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The effects of PPO activity on the proteome of ingested red clover and implications for improving the nutrition of grazing cattle

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Supplementary Material

Spot number	Normalised volumes				Significance			Regulation
	Wild type 0 h	Mutant 0 h	Wild type 4 h	Mutant 4 h	Type	Time	Type vs Time	
1	2,686.6	2,368.2	1309.5	1210	0.956	0.035	0.647	Wild-type and mutant at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
2	377.6	152.94	256.9	196.9	0.982	0.728	0.105	Mutant at 4 hours and wild type at 4 hours are down in abundance compared to wild type at 0 hours
3								Wild-type at 4 hours is more than 2 fold down in abundance and is not present in mutant at 4 hours compared to wild type at 0 hours
4	1,377.4	1,180.8	214.9	<i>np</i>	0.334	<.001	0.965	Wild-type and mutant at 4 hours are more than 2 fold down in abundance compared to wild-type at 0 hours
5	1,106.3	850.4	501.2	290.7	0.278	0.025	0.911	Wild-type at 4 hours is more than 2 fold down in abundance and is not present in mutant at 4 hours compared to wild type at 0 hours
6	711.4	481.4	193.0	<i>np</i>	0.151	0.004	0.777	Mutant at 4 hours is more than 2 fold down in abundance compared to wild type at 0 hours
7	656.6	813.1	652.3	243.9	0.664	0.334	0.341	Wild-type and Mutant at 0 hours down in abundance compared to wild-type and mutant at 4 hours
8	397.6	506.2	293.3	189.7	0.969	0.013	0.129	Wild-type and mutant at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
9	668.4	561.2	572.3	372	0.503	0.533	0.836	Wild-type and mutant at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
10	331.9	236.7	134.6	242.9	0.703	0.649	0.269	Mutant at 4 hours is more than 2 fold up regulated compared to wild-type at 0 hours
11	319.9	<i>np</i>	<i>np</i>	733.4	~	~	~	Wild-type at 4 hours is more than 2 fold down in abundance compared to wild-type at 0 hours
12	400.6	344.7	277.3	221.7	0.684	0.237	0.406	Prominent landscape marker spot present on all gels
13	4,441.1	3,856.1	5,598.6	4,609.9	0.346	0.258	0.804	Wild-type at 4 hours more than 2 fold down in abundance compared to wild type at 0 hours
14	526.7	318.7	221.5	185.5	0.365	0.129	0.516	Mutant at 0 hours more than 2 fold up in abundance compared to wild-type at 0 hours
15	279.4	775.2	501.2	<i>np</i>	0.994	0.426	0.175	Wild-type at 4 hours is more than 2 fold down in abundance and is not present in mutant at 4 hours compared to wild type at 0 hours
16	374.3	354.5	82.2	<i>np</i>	0.477	0.003	0.66	Mutant at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
17	152.4	174.1	291.7	193.8	0.515	0.203	0.321	Present in wild-type and mutant at 4 hours, not present at 0 hours
18	329.6	312.8	267.1	171.3	0.457	0.194	0.598	Only present in the wild-type at 0 hours
19	<i>np</i>	<i>np</i>	527.8	438.5	~	~	~	Wild-type and mutant at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
20	234.4	310.8	<i>np</i>	<i>np</i>	~	~	~	Only present in the wild-type at 0 hours
21	887.3	650.9	352.6	363.3	0.933	0.05	0.874	Only present in the wild-type at 0 hours
22	75.7	<i>np</i>	<i>np</i>	<i>np</i>	~	~	~	Up in abundance at 4 hours in wild-type and mutant, more than 2 fold up in abundance in the mutant after 4 hours
23	334.2	<i>np</i>	<i>np</i>	<i>np</i>	~	~	~	Not present in the wild-type or mutant at 4 hours
24	730.8	581.6	1216.2	1,814.2	0.725	0.492	0.541	Mutant at 0 hours and wild-type and mutant at 4 hours more than 2 fold down in abundance compared to the wild-type at 0 hours
25	128	85	<i>np</i>	<i>np</i>	0.467	0.009	0.467	Wild-type at 4 hours and mutant at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours, not present in the mutant at 4 hours
26	717.6	720.3	463.9	441.6	0.924	0.189	0.934	Not present in the mutant at 4 hours
27	470	149	176	<i>np</i>	<0.001	<0.001	0.026	Mutant at 4 hours more than 2 fold down in abundance compared to mutant at 0 hours
28	147	59	188	<i>np</i>	0.073	0.892	0.458	Wild-type at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours, not present in mutant at 4 hours
29	172	348	266	140	0.853	0.680	0.292	Wild-type at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
30	320	377	145	<i>np</i>	0.580	0.014	0.232	Wild-type at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
31	798	678	219.7	274.8	0.602	0.067	0.579	Wild-type and mutant at 4 hours more than 2 fold down in abundance compared to wild-type at 0 hours
31	1776	1440	907	732	0.330	0.014	0.751	

Table S1. Normalised spot volumes and significance values (P<0.05) dependent on phenotype (type), time and interaction (type vs time) for each gel spot excised and regulation description when comparing each gel type to the wild type at 0 hours (+/- 2 fold abundance).

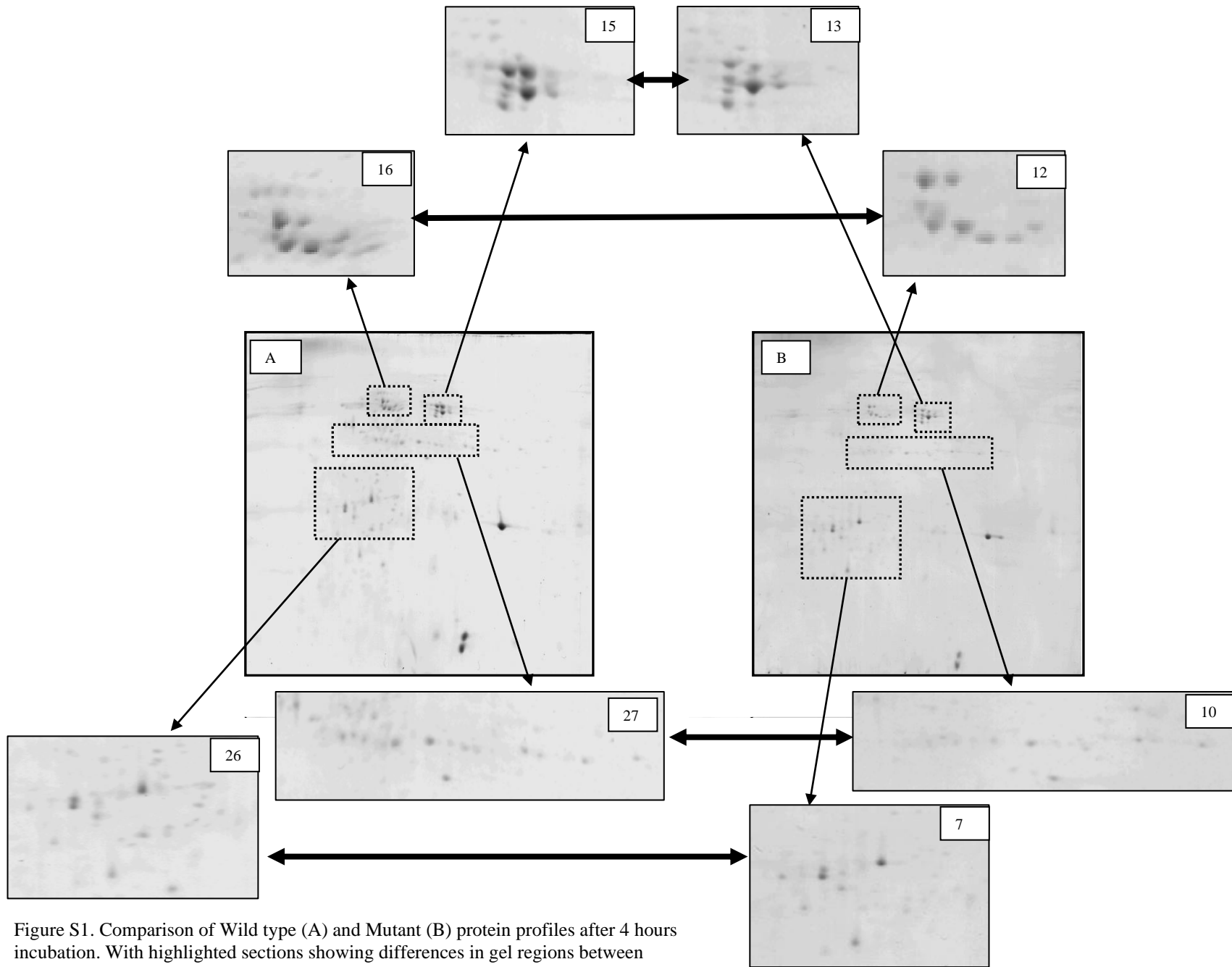


Figure S1. Comparison of Wild type (A) and Mutant (B) protein profiles after 4 hours incubation. With highlighted sections showing differences in gel regions between phenotype and spot count for each region.

Spot Number	Accession	Description	Species	Location	Peptide Sequence	Mascot score	Sequence coverage (%)
1	RBL_BYRCR	Ribulose biphosphate carboxylase large chain	<i>Byrsonima crassifolia</i>	Chloroplast	TFQGPPHGIQVER EITLGFVDLLR DNGLLLHIHR	481	36
2	MDH_PSEM	Malate dehydrogenase	<i>Pseudotsuga menziesii</i>	Mitochondria	LFGVTTLDVVR DDLFINAGIVK	156	100
3	PSBO_PEA	Oxygen-evolving enhancer protein 1,	<i>Pisum sativum</i>	chloroplast	GASTGYDNAVALPAGGR DGIDYAAVTVQLPGGER RLTFDEIQSK	415	19
4	PSBP_PEA	Oxygen-evolving enhancer protein 2	<i>Pisum sativum</i>	chloroplast	EFPGQVLR EFPGQVLR-Pyro-glu	83	15
5	CB215_PEA	Chlorophyll a-b binding protein 215	<i>Pisum sativum</i>	chloroplast	SAPESIWYGPDPRK ELEVIHSR FGEAVWFK	137	11
6	ATPB_HYANO	ATP synthase subunit beta	<i>Hyacinthoides non-scripta</i>	chloroplast	AHGGVSVFGGVGER IFNVLGEPIDNLGPVDTR VALVYGQMNPEGAR	350	37
7	ATPBN_ARATH	ATP synthase subunit beta-2	<i>Arabidopsis thaliana</i>	mitochondria	AHGGFSVFAGVGER FTQANSEVSALLGR	141	11
8	ATPA_BUXMI	ATP synthase subunit alpha	<i>Buxus microphylla</i>	chloroplast	IAQIPVSEGYLGR LIESPAPGIISR EAYPGDVFYLHSR	214	9
9	ATPA_BUXMI	ATP synthase subunit alpha	<i>Buxus microphylla</i>	chloroplast	IAQIPVSEGYLGR LIESPAPGIISR EAYPGDVFYLHSR	163	12
10	CB28_PEA	Chlorophyll a-b binding protein 8	<i>Pisum sativum</i>	chloroplast	VASSGSPWYGPDR KVASSGSPWYGPDR	155	11
11	G3PA_PEA	Glyceraldehyde-3-phosphate dehydrogenase A	<i>Pisum sativum</i>	chloroplast	TFAEEVNEAFR EFPGQVLR QYYSITVLTR HQLITATVADGK KFVENAAGSFSVA	63	6
12	PSBP_WHEAT	Oxygen-evolving enhancer protein	<i>Triticum aestivum</i>	chloroplast	ELEVIHSR VGGGPLGEGLDK VLELGDAIAGLTLEEAR	231	16
13	CB23_ORYSI	Chlorophyll a-b binding protein	<i>Oryza sativa indica group</i>	chloroplast	TEFDVVEVPSSAR	72	7
14	RK12_ORYSJ	50s Ribosomal protein L12	<i>Oryza sativa subsp.japonica</i>	chloroplast	HVIGEDDEFIGK VASPEQAQEVHAAVR GPDFATICNSVTSK	216	16
15	TPIC_SECCE	Triosephosphate isomerase	<i>Secale cereale</i>	chloroplast	GILAMDESATCGK LASIGLENTEANR EAAYYQGGAR TVVSIPNGPSELAVK ALQNTCLK	84	13
16	ALFC_ORYSJ	Fructose-bisphosphate aldolase	<i>Oryza sativa japonica group</i>	chloroplast	ANSLAQLGKYSDGEAAEAK	360	20

17			<i>Oryza sativa japonica group</i>	chloroplast	GILAMDESNATCGK LASIGLENTEANR EAAYYQQGAR TVVSPNGPSELAVK ALQNTCLK ANSLAQLGKYTSDGEAAEAK		
18	ALFC_ORYSJ	Fructose-bisphosphate aldolase	<i>Lolium perenne</i>	chloroplast	KVGIENIGRVVQVGDGIAR GIALNLESK IAQIPVSEAYLGR GEIIASESRLIESPAPSIISR TAVATDTILNQK HTLIYDDLK EAYPGDVFLHSR TSQNQLAR GYLDSLEIEQVNFLLDLRK DTKPQFQEILSSK EAIQEQLER	345	20
19	ATPA_LOLPR	ATP synthase subunit alpha	<i>Triticum aestivum</i>	chloroplast	LLICMGEAMR TASCGGTACVNSFGDEQLAVDMLADK LTGVTGGDQVAAMGIYGP DCPGTHEFLLDEGK MFSPGNLRATFDNPDYDK YTGGMVDPVNQIIVK GIFTNVTSPYAK FEETLYGSSR	643	29
20	S17P_WHEAT	Sedoheptulose-1,7-bisphosphatase	<i>Lolium perenne</i>	chloroplast	ASVGFQAGVK LTYYPPEYETKDTDILAAFR TFQGPPHGIQVER YGRPLLGTIKPK ACYECLRGGLDFTKDDENVNSQPFMR FVFCAEALYKAQAETGEIKGHYLNATAGTCEEMIK DNGLLLHIHRAMHAVIDR MSGGDHIHSGTVVVK EMTLGFVDLLR FEFEPVDTIDN	479	32
21	RBL_LOLPR	Ribulose bisphosphate carboxylase large chain	<i>Triticum aestivum</i>	Apoplast	VDFAPGGTNPP HIHPR GELLVG ILGSLDSGNK AGETFLIPR	485	36
22	GER2_WHEAT	Oxalate oxidase	<i>Triticum aestivum</i>	chloroplast	(K)FLCPCHGSQYNNQGK(V) (K)GDPTYLVVESDK(T)	108	18
23	Q7X9A6	Cytochrome b6-f complex iron-sulfur subunit	<i>Triticum aestivum</i>	chloroplast		42	12
23	RBS_FAGCR	Ribulose bisphosphate carboxylase small chain	<i>Fagus crenata</i>	chloroplast		28	28
24	RBL_LOLPR	Ribulose bisphosphate carboxylase large chain	<i>Lolium perenne</i>	chloroplast	ASVGFQAGVK LTYYPPEYETKDTDILAAFR	627	37

25			<i>Lolium perenne</i>	chloroplast	TFQPPHGIQVER YGRPLLGCTIKPK ACYECLRGGLDFTKDDENVNSQPFMR FVFCAEALYKAQAETGEIKGHYLNATAGTCEEMIK DNGLLLHIHRAMHAVIDR NHGMHFR MSGGDHIHSGTVVGK EMTLGFVDLLR FEFEPVDTIDN ASVGFQAGVK LTYYPPEYETKDTDILA AFR TFQPPHGIQVER YGRPLLGCTIKPK ACYECLRGGLDFTKDDENVNSQPFMR FVFCAEALYKAQAETGEIKGHYLNATAGTCEEMIK DNGLLLHIHRAMHAVIDR NHGMHFR MSGGDHIHSGTVVGK EMTLGFVDLLR FEFEPVDTIDN		
26	RBL_LOLPR	Ribulose biphosphate carboxylase large chain	<i>Lolium perenne</i>	chloroplast	ASVGFQAGVK LTYYPPEYETKDTDILA AFR TFQPPHGIQVER YGRPLLGCTIKPK ACYECLRGGLDFTKDDENVNSQPFMR FVFCAEALYKAQAETGEIKGHYLNATAGTCEEMIK DNGLLLHIHRAMHAVIDR NHGMHFR MSGGDHIHSGTVVGK EMTLGFVDLLR FEFEPVDTIDN	627	37
27	RBL_LOLPR	Ribulose biphosphate carboxylase large chain	<i>Arabidopsis thaliana</i>	Apoplast	YNLSLGLGLNK	627	37
28	SGAT_ARATH	Serine--glyoxylate aminotransferase	<i>Lolium perenne</i>	chloroplast	IPYDMQLK KGGLNVGAVLILPEGFELAPPDR IGNLSFQSYRPDKKNILVIGVPVGGK KYSE IVFPILSPDPATK YPIYVGGNR GQIYPDGSKSNNTVYNATSTGIVK EKGGYEISIVDASDGRQVIDTIPPGPELLVSEGESIK VQLYEMNF	52	2
29	CYF_LOLPR	Apocytochrome	<i>Triticum aestivum</i>	chloroplast	EREFPQQVLR QYYSITVLTR HQLITATVADGK KFVENAAGSFVA	502	47
30	PSBP_WHEAT	Oxygen-evolving enhancer protein 2	<i>Triticum aestivum</i>	chloroplast	VDFAPGGTNPPHIHPRATEIGIVMK	184	16
	GER2_WHEAT	Oxalate oxidase			AGETFLIPR	127	15

31			<i>Triticum aestivum</i>	chloroplast	RLTFDEIQSKT DGIDYAAVTVQLPGGER GDEEELAKENVKNASSTGNITLSVTK	1684	16
	PSBO_WHEAT	Oxygen-evolving enhancer protein 1					

Table S2. Proteins identified by MALDI TOF/TOF mass spectrometry. Gel plugs (fig.1) were excised, trypsin digested and analysed by MALDI TOF/TOF MS. The MS data was queried using the Mascot database.