**Effect of genetic divergence for carcass fatness, slaughter age and body site on the hypertrophy of subcutaneous adipose tissue in beef steers.**

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***Application***

Explain the effect of genetic divergence for carcass fatness, age at slaughter and body site on the hypertrophy of subcutaneous adipose tissue biopsies harvested at slaughter in beef steers

***Introduction***

Carcass fat cover is typically the most challenging market criterion to consistently achieve, particularly where pasture reared beef cattle are slaughtered at a young age (Regan et al., 2018). Whilst it is possible to produce late-maturing beef steers at 24 months from pasture, with an adequate carcass fat score (Doyle et al., 2021), late-maturing steers slaughtered at 19 months from pasture don’t meet the minimum carcass fatness specification (Regan et al., 2018). In Ireland, carcass fat cover is measured at beef processing plants using video image analysis (VIA) and the trait is a key criterion governing the value of the carcass. The Irish cattle breeding federation (ICBF), harness these data to generate estimated breeding values (EBV) for carcass fatness. Previous research has shown that progeny performance, including carcass fat score, can be influenced by the genetic merit of sires (Clarke et al., 2009). At a laboratory scale, histological analysis of subcutaneous adipose tissue is an accurate approach in determining the extent of tissue adiposity including adipocyte hyperplasia (numbers) and hypertrophy (size), which is an objective measure of subcutaneous carcass development to assess subcutaneous fat development. Adipogenesis, and the development of fat, are influenced by genetics, age, diet and body depot location (Jin et al., 2012, Romao et al., 2014). Although it is well accepted that the hypertrophic expansion of adipocytes exists with increasing slaughter age (Hood & Allen, 1973, Du et al., 2013), there is a dearth of information regarding the effect of genetic divergence for carcass fatness, various slaughter ages, on the hypertrophy of subcutaneous adipocytes, in both the shoulder and the loin. Additionally, there is a paucity of information regarding the effect of body location, i.e. the forequarter (shoulder) vs. the hindquarter (loin) on the hypertrophy of subcutaneous adipocytes. Therefore, the objective of this study was to investigate hypertrophic differences of subcutaneous adipocytes in cattle genetically divergent for carcass fatness, with different slaughter ages and of contrasting body sites.

***Materials and methods***

As part of a larger experiment, seventy two weaned spring-born late-maturing crossbred (Limousin and Charolais sired) steers were assigned to the study (Burke et al., BSAS 2025) Cattle selected were genetically divergent for carcass fatness, ‘fat’ cattle had an EBV: 0.14 for carcass fatness, and ‘lean’ cattle had an EBV of -0.55. Cattle were finished at 19, 23 and 26 months, with a target carcass weight of 390kg for each production system. During their first winter, steers were offered silage *ad libitum* and 1.5kg/animal daily of a barley based concentrate, and subsequently turned out to pasture in the spring. The 19 month group were finished at the end of the second grazing season. The 23 and 26 month groups were offered ensiled forage, as well as 5kg and 1kg concentrates/animal daily, respectively. The 23 month group were finished at the end of the second winter, and the 26 month group were finished off pasture after grazing for approximately 2 months of the third grazing season. Prior to slaughter, ultrasonic fat depth was recorded at the rib, lumbar and rump. At slaughter, carcasses were weighed and graded for carcass fatness through VIA. A ~2g biopsy of subcutaneous adipose tissue was harvested from the shoulder and the loin, on the left side of the carcass. Samples were fixed in 10% neutral-buffered formalin for 48 hours, following storage in 70% ethanol <5days. Subcutaneous adipose tissue was then processed, and embedded in paraffin wax in accordance with standard procedures. Sections (5µm thick) were dehydrated in ascending grades of alcohol, followed by clearing with xylene and were stained using haematoxylin and eosin (H&E) stain. Nuclear counts of adipocytes on the depth of colour were conducted using an algorithm developed in Aperio ImageScope (v12.4.6.5003) to count and measure fat cells. Data were analysed using the Mixed Model procedures of SAS (9.4). Partial correlation coefficients of EBV for carcass fatness, live animal measurements, carcass fat scores and subcutaneous adipocyte histology data were derived.

***Results and Discussion***

Results are displayed in Table 1 below. Briefly, ‘Fat’ animals had greater ultrasonic fat depth at the rib, lumbar and rump than ‘Lean’ animals. ‘Fat’ animals had greater carcass fat scores, than ‘Lean’ animals. Increasing slaughter age resulted in increased ultrasonic fat depth at the rib, lumbar and rump, in tandem with increased carcass fatness scores. This trend persisted at the subcutaneous adipocyte level, where ‘Fat’ cattle exhibited greater hypertrophic expansion of adipocytes by increased average adipocyte diameter and nuclei density, than ‘Lean’ cattle, at both the shoulder and the loin. Coincident with increasing slaughter age, increased average adipocyte diameter and increased nuclei density was observed at both sites. There were no differences (P > 0.05) in average adipocyte diameter or nuclei density, between the shoulder and the loin i.e. the forequarter and the hindquarter of the animal. Significant positive correlations were obtained for average adipocyte diameter and EBV (r=0.63). Significant positive correlation values were obtained for average adipocyte diameter and ultrasound fat depth at the rib (r=0.68), lumbar (r=0.66) and rump (r=0.54). Significant positive correlation values were obtained for average adipocyte diameter and VIA measured carcass fatness (r=0.864). Furthermore, the amount of total variation explained in VIA measured carcass fatness was lower for ultrasound fat depth at the rib (R2=0.621) and higher in average adipocyte diameter (R2=0.832). From these results, it can be observed that average adipocyte diameter, at both the shoulder and loin, exhibits the highest correlation values with VIA measured carcass fatness. Therefore, the use of VIA to measure carcass fatness is the greatest predictor of subcutaneous adipocyte diameter, in the shoulder and the loin, in this study.

***Conclusion***

The hypertrophy of subcutaneous adipocytes at both sites were greater in ‘Fat’ vs. ‘Lean’ animals, and with increasing slaughter age. There were no differences in hypertrophy of subcutaneous adipocytes between the loin or the shoulder regions. VIA measurements of carcass fatness explained a greater proportion of the variance in subcutaneous adipocyte diameter at both sites, compared to EBV and ultrasonic fat depth in this study.

**Table 1:**

Effect of genetic divergence for carcass fatness, slaughter age and body site on ultrasound fat depth, carcass traits and hypertrophy of subcutaneous adipose tissue.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genotype****(G)** |  | **Slaughter age****(S)** |  | **Body Site****(B)** |  | **P-value** |
|  | *Fat* | *Lean* | s.e.m. | *19m* | *23m* | *26m* | s.e.m. | *Shoulder* | *Loin* | s.e.m. | **G** | **S** | **B** |
| **Ultrasound fat depth: rib (mm)** | 5.17 | 3.76 | 0.1825 | 3.52b | 4.90a | 5.10a | 0.2235 | - | - | - | \*\*\* | \*\*\* | - |
| **Ultrasound fat depth: lumbar (mm)** | 3.51 | 2.45 | 0.1201 | 2.46b | 3.28a | 3.28a | 0.1470 | - | - | - | \*\*\* | \*\*\* | - |
| **Ultrasound fat depth: rump (mm)** | 7.56 | 4.67 | 0.3642 | 4.12c | 8.11b | 6.37a | 0.4460 | - | - | - | \*\*\* | \*\*\* | - |
| **Final weight (kg)** | 680 | 668 | 4.584 | 616b | 702a | 704a | 5.614 | - | - | - | NS | \*\*\* | - |
| **Carcass fat score (1-15)** | 8.4 | 6.6 | 0.132 | 6.3c | 7.6b | 8.6a | 0.162 | - | - | - | \*\*\* | \*\*\* | - |
| **Average adipocyte diameter (mm)** | 0.194 | 0.165 | 0.003 | 0.163c | 0.175b | 0.201a | 0.004 | 0.180 | 0.180 | 0.003 | \*\*\* | \*\*\* | NS |
| **Nuclei Density (mm)** | 0.030 | 0.022 | 0.001 | 0.021c | 0.024b | 0.032a | 0.001 | 0.026 | 0.026 | 0.001 | \*\*\* | \*\*\* | NS |

\*\*\*P-value is significant at the 0.001 level, \*\*P-value is significant at the 0.01 level, \*P-value is significant at the 0.05 level, NS: Not significant (>0.05).

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