Effect of freezer storage on quality of *M. longissimus lumborum* from fallow deer (*Dama dama* L.)

T. Daszkiewicz[#], T. Lipowski & D. Kubiak

Department of Commodity Science and Processing of Animal Raw Materials, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland

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Abstract

The aim of this study was to analyse changes in the quality of meat (*M. longissimus lumborum*) from 11 fallow deer (*Dama dama* L.) bucks, which had been deep-frozen (-26 °C) for 10 months. The proximate chemical composition, physico-chemical properties and sensory properties of the meat were analysed. Fallow deer aged 17–18 months were hunter-harvested in north-eastern Poland in November and December of the same hunting season. The results revealed that freezer storage did not influence the proximate chemical composition of meat (protein, fat, ash) or the content of water-soluble nitrogen compounds. An analysis of the physico-chemical properties of meat revealed that long-term freezer-stored meat was characterized by higher pH, lower contribution of redness (a*) and yellowness (b*), lower chroma (C*), greater natural drip loss, lower ability to bind added water, and greater cooking loss. Freezer storage improved meat tenderness but reduced meat juiciness. The results of the study also indicated that long-term freezer storage (-26 °C) of vacuum-packaged meat allowed it to maintain its good quality, which indicates that this storage method could help meet consumer demand for fallow deer meat between hunting seasons.

Keywords: Freezing, game meat, meat quality, vacuum packaging [#]Corresponding author: tomasz.daszkiewicz@uwm.edu.pl

Introduction

Red meat is regarded as a source of cholesterol and saturated fatty acids, which increase the risk of various diseases (Binnie *et al.*, 2014; Battaglia Richi *et al.*, 2015). However, this popular belief has not affected the demand for red meat and its products, which continues to be high around the world (OECD/FAO, 2016). Nonetheless, nutrition-conscious consumers are increasingly likely to opt for products that are characterized by high nutritional value and those that deliver health benefits. This trend has increased the demand for lean meat (McNeill, 2014). Game meat is lean (Nuernberg *et al.*, 2009), and is characterized by high protein content and a healthy fatty acid profile (Zomborszky *et al.*, 1996; Hoffman & Wiklund, 2006; Valencak *et al.*, 2015). It is abundant in macronutrients, micronutrients, vitamins, and other bioactive components (Zomborszky *et al.*, 1996; Florek & Drozd, 2013; Strazdina *et al.*, 2014).

Similar to other types of meat, game meat has a limited shelf life owing to the growth of bacterial microflora and lipid peroxidation (Zhou *et al.*, 2010). These processes lead to the gradual deterioration of sensory attributes, processing suitability, eating quality, and safety of meat. The meat of hunter-harvested wild animals is generally available only during the hunting season. Game meat has to be preserved in a manner that ensures its high quality and continued supply outside the hunting season. According to Farouk & Freke (2008), game meat is usually preserved by chilling or freezing.

Freezing is a widely used method of meat preservation (Leygonie *et al.*, 2012a). Freezing induces relatively minor and acceptable changes in meat quality, and preserves meat effectively during prolonged storage (Soyer *et al.*, 2010). Freezing is the preferred method of storage among meat processing and distributing companies (Lagerstedt *et al.*, 2008) and consumers (Muela *et al.*, 2012), who often purchase more meat than can be consumed directly store excess quantities in household freezers.

The changes induced by freezing are related directly to the freezing process and prolonged storage of frozen meat (Leygonie *et al.*, 2012a). Song *et al.* (2017) highlighted that the rate and scope of the changes that occur during freezer storage are influenced by the time and temperature of storage. The optimal temperatures for freezer storage of meat differ in the literature. Zhou *et al.* (2010) determined temperatures for freezer storage at -55 °C, while Leygonie *et al.* (2012a) established them at -40 °C. In practice, frozen

food, including meat, is often stored at a temperature of about -18 °C (0 °F) (James & James, 2014). There is also an assumption that a low storage temperature is always beneficial for storage life. In modern freezers, including household freezers, food products can be stored at temperatures much lower than -18 °C (usually - 25 °C to -26 °C). Freezer storage temperatures are determined by financial considerations, the quality of stored products, and the period of storage. The properties of food products (e.g. unsaturated fatty acid content) should be taken into account during freezer storage. These considerations apply also to game meat, which is characterized by seasonal availability (Schulp *et al.*, 2014) and unique quality attributes (Hoffman & Wiklund, 2006). The meat of wild fallow deer has been researched less extensively than that from farm-raised cervids (Zomborszky *et al.*, 1996; Żochowska-Kujawska *et al.*, 2009; Tešanović *et al.*, 2011; Cifuni *et al.*, 2014; Daszkiewicz *et al.*, 2015). In an attempt to fill this knowledge gap, the aim of this study was to evaluate the effect of 10-month freezer storage at a temperature of -26 °C on the quality of meat from wild fallow deer (*Dama dama* L.).

Materials and Methods

The experimental material comprised 11 samples of the *M. longissimus lumborum* (MLL) from the carcasses of male fallow deer (*Dama dama* L.), that were supplied to a meat-processing plant. The animals were hunter-harvested in the forests of north-eastern Poland (Sępopol Plain, Region of Warmia and Mazury) in November and December during the rutting season. The animals were harvested in accordance with the provisions of the laws on game and hunting enacted by the Parliament and the Government of Poland. The animals was estimated by the hunters based on the appearance of their antlers and the cheek teeth in the lower jaw (tooth eruption and wear of mandibular premolars and molars). The animals were shot (ambush tactics, a heart shot), bled and eviscerated in the field immediately after death. Afterwards, the beheaded carcasses with the skins and legs were transported by hunters within 2–3 hours of harvest to a cold storage, where they were kept at 2–4 °C, and then transported with a mobile fridge to the meat-processing plant. The

Carcasses were skinned and divided into primal cuts, namely neck, saddle, shoulders, legs and ribs, within 48–54 hours of harvesting. The time of harvest was determined from hunter harvest reports. Before cutting, the quality of skinned carcasses was evaluated. Carcasses with signs of bullet damage or evisceration damage to *M. longissimus* (ML), carcasses contaminated with digesta owing to digestive tract damage caused by a bullet or incorrect evisceration, incorrectly chilled carcasses (temperature not higher than 7 °C in the geometric centre of the leg), and carcasses in which the pH value of ML exceeded 5.8 (measured behind the last rib) were discarded to eliminate dark, firm and dry meat. The pH of ML was measured with a double pore electrode (Hamilton Broadus AG, Bonaduz, Switzerland) and a 340i pH meter equipped with a TFK 150/E temperature sensor (WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

Similarly sized samples of MLL were cut out from the right and left ML behind the last rib and were labelled A and B, respectively. Samples were placed in polyethylene bags and transported (within 1 hour) to the laboratory in isothermal containers with refrigerant packs at a temperature of approximately 7 °C. In the laboratory, the A samples were immediately subjected to analyses, namely proximate chemical composition, physico-chemical and sensory properties of meat, and B samples were vacuum-packaged in barrier bags made of ethylene-vinyl alcohol (EVOH) copolymer (gas permeability: $O_2 = 1 \text{ cm}^3/\text{m}^2/24 \text{ h/bar/23 °C}$, $N_2 < 0.1 \text{ cm}^3/\text{m}^2/24 \text{ h/bar/23 °C}$, $CO_2 = 1.6 \text{ cm}^3/\text{m}^2/24 \text{ h/bar/23 °C}$, and $H_2O = 3 \text{ g/m}^2/24 \text{ h/bar/23 °C}$ using the PP-5MG (015) vacuum packaging machine (Tepro S.A.). Vacuum-packaged samples were stored for 10 months in an electronic temperature controlled freezer at -26 °C. The temperature was monitored once a day for five days a week. After 10 months, vacuum-packaged samples were analysed for proximate chemical composition, physico-chemical and sensory properties in the laboratory.

Before analysis, frozen meat samples were thawed overnight at 2 °C until internal temperature reached -1 °C. Samples intended for analyses that required ground meat were passed three times through a 3-mm plate in a meat grinder and were mixed thoroughly. Their chemical composition, physico-chemical and sensory properties were evaluated in laboratory analyses.

Analysis of the proximate chemical composition of meat included determining (AOAC, 1990) dry matter content, total protein content by the Kjeldahl method in the Kjeltec[™] 2200 Auto Distillation Unit (FOSS Analytical, Hillerod, Denmark), crude fat content by Soxhlet extraction with diethyl ether as the solvent in the Soxtec[™] 2050 Auto Fat Extraction System (FOSS Analytical, Hillerod, Denmark), and ash content. The total content of nitrogen compounds and non-protein nitrogen compounds (proteins were

precipitated with trichloroacetic acid) was determined in an aqueous meat extract prepared according to the method of Herring *et al.* (1971).

The pH of MLL samples was determined in water homogenates (10 g meat were homogenized with 10 cm³ redistilled water with an IKA Ultra Turrax® T25 digital homogenizer (IKA[®]-Werke, Staufen, Germany)), a Polilyte Lab electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland) and an inoLab Level 2 pH meter equipped with the TFK 325 temperature sensor (WTW Wissenschaftlich-Technische Werkstätten, Weilheim, Germany).

The extent of lipid peroxidation was estimated based on thiobarbituric-acid-reactive substance (TBARS) values using the method proposed by Pikul *et al.* (1989). Absorbance was measured with the Specord 40 spectrophotometer (Analytik Jena AG, Jena, Germany) at a wavelength of 532 nm. TBARS values were expressed as milligrams o malondialdehyde (MDA) per kg of meat.

Meat colour was determined based on the values of L* (lightness), a* (redness), b* (yellowness), C* (chroma), and h° (hue angle) in the CIELAB system (CIE, 1978). Parameters L*, a* and b* were measured with the HunterLab MiniScan XE Plus spectrocolorimeter (Hunter Associates Laboratory, Reston, Virginia, USA) with standard illuminant D65, 10° standard observer angle and 2.54-cm diameter aperture. The final results were the arithmetic mean of three measurements performed at different points on the surface of muscle cross-sections. The value of parameter C* was calculated from formula $C^* = (a^{*2} + b^{*2})^{1/2}$, and the value of h° from formula h° = arctan (b*/a*).

Water-holding capacity of meat was determined based on thawing loss, natural drip loss (Honikel, 1998) and the ability to bind added water using the centrifugal method described by Piaskowska *et al.* (2016). Thawing loss was determined based on the weight of the packaging (p) with the meat sample (m) and juice (j) after thawing (W_{p+m+j}), packaging with juice (W_{p+j}), and clean and dry packaging (W_p). Thawing loss was expressed as a percentage of weight of a thawed meat sample (m):

% thawing loss =
$$[(W_{p+j} - W_p) / (W_{p+m+j} - W_{p+j})] \times 100$$

The cooking loss of meat samples was determined according to the method described by Honikel (1998). Meat samples were weighed and placed in a thin-walled water-impermeable plastic bag in a water bath at 80 °C, with the opening above the surface. Samples were cooked for approximately 60 minutes to an internal temperature of 75 °C, then removed from the water bath, and cooled in an ice slurry inside their plastic bags. The meat was then blotted dry and weighed. Cooking loss was calculated as the difference in sample weight before (W_1) and after cooking (W_2), and was expressed as a percentage of the initial sample weight:

% cooking loss =
$$[(W_1 - W_2) / W_1] \times 100$$

The sensory properties of meat, namely aroma, taste, juiciness and tenderness, were evaluated after cooking according to the method proposed by Baryłko-Pikielna *et al.* (1964). Fat and muscle epimysium were removed with the perimysium intact, and meat samples were cooked in 0.6% NaCl solution at 96 °C (\pm 2 °C) until internal temperature reached 80 °C. The sensory properties of cooked meat were evaluated by five trained panellists on a 5-point hedonic scale, namely 5 points: most desirable, 1 point: least desirable (Daszkiewicz *et al.*, 2012). Prior to the evaluation, the panellists had been trained in the sensory properties of cooked venison based on cooked beef loin as the reference standard. The panellists evaluated encoded samples composed of 1 cm x 1 cm x 1 cm meat cubes from the centre of each cooked sample, cooled to room temperature. Redistilled water was made available to the panellists for mouth cleansing between samples. All sensory properties of each sample were assessed during a single session. Up to five meat samples were evaluated per session.

The samples used for cooking loss analysis were also used for shear force measurements. The maximum shear force required to cut meat samples (five cylinder-shaped samples measuring 1.27 cm in diameter and 2 cm in height) was determined using a Warner-Bratzler head (500 N, speed 100 mm/min.) attached to the Instron 5542 universal testing machine (Instron, Canton, Massachusetts, USA). The longitudinal axis of the sample was parallel to the direction of the muscle fibres to ensure that the sample was sheared at right angles to the fibre axis (Honikel, 1998).

The statistical analysis was performed with Statistica software version 12 (StatSoft, Inc., 2014, Tulsa, Oklahoma, USA). The data were processed by one-way ANOVA. Bonferroni's test was applied to estimate the significance of differences ($P \le 0.05$ and $P \le 0.01$) in quality of meat from male fallow deer before and after long-term freezer storage (10 months).

Results and Discussion

The results for thawing loss analysis and the chemical composition of fallow deer meat are presented in Table 1. The average thawing loss of the meat samples was determined at 2.30%. Thawing loss is an important parameter that influences weight loss, chemical composition and other properties of meat that are closely linked with the water content (Huff-Lonergan & Lonergan, 2005). An analysis of the proximate chemical composition of meat revealed lower dry matter content in freezer-stored meat than in fresh meat. The difference between mean values in groups was statistically significant at $P \leq 0.05$. No significant differences (P > 0.05) in the average content of protein, fat and ash were observed between samples. No significant differences (P > 0.05) were found in the content of water-soluble nitrogen and non-protein nitrogen compounds in aqueous meat extracts. However, a trend towards a lower content of water-soluble nitrogen compounds and a higher content of non-protein nitrogen compounds was observed in freezer-stored meat. The decrease in the dry matter content of freezer-stored meat that was observed in the current study could be attributed to the loss of water-soluble dry matter components. According to Savage et al. (1990), 1 ml liquid from thawed pork contains up to 112 mg protein on average, mainly water-soluble and sarcoplasmic proteins. The observed reduction in the concentration of water-soluble nitrogen in the aqueous extract from freezer-stored meat probably resulted from partial denaturation of sarcoplasmic proteins (Leygonie et al., 2012a). Natural variations in the chemical composition of ML could contribute to differences in the proximate chemical composition of meat samples before and after freezer storage.

Table 1 Thawing loss and chemical composition of *M. longissimus lumborum* from male fallow deer before and after freezer storage (means ± standard deviations)

Freezer storage period		
11)		
33		
203		
743		
97		
:57		
57		
82		
12		

^{a,b} Mean values in the same row with different superscripts differ significantly at $P \leq 0.05$; -: Not measured

The physico-chemical properties and TBARS values of the MLL from male fallow deer before and after freezer storage are shown in Table 2. Fresh and thawed meat were characterized by similar average pH (Table 2). The average values of pH differed by 0.05 units between groups, and the difference was statistically significant ($P \le 0.01$) owing to minor variations in pH values in the two groups. Farouk & Freke (2008) reported non-significant changes in the pH of the *semimembranosus* muscle of red deer during freezer storage. According to Akhtar *et al.* (2013), the pH of meat is generally lower after freezing and thawing than before freezing. This may be owing to three factors, namely denaturation of protein, thawing loss, and protein deamination. Temperatures below zero lead to the denaturation of protein buffer systems, release of hydrogen ions, and consequently a decrease in pH. The loss of minerals and small protein compounds as exudates, thereby changing the ionic balance in the meat, results in a decrease in pH. Protein deamination by microorganisms and enzymes, leads to the release of hydrogen atoms, which can also change the pH of meat. Despite this, the results of research studies that investigated the influence of freezing time on the pH of meat are inconclusive. For example, Ablikim *et al.* (2016) demonstrated that changes in the pH of lamb meat during freezer storage (30 days, -18 °C) were correlated with animal breed and type of muscle.

The 10-month period of freezer storage did not exert a significant influence (P > 0.05) on TBARS values (Table 2) which were high (0.7 kg mg malondialdehyde/kg of meat) in both groups. Game meat lipids

are rapidly oxidized because of the high content of unsaturated phospholipids (Valencak *et al.*, 2015) and pro-oxidant iron ions in myoglobin (Wiklund *et al.*, 2006). For this reason, game meat should be stored in an oxygen-deficient environment. In the current study, meat samples were vacuum-packaged before freezing, which probably limited the extent of oxidative changes during storage. Similar results were reported by Farouk & Freke (2008) in an analysis of the *semimembranosus* muscle from red deer, in which malondialdehyde concentration was lower in samples that were vacuum-packaged and freezer stored for nine months than in samples that were stored in gas-permeable packaging.

Table 2 Physico-chemical properties and thiobarbituric acid reactive substances values of *M. longissimus lumborum* from male fallow deer before and after freezer storage (means ± standard deviations)

Parameter	Freezer storage period		
Farameter	day 0 (n = 11)	10 months (n = 11)	
На	$5.53^{A} \pm 0.039$	$5.58^{B} \pm 0.045$	
L* (lightness)	30.01 ± 1.853	29.95 ± 1.534	
a* (redness)	16.37 ^A ± 1.566	13.19 ^B ± 1.610	
b* (yellowness)	11.13 ^a ± 1.847	$8.42^{b} \pm 2.848$	
C* (chroma)	19.81 ^A ± 2.299	15.79 ^B ± 2.462	
h [°] (hue angle)	34.02 ± 2.173	31.70 ± 8.919	
Drip loss (%)	2.79 ± 1.004	4.05 ± 1.898	
Cooking loss (%)	30.86 ^A ± 1.161	33.45 ^B ± 1.460	
Water-holding capacity-centrifugal method (%)	$26.51^{A} \pm 6.517$	$18.32^{B} \pm 4.309$	
TBARS value (mg malondialdehyde/kg meat)	0.77 ± 0.107	0.72 ± 0.128	

^{A,B} Mean values in the same row with different superscripts differ significantly at $P \leq 0.01$

^{a,b} Mean values in the same row with different superscripts differ significantly at $P \le 0.05$

TBARS: thiobarbituric acid reactive substances

An analysis of colour parameters in the CIELAB system (Table 2) revealed similar (P > 0.05) values of L^{*} in fresh and freezer stored meat. Thawed meat was characterized by significantly lower a^{*} ($P \le 0.01$), b^{*} ($P \le 0.05$) and, consequently, significantly lower C^{*} ($P \le 0.01$) values. The hue angle (h^o) of thawed meat was lower (P > 0.05) in comparison with fresh meat.

Changes in the colour of thawed meat that were observed in the current study (decrease in a*, b*, and C* values) could be induced by chemical transformation of myoglobin. Myoglobin is denatured during freezing, freezer storage and thawing, which increases its susceptibility to auto-oxidation and provokes undesirable changes in meat colour (Leygonie *et al.*, 2012a). On the other hand, the observed decrease in h^o value of thawed meat relative to fresh meat could be attributed to vacuum-packaging. In vacuum-packaged samples, the absence of oxygen limits auto-oxidation of myoglobin (Jeremiah, 2001). Farouk & Freke (2008) reported that freezer-stored samples of the *semimembranosus* muscle from red deer were characterized by more desirable colour parameters (higher values of a*, b*, C,* and lower values of h^o) in comparison with samples that were stored in gas-permeable packaging.

An analysis of the water-holding capacity of meat (Table 2) revealed that freezer-stored samples were characterized by greater (P > 0.05) natural drip loss and greater ($P \le 0.01$) cooking loss. Meat intended for industrial processing should be able to retain its own water and bind added water. In this study, the capacity to bind added water was significantly ($P \le 0.01$) lower in freezer-stored meat than in fresh meat. The water-holding capacity of the meat samples (natural drip loss, ability to bind added water) was similar to that described in the literature (Leygonie *et al.*, 2012a), which indicates that freezing, freezer storage, and thawing compromise the water-binding ability of meat. These results can be attributed to changes in the structure of meat fibres and protein denaturation. According to Gambuteanu *et al.* (2013), the freezing rate is correlated with the water-holding capacity of thawed meat. During slow freezing, muscle fibres are partially dehydrated owing to the migration of intracellular fluid to extracellular space, where ice crystals are formed. Water expands on freezing, which increases pressure, ruptures connective tissue, and partially damages cell membranes (ice crystals have sharp edges). As a result, intracellular fluid migrates to extracellular space and, on thawing, increases drip loss and stimulates enzymatic activity. The water-holding capacity of thawed

meat also decreases owing to the denaturation of water-binding proteins during freezing and freezer storage. The partial dehydration of muscle cells increases the concentration of water-soluble compounds (Leygonie *et al.*, 2012b).

The influence of freezing on the results of the cooking loss analysis is not as clear as its influence on the results of natural drip loss and the water-holding capacity of meat. Vieira *et al.* (2009) observed that samples of the *longissimus thoracis* muscle from young bulls stored at -20 °C and -80 °C for 30, 75 and 90 days were characterized by greater cooking loss ($P \le 0.01$) than fresh samples, whereas the differences between frozen samples were not significant. In contrast, Leygonie *et al.* (2012b) reported non-significant differences in the cooking loss of fresh and frozen (1 month, -20 °C) ostrich meat (*M. iliofibularis*). The observed differences could be explained because cooking drip is composed of constitutive water (natural drip loss) and chemically bound water, which is released from melting fat and denatured proteins under exposure to heat (Vieira *et al.*, 2009). Cooking loss is also influenced by the volume of thawing loss.

The results of a sensory evaluation revealed that both fresh and thawed meat were characterized by desirable sensory attributes (Table 3). The juiciness of freezer-stored meat was the only sensory parameter that received lower scores. Freezer storage influenced the juiciness and tenderness of the samples. Fresh meat was characterized by higher juiciness ($P \le 0.01$), whereas tenderness scores were higher in freezer stored meat ($P \le 0.01$). Tenderness influences shear force values (Table 3); however, the differences between group means were not statistically significant.

Deservator	Freezer storage period		
Parameter	day 0 (n = 11)	10 months (n = 11)	
Aroma: intensity	4.14 ± 0.778	4.27 ± 0.467	
Aroma: desirability	4.91 ± 0.202	5.00 ± 0.000	
Taste: intensity	4.27 ± 0.410	4.27 ± 0.467	
Taste: desirability	4.86 ± 0.234	4.86 ± 0.234	
Tenderness	$3.86^{A} \pm 0.505$	$4.64^{B} \pm 0.452$	
Juiciness	$4.00^{A} \pm 0.447$	$3.36^{B} \pm 0.234$	
Shear force	22.05 ± 5.472	18.99 ± 3.134	

Table 3 Sensory attributes (points) and shear force (Newton) values of *M. longissimus lumborum* from male fallow deer before and after freezer storage (means ± standard deviations)

^{A,B} Mean values in the same row with different superscripts differ significantly at $P \le 0.01$

Thawed meat was characterized by lower juiciness owing to the loss of water during thawing and cooking (Lagerstedt *et al.*, 2008). A negative correlation between cooking loss and juiciness was reported by Jung *et al.* (2016) and Aaslyng *et al.* (2003) for beef and pork, respectively. According to the literature, the shear force value of meat decreases after freezer storage (Shanks *et al.*, 2002; Farouk & Freke 2008; Lagerstedt *et al.*, 2008), which points to improved tenderness. This is attributed to the enzymatically induced autolysis of muscle fibres during meat ageing before freezing and the loss of structural integrity of muscle fibres caused by ice crystals during freezing (Vieira *et al.*, 2009). However, the results of tenderness assessment are not always reflected in the shear force values of meat. Tenderness is a complex sensation, which is experienced during biting and chewing of meat. The perception of tenderness is determined not only by the structure of muscle tissue, but also by the presence of fat, connective tissue and the water content of muscle fibres. Therefore, greater thawing loss and greater cooking loss can compromise the perceived tenderness of frozen meat in comparison with fresh meat (Lagerstedt *et al.*, 2008).

Conclusion

Freezer-stored meat was characterized by lower dry matter content than fresh meat, which could be attributed to natural variations in the chemical composition of the samples and the loss of water-soluble compounds during thawing. An analysis of the physico-chemical properties of meat revealed higher pH values, lower contribution of redness and yellowness, lower chroma, lower water-holding capacity, lower

ability to bind added water, and higher cooking loss in freezer-stored meat than in fresh meat. Freezer storage for 10 months improved meat tenderness, but reduced its juiciness. The observed changes indicate that freezer storage in vacuum packaging preserves the quality of meat from wild fallow deer adequately and increases its availability between hunting seasons.

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Authors' Contributions

Conception and design: TD; data collection and analysis: TD, TL and DK; drafting of paper: TD and TL; critical revision and final approval of version to be published: TD.

Conflict of Interest Declaration

The authors wish to confirm that no known conflicts of interest are associated with the publication of this manuscript.

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