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Data Article

Digestomic data of proteolysis during whether post rumen digestion after tannin supplementation

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Dataset link: Impact of Tannin Supplementation on Proteolysis during Post- Ruminal Digestion in Wethers (Original data)

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A B S T R A C T

The protein degradation of alfalfa hay after tannin supplementation was monitored during wethers digestion. Three rumen-cannulated wethers were infused a tannin solution, and water for control, through the cannula. The digestion time-points samples were collected *in vivo* in the rumen and *in vitro* in the abomasum, and the small intestine compartments. The digestomic dataset was acquired by identifying and quantifying the peptides resulting from the protein degradation, using high-resolution LC-MS/MS mass spectrometry and label-free quantitation. The digestomic dataset is the compilation of proteomic data acquired in the rumen and peptidomic data acquired in the abomasum and in the small intestine. The proteomic analysis identified 20 *Medicago* proteins in the rumen fluid, based on 169 peptides of which 140 are unique. The peptidomic analysis identified 28 *Medicago* proteins in the abomasum, based on 575 peptides of which 363 are unique, and 11 *Medicago* proteins in the small intestine, based on 94 peptides of which 63 are unique. This digestomic dataset of proteolysis during sheep post rumen digestion after tannin supplementation reveals the protein regions protected by tannin supplementation, and could

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be reused in studies related to the protein use efficiency by ruminants.

Specifications Table

Subject	Agricultural science
Specific subject area	Analysis LC-MS/MS of ruminal digestion products obtained from rumen and a dynamic in vitro digestive system (DIDGI® (abomasum/small intestine)).
Type of data	Figure, Tables
How the data were acquired	NanoLC-MS/MS (Ultimate 3000 nanoRSLC-QExactive HF-X or LTQ-Orbitrap mass spectrometer (Thermo-Fisher Scientific, Villebon-sur-Yvette, France)
Data format	LC-MS/MS Raw data (Thermo-Fisher Scientific, Villebon-sur-Yvette, France) Identification and quantitation data (ProgenesisQI, Nonlinear Dynamics, Waters, Newcastle, UK)
Description of data collection	The mass spectrometry data were recorded with Xcalibur, the mass spectrometer control software. These raw data (.raw) were transformed into reprocessed data (.MZNLD) by the ProgenesisQI quantitation software (Nonlinear Dynamics). The quantified peptides and proteins identified by MASCOT (Matrix Science) and/or Peaks (Bioinformatics Solutions Inc) were analyzed by statistical tools integrated in ProgenesisQI, to give information on the hydrolysis of proteins in each studied compartment. Normalization was applied using a scalar factor on each sample to recalibrate them to a reference run.
Data source location	• *Institution: INRAE* • *City/Town/Region: Saint-Genès-Champanelle* • *Country: France*
Data accessibility	All additional data provided in this article are open access by downloading Excel files:Repository name: Pride project via ProteomeXchange Data identification number: PXD032973, Direct URL to data: https://www.ebi.ac.uk/pride/archive/projects/PXD032973
Related research article	T. Sayd, C. Chambon, M. Popova, D. P. Morgavi, A. Torrent, S. Blinet, L. Théron, V. Niderkorn. Impact of tannin supplementation on proteolysis during post-ruminal digestion in wethers using a dynamic *in vitro* system: a plant (*Medicago sativa*) digestomic approach. Journal of Agricultural and Food Chemistry, 2022, 70, 2221-2230 (https://doi.org/10.1021/acs.jafc.1c07378)

Value of the Data

- The data provide the peptides and proteins identified during sheep digestion in the rumen, and in simulated abomasum and small intestine after tannin supplementation.
- Protein and peptide sequences protected by tannin during sheep digestion are identified and quantified in rumen, abomasum and small intestine.
- This peptidomic and proteomic datasets provide new insights into the animal nutrition and feeding efficiency in ruminants and give new elements to optimise feed supplementation and significantly decrease environmental impact through nitrogen release.
- The digestomic dataset presented can be used to compare with *in vivo* digestion data to further consider this approach as an alternative to animal experiment according to the 'R' principles.

1. Data Description

1.1. Rumen Proteomic

In Rumen, 140 unique peptides were identified from the digestion of 20 medicago proteins with at least two unique peptides (Table 1a and via the PRIDE repository with the dataset identifier PXD032973). The majority of the identified peptides were derived from the 5 most represented medicago sativa proteins. These unique peptides (or peptides without sequence conflicts) for each protein were used for label free quantitation (Table 1b and all the individual raw data acquired during the digestion kinetic in both Control and Tannin groups are available via the PRIDE repository with the dataset identifier PXD032973, https://www.ebi.ac.uk/pride/archive/projects/PXD032973).

1.2. Abomasum Peptidomics

In the abomasum, 363 unique peptides were identified from the digestion of 28 medicago proteins with at least two unique peptides (Table 2a and via the PRIDE repository with the dataset identifier PXD032973). Such as for the rumen proteomic results, the 5 most represented proteins in number of identified peptides account for about 60% of the identified peptides in this digestive compartment. These unique peptides for each protein were used for label-free

Table 1a
Proteomic list of proteins identified in the rumen compartment during wether digestion of alfalfa hay supplemented in tannins.

Protein name	Mass	Accession number	Gene name	Peptide count	Unique peptides	Confidence score
Histone H3.2, partial	14361.8	AAB36495	H3	3	3	79.96
Chlorophyll a/b binding protein	28312.3	AAC25775	CARCAB1	7	7	365.50
Glycolate oxidase, partial	30550.2	AAC32392	GOX	4	4	216.68
Rubisco activase, partial	30018.3	AAN15946	Rca	6	6	420.07
Heat shock protein 70	70996.5	AAV98051	HSP70-1	10	9	674.11
Pentameric polyubiquitin, partial	21187.5	AAZ32851	UBQ11	4	2	164.96
Fructose bisphosphate aldolase	42963.9	ACP40514	FBA	5	5	303.42
Putative glyceraldehyde-3-phosphate dehydrogenase	43000.0	ACV32597	GADPH	13	9	710.20
ATP1, partial	37426.0	ADL63246	atp1	4	2	157.95
Photosystem I P700 chlorophyll a apoprotein A1	83295.9	AMC31418	psaA	2	2	104.76
Ribulose-1,5-bisphosphate carboxylase/oxygenase	52626.9	AMC32005	rbcL	50	37	3952.36
Apocytochrome f	35948.9	AWW91860	petA	7	7	370.41
Rab protein	24012.1	CAA55865	Rab	5	5	138.28
Ferritin	28078.8	CAA65771	FER	2	2	55.43
Ribulose bisphosphate carboxylase small chain, chloroplastic	20251.2	O65194	RBCS	12	11	900.15
Glutamine synthetase leaf isozyme, chloroplastic	47115.5	Q9XQ94	GS2	2	2	42.93
Actin-7	41711.8	XP_003602545	actin	11	11	839.94
Photosystem I P700 chlorophyll a apoprotein A2	82427.3	YP_001381735	psaB	3	3	120.18
ATP synthase CF1 beta subunit	52746.5	YP_009141595	atpB	13	9	747.17
ATP synthase CF1 alpha subunit	55691.8	YP_009141617	atpA	6	4	431.89

For each identified protein, the protein name, accession number, gene name, and confidence score were given by MASCOT or Peaks search engines. Mass is the protein molecular weight in Da. Peptide count is the number of peptides used for identification. Unique peptides are the number of peptides belonging to only one protein, and used for quantitation.

Table 1b
Proteomic list of proteins quantified in the rumen compartment during wether digestion of alfalfa hay supplemented in tannins.

	Condition Tannin			Condition control				
Description	T4	T5	T6	C1	C2	C3	Anova (p)	q Value
Histone H3.2, partial	292999.1	213174.7	200996.4	267962.4	2720471.3	218560.1	0.358	0.337
Chlorophyll a/b binding protein	544875.2	258075.3	632179.4	480376.6	1884755.2	554884.3	0.325	0.322
Glycolate oxidase, partial	314787.0	242773.8	230646.0	28023.1	310523.3	113454.8	0.244	0.285
Rubisco activase, partial	1148583.1	626703.3	614768.3	146612.0	569681.5	217502.5	0.078	0.181
Heat shock protein 70	635331.7	486510.4	411899.0	1740900.1	9479203.8	2562796.2	0.021	0.119
Pentameric polyubiquitin, partial	1649985.1	903976.0	974430.7	2186301.1	10718614.6	5000967.9	0.042	0.132
Fructose bisphosphate aldolase	666929.8	495986.4	493078.6	328746.5	1392891.8	1204722.9	0.436	0.357
Putative glyceraldehyde-3-phosphate dehydrogenase	1121232.6	502703.1	536623.8	145669.6	2062278.1	382199.2	0.713	0.442
ATP1, partial	45306.3	65751.2	30848.5	10737.8	158880.0	127772.6	0.763	0.458
Photosystem I P700 chlorophyll a apoprotein A1	41780.5	21835.3	66875.6	61938.3	220868.5	135880.4	0.082	0.181
Ribulose-1,5-bisphosphate carboxylase/oxygenase	36565782.0	14377637.4	11799474.5	905957.3	6623227.4	2836924.7	0.043	0.133
Apocytochrome f	331301.9	230810.8	367722.6	274847.2	2150485.8	468034.8	0.295	0.318
Rab protein	177677.8	106270.1	94575.1	275243.8	1225792.7	479982.5	0.035	0.129
Ferritin	86707.1	65120.6	43724.1	42948.7	299393.6	132846.9	0.341	0.33
Ribulose bisphosphate carboxylase small chain, chloroplastic	18837450.9	10471723.5	8064851.8	996454.8	4789446.7	2368272.8	0.034	0.129
Glutamine synthetase leaf isozyme, chloroplastic	59995.0	34972.1	30827.6	1723.9	16568.4	8287.3	0.056	0.147
Actin-7	4935346.8	4541437.5	3165078.9	1219273.4	12512883.4	7159569.7	0.85	0.475
Photosystem I P700 chlorophyll a apoprotein A2	41428.5	18559.8	53498.3	41685.5	170371.5	30359.5	0.422	0.355
ATP synthase CF1 beta subunit	661058.2	547722.0	414574.4	66131.4	428958.7	755947.1	0.435	0.357
ATP synthase CF1 alpha subunit	647667.5	426021.6	358597.3	82394.4	695369.1	317001.5	0.432	0.357

For each quantified protein, the normalized abundances are given in the three individuals of each comparative group, i.e. 'Tannin' (T4, T5, and T6) and 'Control' (C1, C2, and C3). The Anova p-value and the power q-value are the results of the quantitative analysis performed using ProgenesisQI software.

Table 2a
Peptidomic list of proteins identified in the abomasal compartment during wether digestion of alfalfa hay supplemented in tannins.

Description	Mass	Accession	Gene	Peptide count	Unique peptides	Confidence score
Aquaporin-like transmembrane channel protein	31230.0	AAB86380	pAFI 8-1	6	4	340.28
Malate dehydrogenase precursor	38397.0	AAB99754	gmdh	2	2	131.57
Chlorophyll a/b binding protein	28351.0	AAC25775	CARCAB1	51	51	3229.86
Glycolate oxidase, partial	30588.0	AAC32392	GOX	6	6	367.63
Rubisco activase, partial	30170.0	AAN15946	Rca	13	13	691.57
Ferredoxin-dependent glutamate synthase, partial	26791.5.0	AAT38954	gltB_C	2	2	89.76
Heat shock protein 70	71351.0	AAV98051	HSP70-1	4	2	215.29
Pentameric polyubiquitin, partial	21187.5	AAZ32851	UBQ	4	4	287.39
Fructose bisphosphate aldolase	43165.0	ACP40514	FBA	9	9	455.95
Putative glyceraldehyde-3-phosphate dehydrogenase	43258.0	ACV32597	GADPH	16	14	990.83
Photosystem I P700 chlorophyll a apoprotein A1	83471.0	AMC31418	psaA	39	38	2018.76
Ribulose-1,5-bisphosphate carboxylase/oxygenase	52993.0	AMC32005	rbcL	205	10	13889.10
Apocytochrome f	36040.0	AWW91860	petA	17	17	1178.71
Photosystem II CP43 chlorophyll apoprotein	50482.0	AWW91876	psbC	5	5	240.01
Histone H3, partial	13915.0	CAA05554	H3-1.1	6	2	270.11
Ferritin	28061.0	CAA65771	FER	2	2	100.18
Photosystem II protein D2	39738.0	NP_054491	psbD	12	12	666.78
Ribulose bisphosphate carboxylase small chain, chloroplastic	20251.2	O65194	RBCS	33	32	2142.95
Photosystem II protein D1	39111.0	P04998	psbA	15	14	905.08
Probable aquaporin TIP-type	25324.4	P42067	MCP1	5	5	322.39
Adenosylhomocysteinase	53744.0	P50246	SAHH	2	2	69.84
Actin-7	41913.0	XP_003602545	actin2	10	9	572.66
Photosystem II 47 kDa protein	56131.0	YP_001381703	psbB	22	22	1360.88
Photosystem II protein V	9400.6	YP_001381711	psbE	2	2	85.46
Photosystem I P700 chlorophyll a apoprotein A2	82489.0	YP_001381735	psaB	45	45	2523.04
Cytochrome b6	24112.5	YP_002149762	petB	2	2	81.96
ATP synthase CF1 beta subunit	52771.0	YP_009141595	atpB	24	21	1271.26
ATP synthase CF1 alpha subunit	55714.0	YP_009141617	atpA	16	16	982.22

For each identified protein, the protein name, accession number, gene name, and confidence score were given by MASCOT or Peaks search engines. Mass is the protein molecular weight in Da. Peptide count is the number of peptides used for identification. Unique peptides are the number of peptides belonging to only one protein, and used for quantitation.

quantitation (Tables 2b, c and all the individual raw data acquired during the digestion kinetic in both Control and Tannin groups are available via the PRIDE repository with the dataset identifier PXD032973(https://www.ebi.ac.uk/pride/archive/projects/PXD032973).

1.3. Small Intestine Peptidomics

In the small intestine, 63 peptides were identified from the digestion of 11 medicago proteins with at least two unique peptides (Table 3a and via the PRIDE repository with the dataset identifier PXD032973). In this compartment, unique peptides from the two Ribulose proteins (gene name rbcL and RBCS) represent more than 35% of the identified and quantified peptides (23/63 unique peptides). As for the other two compartments, all identification and quantitation results of those peptides are available in the Tables 3b, c for protein data and all the individual

Table 2b
Peptidomic list of proteins quantified in the abomasal compartment at 15 minutes of wether digestion of alfalfa hay supplemented in tannins.

	Condition Control			Condition Tannin				
Description	T4	T5	T6	C1	C2	C3	Anova (p value)	q value
Aquaporin-like transmembrane channel protein	1857.3	14873.6	15050.0	73803.0	99424.6	103865.7	0,001	0,002
Malate dehydrogenase precursor	15708.2	83843.4	36613.2	11600.8	16443.0	10775.8	0,001	0,002
Chlorophyll a/b binding protein	5562923.7	2930411.5	3382917.8	5348125.7	5878944.7	7575674.6	0,003	0,003
Glycolate oxidase, partial	442933.8	459805.7	331436.1	90200.2	99275.9	64640.6	0,000	0,000
Rubisco activase, partial	438692.8	813921.2	392757.9	412509.3	535492.9	354148.6	0,101	0,045
Ferredoxin-dependent glutamate synthase, partial	26890.0	64330.0	8489.0	2342.0	15645.9	5215.6	0,003	0,002
Heat shock protein 70	35482.2	36485.2	10186.0	44275.0	38132.3	29724.0	0,158	0,066
Pentameric polyubiquitin, partial	368210.6	590896.4	215780.0	331694.7	269554.2	200907.0	0,106	0,046
Fructose bisphosphate aldolase	342186.0	716798.5	201459.4	176814.6	189979.4	150231.5	0,015	0,010
Putative glyceraldehyde-3-phosphate dehydrogenase	461655.7	932166.5	221701.3	414052.7	569441.1	349280.3	0,097	0,045
Photosystem I P700 chlorophyll a apoprotein A1	571742.2	397239.6	704973.5	855340.1	1106649.5	1323653.0	0,000	0,001
Ribulose-1,5-bisphosphate carboxylase/oxygenase	1722084.7	3338112.2	3658436.2	317144.0	301861.9	209271.9	0,000	0,000
Apocytochrome f	749718.4	853491.8	1483049.0	2039160.4	2526285.3	2263769.7	0,001	0,002
Photosystem II CP43 chlorophyll apoprotein	169847.6	47204.8	203904.8	158969.3	230529.1	235333.9	0,022	0,013
Histone H3, partial	64440.4	126107.5	41682.6	68736.8	92847.9	46001.0	0,453	0,148
Ferritin	107706.4	16375.3	122897.2	25078.1	29882.0	16768.7	0,196	0,075
Photosystem II protein D2	360817.8	419712.0	478604.4	789312.5	948430.9	1086873.6	0,000	0,001
Ribulose bisphosphate carboxylase small chain, chloroplastic	2779311.7	4877198.0	5256231.9	1296395.7	1315805.3	1001594.3	0,000	0,000
Photosystem II protein D1	322122.5	171776.1	295420.7	343115.8	459884.7	526318.9	0,001	0,001
Probable aquaporin TIP-type	208013.4	112229.1	100534.1	315043.5	389293.1	297961.0	0,002	0,002
Adenosylhomocysteinase	114914.1	40278.5	29841.7	70847.1	47868.4	96729.5	0,582	0,173
Actin-7	513312.1	614990.4	272125.6	356455.7	422855.0	436776.3	0,583	0,173
Photosystem II 47 kDa protein	1123112.2	892606.8	1047808.0	1151607.5	1454630.1	1873329.9	0,002	0,002
Photosystem II protein V	38829.1	46227.1	19590.4	52927.9	55935.3	67533.2	0,174	0,069
Photosystem I P700 chlorophyll a apoprotein A2	3493403.0	2583678.5	3431964.2	5972223.7	6671674.3	7296220.5	0,000	0,000
Cytochrome b6	11001.6	3333.6	23939.8	53939.9	36194.8	79898.7	0,001	0,001
ATP synthase CF1 beta subunit	679841.4	1401643.6	1103847.6	472621.9	508287.3	375798.9	0,000	0,000
ATP synthase CF1 alpha subunit	840661.3	1250906.8	372485.8	394510.4	344030.6	256158.5	0,005	0,004

For each quantified protein, the normalized abundances are given in the three individuals of each comparative group, i.e. 'Tannin' (T4, T5, and T6) and 'Control' (C1, C2, and C3). The Anova p-value and the power q-value are the results of the quantitative analysis performed using ProgenesisQI software.

Table 2c
Peptidomic list of proteins quantified in the abomasal compartment at 60 minutes of wether digestion of alfalfa hay supplemented in tannins.

	Condition Tannin			Condition Control				
Description	T4	T5	T6	C1	C2	C3	Anova (p)	q value
Aquaporin-like transmembrane channel protein	11126.8	22693.2	37081.0	74011.8	90190.1	59915.8		
Malate dehydrogenase precursor	36884.3	73079.4	41824.1	14081.2	12192.5	18145.9		
Chlorophyll a/b binding protein	3186637.5	3556093.4	4473847.3	7010609.1	6083171.1	14018136.3		
Glycolate oxidase, partial	233396.4	450099.4	234807.4	85122.3	153123.6	81345.3		
Rubisco activase, partial	483170.2	937792.7	580508.2	443229.8	422350.3	476188.8		
Ferredoxin-dependent glutamate synthase, partial	34418.7	64292.8	38866.7	5625.4	12864.9	4004.9		
Heat shock protein 70	26200.3	40583.3	26010.2	32437.2	34562.0	49039.4		
Pentameric polyubiquitin, partial	267983.8	528200.8	433079.6	322557.6	221439.8	321410.1		
Fructose bisphosphate aldolase	208313.6	884946.7	296949.2	192652.2	127326.0	201823.1		
Putative glyceraldehyde-3-phosphate dehydrogenase	735020.6	1612765.6	966962.1	409861.4	288234.4	431239.8		
Photosystem I P700 chlorophyll a apoprotein A1	439332.4	539277.7	699379.2	1000025.0	1111527.2	1784959.2		
Ribulose-1,5-bisphosphate carboxylase/oxygenase	1951203.6	2429332.8	2413332.0	300774.3	283832.0	194295.1		
Apocytochrome f	660016.3	977287.3	828789.4	1694342.9	1094491.2	2875504.2		
Photosystem II CP43 chlorophyll apoprotein	117568.5	59045.4	124786.3	193296.6	174766.1	341063.8		
Histone H3, partial	101891.0	123710.4	47076.9	70381.1	81289.0	38285.8		
Ferritin	22450.4	174628.1	42922.7	57585.6	48676.7	26881.5		
Photosystem II protein D2	399372.9	491889.0	538907.3	728592.0	671841.7	1615057.1		
Ribulose bisphosphate carboxylase small chain, chloroplastic	2930451.6	4343816.2	3979363.0	1095401.7	1332840.8	1137440.0		
Photosystem II protein D1	195345.6	200489.9	207922.7	331301.5	465001.1	714769.7		
Probable aquaporin TIP-type	178138.0	130487.9	201598.7	340370.4	181964.0	366407.7		
Adenosylhomocysteinase	55913.6	115798.9	81187.8	95923.7	42283.9	152799.7		
Actin-7	304778.7	729244.7	451907.0	363518.8	266950.7	678686.1		
Photosystem II 47 kDa protein	792014.5	888671.8	898258.4	1279347.8	1496798.0	2424064.4		
Photosystem II protein V	26384.5	54253.9	38918.1	66718.9	17525.5	108960.3		
Photosystem I P700 chlorophyll a apoprotein A2	2951805.5	2550061.7	3906789.3	5676007.1	5584737.3	9240266.1		
Cytochrome b6	5720.3	8593.6	20816.5	39401.2	69757.3	50521.5		
ATP synthase CF1 beta subunit	808416.9	1256059.1	969204.2	435569.4	608820.1	346295.8		
ATP synthase CF1 alpha subunit	463554.6	1129156.3	549360.6	324445.3	295022.4	347029.6		

For each quantified protein, the normalized abundances are given in the three individuals of each comparative group, i.e. 'Tannin' (T4, T5, and T6) and 'Control' (C1, C2, and C3). The Anova p-value and the power q-value are the results of the quantitative analysis performed using ProgenesisQI software.

Table 3a
Peptidomic list of proteins identified in the small intestine compartment during wether digestion of alfalfa hay supplemented in tannins.

Description	Mass	Accession name	Gene name	Peptide count	Unique peptides	Confidence score
Ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast)	52626.9	1043523999	rbcL	46	19	2095.15
Photosystem I P700 apoprotein A2 (chloroplast)	82427.3	1043524008	psaB	6	3	230.12
Photosystem II CP43 chlorophyll apoprotein (chloroplast)	51907.9	1043524011	psbC	4	4	127.68
Photosystem II protein D2 (chloroplast)	39535.5	1043524012	psbD	5	5	200.89
Cytochrome f (chloroplast)	35300.9	1043524028	petA	5	5	200.49
Photosystem II 47 kDa protein (chloroplast)	55996.1	1043524040	psbB	5	5	181.49
70 kD heatshockprotein, partial	23312.9	1430887	HSP70	2	2	62.00
Ribulose-1,5-bisphosphate carboxylase small subunit, partial	11914.6	16224234	RBCS	4	4	169.92
Tonoplast intrinsic protein homolog MSMCP1	25324.4	2443836	MCP1	4	4	128.37
Chlorophyll a/b binding protein	28312.3	3293555	CARCAB1	9	9	352.00
ATP synthase CF1 beta subunit	52516.0	ANS57890	atpB	3	3	186.3

For each identified protein, the protein name, accession number, gene name, and confidence score were given by MASCOT or Peaks search engines. Mass is the protein molecular weight in Da. Peptide count is the number of peptides used for identification. Unique peptides are the number of peptides belonging to only one protein, and used for quantitation.

Table 3b
Peptidomic list of proteins quantified in the small intestine compartment at 60 minutes of wether digestion of alfalfa hay supplemented in tannins.

	Condition Tannin			Condition control				
Description	T4	T5	T6	C1	C2	C3	Anova (p)	q value
Ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast)	2687577.2	2818529.4	1226619.2	451227.4	596852.1	379830.1	0,007	0,110
Photosystem I P700 apoprotein A2 (chloroplast)	133604.6	68510.9	88627.7	163463.4	215161.5	184149.7	0,030	0,218
Photosystem II CP43 chlorophyll apoprotein (chloroplast)	2928.9	13558.8	6172.3	3904.2	10309.1	5845.7	0,980	0,788
Photosystem II protein D2 (chloroplast)	33439.6	51182.0	22199.4	38945.7	70052.3	59448.1	0,180	0,496
Cytochrome f (chloroplast)	19619.0	8917.2	18478.9	27646.6	62077.3	55393.5	0,035	0,218
Photosystem II 47 kDa protein (chloroplast)	31756.3	45189.4	26955.8	19559.7	40558.7	29094.3	0,544	0,661
70 kD heatshockprotein, partial	835293.3	1294082.8	477954.3	901645.6	425514.6	861686.5	0,713	0,732
Ribulose-1,5-bisphosphate carboxylase small subunit, partial	39749.9	5976.6	13857.0	10382.3	15178.5	4562.0	0,481	0,661
Tonoplast intrinsic protein homolog MSMCP1	28502.2	30578.8	51972.6	71233.2	45393.7	30251.6	0,457	0,651
Chlorophyll a/b binding protein	349628.0	213941.8	242115.3	185068.8	420252.7	331035.5	0,702	0,732
ATP synthase CF1 beta subunit	45849.6	48924.2	6699.4	1721.9	1317.4	3291.7	0,023	0,218

For each quantified protein, the normalized abundances are given in the three individuals of each comparative group, i.e. 'Tannin' (T4, T5, and T6) and 'Control' (C1, C2, and C3). The Anova p-value and the power q-value are the results of the quantitative analysis performed using ProgenesisQI software.

Table 3c
Peptidomic list of proteins quantified in the small intestine compartment at 180 minutes of wether digestion of alfalfa hay supplemented in tannins.

	Condition Tannin			Condition control				
Description	T4	T5	T6	C1	C2	C3	Anova (p value)	q value
Ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (chloroplast)	146911.2	122501.9	193678.7	91238.0	245590.4	72861.7	0,161	0,729
Photosystem I P700 apoprotein A2 (chloroplast)	50353.7	71072.2	57427.4	53769.6	93352.9	51912.2	0,731	1,000
Photosystem II CP43 chlorophyll apoprotein (chloroplast)	34860.4	16982.5	49548.8	34934.8	4935.1	39881.2	0,552	0,994
Photosystem II protein D2 (chloroplast)	7084.7	17189.1	5698.0	9867.6	38843.0	13022.7	0,288	0,850
Cytochrome f (chloroplast)	7845.0	3759.7	5842.5	5852.9	18523.3	5337.0	0,424	0,983
Photosystem II 47 kDa protein (chloroplast)	5939.2	6256.0	4603.9	2911.6	4532.6	2466.4	0,054	0,729
70 kD heatshockprotein, partial	1949391.3	2740180.3	2339621.9	1902679.8	1195176.9	2751493.7	0,426	0,983
Ribulose-1,5-bisphosphate carboxylase small subunit, partial	1306.9	2669.6	2242.6	758.1	1721.3	1245.2	0,178	0,729
Tonoplast intrinsic protein homolog MSMCP1	8103.3	4525.2	11330.8	10940.8	7761.6	1849.1	0,621	0,994
Chlorophyll a/b binding protein	22083.6	30317.9	19930.5	28820.4	144739.9	99351.7	0,085	0,729
ATP synthase CF1 beta subunit	2423.1	3031.7	5707.4	26.3	786.8	0.0	0,077	0,729

For each quantified protein, the normalized abundances are given in the three individuals of each comparative group, i.e. 'Tannin' (T4, T5, and T6) and 'Control' (C1, C2, and C3). The Anova p-value and the power q-value are the results of the quantitative analysis performed using ProgenesisQI software.

raw data acquired during the digestion kinetic in both Control and Tannin groups are available via the PRIDE repository with the dataset identifier PXD032973 (https://www.ebi.ac.uk/pride/archive/projects/PXD032973).

2. Experimental Design, Materials and Methods

2.1. Experimental Designs

The experiment was conducted at the INRAE Clermont Auvergne Rhône-Alpes center in Theix, France. All animal-related experimental procedures were conducted in accordance with the EU Directive 2010/63/EU, reviewed by the local institutional animal care and use comitee (C2E2A, "Comité d'Ethique pour l'Expérimentation Animale en Auvergne"), and pre-authorized by the French Ministry for Research (approval # 7138-2016092709177605-V5). The protein digestion in the rumen of sheep and in simulated conditions of the abomasum and the small intestine was monitored using a dynamic *in vitro* digestive system DIDGI® [1] coupled to a digestomic approach (Fig. 1).

Three rumen-cannulated wethers were fed alfalfa hay and infused daily through the cannula a tannin solution, while three control wethers were infused with water. Standardized ruminal fluid was introduced into a dynamic *in vitro* digester, which simulated the different digestive compartments in terms of transit rate, pH regulation and digestive enzymes rate [2,3]. Samples were taken along the digestion kinetic and protein degradation in the rumen, the abomasum and the small intestine was determined by the identification and quantitation of peptides after extraction according to Sayd et al. [4].

2.2. LC-MS/MS and Data Analysis

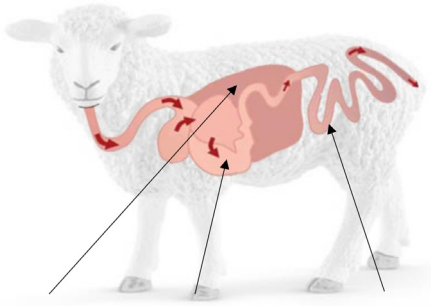
2.2.1. LC-MS/MS Analysis

Peptides (from proteomic (rumen) or digestomic (*in vitro* digester)) were separated at 400 nl/min (40°C) on a nano HPLC column (Acclaim PepMap RSLC 75um x 25 cm, ThermoScientific) using a gradient of 4-35% acetonitrile (v/v) in 0.1 % (v/v) formic acid within 60 min. The eluted peptides were electrosprayed into nanosources of high resolution mass spectrometers used for this study. Thus, for abomasal and intestinal samples, a LTQ Velos Orbitrap (Thermo scientific) was used and raw data acquired at a resolution of 30000 in full scan (400-2000 m/z). For HCD fragmentation, top 10 method (dynamic exclusion enabled, 60 s) was used and only the most abundant precursors ions in full scan with charge ≥ 2 was activated for fragmentation (collision energy at 37%). For rumen samples, a Q-Exactive HFX was used with 60000 resolution in MS1 (375-1600 m/z) and 15000 in MS/MS (NCE 28) with top 18 method and an exclusion dynamic of 20 s.

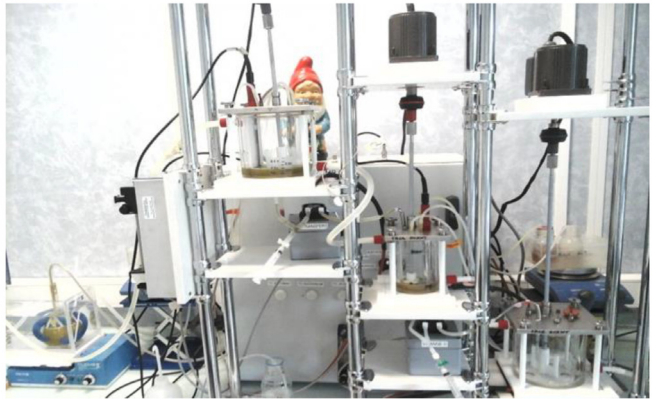
2.2.2. Data Analysis

The raw files were processed for quantitation analysis using Progenesis QI software (Nonlinear Dynamics, Waters). For identification, the MS/MS spectra list was exported from the Progenesis QI software as a mascot file (.mgf) to MASCOT (V 2.5) or Peaks (Peaks X+), using the database "Medicago_sativa" extracted from NCBI (2020,1099 sequences). The search parameters were set as follow: no enzyme for digestomic analyses and trypsin for proteomic (rumen) analyses, the MS mass tolerance was set at 15 ppm for the peptides and 0.02 Da for the fragments, with a possible mass adduct of methionine oxidation. Peptide identification was validated when ion had a significant Mascot or Peaks score with a false positive rate lower than 0.05. Then, the identification results were re-imported into the Progenesis IQ software for quantitation [5]. Only peptides whose sequence is shared by a single protein were used for the abundance calculation. Quantitative data were normalized based on calculation of scalar factor for each

A



B



C

	Abomasum	Small intestine
Digestion sampling (n=6)	15 and 60 minutes (A15 and A60)	60 and 180 minutes (I60 and I180)
Extraction of the peptides resulting from digestion [4]		
Identification by LC-MS/MS high-resolution mass spectrometry analysis (MASCOT and Peaks softwares)		
Quantification by label-free (ProgenesisQI software, Waters, Newcastle, UK)		

Fig. 1. Representation of the experimental design, adapted from Sayd *et al.*, 2022. (A) Schematic representation of the ovine digestive tract and (B) the corresponding compartments in the *in vitro* dynamic digestion system DiDGI®, the abomasum and the small intestine. (C) The sampling and main analysis steps are given for each compartment.

sample which will allow us to recalibrate the sample to a normalization reference run. (normalization method available at: https://www.nonlinear.com/progenesis/qi-for-proteomics/v3.0/faq/how-normalisation-works.aspx. Briefly, this scalar factor, based on all the features intensities required can be represented as αk for each sample: $y'_i=\alpha k y_i$, where yi is the measured peptide ion abundance of peptide ion *i* on sample k, αk is the scalar factor for sample k and y'_i is the normalised abundance of peptide ion i on sample k.

Ethics Statements

The experiment was conducted at INRAE Clermont Auvergne Rhône-Alpes centre in France. The experimental procedures on animals were conducted in accordance with the European Union Directive 2010/63/EU, reviewed by the local ethics committee (C2E2A, "Comité d'Ethique pour l'Expérimentation Animale en Auvergne") and authorised by the French Ministry for Research (no. 7138-2016092709177605-V5).

CRediT Author Statement

Christophe Chambon: Conceptualization, Methodology, Data curation, Writing – original draft preparation, Supervision; **Thierry Sayd:** Conceptualization, Methodology, Data curation, Writing – original draft preparation; **Sylvie Bourillon:** Methodology; **Laetitia Theron:** Methodology, Data curation, Writing – original draft preparation; **Vincent Niderkorn:** Conceptualization, Methodology, Data curation, Writing – Original draft preparation, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Data Availability

Impact of Tannin Supplementation on Proteolysis during Post- Ruminal Digestion in Wethers (Original data) (PUBS ACS)

Digestomic data of proteolysis after tanin supplementation in the rumen (Original data) (Pride).

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