JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2009, 60, Suppl 3, 83-90
www.jpp.krakow.pl

I. CASSAR-MALEK, C. JURIE, C. BERNARD, I. BARNOLA, D. MICOL, J.-F. HOCQUETTE

PASTURE-FEEDING OF CHAROLAIS STEERS INFLUENCES SKELETAL MUSCLE METABOLISM AND GENE EXPRESSION

INRA, UR1213, Herbivore Research Unit, 63122 Saint-Genes-Champanelle, France

Extensive beef production systems on pasture are promoted to improve animal welfare and beef quality. This study aimed to compare the influence on muscle characteristics of two management approaches representative of intensive and extensive production systems. One group of 6 Charolais steers was fed maize-silage indoors and another group of 6 Charolais steers grazed on pasture. Activities of enzymes representative of glycolytic and oxidative (Isocitrate dehydrogenase [ICDH], citrate synthase [CS], hydroxyacyl-CoA dehydrogenase [HAD]) muscle metabolism were assessed in *Rectus abdominis* (RA) and *Semitendinosus* (ST) muscles. Activities of oxidative enzymes ICDH, CS and HAD were higher in muscles from grazing animals demonstrating a plasticity of muscle metabolism according to the production and feeding system. Gene expression profiling in RA and ST muscles was performed on both production groups using a multi-tissue bovine cDNA repertoire. Variance analysis showed an effect of the muscle type and of the production system on gene expression ($P<0.001$). A list of the 212 most variable genes according to the production system was established, of which 149 genes corresponded to identified genes. They were classified according to their gene function annotation mainly in the "protein metabolism and modification", "signal transduction", "cell cycle", "developmental processes" and "muscle contraction" biological processes. Selenoprotein W was found to be under-expressed in pasture-fed animals and could be proposed as a putative gene marker of the grass-based system. In conclusion, enzyme-specific adaptations and gene expression modifications were observed in response to the production system and some of them could be candidates for grazing or grass-feeding traceability.

Key words: *cattle, grazing, muscle, gene expression profiling, selenoprotein W*

INTRODUCTION

Skeletal muscle is the main site of protein deposition in an organism during growth and is an essential actor in metabolism, especially of proteins. In humans, this tissue is known to contribute to the maintenance of health through protein and fuel homeostasis. It is also of major interest in meat-producing farm animals such as cattle. In addition to slaughtering conditions and post-mortem events and ageing, genetic, environmental and production factors influence muscle characteristics, and hence beef meat quality. The influence of production factors (age, sex, and feeding plan) on muscle characteristics has been studied extensively (1, 2). Postnatal nutrition plays a critical role in regulating muscle phenotype and plasticity depending on muscle type (3, 4). In cattle, the quality and the nature of the food supply are determinants for muscle characteristics. For instance, Jurie *et al.* (5) showed that the muscles of 30-month-old Charolais steers fed with grass (grazed or freshly cut) were more oxidative than those of steers fed maize-silage indoors. These muscles also contained lower amounts of intramuscular fat and more soluble collagen.

The advent of transcriptomics has enabled not only new predictors of meat quality to be identified (6), but also beef quality to be monitored through the production systems (7). However, except in extreme situations such as undernutrition (8), there is little knowledge on the impact of production factors on muscle gene expression. In particular, specific and reliable molecular indicators of production systems are still lacking. We have compared the influence of two rearing methods representative of two French production systems (intensive and extensive) on muscle gene expression of 30-month-old Charolais steers using cDNA macro-arrays. This study aimed to explore muscle gene profiles in order to identify differentially expressed genes that may be potential indicators of grass-feeding systems.

MATERIAL AND METHODS

Experimental design

The study was conducted with Charolais steers and the experimental design described in (5). The animals were offspring of pure-bred Charolais cows and bulls of an INRA experimental herd (Le Pin au Haras, F61), weaned at 32 weeks and then housed in open sheds. Thirty steers were initially allotted to two groups (12 animals in a maize group and 18 animals in a grass group). For the second grazing season, five groups of six animals each were formed as follows: twenty-four animals (12 from the grass group and 12 from the maize group) were housed in open sheds with a maximum of 6 animals per pen of 40 m^2 each. Animals on the grass diet were fed freshly cut

grass alone. Animals on the maize diet were fed maize-silage *ad libitum*, with a minimum of wheat straw and rapeseed meal. Half of the indoor animals on each feeding regime were submitted 7 days a week to a 5.2 km walk daily, which represented approximately a 30 min walk before the morning meal plus a 30 min walk before the evening meal. The remaining six animals on grass were left grazing on pasture, with the same perennial rye-grass pasture as that used for the indoor animals. There were therefore five treatment groups: 'grazing', 'cut grass', 'cut grass with walking', 'maize silage' and 'maize silage with walking'. All steers were managed at the same growth rate by adjustment of the amount of maize silage diets offered to the animals. The carcass composition at slaughter was similar between the treatment groups indicating that the observed effects are indeed associated with the type of production and feeding system and not with differences in net energy intake level or growth performances.

The animals were slaughtered at 30 months of age. Two muscles of the carcass were excised from each animal within less than ten minutes after slaughter: *Rectus abdominis* (red and oxidative muscle, RA) and *Semitendinosus* (white and glycolytic muscle, ST). The samples were snap-frozen in liquid nitrogen and stored at -80°C until they were analysed.

Muscle samples from the most extreme groups namely 'grazing' (n=6) and 'maize-silage' (n=6) groups were used for biochemical and transcriptomic (macroarray experiment, qPCR) studies. Samples from all five experimental groups (n=6) were used for Selenoprotein W gene expression studies by Northern-blot analysis.

Muscle metabolism

The maximum activity levels of the following enzymes, reflecting the potential of β-oxidation (β-hydroxyacyl-CoA dehydrogenase, HAD, EC 1.1.1.35), mitochondrial density (citrate synthase, CS, EC 2.3.3.1; isocitrate dehydrogenase, ICDH, EC 1.1.1.42), oxidative phosphorylation (cytochrome-c oxidase, COX, EC 1.9.3.1), and glycolytic metabolic pathway (phosphofructokinase, PFK, EC 2.7.1.11; lactate dehydrogenase, LDH, EC 1.1.1.27) were determined spectrophotometrically, as described elsewhere (4, 5, 9). One unit of enzyme was defined as the amount which catalyses the disappearance of 1 µmol of NADH per min for HAD, PFK and LDH, the appearance of 1 µmol per min of NADPH for ICDH, the liberation of 1 µmol of coenzyme A per min for CS, and the oxidation of 1 µmol per min of cytochrome-c for COX. The results were expressed as units per g muscle fresh weight.

Data of muscle enzymatic activities was analysed using the General Linear Models (GLM) procedure of SAS with three effects: production system (P), animal nested within production system and muscle (M) as previously described (4).

Gene expression

To monitor gene expression in the two extreme groups, we used macroarrays consisting of a cDNA bovine collection from bovine muscle, embryo and mammary gland (10). Briefly, high density filters were constructed from a collection derived from 3 cDNA libraries: a non-normalised directed bovine muscle library (11), a non-normalised 14-day-old embryo library (12) and a lactating mammary library (13). A set of 2304 spots corresponding to 637 muscle, 882 embryo and 377 mammary gland amplified cDNA fragments was printed together with controls (*Arabidopsis thaliana* C554 clone, cDNA from the Amerscham Lucidea kit, and water) onto 8x12 cm nylon membranes at the French National Biological Resources Centre for Animal Genomics (http://www-crb.jouy.inra.fr/BRC/index.html), Jouy-en-Josas, France). Sequences of the cDNAs are available on the SIGENAE website (http://www.sigenae.org/).

Total RNA was extracted from the RA and ST muscles of the six steers of each extreme group respectively. RNA was isolated using a method derived from (14). RNA samples were then pooled to obtain one sample for each muscle and each group (RA/maize silage, RA/grazing, ST/maize silage, ST/grazing). Messenger RNAs were prepared from total RNA following the protocol described in (15). mRNA (500 ng) was reverse-transcribed and labelled by incorporation of [α-^{33}P]dCTP using SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies, Cergy Pontoise, France) as described in (15). Thereafter, the labelled cDNA targets were purified using Quick Spin Columns (Roche Diagnostics, Meylan, France).

Hybridisations were performed in duplicate (at probe labelling or hybridisation levels) in two independent experiments. For each experiment, 4 macroarrays were incubated with each radiolabelled cDNA target as previously described (10). Filters were prehybridised in ExpressHyb™ hybridisation solution (Clontech Laboratories, Palo Alto, USA) for 16 hours at 60°C before hybridisation with labelled targets in ExpressHyb™ overnight at 60°C. Nylon membranes were washed twice for 15 min in SSC 2X, SDS 0.1% at 60°C. After washing, they were exposed 48 hours to phosphor screens before scanning on the PhosphorImager (STORM 840, Molecular Dynamics) imaging plate system for quantitative analysis of signal intensity. After acquisition, the scanned images were analysed using the GenePix Pro V4.1 software (Axon instrument, Inc).

Data were analysed using a standard analysis of variance (ANOVA) with three fixed effects: experiment (hybridisation in duplicate in two independent analyses), production system (maize silage vs grazing), muscle (RA vs ST) using GeneANOVA (16). Raw data were log transformed before variance analysis. The F-value was computed from the ratio of the variance between individual groups to the variance within each experimental group. The higher the F-value, the more significant the difference between groups. Differential expression was considered for a *p-value* <0.05. Expression was declared up- or down-regulated by comparison of the means for RA vs ST or HP *vs* MA. Validation of differential expression was performed by qPCR as described in (6) using the following primers:

ACTA1-Fw: AATGCTTCTAGACGCCTCTC,
ACTA1-Rev: AGAACGGCTGAGTTTAATGC,
AldoA-Fw: CTTTGGCTTCCTCCTCACAG,
AldoA-Rev: GCCTTCTGGTACAGCGTCTC,
CA3-Fw: AGATAGCCAAGCTGCGCACTCT,
CA3-Rev: CTTCACGATCCTGCCCTTGATG,
CKM-Fw: GCTCGTCGGAGGTAGAACAG,
CKM-Rev: GGTTGGAACTCTGGTTGGAA,
CRYAB-Fw: CGCCATTACTTCATCCCTGT,
CRYAB-Rev: TCACTGGTGGGGAACTTTTC,
RPLP0-Fw: CATTTCACAAATAATGCTGGCCT,
RPLP0-Rev: CCCAGCCAAGGTTGAAGCAAAG,
SDHA-Fw: GGAAGCACACGCTCTCCTAC,
SDHA-rev: GGCCTCCTCAGTCTGTCAAG,
SEPW1-Fw: CGGCTTCTTTGAAGTGTTC,
SEPW1-Rev: TCTCTGCCTTTAGGCCACAT,
TNNT1-Fw: TACCTCCTCTGATCCCGCCAAA,
TNNT1-Rev: CTGCTCAAAGTGGACGTCGATG.

RNA levels determined by qPCR were analysed by variance analysis using the general linear models procedure of SAS. The effects tested included production system (two levels), animal nested within production system, muscle (two levels) and muscle × production system interaction. The animal variation

was used as the error term for the production system effect. The residual mean square was used as the error term for other effects (4). Analysis of SEPW1 expression levels was also performed in the muscles of all experimental groups by classic Northern blot hybridisations, as described in (15) using a cDNA probe prepared from the clone used for macroarray spotting.

Functions of differentially expressed genes, notably biological process and metabolic pathway, were determined based on the Gene Ontology (GO) information using the PANTHER classification system (http://www.pantherdb.org/) (17) and FatiGO+ web tool (http://babelomics.bioinfo.cipf.es/index.html) (18).

RESULTS

Changes in muscle characteristics

Muscle type had a significant effect on the activities of metabolic enzymes (*Table 1*). For each muscle type, the activities of glycolytic enzymes (LDH and PFK) did not significantly differ between the two production feeding systems (*Table 1*). By contrast, the activity of oxidative enzymes responded to the production system, except for COX activity: the activities of ICDH (P = 0.08), CS and HAD (P < 0.01) were higher with grazing (*Table 1*). For ICDH, an interaction (P < 0.01) was found between muscle type and production system. A muscle-specific higher ICDH activity was recorded in RA muscle with the grazing system (P< 0.0001), but not in ST muscle.

Changes in muscle gene expression

After controlling the technical variability (effect of the hybridisation experiment), variance analysis of the transcriptomic data showed an effect of the muscle type and of the production system on gene expression (P< 0.001). However, the effect of the production system (grazing vs maize-silage indoors) was less significant on gene expression than the effect of the muscle type (F=1.3 and F= 16.3 respectively, *p*-value < 0.001).

Amongst the most variable clones according to the muscle type, the top 20 were annotated according to library of origin and

Table 1. Influence of the production system on metabolic activities in muscles.

	Maize-silage	Grazing	SEM	Effect
ICDH	1.05	1.26	0.034	M***; Pt; P x M**
CS	4.39	5.16	0.087	M***; P**
HAD	1.53	1.92	0.046	M**; P**
COX	9.99	11.11	0.655	M**
LDH	841	891	14.09	M***
PFK	23.69	22.26	1.532	M***

Values are LSMeans (µmol/min per g fresh tissue) of 6 animals per production group for both muscles. M, effect of muscle; P, effect of production feeding system; P x M, production feeding system x muscle interaction. Level of significance: t, P = 0.08; *, P< 0.05; **, P< 0.01; ***, P< 0.001.

Table 2. Examples of macroarray gene probes from the muscle cDNA library, which were differentially expressed in the RA *vs* ST muscle.

Clone ID	**Gene ID (symbol)**	**F**	***P-value***	**RA/ST**
bcas0004a.e.12	sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2)	415	<0,0001	down-regulated
bcaj0005a.c.02	myosin heavy chain IIx/d (MYH1) clone 1	345	<0,0001	down-regulated
bcaj0003a.h.04	myosin heavy chain IIx/d (MYH1) clone 2	345	<0,0001	down-regulated
bcas0005a.c.03	carbonic anhydrase III (CA3)	327	<0,0001	up-regulated
bcaj0005a.b.09	NADH-ubiquinone oxidoreductase chain 6 (ND6)	255	<0,0001	down-regulated
bcaj0002a.b.08	glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	244	<0,0001	down-regulated
bcaj0009a.g.05	phospholipase C, DKFZP564M182 (chimeric clone)	232	<0,0001	down-regulated
bcas0005a.b.11	fructose-bisphosphate aldolase A (ALDOA), clone 1	205	<0,0001	down-regulated
bcaj0008a.d.01	sarcoplasmic/endoplasmic reticulum calcium ATPase 1 (serca1)	204	<0,0001	down-regulated
bcaj0002a.b.02	ubiquitin-like 3	187	<0,0001	up-regulated
bcaj0005a.b.01	tropomyosin beta chain, skeletal muscle (TPM 2)	179	<0,0001	down-regulated
bcas0006a.e.02	fructose-bisphosphate aldolase A (ALDOA), clone 2	161	<0,0001	down-regulated
bcaj0006a.c.06	glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	161	<0,0001	down-regulated
bcaj0009a.c.08	unknown	158	<0,0001	up-regulated
bcaj0009a.c.02	heat shock protein 27 kDa (HSPB1)	156	<0,0001	up-regulated
bcaj0008a.f.02	troponin I, fast skeletal muscle (TNNI2)	154	<0,0001	down-regulated
bcaj0008a.f.03	myosin light chain 1, skeletal muscle isoform (MYL1)	152	<0,0001	down-regulated
bcas0004a.c.09	NADH-ubiquinone oxidoreductase chain 1	145	<0,0001	down-regulated
bcaj0007a.b.01	myosin regulatory light chain 2, cardiac muscle isoform (MYL22)	143	<0,0001	down-regulated

The F-value is the calculated statistic computed by GeneANOVA. The higher the F-value, the more significant the difference between groups. The *p*-value is the significance level. RA: *Rectus Abdominis* muscle, ST: *Semitendinosus* muscle.

gene ontology (*Table 2*). They all belonged to the subset of cDNA probes derived from the muscle library and were mainly related to metabolic enzyme and contractile protein pathways.

Amongst the 212 most variable clones according to the production system (p-value <0.05), 76 belonged to the muscle cDNA library subset whereas 94 and 42 belonged to the embryo and mammary gland cDNA library subsets respectively. In the muscle subset, 15 clones were unknown sequences and the remaining clones corresponded to 60 different genes. In the embryo and mammary gland subsets, 50 and 39 genes were identified from differential clones as using BLASTN and BLASTX searches respectively. Examples of genes declared to be differentially expressed according to the production system are given in *Table 3*. Differential expression according to the

Table 3. Examples of macroarray gene probes from the muscle cDNA subset declared to be differentially expressed with GenANOVA according to the production system.

Clone ID	Gene ID (symbol)	F	*P-value*	*Grazing/maize silage*
bcaj0008a.h.04	selenoprotein w (SEWP1)	25.44	<0.0001	down-regulated
bcaj0006a.d.04	unknown	18.05	0.0002	up-regulated
bcaj0002a.c.02	creatine kinase M chain (CKM)	14.69	0.0007	up-regulated
bcas0001a.e.12	unknown	14,35	0.0007	up-regulated
bcas0006a.a.05	troponin T1, slow skeletal muscle isoforms (TNNT1)	14.14	0.0008	down-regulated
bcaj0003a.h.04	myosin heavy chain, skeletal muscle, adult 1 (MyHC-2X/D) (MYH1)	14.00	0.0008	up-regulated
bcaj0006a.h.08	60s acidic ribosomal protein p0 (RPLP0)	13.55	0.0010	up-regulated
bcaj0005a.c.11	embryonic nuclear protein 1 (MUSTN1); hypothetical protein (ORF1),	13.46	0.0010	down-regulated
bcas0005a.g.07	retinoic acid-binding protein 2, cellular (CRABP2)	13.36	0.0010	up-regulated
bcas0001a.g.03	skeletal muscle lim-protein 1 (FHL1)	12.60	0.0014	down-regulated
bcas0005a.c.03	carbonic anhydrase III (CA3)	12.02	0.0017	down-regulated
bcaj0005a.e.09	similar to exportin 7 (XPO7)	11.72	0.0019	down-regulated
bcas0006a.h.05	actin, alpha skeletal muscle (alpha-actin 1) (ACTA1)	11.64	0.0020	up-regulated
bcaj0003a.e.09	myosin light chain 1, MLC-1 (MYL1), embryonic muscle/atrial isoform	11.10	0.0024	down-regulated
bcas0003a.d.10	60s ribosomal protein l4 (RPL4)	10.91	0.0026	up-regulated
bcas0001a.e.09	unknown	10.61	0.0030	up-regulated
bcas0004a.e.09	unknown	10.60	0.0030	up-regulated
bcaj0005a.b.01	tropomyosin beta chain, skeletal muscle (tropomyosin 2) (TPM2)	10.56	0.0030	up-regulated
bcaj0008a.e.03	chromosome 14 open reading frame 166 (C14orf166)	10.30	0.0030	up-regulated
bcas0006a.a.08	collagen alpha 1(III) chain (COL3A1)	9.79	0.0041	up-regulated
bcas0003a.g.06	fas-activated serine/threonine kinase (FASTK)	9.71	0.0042	up-regulated
bcaj0009a.b.10	nadh-ubiquinone oxidoreductase chain 3 (NDUFV3)	9.24	0.0051	up-regulated
bcas0006a.f.08	inosine-5'-monophosphate dehydrogenase 1(IMPDH1)	9.20	0.0052	up-regulated
bcaj0006a.f.08	myosin heavy chain, skeletal muscle, adult 2 (MyHC-2A) (MYH2)	9.20	0.0052	down-regulated
bcaj0008a.b.08	60s ribosomal protein l18a (RPL18A)	8.95	0.0057	down-regulated
bcaj0006a.f.11	ferritin heavy chain (ferritin h subunit, FTH1)	8.93	0.0058	up-regulated
bcaj0008a.b.07	SCIRP10-related protein (SCIRP10) membrane associated progesterone receptor component	8.88	0.0059	down-regulated
bcas0004a.e.10	Homo sapiens trafficking protein particle complex 5 (TRAPPC5)	8.45	0.0071	up-regulated
bcaj0002a.a.07	40S ribosomal protein S14 (RPS14)	8.33	0.0074	down-regulated
bcas0005a.a.05	F1Fo-ATP synthase complex Fo membrane domain g subunit (F1F0-ATP synthase g subunit) (ATP5L)	8.28	0.0076	up-regulated
bcas0006a.h.04	cell division protein kinase 4 (cyclin-dependent kinase 4) (CDK4)	8.14	0.0080	up-regulated
bcas0003a.g.04	40s ribosomal protein s24 (RPS24)	8.09	0.0082	up-regulated
bcaj0009a.c.06	unknown	7.37	0.0112	up-regulated
bcaj0007a.c.02	unknown	7.16	0.0123	down-regulated
bcaj0001a.a.02	unknown	7.12	0.0125	down-regulated
bcaj0002a.h.09	ferritin heavy chain (FTH1)	6.94	0.0136	up-regulated
bcas0004a.e.08	nadh-ubiquinone oxidoreductase 18 kda subunit, mitochondrial precursor (NDUFS4)	6.82	0.01430	up-regulated
bcaj0007a.g.09	similar to WAS protein family, member 2 (WASF2)	6.56	0.01612	down-regulated
bcaj0003a.b.05	similar to Homo sapiens ankyrin repeat-containing protein and SOCS box containing 2 (ASB2) mRNA	6.43	0.0171	down-regulated
bcaj0007a.e.11	cytochrome b (CYTB)	6.35	0.0177	up-regulated
bcaj0008a.a.10	serin protease 15 (PRSS15)	6.23	0.0187	up-regulated
bcaj0007a.d.01	6-phosphofructokinase, muscle type (PFKM)	6.09	0.0200	up-regulated

bcaj0002a.a.09	unknown	6.07	0.0201	down-regulated
bcas0002a.e.10	titin (TTN)	5.99	0.0209	up-regulated
bcaj0003a.f.10	vacuolar protein sorting 13C (VPS13C)	5.96	0.0212	down-regulated
bcaj0001a.c.11	unknown	5.92	0.0216	down-regulated
bcas0003a.f.11	Homo sapiens ADP-ribosylhydrolase like 2 (ADPRHL2)	5.82	0.0226	up-regulated
bcaj0003a.a.01	alpha crystallin b chain (alpha(b)-crystallin ; CRYAB)	5.76	0.0233	down-regulated
bcas0006a.f.05	glycerol-3-phosphate dehydrogenase [nad+], cytoplasmic (gpd-c) (GPD1)	5.59	0.0252	down-regulated
bcas0005a.b.06	sparc precursor (secreted protein acidic and rich in cysteine) (SPARC)(osteonectin)	5.58	0.0253	up-regulated
bcaj0005a.g.09	cytochrome oxidase assembly factor pet112 homolog (PET112L)	5.50	0.0263	down-regulated
bcas0005a.a.02	cytochrome b (CYTB)	5.44	0.0271	up-regulated
bcas0003a.a.06	cullin homolog 3 (CUL3)	5.41	0.0275	down-regulated
bcaj0002a.h.11	unknown	5.38	0.0278	down-regulated
bcaj0002a.e.01	anaphase promoting complex subunit 11 (APC11) (hepatocellular carcinoma associated ring finger protein) (protein hspc214)	5.33	0.0285	down-regulated
bcaj0006a.d.07	cytochrome c oxidase I (COX1)	5.28	0.0292	up-regulated
bcaj0009a.c.08	golgi SNAP receptor complex member 2 (GOSR2) variant A	5.22	0.0301	down-regulated
bcaj0004a.e.05	cytochrome c oxidase subunit VIa polypeptide 2 (COX6A2)	5.14	0.0312	up-regulated
bcaj0006a.h.10	unknown	5.13	0.0315	up-regulated
bcas0004a.g.03	succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial precursor (SDHA)	5.10	0.0318	down-regulated
bcas0004a.c.03	similar to cardiomyopathy associated 3 (CMYA3)	5.09	0.0321	down-regulated

The F-value is the calculated statistic computed by GeneANOVA. The higher the F-value, the more significant the difference between groups. The *p*-value is the significance level.

Table 4. Validation of differential expression for some genes in the RA and ST muscles of the 'grazing vs 'maize-silage' animals.

	Production system (P)	Muscle (M)	P x M
TNNT1	NS	0.0001	NS
CRYAB	0.07	0.0001	NS
CA3	0.007	0.0001	0.02
ALDOA	0.001	0.0001	0.0001
RPL0	0.05	0.003	NS
CKM	0.07	NS	NS
SDHA	0.06	NS	0.04
SEPW1	0.0009	NS	0.06
ACTA1	0.0001	NS	NS

Genes were declared as differentially expressed by GeneANOVA according to the muscle type or the production system and their mRNA were quantitated by real-time PCR in the RA and ST muscles of individual animals (n=6 per group). ACTA1: alpha-actin 1, ALDOA: fructose-bisphosphate aldolase A, CKM: creatine kinase muscle chain, CA3: carbonic anhydrase III, CRYAB: alpha crystallin b chain, RPL0: 60s acidic ribosomal protein p0, SEPW1: selenoprotein W, SDHA: succinate dehydrogenase (ubiquinone) flavoprotein subunit, mitochondrial precursor TNNT1: troponin T1, slow skeletal muscle isoform.

muscle type or the production system was checked and validated for some selected genes by qPCR (*Table 4*).

Grazing affected gene expression in many functional categories as shown by the PANTHER classification (*Fig. 1*). Unfortunately 13% of differential genes belonged to unclassified GO "biological processes". However the remaining genes were classified mostly as belonging to "protein metabolism and modification" (22%), "cell cycle" (14%), "developmental processes" (12%), "muscle contraction" (12%), "signal transduction" (10%), "intracellular traffic" (10%), "cell structure and motility" (10%) or "electron transport" (10%). For "protein metabolism and modification", the genes were categorised according to their involvement in "protein modification" (36%), "protein biosynthesis" (28%), "proteolysis" (24%), "translational regulation" (8%) and "protein complex assembly" (4%) (data not shown).

Additional information on gene function was obtained by searching for KEGG pathways using the Babilomics tool "FatiGO+" (http://babelomics.bioinfo.cipf.es/fatigoplus). This allowed us to predict that two main pathways were altered by the production system, the ribosome (path:hsa03010) including 7 genes (UBA52, RPS10, RPS14, RPL4, RPLP0, RPS24, RPL18A) and the oxidative phosphorylation (path:hsa00190) including 6 genes (ATP5L, SDHA, NDUFV3, COX1, NDUFS4, COX6A2) found to be differential.

Identification of a putative gene marker of the production system

Interestingly, a muscle clone corresponding to Selenoprotein W was found to be down-regulated in the muscles of grass-fed compared to maize silage-fed steers using GeneANOVA. In order to validate the differential Selenoprotein W expression according to the production system, we performed a quantitation of SEPW1 mRNA by qPCR in the RA and ST muscles of individual animals (*Fig. 2A*) and a Northern blot analysis of the RA samples of individual animals fed either with a maize- (n=6) or a grass-based diet (n=6) in different experimental conditions (without or with 1h walking per day) (*Fig. 2B*). These analyses confirmed the down-regulation of the Selenoprotein W transcript in the grass-fed animals compared to the maize-fed animals as shown by a higher Ct value in qPCR experiments and a lower hybridisation intensity of the 0.9 kB band in Northern-blot experiments.

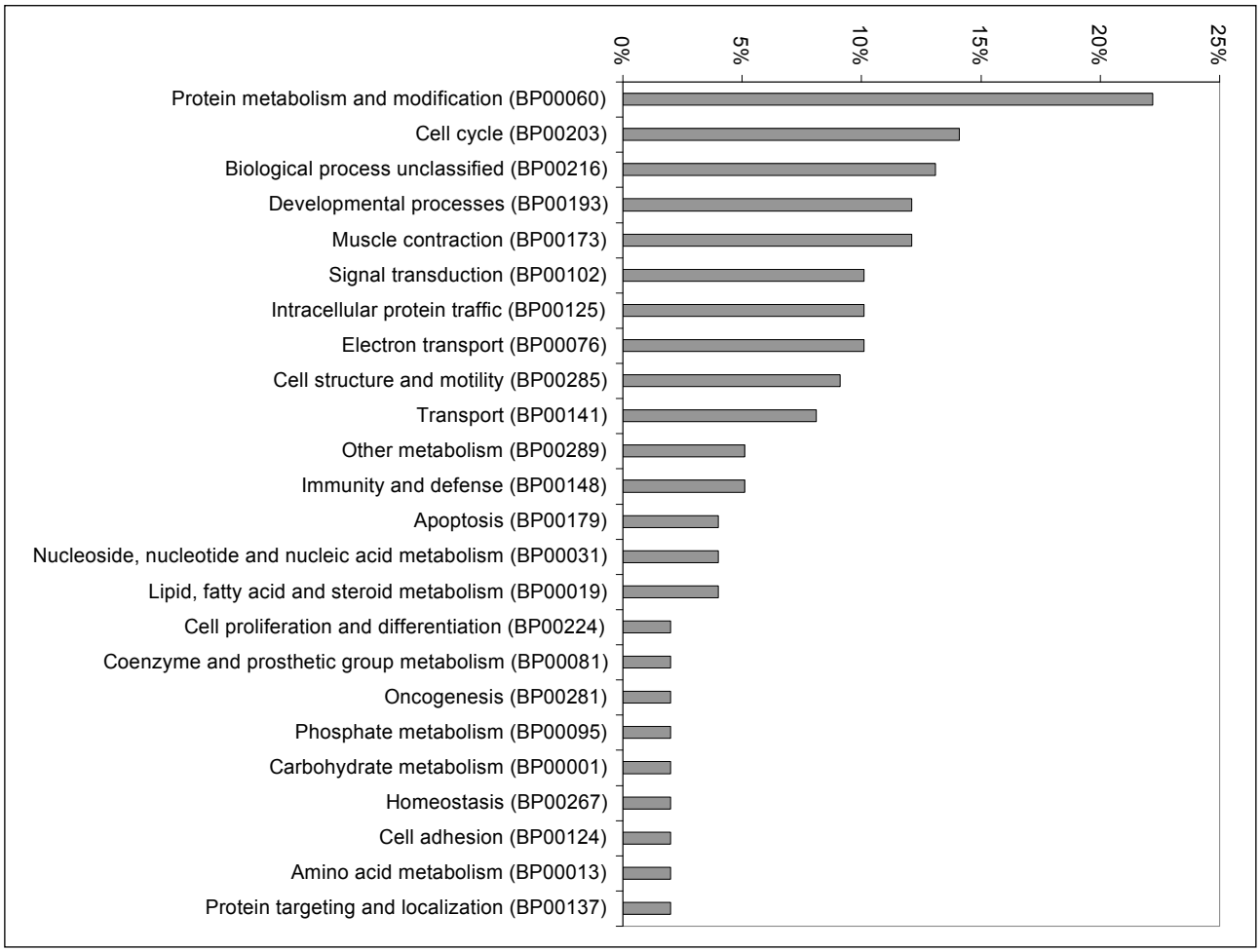

Fig. 1. Biological functions of differential genes according to the production system. Of the 149 detected genes, only 134 genes with GO information, either up- or down-regulated were classified according to the biological process ontology terms using PANTHER. The chart represents the percentage of gene hits against total differential genes relative to GO category name (Biologial Process Accession Number).

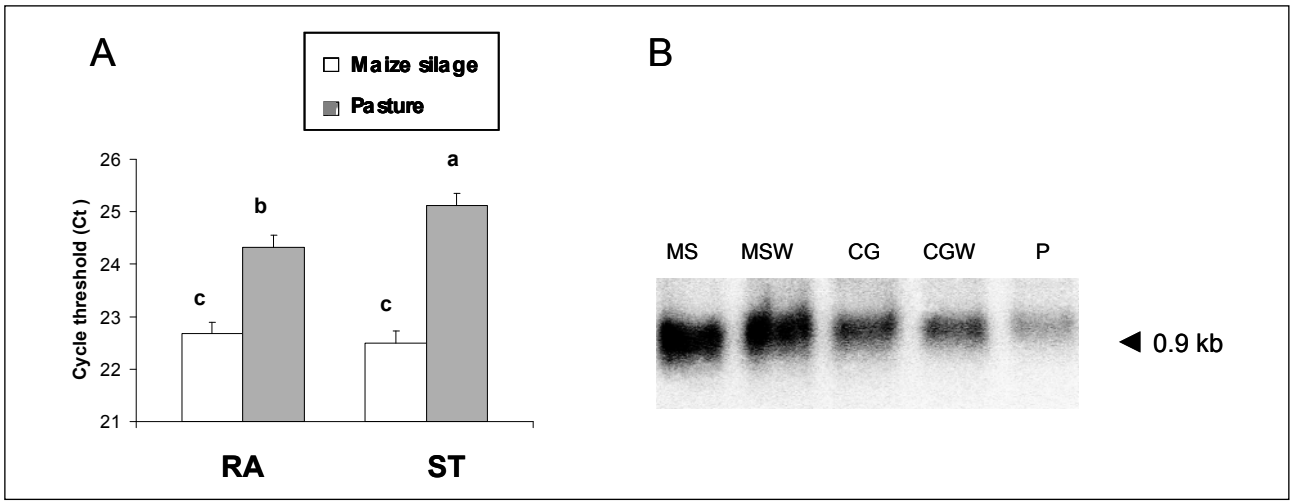

Fig. 2. Selenoprotein W gene expression in the muscles of beef steers according to their production system. (A) Real-time PCR for selenoprotein W mRNA quantitation in the *rectus abdominis* (RA) and *semitendinosus* (ST) muscles of beef steers fed maize-silage indoors (n=6) or grazing on pasture (n=6). Expression levels were quantified by the cycle threshold (Ct). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lowest the Ct level the greatest the amount of mRNA in the sample). Results are means ± SEM. Means without a common superscript letter differ ($P<0.05$). (B) Northern-Blot analysis of Selenoprotein W mRNA (0.9 kb) in the *rectus abdominis* muscle of beef steers grazing on pasture (P), or fed cut grass or maize silage. Animals on the grass diet were fed freshly cut grass alone (CG group). Animals on the maize diet were fed maize-silage (MS) ad libitum, with a minimum of wheat straw and rape-seed meal. Half of the indoors animals on each feeding regime were submitted 7 days a week to a 5.2 km walk (W) daily (CGW and MSW groups).

DISCUSSION

In this study, we have monitored the influence of pasture-feeding on the metabolism and gene expression in the skeletal muscles of 30-month-old Charolais steers. The study was performed on two muscles with different intrinsic properties (the *semitendinosus*, ST, a mixed muscle predominantly glycolytic; the *rectus abdominis*, RA, a mixed muscle predominantly oxidative) and different sensory meat quality scores. While previous data indicated that the carcass composition was not altered (5) we show here that (1) grazing increased the oxidative metabolism of both muscle types (2) and that the muscle type and the production system both influenced muscle gene expression, clearly demonstrating a plasticity of muscle properties in response to the production system.

Plasticity of muscle metabolism according to the production system

As previously observed (19), our results showed that there was an oxidative switch of muscle metabolism when animals were fed on pasture, but no effect on the glycolytic metabolism. Animals were managed at the same growth rate and slaughtered at the same age, and their carcass weight and composition was similar at slaughtering. Thus the effects of mobility and the nature of the diet are likely to be predominant over hypothetical effects of feeding level, age and growth rate. The underlying mechanisms are probably better vascularisation (3) and increased lipid utilisation in grazing animals as observed for moderate exercise (19). In fact Jurie *et al*. (5) showed in the same experimental design that a cut grass diet (vs. maize silage) increased HAD and ICDH activities whereas mobility induced by walking increased HAD and CS activities. Thus the more oxidative metabolic orientation of muscles of grazing steers originates from a combination of increased mobility at pasture and a grass (*vs*. maize-silage)-based diet. These authors speculated that overall moderate daily physical activity as observed at pasture may be sufficient to increase muscle oxidative metabolism. The effect of the grass-based diet was hypothesised to act through polyunsaturated fatty acids (PUFA, which are abundant in grass) since PUFA are known to increase expression of genes encoding proteins involved in lipid oxidation (20). However, the effects of manganese and protein dietary supply which differ between the two groups can not be ruled out (5).

Changes in gene expression according to the feeding system

We conducted this study in order to better understand global muscle gene expression in beef and more specifically muscle gene expression response to the feeding system. Firstly, we detected a potent effect of muscle type on gene expression as expected and previously observed (15). Secondly, although less marked than that of the muscle type, there was an effect of the production system. However, no interaction could be detected by GeneANOVA between the production system and the muscle type, suggesting that eventhough the production system modified gene expression, differences in gene expression still remained between the two studied muscles. The effect of the production system appears to be associated mainly with genes belonging to the muscle and embryo cDNA library subsets. However, only half of the differential clones from the embryo cDNA library subset could be identified (using BLASTN or BLASTX searches). We detected the differential expression of three main categories of genes related to muscle metabolism, namely oxidative phosphorylation, contraction (MYH1, MYH2, ACTA1, ACTC, MYL1, TTN, , CRYAB), and muscle protein metabolism. This confirmed that plasticity of contractile and metabolic properties was occuring in muscle at transcriptional level. Moreover, most of the differentially expressed genes were found to be associated with protein metabolism (*e.g.* biosynthesis and proteolysis), mainly with translation (RPS24, RPL18A, RPS14, RPLP0, RPL4) and intracellular trafficking and secretion. Interestingly, the grass-based diet had a higher protein content that the maize-silage-based diet (5). Thus, since dietary protein is a substrate for cell protein synthesis, changes in skeletal muscle transcript levels probably reflect differences in protein intake. However, the experimental design could not enable us to dissociate the effect of the production system from that of the protein level of the diet.

Could selenoprotein W be a potential indicator of the production system?

Traceability of an animal's identity, breed, diet, and type of production are important indications demanded by consumers. In particular, specific and reliable indicators of pasture or grazing are still lacking. In this study, we monitored muscle gene expression in order to identify molecular indicators of production systems. Interestingly, profiling changes in gene expression on pasture revealed differential expressions of several genes. Amongst them, the expression of selenoprotein W gene (SeWP1) was the most altered and was found to be down-regulated in both the ST and RA muscles of steers grazing on pasture. This gene encodes a glutathione-binding protein containing a selenocysteine at the active site. This protein shows its highest expression in skeletal muscle and heart (21). Although the metabolic function of Selenoprotein W is not yet known, it may be involved in muscle and cardiac metabolism (22) and is a putative antioxidant (23, 24). The abundance of this protein in skeletal muscle and various other tissues is regulated by dietary selenium (25, 26) especially in sheep (27) and human for whom SeWP is highly sensitive to selenium depletion. For instance, SeWP expression levels were significantly low in lambs with white muscle disease, myopathies characterized by alteration of cardiac and skeletal muscle fibres (28). So the differential expression of SeWP may be related to the selenium content or bioavailability in the diet, but this remains to be studied. Lastly, as differential expression of SeWP was observed in muscle atrophy (29), it may also be regulated by muscle activity. SeWP expression could also be influenced therefore by the muscle activity due to the mobility of grazing steers. However, this was not supported by our northern-blot analyses indicating that SeWP expression in grazing steers is more probably linked to the selenium avaibility in the diet than to their muscle activity induced by mobility during grazing. Down-regulation of SeWP expression is also likely a signature of a decrease in the antioxidant potential of muscles.

Our data suggest that, based on the highest differential expression of Selenoprotein W and on its nutritional regulation, expression levels of this gene could be proposed as a putative indicator of a pasture or grass based system. However the usefulness of its expression for traceability purposes has to be fully explored especially according to muscle-types. Further studies including analyses at the post translational level are needed to conclude on the relevance of Selenoprotein W as putative production system marker.

In conclusion, using transcriptomics, we detected genes differentially expressed between two muscle types and two feeding systems. This allowed us to identify the expression levels of the gene encoding Selenoprotein W as a putative correlate of the production system, and consequently as a candidate for grazing traceability. However, further study is needed as to whether variation of Selenoprotein W gene expression is linked more specifically to the selenium status of the diet. Further exploration of the results thanks to the new sequencing data in cattle will also undoubtedly provide additional information on novel putative biomarkers for production systems.

Acknowledgements: The authors thank Genevieve Gentes (URH, INRA, Theix) for her excellent technical assistance, and the Experimental Unit of Le Pin and the slaughterhouse of Theix. This work was supported by the "Commissariat a l'Aménagement et au Développement Economique du Massif Central" (France) and by an INRA grant in the context of internal programmes of the Herbivore Research Unit entitled "Grass and Meat" and "Traceaibility of feeding of ruminants".

Conflict of interest statement: None declared.

REFERENCES

1. Geay Y, Bauchart D, Hocquette JF, Culioli J. Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. *Reprod Nutr Dev* 2001; 41(1): 1-26 (erratum 377).
2. Oddy VH, Harper GS, Greenwood PL, McDonagh MB. Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef. *Aust J Exp Agric* 2001; 41 (7): 921-942.
3. Vestergaard M, Oksbjerg N, Henckel P. Influence of feeding intensity, grazing and finishing feeding on muscle fibre characteristics and meat colour of semitendinosus, longissimus dorsi and supraspinatus muscles of young bulls. *Meat Sci* 2000; 54(2): 177-185.
4. Cassar-Malek I, Hocquette JF, Jurie C *et al*. Muscle-specific metabolic, histochemical and biochemical responses to a nutritionally induced discontinuous growth path. *Anim Sci* 2004; 79: 49-59.
5. Jurie C, Ortigues-Marty I, Picard B, Micol D, Hocquette JF. The separate effects of the nature of diet and grazing mobility on metabolic potential of muscles from Charolais steers. *Livest Sci* 2006; 104(1-2): 182-192.
6. Bernard C, Cassar-Malek I, Le Cunff M, Dubroeucq H, Renand G, Hocquette JF. New indicators of beef sensory quality revealed by expression of specific genes. *J Agric Food Chem* 2007; 55(13): 5229-5237.
7. Cassar-Malek I, Picard B, Bernard C, Hocquette JF. Application of gene expression studies in livestock production systems: a European perspective. *Aust J Exp Agric* 2008; 48(6-7): 701-710.
8. Lehnert SA, Byrne KA, Reverter A *et al*. Gene expression profiling of bovine skeletal muscle in response to and during recovery from chronic and severe undernutrition. *J Anim Sci* 2006; 84(12): 3239-3250.
9. Gondret F, Damon M, Jadhao SB, Houdebine LM, Herpin P, Hocquette JF. Age-related changes in glucose utilization and fatty acid oxidation in a muscle-specific manner during rabbit growth. *J Muscle Res Cell Motil* 2004; 25(4-5): 405-410.
10. Bernard C, Degrelle S, Ollier S *et al*. A cDNA macroarray resource for gene expression profiling in ruminant tissues involved in reproduction and production (milk and beef) traits. *J Physiol Pharmacol* 2005; 56(Suppl. 3): 215-224.
11. Sudre K, Leroux C, Cassar-Malek I, Hocquette JF, Martin P. A collection of bovine cDNA probes for gene expression profiling in muscle. *Mol Cell Probes* 2005; 19(1): 61-70.
12. Degrelle SA, Campion E, Cabau C *et al*. Molecular evidence for a critical period in mural trophoblast development in bovine blastocysts. *Dev Biol* 2005; 288(2): 448-460.
13. Provost Fl, Lepingle A, Martin P. A survey of the goat genome transcribed in the lactating mammary gland. *Mammal Gen* 1996; 7(9): 657-666.
14. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162(1): 156-159.
15. Sudre K, Leroux C, Pietu G *et al*. Transcriptome analysis of two bovine muscles during ontogenesis. *J Biochem* 2003; 133(6): 745-756.
16. Didier G, Brezellec P, Remy E, Henaut A. GeneANOVA-gene expression analysis of variance. *Bioinformatics* 2002; 18(3): 490-491.
17. Thomas PD, Kejariwal A, Campbell MJ *et al*. PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification. *Nucleic Acids Res* 2003; 31(1): 334-341.
18. Al-Shahrour F, Minguez P, Vaquerizas JM, Conde L, Dopazo J. BABELOMICS: a suite of web tools for functional annotation and analysis of groups of genes in high-throughput experiments. *Nucleic Acids Res* 2005; 33(Web Server issue): W460-464.
19. Ortigues-Marty I, Jurie C, Hocquette JF *et al*. Principal component analysis of performances and muscle characteristics of beef steers according to diets and levels of growth. Multi-function grasslands: quality forages, animal products and landscapes. Proceedings of the 19th General Meeting of the European Grassland Federation, La Rochelle, France, 27-30 May 2002; 2002. pp. 584-585.
20. Clarke SD. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. *Br J Nutr* 2000; 83(Suppl. 1): S59-S66.
21. Gu QP, Sun Y, Ream LW, Whanger PD. Selenoprotein W accumulates primarily in primate skeletal muscle, heart, brain and tongue. *Mol Cell Biochem* 2000; 204(1-2): 49-56.
22. Whanger PD. Selenoprotein W: a review. *Cell Mol Life Sci* 2000; 57(13-14): 1846-1852.
23. Jeong D, Kim TS, Chung YW, Lee BJ, Kim IY. Selenoprotein W is a glutathione-dependent antioxidant in vivo. *FEBS Lett* 2002; 517(1-3): 225-228.
24. Loflin J, Lopez N, Whanger PD, Kioussi C. Selenoprotein W during development and oxidative stress. *J Inorg Biochem* 2006; 100(10): 1679-1684.
25. Vendeland SC, Beilstein MA, Yeh JY, Ream W, Whanger PD. Rat skeletal muscle selenoprotein W: cDNA clone and mRNA modulation by dietary selenium. *Proc Natl Acad Sci USA* 1995; 92(19): 8749-8753.
26. Yeh JY, Beilstein MA, Andrews JS, Whanger PD. Tissue Distribution and influence of selenium status on levels of selenoprotein-W. *FASEB J* 1995; 9(5): 392-396.
27. Gu O, Yeh JY, Beilstein MA, Forsberg NE, Whanger PD. Effect of Selenium Status on Selenoprotein-W Distribution in Sheep Tissues. *FASEB J* 1995; 9(3): 158-158.
28. Whanger PD, Vendeland SC, Beilstein MA. Some biochemical properties of selenoprotein W. Trace elements in man and animals - TEMA 8. Proceedings of the 8th International Symposium on Trace Elements in Man and Animals; 1993. pp. 119-126.
29. St-Amand J, Okamura K, Matsumoto K, Shimizu S, Sogawa Y. Characterization of control and immobilized skeletal muscle: an overview from genetic engineering. *FASEB J* 2001; 15(3): 684-692.

Received: September, 10, 2008
Accepted: February 2, 2009

Authors address: Isabelle Cassar-Malek, INRA, UR1213, Herbivore Research Unit, 63122 Saint-Genes-Champanelle, France. Phone: +33-04-73624657; Fax.: +33-04-73624639; e-mail: cassar@clermont.inra.fr