Gene expression phenotypes for cattle and sheep management

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Are measurements of gene expression useful phenotypes for the management and breeding of cattle and sheep?

Two examples of gene expression phenotypes are described below.

- **for the detection of the use of hormone growth promotants (HGP)**
- **for the estimation of intramuscular fat (IMF)%**

Animal resources and datasets

20 sheep, with and without HGP

48 Brahman steers, ~20m old, in two sites (NSW and WA), with and without HGP

4 Wagyu x Hereford, 25m old, high IMF%

4 Piedmontese x Hereford, 25m old, medium IMF%

IMF% measurement by Gas Chromatography → mRNA prepared from longissimus muscle (LM) for Agilent bovine microarray → **Sheep correlation of gene expression with IMF% dataset**

IMF% measurement by **Ultrasound (Live animal) and NIRS[1] (Biopsy or post-mortem)** → mRNA prepared from LM for Agilent bovine microarray → **Cattle correlation of gene expression with IMF% dataset**

mRNA prepared from LM for Agilent bovine microarray → **Cattle differential expression (DE) WxH minus PxH dataset**

Integrated analysis

[1]Near Infrared Reflectance Spectrophotometry

Detecting HGP use

The treated cattle received an HGP dose of 200 mg Trenbolone acetate (TBA) and 20 mg 17β-Estradiol (E2). The treated sheep received a reduced dose based on their body weight. Gene expression in the LM was measured on the bovine Agilent microarray from each set of animals.

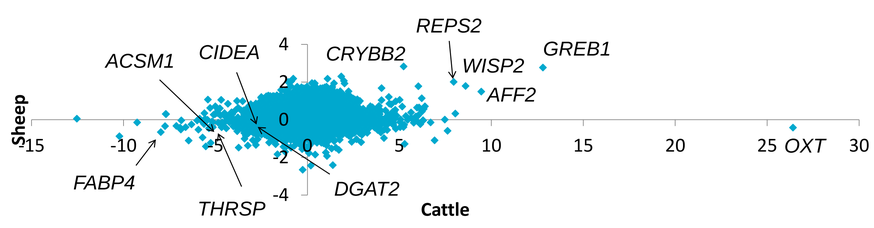

Figure 1. Differential gene expression HGP-treated minus control for cattle and sheep.

Due to sequence differences the bovine Oxytocin (*OXT*) microarray probe did not report from the sheep mRNA, but a QPCR test identified a ~4.4 fold (P = 0.01) increase in OXT expression in LM of HGP-treated sheep. In contrast, cattle exhibited an ~97 fold increase in *OXT* expression in the LM.

Conclusions

At the extreme of increased gene expression cattle and sheep have very similar responses to HGP treatment, albeit apparently much smaller in sheep than in the cattle.

***OXT* expression in LM muscle could be used in both cattle and sheep as a diagnostic of TBA/E2 use. However, in sheep using additional genes, such as *GREB1*, *WISP2*, etc. would reduce the false positive rate.**

Estimating IMF%

1. Identification of genes most correlated with IMF% in both cattle and sheep

Table 1. The top genes most correlated with IMF% were determined based on the rank of their average ranks in the three datasets within the subset of 55 genes in the previously identified, TAG synthesis and storage, FA synthesis and PPARG modules. The top five genes (in green) were defined as the "IMF 5-gene set"

Genes	Rank in the dataset				Description
	average rank	cattle correlation	cattle DE	sheep correlation	
CIDEA	**1**	1	5	1	cell death-inducing dffa-like effector a
THRSP	**2**	5	1	14	thyroid hormone responsive
ACSM1	**3**	12	12	2	acyl-CoA synthetase medium-chain family member 1
DGAT2	**4**	2	15	10	diacylglycerol o-acyltransferase 2
FABP4	**5**	16	9	4	fatty acid binding protein 4,adipocyte
PLIN1	6	6	19	5	perilipin1
ADIPOQ	8	15	13	3	adiponectin
FASN	9	13	8	13	fatty acid synthase
TUSC5	13	22	22	6	tumor suppressor candidate 5
AGPAT2	14	9	30	11	phosphate O-acyltransferase 2
LPL	16	27	44	7	lipoprotein lipase

2. *CIDEA* and the IMF 5-gene set are equally correlated with IMF% in both cattle and sheep

Table 2. Correlation between gene expression and IMF%.

	CIDEA	IMF 5-gene set
20 sheep	0.52	0.51
48 cattle	0.40	0.46

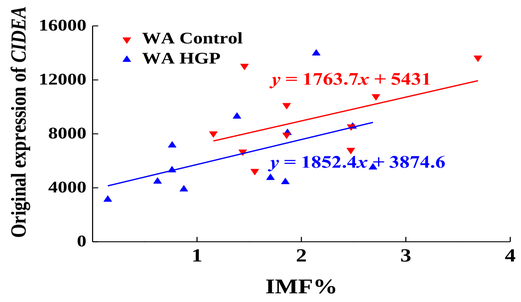

Figure 2. *CIDEA* gene expression shows linear relationship with IMF% in cattle and this relationship is NOT affected by HGP treatment.

3. Applications of gene estimator(s) of IMF% in cattle

Table 3. Using different measures/estimators of IMF% and IMF% related gene expression metrics to discriminate between HGP-treated and control animals.

Method	Animal number	control animals	HGP-treated animals	*P*-value	Predicted experiment size[1]
NIRS measured IMF%	141	2.37±1.00[2]	1.90±0.83	0.001	198
Ultrasound estimated IMF%	173	2.66±0.72	2.93±0.54	0.16	N/A
NIRS measured IMF%	22	2.07±0.77	1.79±0.54	0.34	294
IMF% estimated by *CIDEA* formula	22	2.44±1.33	1.71±1.17	0.03	154
IMF% estimated by IMF 5-gene set formula	22	2.54±1.27	1.40±0.98	0.01	24
Ranking animals using *CIDEA* expression	22	13.2±6.16[3]	9.5±6.62	0.1	156
Ranking animals using IMF 5-gene set exp	22	15.1±4.99	7.2±5.47	0.0026	22
CIDEA DE	22	13.09±0.44[4]	12.83±0.38	0.08	130
IMF 5-gene set DE	22	0.25±0.38[5]	-0.27±0.31	0.0014	24

[1]For $P < 0.05$ and Confidence Interval >95%
[2]Mean and standard deviation of measured or estimated IMF% values
[3]Mean and standard deviation of animal ranks
[4]Mean and standard deviation of gene expression log2
[5]Mean and standard deviation of normalized and standardized gene expression values

4. Why are 5 genes better than one gene?

Although the set of five genes are not more correlated with IMF% than one gene, they are a better discriminator between the two groups, probably because they provide 5 measurements of the gene expression phenotype, reducing the error of the estimation of IMF%.

Conclusions

If you want to make an accurate estimation of IMF% in cattle with low IMF%, the IMF 5-gene set is a better choice than NIRS and much better than ultrasound.

If you want to rank your cattle within a farm/treatment group based on IMF%, or to compare IMF% differences between two farms/different treatment groups, the IMF 5-gene set is the lowest cost, with 7 fold fewer animals required for the same power.

FOR FURTHER INFORMATION
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