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Full Length Research Paper

Health factors associated with persistent konzo in four villages in the Democratic Republic of Congo (DRC)

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Persistent konzo is low level incidence of konzo that is not associated with konzo epidemics due to drought or war. It has been reported from Mozambique and Tanzania. Various health factors associated with persistent konzo in four villages in Kasanji Health Area, Bandundu Province, DRC were studied, where there were 38 konzo cases in a population of 2283, with mean percentage konzo prevalence of 1.7%. Konzo occurred over the years since 1951 with 0 to 3 cases per year. Anthropometric measurements of children aged 0.5 to 14 y showed that 47% were stunted, 24% underweight, 16% had chronic energy deficiency and 16 to 24% suffered malnutrition. There was no significant difference between children from konzo households or from non-konzo households. The % konzo prevalence (%K) calculated from the equation %K = 0.06 %T + 0.035 %M, where %T = percentage of school children with urinary thiocyanate levels >350 µmol/L and %M = percentage of malnutrition, gave %K = 1.6 from non-konzo households and %K = 2.2 from konzo households, which agrees with the actual mean value of 1.7. However, the equation does not apply when either %T or %M is zero or for very high values of %K > 9.5. The value of %K is nearly twice as sensitive to changes in %T as to changes in %M, which partly explains the greater ease of prevention of konzo by reducing %T to zero, using the wetting method to remove cyanogens from cassava flour, than by reducing %M by broad based methods.

Key words: Konzo prevalence, malnutrition, school children, urinary thiocyanate, cassava cyanide.

INTRODUCTION

Konzo is a spastic paraparesis that causes irreversible paralysis of the legs mainly in children and young women, associated with intake of large amounts of cyanide from a diet of bitter cassava that also causes malnutrition (Cliff et al., 1985; Howlett et al., 1990; Nzwalo and Cliff, 2011). It is worst in the Democratic Republic of Congo (DRC), also occurs in Mozambique, Tanzania, Cameroon, Central African Republic, Angola and there are reports of its recent spread to Uganda (Diasolua-Ngudi, 2015) and Zambia (Mbewe, 2015).

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The condition was first described by Trolli (1938) working in the Belgian Congo (now DRC) and it has persisted there up to the present time. Konzo occurs most commonly during the time of the cassava harvest when cassava intake is at a maximum. A study developed a simple equation that relates the % konzo prevalence (%K), with the % malnutrition (%M) calculated from a food consumption survey and the percentage of children with high urinary thiocyanate content (%T), (determined near the time of cassava harvest), which is a good estimate of their cyanide intake (Banea et al., 2015a).

Konzo epidemics occur due to drought when water-stressed cassava plants make 2 to 4 times as much cyanogenic glucoside as normal (Bokanga et al., 1994), and the increased level of cyanide is transferred to the processed cassava flour (Cardoso et al., 2005), which causes cyanide poisoning and konzo (Cliff et al., 2011). Konzo epidemics also result from war when local village people are forced from their homes and gardens and forced to eat unprocessed high cyanide cassava from the bush (Cliff et al., 1997; Nhassico et al., 2008; Chabwine et al., 2011). Konzo outbreaks also occur in Central Africa when peeled cassava roots are not immersed in water for the 3 to 4 days necessary to allow the enzyme linamarase to break down fully the cyanogenic glucoside, but are short soaked for only 1 to 2 days. (Banea et al., 1992). By contrast, konzo can occur at a low level of about one per village per year called persistent konzo, and has been observed in Mozambique (Ernesto et al., 2002) and Tanzania (Howlett, 1994).

The study reports on four villages in the DRC, in which persistent konzo has occurred over many years and various health factors such as the degree of malnutrition, stunting and high cyanide intake from cassava that together are associated with persistent konzo.

MATERIALS AND METHODS

A population census was carried out. People with walking difficulties were examined for konzo following the criteria established by World Health Organisation (WHO, 1996): a spastic visible walk or run, a history of sudden onset within a week of a person in good health, bilateral exagerration of knee jerks and/or Achillian reflexes and non-progressive evolution of the disease. In each household with a case of konzo, the study identified the nearest household with no case of konzo (control household). Anthropometric measurements of weight and height were made on 24 to 31 children aged 6 to 59 months and of 31 children aged 5 to 14 years from konzo households and about 50 children from non-konzo control households. Height for age was recorded and children whose height for age was below minus two standard deviations from the median of WHO standards of child growth were classified as stunted. The weight for age was calculated and those whose weight for age was below minus two standard deviations from the median weight for age of the WHO reference population were classified as underweight. The body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height in metres and those whose BMI for age was below minus two standard deviations from the median BMI for age were considered to have chronic energy deficiency.

Study area and agriculture

The survey was conducted in four villages; Kasanji, Mulopo Luaka, Kidima and Mupepe of Kasanji Health Area in Boko Health Zone on August 21 to 29, 2014 (Banea et al., 2015a). The villages are in the savanna and the main crops are cassava, maize and groundnuts, which are also cash crops. The rainy season extends from October to April and the dry season from May to September. There are major conflicts of succession of leaders and land disputes between several families.

Food consumption survey, food consumption score (FCS) and percent malnutrition (%M)

The number of days in which different foods were eaten during the week preceding the survey was obtained by a survey of 31 konzo and 31 non-konzo households. The food consumption score (FCS) was calculated and interpreted using the methods of the World Food Program (Interagency Workshop Report WFP-FAO, 2008; Banea et al., 2015a). The percentage malnutrition (% M) was calculated (Banea et al., 2015b) for konzo and non-konzo control households. The socio-economic conditions of konzo households and non-konzo households was also compared with respect to type of roof, whether straw or sheet metal on houses, toilet facilities, furniture and other effects within houses.

Urinary thiocyanate analysis, calculation of %T and %K

Urine samples were obtained from 41 school children aged 5 to 14 y in konzo households and 63 school children from non-konzo control families, with oral consent of their parents. These samples were obtained from Kasanji 60, Mulopo Luaka 18, Kidima 19 and Mupepe 7, and were analysed on site using the simple picrate thiocyanate kit D1, http://biology.anu.edu.au/hosted_sites/CCDN/; Haque and Bradbury, 1999). A colour chart with ten shades of colour from yellow to brown was used, which corresponded to 0 to 1720 µmol thiocyanate/L. The percentage of children with urinary thiocyanate levels >350 µmol/ (%T) was calculated for konzo and non-konzo control families. The % konzo prevalence was calculated by the equation % K = 0.06 %T + 0.035 %M (Banea et al., 2015a).

RESULTS

In the four villages there were 47 persons with walking difficulties which included 38 konzo cases with a mean konzo prevalence of 1.7% (Table 1). Detailed examination of konzo cases showed that all had an abrupt beginning of 1 to 7 days, with 82% beginning in one day. The knee jerks bilateral occurred in 92% of cases and Achilles reflexes in 60% of cases. With regard to walking, 84% were mildly disabled not using a stick to walk and 16% were moderately disabled requiring one or two sticks to walk. Speech disorders occurred with 68% of patients and 45% had impaired vision. Of the konzo cases, 63% were women, 37% men; 37% were single, 61% married and 2% widowed. Half of the patients had at least one relative dead or alive with konzo.

The distribution of onset of konzo over the years is shown in Figure 1, two persons did not remember the

Table 1. Konzo prevalence in four villages in Kisanji Health Area, Boko Health Zone, Bandundu Province, DRC.

Village	Population	Number of konzo cases	% Konzo prevalence
Kisanji	1109	23	2.1
Mulopo Luaka	753	6	0.8
Kidima	134	5	3.7
Mupepe	291	4	1.4
Total	2287	38	1.7

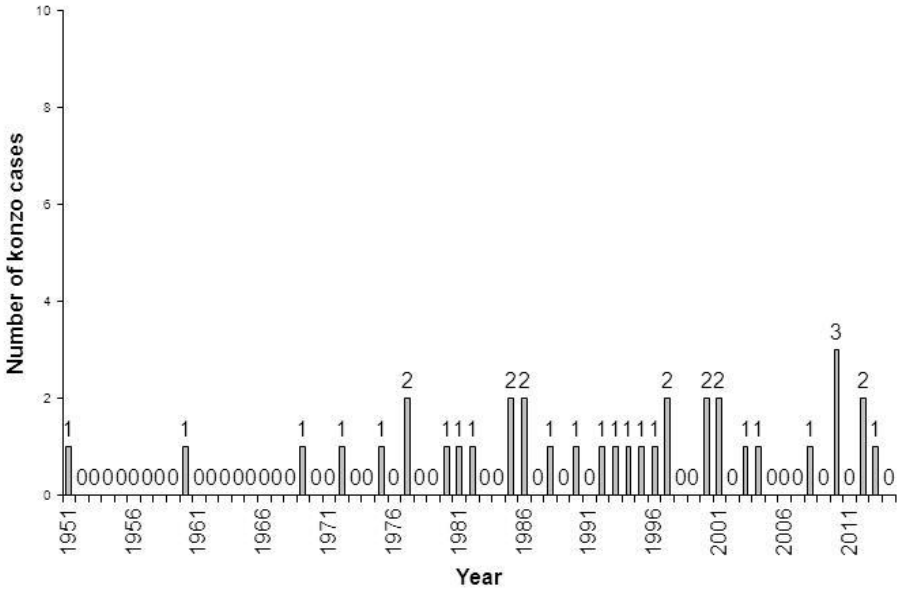

Figure 1. Annual distribution of onset of konzo cases stretching back over 63 years. Data for two cases was not available

year of onset. Only 19 people recalled the exact month of onset of konzo and this data is shown in Figure 2. About 80% of the houses had straw roofs which leaked water in the wet season, nearly all households had unimproved toilets, all households used improved water sources for drinking water, but this involved a 30 min walk. Nearly all houses had chairs and tables, 60% had a radio, 35% a shotgun and two thirds of those from non-konzo households had a telephone compared with only one third from a konzo household.

Anthropometric measurements made on children aged 6 to 59 months from 24 konzo households and 47 non-konzo households showed that 47% were stunted and 24% underweight with no significant differences between those from konzo or non-konzo households. For 31 school children aged 5 to 14 years from konzo households and 53 from non-konzo households the stunting was virtually the same at 48% as from children aged 6 to 59 month. Furthermore, 16% of school children had BMI for age below minus two standard deviations from the median BMI for age, called chronic energy deficiency. There was no significant difference between the % stunting or the % chronic energy deficiency for those children 5 to 14 years from konzo or non-konzo households.

The results of the food consumption survey of 31 konzo households and 31 non-konzo households is given in Table 2, together with the percentage malnutrition (%M) calculated for the konzo and non-konzo households. In Table 3 is shown the percentage of school children with >350 µmol/L urinary thiocyanate levels (%T) from konzo and non-konzo households and also the % konzo prevalence (%K) calculated from the data for %T (Table 3) and %M (Table 2).

DISCUSSION

Persistent konzo, also sometimes known as endemic or sporadic konzo, occurs at the rate of one or two konzo cases per village per year. It has been previously observed in Mozambique (Ernesto et al., 2002) and

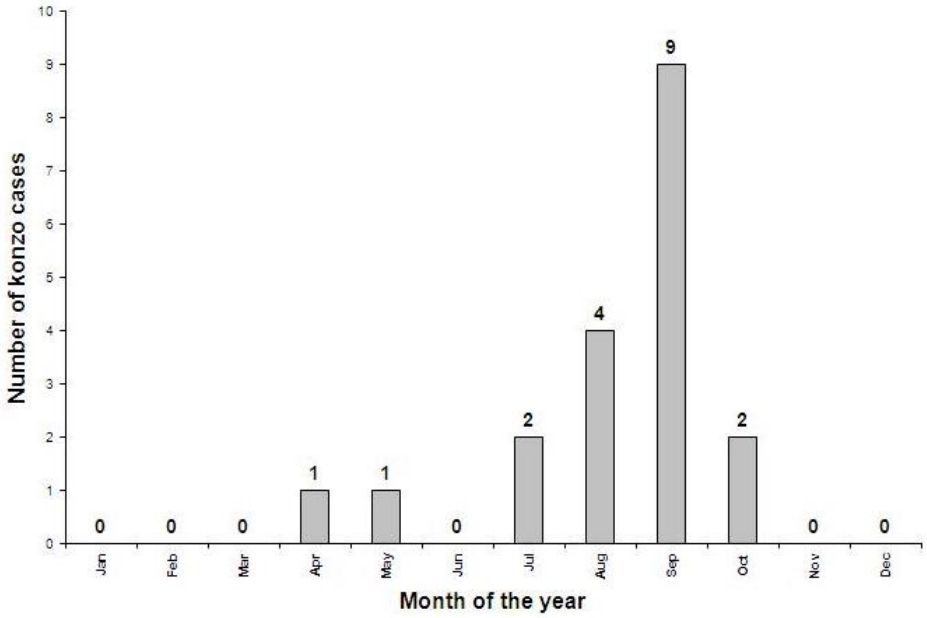

Figure 2. Monthly distribution of onset of konzo for the 19 cases for which informaton was available.

Table 2. Percentage of konzo and non-konzo families with poor, limited and acceptable food consumption scores (FCS) and % malnutrition (%M)[a].

Type of family	Number of families	Percentage of families with			%Malnutrition (%M)
		Poor FCS	Limited FCS	Acceptable FCS	
With konzo case	31	6.5	35.5	58	24
Without konzo case	31	0.0	32.3	67.7	16

[a]% malnutrition calculated from the data by the equation %M = 0.5 [2 (poor FCS) + limited FCS], (Banea et al., 2015a).

Table 3. Percentage of konzo and non-konzo families with high urinary thiocyanate content and calculated % konzo prevalence.

Type of family	% school children with high urinary thiocyanate[a]	% konzo prevalence, calc[b]
With konzo case	22	2.2
Without konzo case	18	1.6

[a]% of children with urinary thiocyanate >350 µmol/L; [b]%K = 0.06 %T + 0.035 %M. %M calculated in Table 2.

Tanzania (Howlett, 1994). The distribution of konzo in these four villages over the years since 1951 is shown in Figure 1 and there are small or zero numbers of konzo cases each year, rather than the variable and increasing numbers found in recent years in our other studies (Banea et al., 2013, 2015b). Lest it is thought that these cases of persistent konzo only amount to a very few cases compared to the many cases that occur during an epidemic due to drought, war or short soaking and it should be noted that a recent survey of thirty villages in Kwilu District of Bandundu Province in which there were 26 villages with low konzo prevalence of 0.11 to 1.1%, accounted for 58% of the total 172 konzo cases (Banea et al., 2015b). It is therefore important to consider persistent konzo where cases are spread thinly over a large number of villages, thus affecting large numbers of people.

The monthly distribution of onset of 19 of the konzo cases who could remember the month of onset is shown in Figure 2. The peak konzo onset (September) also corresponds with the time of the cassava harvest when cassava consumption peaks, as has been found in virtually every study on konzo. This corresponded with

the time of late August for the visit by the study group, which is also the time when the percentage of school children with high urinary thiocyanate (>350µmol/L) levels should be measured (Banea et al., 2015a). The study was designed to show up differences between konzo households and non-konzo households in the same village but no significant differences were observed, except that the ownership of telephones was twice as great in non-konzo households compared with konzo households. Similarly, it has been difficult to establish differences between the urinary thiocyanate levels of school children with konzo and those without konzo, but one study has shown a significant increase amongst konzo children compared with non-konzo children carried out at the time of the cassava harvest (Banea et al., 2013).

Stunting, underweight, chronic energy deficiency, malnutrition

An anthropometric study of 500 children aged 0 to 36 months in a non-konzo area north of the Kasai river and of a konzo area south of the river in Bandundu Province, DRC, showed that the height for age index was significantly lower in children from the southern konzo area (indicative of stunting), but there was no significant differences in weight for height or weight for age indices (Banea et al., 2000). In konzo villages, the level of stunting is about 40% amongst children less than 5 years old (Diasolua-Ngudi, 2015). In these four villages with persistent konzo, the study found 47% of the children aged 0.5 to 14 years were stunted, which agrees with Diasolua-Ngudi (2015). In addition, 24% were underweight and 16% suffered from chronic energy deficiency. The food consumption survey (Table 2) showed that 16 to 24% of the population had malnutrition.

Calculation of % konzo prevalence (%K)

The urinary thiocyanate data from 104 school children from konzo and non-konzo households in Table 3 records the percentage of school children with high urinary thiocyanate content (%T). This is used in equation (1) to calculate %K expected with konzo households (2.2%) and with non-konzo households (1.6%). The actual mean value of %K for the four villages is 1.7% (Table 1). The good agreement between the actual mean value of %K and the calculated value from Equation (1) shows that this simple empirical equation is useful. However, the equation does not apply if either %M or %T is zero (Banea et al., 2015a) or above the upper range of the equation, when %K > 9.5. Recently, Banea et al. (2015b) found in Kongila Ndola village in Kwilu District of DRC a very high konzo prevalence of 17%, perhaps due to very high cyanide intake by the school children which would produce very high urinary thiocyanate levels.

In Equation 1, it is noted that the value of %K is nearly twice as sensitive to a change in %T as it is to an equal change in %M.

Conclusion

The study report shows that %K can be reduced by twice the amount by reducing %T as it can by an equal reduction in %M. This is one reason why it is simpler and more effective to prevent konzo by reducing cyanide intake using the wetting method (Banea et al., 2014b, 2015a) rather than by improving the overall nutrition of village people (Kasongo and Calo, 2011; Delhourne et al., 2012). Indeed once %T is reduced to zero, by daily use of the wetting method by village women that removes cyanogens from the cassava flour, then konzo is prevented (Bradbury et al., 2015). Furthermore, the wetting method is much more direct in its application than a broad based holistic method (Delhourne et al., 2012).

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Residual chlorine and pH influence on hygienic tap quality water consumed in Togo

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Water is used in personal hygiene, but also for food purposes. Unfortunately, the problem of drinking water consumption persists in developing countries. Water supply involves several stages from collection to storage through packaging and transport. During all these steps, the water can undergo various microbiological, physical and chemical contaminations that can transmit waterborne diseases among consumers. The characterization of the water quality is therefore important to protect the health of consumers. The main objective of this study was to assess the influence of residual chlorine and pH on microorganisms in drinking water. To this end, 30 water samples were collected in nine districts of Lomé in Togo. The spores were detected by routine standardized methods of the French Association for Standardization (AFNOR). The results of this study showed the presence of total spores with an average of 1.84 spore/ml in some samples despite the high levels of chlorine.

Key words: Tap water, hygienic quality, residual chlorine, Togo.

INTRODUCTION

Unsafe water kills more humans than all forms of violence. More than 3 million humans die annually from diseases related to water and the environment (WHO, 2005). If globally 2.4 billion people have access to safe drinking water and 600 million to sanitation over the last two decades, 1.1 billion still do not have access, while 3.5 million children die each year of waterborne diseases (Main causes of infant mortality on earth) (Marc, 2003). Water-related diseases are both due to lack of water, especially the lack of drinking water. Several writings including Nanga et al. (2014) and WHO (2005) highlighted the relationship between water quality and waterborne diseases. In Africa, poor quality water consumption is one of the leading causes of death (Bernadette, 2008; Anonymous, 2010). Compared to chemical processes, oxidation by agents such as chlorine and ozone, acts on metals (iron, manganese), on the organic matter and destroyed or inactivates totally or partially living spores, viruses and bacteria (CIEAU, 2008). Kahoul and Touhami (2014) reported that water supply must meet the quality requirements. Thus, it should not contain any microorganism, no noise and no substance presents a potential danger to human health; it must also comply vis-à-vis a set of standards for drinking water.

In Togo, like other sub-Saharan countries, the consumption of poor quality water is one of the causes of death. However the country has ratified various

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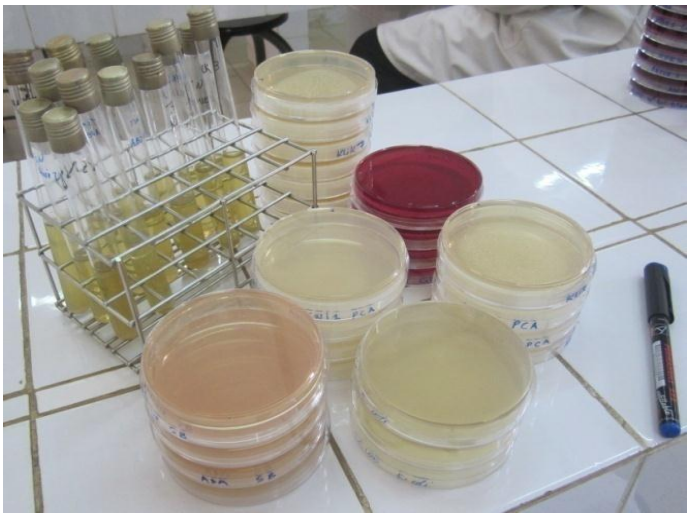

Figure 1. Petri dishes and tubes TSN.

Figure 2. Incubators (Jouan).

conventions and charters on water policy. Also, a lot of people as is the case in several countries in the world do not always have access to potable water. The vulnerability of aquifers to contamination and the high variability of hydrogeological situations require a specific study. Monitoring the hygienic quality of the water produced by the Togolese Water Company of the city of Lomé therefore remains a necessity to ensure safe drinking water to avoid water-borne diseases. The present work aims to analyze the tap water sampled in nine districts of the city of Lomé. For this, microorganisms have been searched, measured residual chlorine and pH in these water samples. The aim of this study is to verify the compliance of drinking water with respect to quality requirements adopted by the standards.

MATERIALS AND METHODS

Sampling took place from May 2 to July 4, 2011. Public tap water was taken at random in nine districts of the city of Lomé. This is the tap water produced by the Togolese Water Company (TdE). Samples collection and transport consist of bottles (Simax) 500 ml test tubes and a cooler (Igloo LEGEND 24, Igloo Products Corporation, USA) with cooling elements. The technical equipment consists of the spectrophotometer (Digitron Elvi 675, Logos Scientific, INC., USA), the pH meter (WTWpH 330i, Wissenschaftlich-Technische Werkstatten GmbH, WTW, Germany), brand Jouan incubators at 30, 37 and 44°C, binocular microscopes (Motic), an electric balance (Mettler P1210N, Mettler Toledo, Switzerland), an autoclave (Leuqueux, Paris). Some materials are illustrated by Figures 1 and 2.

The water samples were placed in a cooler (Igloo LEGEND 24, Igloo Products Corporation, USA) provided with cooling elements. It is advisable to keep the samples at a temperature of about 4°C to slow this bacterial activity (Aminot and Kerouel, 2004). The bacteriological parameters are considered the detection and enumeration of total bacteria, total coliforms, thermotolerant coliforms, *Escherichia coli*, fecal streptococci and sulphite-reducing anaerobic. The seeding technique in mass was used for detection and enumeration of spores. The total spores were detected with Plate Count Agar (PCA). With a sterile pipette, 1 ml of stock solution or one of its dilutions which are placed in petri box was taken. After pouring 20 ml of PCA, agar was incubated at 30°C for 24 to 72 h. Total coliforms were counted with crystal violet agar in neutral Red, the Bile and Lactose (VRBL). Incubation was at 30°C for 24 h. Enumeration of thermotolerant coliforms and *E. coli* is the same as total coliforms; however, the incubation was carried out at 44°C. Enumeration of *E. coli* is from boxes thermotolerant coliforms. Fecal streptococci were detected by the middle Slanetz and Bartley agar, incubated at 37°C for 24 to 48 h. The sulfite- reducing anaerobic bacteria (ASR) were detected by the Tryptone Sulfite Neomycin (TSN) agar tubes. 1 ml of the solution is introduced into 19 ml of TSN; the incubation was carried out at 44°C for 24 to 48 h. A subculture on nutritive agar has achieved the Gram stain. A colony was putted in the solution of hydrogen peroxide to search catalase. The colony was hit with a strip detection of oxidase to produce the oxidase test.

For the dosage of chlorine, residual was done with a spectrophotometer at the wavelength of 440 nm. 0.5 ml of 0.1% orthotolidine was introduced in a test tube and then 10 ml water sample was added. The pH was measured because the chlorine disinfection takes place best when the pH is between 5.5 and 7.5 (Florence, 2007). pH measurement is made using the pH-meter (WTWpH 330i, Wissenschaftlich-Technische Werkstatten GmbH, WTW, Germany).

Statistical analysis

GraphPad Prism 4.00 was used to analyze the results. The difference between the samples was determined by Tukey's test multiple comparison, by a safety factor of 95% and a degree of freedom at risk of 5%.

RESULTS

The results revealed the presence of aerobic mesophilic

Table 1. Assessment of the microbiological results.

Germs sought	Extreme values	General average (n = 30)	Criteria *	% of conformity
			m	Satisfactory
Total spores (30°C)	0 - 19	1.84	100	100
Total coliforms (30°C)	0	0	0	100
Thermotolerant coliforms (44°C)	0	0	0	100
Escherichia coli (44°C)	0	0	0	100
Fecal streptococci (37°C)	0	0	0	100
ASR (44°C)	0	0	2	100

*Criteria of the European Union Council Directive 98/83/EC (m); ASR: Anaerobic sulphite-reducing; n: number of samples analyzed.

Table 2. Distribution of organisms isolated by the catalase and oxidase tests.

Gram's coloration	Catalase	Oxidase	Number of spores	% (n = 30)
B+	+	+	5	17
B+	+	-	19	63
C+	+	-	3	10
C+	-	-	3	10

B^+ : Gram positive bacilli ; C^+ : Gram positive cocci ; +: positive; - : negative.

Table 3. Residual chlorine levels in the different districts.

S/N	Sampling point	Number of samples	Values > than reference (mg/L)	Quality limit/EU*(mg/L)
1	Abové	4	-	0.1
2	Atikoumé	3	0.7	0.1
3	Gbossimé	3	-	0.1
4	Doumassessé	4	0.2	0.1
5	Tokoin Lycée	3	-	0.1
6	Dogbéavou	3	0.2	0.1
7	Klikamé	3	-	0.1
8	Tokoin Trésor	4	-	0.1
9	Akodéssewa	3	-	0.1

*Criteria of the European Union (EU) Council Directive 98/83/EC.

total in 10 samples. On the other hand, other organisms have not been found sought. The results are shown in Table 1.

Total aerobic mesophiles were found whose values are between 1 and 19 microorganisms per ml of water. On the other hand, the absence of coliforms, *E. coli* as well as fecal streptococci and sulfite-reducing anaerobic were noticed.

Gram stain gave us 80% of spores found are Gram positive bacilli and 20% are Gram positive cocci. The catalase tests and oxidase showed that overall 90% of spores are catalase + and 10% are catalase -; 16.66% of spores isolated are oxidase + and 83.33% oxidase - with positive Gram bacilli, catalase + and oxidase +; positive Gram bacilli and catalase +, oxidase -; positive Gram cocci catalase + and oxydase - ; and positive Gram cocci, catalase - and oxidase - (Table 2).

For the 30 measured samples, three samples or 10% have a chlorine residual greater than the reference limit (Table 3).

The analysis of variance (ANOVA) and Tukey's test multiple comparisons showed that the difference of chlorine residual between samples is not significant. In three samples of different districts (Atikoumé, Doumassessé and Dogbéwavou) which have high levels of chlorine, we found the total spores in two of the sample types, Atikoumé (2 spores/ml) and Doumassessé (5 spores/ml). pH values in all 30 samples are within the limits selected by the criteria that is to say values between 6.5 and 9.5 according to Table 4. The

Table 4. Results of measurements of pH values in different districts.

S/N	Sampling point	Extreme values of pH	Quality limits/EU*
1	Abové	6.92 – 7.30	6.5 – 9.5
2	Atikoumé	6.93 – 7.03	6.5 – 9.5
3	Gbossimé	7.09 – 7.34	6.5 – 9.5
4	Doumassessé	7.03 – 7.60	6.5 – 9.5
5	Tokoin Lycée	7.05 – 7.11	6.5 – 9.5
6	Dogbéwavou	6.97 – 7.24	6.5 – 9.5
7	Klikamé	7.10 – 7.16	6.5 – 9.5
8	Tokoin Trésor	6.99 – 7.10	6.5 – 9.5
9	Akodéssawa	7.10 – 7.18	6.5 – 9.5

*Criteria of the European Union Council Directive 98/83/EC, 1998.

disinfection efficiency is determined by the pH of water. The highest value of pH is 7.60 found in a sample of Doumassesse. The lowest value of pH is observed at above with a pH equal to 6.92.

DISCUSSION

According to guidelines set by the European Union, the total bacteria found are below the limit considered. Our results are not similar to those obtained by Mokofio et al. (1991) in Bangui (Republic of Central Africa), that show the presence of faecal bacteria in samples of well water consumed. Degbey et al. (2009) also revealed the presence of bacteria of fecal origin in ten samples of well water consumed at Godomey, Abomey in Benin in 2009. In a study in Côte d'Ivoire in 2006, Odile et al. (2006) found an abnormal concentration of thermotolerant coliforms in the drinking water packaged collected from the market in Abidjan.

However, our results are consistent with Nola et al. (1998) in a study conducted in Yaoundé (Cameroon) on well water for drinking, which found only the total bacteria in their samples.

These results confirm studies of Jabu (2007) in Malawi on household water and Dianou et al. (2002) in Burkina Faso on the bacteriological quality of well water in rural areas, where a lack of fecal bacteria was observed. The presence of these spores proves the vulnerability of water to global pollution, inadequate treatment or an unsafe environment (Camille and Bernard, 2006; Degbey et al., 2010). The water supply must meet the quality requirements. Thus, it should not contain any microorganism, no noise and no substance presents a potential danger to human health; it must also comply vis-à-vis to a set of standards for drinking water (Kahoul and Touhami, 2014). The presence of residual chlorine prevents the breakdown of microbial quality and protects the water during distribution. The bactericidal action of chlorine increases for low values of pH of water, the high pH affects the action of chlorine (François, 2010).

pH values obtained in our study were between 6.5 and 9.5. Note that unlike tap water, in well water, studies (Degbey et al., 2010) showed pH below normal. The values of pH found in our study did not influence the dissolution in water of chlorine, hypochlorous acid and hypochlorite ions in toxic spores. Thus, the pH did not significantly affect the bactericidal action of chlorine. These results are justified in so far as the treated water is not sterile. Moreover, if lower residual chlorine may promote bacterial growth in the network, the study showed that maintaining residual chlorine did not provide completely preventing bacterial growth. The effectiveness of chlorine on microorganisms depends on the type of microorganism, and contact time. That justifies the presence of spores in the samples that have high levels of chlorine.

Conclusion

The study has shown that the bacteriological quality of the water is compliant for human consumption. However, we note the presence of the total mesophilic flora, which implies that tap water should be checked regularly for the well-being of consumers. This study on the hygienic quality of tap water is not exhaustive. It is important to consider other extensive studies in this area given the many parameters to consider in ensuring the quality of drinking water.

Conflict of Interests

The authors have not declared any conflict of interests.

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Influence of means of transportation on the quality of beef from three indigenous cattle breeds in Cameroon

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The influence of transportation conditions on the quality of beef was evaluated on carcasses from three indigenous cattle breeds in Cameroon. Twenty bulls of similar age (3 to 5 years inclusive) from the Gudali, Red Mbororo and White Fulani breeds transported by train and by truck from the same production zone to the abattoir were investigated. Beef samples were used to evaluate technological and chemical properties. Gudali bulls transported by train produced beef with the highest proportion of bright red colour (85%). Beef from Gudali bulls transported by truck had the highest drip loss (9.87±2.82%), White Fulani bulls transported by truck produced beef with the highest freezing and cook-out losses (19.40±3.02 and 28.37±1.90% respectively). Means of transportation did not significantly (p>0.05) influence chemical properties. These results indicate that in Cameroon, animals transported by truck give poor quality beef. Therefore, measures should be taken to minimize animal stress especially during truck transportation.

Key words: Beef quality, indeginouscattlebreed, technological properties, chemical properties, Cameroon.

INTRODUCTION

Livestock production is a rapidly fast growing agricultural subsector in developing countries. Its share of agricultural gross domestic product (GDP) in Cameroon is about 35% and increasing (ECA/SRO-CA, 2012). The growth is driven by rapidly increasing demand for livestock products due to population growth, urbanization and increasing incomes in developing countries (Gebresenbet et al., 2004). There is a steady rise in demand for meat in Cameroon as the national population increases. Cattle contribute approximately 28% of the total animal protein produced in the country with the Gudali, Red Mbororo and White Fulani contributing 45 to 54% of meat consumed in the cities (FAO, 2008).

Transportation of cattle from the production areas to the slaughter house is always accompanied by some degree of stress which subsequently influences the overall quality of the beef. Swanson and Morrow-Tesch (2001) identified the main factors involved with "transport stress" to include pre-transport management, noise, vibration, novelty, social regrouping, crowding, climatic

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factors (temperature, humidity and gases), restraint, loading and unloading, duration oftransit, resting during transport, feed and water deprivation and waiting time after arrival before slaughter. These factors compromise the welfare of the animals and reduce meat quality: lean colour, drip and cook-out losses (Warriss et al., 1995; Grandin, 2000). Incidences of dark-cutting beef provide information about the welfare of cattle during handling, transport and lairage (Broom, 2003). Furthermore, the intensity of stress varies depending on the means of transportation employed. Cattle transported by railcar reportedly were less stressed and lost 4% less liveweight than those shipped through equivalent distance in trucks (Friend et al., 1981).

Putting knowledge into evidence concerning the transportation of animals destined for slaughter can be of assistance in reducing mortality during transportation, reducing skin and carcass damage, increasing the quality of meat supplied to consumers and consequently reducing economic losses to the beef industry (Ljungberg et al., 2007). The main objective of this research was thus to investigate the possible influence of different means of transportation on the technological and chemical properties of beef from the three main indigenous cattle breeds reared in Cameroon.

MATERIALS AND METHODS

Selection of animals and sample collection

Sixty bulls of similar slaughter age (3 to 5 years inclusive) from the three predominant breeds: 20 Gudali, 20 Red Mbororo and 20 White Fulani from the Guinea high savannah agro-ecological zone and reared under the transhumance production system were sampled at the Yaoundé SODEPA abattoir for investigation during May to June, 2014. Among the 20 animals selected from each breed, 10 had been transported to the abattoir by truck and the other 10 by train. An inclined wooden platform supported by a heap of grass is used for on and off loading of the animals. However, cattle movement on and off the train is easier because the angle of inclination is lower than the truck. Averagely, 20 animals are loaded in each train wagon of 24 m^2 area while the same number of animals is loaded on trucks each having an average area of 18m^2. The distance covered by the animals from production site to slaughter house was 673 km by road (duration in transit was 3.5 days) and 622 km by train (duration in transit was 2 days). Most of the road is not tarred, with many potholes. During transit, the animals transported by truck are off loaded once to rest and graze while those in the train are provided water only and do not get off the train until the final destination. All the animals were slaughtered between 12 and 15 h after arrival; they were provided forage and water during this period.

Following slaughter and immediately after carcass dressing, a sample (approximately 1000 g) of *Longissimusdorsi* muscle (between 12^{th} and 13^{th} rib) was removed from the left side by cutting a three-centimetre thick chop from the section dividing the thoracic and lumbar parts of the muscle of each animal. Approximately, 500 g from each sample was used for the evaluation of technological (drip, freezing and cook-out losses) properties and the remaining 500 g was used for chemicalanalysis. Lean colour was assessed on the pelvic region and on the section of the semi-membranous muscles exposed by tail removal immediately after carcass dressing (Nfor et al., 2014).

Evaluation of technological properties

Using a three-point scale, lean colour was assessed visually and graded as pink, bright red or dark red (Baublits et al., 2005). After 24 h storage of meat samples at 4°C, drip loss was calculated as the difference between initial and final weights and expressed as a percentage. Cook-out-loss was evaluated using the method described by Piasentier et al. (2003). 6 h after slaughter, meat samples in ziploc bags were immersed in a thermostatic water bath at 75°C for 15 min. Cook-out-loss was obtained as the difference between the initial and the final weights and expressed as a percentage. Freezing loss was evaluated using the method described by Piasentier et al. (2003). Meat samples were frozen at -20°C for 14 days, then thawed to room temperature (2°C) and reweighed. Freezing loss was calculated as the difference in weight loss before and after freezing and expressed as a percentage.

Evaluation of chemical properties

Meat samples were minced and dried in a ventilated oven at 60°C for 20 to 24 h when a constant weight was attained. Moisture content was calculated as the difference in weight before and after drying. Crude protein, crude lipid and ash contents were estimated on dry matter basis as described (AOAC, 2000). The results were expressed as percentages.

Statistical analysis

Lean colour was expressed in percentage. The effects of transportation means on technological and chemical properties of beef irrespective of breed was evaluated using the Student t-test at a significance level of $p<0.05$. The effects of transportation means on the technological and chemical properties of beef with respect to breed were evaluated using ANOVA (General Linear Model approach; SPSS version 19.0). Means were separated for significant differences ($p<0.05$) using Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS

Technological properties of beef

Lean colour

Lean colour carcass distribution as affected by means of transportation is presented in Figure 1. Within breed analysis revealed, most (85%) of Gudali bulls transported by train gave carcasses with a bright red lean colour compared to only 54% transported by truck. The same trend was observed in carcasses from the other breeds. Between breeds comparison revealed for animals transported by train, the highest percentage bright red colour was found in carcasses from Gudali bulls (85%), pink colour was most predominant in carcasses from White Fulani bulls (22%) while dark red colour was greatest in carcasses from Red Mbororo bulls (22%). For transportation by truck, the highest proportion of lean with bright red colour was recorded in carcasses from Gudali bulls (54%), pink colour was greatest in carcasses from Red Mbororo bulls (67%) while the dark red colour was most predominant in carcasses from White Fulani bulls

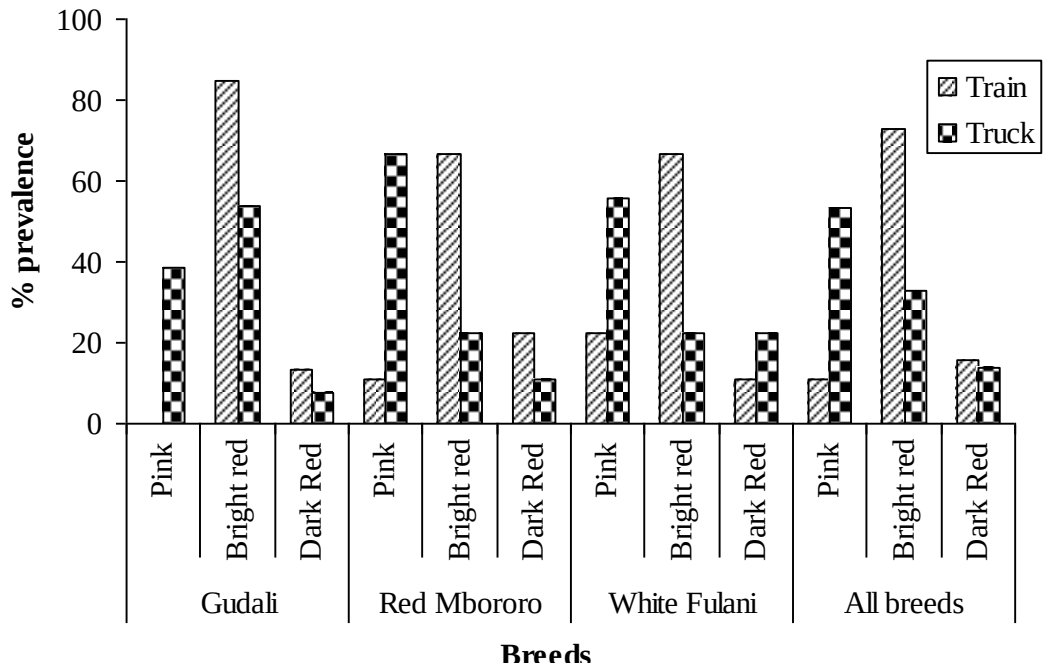

Figure 1. Lean colour distribution of beef from three indigenous cattle breeds in Cameroon.

Table 1. Influence of means of transportation on technological properties of beef with respect to cattle breed (for each breed, n=10 per means of transportation).

Technological property (%)	Transportation means	Gudali	Red Mbororo	White Fulani	All breeds
Drip-loss	Train	8.63±2.65$^{a\mu}$	4.16±2.37$^{a\mu}$	6.60±5.45$^{a\mu}$	6.23±1.89^{a}
	Truck	9.87±2.82$^{a\$}$	4.52±2.19$^{a\mu}$	7.09±4.79$^{a\mu\$}$	8.13±2.51^{a}
Freezing-loss	Train	10.38±5.34$^{a\mu}$	11.38±7.45$^{a\mu}$	13.57±2.91$^{a\mu}$	12.27±2.59^{a}
	Truck	16.92±3.22$^{a\mu\$}$	13.54±2.73$^{a\mu}$	19.40±3.02$^{b\$}$	16.12±6.71^{a}
Cook-out loss	Train	23.04±0.58$^{a\mu}$	24.14±2.25$^{a\mu}$	23.44±1.28$^{b\mu}$	23.19±1.43^{a}
	Truck	24.19±6.72$^{a\mu}$	22.69±5.31$^{a\mu}$	28.37±1.90$^{a\mu}$	24.74±4.91^{a}

$^{a, b}$: Means with different superscript letter on same column for the same technological property are significantly different at 5 %; $^{\mu, \$}$: Means with different superscript letter on same row for the same technological property are significantly different at 5%.

(22%). Irrespective of breed, bright red lean colour was dominant in carcasses from cattle transported by train (73%) while this colour was present in only 33% of carcasses from animals transported by truck. More than half (54%) of all the animals transported by truck were lean with a pink colour.

Drip, cook-out and freezing losses

In general, drip, freezing and cook-out losses were greater in carcasses from animals transported by truck than by train, (Table 1) although the differences were not significant ($p>0.05$). Drip loss was highest in carcasses from Gudali bulls transported by truck (9.87±2.82%) and lowest in Red Mbororo transported by train (4.16±2.37%). For truck transportation, drip loss of carcasses from Red Mbororo bulls (4.52±2.19%) was significantly lower ($p<0.05$) than in carcasses from Gudali bulls (9.87±2.82%). Freezing loss was greatest in carcasses from White Fulani transported by truck (19.40±3.12%) and lowest in carcasses from Gudali transported by train (10.38±5.34%). Freezing loss was significantly higher ($p<0.05$) in carcasses from White Fulani bulls transported by truck (19.40±3.12%) than by train (13.57±2.91%). Furthermore, freezing loss was significantly higher ($p<0.05$) in carcasses from White Fulani bulls (19.40±3.12%) than in carcasses from Red Mbororo bulls (13.54±2.73%) transported by truck. Influence of transportation means on cook-out loss was observed only

Table 2. Influence of means of transportation on chemical properties of beef with respect to breed.

Chemical property (%)	Transportation means	Gudali	Red Mbororo	White Fulani	All breeds
Moisture	Train	67.74±7.69$^{a\,\mu}$	69.25±5.42$^{a\,\mu}$	70.01±7.35$^{a\,\mu}$	69.02±10.29^{a}
	Truck	69.36±4.87$^{a\,\mu}$	73.35±4.14$^{a\,\mu}$	66.75±7.85$^{a\,\mu}$	69.74±.8.84^{a}
Crude ash	Train	1.54±0.33$^{a\,\mu}$	1.41±0.48$^{a\,\mu}$	1.25±0.17$^{a\,\mu}$	1.40±0.48^{a}
	Truck	1.47±0.44$^{a\,\mu}$	1.13±0.12$^{a\,\mu}$	1.45±0.41$^{a\,\mu}$	1.35±0.59^{a}
Crude protein	Train	24.06±5.29$^{a\,\mu}$	21.85±4.58$^{a\,\mu}$	20.19±3.17$^{a\,\mu}$	21.98±4.07^{a}
	Truck	23.02±2.70$^{a\,\mu}$	19.95±2.68$^{a\,\mu}$	25.83±6.87$^{a\,\mu}$	22.93±2.18^{a}
Crude lipid	Train	3.61±1.95$^{a\,\mu}$	3.08±0.42$^{a\,\mu}$	4.04±1.73$^{a\,\mu}$	3.57±1.04^{a}
	Truck	2.54±1.39$^{a\,\mu}$	2.52±0.87$^{a\,\mu}$	3.96±2.22$^{a\,\mu}$	3.00±2.18^{a}

$^{a,\,b}$: Means with different superscript letter on same column for the same chemical property are significantly different at 5 %$^{;\,\mu,\,\$:}$ Means with different superscript letter on same row for the same chemical property and for the same means of transportation are significantly different at 5%.

in carcasses from White Fulani bulls where the loss was significantly higher ($p<0.05$) in carcasses transported by truck (28.37±1.90%) than by train (23.44±1.28%).

Chemical properties of beef

Moisture and crude protein were slightly higher in carcasses transported by truck than by train (Table 2). Conversely, crude ash and crude lipid were slightly higher in carcasses from animals transported by train than by truck. However, no significant differences ($p>0.05$) were noted in the chemical properties with respect to means of transportation. Between breeds comparison, it showed that moisture content was highest in carcasses from Red Mbororo animals transported by truck (73.35±4.14%) and lowest in carcass from White Fulani animals transported by truck (66.75±7.85%). Crude ash was highest in Gudali transported by train (1.54±0.33%) and lowest in bright red Mbororo transported by truck (1.13±0.12%). Crude protein was highest in White Fulani transported by truck (25.83±6.87%) and lowest in bright red Mbororo transported by truck (19.95±2.68%). Lipid was highest in White Fulani transported by train (4.04±1.73%) and lowest in bright red Mbororo transported by truck (2.52±0.87%). Again, there were no significant differences ($p>0.05$) observed between breeds.

DISCUSSION

Technological properties of beef

Lean colour distribution

Irrespective of breed, cattle transported by train produced beef with a better colour (bright red) than those transported by truck. This is because the animals transportation by truck were subjected to many more stress inducing factors such as banging of the animals, more vibrations, changes in velocity, sudden starts and stops due to the poor state of the road, higher stocking density, more difficulties encountered during on and off loading as well as longer transportation time. These same stress factors have been identified by Kadim et al*.* (2007). In an earlier study during which steers were transported for 3 or 16 h, Gallo et al. (2003) reported that the longer journey was associated with a significantly greater increase in the proportion of “dark-cutter” carcasses (reduced bright red colour of lean). Stress promotes accumulation of intracellular water which reflects less light and causes the muscle to appear dark (Page et al., 2001).

Drip, cook-out and freezing losses

Drip, cook-out and freezing losses were higher in animals transported by truck than by train irrespective of breed. This might have been due to differences in the pHu of the carcasses (not measured in this study). Animals that experience more stress will produce carcasses with a higher pHu because very little lactic acid will produced post mortem (Knowles and Warriss, 2000). A high pHu increases drip, freezing and cook-out losses (Grandin, 2000). Gallo et al. (2003) and Awantu (2015) reported high pHu as well as high drip and cook-out losses in carcasses from stressed cattle. Freezing damages cell membranes; and the degree of damage is influenced by the severity of ante mortem stress (Rahelić et al*.,* 1985; Wheeler et al*.,* 1990). The results obtained in this study suggest that in Cameroon, transportation of animals by truck is more stressful than by train. White Fulani bulls transported by truck produced carcasses with higher freezing losses than carcasses from Red Mbororo and Gudali bulls. This implies differences in stress tolerant levels between breeds. Irrespective of breed, average

cook-out loss in carcasses from cattle transported by truck was higher than for those transported by train. This again affirms that transportation of cattle by truck is more stressful than by train within the Cameroonian context. Kadim et al. (2007) obtained similar results with Omani sheep which were subjected to different levels of transportation stress. Increase in drip, freezing and cook-out losses lead to increased loss in organoleptic (texture, juiciness) and nutritive properties (Wheeler et al*.,* 1990; Knowles and Warriss, 2000) and consequently the overall quality of the beef is reduced.

Chemical properties of beef

The chemical properties of beef did not significantly vary either with respect to breed or means of transportation. Maybe the sample size was not sufficiently large. However, the higher average lipid content was recorded in carcasses transported by train than by truck maybe due to the longer time involved in truck transportation. During this period, feed and water supplied to the cattle is grossly inadequate. In such situations, the animals will resort to mobilizing their fat reserves for energy production (Knowles and Warriss, 2000). Loss in lipid results in a lower selling price of the bull as well as reduced organoleptic qualities of the meat especially its taste and flavour (Zhong et al., 2011).

Conclusion

Irrespective of breed, cattle transported by train produced more carcasses with a brighter red colour and better technological properties than those transported by truck. With respect to breed, the breed with the highest proportion of bright red lean colour was Gudali. Lean colour is the primary criterion that most Cameroon consumers use to evaluate beef quality. Therefore, the results from this study imply that bulls transported by train produce better quality beef and that Gudali gives the best beef. Carcasses fromGudali bulls transported by train gave the best quality beef after freezing, while carcasses from White Fulani bulls transported by train gave the best quality after cooking. The findings of this study have shown that ante mortem stress during transportation negatively affects beef quality, and that the stress is more severe when bulls are transported by truck than by train. Therefore, appropriate measures should be taken to minimize animal stress during transportation by improving transportation conditions and/or reducing the duration of transit.

Conflict of Interests

The authors have not declared any conflict of interest.

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