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EFFECT OF INDIGENOUS MICRO-ORGANISM TREATMENT OF DEEP LITTER FLOOR ON NUTRIENT CONTENT OF PORK

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ABSTRACT

Pork meat consumption and its products are constrained by high fat content, particularly high composition of saturated fatty acids. The objective of this study was to investigate the effect of indigenous micro-organism (IMO) treatment of deep litter floor, on nutrient content in pork of pigs raised on IMO treated and untreated deep litter floor. Twenty four three-months old pigs (Large White x Landrace) were raised on deep litter floor; one floor type treated with IMO solution and the control not treated. Significant ($P<0.05$) differences were observed in the amount of saturated, mono-unsaturated, poly-unsaturated, cis, trans and omega-6, 7 and 9 fatty acids in pork of pigs raised on IMO treated and untreated deep litter floor; with more unsaturated fatty acids in pork of pigs raised on deep litter floor without IMO treatment (63%) than pigs kept on deep litter floor with IMO (37%). Therefore, deep litter floor treatment with IMO solution does not affect nutrient contents in pork, but enhances the composition of unsaturated fatty acids. Further research should be conducted on the mechanisms by which deep litter floor whether treated or untreated with IMO solution modifies fatty acids composition in pork.

Key Words: Saturated fatty acids, unsaturated fatty acids

RÉSUMÉ

La consommation de viande de porc et de ses produits est contrainte par une teneur élevée en matières grasses, en particulier une composition élevée d'acides gras saturés. L'objectif de cette étude était d'étudier l'effet du traitement par micro-organismes indigènes (OMI) du planche de litière profonde, sur la teneur en éléments nutritifs du porc de porcs élevés sur un planche de litière profonde traité et non traité par l'OMI. Vingt-quatre porcs de trois mois (Large White x Landrace) ont été élevés sur une litière profonde; un type de planche traité avec une solution IMO et le témoin non traité. Des différences significatives ($P <0,05$) ont été observées dans la quantité d'acides gras saturés, mono-insaturés, poly-insaturés, cis, trans et oméga-6, 7 et 9 dans le porc de porcs élevés sur un planche de litière profonde traitée et non traitée par l'OMI; avec plus d'acides gras insaturés dans le porc de porcs

élevés sur litière profonde sans traitement OMI (63%) que les porcs élevés sur litière profonde avec OMI (37%). Par conséquent, le traitement de planche profond avec une solution IMO n'affecte pas la teneur en nutriments du porc, mais améliore la composition des acides gras insaturés. Des recherches supplémentaires devraient être menées sur les mécanismes par lesquels la litière profonde, qu'elle soit traitée ou non avec une solution IMO, modifie la composition des acides gras dans le porc.

Mots Clés: Acides gras saturés, acides gras insaturés

INTRODUCTION

Pig (*Sus scrofa domesticus*) rearing has the potential for raising incomes of smallholder farmers, especially women and increasingly contributes to improved nutrition and household livelihoods in sub-Sahara Africa (SSA), where pork consumption and pig keeping are culturally acceptable (Ouma *et al*., 2013). Pork consumption in the SSA is still low, but growing at the rate of 1.85 kg/person/year, compared to the global average of 15.05 kg/person/year (Narrod *et al.,* 2011).This growing demand for pork on the continent presents unique business opportunities for pig farmers (Pica and Otte, 2009; FAO, 2011).

Despite the growing demand for pork in the SSA, pork consumption is still constrained by high fat content, with higher composition of saturated fatty acids (Woods and Fearon, 2009). Saturated fatty acids cannot easily be utilised by the body and it is known to elevate blood cholesterol concentrations; thus increasing the risk and prevalence of coronary heart diseases in humans (Dugan *et al.,* 2015). Consuming high amounts of saturated fats found in animal products increases the level of bad low-density lipo protein cholesterol (LDL cholesterol) in the blood which increase the risk of cardio vascular disease or coronary heart diseases (Bellows and Moore, 2013). Nutritionists advocate for decrease in total fat intake, especially saturated fatty acids and trans-fatty acids which are associated with increased risk of cardio vascular disease and some cancers (Mapiye *et al*., 2011; Iqbal, 2014).

Daily application of indigenous micro-organism (IMO) solution on deep litter floors in pig houses has been suggested to improve pig production, and produce lean pork with low fat content. In fact, this is increasingly being practiced in Uganda by smallholder farmers (Nistor *et al.,* 2012; Ndyomugyenyi and Kyasimire, 2015). The objective of this study was to assess nutrient profiles in pork of pigs raised on IMO treated deep litter floor.

MATERIALS AND METHODS

Study area. This study was carried out in Paicho and Koro sub-counties in Gulu and Omoro Districts of Northern Uganda for duration of three months (90 days). These districts were selected because they have the highest population of pig farmers in the region (MAAIF, 2008). The districts are located between longitudes 30° 21' East to longitude 32° East and latitude 2° North to latitude 4° North. The districts receive an average rainfall of 1,500 mm per *annum* with a monthly average ranging between 14 mm in January and 230 mm in August. The wet season normally extends to October with the highest rainfall in May, August and October (Ikwap *et al.,* 2014; Nabukenya *et al.,* 2014).

Treatments and design. Three deep litter floor units with IMO treatment (experimental units) and the other three deep litter floor units without IMO treatment (control) were used in this study. A completely randomised block design (CRBD) was used, whereby twenty four three-months old healthy crosses of Large White and Landrace pigs of mixed sex, were purchased from the existing pig farmers within the two districts. The pigs were randomly distributed into six piggery units within the

treatments. The beddings in all the units were laid systematically in layers on bare soil ground, with charcoal as the first layer; followed by tree shoots, maize stalks, dry red soil mixed with lime and wood shavings. Equal IMO solution amounts were applied on a daily basis in all the experimental units. Restricted feeding method was used and all pigs were fed on growers' mash consisting of maize bran (74 kg), fish meal (5 kg), soybean meal (10 kg), sunflower cake (5 kg), lake shells (4 kg), bone ash (1 kg), salt (0.5 kg) and premix (0.5 kg). In general, the nutrient composition of the feed contained– energy (2971 kcal kg^{-1}), crude protein (13.6%), lysine (0.957%), methionine + cysteine (0.685%), fats (5.1%), crude fibre (6.3%), calcium (1.97%) and phosphorus (0.74%).

Pork nutrient contents. One gilt (young female pig before first service) was randomly selected at the end of the study (three months) from each of the six units, and slaughtered in a humane manner by stunning using mechanical instrument (Council of Europe, 1991). The pork quality attributes measured included fatty acid profile, and the degree of saturated and unsaturated fats. Health examination was done on all the pigs in both IMO treated and untreated deep litter floor units, to identify possible signs of infection before being selected for slaughter. In addition, post-mortem meat inspection was done on all the carcasses by a qualified Veterinarian after slaughtering, before picking pork samples for laboratory analysis.

Chemical analysis of nutrients. Fat quality, which was fatty acids profiling for the degree of saturated fatty acids (SFAs) and unsaturated fatty acids (USFAs), was determined using gas chromatography (Kang and Wang, 2005). Approximately, 100 g of pork sample was removed from various parts of the carcass (ham, loin, shoulder and belly) and placed in a cooler box, vacuum sealed, stored at approximately 4 °C and transported to the laboratory for analysis. A fresh pork sample of 10 g was taken and fats were extracted using ether extraction method per sample. After vapourising off the petroleum ether, 0.5 g of the fat was taken and dissolved in 2 ml of diethyl ether. Approximately, 500 µl of derivatisation reagent (methanolic potassium hydroxide) was added to saponify the fat and derivatise the fatty acids into fatty acid methyl esters (FAMEs). The mixture was allowed to stand for 15 minutes, and then 2 ml of de-ionised (DI) water added; followed by 5ml of hexane and thoroughly mixed using a vortexer.

The mixture was allowed to settle; and thereafter, the organic layer was transferred into another test tube and another 2 ml of DI water was added and mixed well. This was repeated 3 times for precision. The hexane layer was then transferred into a test tube containing anhydrous sodium sulphate, mixed well to remove the water. Then,1 ml of the organic extract layer was transferred into a 1.5 ml Gas Chromatography (GC) vial, and injected onto a Gas Chromatography Flame Ionisation Detection (GC-FID) using, the programme below:

Head pressure	8psi
Column type	Supelco wax-10 (30 m x 0.32 mm x 0.5 µm 5 m film thickness fused silica capillary column).
Temperature program	70 °C Initial 200 °C at 5 °C/min and held for 0 min 280 °C at 2 °C/min and held for 15 mins Total run time is 50 mins.
Injector temperature	220 °C
Detector temperature	250 °C

Protein was determined using the standard Macro-Kjeldahl method, where the samples

were digested with concentrated sulphuric acid in order to release nitrogen, which was determined by a suitable titration technique (Akinsola *et al*., 2016). The proximate amount of protein present was then calculated from the nitrogen concentration of the pork sample (AOAC, 1995).

Ash was estimated by incinerating the pre-weighed test sample in a muffle furnace at 560 °C, for a period of 5 hours. The residue was then weighed and the percentage of ash was calculated (Paine,1964). Dry matter content was estimated by subtracting the dry weight (dried in a hot air oven) of the muscle tissue from the known wet weight of the muscle tissue (AOAC, 1995).

Crude fibre was determined according to AOAC (1995) protocol. Crude fat was determined using Soxhlet Extraction method, which was a solvent-based for determination of fat in meat and meat products that was often considered the standard method by which other methods were evaluated (Habeck *et al.*, 2013). This method involved the use of petroleum ether (a flammable, non-polar solvent with a low boiling point (35°C/95°F) to extract fat from a dried, homogenised meat sample.

Data analysis. All data obtained were analysed using Statistical Package for the Social Sciences (SPSS) version 20.0 and a One-way ANOVA was generated. Significant means were separated by Least Significant Difference (LSD) Tests at 5 % significant level (LSD< 0.05).

RESULTS

Fatty acids composition. Most fatty acids (88.9%) found in pork of pigs raised on both IMO treated and untreated deep litter floor were unsaturated (Table 1). Saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), poly-unsaturated fatty acids (PUFAs), trans-fatty acids (TFAs), cis-fatty acids (CFAs) and omega-6, 7 and 9 fatty acids differed significantly (P<0.05) in pork of raised on IMO treated and untreated deep litter floor. However, no significant (P>0.05) difference was observed in composition of omega-3 fatty acids in pork of pigs raised on both floors. The SFAs, MUFAs, TFAs, Omega-7 and 9 fatty acids were higher in pork of pigs raised on deep floor without IMO treatment; while USFAs, PUFAs, CFAs and omega-6 fatty acids

TABLE 1. Fatty acids composition (%) in pork of pigs raised on deep litter floor treated with IMO incomparison with pigs raised on deep litter floor without IMO treatment

Variables	Litter floor without IMO	Litter floor with IMO	LSD (0.05)	P-value
Saturated fatty acids	3.26± 0.93^{a}	2.93±0.66^{b}	0.00	0.01
Unsaturated fatty acids				
Mono-unsaturated fatty acids	3.83±0.50^{a}	3.74±0.17^{b}	0.02	0.05
Poly-unsaturated fatty acids	2.78±1.00^{b}	3.33±0.50^{a}	0.00	0.00
Trans-fatty acids	0.823±0.04^{a}	0.48±0.06^{b}	0.00	0.00
Cis-fatty acids	6.63±0.40^{b}	7.03±0.66^{a}	0.00	0.00
Omega-3 fatty acids	0.56±0.09	0.44±0.07	0.17	0.13
Omega-6 fatty acids	2.76±0.63^{b}	3.28±0.39^{a}	0.00	0.00
Omega-7 fatty acids	0.50±0.01^{a}	0.47±0.01^{b}	0.03	0.05
Omega-9 fatty acids	3.79±0.25^{a}	3.67±0.11^{b}	0.00	0.00

Means within a row with different superscripts differ significantly (P <0.05)

TABLE 2. Effect of IMO treatment of deep litter floor on fatty acids profile in pork

Variables (%)	Litter floor without IMO	Litter floor with IMO	LSD (0.05)	P-value
Capric acid	0.12±0.02	0.11±0.02	0.63	0.63
Myristic acid	1.35±0.01[a]	1.06±0.07[b]	0.00	0.00
Pentadecanoic acid	0.03±0.02	0.02±.013	0.33	0.36
Palmitic acid	2.21±0.06[a]	1.85±0.56[b]	0.00	0.00
Palmitoleic acid	0.50±0.01[a]	0.47±0.01[b]	0.03	0.04
Heptadecanoic acid	1.26±0.05[a]	0.84±0.05[b]	0.00	0.00
Cis-10-heptadecanoic acid	0.27±0.02	0.23±0.14	0.69	0.65
Steraic acid	7.75±0.23	7.81±0.12	0.63	0.67
Oleic acid	3.71±0.17[a]	3.63±0.13[b]	0.00	0.02
Elaidic acid	0.74±0.07[a]	0.48±0.06[b]	0.00	0.01
Linoleic acid	2.68±0.63[b]	3.17±0.39[a]	0.00	0.00
Alfa-linolenic acid (ALA)	0.67±0.05[b]	0.84±0.05[a]	0.01	0.01
Gamma-linolenic acid	0.53±0.05[a]	0.47±0.12[b]	0.51	0.48
Arachidic acid	0.67±0.00	0.84±0.11	0.06	0.06
Cis-11,14-eicosadienoic acid	0.17±0.05[b]	0.26±0.03[a]	0.02	0.04
Behenic acid	0.02±0.02[b]	0.06±0.00[a]	0.00	0.02

Means within a row with different superscripts differ significantly (P<0.05)

were higher in pork of pigs raised on IMO treated deep litter floor.

Fatty acids profiles. A total of sixteen fatty acids were identified in pork of pigs raised on IMO treated and untreated deep litter floor type (Table 2). Myristic, Palmitic, Palmitoleic, Heptadecanoic, Oleic, Elaidic, Linoleic, Alfa-linolenic (ALA), Cis-11-14-eicosadienoic and Behenic acid significantly (P<0.05) differed in pork of pigs raised on IMO treated and untreated deep litter floor. However, Capric, Pentadecanoic, Cis-10-heptadecanoic, Stearic, Gama-linolenic and Arachidic acid did not show any significant (P>0.05) difference. Myristic, Palmitic, Palmitoleic, Heptadecanoic, Oleic and Elaidic acids were generally higher in pork of pigs raised on untreated deep litter floor than for those treated with IMO solution. In contrast, Linoleic, Alfa-linolenic (ALA), Behenic and Cis-11-14-eicosadienoic acids were higher in pork of pigs raised on IMO treated deep litter floor than those without the IMO treatment.

DISCUSSION

Fatty acids composition and profile. The over 80% of the fatty acids in pork of pigs raised on both IMO treated and untreated deep litter floor being unsaturated (Table 1), suggests that they can easily be utilised by human (Dugan *et al.,* 2015). This also suggests that they are less likely to elevate blood cholesterol concentrations, which increase the risk of coronary heart disease (Nistor *et al.,* 2012; Dugan *et al.,* 2015). There was a wide variation in the composition of fatty acids in pork of pigs raised on both deep litter floor types; where some fatty acids were higher in pork of pigs raised on IMO treated litter floor, and others were higher in pork of pigs raised on untreated litter floor.

These variations could be due to the effect of IMO treatment on litter floor because pigs consumed some of the bedding materials like wood shavings and maize stalk, which contained IMO solution.This might have had an effect on fatty acids composition in pork

of pigs raised on IMO treated litter floor. Another reason for the variation could be due to temperature difference between the two floors, because daily application of IMO solution on deep litter floor further cooled down the deep litter bedding materials on which pigs were resting comfortably most of the time, this might have facilitated ambient body temperature of pigs. This could have also favoured the synthesis of unsaturated fatty acids over saturated fatty acids.

This suggestion concurs with Dooremalen and Ellers (2010), who reported that when temperature decreases, the composition of membrane lipids (phospholipid fatty acids) become more unsaturated to be able to maintain homeoviscosity. In addition, Nurnberg *et al*. (1998) also reported that fatty acid composition in animal is affected by several factors, of which diet and temperature in general, seems to be the most important. Our findings agree with Nilzen *et al*. (2001) and Sundrum *et al.* (2000), who reported that the amount of fat in pork of pigs raised on straw bedding floor and free range land was higher with more composition of unsaturated fatty acids compared to pork of pigs reared traditionally by tethering or on compact soil floor.

In a similarly study, Hogberg *et al*. (2003) and Hansen *et al*. (2006) reported that the higher content of poly-unsaturated fatty acids (PUFAs) in pork of organically kept pigs (concrete floor with litter) may not only be as a result of the different feed and temperature, but also partially caused by the higher lean meat percentage which makes fatty acid composition to vary substantially between the organic (concrete floor, with litter) and conventional (concrete floor) pig rearing system. In addition, Hansen *et al*. (2006) reported that muscles of organically (concrete floor with litter) reared pigs had higher Linolenic acid and lower Palmitic acid and Elaidic acid than those raised on non-organic system (concrete floor). However, our study disagrees with that of Wood and Enser (1997), who reported that in pigs, dietary fatty acids are absorbed unchanged from the intestine and incorporated into tissue lipids which make fatty acid composition of pork fat to be affected by feed composition. In addition, Uemoto *et al*. (2011) reported that Myristic acid and Palmitic acid did not show differences in commercial pork of traditionally kept pigs (compact soil floor).

CONCLUSION

Deep litter floor treatment with IMO solution enhances the composition of unsaturated fatty acids. Further study should be conducted on the mechanisms by which deep litter floor whether treated or untreated with IMO solution modifies fatty acids composition in pork. There is also need to profile amino acids in pork of pigs raised on IMO treated and untreated deep litter floor.

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