Investigating the contributing factors to postmortem pH changes in springbok, eland, red hartebeest and kudu edible offal

Authors:
Kudakwashe Magwedere[1,2]
Fortune Sithole[3]
Louw C. Hoffman[1]
Yvonne M. Hemberger[2]
Francis Dziva[1,4]

Affiliations:
[1]Department of Animal Sciences, Stellenbosch University, South Africa

[2]Division of Veterinary Public Health, Directorate of Veterinary Services, Namibia

[3]Department of Paraclinical Sciences, University of Pretoria, South Africa

[4]School of Veterinary Medicine, University of the West Indies, Eric Williams Medical Sciences Complex, Mt Hope, Trinidad

Correspondence to:
Kudakwashe Magwedere

Email:
gwedas@yahoo.co.uk

Postal address:
Private Bag X1, Maitland 7602, South Africa

Dates:
Received: 07 Aug. 2012
Accepted: 30 Nov. 2012
Published: 13 Mar. 2013

How to cite this article:
Magwedere, K., Sithole, F., Hoffman, L.C., Hemberger, Y.M. & Dziva F., 2013, 'Investigating the contributing factors to postmortem pH changes in springbok, eland, red hartebeest and kudu edible offal', *Journal of the South African Veterinary Association* 84(1), Art. #919, 7 pages. http://dx.doi.org/10.4102/jsava.v84i1.919

The objective of the study was to assess pH measurements between offal organs of different species and the association between pH taken 4 h post-slaughter and different predictor variables in the liver and lungs. A linear regression analysis was conducted on selected variables to identify the main predictors and their interactions affecting the pH of meat 4 h post-slaughter. In an increasing order of magnitude during winter, the pH achieved at 16 h – 36 h post-slaughter in springbok heart, liver, spleen, kidney and lungs was significantly ($p < 0.05$) higher than pH 6.0. The pH attained in springbok carcasses was ($p < 0.05$) below 6.0, whilst no significant differences were observed from the regulatory reference (pH 6.0) in the heart. There was a positive association between the pH of game meat 4 h post-slaughter and liver congestion. The pH of game meat 4 h post-slaughter increased by 0.11 units ($p < 0.05$) per millilitre increase in liver congestion and decreased by 0.04 units ($p < 0.05$) per minute increase in the shooting-to-bleeding interval, irrespective of the species. The lack of a statistically significant association between some selected variables and pH changes in this study suggested that either the factors may have a small effect which is only detectable with large data-sets and/or the effect may be modified by other unidentified factors. As some of the offal organs had final pH readings above 6.0, alternative measures are required to inactivate certain endogenous pathogens in edible wild game offal sourced from endemic areas.

Introduction

Edible offal offers a range of foods which are nutritiously attractive, especially in developing countries. The edible offal is highly prized in South East Asia and Africa, whilst demand is variable and low in Australia and the USA, respectively (Fatma & Mahdey 2010). In slaughtered animals, edible offal contributes 33% of the edible material (Aduku *et al*. 1991; Pearson & Dutson 1988). It is estimated that about 75% of the emerging human infectious diseases arise from animal reservoirs (Allen *et al*. 2012), of which Rift Valley fever (RVF) is placed third on the list of the 17 most dangerous animal threats after foot and mouth disease (FMD) and influenza (Mandell & Flick 2011). A pH value below 6.0 is commonly used to destroy the most dangerous animal pathogens and ensure the safety of livestock products. Microorganisms have optimum, minimum and maximum pH for growth in different foods as a result of the interactions between the pH and other factors which either promote or inhibit microbial growth. In general, microbes do not grow, or else grow very slowly, at pH below 4.6, although there are exceptions. The pH of meat is important for good carcass quality and for inactivating viral and bacterial animal microbes, a number of which are endemic in southern Africa (European Commission [EC] 2001; Falenski *et al*. 2010; Fatma & Mahdey 2010).

A serious zoonotic livestock disease, such as RVF, poses a significant public health threat and can have serious consequences. It is presumed that certain wildlife species (African buffalo, lesser kudu, black rhinoceros, white rhinoceros, lion and impala) may carry the RVF virus as reservoirs between outbreaks and that wildlife meat that is not properly handled may transmit the RVF virus to humans during that period (American Veterinary Medical Association 2010; Evans *et al*. 2008). Preferred samples for isolation of Bunyaviridae (genus *Phlebovirus*), the cause of RVF, are blood (preferably collected in an anticoagulant-containing vessel during the febrile stage of the disease) or liver, spleen and brain tissues of dead animals and organs of aborted foetuses (World Organisation for Animal Health [OIE] 2012a). RVF virus is a relatively unstable agent and at its optimum pH range of 7.4–8.0 and with 2% serum, it has a half-life of 6.8 days at 0 °C and 1.5 h at 37 °C. No references were found on the persistence of RVF virus in carcasses or muscle during the conduct of this study, but the virus would be expected to disappear quickly from the carcass as the pH dropped with *rigor mortis* (Scott 2003).

Inactivation of the FMD virus in skeletal muscle and heart muscle occurs rapidly after animal death as a result of lactic acid formation which accompanies *rigor mortis* and causes the pH to drop to levels between 5.5 and 6.0. The inactivation rate for FMD virus at pH 6.0 is 90% per minute, whilst at pH 5 it is 90% per second (Bachrach *et al*. 1975). In skeletal muscle kept at 4 °C, the FMD virus is regarded as completely inactivated within 48 h, although infectivity may remain for over 4 months in lymph nodes, clotted blood, bone marrow and viscera where the virus is protected from maturation-induced pH changes (Blackwell 1984). The FMD virus is most stable around neutral pH and is sensitive to even mild acidity. Although temperatures above 50 °C destroy most FMD virus infectivity, it is likely that a small proportion of particles are relatively resistant to the effects of heat and pH (Hyslop 1970; MacDiarmid & Thompson 1997). However, the muscle pH of animals slaughtered in a febrile state may not drop below 6.0; hence, some FMD virus may survive in muscle tissue of such animals up to 96 h post-slaughter (MacDiarmid & Thompson 1997). Caution in extrapolating findings between species is recommended because of a reported case of highly myotropic FMD virus surviving beyond 72 h in the carcass of impala when the pH had dropped to 5.6 (Bengis & Veary 1997; Scott 2003).

Brucella melitensis and *Brucella abortus* have been detected at varying counts in edible offal (Fatma & Mahdey 2010). *Brucella* spp. bacteria survival time in chilled meat seems extremely short, except in frozen carcasses where the organism can survive for years (EC 2001). These bacteria often survive desiccation and can survive freezing and near-freezing temperatures for over two years (Iowa State University 2011). The number of *Brucella* organisms per gram of muscle is normally small and rapidly decreases with the drop in meat pH (EC 2001; El-Nesser *et al*. 2007; Sadler 1960). The survival period of *B. abortus* in yoghurt at pH 3.60 and 3 °C is 1 day. The optimal pH range of *Brucella* is between pH 6.6 and 7.4 at 37 °C, whilst the maximum pH is 8.4 and the minimum is 4.1 (Falenski *et al.* 2010; International Commission on Microbiological Specifications for Food 1996; Lerche & Entel 1959; Zobell & Meyer 1932).

Within the animal–human interface, transmission of brucellosis and RVF virus as zoonotic food-borne pathogens frequently depends on factors such as food consumption habits and level of processing (El-Nesser *et al.* 2007; Fatma & Mahdey 2010; Michael *et al*. 2011; United States Department of Agriculture 2011). At present, the OIE *Terrestrial animal health code* (OIE 2012b) has no recommendations to veterinary authorities concerning the importation of edible offal from countries that are not free from *Brucella*, FMD virus and RVF virus. However, the animal and public health requirements for wildlife meat destined for export are sometimes stipulated by the importing country in the import permit. Some importing countries require only carcasses to be submitted to veterinary maturation at a temperature above +2 °C for at least 24 h. The intended goal of this intervention is to attain an electronically measured pH reading from the middle of the *longissimus dorsi* below pH 6.0 (Bengis & Veary 1997; EC 2010; Pharo 2002). Deliberate and natural pH reduction in meat is the cheapest and commonest intervention that can be applied to meat and meat products to destroy or control pathogens to ensure public health and food security (Institute of Food Technologists [IFT] & Federal Drug Administration [FDA] 2003).

Materials and methods

Measurement of pH

The pH and temperature were measured using a testo model 205 (Testo AG, Lenzkirch, Germany) temperature and pH meter with automatic temperature compensation. The selection of farms included in this study was based on application by owners for game harvesting for commercial purposes. For eviscerated carcasses with skin on, meat pH was measured 16 h – 34 h post-slaughter by inserting the probe in the middle of the *longissimus dorsi*, whilst for edible offal organs the pH meter probe was inserted into the organ. Calibration of the testo meter was achieved using a standard calibration solution at the beginning of the measurements and after every five readings.

Winter night harvesting

The springbok (*Antidorcas marsupialis*) were harvested between July and August 2010. Average night temperatures ranged from 0 °C to 17 °C. Average maximum and minimum humidity was 50% and 7%, respectively. Relative humidity was measured using a hygrometer (Hygrocheck; Hanna Instruments, Johannesburg, South Africa) and verified by readings from Intellicast (http://www.intellicast.com/Global/Humidity.aspx). The pH measurements were derived from five consignments of springbok harvested on different days and delivered to the abattoir at a minimum of 10 carcasses per group. In total, 55 harvested springbok carcasses and organs (liver, lung, heart, spleen and kidney) were selected from visually inspected and passed carcasses.

Summer daylight harvesting

The harvesting was conducted on one farm and the random effects of the three harvesting camps on the farm were assumed to be normally distributed. Species under consideration were eland (*Taurotragus oryx*), red hartebeest (*Alcelaphus buselaphus*), springbok and kudu (*Tragelaphus strepsiceros*). All red hartebeest were males except for one female, springbok were females except for two males, kudu were females and eland were males except for one female. All animals were mature. The game harvesting was conducted during the day and during the summer month of February. Average day temperatures ranged from 22 °C to 31 °C. A progressive decrease in the relative humidity from morning to evening was observed, with a maximum humidity of 84% at approximately 07:20 and a minimum humidity of 23% at approximately 17:00. All harvested game included in this study were healthy. The shooting position was consistently the upper neck and head. In springbok, evisceration of the

TABLE 1: Assessment of body condition criteria at bleeding using the rating system outlined below.

Parameter	Point score	Observable measures
Ribs	1	Deep grooves between ribs, even immediately behind shoulder.
	2	Ribs fairly well covered immediately behind shoulder (by the *latissimus dorsi*).
	3	Can still feel ribs, but grooves are not deep.
	4	Ribs nearly flush with tissue between them.
Hips and spine	1	Hip bones very distinct (no back fat). Spine very distinct.
	2	Some padding over hips. Spine very distinct.
	3	Hips fairly well padded. Spine partly covered along each side.
	4	Hips well padded. Spine is flush with or nearly covered with fat.

Source: Gerhardt, K., 2003, *The body condition of caribou*, viewed 29 June 2003, from http://dl1.yukoncollege.yk.ca/caribou/stories/storyReader$2

stomachs and intestines (white offal) was performed 1.25 h after bleeding, whilst in the hartebeest, eland and kudu, it was performed at bleeding soon after shooting (Van Schalkwyk *et al*. 2011).

All harvested animals were given a visual body condition (Table 1). The selection of variables was based on previous postmortem observations during game harvesting inspections. The time (in minutes) taken chasing the identified wild animal until shooting, as well as the time from shooting to severing blood vessels (carotid and jugular) in the neck below the jaw line by means of a deep cut with a sharp disinfected knife, was recorded. The liver and lung temperature and pH were recorded 4 h after the blood vessels in the neck were severed, soon after the red offal (heart, liver, kidney, spleen, lungs, trachea and oesophagus) evisceration. Liver and lung congestion was scored using the rating system of the quantity (mL) of blood collected in a graduated tube from a longitudinal cut across the whole organ followed by organ squeezing for 2 min. The cut was made on the gastric surface of the liver and dorsal surface of the lung. Livers and lungs that had blood on the incision line after 2 min that did not flow into the tube were given a score of 0.09 mL.

Statistical analysis

For winter night harvesting, the Student's *t*-test and one-way analysis of variance (ANOVA) using the *Statistical Analysis Systems* (*SAS*) version 9.1.3 (2007) was used to evaluate pH measurements between organs, and between organs and reference pH value, with significance at $p < 0.05$ and $p < 0.01$, respectively. Data are expressed as mean ± s.d.

For the summer daylight harvesting, all analysis was conducted using *Stata* version 10 (2007). Descriptive statistics describing all variables were used on the data. Linear regression was used to evaluate the association between pH after 4 h (outcome) and the predictor variables. Univariate analyses between pH after 4 h and all the potential predictors (time from chasing to shooting, time from shooting to bleeding, offal temperature at 4 h post-slaughter, liver congestion, lung congestion, body condition score, environmental temperature, humidity, species, liver and lung pH at 4 h post slaughter) were first evaluated to detect those variables that were statistically significant. This was followed by fitting multivariable models evaluating the association between pH after 4 h and the identified predictors of interest and controlling for potential confounder variables. A value of $p < 0.05$ was considered significant. Model diagnostics were conducted to evaluate the assumptions of homoscedasticity and normality of residuals.

Ethical considerations

Harvesting was performed by approved game harvesting teams registered by the Ministry of Environment and Tourism and the Directorate of Veterinary Services of Namibia. Harvesting was conducted following international ethical guidelines stipulated in *Nature Conservation Ordinance* (No. 4 of 1975) (Territory of South West Africa 1975), as amended in the *Guidelines for the harvesting of game for meat export* (Van Schalkwyk & Hoffman 2010). The harvesting process was monitored by officials from the two abovementioned institutions to ensure adherence to the set standards and regulations.

Results

Winter harvesting

Harvesting was conducted at night in the absence of moonlight. The pH measured in all edible offal and the carcasses is summarised in Table 2. In the livers, the pH ranged from 5.89 to 6.57, in the hearts it ranged from 5.43 to 6.89 and for kidneys the pH range was from 6.18 to 6.83. In the spleen and lungs, the pH ranged from 5.94 to 6.67 and 6.69 to 7.76, respectively. In decreasing order, the pH was higher ($p < 0.05$) than the reference pH of 6.0 in the lungs, kidney, spleen, liver and heart. Springbok carcasses had a pH lower ($p < 0.01$) than 6.0. However, there was no difference between the heart pH and the regulatory reference pH of 6.0.

Summer harvesting

Harvesting was conducted during daylight. Descriptive statistics of baseline predictor variables are shown in Table 3. In the univariate linear regression analysis, looking at the independent associations between pH and measured parameters, there were significant associations between liver pH at 4 h and liver congestion (0.08, $p < 0.05$) (the more the congestion, the higher the pH) and shooting to bleeding

TABLE 2: Summary of pH readings (mean ± s.d.) measured 16 h – 34 h ($pH_{16\text{-}24h}$) post-slaughter for springbok edible offal organs and carcasses ($N = 55$) supplied to the export abattoir.

Reference pH value	Lamb carcass	Springbok carcass	Liver	Spleen	Heart	Lung	Kidney
6.00^{a}	$5.83^{b} \pm 0.10$	$5.73^{c} \pm 0.15$	$6.30^{d} \pm 0.14$	$6.35^{d} \pm 0.15$	$6.08^{a} \pm 0.39$	$7.10^{e} \pm 0.23$	$6.54^{f} \pm 0.15$

Mean pH values with differing superscripts within the row were significantly different (at both $p < 0.05$ and $p < 0.01$) from pH 6.0.

TABLE 3: Descriptive statistical values of the measured baseline variables and parameters of the four game species studied.

Parameter	Species							
	Springbok		Red hartebeest		Eland		Kudu	
	N	Mean ± s.d.	*N*	Mean ± s.d.	*N*	Mean ± s.d.	*N*	Mean ± s.d.
Chasing–shooting (min)	21	34.45 ± 20.10	6	27.68 ± 11.50	6	26.91 ± 18.92	3	3.09 ± 1.94
Shooting–bleeding (min)	21	2.91 ± 1.84	6	4.93 ± 1.88	6	2.00 ± 0.40	3	1.55 ± 0.40
Offal temp at 4 h	16	31.72 ± 0.57	-	-	-	-	-	-
Liver pH at 4 h	17	6.15 ± 0.14	6	6.11 ± 0.59	6	6.25 ± 0.20	3	6.22 ± 0.22
Lung pH at 4 h	17	6.53 ± 0.12	6	6.83 ± 0.17	6	6.68 ± 0.18	3	6.71 ± 0.07
Liver pH at 24 h	9	6.10 ± 0.22	-	-	-	-	-	-
Lung pH at 24 h	9	6.61 ± 0.14	-	-	-	-	-	-
Liver congestion (mL)	16	0.61 ± 0.71	6	0.46 ± 0.81	6	0.22 ± 0.19	3	1.16 ± 1.76
Lung congestion (mL)	16	0.57 ± 1.21	6	0.44 ± 0.56	6	0.11 ± 0.04	3	0.09 ± 0.00
Body condition score	21	3.02 ± 0.29	6	3.50	6	3.00 ± 0.55	3	3.00

N, number of game species studied; s.d., standard deviation.

TABLE 4: Results from a multivariable linear regression model of the effect of congestion on pH of game liver 4 h post-slaughter.

Predictor variable	Category	*p*	s.e.	Coefficient	95% CI
Liver congestion	-	0.002	0.032	0 .11	0.05–0.18
Species	Ref: 6.15 ± 0.14	-	-	-	-
	Red hartebeest	0.433	0.084	0.07	-0.11–0.24
	Eland	0.096	0.070	0.12	-0.02–0.27
	Kudu	0.652	0.086	-0.04	-0.22–0.14
Body condition	Ref: 1	-	-	-	-
	2	0.141	0.163	-0.25	-0.59–0.09
	3	0.380	0.143	-0.13	-0.42–0.17
	4	0.079	0.147	-0.27	-0.57–0.03
Constant	-	0.000	0.146	6.26	5.96–6.56

p, probability value; s.e., standard error; CI, confidence index; Ref, reference value.
N = 31.

TABLE 5: Results from a multivariable linear regression model of the effect of shooting-to-bleeding time on the pH of game liver 4 h post-slaughter.

Predictor variable	Category	*p*	s.e.	Coefficient	95% CI
Shooting-to-bleeding time	-	0.060	0.195	-0.0385	-0.079–0.002
	Ref: 3.0 ± 0.2	-	-	-	-
Species	Red hartebeest	0.344	0.104	0.1004	-0.114–0.315
	Eland	0.616	0.078	0.0397	-0.122–0.201
	Kudu	0.997	0.095	0.0003	-0.196–0.196
Body condition	Ref: 1	-	-	-	-
	2	0.175	0.183	-0.2560	-0.634–0 .122
	3	0.458	0.161	-0.1210	-0.453–0.210
	4	0.225	0.164	-0.2044	-0.543–0 .135
Constant	-	0.000	0.168	6.4008	6.053–6.748

s.e., standard error; *p*, probability value; CI, confidence index; Ref, reference value.
N = 31.

(-0.03, $p < 0.05$) (the longer the shooting-to-bleeding time, the lower the pH). The final multiple logistic regression model included only the variables relating to congestion in liver and liver pH at 4 h post-harvesting. Species and body condition were introduced into the multivariate model as potential confounders. In the multivariate linear regression analysis, irrespective of species, the pH of game meat 4 h after slaughter increased by 0.11 units ($p < 0.05$) per millilitre increase in liver congestion (Table 4). Similarly, the pH of game meat 4 h post-slaughter decreased by 0.04 units ($p < 0.05$) per minute increase in the shooting-to-bleeding interval (Table 5).

Discussion

One of the objectives of the study was to assess pH levels in selected chilled edible offal from springbok and other commonly harvested and exported game species so as to establish whether they meet the requirement (pH < 6.0) for safe food (Department of Agriculture 2010). The pH values of the carcasses were all below 6.0, which is consistent with requirements of the EC Regulation 206/2010 (EC 2010), which stipulates the attestation to the effect that the consignment contains boneless meat, obtained only from de-boned meat other than offal that was obtained from carcasses which have been submitted to maturation at a temperature above +2 °C for at least 24 h before the bones were removed. However, significant variations in pH ranges were observed for different offal organs originating from the renal, cardiovascular, respiratory and gastrointestinal systems. These results can be applied directly to a risk management strategy, as the study was run under ideal temperatures and humidity in the presence of competitive microorganisms under field conditions (IFT & FDA 2003; Michael *et al*. 2011).

Muscle pH levels of the springbok in this investigation were similar to those measured for other wild species. Deer shot in the field and deer penned without handling had muscle pH values lower than 5.74 and above 5.74, respectively, with only four of the 66 male deer transported to a slaughterhouse having a muscle pH above 6.0 (Smith & Dobson 1990). In another study, the mean pH_{24h} measurement values were significantly higher in females than males for springbok originating from four production regions in South Africa and ranged between 5.4 and 6.3 (Hoffman, Kroucamp & Manley 2007). In a black wildebeest (*Connochaetus gnou*) meat study, animals harvested during the colder months of winter and autumn had a higher mean muscle pH_{45min}, between 6.7 and 6.8, compared with animals harvested in spring at 6.2, whilst the pH_{24h} values tend to be lower in winter, at 5.4, than in either spring or autumn at 5.6 (Hoffman, Van Schalkwyk & Muller 2009). The rate of pH decline in impala (*Aepyceros melampus*) carcasses harvested at night is slower compared with those harvested during the day (Hoffman, Kritzinger & Ferreira 2003). Male game animals generally tend to have a higher pH than females as a result of a more active response

to disturbances, especially when harvesting occurs during the rutting season (Hoffman 2000; Lewis, Pinchin & Kestin 1997). However, sex was not taken into account during this investigation. Lymph nodes examined by Cottral, Cox and Baldwin (1960) maintained pH readings between 6.4 and 6.9, a favourable range for FMD virus survival (at 4 °C for 72 h). Liver, kidney, rumen, lymph node and blood from diseased cattle have all been shown to be highly infective and to remain so if stored frozen (Henderson & Brooksby 1948).

There are many variables that could have influenced the observed pH variations of the different organs under consideration. However, although these results are subject to potential limitations, the lack of statistically significant association between some selected variables and pH changes in this study suggests that either the factors may have a small effect that is only detectable with large data-sets and/or the effect may have been modified by other unidentified risk-modifying factors that may be elucidated by further investigations. Factors that impact the hunting duration and time from shooting to bleeding include type of hunting terrain, species behaviour, experience and number of the hunters, and size of the harvesting camp in which the animals are found. The role of the environment, genetics and their interactions could also influence the extent of pH decrease in the offal organs (Brown, Bevis & Warriss 1990; Knee *et al*. 2004; Lomiwes 2008; Scanga *et al*. 1998; Young, Thomson, Merhtens & Loeffen 2004; Young, West, Hart & Van Otterdijk 2004; Zuliani *et al*. 2007). The ultimate pH of meat is not only dependent on the muscle glycogen reserves postmortem but also on other factors such as fibre type, which differs between carcasses and offal organs, and antemortem condition of the animal in terms of nutrition, health, stress and behaviour after shooting, all of which may contribute to glycogen depletion, which may cause a high ultimate muscle pH (Atanassova *et al*. 2008; Fink 1992; Forrest 2011; Hoffman *et al.* 2007; Kappelhof 1999; Smith & Dobson 1990; Van Rijswijk & Vorster 1995; Wiklund, Johansson & Malmfors 2003; Young, Thomson, Merhtens & Loeffen 2004). In this study, springbok carcasses and hearts had a pH lower than 6.0 as a result of the fact that both are muscles that have glycogen reserves and thus enter anaerobic metabolism with lactic acid as end product, an effect that could also have influenced shooting-to-bleeding time pH levels in other offal organs.

Observations on seasonal differences and effect of sex and age in ultimate carcass 24 h pH values have been reported previously (Kim *et al*. 2003; Van Schalkwyk 2011; Warriss 2000). Little or no glycogen is found in the lungs, but postmortem pH decline can also be slowed by the buffering effect of blood and ammonium generated by the deamination of adenosine monophosphate (Bendall & Davey 1957), therefore non-pathological haemorrhages (petechial and ecchymotic) and/or congestion sometimes observed at the postmortem inspection of the harvested springbok liver, lung and spleen may have influenced the specific organ pH post-harvest. The pH decline of muscle in a carcass deviates from a linear function because when the muscle enters rigor, hydrogen ion production decreases as the rate of anaerobic glycolysis and myosin ATPase activity decrease during muscle cooling (Bruce, Scott & Thompson 2001). The initial pH fall in muscles does not depend on the glycolytic potential, but at 5 h – 6 h and 24 h postmortem muscles with the lowest glycolytic potential have the highest pH (Maribo *et al*. 1998). In muscles, time taken to reach pH 6.0 from initial pH values of around 7.1 varies between 8 h and 16 h, and as low as 3.5 h in some extreme cases. Some of this variability has been attributed to different rates of cooling between muscles in the same animal (Bendall 1978). The rate of pH decrease is determined by the rate of ATP-turnover, so variability can be to the result of varying intracellular free Ca^{2+} levels exerting a stimulating effect on the actomyosin ATPase, with some of this extra Ca^{2+} arising from calcium released from the mitochondria which become anaerobic after death (Bendall 1978; Honikel, Roncalés & Hamm 1983; Maribo *et al*. 1998). The pH readings 24 h post-slaughter that are greater than 6.5 are indicative of dark, firm and dry (DFD) carcasses, a problem frequently associated with antemortem stress. Apart from lack of visual appeal, dryness and abnormal flavour, DFD meat has increased susceptibility to bacteriological spoilage (Homer & Matthews 1998). Mean pH values have also been reported to correlate linearly with the coliform and *Escherichia coli* counts (Van Schalkwyk 2011).

A linear relationship of lower pH and greater bacteria reduction has been reported in treated organs as a result of induced sublethal injuries through alteration of the pH gradient of the food-borne pathogens (Dorsa, Cutter & Siragusa 1997; Flowers 2006). The effect of lactic acid on food-borne pathogens is ascribed to its ability to penetrate the cell membrane in its non-dissociated form and dissociate within the cell, thereby decreasing the pH and disturbing transmembrane proton motive force and causing an inhibition of acid sensitive enzymes (Dorsa *et al*. 1997; Rajkovic *et al*. 2010). In this study where the hearts are the only edible offal organs likely to have a pH close to or below 6.0, it can be concluded that the organs' natural pH cannot be relied on as a natural mechanism to inactivate endogenous food-borne pathogens, such as RVF virus, in some edible offal. Although international standards set a maximum pH of 6.8 as capable of inactivating RVF virus in carcasses, the primary author observed that this cannot apply to offal as the virus has been isolated in chilled livers (kept for 3–5 days at 4 °C) of healthy sheep that aborted after being transported from the farm to the abattoir. From this study, none of the offal presents a hazard if thoroughly cooked, but the consumption in some cultures of fresh blood, either alone or mixed with milk, raw or undercooked meat and organs should therefore be discouraged (Cima 2012; Corbel 2006; Countries and their Cultures 2011; Fatma & Mahdey 2010; Hall 1991; John *et al*. 2010; Mfinanga *et al*. 2003; Roux 1991).

Conclusion

As part of meat safety risk management, it may be important to consider the use of generally recognised as safe (GRAS) substances to deactivate, by pH changes, susceptible

endogenous and exogenous microbial pathogens in edible offal (Berge *et al*. 2001; Federation of Veterinarians of Europe 2011; Food Safety and Inspection Service 2011; Harrington 2011). Treatment with GRAS should only be carried out after the official meat inspection process has been completed to prevent interference with process hygiene criteria monitoring. Further research should be conducted to establish the normal competitive microflora of the wild game offal that renders the surface of the offal susceptible to preferential growth of pathogens as a result of contamination. Further investigations with large data sets may help elucidate risk-modifying factors for pH changes in harvested wildlife. The prevalence and extent of congested carcasses and organs in harvested wildlife need to be investigated to evaluate the possible maintenance and transmission of some pathogens in different livestock systems caused by the exploding demand for livestock products.

Acknowledgements

The research and the preparation of the article were conducted using personal funds. We would like to thank Dr Nancy Ostiguy from the Department of Entomology, Pennsylvania State University for her assistance with some statistical analysis and data interpretation and the Directorate of Veterinary services for the provision of some of the equipment. Also, we would like to thank the Farmers Meat Market Abattoir HACCP team, especially Victor Mbanga, for their support in pH measurements. Thanks also to Dr E. Midzi and Dr A. Bishi for a critical review of the manuscript. Many thanks to the different game harvesting teams, especially Jan Mostert from Mos-mar game harvesting team, for their kind assistance with the provision and harvesting of some of the game species used in the study.

Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

K.M. (Stellenbosch University) designed and performed all the experiments and wrote the manuscript. F.S. (University of Pretoria) made conceptual contributions, statistical analysis and interpretation of the results, whilst L.C.H. (Stellenbosch University), Y.M.H. (Directorate of Veterinary Services) and F.D. (University of West Indies) were responsible for assistance with the article review, as well as the experimental and project design.

References

Aduku, A.O., Aganga, A.A., Okoh, P.N., Ingawa, S.A. & Phillip, D.O.A., 1991, 'Contribution of offals to the gross value of goat carcasses in Nigeria', *Small Ruminant Research* 6, 179–184. http://dx.doi.org/10.1016/0921-4488(91)90022-I

Allen, L.J., Brown, V.L., Jonsson, C.B., Klein, S.L., Laverty, S.M., Magwedere, K. *et al*., 2012, 'Mathematical modeling of viral zoonoses in wildlife', *Natural Resource Modelling* 25, 5–51. http://dx.doi.org/10.1111/j.1939-7445.2011.00104.x, PMid:22639490

American Veterinary Medical Association, 2010, *Disease precautions for hunters*, viewed 14 June 2010, from https://www.avma.org/public/Health/Pages/Disease-Precautions-for-Hunters.aspx

Atanassova, V., Apelt, J., Reich, F. & Klein, G., 2008, 'Microbiological quality of freshly shot game in Germany', *Meat Science* 78, 414–419. http://dx.doi.org/10.1016/j.meatsci.2007.07.004, PMid:22062460

Bachrach, H.L., Breese, S.S., Callis, J.J., Hess, W.R. & Patty, R.E., 1975, 'Inactivation of foot-and-mouth disease virus by pH and temperature changes and by formaldehyde', *Proceedings of the Society for Experimental Biology and Medicine* 95, 147–152.

Bendall, J.R., 1978, 'Variability in rates of pH fall and of lactate production in the muscles on cooling beef carcasses', *Meat Science* 2, 91–104. http://dx.doi.org/10.1016/0309-1740(78)90010-4

Bendall, J.R. & Davey, C.L., 1957, 'Ammonia liberation during *rigor mortis* and its relation to changes in the adenine and inosine nucleotides of rabbit muscle', *Biochimica et Biophysica Acta* 26, 93–103. http://dx.doi.org/10.1016/0006-3002(57)90059-8

Bengis, R.G. & Veary, C.M., 1997, 'Public health risks associated with the utilization of wildlife products in certain regions of Africa', *Scientific and Technical Review, Office International des Épizooties* 16, 586–593.

Berge, P., Ertbjerg, P., Larsen, L.M., Astruc, T., Vignon, X. & Møller, A.J., 2001, 'Tenderization of beef by lactic acid injected at different times post mortem', *Meat Science* 57, 347–357. http://dx.doi.org/10.1016/S0309-1740(00)00110-8

Blackwell, J.H., 1984, 'Foreign animal disease agent survival in animal products: Recent developments', *Journal of the American Veterinary Medical Association* 184, 674–679. PMid:6327587

Brown, S.N., Bevis, E.A. & Warriss, P.D., 1990, 'An estimate of the incidence of dark cutting beef in the United Kingdom', *Meat Science* 27, 249–258. http://dx.doi.org/10.1016/0309-1740(90)90054-A

Bruce, H.L., Scott, J.R. & Thompson, J.M., 2001, 'Application of an exponential model to early post mortem bovine muscle pH decline', *Meat Science* 58, 39–44. http://dx.doi.org/10.1016/S0309-1740(00)00128-5

Cima, G., 2012, 'Wildlife, trade, susceptibility amplify food risks', *Journal of the American Veterinary Medical Association*, viewed 01 February 2012, from https://www.avma.org/News/JAVMANews/Pages/120215a.aspx

Corbel, M.J., 2006, *Brucellosis in humans and animals*, viewed 01 December 2006, from http://www.who.int/csr/resources/publications/Brucellosis.pdf

Cottral, G.E., Cox, B.F. & Baldwin, D.E., 1960, 'The survival of foot-and-mouth disease virus in cured and uncured meat', *American Journal of Veterinary Research* 21, 228–297.

Countries and Their Cultures, 2011, *Maasai*, viewed 08 December 2011, from http://www.everyculture.com/wc/Tajikistan-to-Zimbabwe/Maasai.html

Department of Agriculture, 2010, *Veterinary health certificate for the importation of venison from Namibia into South Africa for commercial purposes,* RSA Import No. 13/1/1/9/2/10/6–45:2.

Dorsa, W.J., Cutter, C.N. & Siragusa, G.R., 1997, 'Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157:H7, *Listeria innocua*, and *Clostridium sporogenes*', *Journal of Food Protection* 60, 619–624.

European Commission, 2001, 'Brucellosis in sheep and goats (*Brucella melitensis*)', *Report of the Scientific Committee on Animal Health and Animal Welfare*, SANCO.C.2.AH/R23/2001, viewed 12 July 2011, from http://ec.europa.eu/food/fs/sc/scah/out59_en.pdf

European Commission, 2010, 'Commission Regulation (EU) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorized for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements', *Official Journal of the European Union* L73, L146/1–121.

El-Nesser, K.H.A., Mahdey, E., Halaby, A.S. & Deeb, S., 2007, 'Pathological and immunohistochemical studies on *B. melitensis* in cows', *Veterinary Medical Journal Giza* 55, 275–292.

Evans, A., Gakuya, F., Paweska, J.T., Rostal, M., Akopolo, L., Van Vuren, P.J. *et al.*, 2008, 'Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife', *Epidemiology and Infection* 136, 1261–1269. http://dx.doi.org/10.1017/S0950268807009806, PMid:17988425

Falenski, A., Mayer-Scholl, A., Filter, M., Göllner, C., Appel, B. & Nöckler, K., 2010, 'Survival of *Brucella* spp. in mineral water, milk and yogurt', *International Journal of Food Microbiology* 145, 326–330. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.11.033, PMid:21167618

Fatma, H.M. & Mahdey, E.A., 2010, 'Incidence of *Brucella* species in slaughtered food animals and its edible offal at Beni-suef, Egypt', *Global Veterinaria* 5, 248–254.

Fink, H.G., 1992, 'Wild', in K. Fehlhaber & P. Janetschke (eds.), *Veterinärmedizinische Lebensmittelhygiene*, pp. 412–447, Gustav Fischer Verlag Jena, Stuttgart.

Flowers, S.L., 2006, 'Identification and validation of antimicrobial interventions for red meat carcasses processed in very small meat establishments', PhD thesis, Department of Food Science, Pennsylvania State University.

Forrest, J.C., 2011, *Meat quality and safety*, viewed 20 January 2012, from http://ag.ansc.pu rdue.Edu/meat quality/mqfstress.html

Federation of Veterinarians of Europe, 2011, *Decontamination of carcasses*, viewed 19 November 2011, from http://www.fve.org/uploads/publications/docs/fve_11_050%20decontamination%20of%20carcasses_19_nov_2011.pdf

Food Safety and Inspection Service, 2011, *Safe and suitable ingredients used in the production of meat, poultry and egg products*, FSIS directive number 7220.1.1/4/11, United States Department of Agriculture, Food Safety and Inspection Service, Washington, DC.

Gerhardt, K., 2003, *The body condition of caribou*, viewed 29 June 2003, from http://dl1.yukoncollege.yk.ca/caribou/stories/storyReader$2

Hall, W.H., 1991, 'Brucellosis', in A.S. Evans & P.S. Brachman (eds.), *Bacterial infections of humans, epidemiology and control*, 2nd edn., pp. 133–149, Plenum Medical Book Co., New York. PMid:2004204

Harrington, R., 2011, *European Food Safety Authority backs the use of lactic as beef contaminant*, viewed 10 August 2011, from http://www.foodproductiondaily.com/Quality-Safety/EFSA-backs-use-of-lactic-acid-as-beef-decontaminant

Henderson, W.M. & Brooksby, J.B., 1948, 'The survival of foot-and-mouth disease virus in meat and offal', *Journal of Hygiene* 46, 394–402. http://dx.doi.org/10.1017/S0022172400036561

Hoffman, L.C., 2000, 'Meat quality attributes of night cropped impala (*Aepyceros melampus*)', *South African Journal of Animal Science* 30, 133–137. http://dx.doi.org/10.4314/sajas.v30i2.3862

Hoffman, L.C., Kritzinger, B. & Ferreira, A.V., 2003, 'A comparison between the effects of two cropping methods on the meat quality of impala (*Aepyceros melampus*)', *South African Journal of Animal Science* 33, 233–241.

Hoffman, L.C., Kroucamp, M. & Manley, M., 2007, 'Meat quality characteristics of springbok (*Antidorcus marsupialis*) 1: Physical meat attributes as influenced by age, gender and production region', *Meat Science* 76, 755–761. http://dx.doi.org/10.1016/j.meatsci.2007.02.017, PMid:22061254

Hoffman, L.C., Van Schalkwyk, S. & Muller, N., 2009, 'Effect of season and gender on the physical and chemical composition of black wildebeest (*Connochaetus gnou*) meat', *African Journal of Wildlife Research* 39, 170–174. http://dx.doi.org/10.3957/056.039.0208

Homer, D.B. & Matthews, K.R., 1998, 'A repeat national survey of muscle pH values in commercial pig carcasses', *Meat Science* 49, 425–433. http://dx.doi.org/10.1016/S0309-1740(98)00010-2

Honikel, K.O., Roncalés, P. & Hamm, R., 1983, 'The influence of temperature on shortening and rigor onset in beef muscle', *Meat Science* 8, 221–241. http://dx.doi.org/10.1016/0309-1740(83)90046-3

Hyslop, N.S.G., 1970, 'The epizootiology and epidemiology of foot and mouth disease', *Advances in Veterinary Science* 14, 261–307.

International Commission on Microbiological Specifications for Food, 1996, 'Microorganisms in foods 5: Characteristics of microbial pathogens', in T.A. Roberts, A.C. Baird-Parker & R.B. Tompkin (eds.), *Brucella*, pp. 36–44, Springer, Berlin.

Institute of Food Technologists & Federal Drug Administration, 2003, 'Factors that influence microbial growth', *Comprehensive Reviews in Food Science and Food Safety* 2, 21–32. http://dx.doi.org/10.1111/j.1541-4337.2003.tb00048.x

Iowa State University, 2011, *Brucellosis (swine brucellosis)*, viewed 01 March 2011, from http://vetmed.iastate.edu/vdpam/new-vdpam-employees/food-supply-veterinary-medicine/swine/swine-diseases/brucellosis-swine-bru

John, K., Fitzpatrick, J., French, N., Kazwala, R., Kambarage, D., Mfinanga, G.S. *et al.*, 2010, 'Quantifying risk factors for human brucellosis in rural northern Tanzania', *PLoS ONE* 5(4), e9968. http://dx.doi.org/10.1371/journal.pone.0009968, PMid:20376363

Kappelhof, W., 1999, Wildbrethygiene in der jagdlichen Praxis [Game meat hygiene in while hunting]. *Amtstierärztlicher Dienst und Lebensmittelkontrolle* 6, 272–276.

Kim, Y.S., Yoon, S.K., Song, Y.H. & Lee, S.K., 2003, 'Effect of season on colour of Hanwoo (Korean native cattle) beef', *Meat Science* 63, 509–513. http://dx.doi.org/10.1016/S0309-1740(02)00112-2

Knee, B.W., Cummins, L.J., Walker, P. & Warner, R., 2004, 'Seasonal variation in muscle glycogen in beef steers', *Australian Journal of Experimental Agriculture* 44, 729–734. http://dx.doi.org/10.1071/EA03044

Lerche, M. & Entel, H.J., 1959, Über die Säureresistenz von Brucella-Bakterien und ihr Verhalten während der Fleischreifung [On the acid resistance of Brucella bacteria and their behavior during meat maturation]. *Archiv für Lebensmittelhygiene* 9, 194–198.

Lewis, A.R., Pinchin, A.M. & Kestin, S.C., 1997, 'Welfare implications of the night shooting of wild impala (*Aepyceros melapus*)', *Animal Welfare* 6, 123–131.

Lomiwes, D., 2008, 'Rapid on-line glycogen measurement and prediction of ultimate pH in slaughter beef', MAppSc thesis, Faculty of Health and Environmental Sciences, Auckland University of Technology, viewed 30 May 2008, from http://aut.researchgateway.ac.nz/bitstream/handle/10292/970/lomiwesd.pdf?sequence=1

MacDiarmid, S.C. & Thompson, E.J., 1997, 'The potential risks to animal health from imported sheep and goat meat', *Scientific and Technical Review, Office International des Épizooties* 16, 45–56.

Mandell, R.B. & Flick, R., 2011, 'Rift Valley Fever Virus: A Real Bioterror Threat', *Journal of Bioterrorism & Biodefense* 2, 108. http://dx.doi.org/10.4172/2157-2526.1000108

Maribo, H., Olsen, E.V., Barton-Gade, P., Møller, A.J. & Karlsson, A., 1998, 'Effect of early post-mortem cooling on temperature, pH fall and meat quality in pigs', *Meat Science* 50, 115–129. http://dx.doi.org/10.1016/S0309-1740(98)00022-9

Mfinanga, S.G., Mørkve, O., Kazwala, R.R., Cleaveland, S., Sharp, J.M., Shirima, G. *et al.*, 2003, 'Tribal differences in perception of tuberculosis: A possible role in tuberculosis control in Arusha, Tanzania', *International Journal of Tuberculosis and Lung Disease* 7, 933–941. PMid:14552562

Michael, A., Geoghegan, C., Van der Merwe, M., Hlokwe, T. & Marcotty, T., 2011, 'Mycobacteria in the food chain: A problem or not?', *Proceedings of the 1st International One Health Congress*, 14–16 February 2011, Melbourne, Australia.

Pearson, A.M. & Dutson, T.R., 1988, *Advances in meat research*, Elsevier Science Publishers Ltd, London.

Pharo, H.J., 2002, 'Foot-and-mouth disease: An assessment of the risks facing New Zealand', *New Zealand Veterinary Journal* 50, 46–55. http://dx.doi.org/10.1080/00480169.2002.36250, PMid:16032210

Rajkovic, A., Smigic, N., Nielsen, D.S., Arneborg, N., Siegumfeldt, H. & Devlieghere, F., 2010, 'Survival of lactic acid and chlorine dioxide treated *Campylobacter jejuni* under suboptimal conditions of pH, temperature and modified atmosphere', *International Journal of Food Microbiology* 141, S140–S146. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.01.026, PMid:20149937

Roux, J., 1991, 'Public health importance of brucellosis', in E. Tümbay, S. Hilmi & Ö. Ang (eds.), *Brucella and brucellosis in man and animals*, pp. 3–10, Ege Oniversity Press, Yzmir.

Sadler, W., 1960, 'Present evidence on the role of meat in the epidemiology of human brucellosis', *American Journal of Public Health* 50, 504–514. http://dx.doi.org/10.2105/AJPH.50.4.504

SAS version 9.1.3, 2007, SAS Institute Inc., Cary.

Scanga, J.A., Belk, K.E., Tatum, J.D., Grandin, T. & Smith, G.C., 1998, 'Factors contributing to the incidence of dark cutting beef', *Journal of Animal Science* 76, 2040–2047. PMid:9734853

Scott, W., 2003, *Persistence of disease agents in carcasses and animal products*, viewed 30 December 2003, from http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/WilliamsReport.pdf

Smith, R.F & Dobson, H., 1990, 'Effect of pre-slaughter experience on behaviour, plasma cortisol and muscle pH in farmed red deer', *Veterinary Record* 126, 155–158. PMid:2309417

Stata version 10, 2007, Stata Corp., College Station.

Territory of South West Africa, 1975, *Nature Conservation Ordinance* (No. 4 of 1975), Legislative Assembly, Windhoek, viewed 11 February 2013, from http://www.met.gov.na/Documents/Nature%20Conservation%20Ordinance%204%20of%201975.pdf

United States Department of Agriculture, 2011, *Jerky and food safety*, viewed 15 November 2011, from http://www.fsis.usda.gov/factsheets/jerky_and_food_safety/index.asp

Van Rijswijk, A.W. & Vorster, F., 1995, 'The influence of stress on the adrenals of the springbok (*Antidorcas marsupialis*)', *Journal of the South African Veterinary Association* 66, 251–253. PMid:8691417

Van Schalkwyk, D.L., 2011, 'Investigation into selected parameters required to develop a sustainable Namibian game meat industry', PhD dissertation, Department of Food Science, Stellenbosch University.

Van Schalkwyk, D.L. & Hoffman, L.C., 2010, *Guidelines for the harvesting of game for meat export*, Ministry of Agriculture, Water and Forestry, Windhoek.

Van Schalkwyk, D.L., McMillin, K.W., Booyse, M., Witthuhn, R.C. & Hoffman, L.C., 2011, 'Physico-chemical, microbiological, textural and sensory attributes of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*) harvested in Namibia', *Meat Science* 88, 36–44. http://dx.doi.org/10.1016/j.meatsci.2010.11.028, PMid:21185658

Warriss, P.D., 2000, *Meat science: An introductory text*, CABI Publishing, Wallingford.

Wiklund, E., Johansson, L. & Malmfors, G., 2003, 'Sensory meat quality, ultimate pH values, blood parameters and carcass characteristics in reindeer (*Rangife tarandus tarandus*) grazed on natural pastures or fed commercial feed mixture', *Food Quality and Preference* 14, 573–581. http://dx.doi.org/10.1016/S0950-3293(02)00151-9

World Organisation for Animal Health, 2012a, *Manual of diagnostic tests and vaccines for terrestrial animals*, viewed 10 December 2012, from http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

World Organisation for Animal Health, 2012b, *Terrestrial Animal Health Code*, viewed 10 December 2012, from http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

Young, O.A., Thomson, R.D., Merhtens, V.G. & Loeffen, M.P.F., 2004, 'Industrial application to cattle of a method for the early determination of meat ultimate pH', *Meat Science* 67, 107–112. http://dx.doi.org/10.1016/j.meatsci.2003.09.014, PMid:22061123

Young, O.A., West, J., Hart, A.L. & Van Otterdijk, F.F.H., 2004, 'A method for early determination of meat ultimate pH', *Meat Science* 66, 493–498. http://dx.doi.org/10.1016/S0309-1740(03)00140-2

Zobell, C.E. & Meyer, K.F., 1932, 'Metabolism studies on the Brucella Group: IX. Physicochemical requirements in synthetic mediums', *Journal of Infectious Diseases* 51, 361–381. http://dx.doi.org/10.1093/infdis/51.2.361

Zuliani, V., Lebert, I., Augustin, J.C., Garry, P., Vendeuvre, J.L. & Lebert, A., 2007, 'Modelling the behaviour of *Listeria monocytogenes* in ground pork as a function of pH, water activity, nature and concentration of organic acid salts', *Journal of Applied Microbiology* 103, 536–550. http://dx.doi.org/10.1111/j.1365-2672.2007.03283.x, PMid:17714386