Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Mosul, College of Veterinary Medicine

Iraqi Journal of
Veterinary Sciences

A national, scientific and refereed journal
Published by the College of Veterinary Medicine
University of Mosul

ISSN: 1607-2804

VOL. 34 NO. 2 2020

Vol. 33 No.2 2019

Publisher: College of Veterinary Medicine / University of Mosul

Email: vetmedmosul@gmail.com

Editor-in-chief: Professor Dr. Fouad K. Mohammad

Print ISSN: 1607-3894

Online ISSN: 2071-1255

Editorial Board

Editorial Board Page

Editor-in-chief

Professor Dr. Fouad K. Mohammad

Professor, Ministry of Higher Education and Scientific Research, Baghdad, Iraq

h-index: 16

Specialty: Pharmacology

Email Address: ministry_res_deputy@mohesr.gov.iq

Editorial Board

Professor Dr. Muneer S. Al-Badrany

Dean, College of Veterinary Medicine, University of Mosul, Mosul - Iraq

h-index: 4

Specialty: Veterinary Surgery

Email Address: deanvetmed@uomosul.edu.iq

Professor Dr. Najat A. Mohammed

Dean, College of Veterinary Medicine, University of Kirkuk, Kirkuk - Iraq

Specialty: Anatomy of Lymphatic Tissue

Email Address: najatagha@hotmail.com

Professor Dr. Jabbar A. Al-Saad

Dean, College of Veterinary Medicine, University of Al-Qadisiyah, AL-Qadisiyah - Iraq

Specialty: Endocrine Physiology and Reproduction

Email Address: jbr20042002@yahoo.com

Professor Dr. Saleem A. Hasso

Head, Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad - Iraq

Specialty: Internal Medicine

Email Address: saleemaaminhasso@yahoo.com

Professor Dr. Amena N. Al-Thwainy

Institute of Genetic Engineering and Biotechnology for Post Graduate Studies /Baghdad University

h-index: 5

Specialty: Medical Microbiology and Immunogenetic

Email Address: prof.althwani@ige.uobaghdad.edu.iq

Professor Dr. Ihsan M. Alsaqur

Department of Medical Laboratory Sciences, Al-Esraa University College

h-index: 9

Email Address: drihsanalsagur@yahoo.com

Professor Dr. Abdulsattar F. Majeed

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Fallujah, Fallujah - Iraq

Specialty: Animal Reproduction

Email Address: al-majeed@uofallujah.edu.iq

Professor Dr. Majid Sh. Rhymah

Department of Microbiology, College of Medicine, University of Misan, Misan - Iraq

Specialty: Internal and Preventive Medicine

Email Address: mrhaymah@yahoo.com

Volume 34, Issue 2, Summer and Autumn 2020, Page 217-400

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 247-252
DOI: 10.33899/ijvs.2019.125885.1173

Genetic detection to *Aeromonas hydrophila* proteolytic activity in milk samples (cows, buffaloes and goats) in Basra governorate

Rawa B Banay

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 253-258
DOI: 10.33899/ijvs.2019.125888.1174

Molecular identification and phylogenetic analysis of lactic acid bacteria isolated from goat raw milk

Zahra K. Saeed; Basil A. Abbas; Rasha M. Othman

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 259-263
DOI: 10.33899/ijvs.2019.125896.1176

Ovarian morphometric evolution in two consecutive estrous cycles of female rats treated with steroid-free bovine follicular fluid antiserum

Jabber A. Al-Sa’aidi; Areej H. Al-Charack

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 265-271
DOI: 10.33899/ijvs.2019.125923.1186

Effect of steroid-free follicular fluid antiserum on reproductive endocrine profile at estrous and metestrus phases in female rats

Jabber A. Al-Sa’aidi; Areej H. Al-Charack

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 273-278
DOI: 10.33899/ijvs.2019.125925.1187

Anticlastogenic properties of *Quercus infectoria* galls extract against DMBA induced genotoxicity in bone marrow cells of mice in vivo

Sherzad I Amedi; Bushra M. Mohammed

Iraqi Journal of Veterinary Sciences, *2020*, Volume 34, Issue 2, Pages 279-285
DOI: 10.33899/ijvs.2019.125930.1188

Some insights of novel COVID 19 virus: structure, pathogenicity and immunity aspects

Ammar M. Al-Aalim; Mohammad A. Hamad; Ali A. AL-ledani

Iraqi Journal of Veterinary Sciences, *2020*, Volume 34, Issue 2, Pages 287-293
DOI: 10.33899/ijvs.2020.126898.1408

Follow up the antibodies titer against Newcastle disease virus in broiler breeders using ELISA test

Fanar A. Isihak; Salah M. Hassan; Balqees Z. Shaker; Yasir A. Salih

Iraqi Journal of Veterinary Sciences, *2020*, Volume 34, Issue 2, Pages 295-299
DOI: 10.33899/ijvs.2019.125931.1189

Study of *Staphylococcus aureus* isolated from the mouth of canary

Saba A. Hussein

Iraqi Journal of Veterinary Sciences, *2020*, Volume 34, Issue 2, Pages 301-304
DOI: 10.33899/ijvs.2019.125937.1192

Inhibition of *Escherichia coli* biofilm formation by *Streptomyces sdLi* crude extract

Ahmed J. Neamah; Abdul-kareem S. Al-Al-Yassari; Mohammed A. Hamed; Miran A. AlRammahi

Iraqi Journal of Veterinary Sciences, *2020*, Volume 34, Issue 2, Pages 305-310
DOI: 10.33899/ijvs.2019.125965.1202

Detection and pathogenicity of *Listeria monocytogenes* in common carp (*Cyprinus carpio*) fish in Baghdad, Iraq

Nagham M. Al-Gburi

Iraqi Journal of Veterinary Sciences, *2020*, Volume 34, Issue 2, Pages 311-316
DOI: 10.33899/ijvs.2019.125980.1205

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 411-415
DOI: 10.33899/ijvs.2019.126127.1239

Functional morphology of the trunk and paw pad skin of the African palm squirrel (Epixerus ebii)

Chikera S. Ibe; Adanna Elezue; Ekele Ikpegbu; Uchenna Callistus Nlebedum

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 417-425
DOI: 10.33899/ijvs.2019.126047.1216

Using T cell lymphokines to enhance the immune response against Newcastle disease in vaccinated broiler chickens fed naturally contaminated diet with different mycotoxins

Basher H. Saud; Mushtaq T. AL-Zuhariy

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 427-433
DOI: 10.33899/ijvs.2019.125977.1204

Clinical, serological and antigenic study of feline panleukopenia virus in cats in Baghdad, Iraq

Mohammad M. Zenad; Amanee M. Radhy

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 435-439
DOI: 10.33899/ijvs.2019.125960.1201

Molecular detection of Cryptosporidium parvum in chicken in Al-Diwaniya province

Noor I. Jarad

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 441-445
DOI: 10.33899/ijvs.2019.126159.1249

Detection of lead, chromium and cobalt in meats of cattle and buffalo from retails of Mosul city

Hiba S. Al-Naemi; Raad A. Al-Sanjary; Rana A. Faraj; Ahmed ̣adi

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 447-451
DOI: 10.33899/ijvs.2019.126069.1224

The genotype of Entamoeba histolytica in bloody diarrhea samples of humans, cows and sheep

Hassan H. Naser

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 453-458
DOI: 10.33899/ijvs.2020.126135.1242

Detection of the hbl complex genes in Bacillus cereus isolated from cow raw milk in northwest of Iran

R. Alipour Banaei; Saman Mahdavi

Iraqi Journal of Veterinary Sciences, 2020, Volume 34, Issue 2, Pages 459-463
DOI: 10.33899/ijvs.2020.126120.1238

Assessment of the withdrawal period for ractopamine hydrochloride in the goat and sheep

M. Lazuardi[1], B. Hermanto[2] and T.I. Restiadi[3]

[1]Laboratory of Veterinary Pharmacy Science, [3]Departement of Reproduction, Faculty of Veterinary Medicine, [2]Department of Pharmacology, Medical Faculty, University of Airlangga , Surabaya, Indonesia
Email: [1] lazuardi@fkh.unair.ac.id, [2] bambang-h@fk.unair.ac.id,[3] tjukir@yahoo.com

(Received September 18, 2019; Accepted October 13, 2019; Available online July 25, 2020)

Abstract

Ractopamine hydrochloride often used as a bronchodilator, but its β-adrenergic agonist effects on un-striated muscle and its withdrawal time have not been assessed for Etawah goats and sheep. The aim of this study was to determine the safe time to slaughter goats and sheep post-treatment with ractopamine. Five clinically healthy adult goats and sheep (20 kg body weight) were treated with a single dose of ractopamine (1 mg, intravenously). Whole blood was sampled from the jugular vein at 120 min, 180 min and 300 min post-treatment. Ractopamine as a veterinary drug was analysed using HPLC at wavelength 225 nm. The concentrations at 120 min, 180 min and 300 min were 817.156 ± 13.460 µg.mL^{-1}, 554.468 ± 50.157 µg.mL^{-1}, and 294.588 ± 52.845 µg.mL^{-1} in goats and 706.266 ± 89.856 µg.mL^{-1}, 579.194 ± 45.664 µg.mL^{-1}, and 209.36 ± 54.797 µg.mL^{-1} in sheep, respectively. The withdrawal times in goats and sheep were 1141.710 ± 255.85 h and 989.741 ± 167.633 h, respectively, no drug residues detected. The safe time to slaughter goats after administration of 1 mg of ractopamine was approximately 3 months and 5 days post-treatment at a safety factor of 2, which was later than the sheep at 2 months and 22 days by a safety factor of two.

***Keywords*:** β-agonist, Clenbuterol, Vetranal, Residues

DOI: 10.33899/ijvs.2019.126114.1237, ©2020, College of Veterinary Medicine, University of Mosul.

تقييم مدة طرح هيدروكلوريد الراكتوبامين في الماعز والأغنام

محمد لازاردي[١]، بي هيرمانتو[٢] و تي أي ريسفتيادي[٣]

[١]مختبر علوم الصيدلة البيطرية، [٣]فرع علم التكاثر، كلية الطب البيطري، [٢]فرع الادوية، كلية الطب، جامعة ايرلانجا، سورابايا، إندونيسيا

الخلاصة

غالباً ما يستخدم هيدروكلوريد الراكتوبامين كموسع للقصبات الهوائية، ولكن آثاره المنبهة الأدرينالية على العضلات غير المخططة ووقت انسحابها لم يتم تقييمها في ماعز وأغنام إيتاواه. هدفت الدراسة الى تحديد الوقت الآمن لذبح الماعز والأغنام بعد المعالجة بالراكتوبامين. تم علاج خمسة من الماعز والأغنام البالغة (٢٠ كجم من وزن الجسم) بجرعة واحدة من الراكتوبامين (١ ملغم في الوريد). تم أخذ عينات الدم من الوريد الوداجي عند ١٢٠ دقيقة و ١٨٠ دقيقة و ٣٠٠ دقيقة بعد العلاج. تم تحليل الراكتوبامين (كمستحضر بيطري) باستخدام جهاز HPLC على طول موجي ٢٢٥ نانومتر. كانت التراكيز عند ١٢٠ و ١٨٠ و ٣٠٠ دقيقة هي ٨١٧,١٥٦ ±١٣,٤٦٠، ٥٥٤,٤٦٨±٥٠,١٥٧، و ٢٩٤,٥٨٨±٥٢,٨٤٥ ميكروغرام/مل في الماعز على التوالي، و٧٠٦,٢٦٦±٨٩,٨٥٦ و ٥٧٩,١٩٤ ±٤٥,٦٦٤ و ٢٠٩,٣٦ ± ٥٤,٧٩٧ ميكروغرام/مل في الأغنام على التوالي. كانت مدة طرح الدواء في الماعز والأغنام ١١٤١,٧١٠±٢٥٥,٨٥ و٩٨٩,٧٤١±١٦٧,٦٣٣ ساعة، على التوالي، أذ لم يلاحظ وجود بقايا للدواء. الوقت الآمن لذبح الماعز بعد إعطاء ١ ملغم من الراكتوبامين هو ٣ أشهر و ٥ أيام بعد المعالجة باعتماد عامل أمان ٢، والذي كان متأخراً عن الأغنام الذي كان شهرين و ٢٢ يوماً باعتماد عامل أمان ٢.

Introduction

β-Agonists exhibit short-acting effects and long-acting effects and are frequently used to promote growth in livestock, but problems arise when the residues of such veterinary drugs have the potential to influence human health after being consumed in animal products (1-5). Evaluation of β-agonist as a veterinary drug is a new concept to implement the principle using medicines for veterinarian by motto: logic and responsibility. Logic in science has the following characteristics: a methodology, based on evidence and theory update. Responsibility means that a professional veterinarian has a moral responsibility to monitor the effects of administration of a drug that they have recommended (6).

In early 2018, a new concept for calculation of withdrawal time was developed on the basis of pharmacokinetic and bio-analysis data. This improved approach permits the calculation of withdrawal periods for veterinary drugs in any local animal, so it logical to use it to re-assess the current withdrawal period for ractopamine (7).

It was therefore decided to use this updated approach to re-assess the withdrawal period of ractopamine after treatment in goats and sheep, both of which are distributed across Asia, Europe, Australia and Africa, where they are used to produce human food, drugs and cosmetics. They are also closely related to livestock in several countries that are free from adverse drug responses (8). Specifically, we tested whether the withdrawal period of ractopamine, after a single dose was administered intravenously, differs between Indonesian (Etawah) goats and sheep (9). A second goal was to determine the length of time required for β-agonist residues to decrease to safe levels in animals that are to be slaughtered post-treatment.

Materials and methods

Experiment animals and animal ethic clearance

This research was conducted at Airlangga University in collaboration with the Indonesia Veterinary Pharmacy and Pharmacology Association under control by the Indonesia Medical Veterinary Association (IMVA) from August 2016 to August 2018, with rainy conditions and subtropical temperatures of approximately 22-25ºC, and was conducted in compliance with the animal ethics clearance from University of Airlangga. Etawah goats and Javanese thin-tailed sheep, both local Indonesian breeds, were obtained from local farmers in Surabaya, Indonesia. The study was conducted in five of goats and five of sheep. The criteria of experimental animals were from the second generations of the native species of goats and sheep and were healthy adult males aged 1 year (live weight 18-22±1.41kg) raised under controlled veterinarian practices. Before treatment, all animals were adapted in animal housing for approximately 2 weeks.

Research design and experimental protocol

The experimental design consisted of a time series of measurements at 120 min, 180 min and 300 min post-treatment, followed by analysis of the elimination rate constant (K_{el}) and limit of quantification for method analysis (10).

Sample preparation

Certified reference material of Ractopamine-hydrochloride (Vetranal) at HPLC grades was purchased from Sigma-Aldrich in CAS-N0 90274-24-1 at level of purity 95.5%. Pharmaceutical-grade ractopamine-hydrochloride was produced by Shanghai Hannochem International Corporation. (www.hannochem.com). The drug was dissolved in aqua pro injection solution (w/v), and drops of 0.1% phosphoric acid for HPLC grade (Merck Catalog No.7664-38-2) were added until a solution formed. pH was adjusted 6.8-7 using with 0.1 M NaOH. After filtration through a 0.20 µm filter, the solution of ractopamine was ready to use for injection (11). One milligram of the drug was intravenously injected into the jugular veins of goats and sheep. Jugular blood was sampled with a disposable syringe (5 mL) at 120 min, 180 min and 300 min post-treatment. All samples were placed in 10 mL heparinized tubes and centrifuged at 3500 rpm for 30 min. The plasma samples were placed in Nunc tubes (5 mL) and stored at -20ºC until all samples were prepared.

Analysis of ractopamine

Analysis of the drug and drug residues in the liver was performed using a Shimadzu HPLC LC-6AD pump with a PDA detector. Optimization and validation of the HPLC method were modified with the mobile phase consisted of acetonitrile: aqua pro chromatography reagent (30:70) containing 0.1% phosphoric acid at pH 3.8 (11-13). A reverse-phase ODS C_{18} column was used, and the detector was set at 225 nm. Rheodyne® sample loops were configured to perform 2 µL injections (14). Analysis was carried out using isocratic elution without internal standards. Analysis of the method parameters resulted in an optimal flow rate of 0.5 $mL.min^{-1}$ and an analysis time of 20 min. The method was validated for quality assurance at high impact levels according to the new research report (7,8,10).

Analysis of withdrawal time

The withdrawal time was calculated by using new method namely "Lazuardi Theory" base on K_{el} data for ractopamine to determine the elimination half-live ($T_{1/2\beta}$) and the limit of quantification (LOQ), as described by equation at below (7,9,14). The drug residues in goat and sheep in liver were monitored one-month post treatment.

Statistical analysis

The relationship between the ractopamine concentration and HPLC detector response and the differences in the kinetics profiles based on goat versus sheep data were assessed with SPSS 24.0 at 5% significance. The research hypothesis was tested at significance 5% with SPSS 24.0

Results

Validation method and concentration of ractopamine in matrix biology

The optimal UV-Vis wavelength for ractopamine detection was 225 nm, as indicated in Fig. 1. The HPLC retention time was 3.890 min for the ractopamine standard (Fig. 2) but 4.082 min for ractopamine extracted plasma by SPE (Fig. 3).

The peak area of ractopamine increased linearly with the concentration over the evaluated range of 0.242 to 1.601 µg.mL^{-1}, with a regression equation of Y = - 0.205 + 33925175 X and an R-squared value of 0.99. The coefficients of variation (CVs) for the intraday precision and accuracy at concentrations ranging from 0.18 µg.mL^{-1} to 0.956 µg.mL^{-1} were less than 8% (Table 1).

Regarding method sensitivity, the limit of detection (LOD) was 3.5 ×10^{-6} µg.mL^{-1}, and the LOQ was 1.06 ×10^{-5} µg.mL^{-1}. The precision and accuracy had CVs of less than 20%, and the percent recovery in artificial plasma was more than 80% (Table 2). The plasma concentrations of the drug in goats and sheep are detailed in Table 3.

Kinetic parameters of ractopamine

The exponent equation (K_{el}) and $T_{½\beta}$ determined on the basis of the data from goat and sheep between 5- 300 min post-treatment are presented in Table 4. These data show that the drug was eliminated more slowly in the goat than in the sheep (P>0.05).

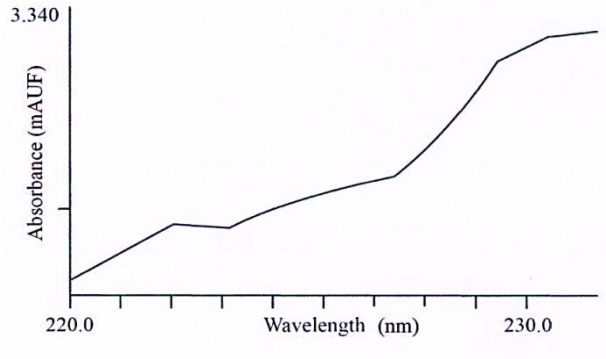

Figure 1: Spectrum of 440 µg.mL^{-1} ractopamine dissolved in acetonitrile:water (30%:70%) containing 0.1% phosphoric acid (pH 3.8), showing the suitable wavelength of 225 nm with a peak of 0.0150 to 0.020 mAUF.

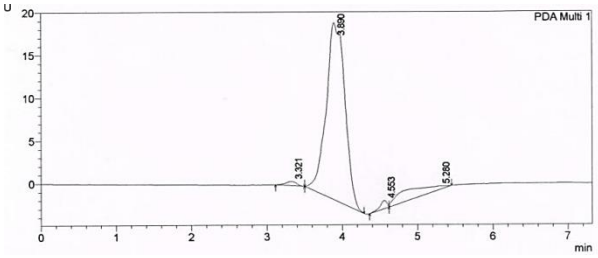

Figure 2: Chromatogram of 50 µg.mL^{-1} ractopamine dissolved in the mobile phase (acetonitrile:water (70:30) containing 0.1% phosphoric acid, pH 3.8) detected at 225 nm, with a retention time of 3.890 min at 25ºC.

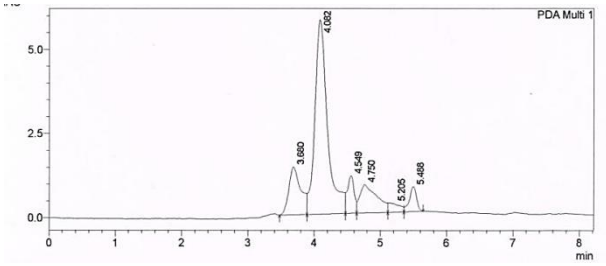

Figure 3: Chromatogram of 0.505 µg.mL-1 ractopamine dissolved in the mobile phase (acetonitrile: water (70:30) containing 0.1% phosphoric acid, pH 3.8) at 225 nm, with a retention time of 4.082 min at 25ºC.

Table 1: Intra-day precision and accuracy for the determination of ractopamine in the eluent mobile phase

Ractopamine concentration (µg.mL^{-1})	Peak area
0.18	6106531
0.19	6116641
0.17	6006429
Average = 0.18	Average = 6076533.667
% CV = 5.555	% CV = 1.002
0.55	18658846
0.54	17957745
0.56	18758856
Average = 0.55	Average = 18458482.33
% CV = 1.818	% CV = 2.365
0.78	28453985
0.74	24413580
0.76	26433782
Average = 0.76	Average = 26433782.33
% CV = 2.631	% CV = 7.642
0.94	35469650
0.97	38499855
0.96	37488754
Means = 0.956	Average = 37152753
% CV = 1.597	% CV = 4.152

Table 2: Analysis precision, accuracy and percent recovery for ractopamine in artificial plasma (µg.mL^{-1})

Drug concentration	Observed drug concentration	Peak area of analyte	Recovery (%)
0.25	0.200	6665032.444	80
	0.220	7610343.295	88
	0.240	8039522.042	96
Average ± % CV	0.22 ± 9.091	7438299.29 ± 9,454	88 ± 0.022
0.50	0.420	14266327.55	84
	0.480	16284083.8	96
	0.530	17980342.55	106
Average ± % CV	0.477 ±11.53	16176917.97 ± 11.494	95.33±11.55
1.00	1.010	34264426.55	101
	0.970	32907419.55	97
	0.980	33246671.30	98
Average ± % CV	0.987 ± 2.107	33472839.13 ± 2.11	98.67 ± 2.11

Table 3: Concentration of ractopamine in goat and sheep plasma

Animal (N)	Time (min)	Drug concentration in plasma samples (µg.mL^{-1})				
		1	2	3	4	5
Goat	5	958.022	985.214	923.071	915.244	899.323
	120	814.540	840.340	805.540	814.320	811.040
	180	600.450	501.530	569.130	600.120	501.110
	300	250.120	351.050	270.230	352.420	249.120
Sheep	5	891.260	910.060	917.060	991.210	887.220
	120	815.150	767.270	718.420	614.230	616.260
	180	599.040	600.010	598.240	497.530	601.150
	300	151.250	249.140	250.430	248.520	147.460

Table 4: Exponential equation, elimination rate constant, elimination half-life, withdrawal time in goat sheep

Animal		Exponent equation	R^2	K_{el} (min^{-1})	$T_{1/2\,\beta}$ (min)	Withdrawal time		
						Safety Factor 1 (hours)	Safety Factor 2 (days)	Safety Factor 3 (days)
Goat	1	$Y=1117.9.e^{-0.005X}$	0.8887	0.005^{a}	138.600	927.882	77.323^{c}	115.985^{e}
	2	$Y=1082.3.e^{-0.004X}$	0.9214	0.004^{a}	173.250	1159.853	96.654^{c}	144.982^{e}
	3	$Y=11051.e^{-0.004X}$	0.8999	0.004^{a}	173.250	1159.853	96.654^{c}	144.982^{e}
	4	$Y=1041.e^{-0.003X}$	0.9162	0.003^{a}	231.000	1546.47	128.872^{c}	193.309^{e}
	5	$Y=1087.1.e^{-0.005X}$	0.8975	0.005^{a}	136.600	914.492	76.208^{c}	114.311^{e}
Mean ± SD						1141.71± 255.85	95.142±21.321	142.71± 31.982
Sheep	1	$Y=1257.6.e^{-0.006X}$	0.8052	0.006^{b}	115.500	773.235	64.436^{d}	96.654^{f}
	2	$Y=1106.1.^{e-0.004X}$	0.8818	0.004^{b}	173.250	1159.852	96.654^{d}	144.981^{f}
	3	$Y=1084.4.e^{-0.004X}$	0.9011	0.004^{b}	173.250	1159.852	96.654^{d}	144.981^{f}
	4	$Y=1058.e^{-0.005X}$	0.9882	0.005^{b}	138.600	927.882	77.323^{d}	115.985^{f}
	5	$Y=1123.e^{-0.005X}$	0.8902	0.005^{b}	138.600	927.882	77.323^{d}	115.985^{f}
Mean ± SD						989.741±167.633	82.478±13.969	123.717± 20.954

Superscript a vs. b in the same column by Independent-Sample t test indicates a significant not-difference at P>0.05.
Superscript c and e vs. d and f in the same column by Independent-Sample t test indicates a significant not-different at 0.05.

Discussion

To determine the ractopamine levels in plasma collected from healthy goats and sheep treated with this agent, we initially utilized the HPLC assay described by other researchers without modification (9,15,16). However, we were unable to reproduce the reported assay so we developed a simpler assay using an isocratic mobile phase. Our protocol

was validated and the CV from linearity analysis was no more than 10%. For intra-day precision and accuracy, CVs were less than 20%. We needed to analyse plasma samples containing low concentrations of ractopamine, below 1.00 $\mu g.mL^{-1}$, because such concentrations are expected in animals receiving a typical dose regimen but measurements at this level are known to be un certainty, primary due to instrument error and human error during sample preparation. The LOQ we achieved (1.06×10^{-5} $\mu g.mL^{-1}$) was lower than the mean ractopamine concentration at 300 min post-treatment in goats and sheep. To produce an accurate, simple assay with high recoveries, sample components were separated on two adsorption columns: an HPLC column and SPE column (17,18). The recovery of ractopamine from plasma was 88-99% (19,20), consistent with reported recoveries of the half-life in these two species (21,22). and supporting suggestions that it is more extensively distributed in goat tissues than in sheep tissues (23,24). Therefore, in goats, ractopamine is not easily metabolized and must be processed more than 1.15 times as much as in sheep. Overall. It is clear that the risk of exposure to ractopamine residues is greater in goat tissues than in sheep tissues (4,25). The residues in liver and became undetectable after 95 h in goat and 82 h in the sheep, similar to delays reported for clenbuterol, a β_2-agonist, in goat tissues (8,26). No ractopamine residues were detected in liver samples at a safety factor of 2 and 3, comparable to the factors reported by Ho and Colleagues the rat (27).

Ractopamine is known for its potential to be distributed into deep tissues in organs such the spleen, liver and kidney, increasing the risk of cancer (28). It is therefore important to acknowledge the risk of residues in animal slaughtered before the end of the withdrawal period. It is known that ractopamine residues do not melt at temperatures 0f 100-120 ^{0}C so imperfectly cooked meat will lead to the consumption of residues so persistent consumption is likely to increase the risk of cancer.

Conclusions

The withdrawal time of 1 mg of ractopamine administered intravenously is longer in goats than that in sheep. In goats, the withdrawal time is 3 months and 5 days post-treatment at a safety factor of two, and 4 months and 8 days post-treatment at a safety factor of three. In sheep, the withdrawal time is 2 months and 22 days post-treatment at a safety factor of two, and 4 months and 4 days post-treatment at a safety factor of three. It is clear that, more research is still needed to improve the detection of ractopamine residues in various tissues. A safety factor of more than 3 will be increase confidence that all residues had disappeared.

Acknowledgements

We thank to Directors of Tropical Diseases Research Center University of Airlangga for supporting the facilities of samples preparations and working by HPLC and spectrometry instruments. This work was financially supported by the Ministry of Research, Technology and Higher Education, Republic of Indonesia by scheme Hibah Kompetisi contract no. 018/SP2H/LT/DRPM/II/2016, February, 17th 2016. Thank you to Prof. Graeme B. Martin from University of Western Australia to correction English editing as a joint Adjunct Professor Program between Faculty of Veterinary Medicine University of Airlangga and University Western of Australia.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Yang F, Yang YR, Wang L, Huang XH, Qiao G, Zeng ZL. Estimating marbofloxacin withdrawal time in broiler chickens using a population physiologically based pharmacokinetics model. J Vet Pharmacol Ther. 2014;37:579-588. DOI: 10.1111/jvp.12137.
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;350:124-129. DOI: 10.1124/jpet. 114.213116.
3. Jiang D, Cao B, Wang M, Yang H, Zhao K, Li J, Li M, Sun L, Deng A. Development of a highly sensitive and specific monoclonal antibody-based enzyme-linked immunosorbent assay for the detection of a new beta-agonist, phenylethanolamine A, in food samples. J Sci Food Agric. 2017;97:1001-1009. DOI: 10.1002/jsfa.7826.
4. Pleadin J, Vulic A, Persi N, Terzic S, Andrisic M, Žarkovic I, Šandor K, Perak E, Mihaljevic Z. Accumulation of ractopamine residues in hair and ocular tissues of animals during and after treatment. J Anal Toxicol. 2013;37:117-121.DOI: 10.1093/jat/bks092 .
5. Regal P, Diaz-Bao M, Barreiro R, Vazquez BI, Cepeda A. Confirmation of beta-agonist residues in bovine retina and liver using HPLC-MS/MS and evaluation of matrix-dependent problems. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2017;34:598-607.DOI: 10.1080/19440049.2016.1271144
6. Lazuardi M. The Generaly Part of Veterinary Pharmacy Sciency. 1st Ed. Jakarta, Indonesia: Ghalia Indonesia.;2016. 29-42 p.
7. Lazuardi M. The Special Part of Veterinary Pharmacy Science. 1st Ed. Surabaya-Indonesia: Airlangga University Press. 2019. 249-250 p.
8. Lazuardi M, Hermanto B. High-performance liquid chromatography ultraviolet-photodiode array detection method for aflatoxin B_1 in cattle feed supplements. Vet World. 2017;10:932-938.DOI: 10.14202/vetworld.2017.932-938
9. Lazuardi M, Hermanto B, Restiadi TI. Calculate of withdrawal times of clenbuterol in goats to obtain safe times of slaughter. Vet World. 2018;11:731-738. DOI: 10.14202/vetworld.2018.731-738
10. Li L, Tang C, Zhang J, Zhao Q, Zhang K.The potential of various living tissues for monitoring clenbuterol abuse in food-producing Chinese simmental beef cattle. J Anal Toxicol. 2016. 40:72-77.DOI: 10.1093/jat/bkv118
11. Chiesa L, Panseri S, Cannizzo FT, Biolatti B, Divari S, Benevelli R, Arioli F, Pavlovic R. Evaluation of nandrolone and ractopamine in the urine of veal calves: liquid chromatography-tandem mass spectrometry approach. Drug Test Anal. 2017;9:561-570.DOI: 10.1002/dta.2026

12. Lazuardi M, Restiadi TI, Hermanto B. Calculated withdrawal time by lazuardi equation method. Proc Natl Indone Vet Pharm Pharmacol Assoc. 2018;3(1):45-48.
13. Ding G, Li D, Qin J, Zhu J, Wang B, Geng Q, Guo M, Punyapitak D, Cao Y. Development and validation of a high-performance liquid chromatography method for determination of ractopamine residue in pork samples by solid phase extraction and pre-column derivatization. Meat Sci. 2015;106:55-60.DOI: 10.1016/j.meatsci.2015.03.016
14. Wang L, Huang L, Pan Y, Wu Q, Xie S, Yuan Z. Simultaneous determination of aditoprim and its three major metabolites in pigs, broilers and carp tissues, and its application in tissue distribution and depletion studies. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2016;33:1299-1311. DOI:10.1080/19440049.2016.1200751.
15. Wu J, Liu X, Peng Y. Determination of ractopamine in pig hair using liquid chromatography with tandem mass spectrometric detection. J Pharmacol Toxicol Methods. 2014;69:211-216.DOI: 10.1016/j.vascn.2014.02.001
16. Pan S, Zhou L, Zhao Y, Chen X, Li X, Jin M. Development and validation of a sensitive method for simultaneous determination of eight beta (2)-agonists in pork by ultrasonic-assisted extraction and liquid chromatography/tandem mass spectrometry. J Chromatogr Sci. 2015;53:104-111.DOI: 10.1093/chromsci/bmu026
17. Du W, Zhao G, Fu Q, Sun M, Zhou H, Chang C. Combined microextraction by packed sorbent and high-performance liquid chromatography-ultraviolet detection for rapid analysis of ractopamine in porcine muscle and urine samples. Food Chem. 2014;145:789-795.DOI: 10.1016/j.foodchem.2013.08.094
18. Freire EF, Borges KB, Tanimoto H, Nogueira RT, Bertolini LC, de Gaitani CM. Monitoring of ractopamine concentration in the mixture of this feed additive with vitamin mineral complex and with swine feed by HPLC. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2013;30:796-803.DOI: 10.1080/19440049.2013.780213.
19. Wang X, Guo T, Wang S, Yuan J, Zhao R. Simultaneous determination of 11 beta-agonists in human urine using high-performance liquid chromatography/tandem mass spectrometry with isotope dilution. J Anal Toxicol. 2015;39:213-218.DOI: 10.1093/jat/bku143.
20. Chan KW. Validation of a straightforward high-performance liquid chromatographic method formorphine quantitation. Egypt J Forensic Sci. 2017;7:1-6. DOI: 10.1186/s41935-017-0003-0.
21. Smith DJ, Shelver WL, Marx A. Detection of residues in urine and tissues of sheep treated with trace levels of dietary ractopamine HCl. J Anim Sci. 2016;94:5423-5433.DOI: 10.2527/jas.2016-0899
22. Zhao Z, Gu X, Su X, Li J, Li J, Dong Y, Yang Y, Yao T, Qin Y. Distribution and depletion of ractopamine in goat plasma, urine and various muscle tissues. J Anal Toxicol. 2017;41:60-64.DOI: 10.1093/jat/bkw102
23. Tang C, Zhang J, Li L, Zhao Q, Bu D. Ractopamine residues in urine, plasma and hair of cattle during and after treatment. J Anal Toxicol 2014;38:149-154.DOI: 10.1093/jat/bku006
24. Huang L, Shi J, Pan Y, Wang L, Chen D, Xie S, Liu Z, Yuan Z. Elimination and concentration correlations between edible tissues and biological fluids and hair of ractopamine in pigs and goats fed with ractopamine-medicated feed. J Agric Food Chem. 2016;64:2012-2020.DOI: 10.1021/acs.jafc.6b00456.
25. Pleadin J, Vulic A, Persi N, Terzic S, Andrisic M, Žarkovic I, Šandor K, Perak E. Comparison of ractopamine residue depletion from internal tissues. Immunopharmacol Immunotoxicol. 2013;35:88-92.DOI:10.3109/08923973.2012.702115
26. Edenburn BM, Kneeskern SG, Bohrer BM, Rounds W, Boler DD, Dilger AC, Felix TL. Effects of supplementing zinc or chromium to finishing steers fed ractopamine hydrochloride on growth performance, carcass characteristics, and meat quality. J Anim Sci. 2016;94(2):771-779.DOI:10.2527/jas.2015-9979
27. Ho JK, Huo TI, Lin LC, Tsai TH. Pharmacokinetics of ractopamine and its organ distribution in rats. J Agric Food Chem. 2014;62:9273-9278.DOI: 10.1021/jf5026168
28. Grandin T. On-farm conditions that compromise animal welfare that can be monitoredat the slaughter plant. Meat Sci. 2017;132:52-58.DOI:10.1016/j.meatsci.2017.05.004