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TRANSCRIPTOMICS PROFILING REVEALS CANDIDATE GENES TO IMPROVE FEED EFFICIENCY IN DAIRY CATTLE

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SUMMARY

Feed is the largest variable cost in milk production industries, thus improving feed efficiency (FE) will give better use of resources. To identify and select animals with high FE, it might be helpful to understand the biological mechanisms and the role of gene expression patterns across the whole genome (transcriptomics). In the present study, RNA sequencing data was used to detect differentially expressed (DE) genes in Danish Holstein and Jersey dairy cows having either a high or low FE (assessed as residual feed intake (RFI). Functional analysis was performed on these genes to identify molecular pathways involved in FE. Ten Jersey and nine Holstein cows were used in the experiment and divided into two RFI groups depending on their calculated RFI. The two RFI groups received a Control (C) and High Concentrate (HC) diet containing 68:32 and 39:61 ratio of forage:concentrate, respectively. This enabled us to compare the interaction between RFI status and diet. The mRNA samples extracted from liver biopsies were paired end sequenced. The RNA-Seq gene expression data was then analyzed using a statistical-bioinformatics pipeline to identify DE genes and perform functional enrichment. We compared gene expressions of the RFI groups, and identified 70 and 19 DE genes in Holstein and Jersey, respectively. An interaction term (RFI x diet) detected two significantly DE genes in Jersey cows. The functional enrichment analysis of the DE genes showed involvement in pathways that might regulate RFI, such as primary immunodeficiency, retinol metabolism, starch and sucrose metabolism, arachidonic acid metabolism and cytochrome P450 drug metabolism. In conclusion, the transcriptomics approach was effective in identifying DE genes and understanding their biological functions. These findings could contribute to the development of biomarkers for RFI and to improving augmented genomic selection procedures that make use of functional information.

INTRODUCTION

Improving feed efficiency of dairy cattle can mean big savings for milk producers. One way to improve feed efficiency is by genetic selection for cows producing more milk for the same amount of feed. Residual feed intake which is the difference between actual and predicted feed intake has been used widely as a measure of feed efficiency in livestock (Berry & Crowley 2012; Connor *et al.* 2013; Lin *et al.* 2013; Tempelman *et al.* 2015).

To understand the mechanisms of action affecting feed efficiency, we suggest the use of system genetics approach including transcriptomics techniques. This might be helpful in supporting genomic selection in the future (Kadarmideen 2014; Zhang *et al.* 2014). The liver plays an important role in the metabolism of nutrients (Partridge *et al.* 2014). Hence, liver transcriptomics might give insight into feed efficiency in dairy cows.

The objective of the study was to identify potential regulatory genes and molecular pathways involved in feed efficiency of dairy cattle by characterising the liver transcriptome based on RNA-Seq technologies.

MATERIALS AND METHODS

Ten Jersey and nine Holstein cows were selected from the research herd of 200 animals in Danish Cattle Research Centre (DCRC), Aarhus University, Denmark. The data from this herd have previously been used in quantitative genetic studies regarding feed or dry matter intake (Li *et al.* 2016). Animals of both breeds were divided into two groups: high- or low-RFI. Residual feed intake was defined using a random regression model (Tempelman *et al.* 2015). Here, the random animal solutions were extracted from a random regression model in which dry matter intake was regressed on the following fixed effects: weeks of lactation, the management group in which the cows were held, and the interaction between weeks of lactation, breed and parity. Fixed linear regressions were applied to adjust for metabolic body weight, daily live weight change and daily body condition score change (fitted with a Legendre polynomial), and energy corrected milk yield. The random effects were cow within the breed and cow within the breed and parity. All cows received a low-concentrate [control (C)] and a high-concentrate (HC) diet in a crossover design with two periods. There was approximately a 30% difference in concentrate proportion on a dry matter (DM) basis between the C and HC diets which were 68:32 and 39:61 ratio of forage:concentrate, respectively.

Approximately 10–20 mg of liver tissue were collected from all the experimental cows at the end of each feeding trial. mRNA was extracted from the liver tissue samples using the Qiazol, RNeasy® Mini Kit and MaXtract High Density and sequenced with Illumina HiSeq 2500. The quality of all mRNA samples was above 8 RIN (RNA Integrity Number).

RNA-Seq data of each cow were analyzed to identify differentially expressed genes. The DE analysis was performed separately for each breed using R package DESeq2 setting all the parameters to default values (Love *et al.* 2014). Two different models were fitted:

Model 1 Y=Parity number + Diet + RFI
Model 2 Y=Parity number + Diet + RFI + Diet*RFI

where: Y is the gene expression counts, RFI is a dummy variable that represents the feed efficiency of the animals (high- and low-RFI), and Parity number was included as a dummy variable to control for potential confounding effects. In Model 1, we assumed an additive effect without interaction between two treatment diet and two RFI groups. In Model 2, we assumed an interaction between two treatment diets by two RFI groups. Differentially expressed genes were considered at a False Discovery Rate (FDR) < 5%.

Finally, functional enrichment analysis on the entire expression profile was performed using Gene Set Enrichment Analysis (GSEA). It has been demonstrated previously that GSEA provide insights into the biology behind a set of genes in terms of how the DEGs interact.

RESULTS AND DISCUSSION

On average, 91% of the read pairs (26,067,856 read pairs) were uniquely mapped to the bovine reference genome UMD 3.1 from Ensembl database release 82. On average, 62% of the read pairs mapped to exonic regions, 20% to intronic regions and almost 18% to intergenic regions.

In total, 12,025 genes in the Holstein breed and 11,905 genes in the Jersey breed were used after removing low expression genes to identify the DEGs. A total of 70 Holstein and 19 Jersey DEGs (Table 1) were identified by comparing between high- and low-RFI directly without accounting for any interaction. The interaction analysis showed low numbers of DEGs in both diet

groups (Table 1). Among the top DEGs in Holsteins were *ACACA, CYP2C9, CYP7A1, CYP11A1, ELOVL6, FOSL2, HCLS1, IFI6, NR1H4, RYR1, SOCS2, TBC1D8, CR2, CTH, DGAT2, FGFR2, SLC20A1* and *TAF6*. The top DEGs in Jerseys were *CYP3A4, EXTL2, TMEM102, FDXR, GIMAP4, GIMAP8, GNG10, HLA-B* and *ZNF613*. Most of the genes identified as DEGs in both breed were also found as DEGs in other RFI divergent study by (Weber *et al.* 2016). In total, 22 Holstein genes and 14 Jersey genes were detected as significant DEGs (p values < 0.05) for the interaction analysis. No significant genes were identified in Holstein cows for the interaction (Table 1). However, two Jersey genes, SEC24 Homolog D (*SEC24D*) and FLT3-Interacting Zinc Finger 1 (*FIZ1*), were differentially expressed (p values < 0.05) in the RFI groups depending on the two diet types.

We identified seven overrepresented pathways for the set of downregulated genes and none for the upregulated genes in high-RFI group Holsteins. In Jerseys, two pathways were overrepresented for genes with negative-fold changes and three pathways for genes with positive-fold changes. The top KEGG pathways for the genes downregulated in the high-RFI group in Holsteins and in Jerseys is the primary immunodeficiency pathway, while the significant pathways identified for genes upregulated in the high-RFI group were only detected in Jerseys. We also identified, that most of the pathways within the strong indications thresholds (FDR q-value <0.05), were related to the metabolism of retinol, starch and sucrose, ether lipid and cytochrome P450 drug metabolism.

Table 1. Number of differentially expressed genes between high- and low-RFI in a separate diet group in the model with interaction term, and without interaction term according to the corrected p values < 0.05

	Control	High Concentrate	With Interaction	Without interaction
Holstein	9	13	0	70
Jersey	6	6	2	19

The functional enrichment and pathway analysis of the DEGs contribute towards understanding the function of these genes in relation to feed efficiency. The steroid hormone biosynthesis pathway was one of the top KEGG pathways identified in the analysis of negative energy balance in dairy cows (McCabe *et al.* 2012). We also discovered that this pathway was overrepresented in the set of genes upregulated in high-RFI group in Jersey cows (FDR < 0.05). Steroid hormone biosynthesis should always occur in the adrenal glands and gonads, while the liver is the site of steroid hormone inactivation. The upregulation of steroid hormone biosynthesis pathway indicated that steroid hormone was inactivated in high-RFI group. Therefore, we would conclude that this pathway plays an important role in RFI. In support, both *CYP11A1* and *CYP7A1* that were upregulated in high-RFI group in Holstein, which function in cholesterol homeostasis, were identified as DEGs in our experiments and they are part of this pathway in KEGG.

Primary immunodeficiency pathway is a heterogeneous group of disorders. This pathway was the top overrepresented pathway detected by GSEA and was significantly enriched in both cattle breeds. The downregulation of the primary immunodeficiency pathway in both breeds of high-RFI cows suggests that the immunity may affect feed efficiency. (Ozuna *et al.* 2012) observed that primary immunodeficiency disorder is consistently inherited by low-feed efficient pigs. Consistently, Kogelman *et al.* (2014) and Do *et al.* (2013) reported a correlation between genes related to immunodeficiency function disorders or immunity-related diseases and low-feed efficiency in pigs.

Notably, the genes identified for the interaction between RFI and diet, were also associated with immunodeficiency. The impact of the diet on genes belonging to the immunodeficiency

pathway and it paves the way for future studies to determine how to improve diet in relation to the genetic background of the animals. Two protein-coding genes, *SEC24D* and *FIZ1*, were differentially expressed in response to diet and were associated with pathways including immune system and transport to the golgi and subsequent modification as well as in transcriptional regulation (www.genecards.org). The lack of a more extensive differential gene expression response to diets indicate that differences in the concentrate proportions between the diets, as tested in this study, may not be able to disturb gene expression levels.

In conclusion, the results reveal differences in biological mechanisms related to residual feed intake in Holsteins and Jerseys. The study provided 70 and 19 candidate genes involved in the regulation of residual feed intake pathways in Holstein and Jersey cattle, respectively. The functional enrichment analysis of the DE genes showed involvement in pathways that might regulate feed efficiency, such as primary immunodeficiency, retinol metabolism, starch and sucrose metabolism, arachidonic acid metabolism and drug metabolism cytochrome P450. The relationship between retinol metabolism and the feed conversion ratio phenotype in Nellore beef cattle has been previously described (de Almeida Santana *et al.* 2016). The candidate genes identified in this study might be useful for explaining biological effects of genomic markers in genomic selection methods utilizing functional information.

REFERENCES

Berry D. and Crowley J. (2012) *Journal of animal science* **90**: 109.

Connor E., Hutchison J., Norman H., Olson K., Van Tassell C., Leith J. and Baldwin R. (2013) *Journal of animal science.* **91**: 3978.

de Almeida Santana M.H., Junior G.A.O., Cesar A.S.M., Freua M.C., da Costa Gomes R., e Silva S.d.L., Leme P.R., Fukumasu H., Carvalho M.E. & Ventura R.V. (2016) *Journal of applied genetics* **57**, 495-504.

Do D.N., Strath, A.B., Ostersen T., Jensen J., Mark T. and Kadarmideen H.N. (2013). *PloS one.* **8**: e71509.

Kadarmideen H.N. (2014) *Livestock Science.* **166**: 232.

Kogelman L.J.A., Cirera S., Zhernakova D.V., Fredholm M., Franke L. and Kadarmideen H.N., (2014) *BMC Med Genomics.* **7:** 57.

Li B., Fikse W., Lassen J., Lidauer M., Løvendahl P., Mäntysaari P. & Berglund B. (2016) *Journal of dairy science* **99**, 7232-9.

Love M.I., Huber W. & Anders S. (2014) *Genome biology* **15**, 550.

Lin Z., Macleod I. and Pryce J. (2013) *Journal of dairy science*. **96**: 2654.

McCabe M., Waters S., Morris D., Kenny D., Lynn D. and Creevey, C. (2012) *BMC genomics*. **13**: 193.

Ozuna A.C., Rowland R.R., Nietfeld J.C., Kerrigan M.A., Dekkers J. and Wyatt C.R. (2012) *Veterinary Pathology*. **50**: 144

Partridge C.G., Fawcett G.L., Wang B., Semenkovich C.F. and Cheverud J.M. (2014) *BMC genomics.* **15**: 1.

Tempelman R., Spurlock D., Coffey M., Veerkamp R., Armentano L., Weigel K., De Haas Y., Staples C., Connor E. and Lu Y. (2015) *Journal of dairy science.* **98**: 2013.

Weber K.L., Welly B.T., Van Eenennaam A.L., Young A.E., Porto-Neto L.R., Reverter A. & Rincon G. (2016) *PloS one* **11**, e0152274.

Zhang Z.H., Jhaveri D.J., Marshall V.M., Bauer D.C., Edson J., Narayanan R.K., Robinson G.J., Lundberg A.E., Bartlett P.F. and Wray N.R. (2014). *PloS one*. **9**: e103207.