36 male (neutered) and female pigs (Landrace x Large White) between 74 and 149 days of age in two temperature conditions of 22.2 to 32.8 ^{o}C and 17.6 to 26.6 ^{o}C. The animals presented cortisol levels of 7.06 and 4.82 mg.dL^{-1}, respectively, which suggests that cortisol may also be an indicator of temperature stress.

The importance of considering the variables of age, weight, sex, and stressor intensity to evaluate stress is emphasized in the literature (BERTOLONI; SILVEIRA, 2003; LUDTKE et al., 2012; PÉREZ et al., 2002). According to Ludtke et al. (2012), growing pigs may show decreased cortisol concentrations with age when the animals are properly managed. Guzik et al. (2006) reported that the use of additives in the diet can reduce plasma and salivary cortisol levels after acute stress in pigs using a diet supplemented with tryptophan. The study also shows that additives may reduce stress caused by management and in the pre-slaughter period,

when the animals are exposed to a variety of potential stressors. This period is the cause of great concern in the production chain (HARTUNG, 2003).

In this context, *L. alba* is currently one of the most promising native medicinal species for the development of new drugs, as has been shown by a series of preclinical studies establishing several actions related to its popular use. However, despite a promising future and similarly to other native species, more studies on *L. alba* are necessary to provide a scientifically validated phytomedicine or herbal medicine. Its essential oil has a chemical constitution of great medicinal importance, including geranial (citral or 3,7-dimethyl-2,6-octadienal or lemonal), carvone, carvacrol (cymophenol), and thymol, which have sedative, tranquilizing, and analgesic actions (BITU et al., 2012). In addition to these compounds, other substances such as alkaloids, tannins, flavonoids, iridoids, and naphthoquinones can also be found in its extracts (SOARES; TAVARES-DIAS, 2013).

In addition to the use of *L. alba* in pig farming proposed in the present study, antimicrobial and antiparasitic effects in horses, cattle, and mice have been widely tested *in vitro* and *in vivo*. Vale et al. (2002) obtained a highly sedative and relaxing effect in mice using different concentrations of citral, limonene, and myrcene obtained from the essential oil of *L. alba*. Ribeiro et al. (2012) also confirmed its activity in aquaculture as an anesthetic, antimicrobial, and antiparasitic drug, emphasizing the importance of this and other plants in phytomedicine due to low production costs and absence of health risks for animals and pharmacists (CUNHA et al., 2011).

Soares and Tavares-Dias (2013) described the use of *Lippia* species as a potential bioactive substance in fish production, showing that the use of 100 to 500 mg/L *L. alba* essential oil was efficient as an anesthetic, inhibiting plasma cortisol levels without changing the odor and flavor of South American silver catfish (*Rhamdia quelen*), and also reducing oxidative stress caused by transport.

Although this and other medicinal plants have been used since the dawn of civilization and their use has been intensified in the last decades, their use and efficacy as a medicine for animals is still little known (ROYER et al., 2013). Therefore, research that elucidates its use and

efficacy is extremely important, since the use of medicinal plants in animal production is promising and tends to grow with advances in technology and with partnerships between research entities and companies. In addition to the benefits related to quality of the final product and animal welfare, there is a reduction of costs related to medication purchase and environmental risks during production. Medicinal plants have proved to be effective in many breeding the animals (SANTANA et al., 2015).

CONCLUSION

The use of *Lippia alba* essential oil had a significant anti-stress effect on growing pigs by decreasing the blood cortisol level, which is an important indicator of animal welfare.

EFEITOS NO NÍVEL SÉRICO DE CORTISOL DE SUÍNOS EM CRESCIMENTO SUPLEMENTADOS COM ÓLEO ESSENCIAL DE *Lippia alba*

RESUMO

O bem-estar animal tornou-se mais importante à medida que o mercado consumidor está cada vez mais exigente com as condições de manejo dos animais de produção, principalmente na suinocultura. Há também evidências científicas de perdas econômicas devido ao estresse animal durante o crescimento e antes do abate, reduzindo o peso corporal, rendimento de carcaça e qualidade da carne. Assim, alternativas terapêuticas naturais e economicamente viáveis foram pesquisadas, como o uso de medicametos à base de plantas, objetivando minimizar esses efeitos negativos em animais. Com destaque à *Lippia alba*, planta comumente utilizada na medicina brasileira, com eficácia comprovada como relaxante e antiparasitário, anti-inflamatório, anestésico e antiestresse para a maioria das espécies animais. O objetivo deste estudo foi avaliar o efeito da suplementação com óleo essencial de *L. alba* na redução dos níveis séricos de cortisol de suínos em crescimento e como parâmetro de bem-estar para avaliar esses animais. Doze suínos castrados F1, com peso médio de 12,3 kg, foram divididos em dois grupos de seis animais. Grupo controle (A1) recebeu solução fisiológica na dose de 1 $mL.kg^{-1}$ e grupo tratamento (A2) 0,5% de óleo essencial de *L. alba* na dose de 1 $mL.kg^{-1}$ nos dias 0, 30, 60 e 90 por gavagem, com coletas de sangue de vaso auricular para dosar os níveis de cortisol usando a técnica de radioimunoensaio (Kit Cort-CT2). A administração de óleo essencial de *L. alba* mostrou efeito antiestresse significativo em suínos em crescimento, diminuindo significativamente os níveis de cortisol sanguíneo, um indicador de bem-estar animal.

Palavras-chave: Cortisol. *L. alba*. Fitoterapia. Suinocultura. Bem-estar.

EFEECTOS EN EL NÍVEL SÉRICO DE CORTISOL DE SUÍNOS EN CRECIMENTO SUPLEMENTADOS CON ÓLEO ESENCIAL DE *Lippia alba*

RESUMEN

El bienestar animal se ha vuelto más importante a medida que el mercado de los consumidores está cada vez más exigente con las condiciones de gestión de los animales de producción, especialmente en la agricultura porcina. También hay evidencia científica de pérdida económica debido al estrés animal durante el crecimiento y antes del sacrificio, reduciendo el peso corporal, el rendimiento de la carcasa y la calidad de la carne. Por lo tanto, se han investigado alternativas terapéuticas naturales y económicamente viables, como el uso de medicamentos herbales, con el objetivo de minimizar estos efectos negativos en los animales. Resaltado *Lippia alba*, planta comúnmente utilizada en la medicina brasileña con eficacia probada como relajante y antiparasitaria, antiinflamatorias, anestésicas y antiestrés para la mayoría de las especies animales. El objetivo de este estudio fue evaluar el efecto de la suplementación con el aceite esencial de *L. alba* en la reducción de los niveles de cortisol de suero de cerdos en crecimiento y el parámetro de bienestar para evaluar estos animales. Doce cerdos castrados F1, con peso promedio de 12,3 kg, fueron divididos en dos grupos de seis animales. Grupo de control (A1) recibió una solución fisiológica a la dosis de 1 $mL.kg^{-1}$ y grupo de tratamiento (A2) 0,5% de aceite esencial de *L. alba* en la dosis de 1 $mL.kg^{-1}$ en los días 0, 30, 60 y 90 por sonda, con muestreo de sangre desde los vasos sanguíneos auriculares para dosificar niveles de cortisol utilizando la técnica de radioinmunoensayo (Kit Cort-CT2). La administración del aceite esencial de *L. Alba* mostró un importante efecto antiestrés en los cerdos en crecimiento, disminuyendo significativamente los niveles de cortisol de sangre, un indicador de bienestar animal.

Palabras clave: Cortisol. *L. alba*. Fitoterapia. Cría de cerdos. Bienestar.

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