Infuluence of Dietary Oils and Cold Storage Conditions on Oxidative Stability of Pork

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Abstract: The effects of dietary oils and varying cold storage conditions on lipid oxidation in pork were evaluated using 24 finishing pigs (crosses of Large White and Duroc) with an average initial live weight of 54.68 ± 3.12 kg. Pigs were group penned (3 boars and 3 gilts) according to dietary oil treatments but individually fed for 5 weeks. Palm kernel oil (PKO), 0.5:0.5 w/w coconut and palm oils (CPO), palm oil (PMO) and soyabean oil (SBO) were incorporated at 5% into an on-farm diet (based on unconventional feed ingredients, 0.5:0.5 w/w palm kernel cake and rice bran plus 0.5% salt). The resultant diet contained 14.21% crude protein and about 13.05 MJ/kg digestible energy. The backfat thickness (P2) of carcass was measured. Muscle samples were subjected to consistent refrigeration (4°C) and inconsistent refrigeration (simulating power outage) for up to 9 days, and consistent frozen storage (-18°C) and inconsistent frozen storage (thawing once, twice and thrice) for up to 60 days. Oxidation in muscle tissues was also induced by heat. Iodine values of feed and muscle lipids, melting point of subcutaneous fat, moisture, lipid contents and extent of oxidation of muscle tissues were determined. The P2 (10.57 \pm 1.71- 12.00 ± 1.41 mm), moisture (69.73 ± 1.72 - 71.97 $\pm 0.80\%$) and lipid (10.23 ± 0.85 -11.05 $\pm 1.01\%$) contents of muscles were not significantly influenced (P>0.05) by dietary treatments. Levels of unsaturation, measured as iodine values were 41.42 \pm 1.16, 45.98 \pm 0.78, 53.70 \pm 1.42 and 78.26 \pm 2.01 (P<0.001) for dietary lipids and 17.96 ± 0.25 , 22.01 ± 0.27 , 23.00 ± 0.22 and 26.44 ± 0.20 (P<0.001) for muscle lipids, for PKO, CPO, PMO and SBO based diets, respectively. Subcutaneous fat melting point decreased with increased unsaturation of dietary lipids (28.23 ± 1.92 , 26.50 ± 0.68 , 25.85 ± 1.32 and 23.08 ± 1.36 , P<0.01 for pigs fed PKO, CPO, PMO and SBO based diets respectively). Muscle tissues from pigs fed PKO diet was most stable to lipid oxidation during refrigerated and frozen storage as well as heat-induced lipid oxidation. This was followed by those from pigs fed CPO and then PMO diets (P<0.05). Muscle tissues from pigs fed SBO diet was most susceptible to lipid peroxidation. Thiobarbituric acid reactive substances (TBARS) contents of muscle lipids increased with increasing length of refrigerated storage irrespective of dietary treatments. TBARS concentrations of muscles stored under consistent refrigeration and frozen storage conditions were lower than those stored under inconsistent conditions. TBARS contents of muscle tissues that were frozen consistently and those thawed once were not significantly different (P>0.05) and were significantly lower (P<0.05) than those thawed twice and thrice. Oxidative susceptibility of muscle tissues increased with increasing unsaturation of muscle lipids (muscle from SBO > PMO > CPO > PKO diets) following heat-induced lipid oxidation.

Key words: Dietary oils, pork, cold storage conditions, lipid oxidation

Introduction

Pork, like other red meat is an excellent source of high quality protein, minerals and the B-series of vitamins. Intensive pig breeding programmes, provision of balanced diets and dietary manipulation of fatty acid composition of pig tissues are practices in developed nations that have led to the improvement of pig performance, development and production of leaner pig breeds and "customised" pork and pork products. In Nigeria, like many developing nations of the world, the economic recession has adversely affected pig production in terms of breed improvement and nutrition. Intensively reared commercial pigs are fed various types of conventional and unconventional feedstuffs, either solely or in combinations, depending on cost and availability. These feedstuffs include bakery wastes, kitchen wastes especially from schools and hotels, swills from vegetable oil processing plants, palm kernel cake, soyabean meal containing variable amount of residual oils, brewers grain, rice bran and cassava peels. Little or no consideration is given to the effect of these feedstuffs, especially the nature of lipids they contain on meat quality and storability. Generally, pigs have more potential to lay down intra- and extra-muscular fat than cattle, sheep, goats and chickens, and fats and oils have had a long history of very bad public image. For example, apart from religious taboos, high lipid content of pork affects its demand especially among the rich class of Nigerian populace.

Various strategies have been advanced in order to achieve a healthy pattern of lipid intake: a reduction in total lipid intake, a low level of saturated fatty acids (SFA) consumption or an increase in intake of unsaturated fatty acids (UFA). High intake of SFA have been related to many human health problems such as obesity, cancers and cardiovascular diseases (CVD) (Neuringer et al., 1984). On the other hand, many studies have shown the

importance of UFA especially polyunsaturated fatty acids (PUFA) as components of a healthy human diet (Onibi, 1997). UFA are important in membrane structure and function, prevention of CVD, hypertension, diabetes, cancer, and inflammatory and autoimmune disorders (Holman et al., 1982; Neuringer et al., 1984 and Yamamoto et al., 1988). For these reasons, dieticians have considered not only the quality of fat or oil, but also the proportions of saturated, monounsaturated, polyunsaturated, total n-6 and n-3 fatty acids in human diets. Pigs and chickens are able to incorporate dietary fatty acids directly into adipose and muscle tissue lipids (Onibi, 1997) and this may be a strategy for increasing human intake of UFA through consumption of UFA-rich meat. Also, the demand for pork may be increased if it is rich in UFA that are of health-benefit to humans. Wiseman and Agunbiade (1998) reported that major changes in fatty acid composition of pig tissues occur within 2 weeks following dietary fat change. The oils incorporated in the present study were palm kernel (85% saturated; Onibi, 1997), coconut (91.2% saturated; Martin et al., 1981), palm oil (58.5% saturated; Madsen et al., 1991) and soyabean (16% saturated; Onibi, 1997). In Nigeria, meat storage either by commercial processors, retailers or household consumers is faced with sporadic electricity supply failure of short- and long-term duration. Most vehicles used in meat transportation too are not refrigerated. Oxidative deterioration of meat occurs during cold storage but the rate is influenced by the cold storage conditions (Onibi, 2001a) and the degree of saturation of tissue lipids (Hamilton, 1989; Onibi and Scaife, 2002). Loss of fresh colour, development of off-flavours, formation of potentially harmful lipid oxidation products and increased exudative loss have been associated with the oxidation of meat lipids (Buckley et al., 1995 and Onibi et al., 2000a). Lipid oxidation products; malonaldehyde (MDA) and cholesterol oxides, have respectively been shown to be carcinogenic (Shamberger et al., 1974) and initiators of atherosclerosis lesions in blood vessels (Peng et al., 1987).

This study was conducted, using Nigeria's situation, to examine the effects of dietary oil types on saturation of lipids in porcine tissues and erratic electricity supply on lipid oxidation in pork during refrigerated and frozen storage.

Materials and Methods

The feeding trial was carried out on-farm, at a medium-scale commercial piggery in Akure, Nigeria. Twenty-four finishing pigs (Large White x Duroc breed) with an average initial live weight of 54.68 ± 3.12 kg (range of 50-57 kg) were used. The pigs were group-penned (3 boars and 3 gilts) according to dietary oil treatments and individually fed to satiety twice daily (8.30-9.30 am and 4.00-5.00 pm) for 5 weeks. Pigs on each treatment were balanced for initial weight and water was provided ad libitum.

Diets: The basal diet was based on the unconventional feed ingredient mixture fed at the commercial piggery (mixture of palm kernel cake and rice bran at 0.5:0.5 w/w with 5% salt). Palm kernel oil (PKO), 0.5:0.5 w/w coconut and palm oils (CPO), palm oil (PMO) and soyabean oil (SBO) was incorporated into the basal feed. The resultant diet contained 14.21% crude protein and about 13.05 MJ/kg digestible energy. Feeds for a maximum of 2 days were mixed at once to avoid rancidity of oil in feed.

Slaughtering of Pigs and Sample Collection: Pigs were killed by electrical stunning and exsanguinations, following overnight feed deprivation. Scalding, dehairing and evisceration were carried out manually. The hot carcass was split into two halves by cutting through the vertebral column. The backfat thickness (P²) was taken at the level of the last rib from the two halves with a NASCO Swine Backfat guage (Fort Atkinson, Wisconsin). From each pig slaughtered, 1 kg muscle tissue from the loin area of each split carcass (2 kg from each whole carcass) and some quantity of subcutaneous (s/c) adipose tissue from this area were collected for laboratory analysis. The s/c fat was frozen prior to determination of melting point..

Meat Storage Conditions: Muscle tissue from the loin area of each carcass was cut to 11 chops of approximately 170g each and separately wrapped in cellophane bags. The samples were treated as follows:

- i One chop was frozen after 3-hour post slaughter chilling.
- ii Three chops were refrigerated consistently for 3, 6 and 9 days.
- Three chops were refrigerated for 3, 6 and 9 days with the refrigerator turned off for 8 hours daily. This represented inconsistent refrigeration condition simulating power outage.
- iv One chop was frozen consistently for 60 days after initial 3-hour post slaughter chilling.
- Three chops were frozen for 60 days after initial 3-hour post slaughter chilling. One chop was thawed once (on 30th day), the second chop was thawed twice (on 20th and 40th days) and the third chop was thawed thrice (on 15th, 30th and 45th days) during frozen storage. The thawing and re-freezing represented inconsistent freezing condition simulating thawing that could occur during prolonged power outage or transportation.

Separate refrigerators were used for samples kept under consistent and inconsistent refrigeration conditions. A

back-up power generator was used to compliment power supply from National electricity grid. Refrigeration and freezing temperatures were set at 4 and -18°C, respectively and these were monitored with a digital thermometer. Temperature change in refrigerator turned off for 8 hours daily averaged 8°C in 2 hours, 13°C in 4 hours, 17°C in 6 hours and 20°C in 8 hours. Thawing of samples under inconsistent freezing conditions was accomplished by keeping frozen samples in a refrigerator for 24 hours. Muscle samples from the same dietary oil treatment and storage conditions were pooled together for laboratory analysis.

Laboratory Analysis: Moisture and lipid contents of muscle samples were determination as described by AOAC (1990). Accessibility to gas-liquid chromatographic equipment was difficult hence level of saturation of lipids in diets and muscle samples was based on quantification of the iodine value using Pearson (1976) procedure after oil extraction with chloroform-methanol (Christie, 1982). The melting point of subcutaneous fat was determined as outlined by Cocks and Rede (1966). Heat-induced (at 40oC) lipid peroxidation of muscle was carried out using the procedure described by Frigg *et al.* (1990) but modified to incorporate incubation period of 0, 60 and 120 min. Extraction and assay procedures for measuring of extent of oxidation were based on the aqueous extraction 2-thiobarbituric acid method described by Pikul *et al.* (1989). Results of the thiobarbituric acid reactive substances (TBARS) were expressed as mg MDA/kg muscle.

Statistical Analysis: Data were subjected to one-way analysis of variance and factorial analysis where appropriate, using the Minitab Statistical Package (v. 10.2, Minitab Inc., P.A., USA). Where significant treatment effects were detected, means were separated using Duncan's new multiple range test (Steel and Torrie, 1980). Graphs were drawn using Microsoft Excel 2000 (Microsoft Corporation).

Results and Discussion

General observation: All the pigs remained apparently healthy throughout the experimental period and no death was recorded. The on-farm basal diet was not improved in term of nutritional value because the objective of the study was the evaluation of the effect of the dietary oils on pork quality.

Carcass P^2 , Moisture and Lipid Contents of Muscle Tissues: The carcass P^2 generally indicated no significant differences (P>0.05) between dietary treatments (Table 1). This showed that the nutritional value of the diets, especially energy and protein contents across treatments were similar as had been intended. The P2 of between 10.75 ± 1.71 and 12.00 ± 1.41 mm for pigs obtained in this study was in agreement with the results of Onibi *et al.* (1998) for pigs of similar live weight. Results of the moisture and lipid contents of muscle tissue (Table 1) revealed no significant effect (P>0.05) due to dietary treatments. This was not unexpected as the diets were similar in composition except for dietary oil types. The moisture content of muscle tissues fell within the range of 65 and 76% reported for meat by Adrian *et al.* (1982) and Ikeme (1990). Muscle lipid content (range, 10.23 \pm 1.01%) was similar to 10.68% reported by Onibi (2003) for muscle tissue from exotic pigs in Nigeria.

Saturation of Dietary and Muscle Tissue Lipids and Melting point of Subcutaneous Fat: Table 2 shows the results of saturation of dietary and muscle tissue lipids: the lower the iodine value, the higher the saturation of the lipids. Iodine values of the diets were in the order of PKO < CPO < PMO < SBO based diets. Similarly, iodine values of the muscle lipids were in the same order and were 17.80 \pm 0.25, 22.01 \pm 0.27, 23.00 \pm 0.22 and 26.44 \pm 0.20 (P<0.001) for PKO, CPO, PMO and SBO based diets, respectively. Thus, with increased degree of unsaturation of lipids in the diets, the level of unsaturation of the muscle lipids also increased. These results corroborate that of Madsen *et al.* (1991) and Onibi *et al.* (1998, 2000b) which reported direct relationship between the fatty acid

Table 1:Carcass P2, moisture and lipid content of muscle tissue of pigs fed diet containing different oils

Sources of dietary oils	Carcass P ² (mm)	Muscle tissue (% fresh muscle)	
		Moisture	Lipid
PKO	11.00 ± 1.16	69.73 ± 1.72	10.23 ± 0.85
CPO	10.57 ± 1.71	69.84 ± 1.52	11.05 ± 1.01
PMO	12.00 ± 1.41	71.97 ± 0.80	10.29 ± 0.53
SBO	11.25 ± 1.95	70.61 ± 0.77	10.60 ± 0.78
Statistical significance	NS	NS	NS

Mean \pm SD, NS = not significant (P>0.05)

N=6 for carcass P^2 (representing 6 pigs/dietary treatment), 4 for moisture and lipid contents of muscle (representing quadruplicate analysis of pooled samples on the same treatment).

Table 2: Saturation of dietary and muscle tissue lipids, and melting point of subcutaneous fat of pigs fed diet containing different oils

Sources of dietary oils	Lipid saturation		
	lodine value of dietary lipids	lodine value of muscle tissue lipids	Melting point (°C) of subcutaneous fat
PKO	41.42 ± 1.16	17.96 ± 0.25°	28.23 ± 1.92°
CPO	45.98 ± 0.78	22.01 ± 0.27 ^b	26.50 ± 0.68^{ab}
PMO	53.70 ± 1.42	$23.00 \pm 0.22^{\circ}$	25.85 ± 1.32 ^b
SBO	78.26 ± 2.01	24.44 ± 0.20^{d}	$23.08 \pm 1.36^{\circ}$
Statistical significance	* * *	* * *	**

Mean \pm SD, NS = not significant (P>0.05), ** = P<0.01; *** = P<0.001

N = 3 for iodine value of dietary and muscle tissue lipids (representing triplicate analysis of pooled samples) and 6 for s/c fat melting point (representing 6 pigs/dietary treatment).

Means with different superscripts within the same column are significantly different (P<0.05).

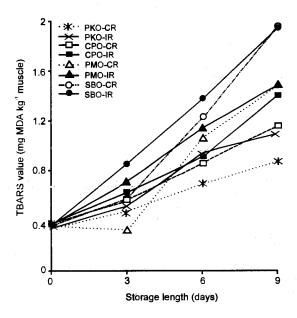


Fig. 1: Oxidative stability of pork from pigs fed different oils during refrigerated storage at 4°C for 9 days (PKO = Palm kernel oil diet, CPO = Coconut/palm oil diet, PMO = Palm oil diet, SBO = Soyabean oil diet, CR = Consistent refrigeration and IR = Inconsistent refrigeration).

composition of deposited fat and amount of UFA in the diet of pigs. Similarly, Pfalzgraf et al. (1995) reported higher unsaturated fatty acid profile in pigs fed soyabean oil than those fed tallow, and Wiseman and Agunbiade (1998) for shoulder fat of pigs fed rapeseed and soyabean oils compared with tallow. Results similar to those of pigs were also reported for chickens by Yau et al. (1991) and Scaife et al. (1994).

The melting point of the depot fat was significantly (P < 0.01) influenced by dietary treatments (Table 2). The melting point of s/c fat from pigs fed PKO based diet ($28.23 \pm 1.92\,^{\circ}$ C) was significantly higher (P < 0.05) than those from pigs on PMO and SBO diets (25.85 ± 1.32 and $23.08 \pm 1.36\,^{\circ}$ C respectively) but not significantly different (P > 0.05) from s/c fat from pigs fed CPO diet ($26.50 \pm 0.68\,^{\circ}$ C). The inclusion of soyabean oil in SBO diet reduced the melting point of s/c fat of pigs fed the diet. Soyabean oil is a rich source of UFA (84%; Onibi, 1997) and this may be attributed to the reduction in melting point of the fat. The CPO diet was not as saturated as the PKO diet and this reflected in lower melting point of s/c fat of pigs fed the former diet. Similar results showing decrease in melting point of s/c fat with increased unsaturation of dietary lipids was reported by Onibi and Scaife (2002).

Oxidative Stability of Pork During Storage

Lipid Oxidation in Muscle Tissue during Refrigeration: Data for oxidative stability of muscle tissue stored at 4°C for up to 9 days were analysed using 4 x 2 factorial analysis (4 dietary oil sources by 2 refrigeration conditions) and are depicted in Fig. 1. There was no significant effect (P>0.05) of dietary treatments on muscle lipid oxidation

at day 0 of storage for both consistent (CR) and inconsistent (IR) storage conditions. At 3 days and beyond, oxidation of lipids in meat was significantly affected (P<0.001) by dietary treatment. Generally, TBARS concentrations in the muscle samples were in order of PKO < CPO < PMO < SBO based diets. Thus, oxidative stability of muscle lipids decreased with increased unsaturation of oils incorporated in the diets. This is in consonant with Monahan et al. (1992) and Pfalzgraf et al. (1995) who reported that meat from pigs fed soyabean oil was more susceptible to lipid oxidation than those from pigs fed beef tallow. Similarly, Lin et al. (1989) reported that meat from broilers fed olive oil was more stable to oxidation than those fed linseed oil after storage at 4oC for 6 days. Muscle lipid oxidation significantly increased (P<0.001) with increased length of refrigerated storage irrespective of dietary treatment or storage conditions. The pattern of lipid oxidation in the muscle tissue stored at 4oC was similar to those reported by Monahan et al. (1992) and Huang and Miller (1993) in pork, lamb and poultry meat showing that TBARS contents increased during storage irrespective of dietary constituent. TBARS concentrations of muscles stored under consistent refrigeration conditions (CR) were lower than those stored under inconsistent refrigeration conditions (IR). On day 9 of refrigeration, there was no significant effect (P>0.05) of refrigeration conditions on oxidation of muscle lipids. This may be attributed to the putrefaction of the meat at this period. The effect of power outage on oxidation of lipids in pork is yet to be documented, probably as a result of uninterrupted supply of electricity in industrialised nations where pork is stored en masse under consistent refrigeration. It was however observed that TBARS concentration was lower in meat stored under the consistent storage condition than those stored under the inconsistent condition. Recently, Onibi (2001b) reported similar result for broiler-chickens. In all the storage periods, there was no significant effect (P>0.05) of interaction between diet and refrigeration condition. This showed that there was a similar oxidative effect of refrigeration condition on the muscle samples irrespective of dietary treatments.

Oxidative Stability of Muscle Tissue during Frozen Storage: Data for oxidative stability of muscle tissue following thawing and refreezing were analysed using one-way analysis of variance and are depicted in Fig. 2. The TBARS concentration of muscle samples under consistent frozen condition for 60 days and those thawed once during this period were not significantly affected (P > 0.05) by dietary treatments. When the pork samples were thawed twice and thrice, there were significant effects (P < 0.01) of dietary treatments on TBARS concentration. Thawing twice resulted in significantly different (P < 0.05) TBARS concentration of muscle from pigs fed PKO diet, and PMO and SBO diets but not of those fed CPO diet. TBARS concentration of muscle from pigs fed CPO, PMO and SBO diets were not significantly different (P > 0.05). Generally, muscle TBARS concentrations (hence oxidative susceptibility) were in order of SBO > PMO > CPO > PKO. This is a reflection of unsaturation of the dietary oils. This is in agreement with the reports of Hamilton (1989) and Onibi *et al.* (1998) that oxidative deterioration of meat occurs

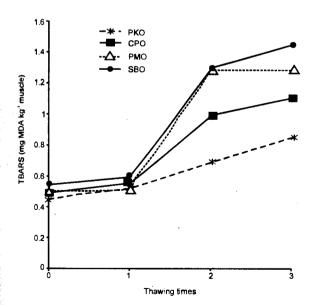


Fig. 2: Oxidative stability of pork from pigs fed different oils during frozen storage at -18°C for 60 days (PKO=Palm kernel oil diet, CPO=Coconut/palm oil diet, PMO=Palm oil diet, SBO=Soyabean oil diet).

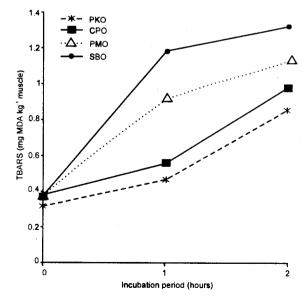


Fig. 3: Oxidative stability of pork from pigs fed different oils following heat-induced oxidation at 40°C for 2 hours (PKO = Palm kernel oil diet, CPO = Coconut/palm oil diet, PMO = Palm oil diet, SBO = Soyabean oil diet)

during refrigerated and frozen storage, but the rate increased with increase in the degree of tissue lipid unsaturation. Storage of the meat under consistent frozen condition produced no significant difference (P>0.05) compared with thawing once across all dietary treatments. However, thawing twice and thrice significantly increased (P<0.05) TBARS concentration. The increase in TBARS concentration as a result of thawing and refreezing of muscle samples showed the effect that epileptic electricity supply could have on meat storage quality.

Oxidative Stability of Muscle Tissue Following Heat-induced Lipid Peroxidation: Results of the oxidative stability of muscle samples when lipid oxidation was induced by heat are shown in Fig. 3, following a one-way analysis of variance. The TBARS concentrations of the unincubated sample (0 hr. of incubation) were not significantly different (P > 0.05) $(0.32 \pm 0.04, 0.38 \pm 0.10, 0.39 \pm 0.08$ and 0.38 ± 0.08 mg MDA/kg muscle for muscle samples from pigs fed PKO, CPO, PMO and SBO diets respectively). At 1 hour of incubation, there were significant differences (P<0.001) in TBARS values due to dietary treatments. Lipid oxidation in muscle samples from pigs fed PKO and CPO diets were significantly different (P<0.05) from those from pigs fed PMO and SBO diets. At 2 hours of incubation, lipid oxidation in muscle samples due to dietary treatment was also significantly influenced (P<0.05). As expected, TBARS concentration of muscle samples (hence oxidative susceptibility) were in order: SBO > PMO > CPO > PKO diets at 1 and 2 hours of incubation. TBARS concentrations significantly increased (P<0.001) with increased length of incubation, irrespective of dietary treatments. The progressive increase in TBARS concentration through 0 to 2 hr. of incubation observed in this study is similar to that reported by Dawson and Gartner (1983) that heat disrupts muscle membranes and break lipoprotein complexes, thereby increase exposure of tissue lipids to attack by oxygen. Also, iron which act as catalyst, is freed from haem pigments of muscle during heating, making them readily susceptible to oxidation (Apte and Morrisey, 1987a,b). Similarly, TBARS content of cooked meat was reported by Monahan et al. (1992), Huang and Miller (1993) and Russell et al. (2003) to be higher than those of uncooked meat. When cooking time was short, Allen and Foegeding (1981) reported that lipid oxidation was minimal.

Conclusion

This study showed that the type of dietary oils did not influence the carcass P², muscle moisture and lipid contents of pigs. This finding suggests a degree of flexibility for farmers in the use of different oils in the diets fed to pigs without adversely affecting the quantity of deposited oils in the muscle. The dietary oil type affects the level of saturation of muscle tissue. This may be valuable in increasing human dietary intake of UFA from pork especially if the oil fed to the pigs is rich in PUFA. However, as the UFA content of the muscle increased, susceptibility of the muscle to lipid oxidation during refrigerated and frozen storage also increased. Lipid oxidation was lower in pork from pigs fed the more saturated oils. Oxidation of muscle lipids also increased with increasing storage length irrespective of the type of oil incorporated in the diets. Therefore, when "customised pork" which is rich in UFA is produced, lipids in the pork should be protected against oxidation by the strategic use of antioxidants in the feed of pigs fed diets rich in PUFA as recommended by Onibi *et al.* (1998, 2000a,b). This would improve meat quality by extending shelf life of pork through reduction in susceptibility to lipid peroxidation.

The influence of power outage on pork during storage showed increased predisposition of pork to lipid oxidation. This shortens the shelf life of the meat and may result in the attendant problems of early development of bad odour, off-flavour and production of toxic oxidation products. The practice of prolonged display of meat on counter in open market should be discouraged, as the resultant elevated temperature is a prooxidant. Individual meat seller should be made to use insulated containers containing ice blocks or flakes. This practice will keep the temperature of retailed meat low thereby reducing the rate of lipid oxidation. Since the electricity supply from the national grid in Nigeria is still not perfectly reliable, owners of meat shops should be made to install back-up power generators in their premises. There should also be responsible health/meat inspectors to ensure strict compliance with standard cold storage display of meat.

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