Comparison of the lipid properties of healthy and pansteatitis-affected African sharptooth catfish, *Clarias gariepinus* (Burchell), and the role of diet in pansteatitis outbreaks in the Olifants River in the Kruger National Park, South Africa

K D A Huchzermeyer[1,2], G Osthoff[3], A Hugo[3] and D Govender[4]

1 Sterkspruit Veterinary Clinic, Lydenburg, South Africa
2 Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa
3 Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa
4 Scientific Services, South African National Parks, Skukuza, South Africa

Abstract

Pansteatitis has been identified in wild populations of sharptooth catfish, *Clarias gariepinus* (Burchell), and Nile crocodiles, *Crocodylus niloticus* Laurenti, inhabiting the same waters in the Olifants River Gorge in the Kruger National Park, South Africa. Mesenteric and pectoral fat tissue was investigated microscopically and by fatty acid analysis in healthy and pansteatitis-affected catfish from both captive and wild populations. Variation in fatty acid composition between pectoral and mesenteric fat was noted. Composition of mesenteric fat differed between fish from various localities as a result of differences in diet. Pansteatitis in the captive population, resulting from ingestion of high amounts of dietary oxidized fat, reflected higher levels of unsaturated fatty acids within the mesenteric fat. Mesenteric fat of pansteatitis-affected wild catfish was characterized by an increase in moisture content, a decrease in fat content and a decrease in stearic and linoleic acids. The n-3 to n-6 fatty acid ratio of mesenteric fat was higher in pansteatitis-affected wild catfish than in healthy catfish from the same locality, reflecting higher polyunsaturated fat intake by pansteatitis-affected fish. The possible role of alien, invasive, phytoplankton-feeding silver carp, *Hypophthalmichthys molitrix* (Valenciennes), in the aetiology of pansteatitis in both catfish and crocodiles in the Olifants Gorge is discussed.

Keywords: *Clarias gariepinus*, lipid properties, pansteatitis, polyunsaturated fat, sharptooth catfish, silver carp.

Correspondence *K D A Huchzermeyer, Sterkspruit Veterinary Clinic, PO Box 951, Lydenburg 1120, South Africa (e-mail: aquavet@telkomsa.net)*

Introduction

During the winter of 2008, carcasses of more than 170 Nile crocodiles, *Crocodylus niloticus* Laurenti, were found close to the confluence of the Olifants and Letaba Rivers in the Kruger National Park (KNP) in South Africa (Anon 2008; Ferreira & Pienaar 2011). The cause of death was established to be a consequence of severe pansteatitis. Further crocodile mortalities occurred during subsequent winters, but in smaller numbers. Both rivers have their catchments in industrial and agricultural areas, before they enter the Kruger National Park. The Letaba River flows 97 km through the Park, and the Olifants River for some 90 km, where they join (confluence 23°59′21.8″S 31°49′35.6″E) and flow a further 9 km through the Olifants River gorge before crossing into Mozambique, entering the upper reaches of Lake Massingir. Extensive sampling of fish from the Olifants River gorge has led to the discovery that pansteatitis also

affected African sharptooth catfish, *Clarias gariepinus* (Burchell), inhabiting this section of the Olifants River (Huchzermeyer *et al.* 2011). Hydrodynamic change in the Olifants River Gorge, brought about by the raising of the sluices of Lake Massingir downstream of the gorge, resulted in the inlet of Lake Massingir extending back into the Olifants Gorge, flooding the previously fast flowing rapids and pools of the gorge. Most of the dead and affected crocodiles were found in both rivers from about 10 km upstream of the confluence, up to the border with Mozambique in the same stretch of river where pansteatitis-affected catfish have been found. It has been speculated that pollutants may be concentrated in the clay deposits trapped by dams along the Olifants River (Heath, Coleman & Engelbrecht 2010) that, when released over time due to local changes in water quality, could be the cause of the crocodile and fish mortalities. High phosphate levels attributed to the discharge of tailings from a phosphate mine and to municipal sewerage spills into the Olifants River near the town of Phalaborwa on the western boundary of KNP prior to 2004 (J. Venter, SANParks, Skukuza, personal communication 2012) have contributed to the nutrient enrichment of Lake Massingir.

Pansteatitis, also called yellow fat disease, has been reported in a variety of animals such as rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Roberts, Richards & Bullock 1979), Sunapee trout, *Salvelinus alpinus oquassa* Girard (Herman & Kircheis 1985), channel catfish, *Ictalurus punctatus* (Rafinesque) (Goodwin 2006), white sturgeon *Acipenser transmontanus* Richardson (Guarda *et al.* 1997), Atlantic halibut, *Hippoglossus hippoglossus* L. (Bricknell *et al.* 1996), northern bluefin tuna, *Thunnus thynnus* (L.) (Roberts & Agius 2008), red-tailed hawk, *Buteo jamaicensis* (Gmelin) (Wong *et al.* 1999), boat billed herons, *Cochlearius cochlearius* (L.) (Pollock *et al.* 1999), the domestic cat (Niza, Vilela & Ferreira 2003), wild rabbit (Jones, Howard & Gresham 1969), marmoset, *Callithrix* spp. (Juan-Sallés *et al.* 2003), Amazon River dolphin, *Inia geoffrensis* (Blainville) (Bonar & Wagner 2003), and Nile crocodile (Huchzermeyer 2003; Osthoff *et al.* 2010). The occurrence and pathology of pansteatitis in sharptooth catfish in the Olifants River has been described by Huchzermeyer *et al.* (2011). The term yellow fat disease may be misleading in this species as sharptooth catfish naturally show a variable fat colour.

Pansteatitis is defined as a nutritional disorder characterized by necrosis and inflammation of adipose tissue and deposition of a ceroid pigment within macrophages in the associated inflammatory reaction. The disease is linked to the consumption of high levels of unsaturated fatty acids and is exacerbated by intake of oxidized fats and depletion of vitamin E (Scott, Miller & Griffin 1995; Fytianou *et al.* 2006). In many species, the condition can be induced by vitamin E deficiency (Farwer *et al.* 1994). Unsaturated fatty acids in the lipids of cell membranes are vulnerable to oxidation through cyclic reduction–oxidation of oxidants resulting in the formation of lipid hydroperoxides (Bus, Aust & Gibson 1976). Lipid free radicals released from decomposition of lipid hydroperoxides initiate the subsequent lipid peroxidation of cell membranes (Bus *et al.* 1976). A lack of vitamin E may result in an accumulation of reactive peroxides in the tissue, which may lead to pansteatitis (Case, Carey & Hirakawa 1995). Treatment with vitamin E may alleviate the disease (Niza *et al.* 2003), but this is not always the case (Bonar & Wagner 2003; Juan-Sallés *et al.* 2003). The effect of pansteatitis on fatty acid composition has not been described by many researchers. Farwer *et al.* (1994) provided evidence that vitamin E depletion affected the fatty acid composition of liver lipids, and Fytianou *et al.* (2006) showed that the fatty acid composition of adipose tissue is affected by pansteatitis. Osthoff *et al.* (2010) have shown that the lipids of adipose tissue of healthy wild crocodiles differed minimally in the fatty acid composition from diseased ones and that the observed hardness of the fat tissue was not due to changes in fat composition, but rather to high moisture content, the result of physiological changes induced by interstitial inflammation similar to that observed by Niza *et al.* (2003).

Analysis of the fat composition of captive-raised African sharptooth catfish (Hoffman & Prinsloo 1995) and a comparison with the neutral lipids of heart muscle of free-living catfish in Ugandan lakes (Kwetegyeka *et al.* 2011) showed that fatty acid composition of the total fat of captive fish and that of the heart muscle fat of free-living fish was similar. Captive fish were, however, found to contain higher levels of 20:4n3 and lower levels of 18:1 isomers than wild fish (Hoffman & Prinsloo 1995), and the fatty acid composition of the heart muscle fat differed between fish of different

localities (Kwetegyeka *et al.* 2011). This comparison could not conclude whether the difference was due to tissue type, locality or diet. However, Steffens (1997) has reported the effect of diet on fatty acid composition. The effect of diet and health conditions on the fat composition of sharptooth catfish has not been reported before. The aim of our study was to determine the impact of pansteatitis on the fat composition of captive and wild African sharptooth catfish and to link this to dietary factors affecting the two populations.

Materials and methods

During November 2009, a minimum of 20 sharptooth catfish were collected from each of three geographically distinct populations: Olifants River Gorge (OG) [23°59′21.8″S 31°49′35.6″E] in the vicinity of the confluence between the Letaba and Olifants rivers in the area regarded as the epicentre of the crocodile mortalities in the KNP; Reenvoël Dam (RV) [23°58′37.2″S 31°19′38.4″E], a rain-filled dam with its entire catchment within KNP; and Lunsklip Fisheries (LK) [25°23′08,9″S 30°15′35″E] on the Lunsklip River near Lydenburg in Mpumalanga Province. Wild fish were caught on baited hook and line. The fish from LK represented remnants of a farmed population of fish and were caught by scoop net.

The LK population of captive catfish was fed an exclusive diet of untreated trout slaughterhouse waste. Since the collapse of the catfish industry many years ago, these fish were no longer used commercially and hence remained in the system reaching a considerable body size. The slaughterhouse waste was made up to a large extent of fat-rich innards of slaughtered trout and was fed in such excess that at any given time a significant amount of waste could be observed uneaten and decomposing in the water.

Fish were killed by an overdose of benzocaine in the holding water. Detailed data sets were collected from all fish and included length and weight measurements, sex determination, macroscopic and histological descriptions of all organs, including adipose tissues. Age determinations were carried out according to the method of Weyl & Booth (2008) using sagittal otolith sections. In addition, samples of the two major adipose tissues, intra-abdominal mesenteric fat and hypodermal fat from the fat cushion behind the pectoral fin, were collected. The sharptooth catfish is unique in that an extension of both the liver and the marrow or haemopoietic part of the anterior kidney extend through the body wall to lie bilaterally under a hypodermal fat cushion just caudal to the pectoral fin. Tissue samples for fat analyses were collected from those fish with adequate fat reserves and were kept on ice until samples could be frozen before being sent to the laboratory. All tissue samples were kept frozen until preparation for fat extraction, which was carried out within 5 days. Tissue samples for histological examination were immediately fixed in 10% buffered formalin before being processed by standard histological techniques. Five-micron-thick tissue sections stained with haematoxylin and eosin and Gomori's aldehyde fuchsin were examined under the light microscope. Severity of pathology in the adipose tissues was scored histologically on a scale of 1–4, with 1 representing no steatitis or the presence of only a few lipopigment-containing macrophages in the fat tissue, but without necrosis of adipocytes and 4 showing the greatest degree of necrosis and inflammation.

Extraction of total fat from tissue samples was performed quantitatively according to Folch, Lees & Sloane-Stanley (1957) using chloroform and methanol in a ratio of 2:1. Total extractable fat content was determined gravimetrically and expressed as g fat per 100 g tissue.

Fatty acids were transesterified to form methyl esters (FAME) using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol (Park & Goins 1994). The FAME were quantified using a Varian 430 flame ionization gas chromatograph (Varian), with a fused silica capillary column, Varian CPSIL 88 (100 m length, 0.25 mm ID, 0.2-μm film thickness) (Varian). The column temperature was 40–230 °C (hold 2 min; ramp 4 °C min^{-1}; hold 10 min). The FAME in hexane (1 μL) was injected into the column using a Varian CP-8400 Autosampler (Varian) with a split ratio of 100:1. The injection port and detector were both maintained at 250 °C. Hydrogen was used as the carrier gas at 45 psi, and nitrogen was the make-up gas. Chromatograms were recorded using Galaxy, Chromatography, Data System (Varian). Identification of sample FAME was made by comparing the relative retention times of FAME peaks from samples with those of standards of all 37 fatty acids obtained from Supelco (Supelco 37 Component Fame Mix 47885-U; Supelco).

Significant differences in means amongst groups were determined using analysis of variance (ANOVA) and multiple comparisons using the Tukey–Kramer test at α = 0.05 (Anon 2007).

Results and discussion

The fish sampled from RV showed no macroscopic signs of pansteatitis and, apart from infestation with a variety of parasites natural to this habitat, appeared healthy. Fish from this population ranged in age from 4 to 19 years, with both sexes being represented in the sampling.

Pansteatitis was found in both male and female fish sampled from the wild population in the OG and from the captive population from LK. In affected fish, lesions were predominantly found in the mesenteric fat reserves. Only a few fish showed lesions in other fat reserves, including the pectoral and hypodermal fat, as well as in fat surrounding the brain. Steatitis was never observed in the pericardial fat. No correlation was observed between age or sex and the presence of pansteatitis in either the wild or captive catfish.

In the OG fish, ratio of adipose tissue to body mass showed little correlation with the presence of pansteatitis, lesions being present in both fat and lean fish. In contrast, easy access to fat-rich food in the captive fish population from LK was reflected in a significantly higher adipose tissue to body mass ratio than in fish from the OG. From the results of the captive fish, it is evident that pansteatitis is not rapidly fatal in these fish, even though the condition may be debilitating. When pansteatitis-affected and healthy fish were kept together in the same tank, clear behavioural differences were observed, the former being slower in finding and taking feed (D. Huchzermeyer, personal observation, 2011).

Comparisons of stomach and intestinal content of fish sampled from the different locations indicated that the sharptooth catfish in OG preyed heavily on other fish, whereas the ingesta of fish from RV reflected a more omnivorous diet consisting of a combination of plants, algae and invertebrates. Stomach content of the LK fish consisted exclusively of decomposing oily fish remnants.

The detailed pathology of pansteatitis in sharptooth catfish from the OG has been described by Huchzermeyer *et al.* (2011). Histological lesions of pansteatitis were similar in the OG and LK fish. Lesions consisted of ruptured adipocytes surrounded by an intense, predominantly macrophage reaction (Figs 1 & 2). Macrophages stained positively for ceroid, a breakdown product of fat, in Gomori's aldehyde fuchsin stained sections (Fig. 1). Coalescing remnants of necrotic fat cells consisted of a refractive yellow staining lipopigment, a fat cell breakdown product, in haematoxylin–eosin-stained preparations (Fig. 2). The same refractive pigment could be observed in macrophages associated with such areas of fat cell necrosis. Fat cell necrosis appeared in distinct foci disseminated through the affected adipose tissue. Such foci were surrounded by macrophages and foreign body giant cells, which in areas coalesced to form epithelioid-like sheaths surrounding lipopigment-containing lacunae. The mononuclear cellular (predominantly macrophage) infiltrate associated with the inflammatory process may have been responsible for the higher moisture content observed in affected fat.

The severest pansteatitis lesions were observed in older, larger fish specimens. In crocodiles, a similar trend was noted with degree of pansteatitis appearing to be related to size and therefore to age (Osthoff *et al.* 2010). On histological examination, the granulomatous inflammation observed surrounding necrotic and ruptured fat cells in pansteatitis-affected catfish was similar to that noted in pansteatitis-affected crocodiles (Osthoff *et al.* 2010).
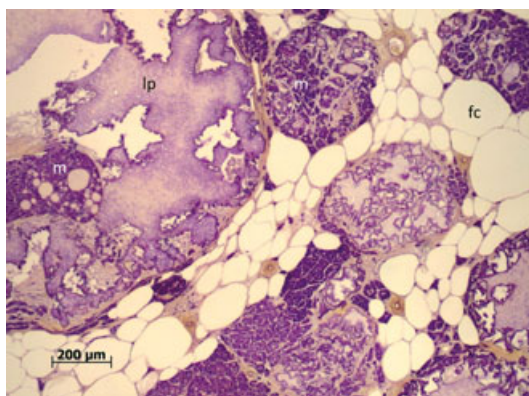

Figure 1 Section of fat from a *Clarias gariepinus* specimen with steatitis, sampled from the Olifants River Gorge during November 2009, stained specifically for the presence of ceroid with Gomori's aldehyde fuchsin. Note ceroid-positive staining of macrophages (m) and fat cell breakdown products. Lipopigment (lp), fat cell (fc).
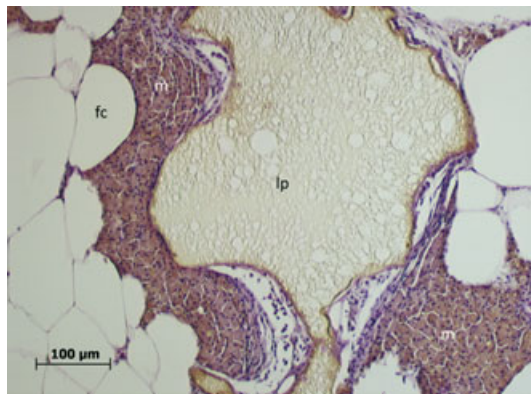
Figure 2 Section of fat from a *Clarias gariepinus* specimen suffering from steatitis sampled from Lunsklip Fisheries during November 2009. Note fat breakdown product with lipopigment (lp) surrounded by macrophages (m). Fat cell (fc). Haematoxylin–eosin.

The fat composition of healthy fish (not affected by pansteatitis) from the three localities, LK, RV and OG is given in Table 1. The mean fatty acid composition of these catfish was in the same order of magnitude as that published by Hoffman & Prinsloo (1995). The monounsaturated acids were found to be higher than the published data, above 36%, mainly due to 16:0, and the levels of polyunsaturated acids were lower, <27% compared with the more than 35% reported. This difference might be ascribed to diet, because the fatty acids of the catfish from the three localities differed significantly (from $P < 0.001$ to $P < 0.05$) amongst each other (Table 1). The greatest differences were observed between the total polyunsaturated fatty acids, mainly due to 18:3c9,12,15(n-3), with 1.75% ± 0.68, 1.11% ± 0.28 and 7.78% ± 1.20 for LK, OG and RV respectively, and 20:4c5,8, 11,14(n-6), with 0.54% ± 0.02, 1.01% ± 0.18 and 2.72% ± 0.66. Significant differences were also noted in monounsaturated fatty acids, the greatest being in 18:1c9, with respectively 23.71% ± 0.48, 30.62% ± 4.35 and 16.03% ± 2.88. Smaller differences were noted in the content of 18:1c7, 15:0, 17:0 and 18:0. At a lower level of statistical significance, several more differences in fatty acid composition were noted, most obviously in the total unsaturated and polyunsaturated fatty acids. Differences in fatty acid content that might be ascribed to locality, and probably a difference in diet, were similarly reported for heart fat of various fish, including the catfish (Kwetegyeka *et al.* 2011). Of interest is that the moisture content of the fat tissue of fish from OG was lower than that of the other two localities ($P < 0.043$), although the fat content and fat-free dry matter were the same. These results show that the fat of healthy and pansteatitis-affected catfish from the three localities have to be interpreted separately.

The fat composition of normal mesenteric and pectoral fat of captive catfish (LK) is shown in Table 2. Small but statistically significant ($P < 0.001$) differences in content of 14:0, 18:0, 18:1c9, 22:0, 20:5c5,8,11,14,17(n-3) and 24:1c15 were observed. At a lower statistical significance, $P < 0.05$, more differences in fatty acid composition became apparent. The total saturated fatty acid content of mesenteric fat was higher than that of pectoral fat, 36.37% ± 3.89 vs. 33.95% ± 3.27, whilst the total polyunsaturated content was lower, 26.70% ± 3.80 vs. 30.70% ± 3.14. Mesenteric fat had a higher content of total monounsaturated and lower content of total omega-3 fatty acids, and also a lower content of the very long-chain fatty acids, longer than 18 carbon length, than pectoral fat. The fat content of mesenteric fat tissue was higher than that of pectoral fat tissue, 78.46% ± 6.92 vs. 69.55% ± 4.19 and the water content lower, 16.01% ± 3.21 vs. 21.86% ± 4.44. Similar differences in fatty acid composition of fat of different body locations have been observed in other animals (Sinclair & O'Dea 1987).

In contrast to mesenteric fat, which frequently showed extensive areas of fat necrosis in pansteatitis-affected fish, the pectoral fat only rarely showed foci of fat necrosis. Little difference, as assessed by histological appearance and fat composition of pectoral fat, was observed between the healthy and pansteatitis-affected catfish. Differences observed between mesenteric and pectoral fat might be due to the specific function of the pectoral fat, which is embedded in a connective tissue matrix giving it a spongy feel. This suggests a protective rather than a metabolic function, as the pectoral fat overlies the area behind the pectoral fin where both the liver and anterior kidney extend through a fine canal in the musculature of the body wall to lie subdermally behind the pectoral fin. This is a unique and peculiar feature of sharptooth catfish that is shared with few other fish species. The higher water content of pectoral fat may explain the slightly glassy gross appearance of pectoral fat when compared to mesenteric fat.

Table 1 Analysis of variance (ANOVA) on the effect of locality on chemical composition, fatty acid composition and fatty acid ratios of mesenteric fat of fish with a steatitis score of 1

Locality		LK (n = 3)	OG (n = 7)	RV (n = 4)	Significance level
Proximate analysis (%)					
% Fat		78.46 ± 6.92	85.75 ± 3.08	79.55 ± 8.08	P = 0.129
% Fat-free dry matter		5.53 ± 4.14	6.92 ± 0.86	8.91 ± 1.30	P = 0.119
% Moisture		16.01 ± 3.21[b]	7.33 ± 2.96[a]	11.54 ± 6.85[ab]	P = 0.043
FAME (% of total fatty acids)					
Common name	Abbreviation				
Myristic	C14:0	3.41 ± 0.59[b]	2.37 ± 0.49[a]	2.05 ± 0.55[a]	P = 0.016
Pentadecylic	C15:0	0.43 ± 0.12[a]	0.43 ± 0.08[a]	1.03 ± 0.18[b]	P < 0.001
Palmitic	C16:0	27.05 ± 3.49	29.09 ± 3.90	28.79 ± 1.75	P = 0.679
Palmitoleic	C16:1c9	8.17 ± 0.64[b]	5.89 ± 0.56[a]	7.53 ± 1.43[b]	P = 0.006
Margaric	C17:0	0.40 ± 0.12[a]	0.64 ± 0.09[a]	2.77 ± 0.35[b]	P < 0.001
Heptadecenoic	C17:1c10	0.38 ± 0.10[b]	0.07 ± 0.02[a]	0.11 ± 0.01[a]	P < 0.001
Stearic acid	C18:0	4.33 ± 0.48[a]	6.93 ± 0.72[b]	6.26 ± 0.26[b]	P < 0.001
Elaidic	C18:1t9	0.31 ± 0.13[b]	0.03 ± 0.02[a]	0.15 ± 0.09[a]	P < 0.001
Oleic	C18:1c9	23.71 ± 0.48[b]	30.62 ± 4.35[c]	16.03 ± 2.88[a]	P < 0.001
Vaccenic	C18:1c7	4.25 ± 0.27[b]	3.47 ± 0.11[a]	6.14 ± 0.71[c]	P < 0.001
Linoleic	C18:2c9,12 (n-6)	13.14 ± 1.80[b]	8.11 ± 2.96[a]	6.78 ± 0.90[a]	P = 0.012
Arachidic	C20:0	0.18 ± 0.02[a]	0.27 ± 0.04[ab]	0.33 ± 0.08[b]	P = 0.009
γ-Linolenic	C18:3c6,9,12 (n-6)	0.35 ± 0.11[ab]	0.20 ± 0.08[a]	0.56 ± 0.23[b]	P = 0.006
α-Linolenic	C18:3c9,12,15 (n-3)	1.75 ± 0.68[a]	1.11 ± 0.28[a]	7.78 ± 1.20[b]	P < 0.001
Heneicosanoic	C21:0	0.49 ± 0.11	0.73 ± 0.55	1.14 ± 0.29	P = 0.167
Eicosadienoic	C20:2c11,14 (n-6)	0.77 ± 0.03[b]	0.41 ± 0.13[a]	0.82 ± 0.15[b]	P < 0.001
Behenic	C22:0	0.08 ± 0.01[a]	0.11 ± 0.02[a]	0.29 ± 0.11[b]	P < 0.001
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.47 ± 0.10[a]	0.44 ± 0.08[a]	0.76 ± 0.16[b]	P = 0.002
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.05 ± 0.02[a]	0.14 ± 0.02[b]	0.90 ± 0.09[c]	P < 0.001
Arachidonic	C20:4c5,8,11,14 (n-6)	0.54 ± 0.02[a]	1.01 ± 0.18[a]	2.72 ± 0.66[b]	P < 0.001
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	2.12 ± 1.29[ab]	0.74 ± 0.25[a]	2.69 ± 0.95[b]	P = 0.004
Nervonic	C24:1c15	0.11 ± 0.02[b]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	P < 0.001
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.81 ± 0.22	1.77 ± 0.71	1.75 ± 0.21	P = 0.990
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.70 ± 1.39	5.41 ± 2.24	2.61 ± 0.63	P = 0.057
Fatty acid ratios					
Total Saturated Fatty Acids (SFA)		36.37 ± 3.89	40.57 ± 4.03	42.67 ± 1.03	P = 0.096
Total Mono-Unsaturated Fatty Acids (MUFA)		36.93 ± 0.50[ab]	40.09 ± 3.85[b]	29.95 ± 4.62[a]	P = 0.004
Total Polyunsaturated Fatty Acids (PUFA)		26.70 ± 3.80[b]	19.34 ± 0.89[a]	27.38 ± 4.25[b]	P < 0.001
Total Omega- 6 fatty acids (n-6)		14.85 ± 1.67[b]	9.88 ± 2.93[a]	11.79 ± 1.77[ab]	P = 0.041
Total Omega- 3 fatty acids (n-3)		11.86 ± 2.13[ab]	9.46 ± 2.86[a]	15.59 ± 2.50[b]	P = 0.012

Means with different superscripts in the same row differ significantly.

Statistical comparison of pansteatitis-affected fish across localities is not possible due to the effects of different diets on fatty acid composition of the fat. Further comparison of fatty tissue in pansteatitis-affected fat will refer only to the mesenteric fat. The comparison of the fat composition of the fish from LK with different scores of pansteatitis is presented in Table 3. No significant differences at P < 0.001 were observed between the fats from healthy and pansteatitis-affected fish. At a lower significance level, the saturated fatty acid content of healthy fish at LK, 36.37% ± 3.89 was higher than that of the fish with pansteatitis score 4, 32.49% ± 1.05, the main difference being in 16:0 and 17:0, whereas the total omega-6 content was lower, 14.85% ± 1.67 vs. 17.81% ± 1.42, with 18:2c9, 12 (n-6) being the main component. Although not statistically significant in all aspects, the fatty acid composition of the fish with pansteatitis scores 2 and 3 resembled that of score 4 and may have been a reflection of the uniform prolonged exposure of the fish to oxidized fish waste in the diet. These results are in agreement with Farwer et al. (1994) who found that pansteatitis in rats, which was induced by a depletion of vitamin E and a diet high in unsaturated fatty acids, was characterized by lower levels of saturated fatty acids and higher levels of unsaturated fats in the liver and serum. However, when pansteatitis was induced in cats due to a diet high in unsaturated fatty acids, a depletion of vitamin E resulted, and an increase in saturated fatty acids and a decrease in unsaturated fatty acids in the subcutaneous adipose tissue was noted (Fytianou et al. 2006). It therefore appears that the changes in tissue fat due to pansteatitis may vary amongst species.

Table 2 Analysis of variance (ANOVA) on the effect of anatomical position (mesenteric vs. pectoral) of fat with a steatitis score of 1 on chemical composition, fatty acid composition and fatty acid ratios of fish from locality LK

Steatitis score		Mesenteric ($n = 3$)	Pectoral ($n = 21$)	Significance level
Proximate analysis (%)				
% Fat		78.46 ± 6.92	69.55 ± 4.19	$P = 0.004$
% Fat-free dry matter		5.53 ± 4.14	8.59 ± 1.57	$P = 0.018$
% Moisture		16.01 ± 3.21	21.86 ± 4.44	$P = 0.040$
FAME (% of total fatty acids)				
Common name	Abbreviation			
Myristic	C14:0	3.41 ± 0.59	5.13 ± 0.71	$P < 0.001$
Pentadecylic	C15:0	0.43 ± 0.12	0.50 ± 0.13	$P = 0.372$
Palmitic	C16:0	27.05 ± 3.49	23.75 ± 2.41	$P = 0.046$
Palmitoleic	C16:1c9	8.17 ± 0.64	9.35 ± 0.56	$P = 0.003$
Margaric	C17:0	0.40 ± 0.12	0.32 ± 0.07	$P = 0.133$
Heptadecenoic	C17:1c10	0.38 ± 0.10	0.53 ± 0.22	$P = 0.255$
Stearic acid	C18:0	4.33 ± 0.48	3.36 ± 0.36	$P < 0.001$
Elaidic	C18:1t9	0.31 ± 0.13	0.39 ± 0.14	$P = 0.362$
Oleic	C18:1c9	23.71 ± 0.48	20.92 ± 0.96	$P < 0.001$
Vaccenic	C18:1c7	4.25 ± 0.27	4.14 ± 0.15	$P = 0.319$
Linoleic	C18:2c9,12 (n-6)	13.14 ± 1.80	15.12 ± 1.44	$P = 0.041$
Arachidic	C20:0	0.18 ± 0.02	0.15 ± 0.02	$P = 0.018$
γ-Linolenic	C18:3c6,9,12 (n-6)	0.35 ± 0.11	0.33 ± 0.10	$P = 0.708$
α-Linolenic	C18:3c9,12,15 (n-3)	1.75 ± 0.68	1.45 ± 0.33	$P = 0.197$
Heneicosanoic	C21:0	0.49 ± 0.11	0.73 ± 0.28	$P = 0.163$
Eicosadienoic	C20:2c11,14 (n-6)	0.77 ± 0.03	0.70 ± 0.12	$P = 0.310$
Behenic	C22:0	0.08 ± 0.01	0.01 ± 0.02	$P < 0.001$
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.47 ± 0.10	0.38 ± 0.06	$P = 0.023$
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.05 ± 0.02	0.06 ± 0.04	$P = 0.535$
Arachidonic	C20:4c5,8,11,14 (n-6)	0.54 ± 0.02	0.66 ± 0.07	$P = 0.011$
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	2.12 ± 1.29	4.02 ± 0.79	$P = 0.001$
Nervonic	C24:1c15	0.11 ± 0.02	0.02 ± 0.03	$P < 0.001$
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.81 ± 0.22	1.64 ± 0.26	$P = 0.284$
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.70 ± 1.39	6.35 ± 1.44	$P = 0.471$
Fatty acid ratios				
Total Saturated Fatty Acids (SFA)		36.37 ± 3.89	33.95 ± 3.27	$P = 0.253$
Total Mono-Unsaturated Fatty Acids (MUFA)		36.93 ± 0.50	35.35 ± 1.04	$P = 0.018$
Total Polyunsaturated Fatty Acids (PUFA)		26.70 ± 3.80	30.70 ± 3.14	$P = 0.056$
Total Omega- 6 fatty acids (n-6)		14.85 ± 1.67	16.86 ± 1.36	$P = 0.028$
Total Omega- 3 fatty acids (n-3)		11.86 ± 2.13	13.83 ± 2.30	$P = 0.175$

Means with different superscripts in the same row differ significantly.

No difference in fat and moisture content was observed between the adipose tissues of healthy and pansteatitis-affected fish from LK. The data differ from results obtained from pansteatitis-affected crocodiles, in which fat tissue was found to have a higher moisture content and contain lower levels of 18:0 and 18:2c9,12(n-6) than fat tissue of healthy crocodiles (Osthoff *et al.* 2010). High moisture content of pansteatitis-affected fat in crocodiles was thought to be associated with the extensive inflammatory reaction in the adipose tissues accompanying advanced pansteatitis. Although the mesenteric adipose tissue of pansteatitis-affected LK fish took on a rubbery consistency, an equivalent degree of inflammation and hardening of the adipose tissues to that observed in crocodiles was not present. According to Farwer *et al.* (1994), Scott *et al.* (1995) and Fytianou *et al.* (2006) a decrease in unsaturated fatty acids may be due to oxidation, either by ingestion of oxidants or low levels of dietary vitamin E. In the case of the LK fish, the natural antioxidant capability of the catfish may have been overwhelmed by the excessively high unsaturated fat content in the trout slaughterhouse waste on which these fish had been fed over long periods of time.

Due to limited representatives of all pansteatitis scores of fish from OG, the data of scores 1 and 2 were pooled in one score group, whilst 3 and 4 were grouped together, and the fat composition of these are shown in Table 4. Similarly to the finding in crocodiles (Osthoff *et al.* 2010), the moisture content of fat in the higher pansteatitis score fish from OG appeared to be higher than that observed in fat with the lower score, 20.87% ± 14.49 vs. 7.33% ± 2.74.

Table 3 Analysis of variance (ANOVA) on the effect of steatitis score of mesenteric fat on chemical composition, fatty acid composition and fatty acid ratios of fish from locality LK

Steatitis score		1 (n = 3)	2 (n = 5)	3 (n = 3)	4 (n = 10)	Significance level
Proximate analysis (%)						
% Fat		78.46 ± 6.92	85.79 ± 2.87	83.70 ± 0.64	81.20 ± 4.80	P = 0.142
% Fat-free dry matter		5.53 ± 4.14	4.07 ± 1.09	3.42 ± 1.48	5.03 ± 1.87	P = 0.537
% Moisture		16.01 ± 3.21	10.13 ± 3.18	12.88 ± 1.23	13.77 ± 3.71	P = 0.122
FAME (% of total fatty acids)						
Common name	Abbreviation					
Myristic	C14:0	3.41 ± 0.59	3.63 ± 0.41	3.26 ± 0.13	3.68 ± 0.53	P = 0.546
Pentadecylic	C15:0	0.43 ± 0.12	0.35 ± 0.04	0.31 ± 0.02	0.34 ± 0.05	P = 0.077
Palmitic	C16:0	27.05 ± 3.49[b]	23.75 ± 1.20[a]	23.66 ± 1.02[a]	23.57 ± 0.70[a]	P = 0.015
Palmitoleic	C16:1c9	8.17 ± 0.64	8.54 ± 0.38	8.37 ± 0.35	8.65 ± 0.61	P = 0.579
Margaric	C17:0	0.40 ± 0.1[b]	0.31 ± 0.04[ab]	0.30 ± 0.02[ab]	0.29 ± 0.02[a]	P = 0.031
Heptadecenoic	C17:1c10	0.38 ± 0.10	0.51 ± 0.06	0.48 ± 0.03	0.47 ± 0.20	P = 0.729
Stearic acid	C18:0	4.33 ± 0.48	4.04 ± 0.28	4.17 ± 0.47	3.81 ± 0.51	P = 0.340
Elaidic	C18:1t9	0.31 ± 0.13	0.39 ± 0.11	0.31 ± 0.07	0.37 ± 0.15	P = 0.750
Oleic	C18:1c9	23.71 ± 0.48	24.28 ± 0.89	24.13 ± 0.69	23.53 ± 1.73	P = 0.753
Vaccenic	C18:1c7	4.25 ± 0.27	4.04 ± 0.34	3.99 ± 0.37	3.93 ± 0.21	P = 0.406
Linoleic	C18:2c9,12 (n-6)	13.14 ± 1.80[a]	16.66 ± 0.91[b]	15.66 ± 0.49[ab]	16.32 ± 1.44[b]	P = 0.008
Arachidic	C20:0	0.18 ± 0.02	0.15 ± 0.03	0.16 ± 0.04	0.15 ± 0.02	P = 0.208
γ-Linolenic	C18:3c6,9,12 (n-6)	0.35 ± 0.11	0.32 ± 0.07	0.30 ± 0.09	0.30 ± 0.04	P = 0.650
α-Linolenic	C18:3c9,12,15 (n-3)	1.75 ± 0.68	0.77 ± 0.24	1.14 ± 0.86	1.19 ± 0.59	P = 0.183
Heneicosanoic	C21:0	0.49 ± 0.11	0.64 ± 0.10	0.57 ± 0.07	0.59 ± 0.25	P = 0.775
Eicosadienoic	C20:2c11,14 (n-6)	0.77 ± 0.03	0.73 ± 0.05	0.75 ± 0.06	0.69 ± 0.06	P = 0.087
Behenic	C22:0	0.08 ± 0.01[b]	0.04 ± 0.02[a]	0.06 ± 0.01[ab]	0.05 ± 0.01[ab]	P = 0.022
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.47 ± 0.10	0.40 ± 0.07	0.43 ± 0.06	0.38 ± 0.04	P = 0.097
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.05 ± 0.02	0.02 ± 0.01	0.03 ± 0.03	0.02 ± 0.02	P = 0.224
Arachidonic	C20:4c5,8,11,14 (n-6)	0.54 ± 0.02	0.48 ± 0.06	0.47 ± 0.05	0.48 ± 0.04	P = 0.134
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	2.12 ± 1.29	2.70 ± 0.46	2.67 ± 0.16	2.90 ± 0.54	P = 0.349
Nervonic	C24:1c15	0.11 ± 0.02	0.09 ± 0.01	0.11 ± 0.02	0.11 ± 0.03	P = 0.461
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.81 ± 0.22	1.58 ± 0.19	1.90 ± 0.18	1.71 ± 0.33	P = 0.414
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.70 ± 1.39	5.60 ± 0.59	6.81 ± 0.90	6.46 ± 1.42	P = 0.424
Fatty acid ratios						
Total Saturated Fatty Acids (SFA)		36.37 ± 3.89[b]	32.90 ± 1.20[ab]	32.47 ± 1.60[ab]	32.49 ± 1.05[a]	P = 0.023
Total Mono-Unsaturated Fatty Acids (MUFA)		36.93 ± 0.50	37.85 ± 0.98	37.39 ± 1.13	37.06 ± 1.37	P = 0.628
Total Polyunsaturated Fatty Acids (PUFA)		26.70 ± 3.80	29.26 ± 1.86	30.15 ± 2.34	30.45 ± 1.99	P = 0.131
Total Omega- 6 fatty acids (n-6)		14.85 ± 1.67[a]	18.21 ± 0.86[b]	17.21 ± 0.51[ab]	17.81 ± 1.42[b]	P = 0.010
Total Omega- 3 fatty acids (n-3)		11.86 ± 2.13	11.04 ± 1.11	12.94 ± 1.84	12.64 ± 1.65	P = 0.299

Means with different superscripts in the same row differ significantly. Steatitis score: 1 = 0–25%; 2 = 25–50%; 3 50–75%; 4 = 75–100%.

The fat content of fish from OG was found to be lower in fish with higher pansteatitis score than in those with lower score, 69.04% ± 18.49 vs. 85.71% ± 2.86, however, only at a significance value of $P = 0.026$. Relative to pansteatitis severity, the greatest differences in fatty acid composition of OG fish were noted amongst the respective levels of 22:5c7,10,13,16,19(n-3), 22:6c4,7,10,13,16,19(n-3) and 18:2c9,12(n-6). The omega-3 polyunsaturated fatty acids made up 9.28% ± 2.70 of total fatty acids in the lower pansteatitis score group compared with 16.79% ± 5.51 ($P = 0.009$) in the higher pansteatitis score group. The opposite trend was observed for the omega-6 acids, with 10.4% ± 2.75 and 5.85% ± 1.08 ($P = 0.017$) in the low and high pansteatitis groups, respectively. A significant difference between the two groups was also noted in the content of 18:0 with 6.95% ± 0.67 vs. 4.06% ± 0.84, respectively. The lower fat and higher moisture content of the adipose tissue and the lower 18:0 and 18:2c9,12(n-6) levels in the catfish with the high pansteatitis score is similar to that observed for the Nile crocodiles from the same waters reported by Osthoff et al. (2010). The increased firmness of the fat tissue observed in crocodiles (Osthoff et al. 2010) and catfish with advanced pansteatitis appears to be unrelated to fatty acid composition and is more likely a reflection of physiological changes associated with interstitial inflammation, as has also been observed in cats with pansteatitis (Niza et al. 2003). In catfish, however, the hardening of affected fat tissue was not as severe as that observed in crocodiles.

Table 4 Analysis of variance (ANOVA) on the effect of steatitis score of mesenteric fat on chemical composition, fatty acid composition and fatty acid ratios of fish from locality OG

Steatitis score		1 + 2 (n = 8)	3 + 4 (n = 4)	Significance level
Proximate analysis (%)				
% Fat		85.71 ± 2.86	69.04 ± 18.49	$P = 0.026$
% Fat-free dry matter		6.96 ± 0.80	10.09 ± 4.23	$P = 0.060$
% Moisture		7.33 ± 2.74	20.87 ± 14.49	$P = 0.023$
FAME (% of total fatty acids)				
Common name	Abbreviation			
Myristic	C14:0	2.44 ± 0.50	2.04 ± 0.88	$P = 0.338$
Pentadecylic	C15:0	0.44 ± 0.08	0.36 ± 0.02	$P = 0.085$
Palmitic	C16:0	28.5 ± 83.89	30.67 ± 2.32	$P = 0.350$
Palmitoleic	C16:1c9	5.87 ± 0.52	7.43 ± 1.55	$P = 0.024$
Margaric	C17:0	0.63 ± 0.09	0.61 ± 0.17	$P = 0.846$
Heptadecenoic	C17:1c10	0.07 ± 0.02	0.15 ± 0.07	$P = 0.018$
Stearic acid	C18:0	6.95 ± 0.67	4.06 ± 0.84	$P < 0.001$
Elaidic	C18:1t9	0.03 ± 0.01	0.06 ± 0.04	$P = 0.133$
Oleic	C18:1c9	31.18 ± 4.32	26.48 ± 4.33	$P = 0.106$
Vaccenic	C18:1c7	3.45 ± 0.12	3.32 ± 1.05	$P = 0.725$
Linoleic	C18:2c9,12 (n-6)	8.29 ± 2.79	4.42 ± 1.05	$P = 0.025$
Arachidic	C20:0	0.26 ± 0.05	0.32 ± 0.09	$P = 0.137$
γ-Linolenic	C18:3c6,9,12 (n-6)	0.19 ± 0.07	0.32 ± 0.07	$P = 0.013$
α-Linolenic	C18:3c9,12,15 (n-3)	1.13 ± 0.27	1.10 ± 0.25	$P = 0.853$
Heneicosanoic	C21:0	0.68 ± 0.52	1.70 ± 0.75	$P = 0.020$
Eicosadienoic	C20:2c11,14 (n-6)	0.42 ± 0.12	0.26 ± 0.07	$P = 0.037$
Behenic	C22:0	0.10 ± 0.03	0.14 ± 0.10	$P = 0.321$
Eicosatrienoic	C20:3c11,14,17 (n-3)	0.44 ± 0.07	0.41 ± 0.16	$P = 0.616$
Eicosatrienoic	C20:3c8,11,14 (n-6)	0.14 ± 0.02	0.23 ± 0.08	$P = 0.010$
Arachidonic	C20:4c5,8,11,14 (n-6)	1.00 ± 0.17	0.62 ± 0.03	$P = 0.002$
Eicosapentaenoic	C20:5c5,8,11,14,17 (n-3)	0.72 ± 0.23	0.79 ± 0.26	$P = 0.655$
Docosapentaenoic	C22:5c7,10,13,16,19 (n-3)	1.90 ± 0.76	3.44 ± 1.35	$P = 0.028$
Docosahexaenoic	C22:6c4,7,10,13,16,19 (n-3)	5.09 ± 2.27	11.06 ± 4.47	$P = 0.010$
Fatty acid ratios				
Total Saturated Fatty Acids (SFA)		40.08 ± 3.98	39.92 ± 3.13	$P = 0.946$
Total Mono-Unsaturated Fatty Acids (MUFA)		40.60 ± 3.84	37.44 ± 6.29	$P = 0.298$
Total Polyunsaturated Fatty Acids (PUFA)		19.32 ± 0.83	22.65 ± 5.52	$P = 0.111$
Total Omega- 6 fatty acids (n-6)		10.04 ± 2.75	5.85 ± 1.08	$P = 0.017$
Total Omega- 3 fatty acids (n-3)		9.28 ± 2.70	16.79 ± 5.51	$P = 0.009$

Means with different superscripts in the same row differ significantly. Steatitis score: 1 + 2 = 0–50%; 3 + 4 = 50–100%.

The sharptooth catfish is a benthic opportunistic scavenger that is also known to hunt and prey actively on other fish (Skelton 2001). Food source varied distinctly between sampling sites, and prevalence of fish in the diet correlated with the presence of pansteatitis in free-living catfish from OG. Fish remnants observed in the stomach content of catfish from OG, often in an advanced stage of digestion, frequently consisted of bones and scales of noticeably large unidentified fish. Factors associated with a fish diet appeared to be associated with the development of pansteatitis in catfish in OG, but these must be distinct from a natural healthy fish diet as documented elsewhere in the literature (Spataru, Viveen & Gophen 1987).

An increase in dietary polyunsaturated fat intake has been reported to result in pansteatitis in various animals. A change in diet from smelt (6.7% fat) to mackerel (29.9% fat) was thought to be the precipitating cause of pansteatitis in captive American alligators (Wallach & Hoessle 1968), whereas a change from Baltic and Mediterranean clupeids to Moroccan Atlantic pilchards was suspected to have been the cause of pansteatitis in northern bluefin tuna (Roberts & Agius 2008). Salmonid diets high in fish oils were found to induce steatitis in channel catfish (Goodwin 2006), and in cats, feeding of an oil-rich fish-based diet similarly induced pansteatitis (Fytianou et al. 2006). As a consequence of raising the dam wall of Lake Massingir, a habitat change in the OG occurred that may have favoured a change in access to certain species of fish not normally consumed in large numbers by crocodiles and catfish. This may have exposed these animals to levels of polyunsaturated fatty acids in the diet to which they were not adapted. Tiger fish, Hydrocynus vittatus Castelnau, sampled from the OG did not

suffer from pansteatitis (D. Huchzermeyer, unpublished data). As obligate piscivores, tiger fish may have developed antioxidant protective mechanisms better enabling them to cope with the consumption of higher levels of dietary polyunsaturated fats than the omnivorous catfish.

The n-6 and n-3 fatty acids derived from linoleic and α-linolenic acids, respectively, are essential fatty acids that cannot be synthesized by animals (Steffens 1997). The relative abundance of these fatty acids in the diet of animals is reflected in the composition of their fat tissues (Hoffman & Prinsloo 1995; Steffens 1997). The fatty acid composition of marine fish oils, and in particular the high n-3 to n-6 ratio of polyunsaturated fatty acids contained in these oils, is a reflection of the fatty acid composition of marine phytoplankton (Steffens 1997). Whereas the ratio of total n-3 to n-6 fatty acids in marine fish oils typically lies between 5 and more than 10 that of freshwater fish is much lower ranging from 1 to 4 (Steffens 1997). In freshwater fish, as in marine fish, these fatty acid ratios are influenced by the composition of the diet. In nutrition trials, the n-3 to n-6 fatty acid ratio in muscle lipid of sharptooth catfish could be manipulated from 0.1 in fish on a sunflower oil diet to 1.8 in fish on a cod liver oil diet (Hoffman & Prinsloo 1995). The fat of captive-farmed crocodiles, receiving a diet of chicken, beef and horse meat, had an n-3 to n-6 fatty acid ratio of 0.08 (Osthoff *et al.* 2010). By contrast, the n-3 to n-6 ratio of fatty acids in the fat of wild crocodiles suffering from pansteatitis from the Olifants and lower Letaba Rivers was found to be 2 (Osthoff *et al.* 2010). This reflected a much higher intake of n-3 fatty acids by crocodiles in the Olifants Gorge. Mean ratios of n-3 to n-6 fatty acids in catfish with mild or no pansteatitis sampled from LK, RV and the OG in November 2009 were 0.8, 1.32 and 0.96, respectively (Table 1). There appeared to be no significant difference in n-3 to n-6 ratio of mesenteric fat between fish from Lunsklip Fisheries with varying degree of severity of pansteatitis. The fish with severe pansteatitis sampled from the OG, however, had an n-3 to n-6 fatty acid ratio of 2.87 compared to 0.92 in fish with only mild or no pansteatitis (Table 4). From these results, it can be inferred that pansteatitis in OG fish was caused by high intake of polyunsaturated fatty acids whereas rancidity rather than high polyunsaturated fatty acid intake was the cause of the pansteatitis

observed in catfish from LK. In the light of absence of observed fish mortality in the OG, it would seem unlikely that rancidity associated with intake of dead rotting fish could have been the cause of pansteatitis in the OG catfish and crocodiles.

A significant proportion of the essential fatty acids derived from the diet are stored in the adipose tissues of animals and of these, docosahexaenoic acid 22:6n-3 (DHA) is deposited into the adipose tissues preferentially over eicosapentaenoic acid 20:5n-3 (EPA) (Lin & Connor 1990). Although the polyunsaturated fatty acids are mobilized more rapidly from the adipose tissues than saturated fats, DHA, the most polyunsaturated fatty acid, has been shown to be poorly mobilized (Connor, Lin & Colvis 1996). The higher levels of DHA found in the mesenteric fat of catfish from the Olifants Gorge with pansteatitis (11.06%) compared with mesenteric fat of those without pansteatitis (5.09%) strongly points to a higher intake of DHA in the diet of those fish that developed pansteatitis at this site. A similar differentiation was not observed in the mesenteric fat of catfish with mild and severe pansteatitis from LK, supporting the argument for a different dietary aetiology, most likely associated with rancidity of fats in the slaughter house waste fed to these fish.

The inlet of Lake Massingir, which prior to 2007 lay in Mozambique beyond the OG, now extends into the OG in the KNP within the boundaries of South Africa, flooding the gorge where this river previously traversed the Lebombo Mountains as fast flowing rapids. Phytoplankton blooms have been observed near the inlet to Lake Massingir (D. Pienaar, SANParks, Skukuza, personal communication 2009). Phytoplankton naturally contain large quantities of α-linolenic acid and other n-3 polyunsaturated fatty acids, in particular EPA and DHA (Steffens 1997). It is proposed that by raising the dam of Lake Massingir, the resulting habitat change that occurred in the OG may have seasonally favoured access by crocodiles and catfish to phytoplankton-feeding fish. Of concern in this respect are silver carp, *Hypophthalmichthys molitrix* (Valenciennes), an invasive species outside of East Asia (Kolar *et al.* 2005) that were introduced into Mozambique and have also escaped into the Olifants River from South Africa and are known to occur in Lake Massingir (Skelton 2001). This fish is a specialized

plankton feeder that by preference feeds off phyto-plankton (Kolar *et al.* 2005) and is known to assimilate n-3 fatty acids (Buchtová & Jezek 2011). It is possible that crocodiles and catfish feed on silver carp when these seasonally migrate from the still waters of the lake into the Olifants River to spawn, and this may provide one explanation for intense intake of excessive polyunsaturated fats by catfish and crocodiles.

Conclusions

The results presented describe and compare the fatty acid composition and pathology found in the adipose tissues of healthy and pansteatitis-affected captive and wild sharptooth catfish. Data indicate possibly differing causes of the pansteatitis observed in the wild and captive fish. A classical nutritional cause, overfeeding of rancid fish waste, adequately explains the pansteatitis found in the captive population of LK fish. Observed fish kills have not been a consistent feature of the Olifants River Gorge, and the results thus strengthen the argument that causes other than consumption of dead fish may be involved in inciting pansteatitis in the OG fish. Similarities in changes in the adipose tissues of catfish and crocodiles inhabiting the same waters were observed. In the OG, the higher n-3 to n-6 fatty acid ratios in the fat of both catfish and crocodiles suffering from pansteatitis, compared with those of healthy catfish and crocodiles, point to an increased intake of polyunsaturated fats as a cause of the observed pansteatitis. The presence of alien invasive, phytoplankton-feeding, silver carp in Lake Massingir and the short seasonal upstream spawning migration of this species through the OG provide one plausible explanation for intense seasonal dietary exposure of catfish and crocodiles to levels of polyunsaturated fats to which they are not adapted. It is proposed that the habitat changes brought about by raising the dam wall of Lake Massingir in 2007 may have improved access of catfish and crocodiles to such fish within the OG, thereby precipitating the pansteatitis outbreaks in these animals.

Acknowledgements

References

Anon (2007) *NCSS, 2007. Statistical System for Windows*. NCSS Statistical Systems, Kaysville, Utah, USA.

Anon (2008) Mystery ailment kills crocs. News in perspective, 2008. *The New Scientist* **199**, 6.

Bonar C.J. & Wagner R.A. (2003) A third report of 'golf ball disease' in Amazon River dolphin (*Inia geoffrensis*) associated with *Streptococcus iniae*. *Journal of Wildlife Medicine* **34**, 296 –301.

Bricknell I.R., Bruno D.W., Bowden T.J. & Smith P. (1996) Fat cell necrosis syndrome in Atlantic halibut, *Hippoglossus hippoglossus* L. *Aquaculture* **144**, 65–69.

Buchtová H. & Jezek F. (2011) A new look at the assessment of silver carp (*Hypophthalmichthys molitrix* Val.) as a food fish. *Czech Journal of Food Science* **29**, 487–497.

Bus J.S., Aust S.D. & Gibson J.E. (1976) Paraquat toxicity: proposed mechanism of action involving lipid peroxidation. *Environmental Health Perspectives* **16**, 139–146.

Case L.P., Carey D.P. & Hirakawa D.A. (1995) Vitamin deficiencies and excesses. In: *Canine and Feline Nutrition. A Resource for Companion Animal Professionals* (ed. by L.P. Case, D.P. Carey & D.A. Hirakawa), pp. 303–308. Mosby-Year Book, St. Louis.

Connor W.E., Lin D.S. & Colvis C. (1996) Differential mobilization of fatty acids from adipose tissue. *Journal of Lipid Research* **37**, 290–298.

Farwer S.R., Haddeman E., Kivits G.A.A., Wiersma A., Danse B.H.J.C. & Derboer B.C.J. (1994) The vitamin E nutritional status of rats fed on diets high in fish oil or sunflower seed oil. *British Journal of Nutrition* **72**, 127–145.

Ferreira S.M. & Pienaar D. (2011) Degradation of the crocodile population of Kruger National Park, South Africa. *Aquatic Conservation and Marine and Freshwater Ecosystems* **21**, 155–164.

Folch J., Lees M. & Sloane-Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry* **226**, 497–509.

Fytianou A., Koutinas A.F., Saridomichelakis M.N. & Koutinas C.K. (2006) Blood α-tocopherol, selenium and glutathione peroxidase changes and adipose tissue fatty acid changes in kittens with experimental steatitis (yellow fat disease). *Biological Trace Element Research* **112**, 131–143.

Goodwin A.E. (2006) Steatitis, fin loss and skin ulcers of channel catfish, *Ictalurus punctatus* (Rafinesque), fingerlings fed salmonid diets. *Journal of Fish Diseases* **29**, 61–64.

Guarda F., Bertoja G., Zoccarato I., Tartari E. & Biolatti B. (1997) Spontaneous steatitis of epicardial fat in farmed white sturgeon (*Acipenser transmontanus*). *Aquaculture* **158**, 167–177.

Heath R., Coleman T. & Engelbrecht J. (2010) Water quality overview and literature review of the ecology of the Olifants River. Water Research Council Report No. TT452/10.

Herman R.L. & Kircheis F.W. (1985) Steatitis in Sunapee trout, *Salvelinus alpinus oquassa* Girard. *Journal of Fish Diseases* **8**, 237–239.

Hoffman L.C. & Prinsloo J.F. (1995) Genetic and nutritional influence on the total lipid fatty acid profile of *Clarias gariepinus* muscle. *Aquatic Living Resources* **8**, 415–421.

Huchzermeyer F.W. (2003) *Crocodiles. Biology, Husbandry and Diseases*. CABI Publishing, Wallingford, Cambridge, pp. 337.

Huchzermeyer K.D.A., Govender D., Pienaar D.J. & Deacon A.R. (2011) Steatitis in sharptooth catfish, *Clarias gariepinus* (Burchell), in the Olifants and Lower Letaba Rivers in the Kruger National Park, South Africa. *Journal of Fish Diseases* **34**, 489–498.

Jones D., Howard N. & Gresham G.A. (1969) Aetiology of 'yellow fat' disease (pansteatitis) in the wild rabbit. *Journal of Comparative Pathology* **79**, 329–334.

Juan-Sallés E., Prats N., Resendes A., Domingo M., Hilton D., Ruiz J.M., Garner M.M., Valls X., Marco A.J. (2003)

Anemia, myopathy, and pansteatitis in vitamin E–deficient captive marmosets (*Callithrix* spp.). *Veterinary Pathology* **40**, 540–547.

Kolar C.S., Chapman D.C., Courtenay W.R. Jr, Housel C.M., Williams J.D. & Jennings D.P. (2005) Asian carps of the genus *Hypophthalmychthys* (Pisces, Cyprinidae) – A biological synopsis and environmental risk assessment. Report to US Fish and Wildlife Service per Interagency Agreement 94400-3-0128, pp. 175.

Kwetegyeka J., Masa J., Kiremire B.T., Mpango G.B. & Grahl-Nielsen O. (2011) Fatty acids of polar lipids in heart tissue are good taxonomic markers for tropical African freshwater fish. *African Journal of Aquatic Science* **36**, 115–127.

Lin D.S. & Connor W.E. (1990) Are the n-3 fatty acids from dietary fish oil deposited in the triglyceride stores of adipose tissue? *American Journal of Clinical Nutrition* **51**, 535–539.

Niza N.M.R.E., Vilela C.L. & Ferreira L.M.A. (2003) Feline pansteatitis revisited: hazards of unbalanced home-made diets. *Journal of Feline Medicine and Surgery* **5**, 271–277.

Osthoff G., Hugo A., Bouwman H., Buss P., Govender D., Joubert C.C. & Swarts J.C. (2010) Comparison of the lipid properties of captive, healthy wild, and pansteatitis affected wild Nile crocodiles (*Crocodylus niloticus*). *Comparative Biochemistry and Physiology A* **155**, 64–69. (Imp 1.56)

Park P.W. & Goins R.E. (1994) *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science* **59**, 1262–1266.

Pollock C.G., Sleeman J.M., Houle C.D. & Ramsay E.C. (1999) Vitamin E deficiency and pansteatitis in juvenile boat-billed herons (*Cochlearius cochlearius*). *Journal of Zoo and Wildlife Medicine* **30**, 297–300.

Roberts R.J. & Agius C. (2008) Pansteatitis in farmed northern bluefin tuna, *Thunnus thynnus* (L.), in the eastern Adriatic. *Journal of Fish Diseases* **31**, 83–88.

Roberts R.J., Richards R.H. & Bullock A.M. (1979) Pansteatitis in rainbow trout *Salmo gairdneri* Richardson: a clinical and histopathological study. *Journal of Fish Diseases* **2**, 85–92.

Scott D.W., Miller W.H. Jr & Griffin C.E. (1995) Nutritional skin diseases. In: *Muller and Kirk's Small Animal Dermatology*, 5th edn (ed. by D.W. Scott, W.H. Miller & C.E. Griffin), pp. 890–901. W.B. Saunders, Philadelphia.

Sinclair A.J. & O'Dea K. (1987) The lipid levels and fatty acid compositions of the lean portions of Australian beef and lamb. *Food Technology in Australia* **39**, 228–231.

Skelton P. (2001) *A Complete Guide to the Freshwater Fishes of Southern Africa*. Struik Publishers, Cape Town, pp. 395.

Spataru P., Viveen W.J.A.R. & Gophen M. (1987) Food composition of *Clarias gariepinus* (= *C. lazera*) (Cypriniformes, Clariidae) in Lake Kinneret (Israel). *Hydrobiologica* **144**, 77–82.

Steffens W. (1997) Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. *Aquaculture* **151**, 97–l 19.

Wallach J.D. & Hoessle C. (1968) Steatitis in captive crocodilians. *Journal of the American Veterinary Medical Association* **153**, 845–847.

Weyl O.L.F. & Booth A.J. (2008) Validation of annulus formation in otoliths of a temperate population of adult African sharptooth catfish *Clarias gariepinus* using fluorochrome marking of wild fish. *Journal of Fish Biology* **73**, 1033–1038.

Wong E., Mikaelian I., Desnoyers M. & Fitzgerald G. (1999) Pansteatitis in a free-ranging red-tailed hawk (*Buteo jamaicensis*). *Journal of Zoo and Wildlife Medicine* **30**, 584–586.