Physiology International 109 (2022) 3, 348–355
DOI: 10.1556/2060.2022.00042

AKADÉMIAI KIADÓ

Effect of ractopamine on the release of dopamine from the striatum dissected from mice

ERIKA G. BÁN*, ESZTER A. DHO-NAGY and ATTILA M. BRASSAI

Department ME1, Faculty of Medicine in English, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures, Targu-Mures, Romania

Received: February 24, 2022 • Revised manuscript received: April 4, 2022 • Accepted: May 16, 2022
Published online: September 5, 2022

ABSTRACT

In the past two decades, ractopamine has been used as a feed additive to increase protein synthesis in farmed animals (swine, cattle, and turkeys) and to produce high-quality meat. However, the excessive feeding of animals with ractopamine may result in its accumulation in animal and human tissues after consuming the meat. Ractopamine is a trace amine-associated receptor1 and β-adrenoceptor agonist banned in the EU but approved in the USA, and it may pose a potential risk to human health. In this paper, the authors, for the first time, provide neurochemical evidence that ractopamine leads to the release of dopamine from nerve terminals of the nigrostriatal pathway in the striatum.

KEYWORDS

ractopamine, transmitter release, noradrenaline, dopamine, β-adrenoceptor, nomifensine, transporter, striatum

INTRODUCTION

Ractopamine, a synthetic biogenic amine (4-(1-hydroxy-2-{[4-(4-hydroxyphenyl)butan-2-yl] amino}ethyl)phenol) (Fig. 1), is approved in the US and several other countries but is banned in more than 100 countries, including the European Union, as a livestock feed additive used to increase muscle quality and limit fat deposition [1]. Ractopamine is a selective TAAR1 agonist [2] and has β-adrenoceptor agonist activity [3].

While most studies with ractopamine have mainly focused on exploring its effects on meat quality, there is very little information on its activities in the central nervous system (CNS) [4].

* Corresponding author. E-mail: banerik@live.com

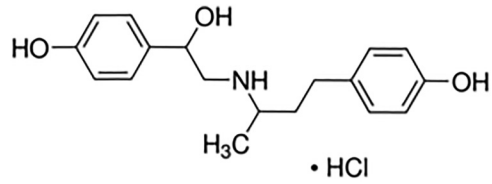

Fig. 1. Structure of ractopamine

The possible link between neurodegenerative diseases such as Parkinson and Alzheimer have been mentioned [5, 6]. Nevertheless, ractopamine does enter into the central nervous system (CNS), as it has been shown that ractopamine increases aggressive behaviour in female pigs [7], indicating its effect on the CNS [8].

It has been reported that there is a link between the chronic use of β-adrenoceptor antagonists, such as propranolol, and an increased risk of Parkinson's disease [5, 9]. This association was supported by clinical observation that chronic β-adrenoceptor agonist administration [10] dose-dependently decreased the risk of neurodegenerative diseases: Parkinson's and Alzheimer's. Furthermore, biochemical evidence was obtained in human tissue that cytosolic sulfotransferase plays an important role in the detoxification of ractopamine [11]. Cytosolic sulfotransferase is a human enzyme involved in the metabolism of catecholamines [12], such as dopamine [11].

In Parkinson's disease (PD) [1], the dysfunction of nigrostriatal dopaminergic and cholinergic interneurons [13–16] is associated with symptoms that develop only after the development of dopamine (DA) deficiency in the extrapyramidal system (caudate, putamen and substantia nigra), suggesting that drugs able to increase the release of DA in the extrapyramidal system would be beneficial in the treatment of PD. A hypothesis was raised that ractopamine, the feed additive, might present protective consequences for Parkinson disease and reduce its incidence in the population ingesting meat containing ractopamine residue [6].

This study aimed to examine the effects of ractopamine on the release of dopamine from nerve terminals of the nigrostriatal pathway in ex vivo striatal slice preparations.

MATERIALS AND METHODS

The experiments were carried out in strict accordance with institutional guidelines, including the European Directive (2010/63/EU). The animals (CD1 mouse of both sexes) are bred and kept in the local animal facility.

The incubation and superfusion medium used in this study contained the following (in mM): NaCl 118, KCI 4.7, $CaCI_2$ 1,4, $NaHCO_3$ 25, KH_2PO_4 1.25, $MgSO_4$ 1.25, glucose 11.5, ascorbic acid 0.2, and pargyline 0.02; the medium was saturated with 5% CO_2 in O_2.

All experiments were performed at 37 °C in a Krebs solution (pH 7.4) containing 118 mM NaCl, 4.7 mM KCI, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 12.5 mM glucose that was continuously saturated with 95% O_2 + 5% CO_2. The Krebs solution

contained 10 μM pargyline. A thermoelectric device (Frigomix, B. Braun, Germany) was used to keep the temperature of the bath solution at 37 °C.

Chemicals

All chemicals were obtained from Tocris Cookson Inc. (Bristol, UK.) The radioactive compound was purchased from American Radiolabeled Chemicals, Inc. (USA) [^{3}H]-dopamine specific activity = 60 Ci mmol^{-1}.

The release of dopamine from mouse striatal slices

The striatum dissected from mice (30–45 g weight) was sliced into 400 μm thick sections, and the slices were incubated in the presence of 3 μCi mL^{-1} (5×10^{-8} M) [^{3}H]-dopamine (dopamine-3,4[7,8-3H(N)], specific activity: 60 Ci mmol^{-1}) at 37 °C for 45 min in 1 mL of Krebs solution. After the loading period, the tissue was washed several times, transferred onto a thermoregulated superfusion apparatus [17], and superfused at 37 °C with a 95% O_2 + 5% CO_2 saturated Krebs solution. After a 30-min preperfusion (flow rate: 0.5 mL min^{-1}), effluent samples were collected every three minutes. During the collection of the 3rd (9 min) and 13th (39 min) samples, the tissue was stimulated with square-wave impulses using a Grass 88 stimulator (30 V, 2 Hz, 1 ms impulse duration for 1 min (120 shocks)). Ractopamine was administered as indicated and kept in the solution until the end of the experiment. The fractional release (FR) due to R1 (FRR1) or S1 (FRS1) served as an internal control. At the end of the experiment, the tritium content of the tissue was determined as previously described [18] and was expressed in terms of disintegrations per gram of tissue (Bq/g). The FRS1 and FRS2 of [^{3}H]-dopamine release were calculated as the percentage of the total radioactivity present in the tissue at rest (S1, S2). The basal resting release was determined during the collection periods before (FRR1) and during (FRR2) ractopamine administration.

The slices were solubilised at the end of the experiment, and radioactivity content was determined [19, 20] to calculate fractional release as a percentage of the radioactivity present in the slices at the beginning of the stimulation period. The effect of ractopamine on the resting release was expressed as the ratio of the fractional release (FR) values for the second (R_2) and first (R_1) samples (FRR_2/FRR_1). The radioactivity of the fraction and the tissue was measured with liquid scintillation spectrometry after the addition of the appropriate scintillation fluids. The evoked release was calculated by subtracting the values of the basal release from the total release during the stimulation period.

Statistical analysis

The statistical significance of the results was determined by Student's *t*-test or by a two-way analysis of variance (ANOVA) with subsequent comparisons by Dunn's test. Statistical significance was set at $P < 0.05$. The data presented are the means ± SEM.

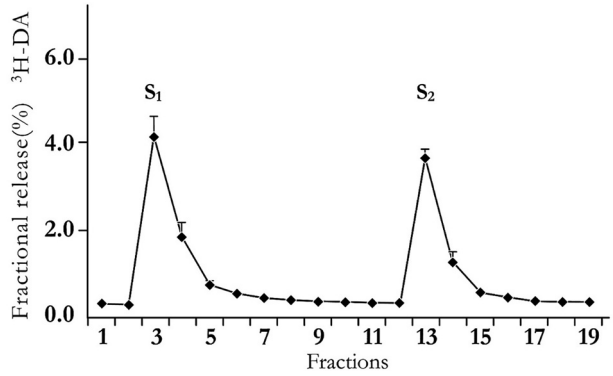

Fig. 2. Resting and stimulation-evoked fractional release of [^{3}H]-dopamine from striatal slice preparations. This tissue was loaded with [^{3}H]DA (for details see Materials and Methods). The stimulation ($2H_2$, 120 shocks)-evoked release of [^{3}H]-dopamine is $[Ca^{2+}]_o$-dependent and N-type of calcium channel is involved [29]

Table 1. Effect of ractopamine on resting release of [^{3}H]-dopamine in *ex vivo* striatal slice preparation

		n	FRR_1%	FRR_2/FRR_1	Signif.
1.	Control	8	0.42 ± 0.04%	0.81 ± 0.05	
2.	Ractopamine, 10 μM	6	0.68 ± 0.09%	2.45 ± 0.16	2:1 < 0.05
3.	Nomifensine, 10 μM	4	0.61 ± 0.03%	0.85 ± 0.12	3:1 > 0.05
4.	Nomifensine 10 μM + Ractopamine 10 μM	6	0.88 ± 0.04%	1.10 ± 0.08	4:2 > 0.05

Ractopamine was administered at 8th fraction after R1 measured as the average of first and second collection and kept in the perfusion fluid until the end of the experiments. Nomifensine was added 6 min before ractopamine and both of them were left in the perfusion Knebb solution throughout the experiments.

For the experimental procedure, see Fig. 2 The %FR of [^{3}H]-dopamine evoked by ractopamine (10 μM) in the striatum.

RESULTS

Release of dopamine induced by ractopamine

The radioactivity uptake was 2.02 ± 0.17 10^6 Bq/g, and the release at rest was 0.42 ± 0.04% (FRR_1). Field stimulation (2 Hz, 120 impulses) increased the fractional (%FR) release of [^{3}H]-dopamine (FRS_1 = 5.43 ± 0.78%, $P < 0.05$) (Fig. 2). Both resting (FRR_2/FRR_1) and stimulation-evoked (FRS_2/FRS_1) releases were maintained. Ractopamine (10 μM) significantly enhanced the resting release (Table 1). The release at rest (C) was significantly augmented (FRR_2/FRR_1 = 2.45 ± 0.16 vs. control value 0.81 ± 0.05, $P < 0.05$, n = 6). Nomifensine (10 μM), a selective DAT blocker able to potentiate stimulation-evoked release [20], significantly prevented the effects of ractopamine on DA release (Table 1), indicating the role of DAT *n* the release.

DISCUSSION

While ractopamine has been approved by the US Food and Drug Administration as a feed additive for use as a growth promoter in farmed animals, this compound is a topic of worldwide debate and is still on the list of banned substances in the European Union and China [21]. Very few behavioural effects were observed in response to ractopamine administration. It was shown that ractopamine increases aggressive behaviour in female pigs [7], indicating its effect on the CNS [8]. There were further hypotheses on the association between the use of Ractopamine and its role in neurodegenerative diseases in humans, such as Alzheimer's and Parkinson's disease [6]. Nevertheless, convincing evidence has been observed that ractopamine is a selective agonist of TAAR1 receptors [2]. Furthermore, TAAR1 receptors agonists (β-phenylethylamine, 3,4-methylenedioxymethamphetamine, mephedrone) have been shown that able to release DA from the nucleus accumbens [22] in a dopamine transporter inhibitable manner.

The fact that our experiments, for the first time, provided neurochemical evidence that ractopamine administration leads to the resting release of dopamine from nerve terminals of the nigrostriatal pathway in the striatum al slice preparations (Table 1) raises the question of whether the consumption of meat obtained from livestock treated with ractopamine will lead to the accumulation of ractopamine in humans, which as ractopamine is a pharmacologically active compound involved in cytosolic sulfotransferase activity, may pose a potential health risk factor or would be beneficial in the disease. Our results with 10 μM ractopamine, although exceeds the concentration calculated for livestock flesh of about 1.25 μM based on feed of 200 mg/head per day [2] is an indication that ractopamine consumed may be able to accumulate in humans.

Nomifensine, a selective dopamine transporter (DAT)-blocking drug, inhibited the effect of ractopamine on the release of DA (Table 1), indicating the important role of this transporter in the nonsynaptic communication in the brain [19]. This interaction is similar to the mechanism described for NMDA [22].

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 0.3% of the general population worldwide and approximately 1% of the older population. It is pathologically characterized by a loss of nigrostriatal dopaminergic neurons that leads to characteristic voluntary motor impairments [23]. There is clinical evidence that levodopa and a few DA agonists mimicking the effect of endogenous DA are beneficial in the treatment of motor impairments in PD [1, 24]. In addition, treatment with β-agonists has shown that there is an inverse correlation between an increased risk of PD and the use of β-adrenoceptor-blocking drugs [25]. It has to be mentioned that ractopamine is also a β-adrenoceptor agonist [21, 26].

In the extrapyramidal system DA release from the nigrostriatal boutons inhibits the release ACh from cholinergic interneurons [27] via stimulation of D_2 receptors [28–30]. A similar interaction was found between NA and acetylcholine (ACh) in the gastrointestinal tract, in which NA inhibits the release of ACh via the activation of α2 heteroreceptors, an observation demonstrated to be the first neurochemical evidence of presynaptic inhibition. In contrast, the release of NA inhibits its own release through α2 autoreceptors but fails to reduce the release of ACh from vagal nerve endings [31, 32]. There is a one-sided interaction between sympathetic and vagal innervation at the level of presynaptic axon terminals. In addition to its negative feedback modulation, ACh inhibits the release of NA through muscarinic receptor stimulation

[31]. This interaction seems to be important in the light of ractopamine with effect on β-adrenoceptors expressed in the heart [3].

The recent finding that ractopamine is a full mTAAR1 agonist constitutes a novel mechanism by which ractopamine can influence the physiology and behaviour of pigs [7, 8].

Dopamine receptor agonists, such as apomorphine given as monotherapy and in combination with levodopa [33], were found to be beneficial in the treatment of Parkinson's disease. MAO-B inhibitors such as selegiline and rasagiline as alternatives to levodopa were also found to be a good choice for treating patients with Parkinson's disease [34]. Hársing et al. have shown that selegiline is capable of inhibiting DA uptake and releasing DA from isolated striatal slice preparations, providing an explanation of its beneficial effect in PD [13].

The central nervous system effects associated to the consumption of meat containing this additive cannot be anticipated, since agent-related food poisonings are rarely encountered, and long-term utilization impact raises multiple questions [35].

The exact aetiology of PD is not yet known, and there is still no preventive strategy available. In this respect, the effect of ractopamine on DA release in the striatum seems to be interesting and raises the question of its application in PD. Nevertheless, its side effects on the CNS when it enters the blood–brain barrier and affects neurochemical transmission increasing DA release would be a potential risk for normal brain function. According to a recent scientific report for European Commission [36] further studies are needed to reach the final conclusion as far as the future of this livestock additive is concerned.

ACKNOWLEDGEMENTS

We would like to thank Ms. Kati Windisch for her technical assistance and the Institute of Experimental Medicine for scientific and technical help in the experiments performed at the institute.

REFERENCES

1. Armstrong MJ, Okun MS. Diagnosis and treatment of Parkinson disease: A review. JAMA 2020; 323(6): 548–60. https://doi.org/10.1001/jama.2019.22360.
2. Liu X, Grandy DK, Janowsky A. Ractopamine, a livestock feed additive, is a full agonist at trace amine-associated receptor 1. J Pharmacol Exp Ther 2014; 350(1): 124–9. https://doi.org/10.1124/jpet.114.213116.
3. Colbert WE, Williams PD, Williams GD. β-adrenoceptor profile of ractopamine HCl in isolated smooth and cardiac muscle tissues of rat and Guinea-pig. J Pharm Pharmacol 1991; 43(12): 844–7. https://doi.org/10.1111/j.2042-7158.1991.tb03192.x.
4. Athayde NB, Dalla Costa OA, Roca RO, Guidoni AL, Ludtke CB, Oba E, et al. Stress susceptibility in pigs supplemented with ractopamine. J Anim Sci 2013; 91(9): 4180–7. https://doi.org/10.2527/jas.2011-5014.
5. Fan FS. Ractopamine residue in meat might protect people from Parkinson disease. Med Hypotheses 2020; 145: 110397. https://doi.org/10.1016/j.mehy.2020.110397.
6. Fan FS. Assessing the possible influence of residues of ractopamine, a livestock feed additive, in meat on Alzheimer disease. Dement Geriatr Cogn Dis Extra 2021; 11(2): 110–3. https://doi.org/10.1159/000515677.

7. Poletto R, Cheng HW, Meisel RL, Richert BT, Marchant-Forde JN. Gene expression of serotonin and dopamine receptors and monoamine oxidase-A in the brain of dominant and subordinate pubertal domestic pigs (Sus scrofa) fed a β-adrenoreceptor agonist. Brain Res 2011; 1381: 11–20. https://doi.org/10.1016/j.brainres.2010.11.035.
8. Marchant-Forde JN, Lay DC, Jr., Pajor EA, Richert BT, Schinckel AP. The effects of ractopamine on the behavior and physiology of finishing pigs. J Anim Sci 2003; 81(2): 416–22. https://doi.org/10.2527/2003.812416x.
9. Chen CL, Wang SY, Chen TC, Chuang CS. Association between β2-adrenoreceptor medications and risk of Parkinson's disease: A meta-analysis. Medicina (Kaunas) 2021; 57(10): 1006. https://doi.org/10.3390/medicina57101006.
10. Hopfner F, Höglinger GU, Kuhlenbäumer G, Pottegård A, Wod M, Christensen K, et al. β-adrenoreceptors and the risk of Parkinson's disease. Lancet Neurol 2020; 19(3): 247–54. https://doi.org/10.1016/S1474-4422(19)30400-4.
11. Ko JH, Strafella AP. Dopaminergic neurotransmission in the human brain: New lessons from perturbation and imaging. Neuroscientist 2012; 18(2): 149–68. https://doi.org/10.1177/1073858411401413.
12. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: A contemporary view with implications for physiology and medicine. Pharmacol Rev 2004; 56(3): 331–49. https://doi.org/10.1124/pr.56.3.1.
13. Hársing LG, Jr., Vizi ES. Release of endogenous dopamine from rat isolated striatum: Effect of clorgyline and (-)-deprenyl. Br J Pharmacol 1984; 83(3): 741–9. https://doi.org/10.1111/j.1476-5381.1984.tb16228.x.
14. Hársing LG, Jr., Vizi ES. Evidence for multiple dopamine receptors involved in the modulation of acetylcholine release in the striatum. Pol J Pharmacol Pharm 1985; 37(3): 383–96.
15. Tárczy M, Mogyorós I, Takáts A, Csanda E. Az esszenciális tremor kezelése Sertannal [Treatment of essential tremor with Sertan]. Orv Hetil 1983; 124(14): 817–20.
16. Vizi ES, Rónai A, Hársing L, Jr., Knoll J. Inhibitory effect of dopamine on acetylcholine release from caudate nucleus. Pol J Pharmacol Pharm 1977; 29(3): 201–11.
17. Vizi ES, Hársing LG, Knoll J. Presynaptic inhibition leading to disinhibition of acetylcholine release from interneurons of the caudate nucleus: Effects of dopamine, β-endorphin and d-Ala2-Pro5-enkephalinamide. Neuroscience 1977; 2(6): 953–61. https://doi.org/10.1016/0306-4522(77)90118-x.
18. Baranyi M, Milusheva E, Vizi ES, Sperlágh B. Chromatographic analysis of dopamine metabolism in a Parkinsonian model. J Chromatogr A 2006; 1120(1–2): 13–20. https://doi.org/10.1016/j.chroma.2006.03.018.
19. Román V, Kedves R, Kelemen K, Némethy Z, Sperlágh B, Lendvai B, et al. Contribution of analog signaling to neurotransmitter interactions and behavior: Role of transporter-mediated nonquantal dopamine release. Physiol Rep 2021; 9(21): e15088. https://doi.org/10.14814/phy2.15088.
20. Bán EG, Brassai A, Vizi ES. The role of the endogenous neurotransmitters associated with neuropathic pain and in the opioid crisis: The innate pain-relieving system. Brain Res Bull 2020; 155: 129–36. https://doi.org/10.1016/j.brainresbull.2019.12.001.
21. Centner TJ, Alvey JC, Stelzleni AM. Beta agonists in livestock feed: Status, health concerns, and international trade. J Anim Sci 2014; 92(9): 4234–40. https://doi.org/10.2527/jas.2014-7932.
22. Zsilla G, Hegyi DE, Baranyi M, Vizi ES. 3,4-Methylenedioxymethamphetamine, mephedrone, and beta-phenylethylamine release dopamine from the cytoplasm by means of transporters and keep the concentration high and constant by blocking reuptake. Eur J Pharmacol 2018; 837: 72–80. https://doi.org/10.1016/j.ejphar.2018.08.037.
23. Frisardi V, Santamato A, Cheeran B. Parkinson's disease: New insights into pathophysiology and rehabilitative Approaches. Parkinsons Dis 2016; 2016: 3121727. https://doi.org/10.1155/2016/3121727.
24. Dietrichs E, Odin P. Algorithms for the treatment of motor problems in Parkinson's disease. Acta Neurol Scand 2017; 136(5): 378–85. https://doi.org/10.1111/ane.12733.

25. Saengphatrachai W, Praditukrit K, Owattanapanich W, Pitakpatapee Y, Srivanitchapoom P. The association between developing Parkinson's disease and β-Adrenoceptor acting agents use: A systematic review and meta-analysis. J Neurol Sci 2021; 430: 120009. https://doi.org/10.1016/j.jns.2021.120009.
26. Vizi ES. Compounds acting on alpha 1- and alpha 2- adrenoceptors: Agonists and antagonists. Med Res Rev 1986; 6(4): 431–49. https://doi.org/10.1002/med.2610060403.
27. Milusheva EA, Dóda M, Baranyi M, Vizi ES. Effect of hypoxia and glucose deprivation on ATP level, adenylate energy charge and [Ca2+]0-dependent and independent release of [3H]dopamine in rat striatal slices. Neurochem Int 1996; 28(5–6): 501–7. https://doi.org/10.1016/0197-0186(95)00129-8.
28. Vizi ES. Interaction between adrenergic and cholinergic systems: Presynaptic inhibitory effect of noradrenaline on acetylcholine release. J Neural Transm Suppl 1974; 11: 61–78. https://doi.org/10.1007/978-3-7091-8341-0_4.
29. Hársing LG, Jr., Sershen H, Vizi SE, Lajtha A. N-type calcium channels are involved in the dopamine releasing effect of nicotine. Neurochem Res 1992; 17(7): 729–34. https://doi.org/10.1007/BF00968013.
30. Vizi ES, Kiss J, Elenkov IJ. Presynaptic modulation of cholinergic and noradrenergic neurotransmission: Interaction between them. News Physiol Sci 1991; 6(3): 119–23. https://doi.org/10.1152/physiologyonline.1991.6.3.119.
31. Manabe N, Foldes FF, Töröcsik A, Nagashima H, Goldiner PL, Vizi ES. Presynaptic interaction between vagal and sympathetic innervation in the heart: Modulation of acetylcholine and noradrenaline release. J Autonom Nerv Syst 1991; 32(3): 233–42. https://doi.org/10.1016/0165-1838(91)90117-L.
32. Löffelholz K, Muscholl E. Die Hemmung der Noradrenalin-Abgabe durch Acetylcholin am sympathisch gereizten, isolierten Kaninchenherzen [The inhibitory effect of acetylcholine on the release of noradrenaline from isolated rabbit hearts stimulated sympathetically]. Naunyn Schmiedebergs Arch Pharmakol Exp Pathol 1969; 263(1): 236–7. https://doi.org/10.1007/bf00549496.
33. Binde CD, Tvete IF, Gasemyr J, Natvig B, Klemp M. A multiple treatment comparison meta-analysis of monoamine oxidase type B inhibitors for Parkinson's disease. Br J Clin Pharmacol 2018; 84(9): 1917–27. https://doi.org/10.1111/bcp.13651.
34. Binde CD, Tvete IF, Gåsemyr JI, Natvig B, Klemp M. Comparative effectiveness of dopamine agonists and monoamine oxidase type-B inhibitors for Parkinson's disease: A multiple treatment comparison meta-analysis. Eur J Clin Pharmacol 2020; 76(12): 1731–43. https://doi.org/10.1007/s00228-020-02961-6.
35. Wu ML, Deng JF, Chen Y, Chu WL, Hung DZ, Yang CC. Late diagnosis of an outbreak of leanness-enhancing agent-related food poisoning. Am J Emerg Med 2013; 31(10): 1501–3. https://doi.org/10.1016/j.ajem.2013.07.001.
36. Bories G, Brantom P, de Barberà JB, Chesson A, Cocconcelli SP, Debski B, et al. Scientific opinion of the panel on additives and products or substances used in Animal Feed (FEEDAP) on a request from the European Commission on the safety evaluation of ractopamine. EFSA J 2009; 7(4): 1041, 52 pp. https://doi.org/10.2903/j.efsa.2009.1041.