ANA MARIA BEZERRA OLIVEIRA LOBO

FATTY ACID AND GLOBAL GENE EXPRESSION PROFILES IN BRAZILIAN HAIR SHEEP

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*

VIÇOSA
MINAS GERAIS – BRASIL
2010

ANA MARIA BEZERRA OLIVEIRA LOBO

FATTY ACID AND GLOBAL GENE EXPRESSION PROFILES IN BRAZILIAN HAIR SHEEP

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de *Doctor Scientiae*.

APROVADA: 16 de dezembro de 2010.

Profª. Concepta M. M. Pimentel

Prof. Fabyano Fonseca e Silva

Pesq. Marta F. Martins Guimarães

Pesq. Samuel Rezende Paiva

Prof. Simone E. Facioni Guimarães
(Orientadora)

“Se realmente estamos pensando em sermos os melhores, não há nada de errado nisso. O problema é querer ser o maior. As pessoas que tentam ser as melhores, em todos os campos, vão para um lugar extremamente gratificante, que é o de ultrapassar barreiras, ver seu esforço verdadeiro ser retribuído. Ser melhor implica disciplina, esforço, trabalho. Muitas pessoas querem ser maiores. O que normalmente implica atalho, mágica, se dar bem, tirar do caminho aqueles que são melhores. Esse sonho de ser o maior é ilusório. Quem quer ser o maior vai descobrir a insignificância humana. Aqueles que continuam na busca da excelência verdadeira por meio do trabalho, do estudo, não para o engrandecimento de si mesmos, mas para cumprir sua função existencial, para dar vazão aos seus dons – terão um retorno maravilhoso. O que você conquistou em busca de sua melhor capacidade, isso é sagrado.”

Nilton Bonder

AGRADECIMENTOS

À Universidade Federal de Viçosa e ao curso de Pós-Graduação em Genética e Melhoramento pela oportunidade de realização de um curso de alto reconhecimento.

Ao CNPq pela concessão da bolsa de estudos que tornou possível minha estadia em Viçosa.

À Embrapa Caprinos e Ovinos pelo financiamento desta pesquisa. E por ter me recebido como bolsista desde a iniciação científica.

Agradeço a minha orientadora, a professora Simone E. Facioni Guimarães; aos meus co-orientadores e aos membros da minha Banca de Tese - pelos ensinamentos, estímulo e colaborações que tornaram possíveis este trabalho.

A todos os amigos do LABTEC pela amizade e inestimável ajuda para realização das minhas atividades.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX

RESUMO

LOBO, Ana Maria Bezerra Oliveira, D.Sc., Universidade Federal de Viçosa, dezembro de 2010. **Perfis de ácido graxo e de expressão gênica global em ovinos deslanados.** Orientadora: Simone Eliza Facioni Guimarães. Co-orientadores: Samuel Rezende Paiva e Raimundo Nonato Braga Lobo.

Para um melhor entendimento de como as variações genéticas contribui para o aumento da produção de carne ovina depende fortemente de identificar e estudar os genes transcritos no músculo esquelético. Neste contexto, buscou-se comparar os perfis de expressão gênica global no músculo *Longissimus* (LD) de quatro grupos genéticos de ovinos em crescimento pós-natal. Foi analisado também o perfil de ácidos graxos do músculo LD e analisamos a correlação desses dados com a expressão gênica de vários genes. Foi utilizado microarrays de oligonucleotídeos (*Sheep oligo microarray*), que contêm 15.744 sondas, para comparar os perfis de transcrição de genes no músculo LD de cordeiros das raças Morada Nova (MO), Somalis Brasileira (SO) e Santa Inês (SI) e os mestiços Dorper e ½ Morada Nova x ½ (F1) criados em pastagem irrigada na região Semi-árida Brasileira. Os resultados mostraram que 262 transcritos foram diferencialmente expressos entre os quatro grupos genéticos. Um total de 26 transcritos de funções conhecida foram diferencialmente expressos em todas as comparações MO-SO (C1), F1-MO (C2), F1-SO (C3), SI-MO (C4), SI-SO (C5) e F1-SI (C6). A abundância de transcritos envolvidos com o desenvolvimento do tecido múscular esquelético e da adipogênese intramuscular foi encontrada em todas as raças. Foi observada forte expressão de fatores de transcrição (MyoD e IGFBP-4), de genes envolvidos com a biossíntese dos ácidos graxos (PGDS e SCD), adipogênese (PPAR e C/EBPδ) e com o metabolismo de carboidratos (ATP5G1, PYGL, GLUT-3 e GGTA1). Os genes altamente transcritos que codificam enzimas do metabolismo energético: PYGL (SI<MO>SO<F1>SI), GLUT-3 (SO<MO<SI>SO>F1<SI) and GGTA1 (MO<SO>SI<F1>MO) sugerem um metabolismo mais glicolítico, o qual indica maior utilização de carboidratos do que de lipídios como substratos energéticos no tecido. Uma maior expressão de genes que controlam a adipogênese intramuscular sugere que a idade em que os animais foram avaliados inicia-se a deposição de gordura intramuscular. A análise de agrupamento demonstrou grupos de genes com expressão semelhante, sugerindo novas funções para alguns genes, por associação com a expressão de outros. Por exemplo, os genes IGFBP-4, PGDS, PPARg, GLUT-3, MyoD,

C/EBPδ, GGTA1, PYGL, DF e ATP5G1 foram agrupados em um único grupo sugerindo que eles provavelmente pertençam a uma mesma via metabólica. Com relação ao perfil de ácidos graxos, as diferenças genéticas entre os grupos estudados foram responsáveis pelas diferenças em seus perfis de ácidos graxos. Uma forte correlação positiva foi encontrada entre os ácidos graxos poliinsaturados (PUFAs) e os transcritos do gene C/EBPδ, o que leva à hipótese de que os PUFAs podem estar envolvidos na ativação da expressão gênica de tal fator, uma vez que os mesmos podem modular a expressão gênica em resposta a fatores dietéticos e do meio ambiente. Transcritos do gene SCD foram positivamente correlacionados com o índice de aterogenicidade. Este é o primeiro estudo que avaliou a expressão gênica em nível global por meio de microarray em ovinos no Brasil. Os dados encontrados revelaram padrões de expressão raça-específicos no músculo esquelético pós-natal de ovinos. Foram identificados importantes genes associados com o desenvolvimento pósnatal do músculo esquelético, com a deposição de gordura intramuscular e com a qualidade da carne. Após a validação, estas informações podem ser aplicadas em programas de melhoramento genético que serão úteis para a caracterização e desenvolvimento de marcadores que podem ser utilizados para a melhoria destas raças.

ABSTRACT

LOBO, Ana Maria Bezerra Oliveira, D.Sc., Universidade Federal de Viçosa, december 2010. **Fatty acid and global gene expression profiles in Brazilian hair sheep.** Adviser: Simone Eliza Facioni Guimarães. Co-advisers: Samuel Rezende Paiva and Raimundo Nonato Braga Lobo.

A better understanding of how genetic variation contributes to increased lamb meat production depends on identifying and studying genes transcribed in skeletal muscle. We aimed to compare global gene expression profiles in *Longissimus* muscle (LD) of four genetic groups of hair sheep during postnatal growth. Fatty acid profile of LD muscle was also analyzed and correlated with expression of several genes. A oligonucleotide microarray consisting of 15,744 probes was used to compare gene transcription profiles of LD from Morada Nova (MO), Brazilian Somali (SO) and Santa Inês (SI) breeds and ½ Dorper x ½ Morada Nova (F1) crossbred lambs raised in irrigated pasture in the Brazilian Semi Arid region. The results showed that 262 transcripts were differentially expressed among the four genetic groups. A total of 26 genes of known function were differentially expressed in MO-SO (C1), F1-MO (C2), F1-SO (C3), MO-SI (C4), SI-SO (C5) and F1-SI (C6) comparisons. The abundance of transcripts involved with skeletal muscle tissue development and intramuscular adipogenesis was found in all breeds. Strong expression of transcriptional factors (MyoD and IGFBP-4), fatty acid biosynthesis (PGDS and SCD), adipogenesis (PPAR and C/EBPδ) and glycolytic metabolism genes (ATP5G1, PYGL, GLUT-3 and GGTA1) was observed. The highly transcribed genes that encode energy metabolic enzymes: PYGL (SI<MO>SO<F1>SI), GLUT-3 (SO<MO<SI>SO>F1<SI) and GGTA1 (MO<SO>SI<F1>MO) suggest a more glycolytic metabolism, that indicates higher use of carbohydrates than lipids as energy substrates in tissue. Cluster analysis revealed groups of genes with similar expression and suggest new roles for some genes by association with the expression of other genes. For example, the genes IGFBP-4, PGDS, PPARγ, GLUT-3, MyoD, C/EBPδ, GGTA1, PYGL, SCD and ATP5G1 were clustered in the same group, suggesting that they probably belong to the same metabolic pathway. With respect to fatty acid profile, the genetic differences among the groups studied were responsible for the differences in their fatty acid profiles. A strong positive correlation was found between polyunsaturated fatty acids (PUFAs) and gene transcripts

C/EBPδ, leading to the hypothesis that PUFAs may is activate the expression of transcription factors, which modulate gene expression in response to dietary factors and the environment. SCD gene transcripts were positively correlated with the index of atherogenicity. This study which was the first evaluated of a whole-genome expression in Brazilian sheep, revealing breed-specific patterns of gene expression in postnatal sheep skeletal muscle. We identified important genes associated with the postnatal development of skeletal muscle, with the deposition of intramuscular fat and meat quality. After validation, this information can be applied in breeding programs that will be useful for characterization and development of markers that can be used in the improvement of these breeds.

GENERAL INTRODUCTION

1. Fatty acid profile

1.1. Fatty acid profile importance

Meat from ruminants is an important part of the human diet in many countries and fatty acids, being essential components of human diet, play an important role in metabolism and growth (Talpur 2007). Although there is evidence that saturated fatty acids increase serum cholesterol level in humans and thus contribute to various heart diseases, the monounsaturated and polyunsaturated fatty acids, including essential fatty acids, such as omega-3 (n-3), have beneficial effect in reducing cholesterol (Wood and Enser 1997). Therefore, the increased interest in enhancing the nutritional quality of the meat has stimulated research on manipulation of their fatty acid profiles through nutritional strategies (Diaz et al., 2011). This stimulation results from the demands of consumers in relation to meat quality, nutritional aspects of food that have been popularized in society. The study of lipid profile of meat from ruminants has been emphasized, due to the worldwide concern with the excessive consumption of unhealthy foods on human health.

Ruminants have a relatively high ratio of saturated to unsaturated fatty acids in their lipids (Sirtori et al., 2009). This is due to the structure of the ruminant digestive tract as well as the process by which their food is digested and absorbed (Wood and Enser 1997; Flux, 2005). However, the ruminant meat has other benefits, such as high levels of polyunsaturated fatty acids (PUFA). These include conjugated linoleic acid (CLA) which as been shown to participate in various metabolic processes beneficial to human health (Ip C et al., 1994). The potential health with consumption of dietary CLA was reviewed by McGuire and McGuire (2000). CLA refers to a range of geometric and positional isomers of linoleic acid.

Ruminant CLA isomers come from two sources: one from biohydrogenation in the rumen, produced during microbial biohydrogenation of dietary linoleic acid (LA; C18:2c9c12) and in the tissues through Δ^9-desaturation (through of enzyme stearoyl-CoA desaturase; SCD) of the rumen-derived trans-vaccenic acid (Corl et al., 2001). However, lipids entering the rumen are acted on initially by microbial lipases, which hydrolyse ester linkages to form free or non-esterified fatty acids. Unsaturated fatty acids may then be acted on by rumen bacteria and the final product is ultimately stearic acid (C18:0). Some of the PUFAs and these intermediate products escape biohydrogenation and are incorporated into milk and body fat (Kepler et al., 1965).

In sheep, adipose tissue is the predominant site of fatty acid synthesis (lipogenesis), while the liver, rumen, abomasums and small intestine tissues combined contributed only 8% of the total fatty acids synthesized (Ingle et al., 1964). The fatty acids present in vertebrates are derived from *de novo* synthesis via acetate or absorbed through of diet in intestine.

Fatty acid composition of meat depends on production system and that the fat deposited is affected by several factors, such as breed, sex, age and nutrition (Smith et al., 2009). Generally the fatty acid composition in lamb meat is more favorable to human health when the animals are grazed in pasture than those fed in feedlot (Aurousseau et al., 2007). As stated by Mir et al. (2004), is necessary provides to animal an appropriate substrate to increase CLA content in muscle.

1.2. Stearoyl-CoA desaturase (SCD) and transcription factors involved in lipid metabolism

The Stearoyl-CoA desaturase (SCD) enzyme catalyzes the removal of hydrogen from saturated fatty acyl chains. SCD always introduces a double bond between carbons

9 and 10, hence its other name, delta-9 desaturase. This enzyme is responsible for some of the variation in CLA concentration in adipose tissues (Dervishi et al., 2010).

The expression of the SCD gene is regulated by dietary, especially PUFA n-6 and n-3 families, hormonal and environmental factors (Miyazaki et al., 2003) through the sterol regulatory element binding protein (SREBP; Horton et al., 2002) and peroxisome proliferator-activated receptor proteins (PPAR; Kersten et al., 2000). Thus three classes of transcriptions factors directly influence adipogenesis, they are: SREBP, PPAR and CCAAT/enhancer binding protein (C/EBP; Hirwa et al 2001). CCAAT/enhancer-binding protein (C/EBP) plays a key role in initiation of adipogenesis in adipose tissue and gluconeogenesis in liver.

PPAR bind small molecular weight ligands and regulate the expression of various genes involved in intra- and extracellular lipid metabolism pathways, such as absorption of fatty acids through membranes and their intracellular binding, and the formation and transport of associated proteins, particularly those involved in peroxisome β-oxidation; Figure 1 (Dreyer et al., 1993; Wahli et al., 1995; Desvergne and Wahli, 1999; Wang et al., 2008). Tonotoz et al. (1995) reported that PPARγ was highly expressed in adipocytes and that its ectopic expression could trigger the entire program of adipogenesis in fibroblast and muscle cells. Moreover, PPARγ expression is activated by C/EBP. This activation results in increased adipogenesis and fatty acid storage (Wu et al., 1995). Fatty acid derivatives are ligands for PPARs and PPARs interact with peroxisome proliferator response elements, which are present in the sequences of genes involved in lipid metabolism.

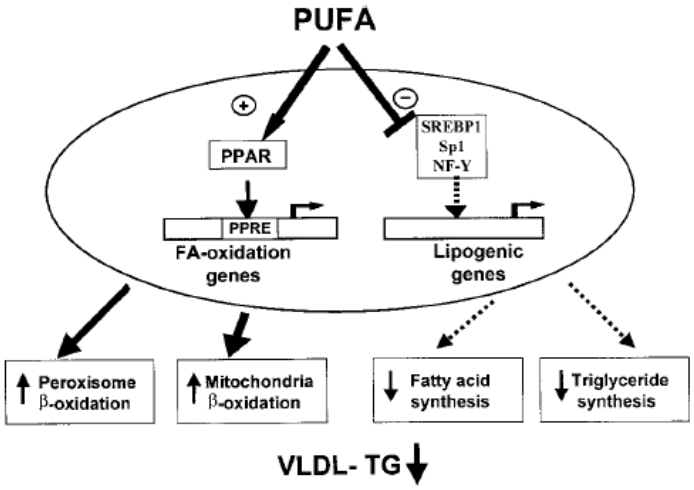


Figure 1. Nuclear mechanism for polyunsaturated fatty acids (PUFA) regulation of gene expression. FA, fatty acids; NF-Y, nuclear factor Y; PPAR, peroxisome proliferators-activated receptor; PPRE, peroxisome proliferator-activated receptor response element; Sp1, stimulatory protein 1; SREBP-1, sterol regulatory element binding protein-1; TG, triglycerides. Source: Clarke, 2001.

1.3. Relations fatty acids and meat quality

The analysis of fatty acids is important because they are involved in various "technological" aspects of meat quality. Because they have very different melting points, variation in fatty acid composition has an important effect on firmess or softness of the fat in meat, especially the subcutaneous and intermuscular (carcass fats) but also the intramuscular fat (*marblin*g; Wood et al., 2003 and 2008).

Characteristics that define the acceptance of lamb meat by consumers, such as odour, flavor and taste panels, were positively correlated with percentage of 18:0 (Stearic acid) and 18:3 (Linolenic acid) and negatively correlated with 18:2 (Linoleic acid [Sanudo et al., 2000]) fatty acid. In pork, beef and lamb the melting point of lipid and the firmness/hardness of carcass fat is closely related to the concentration of stearic acid (Wood et al. 2003). Moreover, the level of 18:2 in subcutaneous fat of lambs

increases with increases in dietary energy (Miller and Rice 1967; Field et al., 1978). 18:0 and 18:3 were positively correlated with overall appraisal for the grass fed lambs panel (subjective evaluation) and negatively correlated with the concentrate fed lambs panel (Sanudo et al., 2000). In cattle, the oleic acid was positively correlated with marbling score (Waldman et al., 1968) and flavor (Westerling and Hedrick, 1979).

The feeding system is one of the main factors affecting fatty acid composition in meat. Due to the particularities of the rumen, the manipulation of diet to improve fatty acid composition in meat of sheep is more difficult than in pigs, for example. Lambs fed the high energy diet had softer, yellower fat than lambs fed the low energy diet (Garton et al., 1972). Fat from lambs fed the low energy diet had higher levels of all the even-numbered fatty acids, except 12:0, while lambs fed the high energy diet had higher levels of all odd-numbered and branched chain fatty acids. The total concentration of branched chain fatty acids is a predictor of lamb fat firmness. It is known that high grain diets will increase levels of circulating propionate in lambs, and it is probable that such diets will increase the level of methylmalonate, a metabolite of proprionate (Busboom et al., 1981).

Summarizing, the interest in meat fatty acid composition not is only from the need to find ways to produce healthier meat, i.e. with a higher ratio of polyunsaturated (PUFA) to saturated fatty acids and a more favorable balance between n-6 and n-3 PUFA, but also because the fatty acid profiling has a direct relationship with meat quality.

1.4. References

Aurousseau, B., Bauchart, D., Faure, X., Galot, A. L., Prache, S. (2007). Indoor fattening of lambs raised on pasture: (1) Influence of stall finishing duration on lipid classes and fatty acids in the longissimus thoracis muscle. *Meat Science*, 76, 241-252.

Busboom, J. R., Miller, G. J., Field, R. A., Crouse, J. D., Riley, M. L., Nelms, G. E., Ferrell, C. L. (1981). Characteristics of fat from heavy ram and wether lambs. *Journal of Animal Science*, 52, 83–92.

Carta, A., Casu, S., Usai, M. G, Addis, M., Fiori, M., et al. (2008). Investigating the genetic component of fatty acid content in sheep milk. *Small Ruminant Research*, 79, 22-28.

Christie, W. W. (1981). *Lipid metabolism in ruminant animals*. 1st ed. Oxford: Pergamon Press. Oxford, New York, 452 p.

Clarke, S. D. (2001). Polyunsaturated fatty acid regulation of gene transcription: a molecular mechanism to improve the metabolic syndrome. *The Journal of Nutrition*, 131, 1129-1132.

Corl, B. A., Baumgard, L. H., Dwyer, D. A., Griinari, J. M. et al., The role of Delta(9)-desaturase in the production of cis-9, trans-11 CLA. (2001). *The Journal of Nutricional Biochemistry*, 12, 622–630.

Dervishi, E., Serrano, C., Joy, M., Serrano, M., Rodellar, C., Calvo, J. H. (2010). Effect of the feeding system on the fatty acid composition, expression of the Δ9-desaturase, peroxisome proliferator-actived receptor alpha, gamma, and sterol regulatory element binding protein 1 genes in the semitendinous muscle of light lambs of the Rasa Aragonesa breed. *BMC Veterinary Research*, 40, 1-11.

Desvergne, B., and Wahli, W. (1999). Peroxisome proliferator activated receptors nuclear control of metabolism. *Endocrine Reviews*, 20, 649–688.

Dreyer, C., Keller, H., Mahfoudi, A., Laud, V., Krey, G., Wahli, W. (1993). Positive regulation of the peroxisomal betaoxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biology of the Cell*, 77, 67–76.

Diaz, M. T., Cañeque, V., Sánchez, C. I., Lauzurica, S., Pérez, C. et al. (2011). Nutricional and sensory aspects of Light lamb meta enriched in n-3 fatty acids during refrigerated storage. *Food Chemistry*, 124, 147-155.

Field, R. A., J, C. Williams, C. L. Ferrell, J. D. Crouse and J. E. Kunsman. 1978. Dietary alterations of palatability and fatty acids in meat from light and heavy weight ram lambs. *Journal of Animal Science*, 47, 858-864.

Flux, C.L. (2005). The effect of conjugated linoleic acid on lipid metabolism in the hamster and the sheep. PhD thesis, University of Nottingham, 241 p. [http://etheses.nottingham.ac.uk/1141/]

Garton, G. A., Hovell, F. D., Duncan, W. R. (1972). Influence of dietary volatile fatty acids on the fatty acid composition of lamb triglycerides, with special reference to the effect of propionate on the presence of branched chain components. *The British Journal of Nutrition*, 28, 409-416.

Hirwa, C. d'Andre., Wallace, P., Xu Shen**,** Qinghua Nie**,** Guanfu Yang, Xiquan Zhang. 2010. Genes Related to Economically Important Traits in Beef Cattle. *Asian Journal of Animal Science*, 5, 34-35.

Horton, J. D., Goldstein, J. L., Brown, M. S. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *The Journal of Clinical Investigation*, 109, 1125-1131.

Ingle, D. I., Bauman, D. E. and Garrigus, U. S. (1964). Lipogenesis in the ruminant: in vivo Site of fatty acid synthesis in sheep. *The Journal of Nutrition*, 102, 617-624.

Ip, C., Scimeca, J. A and Thompson, H. J. (1994). Conjugated linoleic acid—A powerful anticarcinogen from animal fat sources. *Cancer*, 74, 1050-1054.

Kepler, C. R., Hirons, K. P., Mcneill, J.J., Tove, S. B. (1965). Intermediates and products of the biohydrogenation of linoleic acid by butyrivibrio fibrisolvens. *The Journal of Biological Chemistry*, 241, 1350-1354.

Kersten, S., Desvergne, B., Wahli, W. (2000). Roles of PPARs in health and disease. *Nature*, 405, 421-424.

Kuchel, H., Siebert, B. D., Bottema, C. D. K., Webb, G. C., Crawfort, A. M., Duncan, S. J. (2004). Physical mapping of the stearoyl-CoA desaturase (SCD) locus in sheep. *Animal Genetics*, 35, 142-167.

McGuire, M. A. and McGuire, M. K. (2000). Conjugated linoleic acid (CLA): a ruminant fatty acid with beneficial effects on human health. *Journal of Animal Science*, 77, 1-8.

Miller, G. J. and R. W. Rice. (1967). Lipid metabolism in lambs as affected by fattening rations of roughage and concentrate. *Journal of Animal Science*, 26, 1153-1159.

Mir, P. S., McAllister, T. A., Scott, S., Aalhus, J., Baron, V., et al. (2004). Conjugated linoleic acid-enriched beef production. *The American Journal of Clinical Nutrition*, 79, 1207S-1211S.

Miyazaki, M., Ntambi, J. M. (2003). Role of stearoyl-coenzyme a desaturase in lipid metabolism. *Prostaglandins Leukotrienes and Essential Fatty Acids*, 68, 113-121.

Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., María, G., Sierra, I., Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, 54, 339-346.

Sirtori, F., Dal Prà, A., Brajon, G., Pugliese, C., Bozzi, R. (2009). Meat quality of Suffolk and bergamasca lambs slaughtered at 90 days of age. *Italian Journal of Animal Science*, 8, 546-548.

Smith, S. B., Gill, C. A., Lunt, D. K., Brooks, M. A. (2009). Regulation of fat and fatty acid composition in beef cattle. *Asian-Australasian Journal of Animal Sciences*, 22, 1225-1233.

Steinhart, C. (1996). Conjugated linoleic acid – the good news about animal fat. *Journal of Chemical Education*, 73, A302-303.

Talpur, F. N. (2007). Fatty acid composition of ruminant milk, meat and dairy products of livestock in Sindh, Pakistan. Ph.D Thesis, University of Sindh, Jamshoro – Pakistan. http://eprints.hec.gov.pk/2137/1/2054.htm.

Tonotoz, P., Hu, E., Speigelman, B. M. (1995). Regulation of adipocyte gene expression and differentiation by PPARgamma. *Current Opinion in Genetics and Development*, 5, 571–576.

Wahli, W., Braissant, O., Desvergne, B. (1995). Peroxisome proliferator-activated receptors: Transcriptional regulators 174 of adipogenesis, lipid metabolism and more. *Chemistry and Biology*, 2, 261–266.

Waldman, R. C., Suess, G. G., Brungardt, V. H. (1968). Fatty acids of certain bovine tissue and their association with growth, carcass and palatability traits. *Journal of Animal Science*, 27, 632-635.

Wang, Y., Mu, Y., Li, H., Ding, N., Wang, Q. et al. (2008). Peroxisome proliferator-activated receptor-γ gene: a key regulator of adipocyte differentiation in chickens. *Poltry Science*, 87, 226-232.

Westerling, D. B. and Hedrick, H. B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. *Journal of Animal Science*, 48, 1343-1348.

Wood, J. D. and Enser, M. (1997). Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*, 78, Suppl. 1, S49-S60.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., et al. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, 66, 21-32.

Wood, J. D, Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, 78, 343-358.

Wu, Z., Xie Y, Bucher, N. L, Bucher, N. L. R., Farmer, S. R. (1995). Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis. *Genes and Development*, 9, 2350–2363.

2. Gene expression analysis

Recent changes in meat sheep production in Brazil, has stimulated the growth of activity and scientific research. Such research has focused largely on improving the productivity of livestock (Lôbo et al., 2009; Barbosa Neto et al., 2010; Oliveira et al., 2010), as well as quality and quantity of final product, the meat (Bonagurio et al., 2003; Madruga et al., 2005; Cartaxo et al., 2008). In other livestock sectors, meat-producing industries have invested in research, looking for better understanding the physiological and molecular pathways associated with skeletal muscle growth and development (Reecy et al., 2006).

Physiological changes occurring during the different stages of productive life, such as growth and tissue differentiation, exposure to different environmental factors such as nutritional factors, drugs, stress and pathogens, determine changes in gene expression patterns. Gene expression refers to the process in which a gene is used in the construction of a protein or to control the expression of other genes. In a typical cell, about 10,000 to 20,000 genes are expressed simultaneously. The level of gene expression is a number that measures the amount of protein produced (Albert et al. 2002). The techniques for analyze gene expression consist mainly in analysis of RNA obtained during the transcription, enabling to study the "commands" active in different biological states.

To date, the most of the progress made in muscle biology has been accomplished by examining single genes, proteins, or pathways. However, the availability of microarray technology for most production animal species provides new opportunities for researchers to generate global gene expression profiles. These “whole genome” assessments of gene expression have provided new insights concerning changes in gene expression associated with skeletal muscle growth and development, and have identified

novel candidate genes and physiological pathways to target in future hypothesis-based testing (Reecy et al., 2006).

2.1. Methodology for analysis of gene expression on a large scale - *Microarray*

Microarray technology was introduced over a decade by Schena et al. (1995) and since then the high-density microarrays has enabled the rapid expansion of gene expression analysis. These microarrays to measure gene expression based on the intensity of the probe that corresponds to the amount of target RNA. It considers the number of mRNA molecules transcribed from a particular gene, can be considered as an approximation to the level of expression of this gene.

DNA microarrays, also known as DNA chips, are tools that allow the identification and quantification of mRNA transcripts present in the cells. A microarray consists of a solid surface on which strands of polynucleotide called probes have been attached or synthesized in fixed positions. Array platforms most commonly used today are divided into two groups, according to the arrayed material: complementary DNA (cDNA) and oligonucleotide microarrays. Probes for cDNA arrays are usually products of the polymerase chain reaction (PCR) generated from cDNA libraries or clone collections, using either vector-specific or gene-specific primers, and are printed onto glass slides or nylon. For oligonucleotide arrays, short 20–25mers are synthesized *in situ*, either by photolithography onto silicon wafers (high-density-oligonucleotide arrays from Affymetrix - http://www.affymetrix.com) or by ink-jet technology developed by Rosetta Inpharmatics (http://www.rii.com) and licensed to Agilent Technologies (for most details see review Sculze and Downward, 2001). Each probe in an array is printed at a specific point of the slide, known as spot. A single slide can contain hundreds of thousands of spots, which can cover the entire genome.

Schematically, a gene expression microarray experiment works as follows: mRNA is extracted from a sample, converted into cDNA ou cRNA, labeled with a fluorescent dye and hybridized to a platform (*slide*) harboring *probes* corresponding to genes of interest arranged in a coded template (the *array*; Figure 2). When the one-color platforms are used, a single sample is labeled and hybridized in each array, while in two-color platforms a pair of samples is labeled with two fluorescent dyes, such as Cy3 and Cy5 and simultaneously hybridized into array. When the microarray is scanned by a laser with a specific wavelength, the hybridized cDNA or cRNA emits fluorescence. The raw intensities of the fluorescence give an estimation of the level of gene expression (Pariset et al., 2009 and Rosa et al., 2007 are recommended readings). The underlying theory is that the greater the expression of a transcript, the greater the amount of labeled target and, hence, the greater the output signal. (Hiendleder et al., 2005).

According Schulze and Downward (2001) the microarrays can be used to investigate problems in cell biology in various manners. The different experimental approaches fall between two extremes. At one end, the investigator is interested only in finding the single change in gene expression that might be the key to a given alteration in phenotype. The authors emphasize that this is equivalent to looking for a needle in a haystack, and could be thought of as an entirely local approach to analysis of gene-expression changes. At the other extreme, the aim is to look at overall patterns of gene expression in order to understand the architecture of genetic regulatory networks, a global approach that could ultimately lead to complete description of the transcription-control mechanisms in a cell.

As pointed out by Rob Alba et al. (2004) regulatory networks of genes were inferred based on microarray data obtained from a variety of organisms (Hashimoto et

al. 2004; de Hoon et al., 2003; Shmulevich et al., 2003) and related with metabolic processes (de la Fuente et al., 2002; Mendes, 2001). In sheep, Vacuolo et al. (2007) proposed after analysis of gene expression by microarray, a model depicting the regulatory network and the main epigenetic modifications likely explain the changes in fiber types and hypertrophy that characterizes the Callipyge phenotype. Bongiorni et al. (2009) performed the KEGG www.genome.jp/kegg/pathway.html) pathways among the differentially expressed genes in two milk sheep breeds and identified the molecular differences in milk synthesis in two lactation stages.

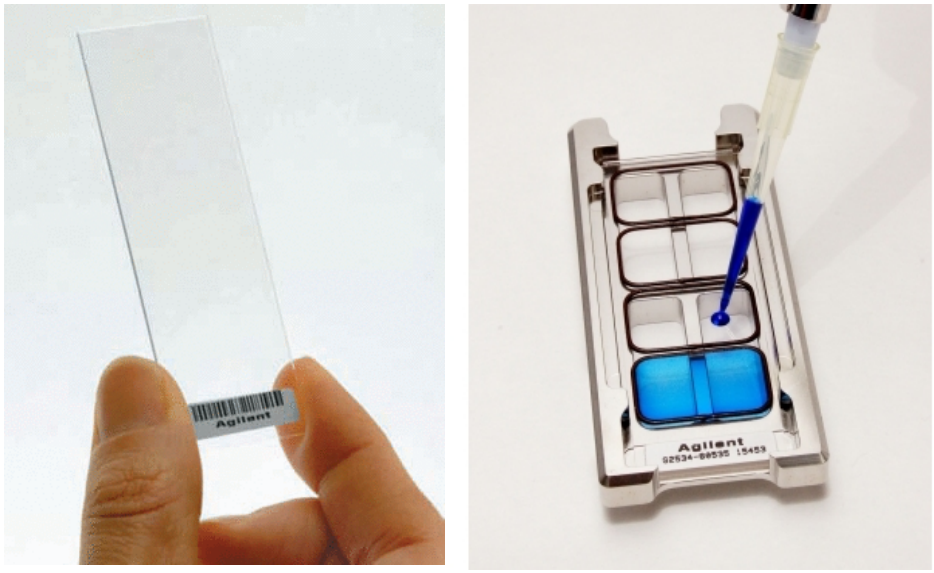
2.2. Gene expression analysis in Sheep using Microarray

The volume of publications using the microarray technology for analyze gene expression is not significant in sheep. The main searches are designs to evaluate the resistance to gastrointestinal parasites using bovine cDNA microarray (Diez-Tascon, 2005; Mackinnon et al. (2009). Keane et al. (2006) constructed cDNA libraries from tissue of the duodenum of sheep to generate ESTs. From these sequences, the authors constructed a microarray, called Ovita ovine array (*University of Otago Genomics Facility*) for analyze the resistance to gastrointestinal nematodes. Bongiorni et al. (2009) built a microarray from sequences deposited in public databases to investigate the expression profile in lactating ewes of different breeds.

The first commercially available microarray for sheep is provided by the company Agilent. This microarray contains all expressed sequences kept in public database. Generally the Agilent uses the ink-jet technology to print oligos and whole cDNAs onto glass slides. Agilent platforms consist of 60-mers probes, longer than 25-mers probes employed by Affymetrix. Probes are synthesized directly on the surface of

the microarray in a maskless manufacturing process, using standard DNA phosphoramidite chemistry (Beaucage and Caruthers, 1981).

The *Sheep Oligo Microarray* (Platform GPL10427 - http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL10427) used in the present study is produced using SurePrint inkjet technology. The Sheep Oligo Microarray have 15,744 sequences from public databases, which contain resources to access well-characterized genes and expressed sequence tags (ESTs) representative of genes of unknown function. About 14,600 sequences in microarray represent the ovine transcriptome. In species where the genome sequences are known, many anonymous sequences have been deposited in gene banks as a result of EST sequencing projects. Thus, the databases represent valuable knowledge that can be exploited by bioinformatics efforts to build species-specific microarray (Pariset et al. 2009).



(A) (B)

Figure 2. *Slide* (A) manufactured by Agilent. The probes are printed in slide in a defined position, *spots*. The slide is hybridized to a *gasket* (B) containing the biological sample, *target*. Source: http://www.agilent.com/about/newsroom/lsca/imagelibrary/images/lsca_160_Array_Slide.jpg

http://www.imgm.com/index.php?id=rna_microarray_technology

2.3. References

Alba, R., Fei, Z., Payton, P., Liu, Y., Moore, S. L., Debbie P., Cohn Jonathan, et al. (2004). ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. *Plant Journal*, 39, 697-714.

Albert, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walter, P. 2002. *Molecular Biology of the Cell*, 4th ed. New York: Garland Science.

Barbosa Neto, A. C., Oliveira, S. M. P., Facó, O., Lobo, R. N. B. (2010). Efeitos genéticos aditivos e não-aditivos em características de crescimento, reprodutivas e habilidade materna em ovinos das raças Santa Inês, Somalis Brasileira, Dorper e Poll Dorset. *Revista Brasileira de Zootecnia*, 39, 1943-1951.

Beaucage, S. L. and Caruthers, M. H. (1981). Deoxynucleoside phosphoramidites – a new class of key intermediates for deoxypolynucleotide synthesis. *Tetrahedron Lett*, 22, 1859-1862.

Bonagurio, S., Pérez, J. R. O., Garcia, I. F. F., Bressan, C., Lemos, A. L. S. C. (2003). Qualidade da carne de cordeiros Santa Inês puros e mestiços com Texel abatidos com diferentes pesos. *Revista Brasileira de Zootecnia*, 32, 1981-1991.

Bongiorni, S., Chillemi, G., Prosperini, G., Bueno, S., Valentini, A., Pariste, L. (2009). A Tool for Sheep Product Quality: Custom Microarrays from Public Databases. *Nutrients*, 1, 235-250.

Cartaxo, F. Q., Sousa, W. H., Cezar, M. F., Gonzaga Neto, S., Cunha, M. G. G. (2008). Efeitos do genótipo e da condição corporal sobre o desempenho de cordeiros terminados em confinamento. *Revista Brasileira de Zootecnia*, 37, 1483-1489.

Diez-Tascón, C., Keane, O. M., Wilson, T., Zadissa, A., Hyndman, D. L. et al. (2005). Microarray analysis of selection lines from outbred populations to identify genes

involved with nematode parasite resistance in sheep. *Physiological Genomics*, 21, 59–69.

Hiendleder, S., Bauersachs, S., Boulesteix, A., Blum, H., Arnold, G. J., Fröhlich, T., Wolf, E. (2005). Functional genomics: tools for improving farm animal health and welfare. *Revue Scientifique et Technique Office International des Epizooties*, 24, 354-377.

Lôbo, A. M .B. O., Lôbo, R. N. B., Paiva, S. R., Oliveira, S. M. P., Facó, O. (2009). Genetic parameters for growth, reproductive and maternal traits in a multibreed meat sheep population. *Genetics and Molecular Biology*, 32, 761-770.

Keane, O. M., Zadissa, A., Wilson, T., Hyndman, D. L., et al. (2006). Gene expression profiling of Naïve sheep genetically resistant and susceptible to gastrointestinal nematodes. *BMC Genomics*, 7, 1-12.

MacKinnon, K. M., Burton, J. L., Zajac, A. M., Notter, D. R. (2009). Microarray analysis reveals difference in gene expression profiles of hair and wool sheep infected with Haemonchus contortus. *Veterinary Immunology and Immunopathology*, 130, 210-220.

Madruga, M. S., Sousa, W. H., Rosales, M. D., Cunha, M. G. G., Ramos, J. L. F. (2005). Qualidade da carne de cordeiros Santa Inês terminados com diferentes dietas. *Revista Brasileira de Zootecnia*, 34, 309-315.

Pariset, L., Chillemi, G., Bongiorni, S., Spica V. R., Valentini, A. (2009). Microarray and high-throughput transcriptomic análisis in species with incomplete availability of genomic sequences. *New Biotechnology*, 25, 272-279.

Reecy, J. M., Spurlock, D. M., Stahl, C. H. (2006). Gene expression profiling: insights into skeletal muscle growth and development. *Journal of Animal Science*, 84, E150-E154.

Rosa, G. J. M., Rocha, L. B., Furlan, L. R. (2007). Estudos de expressão gênica utilizando-se microarrays: delineamento, análise, e aplicações na pesquisa zootécnica. *Revista Brasileira de Zootecnia*, 36, 185-209.

Schena, M., Shalon, D., Davis, R. W., Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, 270, 467–470.

Schulze, A., Downward, J. (2001). Navigating gene expression using microarrays – a technology review. *Nat. Cell Biol*., 3, E190-E195.

Oliveira, K. A. P., Lobo, R. N. B., Facó, O. (2010). Genetic evaluation of partial growth trajectory of Santa Inês breed using random regression models. *Revista Brasileira de Zootecnia*, 39, 1029-1036.

Vuocolo, T., Byrne, K.., White, J., McWiliam, S., Reverter, A., Cockett, N. E., Tellam, R. L. (2007). Identification of a gene network contributing to hypertrophy in callipyge skeletal muscle. *Physiol. Genomics*, 28, 253-272**.**

CHAPTER I
(ARTICLE I)

Fatty acid profile in *Longissimus dorsi* of lambs from four genetic groups raised on irrigated pasture

A.M.B.O. Lôbo[a*], S.E.F. Guimarães[a], S.R. Paiva[c], M.A.D. Bomfim[b], O. Facó[b], G. A. Fernandes Júnior[d], M. F. Ponciano[b], R.N.B. Lôbo[b]

[a] Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, s/n, Viçosa, MG, 36570-000, Brazil

[b] Embrapa Caprinos e Ovinos, Caixa Postal 145, Sobral, CE, 62010-970, Brazil

[c] Embrapa Recursos Genéticos e Biotecnologia, Caixa Postal 02372, Brasília, DF, 70770-917, Brazil

[d] Universidade Federal do Ceará, Avenida da Universidade, 2853, Fortaleza, CE, 60020-181, Brazil

***Corresponding author.** Tel./ Fax: +55 88 3112 7487

E-mail address: oliveiraana@cnpc.embrapa.br /oliveiraana@yahoo.com.br

Brazilian Agricultural Research Corporation - Goats and Sheep

Fazenda Três Lagoas - Estrada Sobral Groaíras, Km 4

Caixa Postal 145 - CEP 62010-970 - Sobral - CE - Brazil

Abstract

Thirty-four unrelated male Morada Nova (MN, n=6), Brazilian Somali (SO, n=7) and Santa Inês (SI, n=13) breeds and ½ Dorper x ½ Morada Nova (F1, n=8) lambs born

during the same season from a single birth and weaned with an average age of 84 d were used in this study to evaluate fatty acid profiles in the *Longissimus dorsi* muscle. The lambs were raised on irrigated pasture and supplemented once a day with concentrate. The lambs average age and weight at slaughter were 200.18±7.54 d and 20.62±3.46 kg, respectively. The MO breed presented the highest values of CLA, PUFA and P:S. MO and F1 lambs had meat with a higher proportion of essential fatty acids (EFA). The atherogenic index and relative activity of the enzyme desaturase were similar in F1, MO and SI lambs. The SO breed showed a less desirable fatty acid profile of from a human health standpoint. Genetic differences among the groups studied were responsible for the differences in the fatty acid profiles.

Keywords: atherogenic index, CLA, desaturase, hair sheep, lipids, meat quality

1. Introduction

The analysis of fatty acids has become increasingly important as more people have become aware of their nutritional and health implications. Meat is the major source of fat in the diet, especially of saturated fatty acids (SFAs), which have been implicated in a number of diseases (Wood et al., 2003). However, research carried out during the last few years has revealed that not only the amount of fat but also its profile should be taken account, as other nutritional benefits of the consumption of ruminant meat with high levels of polyunsaturated fatty acids (PUFA) that participate in various metabolic processes beneficial to human health has been demonstrated. Conjugated linoleic acids (CLA) are the most important among PUFAs and have been shown to occur naturally in ruminant products (Sehat et al., 1998). The most important sources of CLA in the human diet are ruminant-derived products, such as dairy products and meat. CLA

isomers are produced in the rumen during microbial biohydrogenation of dietary linoleic acid (LA; C18:2c9c12) and in tissues through Δ^9-desaturation of the rumen-derived trans-vaccenic acid (trans-11–18:1; Griinari et al., 2000; Palmquist et al., 2004; Corl et al., 2001).

The distribution of CLA isomers in cheese and/or meat shows one major isomer C18:2 c9t11 (CLA9, known as rumenic acid) with nearly 84% total CLA (Steinhart, et al., 2003; and for details, Eder and Ringseis, 2010). However, the fatty acid composition of meat depends fundamentally on the composition of the diet of the animal (Enser et al., 1998). Sãnudo et al. (2000) demonstrated that the production system affects muscle fatty acid composition and the flavor of lamb meat (Spanish and British breeds). Breed, sex and nutrition are factors that affect the levels of deposited fat (Rumsey et al., 1972; Melton et al., 1982; Eichhorn et al., 1986; Huerta-Liedenz et al., 1993; Zembayashi et al., 1995).

The northeast region of Brazil is a large semi-arid area where 54% of the sheep in Brazil. The majority of these animals are hair sheep, mainly of the Santa Inês, Morada Nova and Brazilian Somali breeds, as well as the recently imported Dorper. The consumption of sheep meat in this region is relatively high, mainly in rural areas. These animals are predominantly raised on native pasture ("caatinga") with low production capacity. Alternatives have been proposed to increase production of sheep meat in this region through the use of irrigated pasture. As the demand for sheep meat continues to increase, the evaluation of its quality, especially with respect to its fatty acid profile, under this production system is important, especially as it has not yet been studied. The objective of this study was to determine the fatty acid profile of *longissimus dorsi* in hair sheep of the Morada Nova, Santa Inês and Brazilian Somali breeds and ½ Dorper x

½ Morada Nova crossbreeds raised in a production system with irrigated pasture in a semi-arid region of Brazil.

2. Materials and methods

2.1. Animals and diets

The experiment was carried out using the experimental flock of Embrapa Caprinos e Ovinos, Sobral, CE – Brazil. Thirty-four unrelated male lambs of the Morada Nova (MN, n=6), Brazilian Somali (SO, n=7) and Santa Inês (SI, n=13) breeds and ½ Dorper x ½ Morada Nova (F1, n=8) crossbreeds born in the same season from a single birth and weaned at an average age of 84 days of age were used in this study. The lambs were raised on irrigated pasture composed of Tanzania grass (*Panicum maximun* Jacq cv. Tanzania) with free access to water and mineral salt. The lambs were free to graze and were supplemented once a day with concentrate (corn and soybean meal) at a rate of 1.5% body weight. The lambs were slaughtered at Embrapa Caprinos e Ovinos facilities at an average age and weight of 200.18±7.54 days of age and 20.62±3.46 kg, respectively. A 5-cm length of *Longissimus dorsi* was removed from one side of each carcass. All samples were vacuum-packed, frozen rapidly and stored at -20°C.

2.2. Lipid extraction, profile determination and statistical analysis

The lipids from the samples were extracted using the methodology presented by Bligh and Dyer (1959). The fatty acids were transmethylated according to the method described by Precht & Molkentin (2000). The fat extracted was dissolved in 1 mL of hexane and mixed with 20 μL sodium methylated solution (2N in methanol) in a sample vial. The solution was shaken vigorously for 3 min (vortex mixer) and centrifuged for 1

min (35 x g). After the addition of 10 mg sodium sulfatemonohydrate, the vial was recapped, mixed again for 2 min and centrifuged at same speed for 1 min. The clear supernatant was used for gas chromatography analysis.

The fatty acid profile was determined by gas chromatography according to model modified from Chilliard et al. (2006) under the following conditions: column, SP 2560 (100 m x 0.25 mm x 0.25 μm) - Supelco; patterns, Supelco 37 - Component FAME Mix (10000 μg in CH_2Cl_2) - Supelco cat. 47885-U, linoleic conjugated acid methyl ester - SIGMA Cat. O5632; flow of gas, injection in the split mode (1:100), 1 μL of sample, carrier gas - hydrogen (30 mL/min), synthetic air - 300 mL/min; programming temperature, temperature of injector and detector (FID) - 250°C; initial temperature - 70°C, held for 1 min; increase at 5°C/min to 100°C, held for 2 minutes; increase at 10°C/min up to 175°C, held for 40 minutes; increase at 5°C/min up to 225ºC; increase at 20°C/min to 245°C, held for 20 minutes; total run - 87.50 minutes. The analyses were performed in the Animal Nutrition Laboratory of Embrapa Caprinos e Ovinos.

The data were analyzed as a completely randomized design with a model that included breed effects and experimental error by the least-squares method using the GLM procedure of SAS Institute, Inc. (1999). The atherogenic index, calculated as (C12:0+(4*C14:0)+C16:0)/total unsaturated fatty acids (Chilliard et al., 2003), and the indices used to predict the activity of fatty acid desaturase (C18:1n9c/C18:0 and C16:1/C16:0) were also evaluated.

3. Results and Discussion

The intramuscular fatty acid profiles of the studied lambs are presented in Figure 3 and Table 1. The main fatty acids present in the *Longissimus dorsi* of lambs from all

genetic groups were C18:1n9c (oleic acid - 28%), C18:0 (estearic acid - 25%) and C16:0 (palmitic - 24%), which represented approximately 77% of the total. Similar percentages of oleic, estearic and palmitic acids were reported in meat from culled ewes (Pelegrini et al., 2007) and lambs grazed on pasture (Enser et al., 1998). These three are the predominant fatty acids in the meat of ruminants. In general, the results demonstrated the significant effect of breed type on fatty acid profile. This effect has been well reported in the literature (Fisher et al., 2000; Choi et al., 2000; Warren et al., 2008).

Figure 3. Fatty acid profile of ½ Dorper x ½ Morada Nova (F1), Morada Nova (MO), Santa Inês (SI) and Brazilian Somali (SO) lambs raised on irrigated pasture

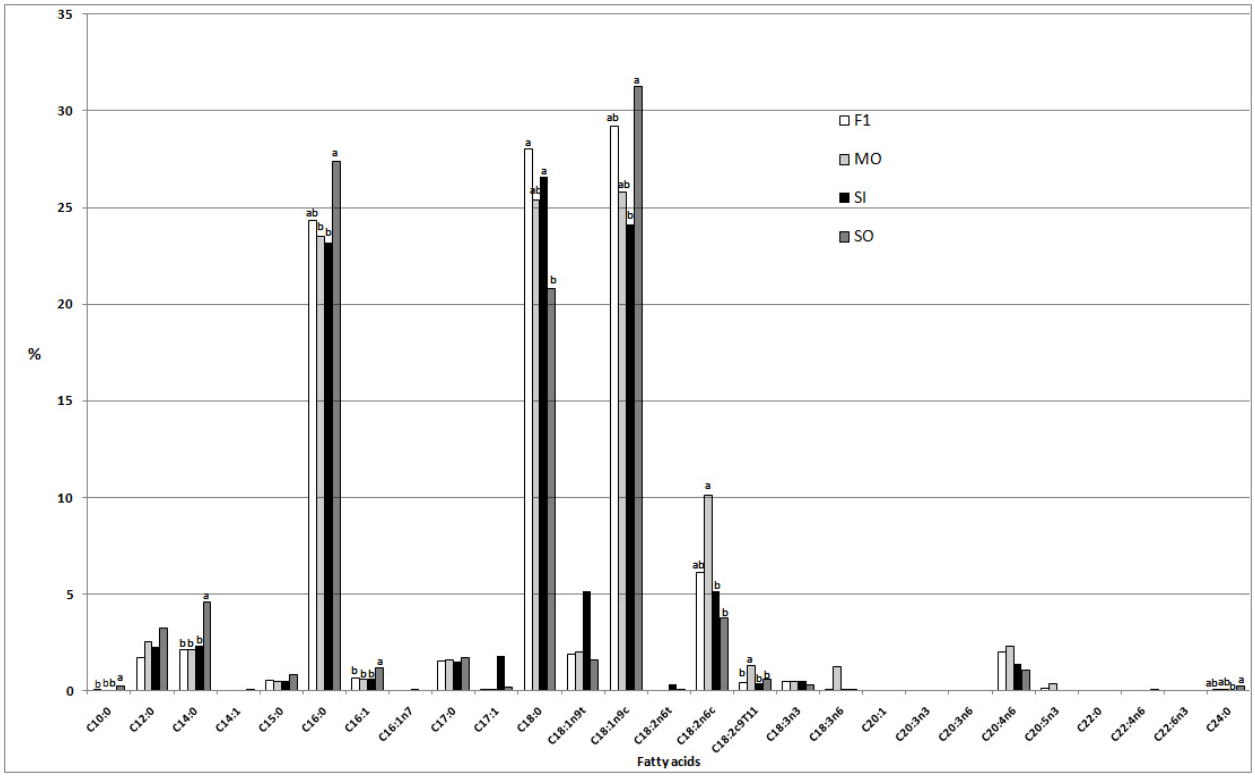


Table 1. Least-square means for fatty acid composition (% of total) in *Longissimus dorsi* of ½ Dorper x ½ Morada Nova (F1), Morada Nova (MO), Santa Inês (SI) and Brazilian Somali (SO) lambs raised in irrigated pasture

	Genetic groups			
Fatty acids	**F1**	**MO**	**SI**	**SO**
Capric acid (C10:0)	0.07 b	0.00 b	0.06 b	0.27 a
Lauric acid (C12:0)	1.71 a	2.56 a	2.28 a	3.22 a
Myristic acid (C14:0)	2.10 b	2.12 b	2.31 b	4.57 a
Myristoleic acid (C14:1)	0.00 a	0.00 a	0.04 a	0.03 a
Pentadecanoic acid (C15:0)	0.52 a	0.49 a	0.50 a	0.82 a
Palmitic acid (C16:0)	24.31 ab	23.48 b	23.20 b	27.39 a
Palmitoleic acid (C16:1)	0.66 b	0.61 b	0.65 b	1.20 a
Palmitoleate (C16:1n7)	0.00 a	0.00 a	0.08 a	0.00 a
Heptadecanoic acid (C17:0)	1.51 a	1.58 a	1.50 a	1.71 a
cis-10Heptadecanoic acid (C17:1)	0.09 a	0.04 a	1.80 a	0.18 a
Stearic acid (C18:0)	28.05 a	25.38 ab	26.57 a	20.80 b
Elaidic acid (C18:1n9t)	1.87 a	2.01 a	5.14 a	1.59 a
Oleic acid (C18:1n9c)	29.23 ab	25.80 ab	24.14 b	31.28 a
Linolelaidic acid (C18:2n6t)	0.00 a	0.00 a	0.32 a	0.02 a
Linoleic acid (C18:2n6c)	6.14 ab	10.12 a	5.16 b	3.74 b
CLA – *Conjugated linoleic acid* (C18:2c9T11)	0.40 b	1.28 a	0.39 b	0.57 b
Linolenic acid (C18:3n3)	0.50 a	0.47 a	0.50 a	0.31 a
Gamma-linolenic acid (C18:3n6)	0.02 a	1.22 a	0.08 a	0.02 a
Eicosenoic acid (C20:1)	0.00 a	0.00 a	0.03 a	0.00 a
cis-11, 14, 17 Eicosatrienoic acid (C20:3n3)	0.00 a	0.00 a	0.04 a	0.00 a
cis-8, 11, 14 Eicosatrienoic acid (C20:3n6)	0.00 a	0.00 a	0.01 a	0.00 a
Arachidonic acid (C20:4n6)	2.00 a	2.29 a	1.36 a	1.05 a
Eicosapentaenoic acid (C20:5n3)	0.11 a	0.33 a	0.05 a	0.00 a
Behenic acid (C22:0)	0.00 a	0.00 a	0.01 a	0.00 a
Adrenic acid (C22:4n6)	0.00 a	0.00 a	0.01 a	0.02 a
Docosahexaenoic acid (C22:6n3)	0.00 a	0.00 a	0.01 a	0.00 a
Lignoceric acid (C24:0)	0.02 ab	0.08 ab	0.00 b	0.26 a

Means followed by different letter in the row are statistically different by t test ($P<0.05$).

Table 2. Least-square means for fatty acid composition (%) in *Longissimus dorsi* of ½ Dorper x ½ Morada Nova (F1), Morada Nova (MO), Santa Inês (SI) and Brazilian Somali (SO) lambs raised on irrigated pasture according to classification of saturation

	Genetic groups				
	F1	**MO**	**SI**	**SO**	**Mean ± SD**
SFA	58.31 ± 3.18 a	55.69 ± 3.18a	56.45 ± 2.16 a	59.04 ± 2.76 a	56.93 ± 7.70
MUFA	31.86 ± 3.39 a	28.47 ± 3.39 a	31.90 ± 2.31 a	34.28 ± 2.94 a	31.79 ± 8.02
PUFA	9.19 ± 1.97 ab	15.71 ± 1.97 a	7.94 ± 1.33 b	5.75 ± 1.70 b	9.50 ± 6.21
UFA	41.05 ± 3.30 a	44.18 ± 3.30 a	39.84 ± 2.24 a	40.03 ± 2.86 a	41.30 ± 7.11
UFA/SFA	0.71 ± 0.12 a	0.82 ± 0.12 a	0.75 ± 0.08 a	0.69 ± 0.10 a	0.75 ± 0.28
P:S	0.16 ± 0.04 ab	0.30 ± 0.04 a	0.14 ± 0.03 b	0.09 ± 0.03 b	0.17 ± 0.13
n6	8,16 ± 3,91 ab	13,63 ± 7,37 a	6,95 ± 4,21 b	4,86 ± 2,06 b	8,29 ± 5,69
n3	0,61 ± 0,43 a	0,80 ± 0,72 a	0,61 ± 0,50 a	0,31 ± 0,38 a	0,58 ± 0,51
n-6/n-3	12.30 ± 1.80 a	11.86 ± 2.01 a	7.07 ± 1.34 a	6.89 ± 2.01 a	9.80 ± 5.56
EFA	6,64 ± 2,46 ab	10,59 ± 5,83 a	5,65 ± 3,39 b	4,05 ± 1,53 b	6,56 ± 4,14
IA	0,86 ± 0,28 b	0,82 ± 0,36 b	0,92 ± 0,22 b	1,24 ± 0,31 a	0,95 ± 0,32
C18:1n9c/C18:0	1,06 ± 0,30 b	1,04 ± 0,27 b	0,87 ± 0,40 b	1,55 ± 0,37 a	1,11 ± 0,43
C16:1/C16:0	0,03 ± 0,00 b	0,03 ± 0,00 b	0,03 ± 0,01 b	0,04 ± 0,02 a	0,03 ± 0,01

Means followed by different letter in the row are statistically different by t test ($P<0.05$);
SFA = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C22:0 + C24:0; SFA was analyzed as SFA^2;
MUFA = C14:1 + C16:1 + C16:1n7 + C17:1 + C18:1n9t + C18:1n9c + C20:1;
PUFA = C18:2n6t + C18:2n6c + C18:3n6 + C18:2c9T11 + C18:3n3 + C20:3n6 + C20:3n3 + C20:4n6 + C20:5n3 + C22:4n6 + C22:6n3; PUFA was analyzed as log10(PUFA);
UFA (total unsaturated fatty acids) = MUFA + PUFA; It was analyzed as 1/UFA;
P:S = PUFA/SFA; P:S was analyzed as log10(P:S);
n-6 = C18:2n6t + C18:2n6c + C18:3n6 + C20:3n6 + C20:4n6 + C22:4n6; n6 analyzed as log10(n6);
n-3 = C18:3n3 + C20:3n3 + C20:5n3 + C22:6n3; n3 analyzed as log10(n3+0.1);
n-6/n-3 was analyzed as log10(n-6/n-3);
EFA = C18:3n3 + C18:2n6c; analyzed as sqrt(EFA+0.1);
IA = (C12:0 + (4*C14:0) + C16:0)/ UFA;
C18:1n9c/C18:0; analyzed as x+0.1;
C16:1/C16:0; analyzed as x+0.1;

SO animals showed higher percentages of palmitic acid (C16:0) than those of the SI and MN breeds and had similar percentages to F1 animals. This similarity can be explained as the F1 is ½ Dorper, and the Dorper breed is ancestrally linked to the BlackHead Persian, the same breed that originated the SO in Brazil. Palmitic acid is one of the more common monounsaturated fatty acids present in animal tissues.

Higher percentages of myristic acid (C14:0) were also observed in the SO breed compared to the other genetic groups. SO is a fat-tailed breed that accumulates fat in its tail as a reserve for use in periods when food is scarce. The higher proportion of short-chain and saturated fatty acids can be associated with this traits. The tail adipose tissue was not analyzed here, but its content can be predicted from the intramuscular fat, as the SFA, UFA and P:S composition between meat and caudal fat is the same (Moharrery, 2009).

Despite the fact that myristic and palmitic acids are considered undesirable to human health, from the adaptation and survival viewpoint of the animal, the storage of fat in its tail is very important. Tail fat storage is an energy stock that can be mobilized when body energy requirements exceed energy intake. Thus, the fat is hydrolyzed to form glycerol and non-esterified fatty acids that can then be used to meet the energy requirements of the animal.

The SO breed had lower percentages of stearic acid (C18:0) than the SI and F1 genetic groups. Stearic acid is the primary determinant of fat hardness in cattle (i.e., lipid melting point; Smith et al., 1998). Fat hardness is the most notable physical effect of fat and the fat is harder when the proportions of UFA/MUFA and PUFA/SFA are lower. Results found by Westerling and Hedrick (1979) in beef cattle related that stearic and palmitic acids had a negative effect on flavor. Flavour in meat derives from volatile compounds produced during cooking. Muscle samples of beef cattle and sheep fed with

similar diets were similar in relation to lipid oxidation products (Elmore et al., 1999 and Elmore et al. 2000).

Higher percentages of oleic acid (C18:1n9c) were observed in SO, MO and F1. In beef cattle, a highly significant association was found between age and oleic content in outer-subcutaneous and intramuscular fat in *Longissimus dorsi* (LD). A positive correlation was also identified between LD marbling score and concentrations of oleic in outer-subcutaneous fat (Waldman et al., 1968). Relation positive between oleic acid and flavor was observed in beef cattle (Westerling and Hedrick, 1979). Here we mentioned references with other species due other ruminants such as cattle and goats have almost the same profile of sheep (Macedo et al., 2008). Agreeing with Enser et al. (1996), oleic acid was the main unsaturated fatty acid found in the *Longissimus* of lambs.

Except for the observed concentration of linolenic acid, our results are different from those results reported by Costa et al. (2009) who compared three genetic groups (Morada Nova, Santa Inês and ½ Dorper x ½ Santa Inês) with respect to feedlot and two diets with different energy levels. In relationship to the main SFAs, myristic and stearic acids had higher concentrations in the meat of SI animals, though the palmitic concentration was higher in the meat of animals of the MO breed. Similar levels of oleic and linolenic acids were observed in these breeds. However, it is important to highlight that the authors cited above did not fit the effect of the animals' age (at the beginning and at the end of the experiment), which could cause bias in the analysis. These observations demonstrate that the major factors that influence the fatty acid composition are: i) age, ii) diet, and iii) breed type (Smith et al., 2009). As the age of the animal increases, the levels of saturated fatty acids (SFAs) increase, and the levels of polyunsaturated fatty acids (PUFA) decrease (Nürnberg et al, 1998).

Among the main fatty acids of interest to human health, linolenic (C18:3n3), linoleic (C18:2n6), those with unsaturation at carbon 3, and conjugated linoleic acid (C18:2c9T11; CLA), can be highlighted. Linolenic acid percentage was not influenced by breed type ($P>0.05$). Considering all genetic groups, 6% of all the lipids in the muscle were linoleic acid. The MN breed had a higher percentage of linoleic (10.12%; $P<0.05$) than the SI and SO breeds. The CLA percentage was also higher in MO *longissimus* (1.28%) than in other breeds (Table 1 and Figure 3). MO is an important local breed with high adaptation to the region, and this aspect will be important for its conservation and improvement.

All animals received the same management on irrigated pasture with concentrate supplementation simulating commercial conditions. Should be noted that supplementation used here, only seeks to attend the maintenance requirements of animals and not to confer a higher weight gain. However, different sources for supplementation can be interesting alternatives for evaluation in future studies. Jerónimo et al. (2009) analyzed the replacement of dietary sunflower oil (SO) with linseed oil (LO) on fatty acid composition of lambs meat and suggested that the utilization of blends of these oils is a good approach to obtain lamb meat enriched with CLA. Fatty acid composition in muscle is strongly influenced by an animal's diet (Enser et al., 1998; Rowe et al., 1999; Fisher et al., 2000). The fatty acid composition in lamb meat is more favorable to human health when the animals graze on pasture than when they are fed in feedlots (Aurousseau et al., 2007). This aspect is probably because green forages contain a higher content of linolenic acid than grains (Diaz et al., 2002). Mir et al. (2000) found that the CLA content in sheep muscle increases with supplementation with oils rich in linoleic acid. These results indicate that the biohydrogenation of fatty acids in the rumen is not complete and that the extent of this process varies with the

type of diet (Pelegrini et al., 2007). French et al. (2000) observed that the fatty acid profile of intramuscular fat in beef cattle can be improved from a human nutrition perspective by the inclusion of grass in the animal's diet. This aspect reinforces the results of this study with respect to animals being raised at pasture.

The main polyunsaturated fatty acids (PUFA) arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were identified in this study. AA was present in higher proportion (Table 1). The percentage of these fatty acids were not influenced by breed type ($P>0.05$).

There were no significant differences between genotypes for total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), total unsaturated fatty acid (UFA), and UFA/SAT and n6/n3 ratios (Table 2). Approximately 41% of total fatty acids were present as UFA. The P:S and n6/n3 ratios are indices used to evaluate the nutritional value of fat for human consumption, as n-3 and n-6 are essential to the human diet. There was no difference in n3 across the genetic groups; however, differences in n6 were observed ($P<0.05$). Omega-3 and omega-6 are essential in the production of eicosanoids and are recruited as the first line of defense against infections.

The percentage of PUFA (15.71 ± 1.97 %) and the P:S ratio (0.30 ± 0.04 %) in the MO breed were significantly higher than those for the SO and SI breeds ($P<0.05$). The values of PUFA (9.19 ± 1.97 %) and P:S ratio (0.16 ± 0.04 %) for the F1 breed were similar to those for the MO breed, probably due to the participation of this breed in the cross (½ Dorper x ½ Morada Nova). These traits did differ between this crossbreed and the SI and SO breeds. Although ruminant meat normally has a low P:S ratio, the muscle contains a range of C20 and C22 PUFA of both the n-6 and n-3 series of potential significance in human nutrition (Enser et al., 1998). The two series of PUFAs

n-6 and n-3 and their derivatives originate from linoleic acid (cis-9, cis-12) and linolenic acid (cis-9, cis12, cis-15), respectively.

Studies verified that the increase in grass intake and forage caused a linear decrease in the concentration of SFA and in the n-6/n-3 PUFA ratio and a linear increase in the PUFA:SFA ratio and the CLA concentration (Fisher et al., 2000 and French et al., 2000). This increases the importance of animals in pasture.

The analysis of essential fatty acids (*Linolenic acid* - C18:3n3 + *Linoleic acid* - C18:2n6c) demonstrated differences across genetic groups. MO lambs showed meat with higher contents of essential fatty acids (EFA), similar to that observed in the F1 crossbreeds. These fatty acids cannot be biosynthesized in animals, including humans, and are necessary for health.

In addition, the atherogenic index was similar in F1, MO and SI lambs and different in SO, which showed the highest index due to a higher concentration of C12:0, C14:0 and C16:0 (Table 2). From the human health standpoint, meat with fewer short-chain saturated fatty acids content is desirable. The atherogenic index was proposed as a measure of the tendency of the food to influence the incidence of coronary heart disease.

The Δ9 desaturase indices provide estimates of stearoyl-CoA desaturase (SCD) enzyme activity (Corl et al., 2001) and determine the ratio substrate/product of this enzyme. As shown in Table 2, these indices (C18:1n9c/C18:0 and C16:1/C16:0) were lowest in F1, MO and SI lambs and highest in SO lambs ($P<0.05$). These results suggest lower activity of the enzyme with larger amounts of substrate in SO lamb meat. The ratio of product/precursor of Δ9 desaturase is correlated with the abundance of mRNA and enzyme activity (Bernard et al., 2005). The expression of C/EBPδ and PPARγ that

regulate the transcription of genes involved in lipid metabolism pathways and the SCD enzyme was observed in animals of this study (Lobo et al., 2010 – Article 2).

The study of fatty acids is of extreme importance, not only with respect to human health but also due their influence on the quality and acceptability of meat. Fatty acid profiles should be considered in breeding programs for sheep meat, such as they are related to quality factors of the meat, such as palatability and tenderness. According to the literature review by Wood et al. (2008), fatty acid composition determines the firmness/oiliness of adipose tissue and the oxidative stability of muscle, which in turn affects flavor and muscle color. The variation in fatty acid content among the breeds studied here can be considered an advantage because it offers opportunities to explore it in selection programs for the improvement of these breeds or in crossbreeding for the improvement of lamb meat production systems.

4. Conclusion

Genetic differences between the animals studied were responsible for the differences in the fatty acid profiles. The Morada Nova breed, which had the highest values of CLA, PUFA, P:S and EFA, showed better meat quality parameters with regard to the fatty acid profile. On the other hand, the Brazilian Somali breed exhibited the lowest meat quality, with the highest proportion of saturated and short-chain fatty acids.

The profiles observed for the genetic groups studied here reinforce the importance of raising lambs at pasture for the production of healthy meat for human consumption.

Acknowledgements

The authors acknowledge Embrapa for the financial support. The first author thanks CNPq and Universidade Federal de Viçosa for the scholarship.

5. References

Aurousseau, B., Bauchart, D., Faure, X., Galot, A. L., Prache, S. (2007). Indoor fattening of lambs raised on pasture: (1) Influence of stall finishing duration on lipid classes and fatty acids in the longissimus thoracis muscle. *Meat Science, 76,* 241-252.

Bernard, L., Rouel, J., Leroux, F., Ferlay, A., Faulconnier, Y., Legrand, P., Chilliard, Y. (2005). Mammalian lipids metabolism and milk fatty acid secretion in alpine goats fed vegetable lipids. *Journal of Dairy Science, 88,* 1478-1489.

Bligh, E. C., Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry Physiology***,** *37,* 911-917.

Chilliard, Y., Rouel, J., Lerox, C. (2006). Goat's alpha-s1 casein genotype influences its milk fatty acid composition and delta-9 desaturation ratios. *Animal Feed Science and Technology***,** *131,* 474-487.

Chilliard, Y., Ferlay, A., Rouel, J., Lamberet, G. (2003). A review of nutritional and physiological factors affecting goat milk synthesis and lipolysis. *Journal of Dairy Science, 86,* 1751–1770.

Choi, N. J., Enser, M., Wood, J. D., & Scollan, N. D. (2000). Effect of breed on the deposition in beef muscle and adipose tissue of dietary n-3 polyunsaturated fatty acids. *Journal of Animal Science, 71,* 509–519.

Corl, B. A., Baumgard, L. H., Dwyer, D. A., Griinari, J. M., Phillips, B. S., Bauman, D. E. The role of 9-desaturase in the production of cis-9, trans-11 CLA. (2001). *Journal of Nutritional Biochemistry, 12,* 622-630.

Costa, R. G., Batista, A. S. M., Azevedo, P. S. de, Queiroga, R de C. R. E., Madruga, M. S., Filho, J. T. A. (2009). Lipid profile of lamb meat from different genotypes submitted to diets with different energy levels. *Brazilian Journal of Animal Science, 38,* 532-538.

Daz, M.T., Velasco, S., Caneque, V., Lauzurica, S., Ruiz de Huidobro F. et al. (2002). Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. *Small Ruminant Research, 43,* 257-268.

Eder, K. & Ringseis, R. (2010). Metabolism and actions of conjugated linoleic acids on atherosclerosis-related events in vascular endothelial cells and smooth muscle cells. *Molecular Nutrition & Food Research, 54,* 17-36.

Eichhorn, J. M., Wakayama, E. J., Blomquist, G. J., Bailey, C. M., Bailey, C. M. (1986). Cholesterol content of muscle and adipose tissue from crossbred bulls and steers. *Meat Science, 16,* 71-78.

Elmore, J. S., Mottram, D. S., Enser, M., Wood, J. D. (1999). Effect of polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of Agricultural and Food Chemestry*, 457, 1619-1625.

Elmore, J. S., Mottram, D.S., Enser, M., Wood, J. D. (2000). The effects of diet and breed on the volatile compounds of cooked lamb. *Meat Science*, 55, 149-159.

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A. J., Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science*, 42, 443-456.

Enser, M., Hallett, K. G., Hewett, B., Fursey, A. J., Wood, J. D., Harrington, G. (1998). Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Science, 49,* 329-341.

Fisher, A. V., Enser, M., Richardson, R. I., Wood, J. D., Nute, G. R., et al. (2000). Fatty acid composition and eating quality of lamb types derived from four diverse breed x production systems. *Meat Science, 55,* 141-147.

French, P., Stanton, C., Lawless, E. G., O'Riordan, F. J. Monahan, P. J., et al. (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage, or concentrate-based diets. *Journal of Animal Science, 78,* 2849-2855.

Griinari, J. M., Corl, B. A., Lacy, S. H., Chouinard, P. Y., Nurmela, K.V.V., Bauman, D. E. (2000). Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9 -desaturase. *Journal of Nutrition, 130,* 2285- 2291.

Huerta-Leidenz, N. O., Cross, H. R., Savell, J. W., Lunt, D. K., Baker, J. F., Pelton, L. S., Smith, S. B. (1993). Comparison of the fatty acid composition of subcutaneous adipose tissue from mature Brahman and Hereford cows. *Journal of Animal Science, 71,* 625–630.

Jerónimo, E., Alves, S. P., Prates, J. A. M., Santos-Silva, J., Bessa, R. J. B. (2009). Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat. *Meat Science, 83,* 499–505.

Macedo, V. P., Garcia, C. A., Silveira, A. C., Monteiro, A. L. G., Macedo, F. A. F., Spers, R. C. (2008). Composição tecidual e química do lombo de cordeiros alimentados com rações contendo sementes de girassol em comedouros privativos. *Revista Brasileira de Zootecnia*, 37, 1860-1868.

Melton, S. L., Amiri, M., Davis, G. W., Backus, W. R. (1982). Flavor and Chemical Characteristics of Ground Beef from Grass-, Forage-Grain- and Grain-Finished Steers. *Journal of Animal Science, 55,* 77-87.

Mir, Z., Rushfeldt, M. L., Mir, P. S., Paterson, L. J., Weselake, R. J. (2000). Effect of dietary supplementation of either conjugated linoleic acid (CLA) or linoleic acid rich oil on CLA content of lamb tissues. *Small Ruminant Research, 36,* 25–31.

Moharrery, A. (2009). Effect of Docking and Diet Energy on Carcass Fat Characteristics in Fat-Tailed Baluchian Sheep. *Turkish Journal of Veterinary and Animal Sciences*, *33,* 95-103

Nurnberg, K., Wegner, J., Ender, K. (1998). Factors effecting fat composition in muscle and adipose tissue of farm animals. *Livestock Production Science, 56,* 145–156.

Palmquist, D. L., St-Pierre, N. R., McClure, K. E. (2004). Tissue fatty acid profiles can be used to quantify endogenous rumenic acid syntehsis in lambs. *Journal of Nutrition*, *134,* 2407-2414.

Pelegrini, L. F. V., Pires, C. C., Kozloski, G. V., Terra, N. N., Baggio, S. R., et al. (2007). Perfil de ácidos graxos da carne de ovelhas de descarte de dois grupos genéticos submetidos a dois sistemas de manejo. *Ciência Rural*, *37,* 1786-1790.

Precht, D., Molkentin, J. (2000). Recent trends in the fatty acid composition of German sunflower margarines, shortenings and cooking fats with emphasis on individual C16:1, C18:1, C18:2, C18:3 and C20:1 trans isomers. *Die Nahrung*, 44, 222-228.

Steinhart, H., Rickert, R., Winkler, K. (2003). Identification and analysis of conjugated linoleic acid isomers (CLA). *European Journal of Medical Research*, *8,* 370–372.

Rowe, A., Macedo, F. A. F., Visentainer, J. V., Souza, N. E., Matsuhita, M. (1999). Muscle composition and fatty acid profile in lambs fattened in drylot or pasture. *Meat Science, 51,* 283-288.

Rumsey, T. S., Oltjen, R. R., Bovard, K. P., Priode, B. M. (1972). Influence of widely diverse finishing regimens and breeding on depot fat composition in beef cattle. *Journal of Animal Science, 35,* 1069-1074.

Sañudo, C., Enser, M. E., Campo, M. M., Nute, G. R., María, G., Sierra, I., Wood, J. D. (2000). Fatty acid composition and sensory characteristics of lamb carcasses from Britain and Spain. *Meat Science*, *54,* 339-346.

SAS Institute Inc (1996) SAS/STAT. User's Guide,v. 6.11. 4th ed., v.2. SAS Institute Inc., Cary, 842 pp.

Sehat, N., Kramer, J. K. G., Mossoba, M. M., Yurawecz, M. P., Roach, J. A. G., Eulitz, K., Morehouse, K. M., Ku, Y. (1998). Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. *Lipids*, *33,* 963-971.

Smith, S. B., A. Yang, T. W. Larsen and R. K. Tume. (1998). Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. *Lipids*, *33,*197-207.

Smith, S. B., Gill, C. A., Lunt, D. K., Brooks, M. A. (2009). Regulation of Fat and Fatty Acid Composition in Beef Cattle. *Asian-Australasian Journal of Animal Sciences*, *22,* 1225-1233.

Waldman, R. C., Suess, G. G., Brungardt, V. H. (1968). Fatty acids of certain bovine tissue and their association with growth, carcass and palatability traits. *Journal of Animal Science*, *27,* 632-635.

Warren, H. E., Scollan, N. D., Nute, G. R., Hughes, S. I., Wood, J. D., Richardson, R. I. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. II: Meat stability and flavour. *Meat Science*, *78,* 270-278.

Westerling, D. B. & Hedrick, H. B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. *Journal of Animal Science*, *48,* 1343-1348.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R., Enser, M. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, *66,* 21-32.

Wood, J. D, Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, *78,* 343-358.

Zembayashi, M., Nishimura, K., Lunt, D. K., Smith, S. B. (1995). Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. *Journal of Animal Science*, *73,* 3325-3332.

CHAPTER II

(ARTICLE II)

Differentially transcribed genes in skeletal muscle of lambs in postnatal growth of four genetic groups raised in Brazilian Semi Arid

A.M.B.O. Lôbo[a*], S.E.F. Guimarães[a], S.R. Paiva[b], F.C. Flores[c], F.F. Silva[a], R.N.B. Lôbo[d]

[a] Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, s/n, Viçosa, MG, 36570-000, Brazil

[b] Embrapa Recursos Genéticos e Biotecnologia, Caixa Postal 02372, Brasília, DF, 70770-917, Brazil

[c] Embrapa Pecuária Sul, Caixa Postal 242, Bagé, RS, 96401-970, Brazil

[d] Embrapa Caprinos e Ovinos, Caixa Postal 145, Sobral, CE, 62010-970, Brazil

***Corresponding author.** Tel./ Fax: +55 88 3112 7487

E-mail address: oliveiraana@cnpc.embrapa.br/oliveiraana@yahoo.com.br

Embrapa Caprinos e Ovinos

Fazenda Três Lagoas - Estrada Sobral Groaíras, Km 4

Caixa Postal 145 - CEP 62010-970 - Sobral - CE - Brazil

Abstract

In the present study the oligonucleotide microarray technique was used to compare global gene expression profiles in *Longissimus dorsi* of Morada Nova (MO), Brazilian

Somali (SO) and Santa Inês (SI) and ½ Dorper x ½ Morada Nova (F1) crossbred lambs raised on irrigated pasture in the Brazilian Semi Arid region, and thus to prospect genes responsible for phenotypic differences between these genetic groups. The lambs were unrelated and contemporaries. Over 260 transcripts were differentially expressed among the comparisons. A total of 26 genes of known function, involved in skeletal muscle development (MyoD1 and IGFBP-4), lipogenesis (C/EBPδ and PPARγ) and fatty acid biosynthesis (PGDS), were differentially expressed at least in one comparison. Clustering analysis grouped all transcripts in 6 clusters by expression similarity. This analysis also revealed groups of genes with more expression similarities between MO and SI. This same profile was observed between F1 and SO. A heatmap with all differentially expressed genes suggests more similarities among MO, SI and F1, with SO breed presenting a more distinct expression pattern. Our study provides comprehensive knowledge on global gene expression and biological functionalities of differentially expressed genes in the main hair sheep breeds raised in Brazil. This information could be used for characterization and development of markers that can be used to the improve these breeds.

Keywords: Cluster, differential expression, IGFBP-4, MyoD1, Ovis aries

1. Introduction

The traits that determine productivity in beef cattle, and sheep have their basis in prenatal development and postnatal growth of skeletal muscle. In particular, yield of saleable meat and quality characteristics of the product are influenced by growth during the postnatal period (Harper, 1999). The understanding of growth and development of

skeletal muscle is one of the most important goals in animal and meat science and is also related to particular aspects of human medicine (te Pass et al., 2004).

Myogenesis in sheep is completed between 80 and 125 days of gestation (Ashmore et al., 1972a), although some muscles are completely formed at 140 days of gestation (Finkelstein et al. 1992). However, muscle fibers (myofibers) acquire myonuclei during embryonic myogenesis from myoblasts, or during postnatal myogenesis from satellite cells (Cardasis and Cooper 1976). Sheep satellite cell-derived myotubes produce both fast and slow myosin heavy chain (Molnar and Dodson 1992). Animals born with a higher number of muscle cells have greater muscling potential. Animals in which satellite cells are more active could potentially have greater muscle mass. The majority of DNA content of the muscle is accumulated during postnatal muscle growth and development, which is the direct result of satellite cell activity (Koohmaraie et al. 2002). However, postnatal skeletal muscle growth occurs through proliferation of satellite cells followed by differentiation and fusion with existing muscle fibers (Moss and LeBlond et al., 1971).

Relatively little is known about the postnatal skeletal muscle growth in sheep and there is need to identify the genes that play critical roles during this phase. Identification of key genes involved in specific developmental processes requires an understanding of the global patterns of gene expression in the specific tissue at the specific time. Gene transcript profiling technologies, such as microarrays, allow for genome-wide or tissue-specific examination of global changes in gene transcription. However, particularly in Brazil, little is known about the growth potential of sheep. To our knowledge, the global transcription profile of growing hair sheep in growing has not been previously reported.

In Brazil, the Northeast region is a large semi-arid area which has 54% of the Brazilian sheep population. The great majority of these animals are hair sheep, mainly from Brazilian local breeds, such as Santa Inês, Morada Nova and Brazilian Somali, as well as the recently imported Dorper. The consumption of sheep meat in this region is relatively high, mainly in rural areas. These animals are predominantly raised on native pasture ("Caatinga") that has a low production capacity. Alternatives are being established to increase the production of sheep meat in this region such as the use of irrigated pasture. As the meat demand from these animals by human population is increasing, the evaluation of their growth potential is necessary.

Differences among the three breeds cited above are evident. There are differences in size at birth, and development and muscle growth (Figueiredo et al. 1982). The Santa Inês (SI) breed is commercially important for meat production in Brazil and commonly used in crossbreeding as maternal breed in all regions of Brazil. Brazilian Somali and Morada Nova are important genetic resources and are characterized by small body size and slow growth when compared with SI (Rajab et al. 1992). On the other hand, the Dorper breed, recently introduced into Brazil from South Africa, has been extensively used in crossbreeding aiming to increase meat production.

The objective of this study was compare gene transcription profiles of *Longissimus dorsi* of lambs in postnatal growing in sheep from the Morada Nova, Brazilian Somali, Santa Inês, and ½ Dorper x ½ Morada Nova genetics groups using a *Sheep Oligo Microarray*.

2. Material and methods

2.1. Sampling

The field experiment was carried out the Experimental Research Station of Embrapa Caprinos e Ovinos, Sobral, CE – Brazil. A total of twenty-four unrelated males lambs (six of each breed) of Morada Nova (MO), Brazilian Somali (SO) and Santa Inês (SI) breeds and ½ Dorper x ½ Morada Nova (F1) born in the same season, from single births, weaned at an average of 84 d of age, were used in this study. All lambs were weaned on the same date and kept under the same feeding management on grass pasture, with efforts taken to prevent parasite infection. The lambs were raised on irrigated Tanzania (*Panicum maximun* Jacq cv. Tanzania) pasture with free access to water and mineral salt. The lambs were free to graze and they were supplemented once a day with concentrate (corn and soybean meal) at a rate of 1.5% of body weight. The animals underwent standard sanitary care; periodical faecal egg counts and the Famacha method were used to control gastrointestinal nematodes. Anthelmintic treatments were used when necessary. The experimental design was completely randomized. The conditions analyzed here seek to simulate the present conditions of production system for lambs in Brazil, where animals are exposed to various challenges such as rain, heat, parasites, etc. We seek to identify expressed markers for commercial production of meat.

The lambs used in this study were slaughtered at Embrapa Caprinos e Ovinos facilities with average of 200.18±7.54 days of age (young immature lambs) and 20.62±3.46 kg. Samples of tissue from *Longissimus dorsi* (LD) muscle (approximately 3 g) were collected immediately post slaughter and conserved in RNA stabilization solution (*RNA Holder*, BioAgency).

2.2. RNA isolation and labeling

The samples of tissue were kept in the RNA stabilization solution at 4°C overnight, thereafter excess solution was removed and stored at -20°C. After that, they were sent on dry ice to Laboratório de Biotecnologia Animal, at the Universidade Federal de Viçosa for processing. Total RNA was obtained using the RNeasy Miniprep kit (Qiagen Inc., Valencia, CA), according to the instructions of the manufacturer. Purified total RNA samples were stored at -80ºC for microarray experiments. RNA quality and integrity were determined using the Eukaryote Total RNA Nano 6000 assay (Agilent RNA 6000 Nano LabChip® Kit) on the Agilent Technologies 2100 Bioanalyzer and quantified by measuring A260nm on a UV/Vis spectrophotometer. Only samples with RNA Integrity Number (RIN) above 7.0 (Appendix 1) were used. Fifty nanograms (ng) of total RNA were reverse-transcribed to cDNA with a T7 sequence. T7 RNA polymerase-driven RNA synthesis was used for the preparation and labeling of RNA with Cy3 dye (Agilent Technologies).

RNA spike-in (Agilent RNA Spike-In kit) controls were used in the reverse transcription reaction to adjust possible dye effects following manufacturer's instructions. The Spike-in controls represent two sets of ten synthesized RNA mixtures derived from the Adenovirus E1A transcriptome with different concentrations in each set. These spike-in sets were mixed with samples and co-hybridized to arrays. The fluorescent cRNA probes were purified using Qiagen RNeasy Mini Kit (Qiagen Inc., Valencia, CA) and the mass yields and specific activities of the labeled cRNA targets were determined by measuring the absorbance spectra on a UV/Vis spectrophotometer. Labeled cRNA samples were sent on dry ice to Laboratório de Genômica Pediátrica from the Faculdade de Medicina – Universidade de São Paulo for hybridization, washing and scanning (described below).

2.3. Microarray experiment design

As the main objective of this experiment was to identify which transcripts were differentially expressed among the genetic groups (MO, SO, SI and F1) a design was used to provide six different comparisons: F1-MO, F1-SI, F1-SO, MO-SI, MO-SO, SI-SO. Six 8x15K slides were used and each array was considered as a block (48 arrays in total). Each slide contained all genetic groups, so the microarray design was randomized complete-block. Six biological replicates and two technical replicates were provided in each comparison. The total RNA collected from each sample (animal, n=6) of each genetic group (n = 4) were hybridized in two slides (technical replicates). Each array of one slide (8 per slide) was considered one block.

2.4. Hybridization and scanner

Equal amounts (600 ng) of Cy3 labeled cRNA probes were hybridized on an 8x15 K Sheep Agilent array (GEO accession: G4813A). The Agilent Gene Expression Hybridization Kit was used to hybridize fluorescently-labeled cRNA to microarrays. The hybridized slides were washed using a commercial kit package (Agilent Technologies, Palo Alto, CA, USA) and scanned using Agilent's DNA microarray scanner. To extract data from the probe, the Agilent Feature Extraction Software (www.agilent.com/chem/fe) was used.

2.5. Microarray data collection and analysis

Raw data sets were normalized for total fluorescence, which represents the total amount of cRNA hybridized to a microarray. The background correction was applied to the raw data (using LIMMA normexp + offset 50 method) and then the corrected values were normalized (within arrays) using the “quantile” method. After normalization, data

were log2-transformed. Quality control was carried out using a plot density tool. Spots from duplicate probes were averaged and the normalized data were analyzed using R version 2.11.1 with MAANOVA package (Bioconduction, R/ MAANOVA). The microarray analysis of variance (MAANOVA; Kerr et al. 2000) for detecting genes with differential expression was implemented. The model used was:

$$Y_{ijk} = \mu + gg_i + s_j + e_{ijk}$$

where is μ is overall mean, gg_i is the fixed effect of genetic group (i=1 to 4), s_j is the random effect of sample (j=1 to 24) and e_{ijk} the random residual effect.

The *t* test was used to estimate the significant differences for each transcripts in each comparison, where $P<0.05$ was considered as statistically different. The Fs statistic, based on the James-Stein shrinkage estimates of the error variance, was used. The false discovery rate was considered by the multiple test adjustment approach (FDR, Benjamin and Hochberg, 1995). The null versions of the test statistics was simulated by 1000 permutations with sample shuffling.

Heatmaps and clusters images were developed using specific functions of R and MAANOVA.

2.6. Correlation Analysis

Transcripts of known function gene and that were significant in microarray analysis were chose for the correlation analysis. The values for the expression of these genes in each animal predicted by the model cited above in microarray analysis were correlated with the traits related to fatty acid profile, carcass and meat quality of the same animal. Analyses were carried out with CORR procedure of SAS Institute, Inc. (1999), using Pearson coefficient ($P<0.05$).

3. Results and Discussion

The slaughter weight was highest in SI lambs (P<0.05) and similar to F1 lambs (P>0.05). F1 lambs had similar slaughter weight to SO lambs and MO lambs had the lowest slaughter weight (SI≥F1≥SO>MO; Table 3).

Table 3. Least-square means for slaughter weight (kg) of ½ Dorper x ½ Morada Nova (F1), Morada Nova (MO), Santa Inês (SI) and Brazilian Somali (SO)

Genetic groups	**Mean ± Standard Error**
½ Dorper x ½ Morada Nova	20,82 ± 1,31 ab
Morada Nova	14,38 ± 1,41 c
Santa Inês	23,98 ± 0,96 a
Brazilian Somali	19,54 ± 1,31 b

Means followed by different letter in the row are statistically different by t test (P<0.05);

The genome-wide expression profiling of each element (probe) was assigned to six different comparisons F1-MO, F1-SI, F1-SO, MO-SI, MO-SO, SI-SO. Gene expression analyses were performed using 39 array post background correction and normalization of raw data set (Appendix 2).

The probability test for differentially expressed transcripts, for all contrasts are show in the Volcano plots in the Figure 4. In graph, the red dots are the significant transcripts using multiple test adjustment for false discovery rates (FDR, Benjamin and Hochberg, 1995). A total of 262 differentially expressed transcripts were found among the four genetic groups (Table 4). A heatmap with the 262 differentially expressed genes is presented in Appendix 3 while Appendix 4 to 9 show heatmaps with differentially expressed genes in each of the six comparisons. Of the total transcripts, 239 are non-characterized and thus the BLASTn tool was used to search annotations of

homologous sequences (*Bos taurus*) which present the same correspondence at level of nucleotide (Table 4). A total of 23 transcripts of known function were differentially expressed in MO-SO (C1), F1-MO (C2), F1-SO (C3), MO-SI (C4), SI-SO (C5) and F1-SI (C6) comparisons (Table 5 and 6).

3.1. Genes differentially expressed in comparison MO-SO

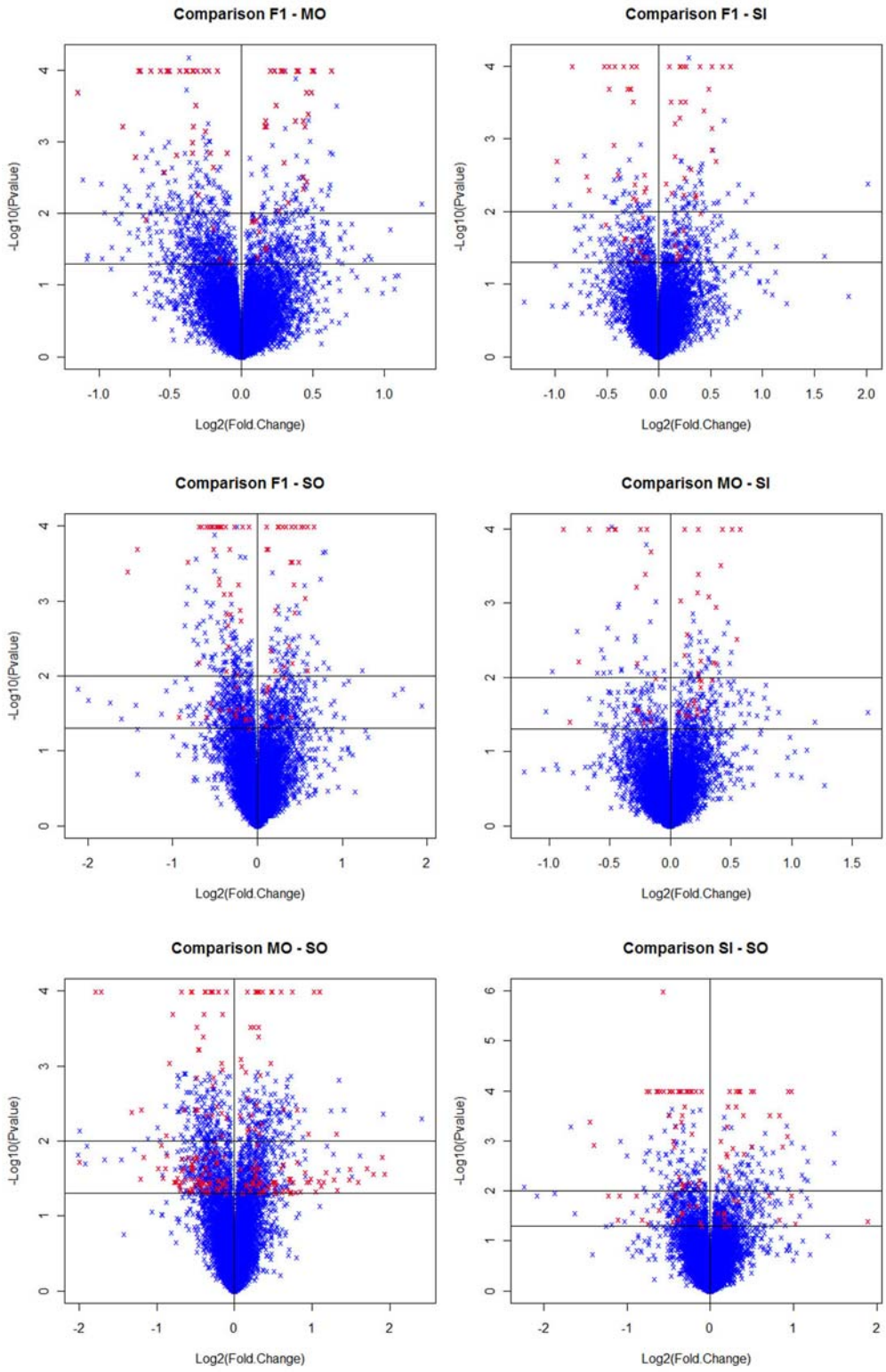
Ten transcripts of known function gene were differentially expressed only in the MO-SO comparison (Table 5). They were classified into eight categories according to their biological processes: innate immune response, transcription, chitin catabolic process, fatty acid biosynthesis, skeletal muscle tissue development, copper transport, apoptosis and ATP synthesis (Table 5).

The transcription factor CCAAT/enhancer binding protein δ (also known as C/EBPδ, CRP3, CELF, NF-IL6β) exhibited 2.2-fold higher differential expression in the LD of MO lambs compared to SO lambs. The role of the family of transcription factors, of which C/EBPδ is member, in preadipocyte differentiation processes in farm animals has been well described (Hirwa et al., 2010; Wang et al., 2009).

The higher expression of C/EBPδ transcripts in MO lambs (about 200 days of age), may indicate that the animals these breeds are starting the deposition of intramuscular, while SO lambs are still deposition muscle tissue. The C/EBPδ is active in the early adipogenesis and is required for subsequent activation of PPARg that is expressed during fat accumulation. The PPARγ was over expressed in F1 lambs compared to others breeds (discussed below). The synthesis of adipose tissue increases with the reduction in the synthesis of muscle tissue (Owens et al., 1995). In fact the expression of C/EBPδ in lambs in early postnatal life is practically zero and increases to 230 days (Byrne et al., 2010). In addition, fat development in muscle begins at the early

stage of animal development and the intramuscular fat content at these early stages is an essential factor in determining the final level of marbling in the adult animal (Hirwa et al., 2010).

Figure 4. The Volcano plot for all contrast (F1-MO; F1-SI; F1-SO; MO-SI; MO-SO; SI-SO). Genes represented by red dots above the second row are significant to P<0.01, and those genes represented by red dots between the two rows are significant to P<0.05 using multiple test adjustment for false discovery rates (FDR).



The 2.2-fold higher differential expression of MyoD1 in SO lambs suggests that proliferative activity of satellite cells, which is the source of new nuclei embedded the muscle fibers, is occurring at a higher rate than in MO. Similar to observed for C/EBPδ, it is possible that this higher activity of MyoD1 is due to lower SO precocity. This suggests that the higher expression of transcripts involved in the proliferation of satellite cells (MyoD; Table 5) in SO lambs is higher that the transcripts involved with deposition of intramuscular fat. Although the deposition of subcutaneous fat in this breed is great (principally in the tailed), according to Petrick et al. (2004) the deposition of fatty tissue is not uniform and intramuscular fat is in turn the type of fat deposited later.

Three transcripts involved with metabolism of fatty acid, prostaglandin D2 synthase (Table 5), stearoyl-CoA desaturase (SCD; sequence similar to *Bos taurus*, Table 4) and acetyl-CoA acyltransferase 1 (ACAA1; sequence similar to *Bos taurus*, Table 4) were higher in SO lambs compare to MO. Although the SCD has been over expressed the desaturase indexes (C18:1n9c/C18:0 and C16:1/C16:0), that provided an estimate SCD enzyme activity, was less active in SO lambs (Lobo et al., 2010 – Article I). In sheep, SCD gene expression is affected by feeding system, conjugated linoleic acid (CLA) and possibly by n6/n3 PUFAs (Dervishi et al., 2010).

3.2. Genes differentially expressed in the comparisons F1-MO, F1-SO, F1-SI and MO-SI

Transcripts IGFBP-4 and PPAR GAMMA (PPARγ) were over expressed this study. Insulin-like growth factor-binding proteins (IGFBPs) regulate the half-life of circulating IGFs. In addition to their endocrine function, IGFBPs also modulate IGF availability and biological activity in local tissues (Duan et al., 2010). The interaction

between IGF-I and IGFBPs is bidirectional. IGFBPs modulate the effects of IGF-I, but IGF-I also regulates the expression and/or production of IGFBPs (Durham et al., 1995).

Peroxisome proliferator-actived receptors (PPARs) are ligand-activated nuclear transcription factors that belong to the nuclear receptor superfamily. Three isoforms of PPAR have been identified, α, δ and γ, which play distinct roles in the regulation of key metabolic processes, such as glucose and lipid redistribution. PPARγ is mainly associated with adipose tissue, where it controls adipocyte differentiation and insulin sensitivity (Robinson and Grieve, 2009). Inducing PPARγ during differentiation is responsible for activating a number of genes involved in binding, uptake, and storage of fatty acids (Wu et al., 1995).

IGFBP-4 and PPARγ were more highly represented in F1 than MO, SO and SI lambs (Table 6). IGFBP-4 gene transcripts were of greater magnitude in LD of SI lambs compared to MO (C4; Table 6). The F1>SI>MO expression sequence for IGFBP-4 is consistent to the observed growth potential of these breeds. In addition the highest expression of PPARγ in F1 lambs suggest that deposition of intramuscular fat is higher in these animals than in others breeds. Higher expression of PPARγ in LD muscle of crossbred lambs has been found at 230 days of age (Byrne et al., 2010). In cattle, the mRNA expression for PPARγ displayed a peak of expression occurring between 25 e 30 months of age for Wagyu x Hereford animals, while in Piedmontese x Hereford the mRNA expression remained constant (Wang et al., 2009).

The fat content in cattle may be regulated by the PPARγ in a depot-specific manner (Wang et al., 2009). As demonstrated by Wu et al. (1995) in the early stages of adipogenesis, PPARγ expression is activated by CCAAT/enhancer binding protein β. CEBPβ gene transcripts were not differentially expressed in our study, only CEBPδ. On the other hand, there are evidences that Conjugated Linoleic Acid (CLA) induces

PPARγ and isomers of CLA have moderate affinity for binding and activating PPARγ (Belury et al., 2002; Evans, et al., 2000). We have found that the LD of F1 lambs of this study contain significant proportion of CLA (Lobo et al., 2010 – Article I), although we found no correlation between them (discussed below).

3.3. Transcrits related to energy and carbohydrate metabolism

Gene transcripts involved in glycolytic metabolism were differentially expressed in some comparisons. ATP5G1 and GGTA1 were highly transcribed in SO compared with MO lambs (Tables 1 and 2, respectively) that expressed more PYGL and GLUT-3 gene transcripts. PYGL and GLUT-3 were also more expressed in LD of F1 than SO lambs. GLUT-3 was higher expressed in LD of SI compared with MO, SO and F1. According Ashmore and Doerr (1971) the fibers can be classified based upon their energy-producing enzyme patterns. In addition, "red" fiber types metabolize and store more lipid than "white" fibers (Ashmore et al., 1972b), and that the quantity of intramuscular lipid is correlated with the proportion of fibers adapted for oxidative metabolism (Ashmore et al., 1973).

Additionally, there is evidence that in the genetics groups analyzed here the LD of the growing animals have more glycolytic and less oxidative fiber types. The presence of PYGL gene transcripts may indicate higher glycogen content. Glycolytic-type fibers generally contain less intramuscular fat and are implicated in meat aging after slaughter, yielding meat with greater tenderness (Hocquette et al., 1998). Lin and Hsu (2005) verified that transcripts involved in glycolytic metabolism, among them ATP synthases (H^+ transporting, mitochondrial F_1 or F_0), were more highly expressed in Duroc than Taoyuan pigs. The authors justified that Duroc loin muscle rely more on

glycolitic metabolism, using more carbohydrates and less lipids as fuel, relative to Taoyuan muscle.

3.4. Genes related to stress response and others

Gene related to innate immune and stress response (TRL6, CLEC6, and VDUP1) and acute-phase response (FVIII) were more highly expressed in muscle of MO lambs in relation to SO (Tables 5 and 6) and SI (Table 6), respectively. This trend was also observed in F1, which is a ½ MO.

TRL6 encodes a protein member of the tool-like receptor family which plays a fundamental role in pathogen recognition and activation of innate immunity. Toll-like receptor 6 (TLR6) is one of a series of highly conserved innate immune receptors (Tantisira et al., 2004). CLEC6A may be involved in regulating immune reactivity. VDUP1 is a stress-responsive gene and act as an oxidative stress mediator by inhibiting thioredoxin activity or by limiting its bioavailability.

VDUP1 gene is up-regulated by various stresses including H_2O_2, irradiation, heat shock, serum starvation, and transforming growth factor-β (TGF- β). Research suggest that up-regulation of VDUP1 expression is accompanied by the induction of apoptosis triggered by various agents, among them peroxisome proliferator-activated receptor gamma (PPARγ).

Over expression of genes related to immune and stress response may be related to a higher degree of adaptation of the MO breed that can be activing genes involved with a response to parasitism infection, since it was observed that the MO lambs of this study showed greater resilience to gastro-intestinal parasitism (Fernandes Junior, 2010).

These genes may also be actived by environmental factors that do not know and were not investigated in this study, such as rain, pasture humidity and heat.

Higher gene expression of signal processing protein or chitinase-3-like protein 1 (CHI3L1) was observed in the muscle of MO compared to SO and F1. For this same gene, the expression in SI was 1.16-fold and 1.20-fold higher than those for SO and F1, respectively (Table 6). This gene may play an important role in the capacity of cells to respond to and cope with changes in their environment. Mammalian chitinases and chitinase-like proteins are a group of molecules known to be upregulated and secreted in Th2-induced inflammatory responses, such as asthma, allergy and nematode infection (Knight et al., 2007). These authors verified that CHI3L1 transcripts are upregulated in abomasum and gastric lymph nodes in response to nematode infections (*Telardorsagia circumcincta*) in sheep. Here, despite of gastrointestinal nematodes control, high levels of infestation were observed due the system of management in irrigated pasture with high humidity.

Stem cell factor (also known as kit ligand [KITLG], mast cell growth factor, or steel factor) had higher expression in F1 compared to SO (C3; Table 6). On the other hand, SI presented lower expression for this gene in relation to MO, SO and F1 (C4, C5 and C6, respectively; Table 6). KITLG is a cytokine that triggers its biological effects by binding to its receptor, *c-kit* (Huang et al., 1990). Cytokines are mainly involved in regulation of immune response; however, recent evidence suggests they are involved in different physiologic processes such as cell growth and differentiation, tissue repair and remodeling and aging (Roubenoff et al., 1998). Cytokines that affect muscle cell function can be produced in the muscle intrinsically or produced by non-muscle cells, either locally in the muscle (extrinsic-local) or elsewhere (extrinsic-distant). There are limited studies of the intrinsic production of cytokines by muscle cells, but all of these

data point to the capacity and response to a variety of inflammatory and no inflammatory stimuli (details in review Zoico and Roubenoff, 2002).

OXTR gene transcripts were more highly expressed in LD of SO lambs in all comparison involving this breed. Oxytocin receptors are widespread throughout the central nervous system and modulate a variety of behaviors. These include responses to stress and anxiety, social memory and recognition, bonding, sexual and maternal behaviors. ATXO1 could bind and deliver cytosolic copper to the copper ATPase proteins. This gene may be important in cellular antioxidant defense. Additional evidence suggests that oxytocin regulates inflammatory processes in other tissues given the ubiquitous expression of the oxytocin receptor (Amrani et al., 2010).

Summarizing, the results suggest that each breed activates its immunological defense system differently and larger number of genes were more express in MO lambs than in other breeds. MO and SO breeds are well adapted to semi-arid region and grazing on native pasture ("caatinga").

3.5. Cluster Analysis

Cluster analysis (Figure 5) was carried out for all differentially expressed transcripts. The goal of cluster analysis was to group genes into clusters with similar profiles. The pattern of differential gene expression in the comparisons F1-MO, F1-SI, F1-SO, MO-SI, MO-SO, SI-SO can provide insights into biologically functional relevance among genes and uncover important trends. The genes were placed into six groups. The lists of genes within each cluster group can be seen in Appendix 10. Group 1 (G1) and group 2 (G2) represent genes that were expressed more in MO and SI, with higher magnitude in G2. Group 3 (G3) and group 4 (G4) represent genes that were more highly expressed in F1 and SO. There is a reversal magnitude between groups G1/G2

and G3/G4 groups. Group 5 (G5) represents genes of similar expression in all genetic groups while group G6 contain genes not covered by other groups, without a standard pattern of expression. The overall analyses of the behavior of the genome reveal groups of genes with similar expression and suggest new roles for some genes by association with the expression of other genes. The genes IGFBP-4, PGDS, PPARγ, GLUT-3, MyoD, C/EBPδ, GGTA1, PYGL, SCD and ATP5G1 were clustered in the same group, suggesting that they probably belong to the same metabolic pathway.

On the other hand, a heatmap with all differentially expressed genes (Appendix 3) suggests higher similarities between MO, SI and F1, with SO breed presenting a more distinct profile of expression. Actually, the origin of the breeds could explain this. MO and SI are local breeds from Brazil, originated from Portuguese and Spanish genetic groups and those brought from Africa with the slave trade. Despite the fact that the F1 has Dorper participation, it is a ½ MO. SO originated from BlackHead Persian breed brought to Brazil in more recent years (1939). This same breed gave origin the Dorper breed, which could explain some similarity between F1 and SO observed here.

Table 4. List of 236 differentially expressed transcripts. Numeric values represent fold change: values >1 indicates that the first genetic group has higher expression and values <1 indicates that the second genetic group has higher expression. Numeric values in bold corresponding P<0.05 and no bold values corresponding P<0.01.

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
EE751947	shisa homolog 2 (Xenopus laevis)	0.0	SHISA2					**0.624**	
EE752085								**0.864**	
EE752173	aminopeptidase B-like (LOC531757)	4E-28						**1.635**	
EE854466								**1.206**	
EE825881	retinoic acid induced 14-like	0.0	RAI14					**0.774**	
EE835610	SH3-domain GRB2-like endophilin B1	0.0	SH3GLB1					**1.449**	
FE029003	deoxynucleotidyltransferase, terminal, interacting protein 1	0.0	DNTTIP1					**1.256**	
EE812374								**3.825**	
EE832089								**0.734**	
DY496371	mitochondrial ribosomal protein S26	0.0	MRPS26					**0.630**	
CO202391	growth and transformation-dependent protein	0.0	LOC617104					**0.718**	
EE819911	chromosome 9 open reading frame 72 ortholog	0.0	C8H9orf72					**1.453**	
EE808996	origin recognition complex, subunit 6 like	0.0	ORC6						**0.917**
EE811348	5,10-methenyltetrahydrofolate synthetase	0.0							**0.796**
EE776683	zinc finger protein 768	0.0	ZNF768					**0.686**	
EE833255	solute carrier family 15, member 4	0.0	SLC15A4					**1.404**	
EE814522								**2.449**	
DY495383	amine oxidase, copper containing 3 (vascular adhesion protein 1)	0.0	AOC3					**0.676**	
EE808114	NIN1/RPN12 binding protein 1 homolog (S. cerevisiae)	0.0	NOB1					**1.442**	
EE832209	interferon-related developmental regulator 2	0.0	IFRD2					**1.174**	
FE032492	zinc finger protein 570-like	0.0	ZNF140					**1.166**	
EE755844	ATPase, H+ transporting, lysosomal accessory protein 2	0.0	ATP6AP2			**0.835**		**0.855**	**0.841**
EE851871								**0.623**	
EE752732						**1.229**			
EE772267	nucleoporin 43kDa	0.0	NUP43					**0.743**	
EE772498								**0.719**	

Continue

Continued

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
EE773271	nudix (nucleoside diphosphate linked moiety X)-type motif 3	0.0	NUDT3					**0.789**	
CN821697								**0.759**	
FE024992	inhibitor of growth family, member 3	3E-109	ING3					**0.800**	
FE025124	ribosomal protein L22-like 1	0.0	RPL22L1					**1.699**	
EE827449	heme oxygenase (decycling) 1	0.0	HMOX1					**1.201**	
EE829459	N-myc downstream regulated 1	0.0	NDRG1					**1.224**	
EE854994	polyhomeotic homolog 2 (Drosophila)	0.0	PHC2					**1.436**	
EE851357	shisa homolog 2 (Xenopus laevis)	0.0	SHISA2					**0.640**	
EE781147								**0.692**	
EE829264	transducin-like enhancer of split 4 (E(sp1) homolog (Drosophila)	0.0	TLE4					0.821	
EE824995	DDB1 and CUL4 associated factor 4	0.0	DCAF4					**1.152**	
EE814258	non-POU domain containing, octamer-binding	3E-29	NONO					**0.551**	**0.594**
EE785523								2.494	
EE868405								**0.662**	
EE834723	SDCCAG33 variant protein-like	0.0	TSHZ1					**1.365**	
EE804140	nei endonuclease VIII-like 2 (E. coli)	0.0	NEIL2				**0.564**		
EE791309	vacuolar protein sorting 24 homolog (S. cerevisiae)	0.0	VPS24					**0.777**	**0.768**
EE848777	RAN binding protein 10	0.0	RANBP10					**1.662**	
EE856300								**1.208**	
EE852749								**0.618**	
EE803910								**1.611**	
FE037117								**0.531**	
FE022000									**1.241**
EE774871								**1.660**	
EE792509	protein phosphatase, Mg2+/Mn2+ dependent, 1B	0.0	PPM1B					**1.535**	
EE795286	heterogeneous nuclear ribonucleoprotein D-like	2E-121	HNRPDL					**1.732**	
EE765035	anaphase promoting complex subunit 13	0.0	ANAPC13					**0.751**	
EE800480	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	0.0	PFKFB3					**2.510**	

Continue

Continued

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
EE770260	tRNA splicing endonuclease 34 homolog (S. cerevisiae)	0.0	TSEN34					**0.931**	
EE834850								**3.464**	**3.730**
EE848572	heme binding protein 2	0.0	HEBP2			0.625		0.560	
EE812615								**3.280**	
FE031903	chromosome 6 open reading frame 203 ortholog (C9H6ORF203)	0.0						**0.586**	
FE031762	nucleoporin 205kDa	0.0	NUP205					**0.849**	
EE780219	coiled-coil domain containing 152	8E-115	CCDC152					**2.135**	
EE838168						**0.739**		0.721	**0.751**
EE753128	DEK oncogene	0.0	DEK			**0.528**			
EE812141	GABA(A) receptor-associated protein like 1	0.0	GABARAPL1					**2.178**	
DY492665	guanine nucleotide binding protein (G protein), alpha	2E-116	GNAI2					**0.901**	
EE864160	matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase,72kDa type IV collagenase)	0.0	MMP2						**1.975**
FE022009	ubiquinol-cytochrome c reductase binding protein	0.0	UQCRB					**0.693**	
FE037577	cytokine receptor-like factor 3	3E-119	CRLF3					**0.766**	
EE778937	sel-1 suppressor of lin-12-like 3 (C. elegans)	0.0	SEL1L3					**0.862**	
EE779283	nuclear autoantigenic sperm protein (histone-binding)	2E-86	NASP					**1.559**	
EE848938	S100 calcium binding protein A14	3E-64	S100A14					**0.508**	
EE864166	mediator of RNA polymerase II transcription, subunit 8 homolog (yeast) (predicted)-like (MED8)	0.0						**1.261**	
EE811400	STAM binding protein-like 1	0.0	STAMBPL1					**0.804**	
EE748095	stearoyl-CoA desaturase (delta-9-desaturase)	0.0	SCD					**0.624**	
EE753590	nuclear prelamin A recognition factor-like	1E-132	NARFL					**0.599**	
EE753915								0.544	**0.541**
EE859098	suppressor of cytokine signaling 2	0.0	SOCS2					**1.411**	
EE754015	similar to Uncharacterized protein C18orf8	0.0						**1.226**	
EE754172	G protein-coupled receptor 125-like	0.0	GPR125					**1.448**	
FE034423								**1.917**	

Continue

Continued

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
EE874417								1.941	
EE801143	chromosome 10 open reading frame 119 ortholog (C26H10ORF119)	0.0						**1.223**	
EE783629	toll-like receptor 6	0.0	TLR6					**1.890**	
EE755637	transducer of ERBB2, 1	0.0	TOB1					0.437	**0.472**
EE773471	membrane metallo-endopeptidase	0.0	MME					**0.787**	
CF117640								**0.650**	
EE775850	archaelysin family metallopeptidase 2	0.0	AMZ2					**0.692**	
EE856892	hypothetical protein LOC539712	0.0	FAM122A				**1.270**	**1.179**	
EE822858	cardiolipin synthase 1	0.0	CRLS1					**0.683**	
EE832312	growth arrest and DNA-damage-inducible, alpha	0.0	GADD45A					**1.654**	
EE785588	Predicted hypothetical LOC513300	0.0					0.594	**0.687**	
EE854460	tumor necrosis factor, alpha-induced protein 8-like 3-like	0.0	TNFAIP8L3					**0.925**	
FE038466								**0.591**	
CN824409								**0.602**	
EE828696								**0.750**	
FE023103								**0.697**	
EE799584								**1.811**	**2.037**
EE748531	ribosomal RNA processing 1 homolog B (S. cerevisiae)	0.0	RRP1B					**1.273**	
DY505337	monoamine oxidase A	0.0	MAOA					**1.153**	
DY508649	glutathione S-transferase alpha 4	0.0	GSTA4					**0.687**	
EE746182	eukaryotic translation initiation factor 3, subunit D	0.0	EIF3D					**1.295**	
EE773337	secreted frizzled-related protein 2	0.0	SFRP2			**0.843**		**0.855**	
EE801047	major histocompatibility complex, class II, DM beta-chain	0.0	BOLA-DMB					**1.112**	
CN823486	glutamate-ammonia ligase	0.0	GLUL					**2.817**	
EE751998								**1.551**	
EE803089	transcription factor A, mitochondrial	0.0	TFAM					**0.796**	
EE757038								**1.188**	
EE801816	ATPase, Na+/K+ transporting, beta 3 polypeptide	0.0	ATP1B3					**1.298**	

Continue

Continued

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
EE757603	transcription factor MAFB-like	0.0	MAFB					**0.434**	
EE756575	ring finger protein 139	0.0	RNF139			**0.793**			
EE766196	NAD(P)H dehydrogenase, quinone 2	0.0	NQO2					**1.633**	**1.785**
DY486333	dynein light chain LC8-type 2	2E-171	DYNLL2					**0.810**	
EE752505	WD repeat domain 75	0.0	WDR75					1.761	**1.636**
EE750731	ring finger protein 215-like	0.0	RNF215					**0.746**	
EE784781	protein tyrosine phosphatase, mitochondrial 1	0.0	PTPMT1					**0.772**	
FE033253								**0.662**	**0.655**
EE797982	fumarylacetoacetate hydrolase domain containing 2A	0.0	FAHD2A					**0.673**	
EE793621	hypothetical protein LOC100335754	8E-135						**1.984**	
EE773802	zinc finger protein 22 (KOX 15)	0.0	ZNF22				1.463		0.675
EE774767	protein phosphatase 1, regulatory (inhibitor) subunit 3C	0.0	PPP1R3C					**0.251**	
EE794100	epidermal growth factor receptor pathway substrate 15	0.0	EPS15			**1.313**			
EE827115	GABA(A) receptor-associated protein like 1	0.0	GABARAPL1					**2.064**	
EE807729	SNAP-associated protein	0.0	SNAPIN					**3.765**	
EE835115						**0.662**		**0.719**	
FE037658	chromosome 18 open reading frame 55 ortholog (C17H18orf55)	0.0						**0.613**	
EE752685	testis expressed 2	0.0	TEX2					**0.685**	
EE800098	PX domain-containing protein C6orf145 homolog(LOC782137)	0.0						**1.431**	
EE746925	acetyl-CoA acyltransferase 1	0.0	ACAA1					**0.706**	
EE773642	similar to cytochrome P450 isoform 2J (LOC511936)	7E-41						**0.853**	
FE036588	S100 calcium binding protein A14	0.0	S100A14					**0.524**	**0.569**
FE022664	ERBB receptor feedback inhibitor 1	0.0	ERRFI1					**2.274**	
EE781069	chromosome 1 open reading frame 93 ortholog (C16H1orf93)	0.0						0.709	**0.769**
FE027121	hypothetical LOC783760	0.0	EIF2B5					**1.245**	
EE754694	Os05g0242100-like (LOC100335189)	0.0						**1.957**	
FE022752								**1.118**	

Continue

Continued

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
FE036768	TBC1 domain family, member 17	0.0	TBC1D17					**1.520**	
FE022668								**1.348**	
EE752544	zinc finger, AN1-type domain 5	0.0	ZFAND5					**1.752**	
EE752716	NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa (NADH-coenzyme Q reductase)	0.0	NDUFS1					**0.706**	
FE038561	HMG-box transcription factor 1	0.0	HBP1					**1.662**	
FE027322	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa	5E-37	EIF2S2	1.203	1.148	1.184			
EE783894	thioredoxin interacting protein	0.0	TXNIP			1.579		1.518	1.399
AY392761	chitinase 3-like 1 (cartilage glycoprotein-39)	0.0	CHI3L1	0.792	0.829			1.215	1.161
EE777898	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1	0.0	ATP6V0D1	0.695	0.718	0.776			
FE021265	PREDICTED:SDCCAG33 variant protein-like	0.0	TSHZ1	0.856	0.906			1.122	
EE810887	replication protein A3, 14kDa	0.0	RPA3		1.382		1.458		0.647
EE834974	general transcription factor IIH, polypeptide 5	0.0	GTF2H5		1.092	**0.940**	1.089	**0.937**	0.861
DY519030				**1.123**	0.806	**0.889**	0.717	0.792	
EE872389	very low density lipoprotein receptor	0.0	VLDLR			0.750		0.687	0.687
FE036016	erythrocyte membrane protein band 4.1 like 4A	0.0	EPB41L4A	1.392	1.374	1.369			
EE828327	squamous cell carcinoma antigen recognized by T cells 3	0.0	SART3	0.936			1.090	1.070	
EE818108	PREDICTED: methionine sulfoxide reductase B2-like	0.0	MSRB2	1.414	1.416			0.785	0.784
CN821865	CCAAT/enhancer binding protein (C/EBP), zeta	0.0	CEBPZ	0.563	0.629	**0.686**			
EE850133						0.569		0.578	**0.747**
EE845177	PREDICTED: Bos taurus ubiquilin 2-like, transcript variant 3	0.0	UBQLN2	0.786	0.700	0.726			
EE780115					**1.128**	1.329		1.245	1.183
EE801700	twinfilin, actin-binding protein, homolog 2 (Drosophila)	0.0	TWF2	0.701	**0.751**			1.690	1.577
EE831262	family with sequence similarity 92, member A1	8E-165	FAM92A1			0.875	**1.085**	0.891	0.821
FE033889	PREDICTED: hypothetical LOC513129	0.0			**0.904**		0.868		1.177
EE826084	PREDICTED: protein tyrosine phosphatase, receptor type, M	0.0	PTPRM	1.193	1.200	1.270			
DY500857	quinoid dihydropteridine reductase	0.0	QDPR		0.542		0.544		1.940

Continue

Continued

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
DY508106	fibronectin 1	0.0	FN1		**0.834**			1.254	1.421
EE751512	proteasome (prosome, macropain) subunit, alpha type 1	0.0	PSMA1	0.603	0.731	0.777	**1.214**	1.289	
FE029473	microsomal glutathione S-transferase 2	0.0	MGST2	1.202		1.455		1.211	1.314
EE792573	microfibrillar-associated protein 1	0.0	MFAP1	1.363	1.621	1.293	**1.189**		0.798
EE823492	mitochondrial ribosomal protein L14	0.0	MRPL14			0.696		0.758	0.719
EE863568	mannose-6-phosphate receptor (cation dependent)	0.0	M6PR			0.347		0.301	0.372
DY504785	aminoacyl tRNA synthetase complex-interacting multifunctional protein 2	0.0	AIMP2	0.642	0.783	0.762	**1.220**	**1.187**	
EE806421	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1	2E-81	ATP6V0E1	0.844			1.178	1.225	
EE832340	translocase of inner mitochondrial membrane 10 homolog(yeast)	0.0	TIMM10	0.810	0.699	**0.842**	**0.863**		**1.204**
EE857255	hypothetical LOC522091	0.0	ASXL1			1.169		1.189	1.232
EE856254						1.512		1.550	1.787
EE755227	cellular repressor of E1A-stimulated genes 1	0.0	CREG1	0.604	**0.793**	0.634	1.311		**0.800**
EE824976	transforming growth factor, beta receptor II (70/80kDa)	0.0	TGFBR2		1.055	1.079		1.053	
EE827364	zinc finger, MYND-type containing 8	0.0	ZMYND8	1.240	**1.128**	**1.118**		**0.902**	
EE855739	flavin containing monooxygenase 4	0.0	FMO4	0.702		0.786	1.370		0.817
EE766889	ubiquitin C, transcript variant 12	0.0	UBC	**0.631**				2.098	1.854
EE754710				1.415	**1.209**	1.417			
EE825378	bladder cancer associated protein	0.0	BLCAP	0.794	0.843	0.662		0.834	0.786
FE025048	hypothetical protein LOC100335789	0.0				0.327		0.283	0.376
EE756197	DnaJ (Hsp40) homolog, subfamily B, member 2	0.0	DNAJB2		**1.179**	0.830	1.192	**0.839**	0.704
CN824778	Metadherin	0.0	MTDH	0.786	0.808	0.801			
DY506359	COMM domain containing 9	0.0	COMMD9	0.827	0.817	0.732		0.886	**0.897**
EE752917	Ras and Rab interactor 2	0.0	RIN2	**1.097**		1.237		1.128	1.155
EE813874	proteasome (prosome, macropain) 26S subunit, ATPase, 3	0.0	PSMC3	0.799		0.712	**1.158**		0.770
EE831647	cellular repressor of E1A-stimulated genes 1	0.0	CREG1	0.684		0.679	1.248		0.795

Continue

Continued

ID	Gene Name	E-value	Symbol	F1-MO	F1-S1	F1-SO	MO-SI	MO-SO	SI-SO
CO202210	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	0.0	GNAI2	1.379	**1.329**	1.482			
EE828507	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	0.0	ATIC	**0.877**	0.858			1.251	1.278
EE804181	PREDICTED: unc-51-like kinase 1 (C. elegans)	0.0	ULK1		**0.929**	**1.077**		1.149	1.159
EE772219	PREDICTED: TBC1 domain family, member 4-like	0.0	TBC1D4			0.656		0.723	0.616
FE030661				**1.100**	0.808	**0.897**	0.735	0.816	**1.110**
EE746369	PREDICTED: Bos taurus RAB5B, member RAS oncogene family-like	0.0	RAB5B		0.865	**0.946**	0.875		1.094
CO202836	ubiquitin carboxyl-terminal esterase L1	0.0	UCHL1	0.954		0.890	1.052	0.886	

Table 5. Genes of known function differentially expressed in the MO-SO comparison

			Fold Change[2]	
GenBank ID	**Gene Symbol**	**Description**	**MO**	**SO**
Genes related to innate immune response[1]				
AM231302	Tlr6	Toll-like receptor 6	**1.081**	
AM167931	CLEC6A	C-type lectin domain family 6 member A	**1.056**	
Genes related to transcription				
DY495847	CEBPD	c/EBP delta gene for CCAAT/enhancer binding protein delta	**2.219**	
NM_001009426	C-MYC	Cellular myelocytomatosis oncogene	**2.807**	
Genes related to chitin catabolic process				
EF581383	LOC100101235	Stabilin-1 interacting chitinase-like protein		**1.360**
Genes related to fatty acid biosynthesis				
NM_001009257	PGDS	Prostaglandin D2 synthase		1.565
Genes related to skeletal muscle tissue development				
NM_001009390	MYOD1	Myogenic differentiation 1		**2.237**
Genes related to copper transport				
NM_001009429	SAH	Copper chaperone or Copper transport protein ATOX1 (ATOX1)		**1.362**
Genes related to apoptosis				
EE748871	ENDOG	Endonuclease G		**1.852**
Genes related to ATP synthesis and hydrogen transport				
NM_001009396	ATP5G1	ATP synthase, H+ transporting. mitochondrial Fo complex, subunit C1 (subunit 9)		**1.242**

[1]GO biological processes are described in the GeneRIF (Gene Reference Into Function); UniProt (Universal Protein Resource); DAVID (Database for Annotation, Visualization and Integral Discovery).

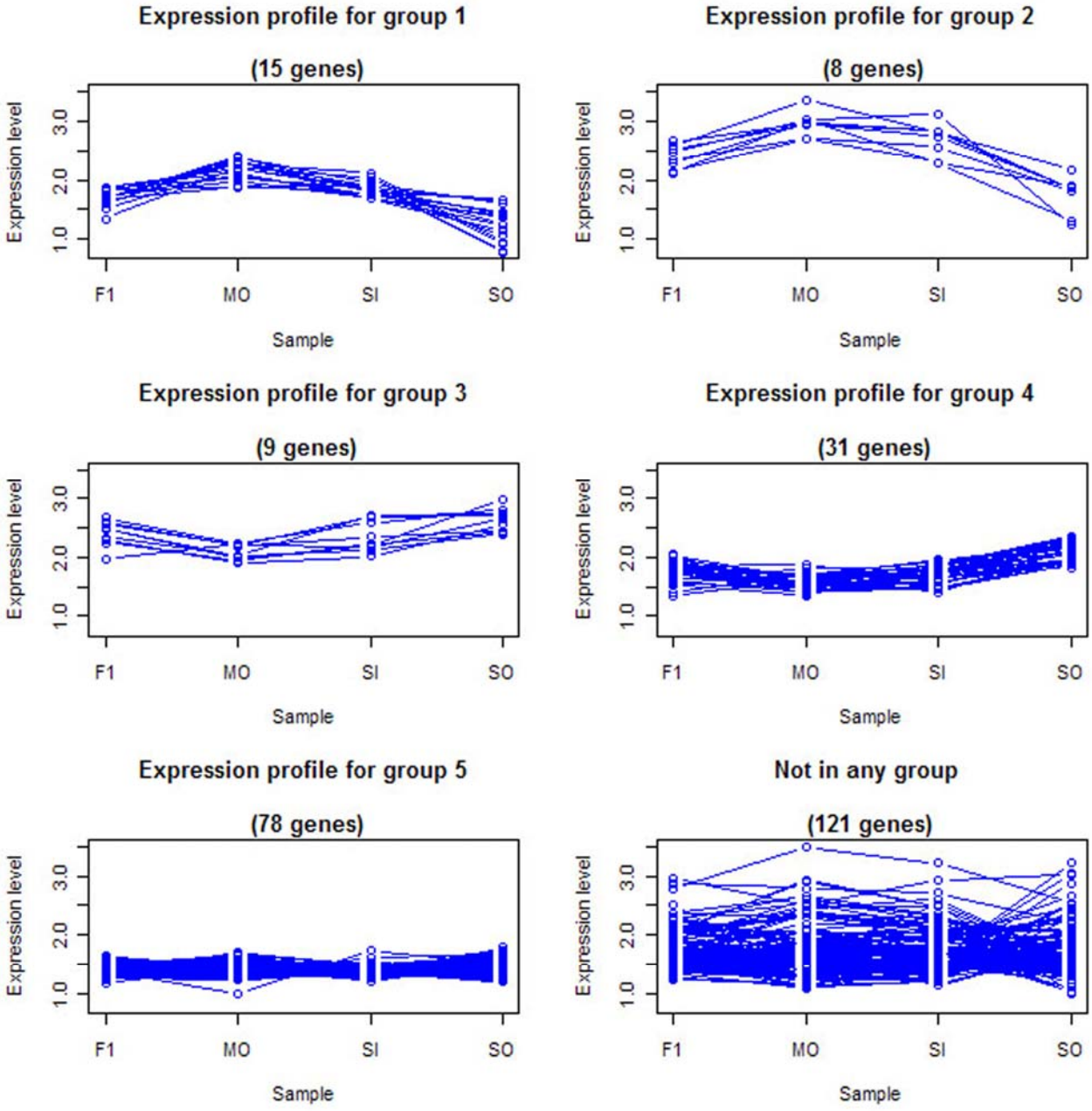
[2] Indicates the group with greater expression; numeric values in bold corresponding $P<0.05$ and no bold values corresponding $P<0.01$

Table 6. Genes of known function that were differentially expressed in six comparisons (C1-C6)

			FC-C1[2]		FC-C2[2]		FC-C3[2]		FC-C4[2]		FC-C5[2]		FC-C6[2]	
GenBank ID	**Gene Symbol**	**Description**	**MO**	**SO**	**F1**	**MO**	**F1**	**SO**	**MO**	**SI**	**SI**	**SO**	**F1**	**SI**
Gene related to immune and stress responses[1]														
EE783894	VDUP1	TO-DOWN-G1-4 vitamin D3 up-regulated protein 1	1.52				1.58				1.40			
Gene related to acute-phase response														
AF180523	FVIII	Factor VIII sequence	1.06				1.08		1.06				1.08	
Gene related to enzyme activator activity														
NM_001105261	LOC100125620	Guanylin precursor	**1.28**			1.23			**1.11**		**1.11**			**1.11**
Genes related to carboydrate metabolism														
NM_001024861	PYGL	Phosphorylase, Glycogen	1.27				1.33		**1.19**				1.25	
NM_001009770	GLUT-3	Glucose transporter type 3 or Solute carrier family 2 (SLC2A3)	**1.17**				1.23			1.22	1.43			**1.16**
NM_001009764	GGTA1	Alpha-1,3-galactosyltransferase		1.30	1.35							1.19	1.35	
Gene related to chitin catabolic process														
AY392761	LOC443279	Signal processing protein or Chitinase-3-like protein 1 (CHI3L1)	1.21			1.26					1.16			1.20
Genes related to amino acid metabolism														
AY162433	LAT2	L-type amino acid transporter subunit LAT2	1.12			1.12					1.09			1.09
Genes related to signaling														
NM_001009752	OTR	Endometrial oxytocin receptor (OXTR)		1.09				1.09				1.07		
DQ239650	LOC100037678	TO-UP-C22-8 serine protease 23 precursor		**1.18**				1.35	1.35			1.56	1.17	
Genes related to regulation of cell growth														
S77394	IGFBP-4	Insulin-like growth factor-binding protein-4			1.57		1.37			**1.22**			1.29	
Genes related to transcription														
NM_001100921	PPARγ	Peroxisome proliferator activated receptor gamma 1 protein			1.36		1.33						1.43	
Genes related to cell adhesion														
U89874	KITLG	Stem cell factor or Kit Ligand					**1.09**		1.10			**1.06**	1.16	

[1]GO biological processes are described in the GeneRIF (Gene Referende Into Function); UniProt (Universal Protein Resouce); DAVID (Database for Annotation, Visualization and Integral Discovery); [2]FC=Fold Change: Indicates the group with greater expression; numeric values in bold corresponding P<0.05 and no bold values corresponding P<0.01.

Figure 5. Patterns of differential expression. The mean value of each genetic group was independently plotted in graph.



3.6. Correlation between and phenotypic traits

Correlation coefficients among gene expression and phenotypic traits are presented in Table 7. A medium negative correlation was found between IGFBP4 and CLA percentage. The relationship between IGFBP-4 and CLA has been investigated, and studies suggest that inhibition of cancer cell growth by CLA may be mediated by decreasing IGF-II secretion in these cells. Cho et al. (2003) suggested that the production of IGFBP-4 is decreased by CLA.

In general, levels of MyoD gene expression were strongly correlated with the activity desaturase index, as well as saturated and monounsaturated fatty acids percentages. The Δ9 desaturase indices provide an estimate of stearoyl-CoA desaturase (SCD) enzyme activity (Corl et al., 2001) and determine the ratio substrate/product of this enzyme. This result implies that the highest expression of MyoD increases SCD enzyme activity and consequently increases the production of saturated fatty acids.

In the same, the expression of PGDS was directly related to the percentage of saturated fatty acids (C16:1) and the desaturase activity index. A contrary relationship between the expression of PGDS and percentages of polyunsaturated fatty acids (PUFA, P:S and n6) was observed.

MyoD expression was negatively correlated with daily weight gain (r = -0.45) and positively correlated with cold carcass yield (r = 0.66). These results clearly emphasize the role of MyoD in proliferating myoblast and its direct relationship with muscle mass since cold carcass yield is related to quantity of meat in carcass. The negative correlation between myoD and daily weight gain (GWD) could be justified since the GWD is not only related to muscle mass, but also with fat deposition, weight and growth of viscera and bones. Thus bias may be present in this correlation. MyoD is only expressed when satellite cells are activated to proliferate and differentiate into

primary myoblasts, which will then differentiate into cells of the myofibers. Muscle satellite cells possess multipotential mesenchymal stem cell activity and are capable of forming osteocytes and adipocytes as well as myocytes (Asakura et al., 2001).

The correlations of mRNA transcripts of the C/EBPδ with the percentages of saturated fatty acids (C14:0; myristic acid), monounsaturated (C16:1; palmitoleic acid), total monounsaturated fatty acid (MUFA) and desaturase indexes were negative, while the correlations of the expression of this gene with the percentages of polyunsaturated fatty acids, P:S ratio and EFA were strongly positive. This suggests that there is a direct relationship between expression of C/EBPδ and the percentage of polyunsaturated fatty acids in meat. The PUFAs may activate the expression of transcription factors. PUFAs are known to activate nuclear transcription factors such as PPARs, which modulate gene expression in response to environmental and dietary factors (Ntambi, 1999; Dervishi et al., 2010). Although we have not observed any correlation of the PPARγ with the traits analyzed, the C/EBP transcription factors (C/EBPδ and β) act synergistically with PPAR for triggering the adipocyte differentiation program (Rosen and Spiegelman, 2000). C/EBPδ expression was negatively correlated with traits involved in muscle mass and positively correlated with the quality of the meat.

SCD gene transcripts were positively correlated with the percentage of myristic fatty acid (C14:0), the atherogenicity index and index that estimates its activity. This suggests that the C18:1n9c/C18:0 index can be considered as a good predictor of desaturase activity in sheep under conditions of feeding management of this study. The atherogenic index has been proposed as a measure of the capacity of the diet to influence the incidence of coronary heart disease. Here, we found no correlation between SCD and CLA. Daniel et al. (2004) reported that the increased concentration of

Table 7. Significant correlations coefficients among the expression of some genes and the fatty acid composition, carcass and meat traits of lambs raised in cultivates pasture

	PGDS	**MYOD1**	**IGFBP4**	**GGTA1**	**PYGL**	**GLUT3**	**C/EBPδ**	**ATP5G1**	**SCD**
Meat luminosity							0.42		
Red intensity of meat					0.47	0.42	0.50		-0.52
Daily weight gain		-0.45						-0.41	
Slaughter weight							-0.47		
Cold carcass weight							**-0.56**		
Loin eye area							-0.44		
Fat Thickness					**-0.59**	-0.49	-0.51		
Cold carcass yield	0.49	**0.66**					**-0.74**		
				Fatty acids					
Saturated									
C10:0	**0.62**	**0.64**		0.49					
C14:0	**0.69**	**0.71**				-0.44	-0.50	0.45	0.50
C16:0	0.45	0.46							
Monounsaturated									
C16:1	**0.58**	**0.59**				-0.52	**-0.56**		
C18:1n9c		0.41				**-0.55**			
MUFA		0.40					-0.51		
Polyunsaturated									
C18:2n6c		-0.45		-0.36			**0.55**		
C18:2c9T11 CLA			-0.45						
PUFA	-0.46	-0.47		-0.36			**0.55**		
P:S	-0.45	-0.44		-0.30			0.50		
Essential Fatty Acid		-0.43					**0.55**		
n6	-0.46	-0.47		-0.36			**0.54**		
Atherogenic Index									0.48
C18:1n9c/C18:0	**0.55**	0.51				**-0.63**	-0.43		0.45
C16:1/C16:0	0.52	**0.55**				-0.53	**-0.54**		

Numeric values in bold corresponding P<0.01 and no bold values corresponding P<0.05;
MUFA = Total monounsaturated fatty acids (C14:1 + C16:1 + C16:1n7 + C17:1 + C18:1n9t + C18:1n9c + C20:1);
PUFA = Total polyunsaturated fatty acids (C18:2n6t + C18:2n6c + C18:3n6 + C18:2c9T11 + C18:3n3 + C20:3n6 + C20:3n3 + C20:4n6 + C20:5n3 + C22:4n6 + C22:6n3);
P:S = PUFA/(SFA - C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C22:0 + C24:0);
IA = (C12:0 + (4*C14:0) + C16:0)/(UFA - total unsaturated fatty acids);
n-6 = C18:2n6t + C18:2n6c + C18:3n6 + C20:3n6 + C20:4n6 + C22:4n6;
PGDS = Prostaglandin D2 synthase**; MYOD1** = Myogenic differentiation 1**; IGFBP4** = Insulin-like growth factor-binding protein-4**; GGTA1** = Alpha-1,3-galactosyltransferase**; PYGL** = Phosphorylase, glycogen**; GLUT3** = Glucose transporter type 3**; PPARγ** = Peroxisome proliferator activated receptor gamma 1 protein**; C/EBPδ = ; ATP5G1** = ATP synthase, H+ transporting, mitochondrial Fo complex, subunit C1 (subunit 9)**; SCD** = Stearoyl-CoA desaturase.

CLA in lambs fed with forage-based diets was associated with increase in the substrate for conversion to CLA and decrease in SCD gene expression. Dervishi et al. (2010) found, in the semitendinous muscle of lambs, that the SCD gene expression was low in the presence of CLA and low levels of PUFA n-6/n-3. These authors also observed positive correlation between oleic acid content and SCD gene expression in the muscle of lambs of the Rasa Aragonesa breed (data obtained by qRT-PCR).

Transcripts of the gene PYGL and GLUT3 had positive and negative correlation with red intensity of meat and fat thickness, respectively. In addition, the GLUT3 gene transcripts and the percentages of saturated and monounsaturated fatty acids and indices of desaturase were negatively correlated.

Negative relationship between GGTA1 transcripts and polyunsaturated fatty acid composition was found. The genes PYGL, GLUT3 and GGTA1 (glycolitic enzymes) are related to the metabolic type of muscle fibers. Glycolitic type fibers contain less intramuscular fat and are implicated in meat aging after slaughter, yielding meat with higher tenderness (Hocquette et al., 1998).

We found no studies that correlate the expression of these same genes. Dervishi et al. (2010) found moderate correlation (0.554) between the expression of SCD gene and the expression of PPARγ.

4. Conclusions

The results of this study provide comprehensive knowledge on global gene expression, and biological functionalities of differentially expressed genes in Brazilian local hair sheep. To our knowledge, this is the first report of global gene expression profile in hair sheep by oligonucleotide microarray.

We used oligo microarray to differentiate skeletal muscle gene transcription across four genetic groups of the hair sheep. Several genes in the skeletal muscle of genetic groups displayed significantly different transcription for complex traits.

The genes differentially expressed identified here are involved in various biological processes, among them fatty acid biosynthesis, skeletal muscle tissue development, transcription and immune and stress responses. Our study showed that lambs raised on pasture not only express differential genes related to muscle growth but also those activated by environmental conditions such as pathological stimulation, infection, exposure to injury, etc.

The expression of genes involved in adipogenesis in the *Longissimus* muscle can give a greater amount of marbling and shear force, since the amount of intramuscular fat directly affects these characteristics.

The myogenic factors (MyoD1 and IGFBP-4) differentially transcribed suggest proliferative activity of satellite cells. Cluster analysis grouped these two transcripts in the same group of transcritos involved in fat deposition and fatty acid synthesis, suggesting that they probably belong to the same metabolic pathway. These findings are important since until now the genes have been studied individually and genes may behave differently when analyzed together. The genes found here can be considered important candidates genes involved in production traits.

Importantly, these livestock studies demonstrate the utility of gene expression profiling as a tool for the discovery of genes contributing to quantitative variation among breeds with respect to the analyzed trait. The differentially expressed genes could be directly responsible for the differences observed these breeds.

The relationship observed here between IGFBP4 gene transcripts and percentage of CLA in muscle of grass fed lambs, suggested that CLA may inhibit gene expression

of IGFBP4. More studies will be necessary to elucidate the mechanisms by which CLA affect the IGFBP4 expression.

We identified strong relation between MyoD and important trait related to carcass yield, therefore, is considered as candidate gene for meat production traits in hair sheep.

The results indicate that the manipulation of the expression of the genes studied here may contribute to a better fatty acid profile and meat quality of lambs. The results provide more information about to potential of production of hair sheep and can be used for to improvement these breeds. However, the validation of these results by quantification of transcripts using real time PCR is needed.

In addition, our results provide important information that could be used for characterization and development of markers that can be used to the improvement of these breeds which are important genetic resources of the Brazil. The breeding of these breeds, particularly in relation to meat quality, is necessary since quality is a major factor limiting the consumption of sheep meat.

Acknowledgements

The authors acknowledge Embrapa for the financial support. The first author thanks CNPq and Universidade Federal de Viçosa for the studentship and Regina Maki Sasahara (Product Specialist, GE Healthcare) for their technical and scientific support for the experiment of microarray. Thanks to all employees, researchers and students of the Laboratório de Genômica Pediátrica (Faculdade de Medicina da Universidade de São Paulo) for your generous hospitality during my stay in the laboratory and the enormous contribuition of the microarray experiment.

5. References

Amrani, Y., Syed, F., Huang, C., Li, K., Liu, V., Jain, D. et al. 2010. Expression and activation of the oxytocin receptor in airway smooth muscle cells: Regulation by TNFa and IL-13. Respir. Res., 104, 1-12.

Asakura, K., Rudnicki, M. A., Komaki, M. (2001). Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic, and adipogenic differentiation. Differentiation, 68, 245-253.

Ashmore, C. R., Doerr, L. 1971. Comparative aspects of muscle fiber types in different species. Exp. Neurol. 31, 408-418.

Ashmore, C.R., Robinson, D.W., Rattray, P., Doerr, L. 1972a. Biphasic development of muscle fibers in the fetal lamb. Exp. Neurol. 37, 241-255.

Ashmore, C.R., Thompkins, G., Doerr, L. 1972b. Postnatal development of muscle fiber types in domestic animals. J. Anita. Sci. 34, 37-41.

Ashmore, C.R., Addis, P.B., Doerr, L. 1973. Development of muscle fibers in the fetal Pig. J. Anim. Sci. 36, 1088-1093.

Belury, M.A., Moya-Camarena, S.Y., Lu, M., Shi, L., Leesnitzer, L.M., Blanchard, S.G. 2002. Conjugated linoleic acid is an activator and ligand for peroxisome proliferator-activated receptor-γ (PPARγ). Nutr. Res. 22, 817-824.

Benjamini, Y., Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Statist. Soc. B. 57, 289-300.

Billiet, L., Furman, C., Larigauderie, G., Copin, C., Page, S., Fruchart, J.C. et al. 2008. Enhanced VDUP-1 gene expression by PPARγ agonist induces apoptosis in human macrophage. J. Cell. Physiol. 214, 183-191.

Byrne, K., Vuocolo, T., Gondro, C., White, J.D., Cockett, N.E., Hadfield, T. et al. 2010. A gene network switch enhances the oxidative capacity of ovine skeletal muscle during late fetal development. BMC Genomics 11, 1471-2164.

Cardasis, C.A., Cooper, G.W. 1976. An analysis of nuclear numbers in individual muscle fibers during differentiation and growth: a satellite cell-muscle fiber growth unit. J. Exp. Zool. 191, 347-358.

Cho, H.J., Lee, H.S., Cheng, C.K., Kang, Y.H., Ha, Y.L., Park, H.S., Park, J.H. (2003). Trans-10, cis-9 conjugated linoleic acid reduces insulin-like growth factor-II secrection in HT-29 human colon cancer cells. J. Med. Food, 6, 193-199.

Corl, B.A., Baumgard, L.H., Dwyer, D.A., Griinari, J.M., Phillips, B.S., Bauman, D.E. (2001). The role of 9-desaturase in the production of cis-9, trans-11 CLA. J. Nutrit Biochem., 12, 622-630.

Daniel, C.T.R., Wynn, R.J., Salter, A.M., Buttery, P.J. (2004). Differing effects of forage and concentrate diets on the oleic acid and conjugated linoleic acid content of sheep tissues: The role of stearoyl-CoA desaturase. J. Anim Sci, 82, 747-758.

Dervishi, E., Serrano, C., Joy, M., Serrano, M., Rodellar, C., Calvo, J.H. 2010. Effect of the feeding system on the fatty acid composition, expression of the Δ9-desaturase, peroxisome proliferator-actived receptor alpha, gamma, and sterol regulatory element binding protein 1 genes in the semitendinous muscle of light lambs of the Rasa Aragonesa breed. BMC Vet. Res. 40, 1-11.

Duan, C., Ren, H., Gao, S. 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: Roles in skeletal muscle growth and differentiation. Gen. Comp. Endocr. 167, 344–351.

Durham, S.K, Leon, D.D, Okazaki, R., Riggs, B.L, Conover, C.A. 1995. Regulation of insulin-like growth factor (IGF)-binding protein-4 availability in normal human osteoblast-like cells: role of endogenous IGFs. J. Clin. Endocrinol. Metab. 80, 104–110.

Evans, M., Pariza, M., Park, Y., Curtis, L., Kuebler, B., McIntosh, M. 2000. Trans-10*cis*-12 conjugated linoleic acid reduces triglyceride content while differentially affecting peroxisome proliferator activated receptor-γ2 and aP2 expression in 3T3–L1 preadipocytes. Lipids 36, 1223-1232.

Fernandes Junior, G.A. (2010) Desempenho produtivo e qualidade da carne de ovinos terminados em pastagem irrigada no semi árido nordestino. [Productive performance and meat quality of sheep finished in irrigated pasture in the northeastern semi arid]. Dissertação de Mestrado, Universidade Federal do Ceará, 88 p.

Figueiredo, E.A.P., Simplicio, A.A., Pant, K.P. 1982. Evaluation of sheep breeds for early growth in tropical North-East Brazil. Trop. Anim. Health Prod. 14, 219-223.

Finkelstein, D.I., Andrianakis, P., Luff, A.R., Walker, D.W. 1992. Development changes in hindlimb muscles and diaphragm of sheep. Am. J. Physiol. 4, R900-908.

Harper, G.S. 1999. Trends in skeletal muscle biology and the understanding of toughness in beef. Aust. J. Agric. Res. 50, 1105–1129.

Hirwa, C. d'Andre., Wallace, P., Xu Shen**,** Qinghua Nie**,** Guanfu Yang, Xiquan Zhang. 2010. Genes Related to Economically Important Traits in Beef Cattle. Asian J. Anim. Sci. 5, 34-35.

Hocquette, J. F., Ortigues-Marty, I., Pethick, D. W., Herpin, P., Fernandez, X. 1998. Nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat-producing animals. Livest. Prod. Sci. 56, 115–143.

Huang, E., Nocka, K., Beier, D.R, Chu, T.Y, Buck, J., Lahm, H.W. et al. 1990. The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. Cell. 63, 225–233.

Ikai, K., Kudo, H., Toda, K., Fukushima, M. 1998. Induction of apoptosis, p53 and heme oxygenase-1 by cytotoxic prostaglandin Δ12–PGJ2 in transformed endothelial cells. Prostaglandins Leukot. Essent. Fatty Acids 58, 295–300.

Kerr, M.K., Martin, M., Churchill, G.A. 2000. Analysis of variance for gene expression microarray data. J Comput. Biol. 7, 819-837.

Kim, S.Y., Suh, H-W., Chung, J.W., Yoon, S-R., Choi, I. 2007. Diverse Functions of VDUP1 in Cell Proliferation, Differentiation, and Diseases. Cell. Mol. Immunol. 5, 345-351.

Knight, P.A., Pate, J., Smith, W.D., Miller, H.R.P. 2007. An ovine chitinase-like molecule, chitinase-3 like-1 (YKL-40), is upregulated in the abomassum in response to challenge with the gastrointestinal nematode, Teladorsagia circumcincta. Vet. Immunol. Immunopathol. 120, 55–60.

Koohmaraie, M., Kent, M.P., Shackelford, S.D, Veiseth, E., Wheeler, T.L. Meat tenderness and muscle growth: is there any relationship? Meat Sci. 62, 345-352.

Lin, C.S., Hsu, C.W. 2005. Differentially transcribed genes in skeletal muscle of Duroc and Taoyuan pigs. J. Anim. Sci. 83, 2075-2086.

Molnar, G., Dodson, M.V. 1992. Characterization of sheep semimembranosus muscle and associated satellite cells: expression of fast and slow myosin heavy chain. BAM 2, 183-190.

Moss, F.P, LeBlond, C.P. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170, 421–436.

Ntambi, J.M. (1999). Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid Res., 40, 1549-1558.

Pethick, D.W. et al. (2004). Growth, development and nutritional manipulation of marbling in cattle: A review. Aust. J. Exper. Agric., 44, 705-715.

R Development Core Team: [http://www.R-project.org]. 2004. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rajab, M.H., Cartwright,T.C., Dahm, P.F., Figueiredo, E.A. 1992. Performance of three tropical hair sheep breeds. J. Anim. Sci. 9, 3351-3359.

Reecy, J. M., S. A. Miller, Webster, M. 2003. Recent advances that impact skeletal muscle growth and development research. J. Anim. Sci. 81, E1–E8.

Robinson, E., Grieve, D.J. 2009. Significance of peroxisome proliferator-activated receptors in the cardiovascular system in health and disease. Pharmacol. Ther. 122, 246-263.

Rosen, E.D., Walkey, C.J., Puigserver, P., Spiegelman, B.M. 2000. Transcriptional regulation of adipogenesis. Genes Dev. 14, 1293–1307.

Roubenoff, R., Harris, T.B, Abad, L.W, Wilson, P.W.F., Dallal, G.E., Dinarello, C.A. 1998. Monocyte cytokine production in an elderly population: effect of age and inflammation. J. Gerontol. 53, M20–26

Tantisira, K., Klimecki, W.T., Lazarus, R., Palmer, L.J., Raby, B.A., Kwiatkowski, J. et al. 2004. Toll-like receptor 6 gene (TLR6): single-nucleotide polymorphism frequencies and preliminary association with the diagnosis of asthma. Genes Immun. 5, 343-346.

te Pas, M.F.W., Everts, M.E., Haagsman, H.P. (editors). 2004. Muscle development of livestock animals: physiology, genetics, and meat quality. CABI Publishing, Netherlands, 432p.

Urade, Y., Hayaishi, O. Prostaglandin D synthase: structure and function. 2000. Vitam. Horm. 58, 89-120.

Wang, Y.H., Bower, N.I., Reverter, A., Tan, S.H., De Jager, N., Wang, R., et al. 2009. Gene expression patterns during intramuscular fat development in cattle. J Anim Sci 87, 119-130.

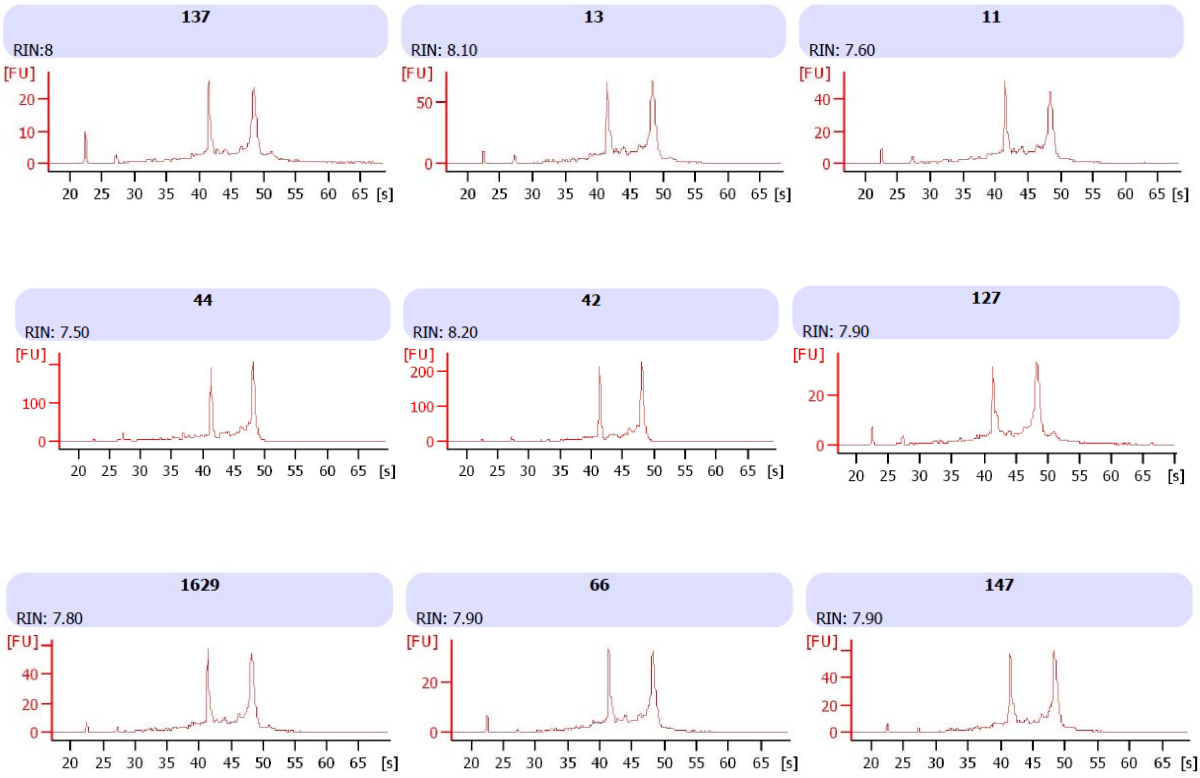
Weintraub, H, Davis, R., Tapscott, S., Thayer, M., Krause, M., Benezra, R., et al. 1991. The myoD gene family: nodal point during specification of the muscle cell lineage. Science 251, 761-766.

Wu, Z., Xie Y, Bucher, N.L, Bucher, N.L.R., Farmer, S.R. 1995. Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis. Genes Dev. 9, 2350–2363.

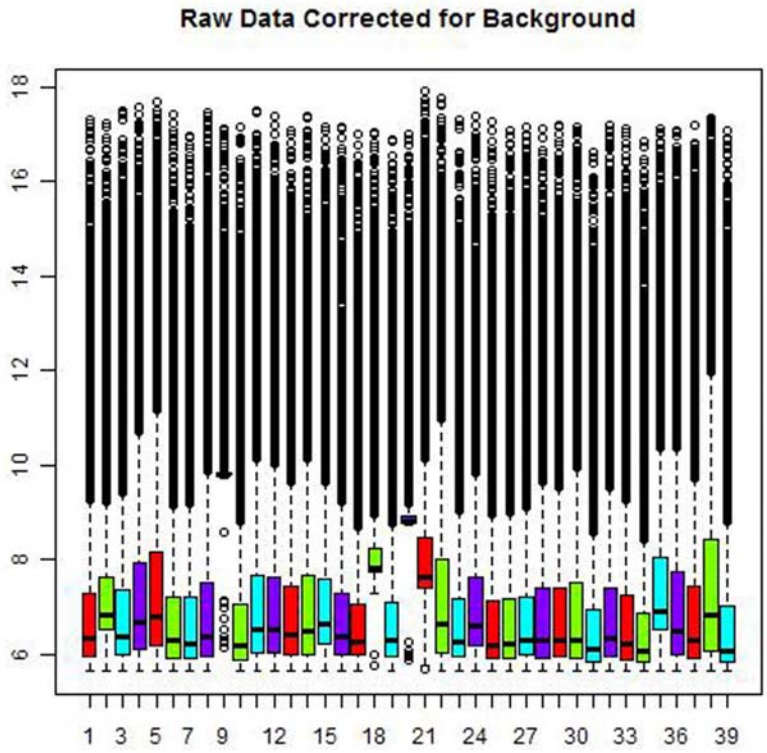
Yang, H., Mammen, J., Wei Wei, Menconi, M., Evenson, A., Fareed, M., et al. 2005. Expression and activity of C/EBPβ and δ are upregulated by dexamethasome in skeletal muscle.

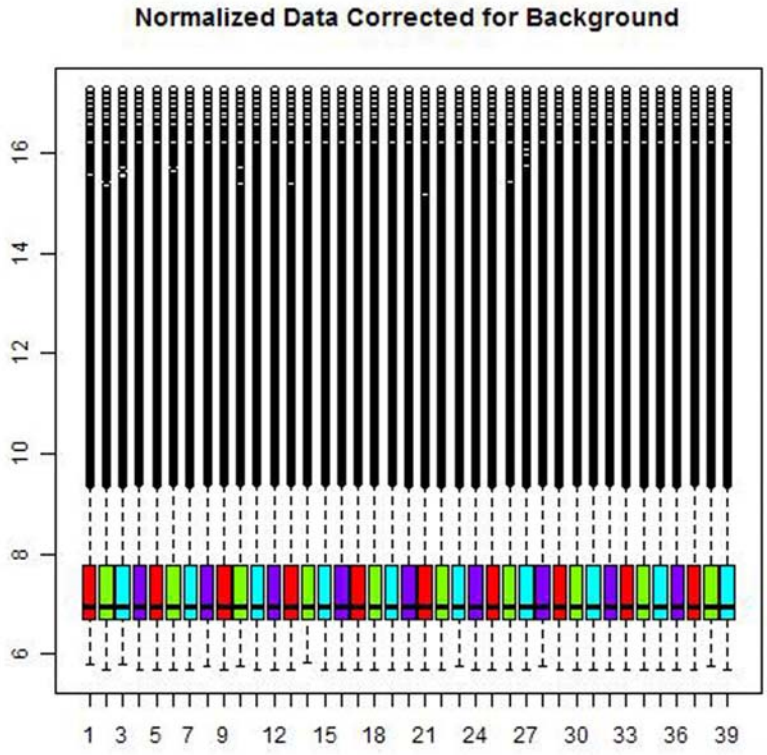
Zoico, E.M.D., Roubenoff, R.M.D. 2002. The role of cytokines in regulating protein metabolism and muscle function. Nutr. Reviews 60, 39-51.

Appendix 1. Electropherogram – RNA Integrity Number (RIN) of some samples used in the synthesis of RNA.

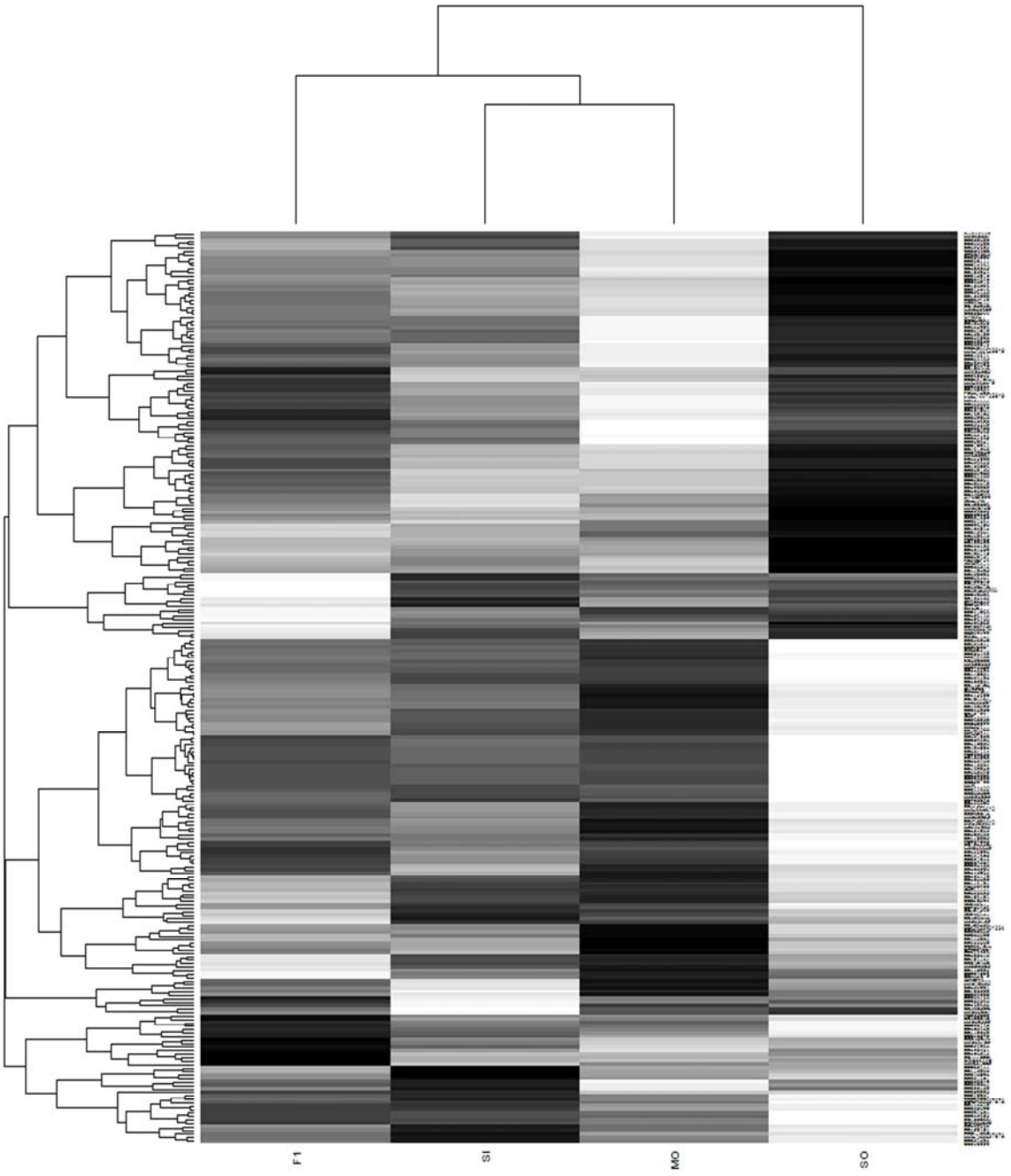


Appendix 2. Microarrays data after background correction and normalization.

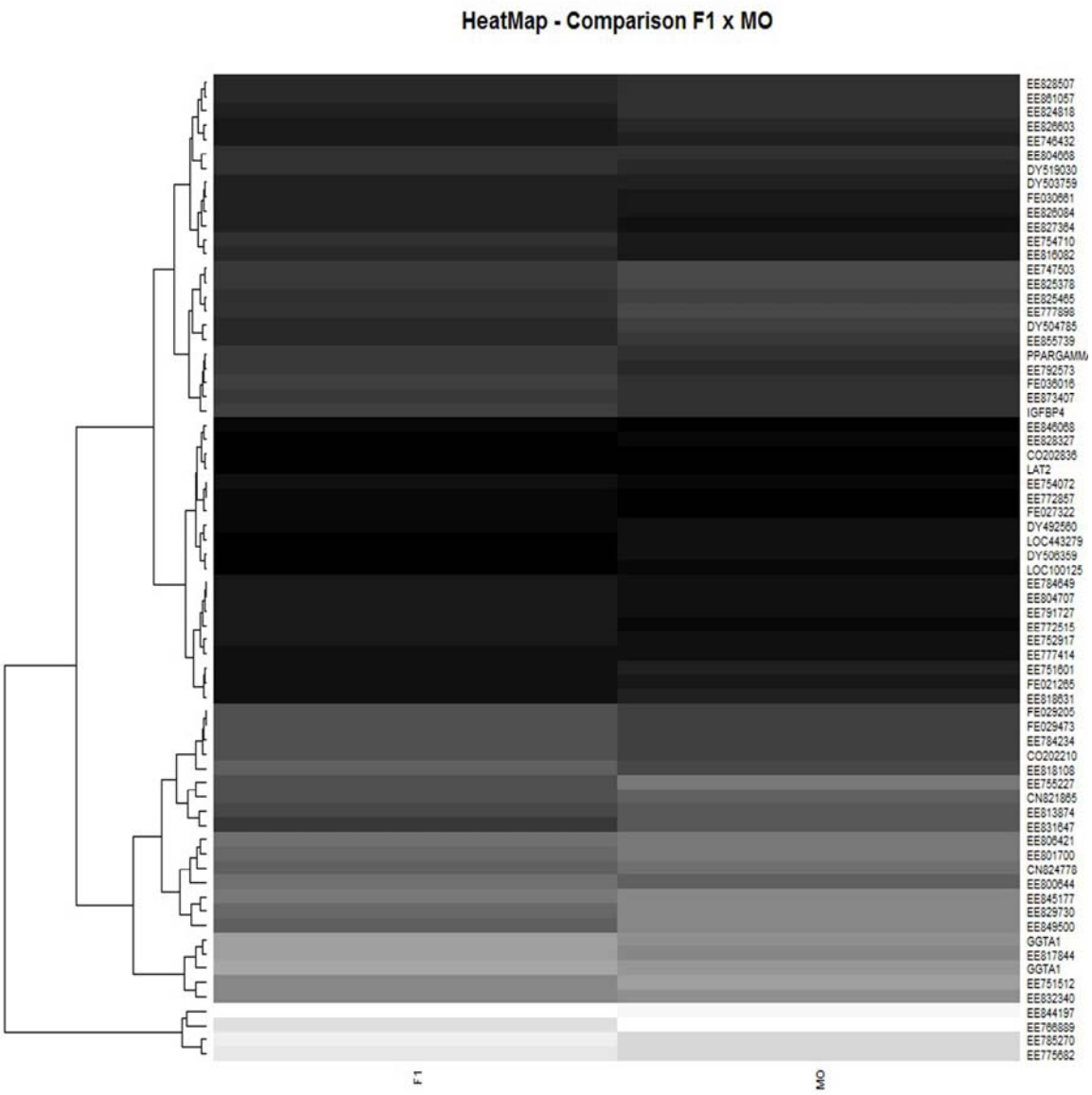




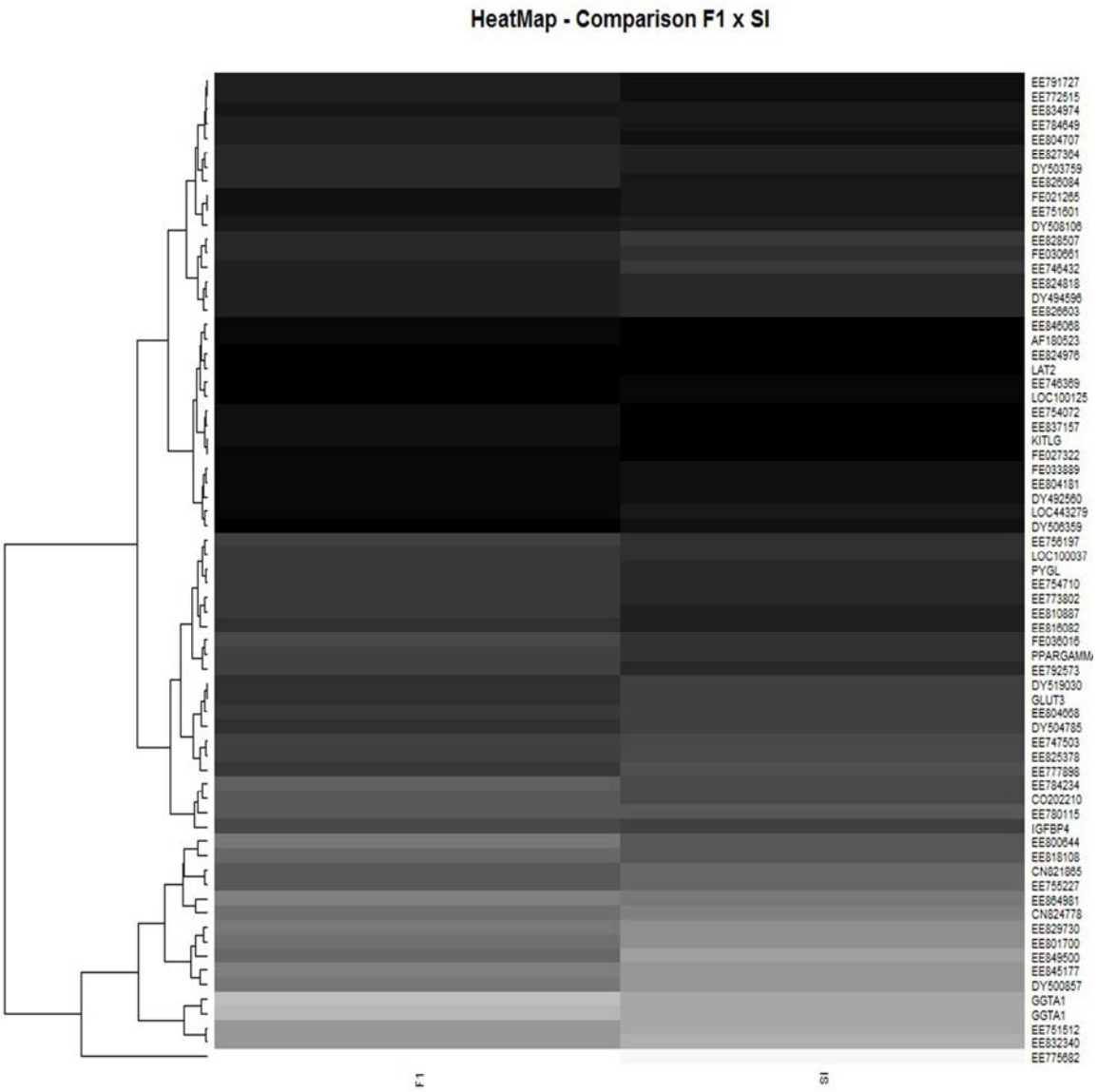
Appendix 3. Heatmap with 262 differentially expressed genes. Genes that are expressed at higher levels are assigned progressively brighter of white in the group, while genes expressed at low levels are assigned shades of black in the group.



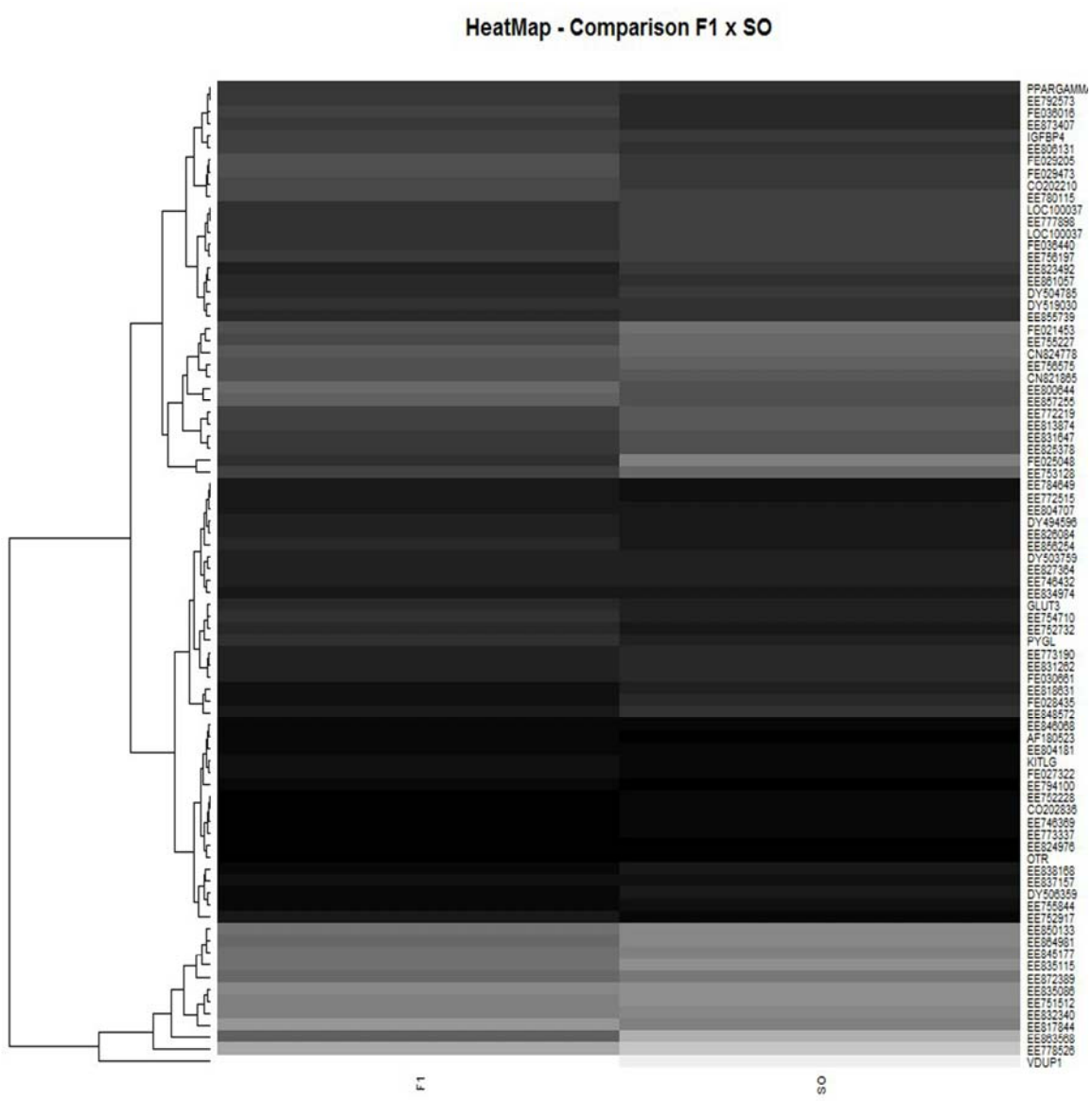
Appendix 4. Heatmap with genes differentially expressed in the comparison F1-MO. Genes that are expressed at higher levels are assigned progressively brighter of white in the group, while genes expressed at low levels are assigned shades of black in the group.



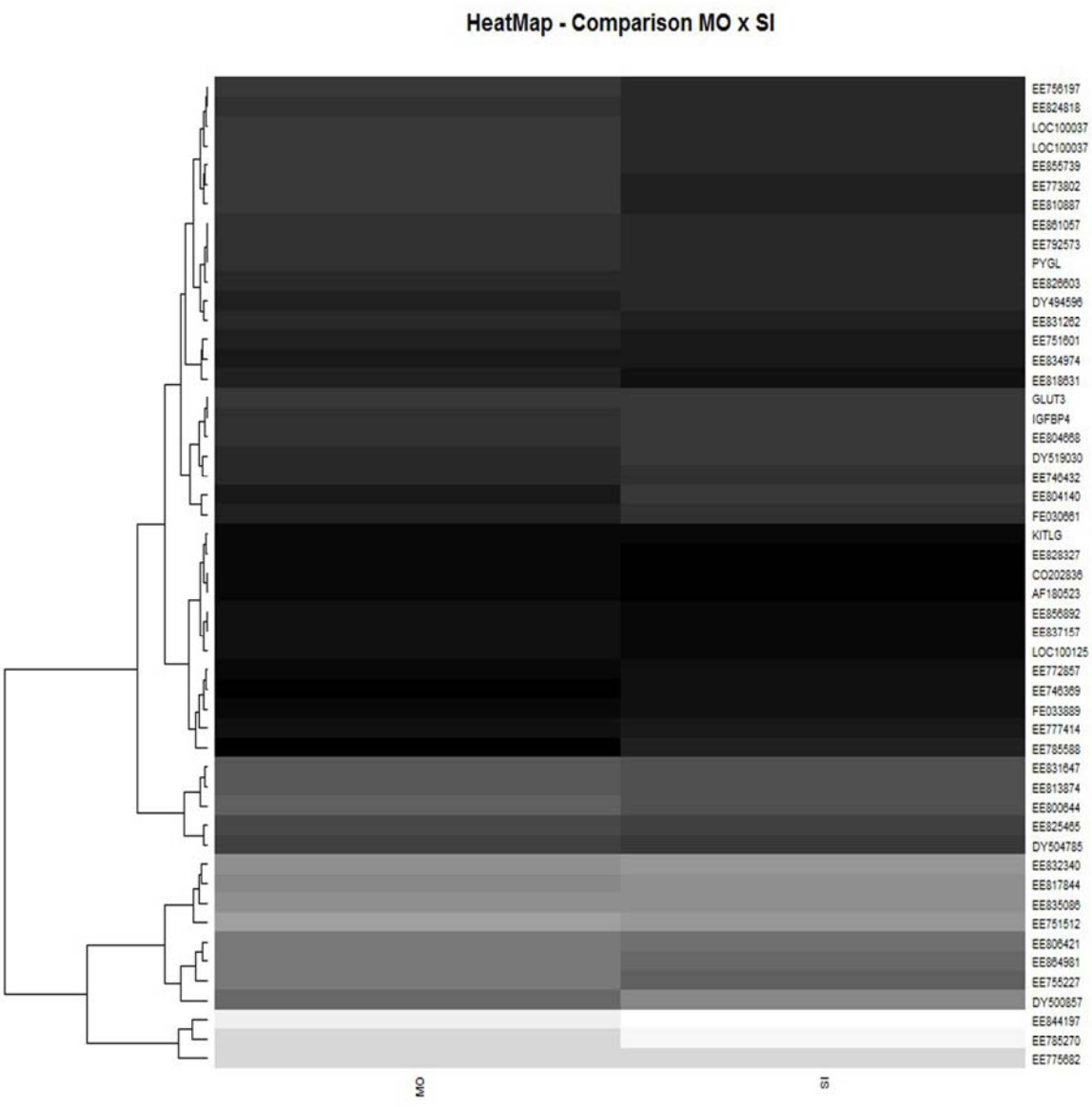
Appendix 5. Heatmap with genes differentially expressed in the comparison F1-SI. Genes that are expressed at higher levels are assigned progressively brighter of white in the group, while genes expressed at low levels are assigned shades of black in the group.



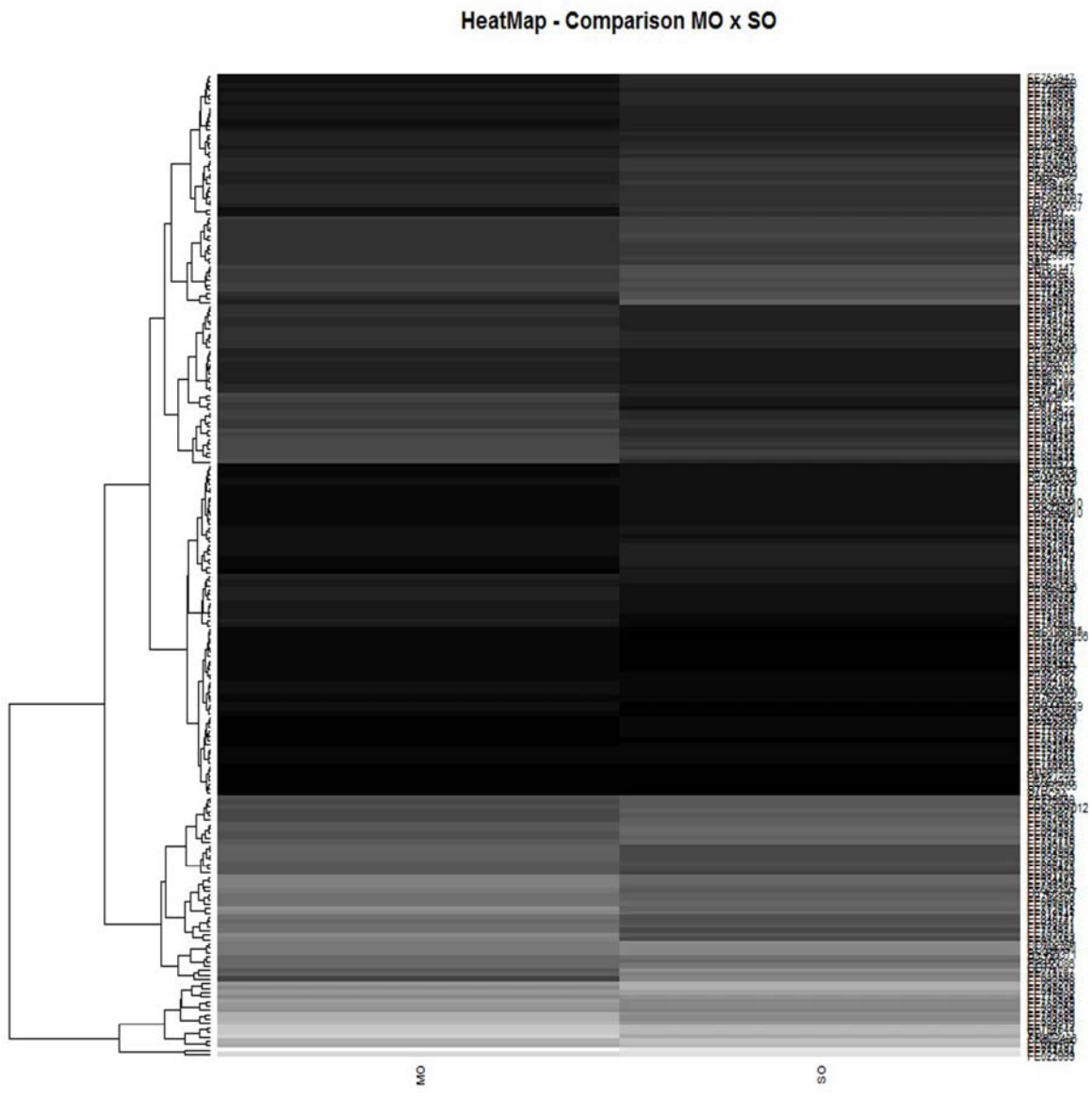
Appendix 6. Heatmap with genes differentially expressed in the comparison F1-SO. Genes that are expressed at higher levels are assigned progressively brighter of white in the group, while genes expressed at low levels are assigned shades of black in the group.



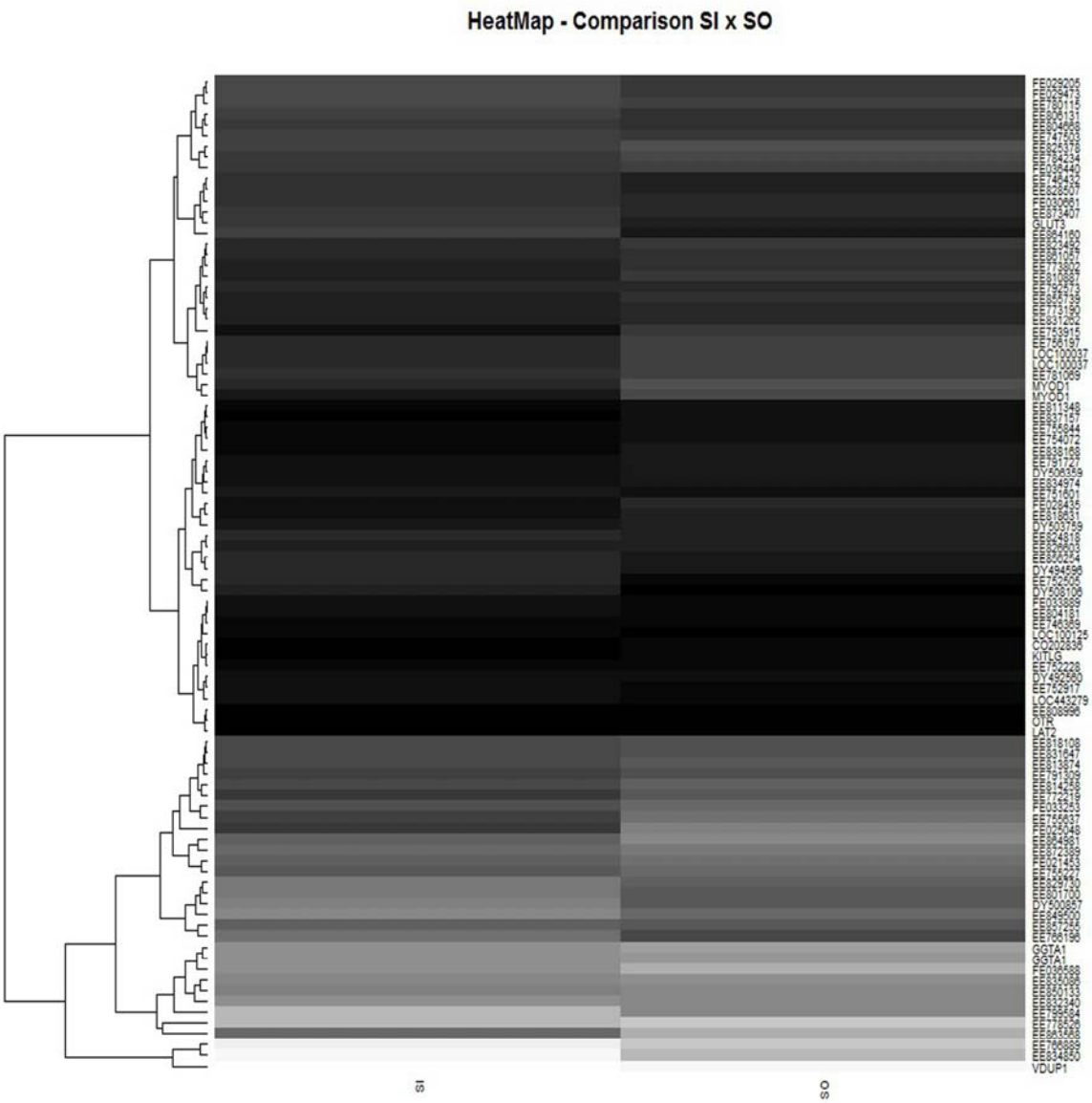
Appendix 7. Heatmap with genes differentially expressed in the comparison MO-SI. Genes that are expressed at higher levels are assigned progressively brighter of white in the group, while genes expressed at low levels are assigned shades of black in the group.



Appendix 8. Heatmap with genes differentially expressed in the comparison MO-SO. Genes that are expressed at higher levels are assigned progressively brighter of white in the group, while genes expressed at low levels are assigned shades of black in the group.



Appendix 9. Heatmap with genes differentially expressed in the comparison SI-SO. Genes that are expressed at higher levels are assigned progressively brighter of white in the group, while genes expressed at low levels are assigned shades of black in the group.



Appendix 10. GenBank accession numbers for the six clusters groups of expression patterns (Figure 5)

ID	Symbol	Group	ID	Symbol	Group
EE835610		1	EE752716		4
EE814522		1	EE784234		4
FE025124		1	FE031903		4
EE785523		1	EE864981		4
EE834723		1	FE021453		4
EE857255		1	EE753128		4
EE803910		1	EE748871	ENDOG	4
NM_001009426	C-MYC	1	EE773471		4
FE022664		1	EE775850		4
EE792509		1	EE822858		4
EE800480		1	FE027322		5
EE779283		1	AY392761	LOC443279	5
EE874417		1	FE021265		5
EE832312		1	EE834974		5
EE766889		2	EE854466		5
CN823486		2	EE825881		5
EE752544		2	EE828327		5
EE795286		2	EE808996		5
EE834850		2	EE811348		5
EE812615		2	AM167931	CLEC6A	5
EE780219		2	EE831262		5
EE783629		2	FE033889		5
DY496371		3	EE832209		5
CO202391		3	FE032492		5
EE868405		3	EE755844		5
EE784781		3	EE773271		5
EE775682		3	FE024992		5
EE785270		3	EE827449		5
EE778526		3	EE829459		5
EE848938		3	EE857179	LOC44341	5
EE844197		3	U89874	KITLG	5
EE832089		4	EE829264		5
EE872389		4	EE824995		5
EE818108		4	EE824976		5
EE851871		4	EE827364		5
EE772498		4	EE856300		5
CN821697		4	FE022000		5
EE781147		4	AY162433	LAT2	5
EE814258		4	DY506359		5
EE791309		4	EE752917		5
EE825378		4	FE023103		5
DY508649		4	EE748531		5
EE756575		4	DY505337		5
EE750731		4	EE773337		5

Continue

Continued

ID	Symbol	Group	ID	Symbol	Group
EE772219		4	EE801047		5
FE033253		4	EE803089		5
EF581383	LOC100101235	4	EE757038		5
NM_001009429	SAH	4	DY486333		5
FE037658		4	DY486333		5
EE753590		4	EE804181		5
EE752685		4	EE746369		5
CO202836		5	EE812374		6
EE861057		5	EE819911		6
EE794100		5	FE036016		6
DY503759		5	CN821865		6
EE772857		5	EE776683		6
AF180523	FVIII	5	EE833255		6
EE791727		5	EE850133		6
EE773642		5	EE845177		6
FE028435		5	EE780115		6
EE752228		5	EE801700		6
FE022752		5	DY495383		6
NM_001105261	LOC100125619	5	EE826084		6
EE854460		5	EE808114		6
NM_001009752	OTR	5	DY500857		6
EE784649		5	DY508106		6
EE765035		5	EE751512		6
EE770260		5	FE029473		6
DY492560		5	EE792573		6
EE837157		5	EE752732		6
EE846068		5	EE823492		6
FE031762		5	EE772267		6
EE818631		5	S77394	IGFBP-4	6
EE838168		5	EE863568		6
DY492665		5	EE854994		6
FE037577		5	DY504785		6
EE777414		5	EE851357		6
EE778937		5	EE806421		6
EE804707		5	EE832340		6
EE811400		5	EE804140		6
EE754072		5	EE856254		6
EE772515		5	EE755227		6
EE801143		5	EE855739		6
AM231302	TRL6	5	EE848777		6
EE856892		5	EE852749		6
EE785588		5	FE037117		6
EE751601		5	EE754710		6
CO202210		6	FE038466		6
EE751947		6	FE025048		6
EE752085		6	CN824409		6
EE783894	VDUP1	6	EE756197		6

Continue

Continued

ID	Symbol	Group	ID	Symbol	Group
EE752173		6	CN824778		6
EE777898		6	EE828696		6
EE810887		6	EE799584		6
DY519030		6	EE813874		6
FE029003		6	DY495847	CEBPD	6
EE746182		6	EE831647		6
EE751998		6	NM_001009764	GGTA1	6
EE801816		6	NM_001024861	PYGL	6
EE757603		6	DQ239650	LOC100037678	6
EE766196		6	EE806131		6
EE752505		6	FE038561		6
EE828507		6	EE774871		6
FE030661		6	EE873407		6
EE797982		6	EE829730		6
EE793621		6	EE824818		6
EE773802		6	EE848572		6
EE774767		6	EE773190		6
EE827115		6	EE825465		6
EE807729		6	EE800644		6
EE835115		6	EE812141		6
NM_001009257	PGDS	6	EE864160		6
EE835086		6	FE022009		6
EE804668		6	EE816082		6
DY494596		6	EE826603		6
EE800098		6	EE864166		6
EE746925		6	EE748095		6
FE029205		6	EE753915		6
EE746432		6	EE859098		6
FE036588		6	EE754015		6
EE781069		6	EE754172		6
FE027121		6	FE034423		6
EE754694		6	EE817844		6
FE036768		6	EE755637		6
FE022668		6	CF117640		6
NM_001100921	PPARGAMM	6	EE849500		6
NM_001009770	GLUT-3	6	FE036440		6
NM_001009390	MYOD1	6	DQ239650	LOC10003	6
EE747503		6			

GENERAL CONCLUSIONS

In this study we analyzed the fatty acid and gene expression profiles in *Longissimus dorsi* of Morada Nova (MO), Brazilian Somali (SO) and Santa Inês (SI) and ½ Dorper x ½ Morada Nova (F1) lambs. We also analyzed the relationship between fatty acid content and gene expression.

Regarding the profile of fatty acids, we conclude that breed is important sources of variation for fatty acid composition of LD.

Microarray analysis revealed several transcripts involved with skeletal muscle growth, fatty acid biosynthesis and other biological processes. This study, which was the first to evaluate whole-genome expression in Brazilian sheep, revealed breed-specific patterns of gene expression in postnatal sheep skeletal muscle. In conclusion, the result suggests more similarities among MO, SI and F1, with SO breed presenting more distinct expression pattern.

We prospect genes involved in the activation mechanism and maintenance of myonucleic from satellite cells and fiber type, intramuscular fat deposition and fatty acid biosynthesis. These transcripts are important expressed markers for muscle deposition and meat quality and may be responsible for differences of muscle deposition and intramuscular fatty acid between genetic groups.

Our study provides comprehensive knowledge on global gene expression and biological functionalities of differentially expressed genes in the main hair sheep raised in Brazil. This information could be used in characterization and improvement for these breeds. More studies evaluating various age during postnatal life and other management systems are necessary.