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The influence of growth stage on carcass composition and factors associated with marbling development in beef cattle[1]

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SUMMARY

There are many cellular regulatory factors that ultimately determine the intramuscular fat, or marbling content and quality of beef carcasses. Identifying factors which play a critical role in the development of intramuscular fat throughout the feeding period and determining how cattle feeders can manipulate these factors will be crucial to continue improving beef quality. Ideally, marbling must increase without excess accumulation of adipose in depots that are undesirable and economically detrimental (subcutaneous and visceral). The results of this study are novel as they show not only what cellular factors play a role in marbling development, but also how their expression and presence change as an animal grows in an American-style production system. The increase in both expression and presence of peroxisome proliferator-activated receptor γ (**PPARγ**) at the end of the feeding phase suggest the proliferation and differentiation of additional cells to adipocytes is required in order to increase intramuscular fat content. This does not mean that adipocyte filling (lipogenesis) does not play a key role as well. However marbling content will reach a plateau without the recruitment of additional adipocytes. While it has been previously established that intramuscular adipocytes have a pattern of metabolism unique to other adipocytes, further research into how the metabolism of intramuscular fat differs from other fat depots and how this metabolism changes throughout the feeding phase will enhance the ability to produce high quality carcasses while limiting undesirable carcass fat.

INTRODUCTION

Intramuscular fat, or marbling, in the cross sectional area of the *Longissimus* muscle (**LM**) at the 12th rib is the primary factor used to determine the quality grade of young beef animals in the United States. Consumers are willing to pay a premium for beef products with increased marbling content (Platter et al., 2005) as marbling positively impacts flavor and juiciness (Mcbee and Wiles, 1967). However, a strong correlation between marbling content and subcutaneous fat thickness has been identified (McPhee et al., 2006) and excess subcutaneous fat serves as an economic detriment to the beef industry. Postnatal adipose tissue development was previously thought to occur in the following order: internal, intermuscular, subcutaneous, and lastly intramuscular (Andrews, 1958). Yet, this has been contested by recent studies suggesting postnatal adipose tissue deposition occurs simultaneously among these depots during growth (Wang et al., 2009) and marbling is not a late developing tissue as previously believed (Bruns et al., 2004). A better understanding of how and what causes marbling to develop throughout the finishing phase is required to continue to improve this economically important trait.

Previous marbling research in cattle has explored the effect of various enzymes and transcription factors and how their expression or presence impacts marbling. However, many of these factors identified were

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only measured near the end of the feeding phase, so a lack of knowledge remains as to how these factors change throughout the finishing phase. Therefore, our hypothesis is that marbling is an early developing tissue and cellular factors influencing marbling development are growth stage dependent. The objective of this study was to determine whether cellular factors associated with marbling development change with growth stage throughout the feeding period and whether they are related to marbling relative to carcass composition.

MATERIALS AND METHODS

All animal procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee. In November 2011, 66 steer calves from a single source with predominately Angus breeding were received at the South Dakota State University Ruminant Nutrition Unit (Brookings, SD). Backgrounding and finishing diets are presented in Table 1. Seven days after arrival, blood was collected from the jugular vein for leptin genotype determination. The leptin c.73 C>T polymorphism was genotyped by PCR-restriction fragment length polymorphism according to the procedure by Buchanan et al. (2002). Only steers possessing the CT genotype (heterozygous genotype) were selected for this study. Twenty four steers were selected and randomly assigned to one of three harvest groups based upon harvest point (8 steers per harvest group, one harvest group per pen): early-feeding period (**EF**), mid-feeding period (**MF**), and late-feeding period (**LF**). Point of harvest was predetermined for each treatment as follows: 35 days on feed (EF), average live weight (**LW**) of 1000 lb (MF, 111 days on feed), and 0.4 inch 12th rib subcutaneous fat thickness (LF, 188 days on feed). Individual weights were measured every 28 days to monitor growth throughout the feeding period. Steers were implanted with a Synovex-S implant on d 29 and reimplanted with a Revalor-S implant on d 113.

Table 1. Diet formulations for backgrounding and finishing phases[a]

	Backgrounding Diets			Finishing Diets		
Ingredients	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 1**	**Diet 2**	**Diet 3**
Dry rolled corn	40.01	40.01	31.00		45.00	42.20
Chopped ear corn			38.00			
High moisture ear corn				80.00	40.75	40.85
WDGS[b]	14.00					
DDGS[c]		14.00	12.00	12.55	10.00	10.00
Sorghum silage	31.49	31.49	15.00	3.20		
Alfalfa hay	10.00	10.00				
Dry supplement 1[d]	4.50	4.50				
Dry supplement 2[d]						6.95
Liquid supplement[d]			4.00	4.25	4.25	
Days fed	34	27	33	19	38	37

[a] Percent inclusion; dry matter basis

[b]WDGS = Wet distillers grains with solubles

[c]DDGS = Dried distillers grains with solubles

[d] Supplements fortified to meet or exceed NRC (1996) requirements for vitamins and minerals. Monensin added to provide 22g/T in backgrounding diets and 29g/T in finishing diets.

The six steers most representative of each harvest group based upon LW were harvested at the South Dakota State University Meat Laboratory (Brookings, SD) using accepted slaughter methods. Immediately following exsanguination, a 3-inch section of the LM was excised from the left side of the carcass between the 12th and 13th rib. A portion of the LM section was cut into small pieces, snap frozen

in liquid nitrogen, and stored at -80°C for western blot and real-time PCR analysis. The remaining LM section was stored at -20°C for later determination of water, fat, protein, and ash.
After cooling for 48 h postmortem at 4°C, the right side of each carcass was ribbed between the 12th and 13th rib. REA and 12th rib subcutaneous fat (**FT**) were measured and USDA Yield Grades were calculated. Marbling scores, lean and skeletal maturity were assessed by trained university personnel. Additionally, the 9-10-11 rib section was fabricated from the right side of each carcass as described by Hankins and Howe (1946). Soft tissue was separated from bone and both were weighed. The soft tissue was mixed and homogenized using a bowl chopper. Water, fat, protein, and ash content of the soft tissue were determined. From these values, Hankins and Howe (1946) equations for steers were used to predict composition of the carcass soft tissue as well as total carcass composition.

Real-Time PCR
Muscle tissue from the 12th rib LM was frozen in liquid nitrogen and powdered using a mortar and pestle. RNA was extracted from the powdered muscle tissue using TRI Reagent® RT (Molecular Research Center, Inc. Cincinnati, OH). RNA concentration was determined and samples were diluted to a standard RNA concentration. RNA was converted to cDNA through a high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA) using a thermal cycler (My Cycler, Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. Real-time quantitative PCR analysis using reverse transcribed cDNA was performed in triplicate using a SYBR Green RT-PCR kit (Bio-Rad Laboratories, Hercules, CA) to determine the relative expression of; AMP activated protein kinase α (**AMPKα**), CCAAT/enhancer binding protein β (**C/EBPβ)**, lipoprotein lipase (**LPL**), myostatin, PPARγ, and stearoyl-CoA desaturase (**SCD**).

Western Blot Analysis
Frozen LM tissue was subsequently powdered using a mortar and pestle. Powdered LM samples were added to a homogenizing solution and protein concentration was determined. Samples were diluted to a constant protein concentration, mixed with sample buffer, denatured at 100°C, and stored at -20°C. Samples were subjected to SDS-PAGE and western blotting to determine the relative abundance of AMPKα, C/EBPβ, LPL, myostatin, PPARγ, and SCD.

Warner-Bratzler Shear Force (WBSF)
Strip loins were excised from the right side of each carcass. Four 1-inch steaks were cut and allotted for WBSF at 2, 7, 14, and 21 d postmortem aging. Steaks were stored at -20°C after their predetermined postmortem aging period. Shear force was then conducted as described by Mohrhauser et al. (2011).

Statistical Analysis
Carcass characteristics, carcass composition, muscle composition, and western blot data were analyzed as a complete randomized design using GLM (General Linear Model, SAS Inst. Inc., Cary, NC) with individual animal as the experimental unit. Orthogonal contrasts were used to determine linear and quadratic effects for harvest groups. WBSF data were analyzed using repeated measure (PROC MIXED DATA) procedures (SAS Inst. Inc., Cary, NC). Means for each harvest group, postmortem aging period and their interactions were compared by the Tukey's multiple comparison. The relationships between the relative quantity of proteins of interest as determined by western blotting and 12th rib LM fat content (**IMF**), as well as the relationship between HCW and IMF, were determined using PROC REG (SAS Inst. Inc., Cary, NC). REST 2008 (Relative Expression Software Tool V2.0.07, Corbett Research Pty, Ltd., Sydney, Australia) was utilized to calculate the fold change in gene expression through the incorporation of reaction efficiencies, reference gene normalization, and cycle thresholds values to determine

statistical differences. Statistical significance was considered as $P < 0.05$ with trends considered at $P < 0.10$.

RESULTS

Actual points of harvest corresponded well with predetermined harvest points as MF was harvested at 1023 lb LW (target was 1000 lb) and LF at 0.46 in FT (target was 0.4 inch FT, Table 2). The time between harvest for EF and MF steers was 76 d with a live weight difference of 273 lb and the time difference between MF and LF was 77 d with a difference of 280 lb. Therefore, slaughter points were not only equally distributed between time on feed, but by LW as well. Both FT and KPH increased at similar rates between harvest points. Moreover, estimated carcass fat increased at a steady rate between harvest points and estimated carcass protein percentage only differed for EF carcass (Table 2). Collectively, these data contradict standard growth curves where lean muscle accretion slows and adipose deposition accelerates as an animal approaches its mature body size.

Table 2. Carcass data and estimated carcass composition by growth stage[a]

	Growth Stage				Significance of contrasts	
	EF	**MF**	**LF**	**SEM**	**Linear**	**Quadratic**
Live weight, lb	750	1023	1303	14.24	**<0.0001**	**<0.0001**
HCW, lb	417	609	810	7.25	**<0.0001**	**<0.0001**
Dressing percent	55.56	59.65	62.19	0.52	**<0.0001**	**<0.0001**
REA, in^2	9.1	10.3	13.0	0.34	**<0.0001**	**0.0227**
12^{th} rib fat thickness, in	0.11	0.29	0.46	0.04	**<0.0001**	**0.0075**
Adjusted fat thickness, in	0.10	0.32	0.48	0.04	**<0.0001**	**0.0007**
KPH, %	2.10	2.37	2.88	0.18	**0.0070**	0.2944
USDA Yield Grade	1.88	2.85	3.26	0.13	**<0.0001**	**<0.0001**
Marbling score[b]	282	322	427	16.57	**<0.0001**	0.1084
Intramuscular fat, %	1.85	3.53	5.40	0.27	**<0.0001**	**0.0005**
M-ratio[c]	0.17	-0.32	0.15	0.30	0.9684	0.2620
Edible Portion,%[d]	83.53	85.26	86.49	0.36	**<0.0001**	**0.0042**
Water, %	52.06	48.47	44.89	0.63	**<0.0001**	**0.0011**
Fat, %	15.97	22.13	27.72	0.88	**<0.0001**	**0.0002**
Protein, %	14.43	13.75	13.22	0.18	**0.0003**	**0.0180**
Ash, %	0.85	0.75	0.66	0.02	**<0.0001**	**0.0021**
Bone, %	16.47	13.75	13.22	0.36	**<0.0001**	**0.0042**

[a] Least square means.

[b] Marbling score: 200=Traces[0], 300=Slight[0], 400=Small[0], 500=Modest[0].

[c] M-ratio calculated by the following equation where means and standard deviations used were for the whole population; variable 1 = marbling score and variable 2 = 12^{th} rib fat thickness

$$\left[\frac{(\text{Obs Var}_1 - \text{Var}_1\ \bar{x})}{\text{Var}_1\ S_d} \right] - \left[\frac{(\text{Obs Var}_2 - \text{Var}_2\ \bar{x})}{\text{Var}_2\ S_d} \right]$$

[d] Values calculated from proximate analysis of 9-10-11 rib sections using equations for carcass composition outlined by Hankins and Howe (1946).

Recent studies have conflicted with more classical research in regard to the timing of intramuscular fat deposition. Evidence from this study supports the theory that there is an increased priority for intramuscular fat deposition and it is subsequently accumulated throughout the feeding period. Both

marbling score and IMF (Table 2) began to increase from the time steers were placed on feed. Marbling score increased linearly throughout the finishing phase ($P < 0.0001$, Table 2). Additionally, IMF, an objective and less variable measure of intramuscular fat, revealed a both a linear and quadratic increase throughout the feeding period ($P < 0.0001$ and $P = 0.0005$, Table 2). Furthermore, IMF was found to increase in a linear fashion with HCW ($P < 0.01$; $r^2 = 0.8373$), giving additional evidence of the linear nature of IMF deposition throughout the feeding phase.

Gene expression of PPARγ and SCD was down-regulated in EF steers relative to the other two harvest groups ($P = 0.046$ and $P < 0.01$, respectively). This indicates increased adipogenesis (PPARγ) and lipogenesis (SCD) later in the feeding period relative to the early stages. Furthermore, expression of SCD was up-regulated in LF steers relative to the first two harvest groups ($P = 0.02$) indicating increased lipogenesis and adipose cell hypertrophy at the end of the feeding phase. Additionally, AMPKα expression was down-regulated in the LF group relative to the two earlier growth stages ($P < 0.01$). AMPK acts as the "fuel gauge" of the cell and its presence and subsequent activation acts to cease ATP consuming processes (i.e. lipogenesis) and activate ATP regeneration pathways. Thus, this decreased expression of AMPKα would indicate that anabolic pathways in LM cells were active resulting in energy storage and adipose accumulation.

The previous changes in gene expression did not all result in a difference in the relative amount of the respective protein present. There was a linear increase in PPARγ throughout the feeding period ($P = 0.03$) driven by a sharp increase in PPARγ present in the LF group relative to the first two growth stages. This agrees with the gene expression results presented previously, which indicates an up regulation of PPARγ in both MF and LF groups. There was a linear ($P = 0.07$) and quadratic ($P = 0.08$) trend for myostatin to increase throughout the feeding period. Myostatin acts as an inhibitor of skeletal muscle growth. Therefore, the increased presence of myostatin at the end of the feeding phase would coincide with the standard growth curve in which muscle growth begins to slow and fat accumulation rapidly increases, however, these findings are somewhat contradicted by the large increase in REA between the MF and LF groups (Table 2). Nonetheless, this may be due to the fact that steers in this study were implanted which may have resulted in a skewed growth curve. The relative abundance of the other proteins of interest did not differ between other growth stages. Only PPARγ was found to have a significant correlation to IMF ($P = 0.04$, $R^2 = 0.2382$). Therefore, the relative abundance of PPARγ present in muscle at harvest may serve as an indication of the marbling content of the LM. Additionally, targeting and increasing PPARγ expression may serve as a mechanism to increase marbling deposition.

Tenderness improved between two and seven days postmortem ($P = 0.03$), but no significant decrease in WBSF was detected when postmortem aging was extended beyond seven days. This suggests that much of the postmortem proteolysis relevant to tenderness improvement was complete after seven days of aging. Steaks from the chronologically oldest, most mature harvest group, LF, were found to be the most tender ($P = 0.01$). This differs from past findings, which support a decrease in tenderness with increased physiological maturity, but concurs with other research which supports increased tenderness with increased days on feed.

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