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The effect of treating growing gilts with either Ractopamine or Reporcin on pork characteristics

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MHDM conceived idea, collected data, wrote manuscript.

ABSTRACT

The pork industry is constantly seeking economical and sustainable strategies that will improve production efficiencies and meat quality. Such successful improvements will be of value even here in Botswana to improve food security. The evaluation of pork characteristics after use of these strategies is important. This study aimed at evaluating the effect of a beta-agonist (BA - Ractopamine) and porcine growth hormone (rpGH - Reporcin) on the pork characteristics of the longissimus dorsi (LD) muscle of adult gilts (starting weights of 85±5kg) treated for 27 days under three treatment groups (15 animals per treatment group). The control group was fed a standard commercial diet ad-libitum, while the beta-agonist (BA) group were also fed ad-libitum the standard commercial diet containing Ractopamine (20mg/kg) and the growth hormone (rpGH) group were fed the commercial diet ad-libitum and administered Reporcin (10mg) intramuscularly every other day until the day before slaughter. No significant differences were observed between treatment groups regarding the gilts' carcass weights after 27 days of treatment (control – 81.98kg; BA – 86.97kg and rpHG – 84.51kg). However, LD muscle from gilts of the growth hormone (1.569%) and Ractopamine (1.502%) groups had significantly lower lipid content compared to the control group at 1.873% ($p < 0.001$), but no significant differences were observed between groups regarding fatty acid profiles. The two growth promoters can thus be locally adopted for use to improve feed utilization, pork production and to lower lipid content in pork of growing gilts without deleteriously affecting fatty acid composition.

Keywords: Ractopamine, Reporcin, carcass weight, total fat, fatty acid composition

INTRODUCTION

The pork industry is constantly seeking economical and sustainable methods to increase production efficiencies and carcass/meat quality. Meat in general represents an energy-dense, protein-rich food source that contributes significantly to the required intake of a range of micronutrients (Salter, 2013). According to Salter (2013), with increasing economic prosperity, populations tend to increase their consumption of such animal products. However, in developed countries, there has been a significant shift in intake of red meat towards white meat products. Thus, the evaluation and assessment of meat's fat content and fatty acid composition has become increasingly important since more consumers have become aware of their nutritional and health implications (O'Fallon et al., 2007). While death from cardiovascular disease has decreased dramatically in many developed countries, the prevalence of the disease remains high in developing countries (Salter, 2013). High intake of saturated fatty acids is a major contributor to the development of cardiovascular disease through increased plasma cholesterol (Salter, 2013). At farm level, opportunities to manipulate fat content and fatty acid composition management, feeding and feed additives exist. However, this can also be achieved through the use of chemicals. Beta-agonists, amongst them Ractopamine, are reported as powerful stimulants of muscle growth in livestock (McElligott et al., 1988; Sillence, 2004). It has been realized that some beta-agonists increase skeletal muscle mass and decrease body fat (Sillence, 2004). The nutrient repartitioning effects of these stimulants have proven desirable for the livestock industry trying to improve feed efficiency, meat production and quality (Lynch and Ryall, 2008). With improved production efficiencies, food security at household level across the world may be improved. These developments may be even welcomed locally. However, in Botswana the use of stimulant drugs in meat production is not favoured due to sanitary requirements of the EU. Ractopamine has a repartitioning effect, promoting lean tissue deposition and also improve average daily gain (ADG), feed conversion efficiency (FCE), dressing percentage and carcass lean content (Sillence, 2004; Apple et al., 2007; Kutzler et al., 2010). This agonist tends to channel nutrients away from adipose tissues into more lean tissue material through increased protein synthesis and muscle protein accretion (Aalhus et al., 1990; Apple et al.,

2007; Kutzler et al., 2010). The effects of exogenous growth hormone (GH) on animal growth and meat quality have also been reported before (Fabry et al., 1991; Brameld et al., 1996). It has been reported that exogenous GH can improve animal daily weight gain, feed efficiency and lean accretion rate (Campbell et al., 1991; Brameld et al., 1996). In contrast to cattle, the anabolic effects of exogenous growth hormone and/or Reporcin (rpGH) in pigs are more pronounced, being constrained only by the availability of dietary protein and energy, or in the case of highly improved breeds, the level of intake (Campbell et al., 1991). Therefore, the objective of the current study was to evaluate the effect of a beta-agonist (BA - Ractopamine) and porcine growth hormone (rpGH - Reporcin) on the carcass characteristics and fatty acid profiles of the Longissimus muscle from adult gilts treated for 27 days.

MATERIAL AND METHODS

Animals and their management

White Duroc x (Landrance x Large White) gilts, weighing 85±5kg, aged about 3 months, were sourced from PIC (Alpha Building, Nantwich, Cheshire, UK) and acclimatized to the feed and environment for five days. Upon arrival at The University of Nottingham (Sutton Bonnington campus) Animal Unit, the pigs were individually penned to prevent any stress effects associated with group housing. Management practices at Sutton Bonnington comply with UK Home Office regulations. Pigs' individual pens measured 3 m^2 per animal. Animals were watered *ad lib* and fed *ad lib* and temperature and relative humidity were monitored daily.

Animal welfare

All pigs were observed at least twice daily and any abnormal occurrences were noted. Identical handling practices were used for all animals, with each pig handled with due regard for their welfare. In compliance with UK Home Office regulations, veterinary care was available during the entire course of the study.

Trial design and sample collection

Forty five (45) gilts were randomly allocated to one of the three treatment groups (Control, BA or GH) and slaughtered at Day 27 (n = 15). The Control group were fed a standard commercial diet (Table 1) *ad-lib*, while the β-agonist (BA) group were also fed *ad-lib* the standard commercial diet containing Ractopamine (20 mg/kg in feed) and the growth hormone (rpGH) group were fed the commercial diet *ad-lib*, and each animal injected with 10 mg Reporcin intramuscularly every other day until the day before slaughter. In short the standard commercial diet's calculated chemical composition was; 14 MJ/kg digestible energy, 16.7% crude protein, 3.4% crude fibre and 5.2% total ash (Table 1).

Table 1. Composition of the standard commercial diet fed to the gilts

Ingredient	g/kg	Calculated composition	
Wheat	600.0	Digestible energy	14MJ/kg
Barley	185.5	Crude Protein	16.7%
Soyabean meal	125.0	Crude Fibre	3.4%
Rapeseed meal	35.0	Ash	5.2%
Lysine	4.0		
Methionine	0.9		
Threonine	0.9		
Soya Oil	20.2		
Dicalcium phosphate	12.5		
Limestone	10.0		
Salt	3.5		
Premix (vitamins)	2.5		

At the start of each week, all animals were weighed and feed quantities were adjusted based on body weight measurements taken at the start of each week or increased by 30% (based on previous studies on a similar pig breed, feed intake levels calculated from the Akey company feeding protocol guide (Akey Manufacturing, 2010), to ensure pigs were fed close to *ad lib* levels. The feed quantity remained unchanged for the duration of that week. Water was always available.

On the day of slaughter, pigs were calmly moved to the abattoir from their pens, and they were slaughtered humanely according to a schedule 1 procedure (UK Home Office), which involved initial stunning with low voltage electricity and then exsanguinated by severing the carotid artery. A registered slaughter man was engaged to perform all slaughter and evisceration procedures. Within fifteen (15) minutes post-mortem, carcass weights and Longissimus dorsi (LD) muscle samples were collected and snap frozen in liquid nitrogen for total lipid and fatty acid evaluation.

Fat content determination

Fat content of tissue samples were performed according to the Soxtherm Fat Extraction method (AOAC, 2000). The final weight of the extracted fat was then recorded and the fat content of the sample calculated as follows;

Soxlet Fat (%) = [weight of extract (g)/sample weight (g)] x100.

Fatty acid analysis

Direct synthesis of fatty acid methyl esters

Fatty acid profiling was carried out on tissue samples using gas chromatography (GC) of fatty acid methyl esters (FAME) according to O'Fallon et al. (2007). This was done on the Longissimus dorsi (LD) samples collected at 0hr and

snap frozen in liquid nitrogen. Ground 1g tissue sample were weighed into 15ml methylation tubes, to which 0.7ml 10M potassium hydroxide in water and 5.2ml methanol were added. These were incubated in a water bath at 55^{o}C for 1.5 hours with vigorous shaking for 5 seconds every 20 minutes. The samples were thereafter cooled to below room temperature in cold tap water for 10 minutes before adding 0.58ml 12M sulphuric acid and mixing by inversion before putting them back into the water bath for further incubation (55^{o}C) for another 1.5 hours with intermittent vigorous shaking after every 20 minutes for 5 seconds. They were then cooled below room temperature again for 10 minutes before adding 3ml of hexane with vortex mixing for 30 seconds. Samples were finally centrifuged at 500g for 5 minutes at room temperature and the top hexane layer was then transferred into solvent resistant 4ml tubes of which 1ml was thereafter placed into GC vials (VWR International Ltd), capped and stored at –20^{o}C awaiting gas chromatography.

Gas chromatography analysis of fatty acid methyl ester

FAME analysis was carried out using a Perkin Elmer Clarus 500 Gas Chromatograph fitted with an autosampler and flame ionization detector (FID) running on TotalChrom software. Analyses were carried out using a 100m CP-Sil 88 column. Samples were contained in 1.5ml vials sealed with an aluminium cap. The different fatty acid components were identified by reference to a C4 – C24 standard of known composition (Supelco 189-19 AMP), and the output report contained the proportion of each individual FAME as a percentage of the total reported. This allowed for quick, easy comparison across samples. In case of fatty acids not contained in the C4 – C24 standard, individual FAME were purchased (Supelco) and added to the standard.

Statistical analyses

Data were analyzed using two way ANOVA and Post Hoc Dunnett's test, with significance level set at $P<0.05$ to determine the effect of day of slaughter and to identify differences between treatment groups on carcass weight, lipid content and fatty acids profiles (Statistical Software for Windows, Genstat 15th Edition, Hemel Hemstead, U.K).

RESULTS

Carcass weights

No significant differences were observed between treatment groups regarding the gilts' carcass weights after 27 days of treatment, although gilts treated with Ractopamine tended to have numerically higher weights ($P = 0.146$, Table 2). Neither day of slaughter nor initial carcass weight had effects on the results.

Total lipid and fatty acid composition

Total lipid content and fatty acid composition are reported in Table 2. As shown in Table 2, LD muscle from gilts treated with either growth hormone or Ractopamine had significantly lower lipid content, compared to the control group ($P <0.001$). There were no significant differences for any of the fatty acid across treatments (Table 2), but there was a trend for higher ($P = 0.086$) palmitic fatty acid (C16:0) in control group compared to both the treatment groups. There was also tendency for lower Linolenic/arachidonic ratio ($P = 0.092$) in BA treated gilts compared to other groups.

DISCUSSION

Carcass Weights

Beta-agonists, amongst them Ractopamine, have been reported as powerful stimulants of muscle growth in ruminants, swine, rodents, and avian species (McElligott et al., 1988; Sillence, 2004). Ractopamine has a nutrient repartitioning effect, promoting lean tissue deposition and also improvements in average daily gain (ADG), feed conversion efficiency (FCE), dressing percentage and carcass lean content (Sillence, 2004; Apple et al., 2007; Kutzler et al., 2010).

Ractopamine tends to channel nutrients away from adipose tissues into lean tissue through increased protein synthesis and muscle protein accretion without deleteriously affecting pork quality, thus leading to more quality meat in high value cuts (Apple et al., 2007; Kutzler et al., 2010). Pigs fed Ractopamine, regardless of dietary inclusion level have been found to gain weight faster, being more efficient and producing heavier muscular carcasses than untreated ones (Carr et al., 2005; Weber et al., 2006).

In the current study Ractopamine treated gilts had higher, although not significantly different, carcass weights than other groups. However, Ractopamine treated gilts had significantly lower total lipid content compared to those from the untreated lot (control group). According to Armstrong et al. (2004), feeding 5 ppm Ractopamine results in improved live animal performance, with small improvements in carcass composition. Increased Ractopamine concentrations (10 to 20 ppm) and longer feeding durations such as up to 28 - 35 days usually result in further improvements in carcass characteristics (Armstrong et al., 2004). This was corroborated in the present work with a similar feeding duration of 27 days. According to Baker et al. (2006), chronic administration of a β2-agonist, such as clenbuterol or fenoterol induces muscle fibre hypertrophy in animal models, resulting in heavier carcasses.

Different studies (Fabry et al., 1991; Brameld et al., 1996; Sillence, 2004; Yang et al., 2007) have reported positive effects of exogenous growth hormone (GH) on animal

Table 2. Mean carcass weight (kg), total lipid/fat content and fatty acid profiles (% and ratios) of the LD from gilts treated with BA or GH for 27 days (n=15).

Measurement	Control (n=15)	BA* (n=15)	rpGH$^{\gamma}$ (n=15)	SED[1]	P-value
Carcass weight	81.98	86.97	84.51	2.500	0.146
Total lipid/fat	1.873	1.502	1.569	0.071	0.001
Fatty Acids					
Lauric (C12:0)	0.093	0.103	0.091	0.006	0.123
Myristic (C14:0)	1.409	1.470	1.352	0.070	0.233
Palmitic (C16:0)	27.490	26.040	25.890	0.768	0.086
Palmitoleic (C16:1)	3.446	3.287	3.055	0.254	0.300
Stearic (C18:0)	14.770	14.610	14.420	0.406	0.701
Elaidic (C18:1trans9)	0.185	0.167	0.194	0.027	0.589
Oleic (C18:1cis9)	34.960	37.550	36.540	1.804	0.374
Linoleic (C18:2)	13.940	13.600	14.850	0.903	0.397
Linolenic ((C18:3n3)	1.243	1.423	1.373	0.100	0.172
Arachadonic (C20:4n6)	2.465	1.744	2.211	0.356	0.131
Saturated/unsaturated ratio	0.737	0.787	0.721	0.034	0.139
Linoleic/arachadonic ratio	1.143	0.563	0.957	0.268	0.092
Linoleic/linolenic ratio	9.82	11.37	11.99	1.401	0.312
Oleic/(oleic+stearic) ratio	0.716	0.698	0.715	0.015	0.421
Palmitoleic/(Palmitoleic+Palmitic)	0.112	0.111	0.104	0.007	0.449

BA* = beta-agonist; rpGH$^{\gamma}$ = growth hormone; SED[1] = standard error of the differences of the means

growth and meat quality. GH has been associated with improved daily weight gain, feed efficiency and lean accretion rate in animals (Campbell et al., 1991; Brameld et al., 1996). In contrast to cattle, the anabolic effects of exogenous growth hormone and/or Reporcin (rpGH) in pigs are more pronounced, being constrained only by the availability of dietary protein and energy, or in the case of highly improved breeds, the level of intake (Campbell et al., 1991). But some studies have reported no such improvements (Elsasser and Drath, 1995), only noticing such increases on internal organs. As has been reported previously by Brameld et al. (1996), GH in the current study only significantly increased liver weights after 27 days of treatment (data not shown). This observation is in agreement with that of Klindt et al. (1992), who stated that animal organs respond more to growth hormone than carcass components.

Although in the current study no differences were observed in weights compared to other treatment groups, other studies have reported significant increases in average daily weight gain of up to 26.1% (749.66g vs 594.66g) for rpHG treated pigs compared to the control (Yang et al., 2007). Although the current study period was also similar to that of Yang et al. (2007), no improved gains were observed with GH treatment. The contradiction could perhaps be attributed to the fact that Yang et al. (2007) used castrates instead of gilts. This may indicate differences in response to growth hormone due to sex (Sillence, 2004).

Using an improved pig breed, Belgian Landrace that has been intensively selected for superior carcass quality, Fabry et al. (1991) also reported stimulated growth rate (16.3 to 25.4%), improved feed efficiency (16.9 to 29.4%) and reduced feed consumption (12%). Sillence (2004) has observed that treatment of neonatal pigs with rpGH or a β-agonist, or a combination of both, causes a lasting improvement in growth rate, body composition, and feed efficiency in pigs. Unfortunately, the principal drawback to the commercial use of rpGH is the need for frequent administration of the product.

Total Lipid and Fatty Acid Composition

The ratios in Table 2 above are meant to indicate the desaturation of short chain fatty acids which are precursors for the synthesis of long chain fatty acids. Fatty acids consist of a carboxyl (COOH) group with a long hydrocarbon chain, which can be classed as either saturated and/or unsaturated, depending on the number of double bonds between the carbon atoms. According to Apple et al. (2007), the use of faster growing, leaner genetics after years of selection has given rise to increased unsaturation of fat in pork carcasses. There are health benefits to consumers associated with the consumption of poly-unsaturated fatty acids (PUFA), especially linoleic and linolenic acids (Sillence, 2004; Salter, 2013). But unsaturated fatty acids also cause soft fat which results in problems in handling and processing of pork products. Pork of this quality has reduced shelf life.

The treatment of gilts with the two growth promoters in the present study led to significantly lower lipid contents versus the control group. β2-adrenergic agonists such as Ractopamine tend to increase lipolysis in adipose tissues, decreasing lipogenesis and thus improving lean mass deposition, with hypertrophied muscle fibres that switch towards a fast-twitch, that are more glycolytic in character (Depreux et al., 2002; Sillence, 2004; Gunawan et al., 2007; Baxa et al., 2010; James et al*.*, 2013). According to James et al*.* (2013) and Watt et al. (1991), both rpGH and Ractopamine also tend to up-regulates β-adrenoceptors in adipose tissue, and thus making them sensitive to catecholamines, leading to improved lipolysis and leaner carcasses. Increased hydrolysis of adipose tissue triglycerides leads to increased production of free fatty acids (FFA), which are exported from fat cells to be used as oxidative fuels by other tissues (James et al., 2013), and this results in low carcass fat.

As corroborated by the present study, several studies also reported minimal effects on fatty acid composition after Ractopamine supplementation (Carr et al., 2005; Weber et al., 2006), but a significantly decreased carcass fat was observed (Baker et al., 2006). Engeseth et al. (1992) have reported percentage decreases in saturated fatty acids palmitic acid (16:0), stearic acid (18:0) and increases in poly-unsaturated fatty acids linoleic acid (C18:2n6) in LD muscle after feeding Ractopamine (20 mg/kg) to pigs for up to 6 weeks, but in the current study there was a tendency for decrease in palmitic acid percentage in both the Ractopamine and growth hormone treated groups relative to the control group. A tendency for decrease in the alpha Linolenic/arachidonic ratio was observed, indicating a slow or impairment of desaturation from the alpha linolenic acid into the synthesis of a longer chain fatty acid arachidonic acid in the Ractopamine treated gilts.

According to Apple et al. (2007), Ractopmaine tends to alter fatty acid composition of the subcutaneous fat. It increases the proportions of the essential PUFAs, linoleic and alpha linolenic acids. According to the authors, alterations in the muscles, more especially LD muscle, only occurs when Ractopamine is fed to finishing pigs at higher levels of about 20 mg/kg. Regarding the GH effect on fatty acid composition, Clark et al. (1992) reported no effect of porcine growth hormone on the LD muscle lipid profile, an observation that agrees with the results of the current study where no effect was observed due to GH treatment. Sillence (2004) has also reported that the combined use of rpGH and Clenbuterol, a β2-agonist in finishing pigs, produced a pronounced decrease in carcass fat.

CONCLUSIONS

The two growth promoters (Ractopamine and Reporcin) used in this study in the 27 day period of treatment at their respective dosages significantly lowered the total lipid/fat content in LD muscle of the gilts, but did not have much influence on the composition of the fatty acids. This means that at the current dosages, the two promoters can be used in improving feed efficiency in pig production enterprises to lower carcass lipid/fat content in pork without deleteriously affecting fatty acid composition. This will help in production of much healthier pork regarding low fat content.

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CONFLICT OF INTEREST

None.

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