EFFECT OF *MATRICARIA CHAMOMILLA* CH_{12} WEANING STRESS IN PIGS

Luis Souza Lima de Souza Reis[1], Paulo Eduardo Pardo[2], Eunice Oba[3], Sérgio do Nascimento Kronka[4], Giovane Rodrigues[1], Rodrigo Camacho Bezerra[1]

[1]Universidade do Oeste Paulista – UNOESTE, Mestrado em Medicina Veterinária e Bem-Estar Animal, Curso de Medicina Veterinária, Presidente Prudente, SP. [2]Médico Veterinário, Presidente Prudente, SP. [3]Universidade Estadual Paulista – UNESP, Faculdade de Medicina Veterinária e Zootecnia, Botucatu, SP. [4]Universidade Estadual Paulista – UNESP, Faculdade de Ciências Agrárias e Veterinária, Jaboticabal, SP

ABSTRACT

Weaning is a phase of life that creates stress in pigs causing damage to the welfare and health of pigs. The *Matricaria chamomilla* CH_{12} reduced stress management in cattle, but has not been tested in pigs. Study evaluated the effect of *Matricaria chamomilla* CH_{12} in the stress of weaning. We used 16 male pigs Landrace animals with 28 days of age, randomly divided into two groups (8 animals/group), the control group (G_C) received concentrate feed without and other group (G_{Mc}) received concentrate feed containing addition *Matricaria chamomilla* CH_{12}. Blood samples were collected from pigs on days 1, 14, 24 and 30 after weaning for determination of serum cortisol radioimmunoassay. Piglets were stressed out the next day weaning with a significant increase ($P < 0.05$) in serum cortisol concentration above the baseline. But piglets adapted to it, 30 days after weaning, the serum cortisol showed a significant linear decrease (R^2=0,88; $P<0.01$) returning the basal concentration. However, piglets GMc the group at any time after weaning showed up stressed and serum cortisol was 61.8 in group GMc %, 43.5%, 77.2% and 32.0% lower than the GC on days 1, 14, 24 and 30 after weaning, even so, the serum cortisol did not differ significantly between ($P>0,05$) groups. But these results show the physiological effect of chamomilla. *Matricaria chamomilla* CH_{12} can be used for the decrease of the stress of weaning pigs and the weaning acts as stressor agent, but the animals adapt.
Keywords: swine; cortisol; homeopathy; animal health.

EFEITO DA *MATRICARIA CHAMOMILLA* CH_{12} NO ESTRESSE DA DESMAMA EM SUÍNOS

RESUMO

O desmame é uma fase da vida que gera estresse nos suínos, causando danos ao bem-estar e a saúde desses animais. A *Matricaria chamomilla* CH_{12} reduz o stress nos bovinos, mas ainda não foi testado em suínos. O presente estudo avaliou o efeito da *Matricaria chamomilla* CH_{12} no estresse do desmame dos suínos. Foram utilizados 16 suínos, machos da raça Landrace, com 28 dias de idade, divididos aleatoriamente em dois grupos (8 animais/grupo), o grupo controle (Gc) que recebeu concentrado sem *Matricaria chamomilla* CH_{12} e outro grupo (GMc) que consumiu concentrado contendo a *Matricaria chamomilla* CH_{12}. As amostras de sangue dos animais foram colhidas nos dias 1, 14, 24 e 30 após o desmame para a determinação de cortisol sérico que quantificado por meio de radioimunoensaio em fase sólida. Os leitões ficaram estressados no dia seguinte ao desmame com um aumento significativo ($P < 0,05$) na concentração de cortisol sérico. Mas os leitões adaptaram-se ao estresse causado pela desmama após 30 dias, apresentando redução linear significativa no cortisol sérico (R^2=0,88, $P < 0,01$), retornando a concentração basal. No entanto, os leitões do grupo GMc após o desmame também ficaram estressados, mas a concentração sérica de cortisol nesses animais era 61,8%; 43,5%; 77,2% e 32,0% inferior ao animais do grupo Gc nos dias 1, 14, 24 e 30 após o desmame, mesmo assim, o cortisol sérico não diferiram significativamente entre ($P> 0,05$) entre os grupos. Mas estes resultados mostram o efeito fisiológico da *Matricaria chamomilla* cH12 mostrando que pode ser usada para diminuir o stress dos leitões após o desmame.
Palavras-chave: suíno; desmame; cortisol; homeopatia; *Matricaria chamomilla*.

INTRODUCTION

Homeopathic complexes have being efficient in pig production, improved production indices of the animals, when coupled with appropriate health management (SOTO et al., 2008).

Weaning is a critical step by stressing the pigs (HESSEL et al., 2006) due to the presence of

stressing factors such as physical and psychosocial agents, the abrupt separation from the sow, changes in the diet and social hierarchy among the different litters that are joined together (MOESER et al., 2007). Thus, there is activation of the hypothalamic-pituitary-adrenal axis (HPA) resulting in a higher blood concentration of the hormones cortisol, adrenaline and noradrenalin (CHARMANDARI et al., 2005) with negative effects over the welfare, health and production of pigs (SUTHERLAND et al., 2005), among them, immunosupressed leaving them susceptible to diseases, especially the enteric which cause high mortality of piglets and depressed growth performance causing enormous economic losses to the pig farmers (MOESER et al., 2007).

Phytotherapy or homeopathic produtcs, such as, the *Matricaria chamomilla*, have been used to decrease stress. Moreover, apigenin the active principle of chamomilla, decreases cortisol decreases (YAMADA et al., 1996), and has sedative, anxiolytic (AVALLONE et al., 2000) and immunomodulatory effects (GHONIME et al., 2011).

To reduce the stress of animals in production systems, phytoterapic or homeopathic products have been used. Among them is *Matricaria chamomilla* CH_{12} that reduced by 38.4% the stress in cattle that was handling repeatedly in the corral (REIS et al., 2006).

Despite the knowledge of the effects of *Matricaria chamomilla* CH_{12} in reducing the stress in cattle, that effect has not been tested in pigs. Therefore, a trial was designed to evaluate the effect of *Matricaria chamomilla* CH_{12} in the stress of weaning in pigs.

MATERIAL AND METHODS

The experiment was conducted in the Swine Experimental Farm of the Universidade do Oeste Paulista, Presidente Prudente, SP, Brazil, with annual average temperature of 25 ºC in intensive production system.

We used 16 male pigs Landrace animals with 28 days of age, adapted to the local environmental conditions and fed *ad libitum* with a balanced feed according to the National Research Council (1998) randomly divided into two groups (8 animals/group), the Control group (Gc) received diet without addition *Matricaria chamomilla* CH_{12} and Treated group (GMc) received diet containing *Matricaria chamomilla* CH_{12}. The average daily consumption of *Matricaria chamomilla* CH_{12} per pig was 2.0 grams. The quantity of this diet consumed by the animals was estimated by incorporating *Matricaria chamomilla* CH_{12} into diet every and calculating the mean individual diet consumption (daily difference between diet weight offered and amount left after 24 h decided by the number of animals).

Fourteen days before weaning, pigs in the group G_{Mc} began receiving *Matricaria chamomilla* CH_{12} (Fator Estresse®) produced by Arenales Fauna e Flora, Presidente Prudente, SP, Brazil) for adaptation and adjustment of the intake of the product and after that began the experimental period of 30 day.

Matricaria chamomila CH_{12} (Fator Estresse; Arenales Fauna e Flora) is composed of *Matricaria chamimilla* CH_{12}, milk CH_{12}, *Bixa orellana* and sucrose (100 g).

Blood samples were collected by jugular vein from pigs on days 1, 14, 24 and 30 after weaning, always in the morning and centrifuged at 525 g for 10 minutes to obtain serum samples for determination of serum cortisol using a commercial kit for solid phase radioimmunoassay (DPC-Diagnostic Products Corporation, USA) and counted in an Auto-Gamma Counter Cobra II.

The serum cortisol concentration between groups was compared and the analysis of variance was applied by F test and means compared by the Tukey's test. For the comparison of serum cortisol, was applied the regression analysis of the data of cortisol (Y) in terms of days of collection (X). In all tests was considered the probability of error of 5% (BANZATTO; KRONKA, 2006).

RESULTS AND DISCUSSION

The serum cortisol concentration has been used as an indicator of stress in pigs (SHEN et al., 2012; WESCHENFELDER et al., 2012). Hessel et al. (2006), Radostits et al. (2007) considered the concentration of cortisol basal/normal for the pigs in good health up to 3.3 µg/dL and the animals that have concentrations above this number are considered as stressed.

In **Figure 1** shows that the piglets were stressed out the next day (day 1) weaning because the serum cortisol concentration was considered above the baseline (≤ 3.3 mg/dL), indicating neuroendocrine stress response consists of the activation of the HPA axis, witch results in the secretion of cortisol by the adrenal gland (CHARMANDARI et al., 2005; MATTERI et al., 2000). Therefore, weaning piglets can be considered the stressor (KICK et al., 2012), but by a natural condition of life, there was also an adaptation of animals to this condition due to significant linear reduction (R^2 = 0.88, P < 0.01) in the serum concentration of cortisol during the experimental period (HOPSTER et al., 1999; COOK et al., 2000).

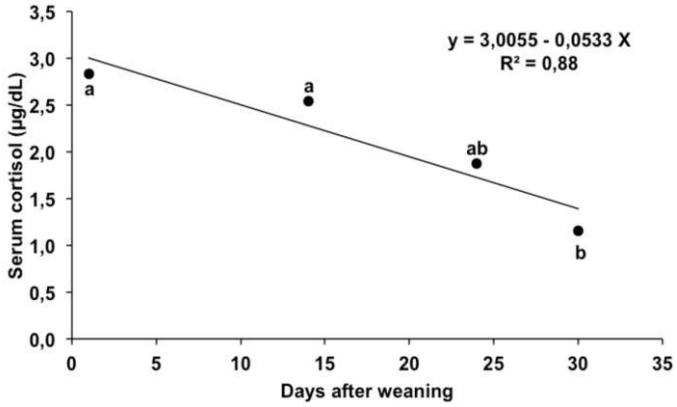

Figure 1. Effect of days after weaning in serum cortisol. Different letters indicate significant difference between days after weaning (F = 5.66, P < 0.01).

Despite the animals to adapt to adverse conditions of weaning, it is observed in **Figure 2** that the addition of *Matricaria chamamilla* CH_{12} (Fator Estresse®) in the diet of piglets is beneficial to decrease stress, because serum cortisol piglets group GMc remained within the normal range (≤ 3.3 mg/dL) during the experimental period, showing up well, no stress.

Other interesting results and biological effect that reinforces the ability of *Matricaria chamomilla* CH_{12} (Fator Estresse®) in decreasing the stress of weaning pigs, concentration cortisol shown was 61.8%, 43.5%, 77.2% and 32.0% lower than the Gc group on days 1, 14, 24 and 30 after weaning, respectively (**Figure 2**). Similar response to that was described by Reis et al. (2006) as a reducer 38,4% of the stress by management of cattle. Otherwise, there was no significant difference (P>0.05) in serum cortisol of pigs in both groups Gc and GMc (**Figure 2**), probably due to the high coefficient of variation of 138.9% for the experimental groups.

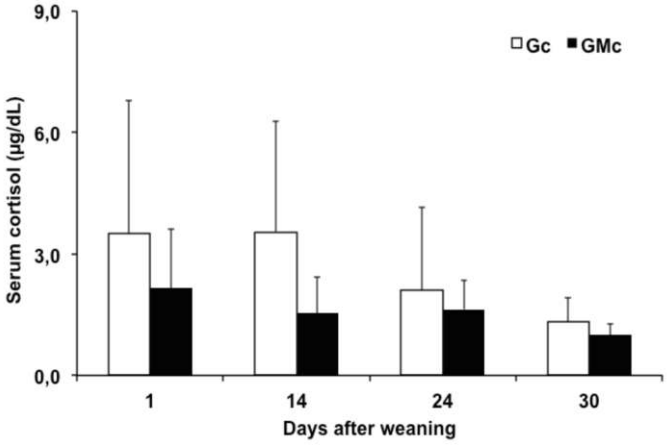

Figure 2. Average serum cortisol concentrations (±SD) of pigs after weaning without supplemental (Gc) or supplemented with *Matricaria chamomilla* CH_{12} (Fator Estresse ®, Arenales, SP, Brazil) (GMc) group. There was no significant difference (P>0.05) in serum cortisol between the experimental groups.

An interesting fact that was also observed that with the use of *Matricaria chamomilla* CH_{12} (Fator Estresse®) became the animals more docile, facilitating their management.

The physiological mechanisms that are involved in the biological action of *Matricaria chamomilla* CH_{12} resulted in decreased serum cortisol concentrations of pigs in the GMc group, was not determined in this experiment due to limited work carried out. However, in other animals, it is known that the flavonoid apigenin contained in *Matricaria chamomilla* herbal (PALADINE et al., 1999; WASOWSKI et al., 2002; MARDER et al., 2003) was found to act on the nervous system by diverse and complex mechanism ligands for the benzodiazepine receptors (MEDINA et al., 1998; GOUTMAN et al., 2003).

It is very important to the success in swine production the animals left without stress because the stress of weaning, causes great damage in the welfare and health of the pigs to make them immunosupressed leaving them susceptible to diseases, especially the enteric ones caused by *Escherichia coli* and *Clostridium difficile*, which cause high mortality of piglets, causing enormous economic losses to the pig farmers (MOESER et al., 2007; BLIKSLAGER, 2007).

CONCLUSION

The conditions of the experiment that was carried out allowed to conclude that: *Matricaria chamomilla* CH_{12} showed biological effect for the reduction of the stress of weaning pigs.

ACKNOWLEDGMENTS

The author would like to thank the "Homeopatia Arenales Fauna e Flora, Presidente Prudente, SP, Brazil.

REFERENCES

AVALLONE, R.; ZANOLI, P.; PUIA, G.; KLEINSCHNITZ, M.; SCHREIER, P.; BARALDI, M. Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. **Biochemical Pharmacology**, v.59, n.11, p.1387-1397, 2000. doi:10.1016/S0006-2952(00)00264-1

BANZATTO, D.A.; KRONKA, S.N. **Experimentação agrícola**. Jaboticabal: FUNEP, 2006.

BLIKSLAGER, A.T. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. **American Journal of Physiology. Gastrointestinal and Liver Physiology**, v.292, n.1, p.173-181, 2007. http://dx.doi:10.1152/ajpgi.00197.2006

CHARMANDARI, E.; TSIGOS, C.; CHROUSOS, G. Endocrinology of the stress response. **Annual Review Physiology**, v.67, p.259-284, 2005. http://dx.doi:10.1146/annurev.physiol.67.040403.120816

COOK, C.J.; MELLOR, D.J.; HARRIS, P.J.; INGRAM, J.R.; MATTHEWS, L.R. Hands-on and hands-off mensuriment of stress. In: MORBERG, G.P.; MENCH, J.A. **The biology of animals stress: basic principles and implications for animal welfare**.

New York: CABI Publishing, 2000. p.123-146. http://dx.doi.org/10.1079/9780851993591.0123

GOUTMAN, J.D.; WAXEMBERG, M.D.; DOÑATEI-OLIVER, F; POMATAA, P.E.; CALVO, D.J. Flavonoid modulation of ionic currants mediated by $GABA_A$ and $GABA_C$ receptors. **European Journal of Pharmacolology**, v.461, n.2-3, p.79-87, 2003. http://dx.doi.org/10.1016/S0014-2999(03)01309-8

GHONIME, M.; ELDOMANY, R.; ABDELAZIZ, A.; SOLIMAN, H. Evaluation of immunomodulatory effect of three herbal plants growing in Egypt. **Immunopharmacology and immunotoxicology**, v.33, n.1, p.141-145, 2011. doi: 10.3109/08923973.2010.487490.

HESSEL, E.F.; REINERS, K.; VAN DEN WEGH, H.F. Socializing piglets before weaning: effects on behavior of lactating sows, pre and post weaning behavior, and performance of piglets. **Journal of Animal Science**, v.84, n.10, p.2847-2855, 2006. http://dx.doi:10.2527/jas.2005-606

HOPSTER, H.; VAN DER WERF, J.T.N.; ERKENS, J.H.F.; BLOKHUIS, H.J. Effects of repeated jugular puncture on plasma cortisol concentrations in loose-housed dairy cows. **Journal of Animal Science**, v.77, n.3, p.708-714, 1999. http://dx.doi.org/10.2527/1999.773708x

KICK, A.R.; TOMPKINS, M.B.; FLOWERS, W.L.; WHISNANT, C.S.; ALMOND G.W. Effects of stress associated with weaning on the adaptive immune system in pigs. **Journal of Animal Science**, v.90, p.649-656, 2012. http://dx.doi:10.2527/jas.2010-3470

MARDER, M.; VIOLA, H.; WASOWSHI, C.; FERNÁNDEZ, S.; MEDINA, J.H.; PALADINI, A.C. 6-methylapigenin and hesperidin: new valeriana flavonoids with activity on the CNS. **Pharmacology, Biochemistry, and Behavior**, v.75, n.3, p.537-545, 2003. http://dx.doi.org/10.1016/S0091-3057(03)00121-7

MATTERI, R.L.; CARROLL, J.A.; DYER, C.J. Neuroendocrine responses to stress. In: MORBERG, G.P.; MENCH, J.A. **The biology of animals stress: basic principles and implications for animal welfare**. New York: CABI Publishing, 2000. p.43-76. http://dx.doi.org/10.1079/9780851993591.0043

MEDINA, J.H.; VIOLA, H.; WOLFMAN, C.; MARDER, M.; WASOWSKI, C.; CALVO, D.; PALADINI, A.C. Neuroactive flavonoids: new ligands for the benzodiazepine receptors. **Phytomedicine**, v.5, n.3, p.235-243, 1998. http://dx.doi:10.1016/S0944-7113(98)80034-2

MOESER, A.J.; KLOK, C.V.; RYAN, K.A.; WOOTEN, J.G.; LITTLE, D.; COOK, V.L.; BLIKSLAGER, A.T. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. **American Journal of Physiology. Gastrointestinal and Liver Physiology**, v.292, n.1, p.173-181, 2007. http://dx.doi:10.1152/ajpgi.00197.2006

NATIONAL RESEARCH COUNCIL. **Nutrient requirements of swine**. National Washington: Academy Press, 1998.

PALADINE, A.C.; MARDER, M.; VIOLA, H.; WOLFMAN, C.; WASOWSKI, C.; MEDINA, J.H. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. **Journal of Pharmacy and Pharmacology**, v. 51, n.5, p.519-526, 1999. http://dx.doi.org/10.1211/0022357991772790

RADOSTITS, O.M.; GAY, C.C.; HINCHCLIFF, K.; CONSTABLE, P.D. 2007. **Veterinary medicine**. London: Sauders Elsever, 2007. p.107-110.

REIS, L.S.L.S.; PARDO, P.E.; OBA, E.; KRONKA, S.N.; FRAZATTI-GALLINA, N.M. *Matricaria chamomilla* CH_{12} decreases handling stress in Nelore calves. **Journal of Veterinary Science**, v.7, n.2, p.189-192, 2006. http://dx.doi:10.4142/jvs.2006.7.2.189

SHEN, Y.B.; VOILQUÉ, G.; ODLE, J.; KIM, S.W. Dietary L-Tryptophan supplementation with reduced large neutral amino acids enhances feed efficiency and decreases stress hormone secretion in nursery pigs under social-mixing stress. **Journal of Nutrition**, v.142, n.8, p.1540-1546, 2012. http://dx.doi:10.3945/jn.112.163824

SOTO, F.R.M.; VUADEN, E.R.; BENITES, N.R.; AZEVEDO, S.S.; PINHEIRO, S.R.; COELHO, C.P.; VASCONCELLOS, S.A. Avaliação dos índices zootécnicos de uma granja comercial com a utilização do tratamento homeopático. **Veterinária e Zootecnia**, v.15, p.100-115, 2008.

SUTHERLAND, M.A.; RODRIGUEZ-ZAS, S.L.; ELLIS, J.L.; SALAK-JOHNSON, M. Breed and age affect baseline immune traits, cortisol, and performance in growing pig. **Journal of Animal Science**, v.83, n.9, p.2087-2095, 2005. http://dx.doi.org/10.2527/2005.8392087x

WASOWSKI, C.; MARDER, M.; VIOLA, H.; MEDINA,

J.H.; PALADINI, A.C. Isolation and identification of 6-methylapigenin, a competitive ligand for the brain $GABA_A$ receptors, from *Valeriana wallichii*. **Planta Medica**, v.68, n.10, p.934-936, 2002. http://dx.doi:10.1055/s-2002-34936

WESCHENFELDER, A.V.; TORREY, S.; DEVILLERS, N.; CROWE, T.; BASSOLS, A.; SACO, Y.; PIÑEIRO, M.; SAUCIER, L.; FAUCITANO, L. Effects of trailer design on animal welfare parameters and carcass and meat quality of three Pietrain crosses being transported over a long distance. **Journal of Animal Science**, v.90, n.9, p.3220-3231, 2012. http://dx.doi:10.2527/jas.2012-4676

YAMADA, K.; MIURA, T..; MIMAKI, Y.; SASHIDA T. Effect of inhalation of Camomilla oil vapour on plasma ACTH level in ovariectomized-rat under restriction stress. **Biological & Pharmaceuthical Bulletin**, v.19, n.9, p.1244-1246, 1996. http://dx.doi.org/10.1248/bpb.19.1244

Recebido para publicação em 13/04/2014
Revisado em 17/03/2015
Aceito em 23/05/2016