Impact of weight recovery on transcriptome and meat phenotypes of adult Nellore cows

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Summary

The speed of renewal of muscle proteins defines muscle growth, and can affect meat quality, as well as collagen turnover and proteolytic rate. There is little information in the literature about changes in the muscle protein remodeling process in response to the rate of recovery of weight gain after malnutrition in adult animals. The use of RNA sequencing may indicate changes in muscle tissue through a differential expression profile that explains changes in phenotypes associated with muscle growth. Therefore, the objective of this study was to evaluate changes in the transcriptome associated with phenotypes of meat of Nellore adult cows submitted to different rates of weight recovery. In this experiment, 28 adult cows aged 5 to 16 years were used. With the exception of 5 animals, the others had low body score and were randomized into 3 groups: T1-Control animals (slaughtered with low body score, n=4); T2-Slow weight gain (0.6kg/day, n=9); T3 - Accelerated weight gain (1.2 kg/day, n=10). The group T4-Maintenance of body weight (animals with high body score, kept in confinement until the end of the experiment, n=5), were allocated the other animals. The animals were slaughtered in 4 periods (S1=T1; S2=T2 and T3; S3=T3; S4=T2 and T4), and *Longissimus* muscle samples were collected to obtain the total RNA. After obtaining the RNA and preparation of the libraries, the sequencing of the samples was done in a HiScanSQ equipment. Twenty-four hours after slaughter, the pH and instrumental color (L*, a*, b*) were measured in the *Longissimus* muscle, and 2 steaks were collected for shear force analysis at 24h and 21 days. For comparisons of the phenotypes and genes, contrasts were made using the T1 group as a reference. Statistical differences between treatments were analyzed in R software, using ANOVA and Tukey test. There were significant ($P<0.05$) changes in shear force at 21 days in the group T2 (S4) e T4 (S4) and values L*, a*, b* in the T3 group (S3). Seven differentially expressed genes were found in both contrasts analyzed, D-aspartate oxidase (DDO), Serpin family H member 1 (SERPINH1), Neural precursor cell expressed, developmentally down-regulated 4 (NEDD4), novel gene (ENSBTAG00000030593), Collagen type IV alpha 1 chain (COL4A1), Activating transcription factor 5 (ATF5), and the novel gene (ENSBTAG00000018451). The differences found between phenotypes and genes indicate that growth rates impact differently on the phenotypes associated with meat quality. In addition, it is possible to state that feedback in adult animals may be an alternative to improving quality of meat, since some differentially

expressed genes are associated with important modifications in muscle.

Keywords: meat quality, nellore, adult, muscle growth, renowal

Introduction

It is a fact that growth alters DNA, RNA, providing a protein accretion affecting skeletal muscle and turnover of myofibrillar proteins (Koohmaraie *et al*., 2002), being this associated with cellular routes related the tenderness of the meat. Studies have evaluated changes in the transcriptome of genes associated with muscle remodeling in response to nutritional strategies (Lee & Hossner, 2002; Byrne *et al*., 2005).

These changes indicate the possibility of modifying proteins that are part of the muscular structure involved in meat tenderness, such as components of the cytoskeleton and extracellular matrix. However, there is almost no information on muscle remodeling in adult cows, which would be important for Brazil, since this category occupies a significant portion of slaughter and meat production. Therefore, the objective of this study was to evaluate the effect of compensatory growth in meat quality traits of *Longissimus* muscle from adult Nellore cows and describe the transcriptomic changes that lead the differences in growth and meat quality.

Material and methods

The experiment was conducted at the National Center for Research in Beef Cattle (CNPGC) of Emprapa (Brazilian Agricultural Research Company), in Brazil. In this experiment, 28 adult Nellore cows were used and aged between 5 and 16 years. The recovery of weight was assessed in four treatments using diets with different energy density at different periods of recovery: T1. Control animals (lost weight due to a feed restriction event); T2. Slow repository of weight gain (estimate gain of 0.6kg/day); T3. Accelerated repository of weight gain (estimate of gain of 1.2kg/day); and T4. maintenance of weight (cows with high body score were keep on maintenance diet until the end of the experiment).

The animals T1, T2 and T3 had an initial mean weight of 383.1±33.4 kg, while the group T4 had a mean of 500±20.1 kg. All animals were introduced in confinement for an adapting period of 7 days receiving their respective diets and were weighed one day before of the slaughter (live weight), besides receiving score values. The slaughters occurred in 4 periods (S1, S2, S3, S4) at the Embrapa Gado de Corte carcass laboratory following the normal flow of the establishment.

Meat quality evaluation

Twenty-four hours after slaughter, phenotypic measurements such as pH, instrumental color (CIELAB) were done at the Embrapa Gado de Corte carcass Laboratory. Shear force was analyzed in the *Longissimus* muscle at the 12th rib of the left sides of carcasses after 24h of slaughter and after 21 days of aging at 4°C.

Library preparation and RNA-seq analysis

For extraction of total RNA, was use TRIzol (Life Technologies, Carlsbad, CA, USA) method. The samples were selected according to RNA Integrity Number (RIN), being 2µg of

each use to prepare the libraries in HiScanSQ, equipment of the Center for Genomics of ESALQ/USP. The quality of the sequences was assessed with FASTQC, while Seqclean and Univec were used in the preprocessing step to remove the low quality reads and adaptors. The cleaned reads were mapped to the bovine reference genome (*Bos taurus* UMD3.1) using TopHat. We perform 5 contrasts to find the differentially expressed (DE) genes (adjust p-value <0.05 and $\log_2$FC≥1). The pathway enrichment analysis was done with DAVID (Database for Annotation, Visualization and Integrated Discovery, version 6.8).

Statistical analysis

The experiment was a completely randomized design (CRD). Statistical differences between treatments were analyzed in R software, using ANOVA and Tukey test. Data is presented as mean ± SD (standard deviation) with statistical significance at P<0.05.

Results

With the exception of 24h shear force e pH, all other phenotypes (Table 1) showed changes. The groups T2 and T3 show significant differences between the live weight and body score of the animals, but no differences were observed between slaughterings. Considering that there was a significant difference for 24h shear force, we can observe an improvement in the meat tenderness of the animals submitted to re-feeding (T2 and T3) and maintenance (T4) of body weight. However, this reduction in shear force seems to be associated more with a biggest period of re-feeding, than the type of diet offered, as we can observe in slaughter groups 4.

The color variables were different (P<0.05) between treatments, especially compared to T3 group, where the values of L*, a*, b* in muscle were higher, suggesting a color change to a lighter and more alive profile of red. The increase in these values indicates that there was actually a tissue change during the animal weight gain, and these changes are important, since the color of the meat is one of the determining factors for consumer choice.

Phenotypes	T1	T2		T3		T4	Value P<
	S1	S2	S4	S2	S3	S4	
Number of Cows	4	5	4	4	6	5	-
Live weight(kg)	324±24^{a}	447±35^{b}	440±34^{b}	452±15^{bc}	520±59^{cd}	583±35^{d}	>0.0001
Body score	2.8±1.0^{a}	5.8±0.8^{b}	6.3±1.0^{bc}	6.5±0.6^{bc}	7.5±0.5^{cd}	8.8±0.4^{d}	>0.0001
pH 24h	5.78±0.3	5.72±0.1	5.65±01	5.62±0.1	5.42±0.3	5.58±0.1	0.1000
SF 24h(kgf)	8.62±1.7	6.82±1.9	6.53±0.9	7.04±1.8	7.19±1.0	5.99±1.1	0.2000
SF 21 days(kgf)	8.89±3.0^{a}	5.99±1.4^{ab}	5.72±0.5^{b}	6.38±1.5^{ab}	6.15±1.3^{ab}	4.89±1.0^{b}	0.0140
L* Muscle	35.0±2.4^{a}	36.6±1,7^{a}	38±1.7^{ab}	37.7±1.8^{ab}	41.0±1.8^{b}	38.1±2.5^{ab}	0.0032
a* Muscle	22.0±1.5^{a}	23.6±0.9^{a}	23.9±1.1^{ab}	24.2±1.2^{ab}	25.8±1.3^{b}	24.3±0.9^{ab}	0.0021
b* Muscle	13.9±1.7^{a}	14.7±0.9^{a}	15.1±0.9^{ab}	15.8±0.9^{ab}	17.3±1.6^{b}	15.6±0.9^{ab}	0.0071

Table 1. Comparison of carcass traits and beef quality on Longissimus muscle from Nellore cows between feeding treatment and slaughter points.

Treatments= T1 (Control); T2 (Low gain); T3 (Accelerated gain); T4 (Maintenance of weight)

Slaughters= (S1) 0 days of feeding; (S2) 51 days of feeding; (S3) 74 days of feeding; (S4) 104 days of feeding

SF = Shear Force

Results are expressed as mean ± standard error.

$^{a–d}$Means in a row without common superscripts are different (P<0.05).

There were 16, 186, 3, 78, and 295 differentially expressed genes presented uniquely in the contrasts T1vsT4 (S4), T1vsT2 (S2), T1vsT2 (S4), T1vsT3 (S2), T1vsT3 (S3) respectively. Seven overlapped genes were found between contrasts, D-aspartate oxidase (DDO), Serpin family H member 1 (SERPINH1), Neural precursor cell expressed, developmentally down-regulated 4 (NEDD4), novel gene (ENSBTAG00000030593), Collagen type IV alpha 1 chain (COL4A1), Activating transcription factor 5 (ATF5), and the novel gene (ENSBTAG00000018451).

The genes COL4A1, SERPINH1, ATF5 were up-regulated for animals in re-feeding, and confirm a modification in connective tissue associated with a process of cellular stress during the recovery of weight. These changes in connective tissue are associated with the renewal of endomysial proteins and changes in the collagen crosslinks (Purslow, 2005; Lindert *et al.*, 2005). In addition, DDO and NEDD4 were up-regulated for the control group (T1), these genes show that these animals were physiologically regulating energy expenditure through muscle protein turnover, which reflects low body condition.

Conclusions

The beef quality of adult Nellore cows can be improved by renewal of muscle. The modifications appear to be associated with modifications in connective tissue and the development of new muscle cells. In addition, changes in meat phenotypes appear to be affected by the rate of weight gain.

Acknowledgement

CG was supported by a grant from the Next-Generation BioGreen 21 Program PJ01134906, and PJ012611 Rural Development Administration, Republic of Korea.

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