**Review: Microbial endocrinology: Intersection of microbiology and neurobiology matters to swine health from infection to behavior**

Joshua M. Lyte and Mark Lyte

*Animal* journal

**Supplementary Material S1: Microbial endocrinology-based mechanisms in society and culture**

Non-nutritional feed additives are frequently regulated by governmental bodies, which impose strict no-tolerance policies of considerable economic impact on the import of pork tainted by banned substances. Ractopamine, one such non-nutritional feed additive, is banned in mainland China and the European Union but permitted in the United States, Japan, and some other countries. A beta-adrenergic agonist, ractopamine offers numerous benefits of economic value to the swine producer, including improved carcass leanness and feed efficiency (Apple, 2007). The effects of ractopamine on different stress parameters in swine, including norepinephrine and epinephrine are inconsistent. For example, although in-feed ractopamine treatment has been reported to more than double porcine resting plasma catecholamine concentrations (Marchant-Forde *et al.*, 2003), others have reported no such effect (Poletto *et al.*, 2010, Puls *et al.*, 2015). Moreover, dose-dependent effects of ractopamine were found to increase epinephrine but not norepinephrine following a stressor, such as handling or transport. After 34 days of in-feed ractopamine treatment, pigs were experimentally infected with *Salmonella typhimurium* and studied for 4 days post-infection (Edrington *et al.*, 2006). Interestingly, fecal shedding of *S. typhimurium* was lower each day post-infection in ractopamine-fed pigs compared to control animals which did not receive ractopamine. In addition, the authors found non-statistically significant effects of ractopamine on reducing *Salmonella*-positive ileocecal lymph nodes and liver samples. Importantly, *in vitro* incubation with 2μg/mL ractopamine was found to increase the growth of *S. choleraesuis* but not *S. typhimurium* in pure culture, an effect which likely requires further investigation in a medium similar to the porcine gastrointestinal *in vivo* environment. Not a single study was identified in the construction of this review which examined an effect of ractopamine on the swine microbiome, either from a compositional or functional standpoint.

 Relatedly, one of the world’s largest pork producers, Smithfield Foods, once an American company was recently acquired by WH Group, formerly known as Shuanghui International Holdings Ltd., a Chinese entity. While Smithfield still receives ractopamine-fed pigs from outside suppliers, on the Smithfield company site it is stated that ractopamine has been eliminated entirely from the feed of company-owned animals (Smithfield Foods, 2015). Although ractopamine is officially banned in China, Chinese swine can be exposed to other, likewise banned, in-feed beta-adrenergic agonists such as clenbuterol (Yan, 2015). Like ractopamine, clenbuterol provides economic incentive to swine producers as it increases lean gain in pigs. Clenbuterol has been shown to increase norepinephrine in swine (Mersmann, 1989), however any effect on host-microbe interaction, pathogen growth or invasion, or microbiome is unknown. Therefore, unique socio-cultural and economic motivations in different countries may expose swine to distinct in-feed non-nutritive additives which can impact porcine stress physiology and potentially alter host-microbe interactions.

 Farm structure and feed supply sources can vary significantly between countries. This is important as the nutritional composition of grains, soybeans, and other feed components may differ depending on crop growing conditions, and the effect of diet on microbiome composition and function is well-known. Global and regional leaders in pork production often source feed supplies from vastly different geographical origins. For example, within the European Union, France and Germany export feed grain to the Netherlands which has been estimated to import approximately 90% of its feed grain whereas 80% of feed grain in Denmark is domestically-sourced (Willems, 2016). China utilizes several feed components, including maize, dried distiller’s grains, wheat, soybean, and others which rely on both domestic-production and importation (Hoste *et al.*, 2013). Although little is known regarding how the chemical compositions of feed components grown in China vary between domestic regions (Li *et al.*, 2015) and compare to those grown in other countries (Huang, 2017), such comparisons may illuminate a relationship between incidence of swine disease and feed composition. Indeed, several naturally-occurring amino acids, minerals, prebiotic carbohydrates, and other components of feed ingredients have demonstrated roles in affecting host stress (Shen *et al.*, 2012), immune response (Medardus *et al.*, 2014, Schulte *et al.*, 2016), as well as microbiome composition (Umu *et al.*, 2015).

**References**

Apple JK, Rinckert PJ, McKeith FK, Carr SN, Armstrong TA and Matzat PD 2007. Meta-Analysis of the Ractopamine Response in Finishing Swine. The Professional Animal Scientist 23, 179-196.

Edrington TS, Callaway TR, Smith DJ, Genovese KJ, Anderson RC and Nisbet DJ 2006. Effects of ractopamine HCl on Escherichia coli O157:H7 and Salmonella in vitro and on intestinal populations and fecal shedding in experimentally infected sheep and pigs. Current Microbiology 53, 82-88.

Hoste R, Hu D and Tolkamp J 2013. Investment and export opportunities in a sustainable pig supply chain in China. LEI Wageningen UR, The Hague, Netherlands.

Huang CF, Stein HH, Zhang LY, Li D and Lai CH 2017. Concentrations of minerals in pig feed ingredients commonly used in China. Translational Animal Science 1, 126-136.

Li P, Li DF, Zhang HY, Li ZC, Zhao PF, Zeng ZK, Xu X and Piao XS 2015. Determination and prediction of energy values in corn distillers dried grains with solubles sources with varying oil content for growing pigs. Journal of Animal Science 93, 3458-3470.

Marchant-Forde JN, Lay DC Jr., Pajor EA, Richert BT and Schinckel AP 2003. The effects of ractopamine on the behavior and physiology of finishing pigs. Journal of Animal Science 81, 416-422.

Medardus JJ, Molla BZ, Nicol M, Morrow WM, Rajala-Schultz PJ, Kazwala R and Gebreyes WA 2014. In-feed use of heavy metal micronutrients in U.S. swine production systems and its role in persistence of multidrug-resistant salmonellae. Applied and Environmental Microbiology 80, 2317-2325.

Mersmann HJ 1989. Influence of infused beta-adrenergic agonists on porcine blood metabolites and catecholamines. Journal of Animal Science 67, 2633-2645.

Poletto R, Meisel RL, Richert BT, Cheng HW and Marchant-Forde JN 2010. Behavior and peripheral amine concentrations in relation to ractopamine feeding, sex, and social rank of finishing pigs. Journal of Animal Science 88, 1184-1194.

Puls CL, Trout WE, Ritter MJ, McKeith FK, Carr SN and Ellis M 2015. Impact of ractopamine hydrochloride on growth performance, carcass and pork quality characteristics, and responses to handling and transport in finishing pigs. Journal of Animal Science 93, 1229-1238.

Schulte JN, Brockmann GA and Kreuzer-Redmer S 2016. Feeding a high dosage of zinc oxide affects suppressor of cytokine gene expression in Salmonella Typhimurium infected piglets. Veterinary Immunology and Immunopathology 178, 10-13.

Shen YB, Voilque G, Odle J and Kim SW 2012. 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 142, 1540-1546.

Smithfield Foods 2015. 2015 Sustainability & Financial Report: Ractopamine. In Smithfield Foods, Smithfield, VA USA.

Umu OC, Frank JA, Fangel JU, Oostindjer M, da Silva CS, Bolhuis EJ, Bosch G, Willats WG, Pope PB and Diep DB 2015. Resistant starch diet induces change in the swine microbiome and a predominance of beneficial bacterial populations. Microbiome 3, 16.

Willems J, van Grinsven H, Jacobsen BH, Jensen T, Dalgaard T, Westhoek H and Kristensen IS 2016. Why Danish pig farms have far more land and pigs than Dutch farms? Implications for feed supply, manure recycling and production costs. Agricultural Systems 144, 122-132.

Yan H, Xu D, Meng H, Shi L and Li L 2015. Food poisoning by clenbuterol in China. Quality Assurance and Safety of Crops & Food 7, 27-35.