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Abstract. The citrus industry produces a wide amount of citrus pulp which can represent an alternative feed resource forfeeding ruminants. However, citrus pulp also contains chemicals such aspolyphenols, which can cause toxicity, limiting its use. We investigated the potential of replacing barley by dehydrated citrus pulp (DCP), at two levels of inclusion (24% and 35% on an as-fed basis), in a lamb fattening diet and monitored the performance, feeding pattern and serum parameters of the experimental lambs. The consumption of a diet containing up to 35% of DCP resulted in equivalent performance, feedefficiencyandcarcassweightandyieldascomparedwithanimalsingestingacereal-based diet(control). The daily feed consumption pattern was slightly affected by the inclusion of citrus pulp in the diet. In terms of serum haematochemical profile, DCP ingesting animals had similar levels to control lambs. Pertaining to the serum protein profile, DCP addition had minor effects. A significant increase in the albumin content and in the albumin to globulin ratio was observed in the animals ingesting 35% DCP compared with the control-fed ones; but the values were not at a level to cause metabolic distress. The use of high levels of DCP in small ruminant fattening can ensure equivalent animal performances and metabolic welfare while providing a value addition to a local by-product.

Additional keywords: dehydrated citrus pulp, polyphenols, serum metabolites, serum protein profile.

Introduction

Ruminants have an intrinsic capability to convert several agroindustrial by-products into valuable products, namely meat and milk, allowing a waste to become a feed resource. Citrus pulp is an agro-industrial by-product of juice extraction from fresh fruits. Citrus are widespread in the Mediterranean region, which produces a fifth of world citrus production; Sicily is the main Italian producer accounting for ~50% of the country's total production (ISTAT 2015). In livestock production, the cost of feeding is, in most cases, the main expenditure incurred by breeders; considering the concerns about the high dependence on cereals, and their highly volatile prices, the potential use of relatively cheap and locally available by-products as a cereal substituteishighlyattractive.Severalstudiesdealtwiththeuseof citrus by-products in ruminant feeding (Bampidis and Robinson 2006; Arthington *et al.* 2002; Wadhwa and Bakshi 2013). However, due to wide variation in citrus species and varieties, agronomicsystemsofproductionaswellasprocessingconditions, which affect the nutritional composition of the by-product (Kale and Adsule 1995; Bampidis and Robinson 2006; Wadhwa and Bakshi 2013) there is no universally recognised formula for its inclusion in animal rations, only guidelines.

Citruspulpisknowntobeagoodsourceofdietaryfibre(Welch and Smith 1971) and soluble sugars (Wadhwa and Bakshi 2013); it is also rich in plant secondary compounds such as phenolic compounds (Balasundram *et al.* 2006) and essential oils (for example, limonene) (Tao *et al.* 2009). The inclusion of citrus pulp in ruminant feed formulation may confer advantages such as improved animal health status (Callaway *et al.* 2011*a*), reduced nematodeseggshatchingsuggestingatendencytoshedfewereggs to the environment (Nordi *et al*. 2014) or improved meat shelf life (Gravador *et al*. 2014; Inserra *et al.* 2014) and intramuscular fatty acid composition (Lanza *et al.* 2015), but may also confer disadvantages such as hindered performance and metabolic discomfort (Durmic and Blache 2012). The present study aims at investigating whether the inclusion of locally available dried citrus pulp in a total mixed ration, as a substitute for barley, affects lamb productivity and metabolic welfare.

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However, due to wide variation in citrus species and varieties, agronomic systems of production as well as processing conditions, which affect the nutritional composition of the by-product (Kale and Adsule 1995; Bampidis and Robinson 2006; Wadhwa and Bakshi 2013) there is no universally recognised formula for its inclusion in animal rations, only guidelines.

Citrus pulp is known to be a good source of dietaryfibre(Welch and Smith 1971) and soluble sugars (Wadhwa and Bakshi 2013); it is also rich in plant secondary compounds such as phenolic compounds (Balasundram *et al.* 2006) and essential oils (for example, limonene) (Tao *et al.* 2009). The inclusion of citrus pulp in ruminant feed formulation may confer advantages such as improved animal health status (Callaway *et al.* 2011*a*), reduced nematodes eggs hatching suggesting a tendency to shed fewer eggs to the environment (Nordi *et al*. 2014) or improved meat shelf life (Gravador *et al*. 2014; Inserra *et al.* 2014) and intramuscular fatty acid composition (Lanza *et al.* 2015), but may also confer disadvantages such as hindered performance and metabolic discomfort (Durmic and Blache 2012). The present study aims at investigating whether the inclusion of locally available dried citrus pulp in a total mixed ration, as a substitute for barley, affects lamb productivity and metabolic welfare.

Materials and methods

Twenty-six Comisana male lambs of 90 (10) days of age were selected. The animals were weighed, divided into three homogeneous groups and housed in individual pens. Each group wasallocatedtoanexperimentaldiet(Table1).Thecontrolgroup (Control, $n = 8$) was fed a total mixed diet mainly consisting of barley and lucerne hay, coarsely ground in order to avoid feed selection. Two groups received a mixed diet with the same ingredients as the Control group with the addition of different proportionsofdehydratedcitruspulp(DCP),respectively24%in Cp24group(n=9)and35%inCp35group(n=9).Therespective diets were given on an *ad libitum* basis from 9 a.m. to 6 p.m. and thissystemwasmaintainedthroughoutthedurationofthefeeding trial, which lasted for 56 days. A 10-day adaptation period was used; during this period lambs were fed the pre-experimental dietreplacedbygraduallyincreasingamountsoftheexperimental diets.

The daily intake was measured by weighing the refused feed, which was then discarded. Water was always available.

Animals were weighed weekly using an electronic weighing scale before feeding. The animals were slaughtered after 56 days of feeding trial by captive bolt followed by exsanguination. The experimentalprotocolwasapprovedbytheUniversityofCatania; the animals were handled by specialised personnel following the European Union Guidelines (2010/63/EU Directive).

Feed and blood sampling

Fresh feed samples (i.e. the mixed diets for Control, Cp24 and Cp35 groups) were collected four times during the trial (on Days 9, 30, 44 and 51 respectively) for subsequent laboratory analysis, carried out on a pooled sample. The feeds were stored at 30C until analyses.

Individual blood samples (10 mL) were collected from the external jugular vein using Vacutainertubes(TerumoCorporation, Tokyo, Japan) with no additive. Trained professionals were assigned to carry out this operation to minimise stress. The blood samples were collected, before feed allocation, on Day –10 (i.e. the day in which the adaptation period was begun) and on Day 55 of the feeding trial. The blood samples were allowed to clot at room temperature (20C) and centrifuged at 2081*g* for 15 min at 4C to separate the serum. The serum samples obtained were neither lipemic nor haemolysed and were dispensed into plastictubesandstoredat80Cbeforeanalyses,performedwithin 2 months. At the time of analysis serum samples were thawed at 20C for 30 min before assessing serum haematochemical and electrophoretic parameters.

Laboratory analyses Feed analyses

Feeds were analysed for dry matter (DM); the AOAC (1995) methods were used for the analyses of crude protein (method 984.13) and crude fat (method 935.38) extracted with petroleum ether. Neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined according to Van Soest *et al*. (1991)with sodium sulfite, without aamylase and expressed with residual ash

method. The metabolis able energy was estimated by the use of 'ASSIST.T Alimentazione' software, version 1.3.1 developed by CRPA spa, Italy (www.CRPA.it, verified 10 August 2015).

For the analysis of total phenols in the feed, samples were first treated as described by Makkar *et al*. (1993): total phenolic compounds were extracted from the feeds using aqueous acetone (70% v/v), analysed by means of the Folin–Ciocalteau reagent and expressed as tannic acid equivalents.

Blood analyses

Serum haematochemical and electrophoretic parameters were selected as indicators of metabolic welfare.

Table 1. Ingredients and chemical composition of the diets (Control, Cp24 and Cp35 groups)

Item	Diet		
	Control	Cp24	Cp35
Ingredients (% as fed)			
Barley	60	35	23
Citrus pulp	0	24	35
Dehydrated lucerne	20	19	20
Soya bean meal	9	12	13
Wheat bran	11	10	9
DM (g/100 g fresh weight)	88.9	89.4	90.6
Crude protein[A]	18.0	18.5	17.8
Neutral detergent fibre[A]	34.6	31.8	33.1
Acid detergent fibre[A]	13.7	16.0	18.0
Hemicellulose[A]	20.9	15.8	15.1
Cellulose[A]	5.4	5.9	6.5
Acid detergent lignin[A]	8.3	10.0	11.5
Ether extract[A]	2.2	1.6	2.2
Total phenols[B]	4.0	6.7	7.9
Metabolisable energy (MJ/kg)[C]	10.49	10.52	10.51

[A] Expressed as g/100 g of DM.
[B] Expressed as mg of tannic acid equivalents/g of DM.
[C] Expressed as fresh weight basis.

In particular, among the haematochemical parameters total protein, total cholesterol, urea, total bilirubin, non-esterified fatty acids, triglycerides and iron were included. Electrophoretic parameters included albumin, a1, a2, b and gamma globulins. Total protein was measured by biuret method using an automated analyser (Knoelab20, Dasit, Helsinki, Finland). Electrophoresis was performed using a semiautomated AGE system (HelenaLaboratories,HelenaBiosciences, Gateshead, UK) according to the manufacturer's procedure.

Foreachserumsample10mLwereappliedtonumberedsample wells containing agarose gel previously prepared. Each gel could accommodate up to 24 samples. Films were electrophoresed for 28 min at 450 V. After electrophoresis, films were simultaneously fixed using an automated system (SAS2, Helena Biosciences), stained in blue stain acid solution (Coomassie Blue Brillant R250, Helena Biosciences) for 10 min, and then dried at 37C. After destaining in acetic acid and drying completely for 15 min, films were scanned on a densitometer (EZ-Scan, Helena Biosciences). Using the computer software Phoresis (Helena Biosciences), electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/dL) were calculated using the total serum protein concentration. Values obtained were multiplied by 10 in order to express protein fraction concentration in g/L.

Triglycerides and total cholesterol were assessed by means of a spectrophotometer (SEAC, Florence, Italy). Triglycerides and total cholesterol were determined after enzymatic hydrolysis by means of an enzymatic colourimetric test. Briefly, triglycerides were determined after enzymatic hydrolysis with lipoprotein lipase. The indicator was a coloured phenazone formed from hydrogen peroxide, 4-aminoantipyrine, and 4-chlorophenol under the catalytic influence of peroxidase. Total cholesterol was determined

after enzymatic hydrolysis and oxidation. Hydrogen peroxide produced formed a red dyestuff by reacting with 4-aminoantipyrine in the presence of phenol and peroxidase. The colour intensity is directly proportional to the concentration of total cholesterol. Non-esterified fatty acids were measured enzymatically with a commercially available kit (Randox Laboratories, Crumlin, UK). Samples exhibited parallel displacement to the standard curve; the intra-assay coefficient of variation was less than 8%.

Urea, total bilirubin and iron were determined with the use of commercial kits (Centronic GmbH, Wartenberg, Germany) and finally measured using the UV Spectrophotometer (SEAC, Slim, Florence, Italy).

Statistical analyses

Analysis of variance (ANOVA) was used to determine the dietary effect on performance indicators, namely intake, live weight gain, feed efficiency, live and carcass weights and on serum heamatochemical and electrophoretic parameters. Data were analysed as a completely randomised design, with a model that included the diet as treatment effects. When the ANOVA was significant ($P < 0.05$), means were separated by pairwise comparison. Serum data were analysed separately on each day of sampling(i.e.Day–10andDay55); when the dietary effect was significant at Day –10, statistical analysis for Day 55data included the value at Day –10 as covariate.

Individual data of the feed intake pattern in terms of DMintake (DMI) during the day were analysed by including the fixed effects of diet (Control, Cp24 and Cp35), as between-subject factor, and the experimental day (20, 33, 47 and 53) and period of the day (interval 1 = 0900–1200 hours; interval 2 = 1200–1500 hours; interval 3 = 1500–1800 hours) as within-subject factors. The model also considered the interactions. Animals, nested within the group, were included as random factor. The effect of the experimental day was not significant, so as implified model considering only diet, period of the day and their interaction was used. Pairwise comparisons allowed comparison of the mean values ($P < 0.05$). Data were analysed by Minitab software (version 16.0).

Results

Diet composition and lamb performance

Composition and chemical profile of the Control, Cp24 and Cp35 diets are displayed in Table 1. The effects of the diets on *in vivo* and post mortem performances, presented in Table 2, were not significant for the most part of the parameters, but statistical differences were observed in nutrient intake. The total fibre content of the various diets was similar but, as expected, differed in terms of composition mainly due to the substitution of barley by DCP in the Cp24 and Cp35 diets. As a consequence, the intake of the fibre fractions was significantly affected by the experimental diet. In particular, acid detergent fibre and acid detergent lignin fractions intake was highly affected by DCP inclusion (P=0.044and$P$=0.016respectivelyforaciddetergent fibre and acid detergent lignin) with the Cp35 group ingesting significantly higher(P<0.05) amount than Control lambs. On the other side, the highest intake of the hemicellulose has been observed in the Control group when compared with both Cp24 andCp35 groups. According to the different polyphenols content of the three diets (Table1), lamb total phenol sintake(Table2)was significantly affected by the diet (P < 0.0005), showing citrus pulp-fed lambs significantly (P < 0.05) higher values than control-fed lambs.

Feed intake pattern

The daily distribution of the DMI was significantly affected by the period of the day(P<0.0005)and the interaction diet*period (P < 0.0005), whereas the effect of the diet was not significant (P > 0.05). On the whole, considering all three experimental groups, the lambs ingested the higher quantity of feed immediately after its supply, i.e. in period 1 (0900–1200 hours). They showed a decrease in intake consumption in period 2 (1200–1500 hours) followed by an increased intake in the last period of feed availability (1500–1800). The percentages of the total daily DMI were equal to 48% (1.08 s.e.), 21% (1.03 s.e.) and 31% (0.69 s.e.) for periods 1, 2 and 3 respectively; all these values were significantly different (P < 0.05).

Table 2. Performance and intake of lambs fed on Control (C) diet or two citrus pulp-based diets (Cp24 and Cp35) a,b, Within a row, means with unlike letters differ (P < 0.05)

	Control (C)	Citrus 24 (Cp24)	Citrus 35 (Cp35)	s.e.m.	*P*-value
		Performance			
No. of lambs	8	9	9		
Bodyweight Day 0 (kg)	19.3	19.2	18.2	0.653	0.763
Bodyweight Day 57 (kg)	29.6	29.6	28.5	1.04	0.876
Average daily gain (Day 0 to Day 57, g/day)	181	181.3	179.2	8.37	0.994
Dry matter intake (DMI, g/day)	749.2	767.0	756.4	30.2	0.886
Feed efficiency (kg weight gain/kg DMI)	0.24	0.25	0.25	0.005	0.851
Carcass yield (%)	43.8	44.0	43.7	0.416	0.953
Carcass weight (kg)	13.0	13.1	12.49	0.530	0.882
		Nutrient intake (*g*/*day*)			
Crude protein	135.1	141.8	134.2	5.29	0.824
Neutral detergent fibre	259.4	243.6	250.3	9.71	0.824
Acid detergent fibre	102.8a	122.5ab	136.4b	5.58	0.044
Hemicellulose	156.6b	121.1a	113.9a	5.97	0.005
Cellulose	40.6	45.5	49.2	1.93	0.199
Acid detergent lignin	62.2a	77.01ab	87.21b	3.69	0.016
Ether extracts	16.7b	11.96a	17.04b	0.761	0.005
Total phenols[A]	2996a	5156b	5950 b	315	0.0005

[A] mg tannic acid equivalents/day.

The same pattern was observed when comparing the proportion of DMI between the periods of the day within each dietary treatment (Fig. 1). Indeed the highest value was always found in period 1 and the lowest in period 2 whatever the diet supplied to lambs. However, it is worthy to note that within each period of the daya different pattern among the experimental diets was observed. The proportion of the ingestion in the first 3 h following feed distribution was significantly lower in the Cp35 group compared with the Control group (45% vs 51%; P < 0.05). In the interval 1200–1500 hours the Cp35 lambs ingested more DM than Control lambs (25% vs 18%, P < 0.05). In both periods 1 and 2 Cp24 lambs showed no different DMI percentages compared with the Control and Cp35 groups. In the last period of feed availability (1500–1800 hours) no significant differences in the proportion of DMI among groups have been detected. Overall, in

our experimental conditions, citrus pulp inclusion in the experimental diets offered to lambs did not affect total DMI (Table 2) but differently modulated the rate of feed consumption when added at the highest level.

Serum haematochemical parameters

The concentrations of serum haematochemical parameters observed in all the animals before and at the end of the experimental trial are shown in Table 3.

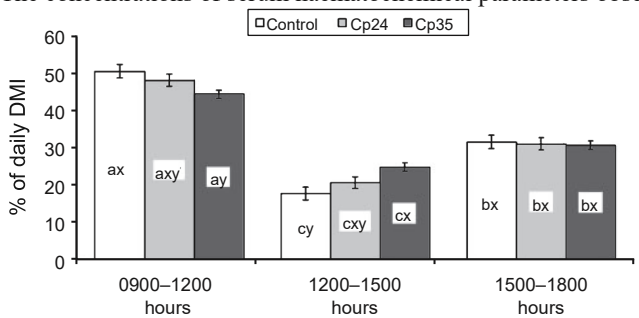

Fig.1. ThepatternofDMIthroughoutthedayintermsof%ofthetotaldaily intake (means s.e.). a,b, Within each dietary treatment, different letters indicate differences between periods of the day (*P* 0.05). x,y, Within each period of the day, different letters indicate differences between dietary treatments (*P* 0.05).

There was no effect ($P > 0.05$) of the diets on all the studied parameters measured after 55 days of experimental diets being supplied. It can be summarised that, in our experimental conditions, the ingestion of citrus pulp did not affect the serum metabolic indicators.

Serum protein electrophoresis

The results of serum electrophoretic profile are shown in Table 4. Statistically significant differences were found among the groups atthebeginningoftheexperimentaltrial(i.e.10daysbeforeonset of trial) despite the randomisation of animals, which came from the same flock and were subjected to the same pre-experimental nutritionandhusbandrypractices.Consideringthatserumprotein fractions quickly change in response to nutritional and health statusandthatthecovariatestatisticalprocedureallowstoremove

Table 3. Haematochemical parameters before the feeding experimental trial (Day –10) and at the end of the study (Day 55)

a,b, Within a row, means with unlike letters differ ($P < 0.05$). *Statistical analysis includes the value at Day –10 as covariate and the interaction diet * covariate. The interaction diet * covariate was not significant ($P > 0.05$). Adjusted means are shown

	Control (C)	Citrus 24 (Cp24)	Citrus 35 (Cp35)	s.e.m.	*P*-value
Total protein at Day –10 (g/100 mL)	5.89b	4.33a	6.48b	0.224	0.0005
Total protein at Day 55 (g/100 mL)*	6.64	6.95	6.87	0.0976	0.582
Urea at Day –10 (mmol/L)	6.65	5.24	5.73	0.275	0.120
Urea at Day 55 (mmol/L)	7.12	8.28	7.96	0.247	0.159
Total cholesterol at Day –10 (mg/100 mL)	44.25	40.22	53.80	2.81	0.110
Total cholesterol at Day 55 (mg/100 mL)	46.13	42.56	43.00	1.73	0.691
Triglycerides at Day –10 (mg/100 mL)	17.63a	15.78a	24.30b	1.15	0.002
Triglycerides at Day 55 (mg/100 mL)*	25.83	22.24	20.62	1.17	0.778
NEFA at Day –10 (mmol/L)	0.11	0.14	0.14	0.0089	0.422
NEFA at Day 55 (mmol/L)	0.18	0.32	0.23	0.0334	0.247
Total bilirubin at Day –10 (mM/L)	0.28	0.22	0.35	0.033	0.246
Total bilirubin at Day 55 (mM/L)	0.33	0.33	0.45	0.039	0.347
Iron at Day –10 (mg/100 mL)	146.5a	165.7ab	172.8b	4.27	0.031
Iron at Day 55 (mg/100 mL)*	232.0	188.6	225.0	10.7	0.541

Table 4. Serum protein profile before the feeding experimental trial (Day –10) and at the end of the study (Day 55)

a,b, Within a row, means with unlike letters differ ($P < 0.05$). *Statistical analysis includes the value at Day –10 as covariate and the interaction diet * covariate. The interaction diet * covariate was not significant ($P > 0.05$). Adjusted means are shown

	Control (C)	Citrus 24 (Cp24)	Citrus 35 (Cp35)	s.e.m.	*P*-value
Albumin at Day –10 (g/100 mL)	3.36b	2.21a	3.06b	0.115	0.0005
Albumin at Day 55 (g/100 mL)*	2.76	3.23	3.43	0.0791	0.905
a1 globulins at Day –10 (g/100 mL)	0.24a	0.26a	0.46b	0.0329	0.003
a1 globulins at Day 55 (g/100 mL)*	0.23a	0.33b	0.19a	0.0178	0.004
a2 globulins at Day –10 (g/100 mL)	0.75	0.67	0.81	0.0468	0.488
a2 globulins at Day 55 (g/100 mL)	0.93b	0.89ab	0.83a	0.0185	0.046
b globulins at Day –10 (g/100 mL)	0.40a	0.34a	0.83b	0.0572	0.0005
b globulins at Day 55 (g/100 mL)*	0.51	0.51	0.40	0.0236	0.887
g globulins at Day –10 (g/100 mL)	1.13ab	0.86a	1.32b	0.0622	0.003
g globulins at Day 55 (g/100 mL)*	2.15	2.24	1.84	0.105	0.469
Ratio albumin/globulin (Day –10)	1.38b	1.07a	0.94a	0.0567	0.003
Ratio albumin/globulin (Day 55)*	0.72a	0.86ab	1.07b	0.0454	0.044

any possible inference due to initial differences between groups, the results observed at Day 55 suggest that the experimental dietary treatments affected most of the selected parameters. Indeed, at the end of the experimental feeding trial, i.e. at Day 55, the globulin fractions a1 and a2 and the ratio of albumin to globulin (AG ratio) were significantly modified by the diet supplied to lambs. In particular, for the AG ratio the Cp35-fed lambs showed significantly higher values compared with Control-fed lambs ($P < 0.05$) whereas Cp24 lambs maintained average values that were not different from the other two groups. Among the globulin fractions, a significant(P=0.046)decreasing trend due to citrus pulp inclusion in the diet has been observed for a2 globulins, which were significantly lower in the case of Cp35-fed lambs compared with the Control ones.

Discussion

Intake and animal performance

In this study substitution of barley by DCP, to up to 35%, did not affect DMI and all performance indicators providing an equivalent output, as exhibited by the similar carcass weight and yield. Literature shows similar results for calves(Ahooei*etal*. 2011) and sheep (Morales *et al.* 2010; Gilaverte *et al*. 2011); whereas an improvement has been observed in kids (Bueno *et al*. 2002) and in dairy cows (Miron *et al*. 2002).

Our results suggest that, in our experimental conditions, the organic matter derived from DCP was equivalently degraded as compared with barley. Despite the different ingestion in terms of fibre fractions, in particular the much higher acid detergent lignin ingested, and polyphenols, there were no detrimental effects on feed intake and even on feed conversion efficiency in DCP ingesting animals. Thus it can be hypothesised that inclusion of DCP in this study did not cause nutritional inadequacies. These observations tend to agree with findings by Ben-Ghedalia *et al*. (1989) and Madrid *et al*. (1997) who attributed high digestibility to cell walls from citrus by-products which, to some extent, justify the similar carcass weight in the DCP ingesting animals when compared with those eating the Control diet. Nevertheless, the advantage that inclusion of DCP confers in terms of improved feed digestibility due to improved rumen microflora activity (Ben-Ghedalia *et al*. 1989; Moss 1994) was limited in the present trial as the latter is much more evident in a straw based diet, which was not the case in the present trial.

This study seems to confirm that the restriction of feeding to only daytime (from 0900 hours to 1800 hours) does not adversely affect the animals in terms of meeting their nutritional requirements, as already observed in a similar research focusing on carob pulp inclusion in lamb diets (Gobindram *et al*. 2014). According to Gill (2004), this could be explained by the fact that sheep are diurnal animals predominantly eating during daytime. Another interesting aspect was that intake was not abated despite negative palatability issues (Battacharya and Harb 1973) sometimes associated with DCP. However, it could be hypothesised that the differences observed in the pattern of ingestion among the three groups throughout the day could be due to a negative effect of the highest level of citrus pulp inclusion on diet palatability. However, theshiftproducedinthefeedconsumptionintheCp35groupmade DMI more even along the day; this could be positively related to welfare (Villalba *et al.* 2011).

Dehydrated citrus pulp has potential toxic effects in ruminants (Rihani 1991; Saunders *et al*. 2000) but, by virtue of the highly variable amount and types of substances which may cause such toxicity, it is very difficult to pinpoint the causing agent. Polyphenols are one of the group of secondary compounds that are present in citrus by-products (Balasundram *et al*. 2006). In our study we focussed on determination of polyphenols content to try to surmise any observable differences between the various diets; as expected, the polyphenols ingestion was strongly affected by the substitution of barley with dried citrus pulp. Frutos *et al*. (2004) suggested that there is a threshold level at which polyphenols adverse levels overcome positive effects and thence result in toxic symptoms and that threshold is variable depending on a range of factors partly due to the complexity of the polyphenols that can be found in feeds. It can be hypothesised

that the nature and the amount of ingested polyphenols, even by Cp35-fed lambs, may be not at a level for toxic symptoms to be observed, as reflected in the assessed parameters, or else that its effects have been countered by other substances present in the diet. Moreover, a possible effect of essential oils could be excluded. Indeed, the limonene content oftheDCPusedinthepresentstudywas~48mg/kgDM (W.Diani, unpubl.data),whichisatleast10000timeslessthanwhatcouldbe expected in citrus pulp (Amparo *et al*. 1998).

Serum haematochemical profile

Serum haematochemical profile can be considered as one of the means that can be used to detect animal welfare/animal health discrepancies (Broom1991;OhlandVander Staay2012). In this study, it was observed that in all of the measured metabolic parameters there was no significant difference among the diets. This is a promising result as it showed that even at the highest levels of DCP inclusion in lamb diets, i.e. at the highest polyphenols daily intakes, metabolic discomfort could reasonably be excluded. To our knowledge, this is the first paper in which the effect of high DCP level in the diet on ruminant metabolic parameters has been evaluated. Indeed, similar results have been observed in calves (Ahooei *et al*. 2011) and in dairy cows (Belibasakis and Tsirgogianni 1996) but in those studies, DCP percentages in the experimentaldietswere12%and20%respectively.Citruspulpcan contain also other secondary compounds, such as saponins and steroids (Mathur *et al*. 2011) that could have interfered with the polyphenols, thus limiting their effect. This would be in agreement with Provenza *et al*. (2003) who reported on the antagonistic, synergistic and complementary relationship existing between various plant secondary compounds (PSCs) and nutrients. It could have been expected that DCP diets would have provided a higher energy than the Control diet as DCP is often considered as an energy supplement in ruminant ration (Kim *et al*. 2007); however, this was not the case in the present study (Table 2). The composition of DCP may indeed vary a lot, mainly depending on the processing conditions thereby leading to variable composition of nutrients and secondary compounds both in quality and in quantity (Kale and Adsule 1995). In conclusion, it can be hypothesised that, in our experimental conditions, the higher polyphenols intake, the different fibre fractions ingestion and the potential presence of other PSCs due to citrus pulp addition to the diet did not affect protein and carbohydrates utilisation by the experimental lambs.

Serum protein electrophoresis

Protein is the most abundant component in plasma and its levels and its profile can assist in the diagnosis of immune system disruption (Eckersall 2008). In particular, the levels of a group of serum proteins called acute phase proteins are correlated with infection, trauma and inflammation(Eckersall2008).These acute phase proteins are termed as positive or negative depending on whether they increase or decrease in case of distress (Ceciliani *et al*. 2012). For example, albumin decreases with inflammation and is therefore a negative acute phase protein (Ceciliani *et al*. 2012). In this study, the experimental diets caused an increase in the AG ratio in DCP groups, probably linked to the higher polyphenol ingestion. Several authors have reported that AG ratio can be used as an indicator of metabolic disorders (Alberghina *et al*. 2010; Waziri *et al*. 2010) but seasonal variations have also been observed in sheep and goats (Piccione *et al.* 2011). Usually high AG ratio may be linked to impaired protein utilisation efficiency and liver dysfunction, whereas low AG ratio may indicate infection (Farver 1997; Ndlovu *et al*. 2009). The values of the AG ratio recorded in this study were quite far from those observed in Valle del Belice sheep, ranging from 1.2 to 1.7 (Piccione *et al.* 2011). However, the breed, age and sex effects may justify such variations. Considering that no symptoms of any pathology in the animals during the course of the trial have been observed, such significantly different levels of AG ratio can be attributable to the inclusion of DCP, which was the only variable factor among the three groups of animals. However, the reason why this occurs is relatively obscure as there is little knowledge on the composition of the diets in terms of secondary compounds, other than total polyphenols. DCP are known to have antioxidant properties (Middleton and Kandaswami 1994; Fattouch *et al*. 2007) and have the inherent capacity to reduce pathological bacteria like *E. coli* O157:H7 (Callaway *et al*. 2011*b*) and salmonella (Callaway *et al*. 2011*c*). It may be inferred here that somehow the inclusion of DCP affected the protein profile but not to a level to show any apparent signs of distress.

Conclusions

This study showed that DCP may be confidently used up to 35%, on an as-fed basis, as a substitute for barley in diets fed to fattening lambs. This can potentially lead to a significant reduction in feeding cost and provide a value addition to a local by-product while avoiding any metabolic distress to the animals. Moreover, complementary studies have demonstrated an improved meat quality in lambs fed citrus pulp diets. The highly variable nature of citrus pulp requires detailed analyses on nutrient composition and, above all, on secondary compounds in order to fully understand the metabolic reasons of the experimental results. Further studies could be advisable on this issue.

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References

Ahooei GR, Foroughi AR, Tahmasbi AM, Shahdadi AR, Vakili R (2011) Effects of different levels of dried citrus pulp and urea on performance of fattening male calves. *Journal of Animal and Veterinary Advances* **10**, 1811–1816. doi:10.3923/javaa.2011.1811.1816

Alberghina D, Casella S, Vazzana I, Ferrantelli V, Giannetto C, Piccione G (2010) Analysis of serum proteins in clinically healthy goats (*Capra hircus*) using agarose gel electrophoresis. *Veterinary Clinical Pathology* **39**, 317–321. doi:10.1111/j.1939-165X.2010.00226.x

AmparoC,Martinez-MonzoJ,ChaferT,FitoP(1998)Limonenefromcitrus. In Mazza, Giuseppe, ed. *Functional foods: biochemical and processing aspects*. Vol. 1. CRC Press, 1998.

AOAC (1995) 'Official methods of analysis.' 16th edn. (Association of Official Analytical Chemists: Arlington, WA)

Arthington JD, Kunkle WE, Martin AM (2002) Citrus pulp for cattle. In 'The veterinaryclinicsofNorthAmerica-foodanimalpractice'.(EdsGRogers, M Poore) pp. 317–328. (W.B. Saunders Company: Philadelphia, PA)

BalasundramN,SundramK,SammanS(2006)Phenoliccompoundsinplants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chemistry* **99**, 191–203. doi:10.1016/j.foodchem. 2005.07.042

Bampidis VA, Robinson PH (2006) Citrus by-products as ruminant feeds: a review. *Animal Feed Science and Technology* **128**, 175–217. doi:10.1016/j.anifeedsci.2005.12.002

Battacharya AN, Harb M (1973) Dried citrus pulp as grain replacement for Awasi lambs. *Journal of Animal Science* **36**, 1175–1180.

Belibasakis NG, Tsirgogianni D (1996) Effects of dried citrus pulp on milk yield, milk composition and blood components of dairy cows. *Animal Feed Science and Technology* **60**, 87–92. doi:10.1016/0377-8401(95) 00927-2

Ben-Ghedalia D, Yosef E, Miron J, And Est Y (1989) The effects of starch and pectin-rich diets on quantitative aspects of digestion in sheep. *Animal Feed Science and Technology* **24**, 289–298. doi:10.1016/0377-8401(89) 90150-8

Broom DM (1991) Animal welfare: concepts and measurement. *Journal of Animal Science* **69**, 4167–4175.

BuenoMS,Ferrari E Jr,BianchiniD, LeinzFF, Rodrigues CFC(2002)Effect of replacing corn with dehydrated citrus pulp in diets of growing kids. *Small Ruminant Research* **46**, 179–185. doi:10.1016/S0921-4488(02) 00184-0

Callaway T, Carroll JA, Arthington JD, Edrington TS, Anderson RC, Ricke SC, Crandall P, Collier C, Nisbet DJ (2011*a*) Citrus products and their use against bacteria: potential health and cost benefits. In: 'Nutrients, dietary supplements, and nutriceuticals: cost analysis versus clinical benefits'. (Eds RR Watson, JK Gerald, VR Preedy) pp. 277–286. (Springer Sciences – Business Media LLC: New York, NY)

Callaway TR, Carroll J, Arthington JD, Edrington TS, Anderson RC, Rossman ML, Carr MA, Genovese KJ, Ricke SC, Crandall P, Nisbet DJ (2011*b*) Orange peel products can reduce Salmonella populations in ruminants. *FoodbornePathogens and Disease***8**, 1071–1075.doi:10.1089/ fpd.2011.0867

Callaway TR, Carroll J, Arthington JD, Edrington TS, Rossman ML, Carr MA, Krueger NA, Ricke SC, Crandall P, Nisbet DJ (2011*c*) Research Note: *Escherichiacoli* O157:H7 populations in ruminants can be reduced by orange peel product feeding. *Journal of Food Protection* **74**(11), 1917–1921. doi:10.4315/0362-028X.JFP-11-234

CecilianiF,CeronJJ,EckersallPD,SauerweinH(2012)Acutephaseproteins in ruminants. *Journal of Proteomics* **75**, 4207–4231. doi:10.1016/j. jprot.2012.04.004

Durmic Z, Blache D (2012) Bioactive plant and plant products: effects on animalfunction,healthandwelfare.*AnimalFeedScienceandTechnology* **176**, 150–162. doi:10.1016/j.anifeedsci.2012.07.018

Eckersall PD (2008) Proteins, proteomics, and the dysproteinemias. *Clinical Biochemistry of Domestic Animals* **6**, 114–155.

Farver TB (1997) Concepts of normality in clinical biochemistry. II. Reference interval determination and use. In 'Clinical biochemistry of domestic animals'. 5th edn. (Eds J Kaneko, J Harvey, M Bruss) pp. 2–9. (Academic Press: San Diego, CA)

FattouchS,CaboniP,CoroneoV,TuberosoC,AngioniA,DessiS,Marzouki N, Cabras P (2007) Antimicrobial activity of Tunisian quince (*Cydonia oblonga*Miller)pulpandpeelpolyphenolicextracts.*JournalofAgricultural and Food Chemistry* **55**(3), 963–969. doi:10.1021/jf062614e

Frutos P, Hervás G, Giráldez FJ, Mantecón AR (2004) Review: tannins and ruminant nutrition. *Spanish Journal of Agricultural Research* **2**(2), 191–202. doi:10.5424/sjar/2004022-73

Gilaverte S, Susin I, Pires AV, Ferreira EM, Mendes CQ, Gentil RS, Biehl MV, Rodrigues GH (2011) Diet digestibility, ruminal parameters and performance of Santa Ines sheep fed dried citrus pulp and wet brewer grain. *Revista Brasileira de Zootecnia* **40**, 639–647. doi:10.1590/S15163598201100030024

GillW(2004)Appliedsheepbehavior.AgriculturalExtensionService,1–24. Availableathttp://animalscience.ag.utk.edu/sheep/pdf/appliedsheepbehaviorwwg-2-04.pdf [Verified 1 July 2014]

Gobindram NEM, Bognanno M, Luciano G, Lanza M, Biondi L (2014) Carob pulp inclusion in lambs' diet: effect on intake, performance, feeding behaviour and blood metabolites. *Animal Production Science* doi:10.1071/AN14733

Gravador RS, Jongberg S, Andersen ML, Luciano G, Priolo A, Lund MN (2014) Dietary citrus pulp improves protein stability in lamb meat stored under aerobic conditions. *Meat Science* **97**, 231–236. doi:10.1016/ j.meatsci.2014.01.016

InserraL, Priolo A, BiondiL, LanzaM,BognannoM,Gravador RN,Luciano G (2014) Dietary citrus pulp reduces lipid oxidation in lamb meat. *Meat Science* **96**, 1489–1493. doi:10.1016/j.meatsci.2013.12.014

ISTAT (2015) Data warehouse 'Censimento Agricoltura 2010'. Available at http://dati.istat.it/Index.aspx?lang=en&SubSessionId=a9921983-14c44a08-842e-50323c892151&themetreeid=101 [Verified 22 June 2015]

Kale PN, Adsule PG (1995) Citrus. In 'Handbook of fruit science and technology: production, composition, storage and processing'. (Eds DK Salunkhe, SS Kadam) pp. 39–65. (Marcel Dekker, Inc.: New York, NY)

KimSC,AdesoganAT,ArthingtonJD(2007)Optimisingnitrogenutilization in growing steers fed forage diets supplemented with dried citrus pulp. *Journal of Animal Science* **85**, 2548–2555. doi:10.2527/jas.2007-0059

LanzaM,ScerraM,BognannoM,BuccioniA, CilioneC, BiondiL, Priolo A, LucianoG(2015) Fatty acidmetabolism in lambsfed citrus pulp. *Journal of Animal Science* doi:10.2527/jas.2014-8708

MadridJ,HernándezF,PulgarMA,CidJM(1997)Ureaandcitrusby-product of straw-based diets for goats: effect on barley straw digestibility. *Small Ruminant Research* **24**, 149–155. doi:10.1016/S0921-4488(96)00957-1

Makkar HPS, Blümmel M, Borowy NK, Becker K (1993) Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of the Science of Food and Agriculture* **61**, 161–165.

MathurA,VermaSK,PurohitR,GuptaV,DuaVK,PrasadGBKS,MathurD, Singh S (2011) Evaluation of in vitro antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. *Journal of Biotechnology and Biotherapeutics* **1**(2), 1–17.

Middleton E, Kandaswami C (1994) Potential health-promoting properties of citrus flavonoids. *Food Technology* **48**, 115–119.

MironJ,YosefE,Ben-GhedaliaD,ChaseLE,BaumanDE,SolomonR(2002) Digestibility by dairy cows of monosaccharide e constituents in total mixed rations containing citrus pulp. *Journalof Dairy Science* **85**, 89–94. doi:10.3168/jds.S0022-0302(02)74056-3

Morales H, Stuart JR, González J, Gutiérrez E, Bernal H, Colin J (2010) Productive performance and dynamics of blood metabolites in Pelibuey lambs, fed cereals, dehydrated citrus pulp and urea. *Cuban Journal of Agricultural Science* **44**(2), 129–133.

Moss AR (1994) Methane production by ruminants-literature review of I dietary manipulation to reduce methane production and II Laboratory procedures for estimating methane potential of diets. *Nutrition Abstracts and Reviews (Ser. B)* **64**, 785–806.

Ndlovu T, Chimonyo M, Okoh AI, Muchenje V, Dzama K, Dube S, Raats JG (2009) A comparison of nutritionally-related blood metabolites among Nguni, Bonsmara and Angus steers raised on sweet-veld. *Veterinary Journal (London, England)* **179**, 273–281. doi:10.1016/j.tvjl.2007.09. 007

Nordi ECP, Costa RLD, David CMG, Parren GAE, Freitas ACB, Lameirinha LP, Katiki LM, Buenoa MS, Quirino CR, Gamac PE, Bizzo HR, Chagas ACS (2014) Supplementation of moist and dehydrated citrus pulp in the diets of sheep artificially and naturally infected with gastrointestinal nematodes on the parasitological parameters and performance. *Veterinary Parasitology* **205**(3–4), 532–539. doi:10.1016/j.vetpar.2014. 09.015

Ohl F, Van der Staay FJ (2012) Animal welfare: at the interface between science and society. *Veterinary Journal (London, England)* **192**, 13–19. doi:10.1016/j.tvjl.2011.05.019

Piccione G, Messina V, Giannetto C, Casella S, Assenza A, Fazio F (2011) Seasonal variations of the serum proteins in sheep and goats. *Archiv Tierzucht* **54**(4), 399–405.

Provenza FD, Villalba JJ, Dziba LE, Atwood SB, Banner RE (2003) Linking herbivore experience, varied diets, and plant biochemical diversity. *Small Ruminant Research* **49**, 257–274. doi:10.1016/S0921-4488(03) 00143-3

Rihani N (1991) Valeur alimentaire et utilisation des sous-produits des agrumes en alimentation animale. *Options Méditerranéennes-Série Séminaires* **16**, 113–117.

Saunders GK, Blodgett DJ, Hutchins TA, Prater RM, Robertson JL, Friday PA, Scarratt WK (2000) Suspected citrus pulp toxicosis in dairy cattle. *Journal of Veterinary Diagnostic Investigation* **12**, 269–271. doi:10.1177/ 104063870001200314

Tao NG, Liu YJ, Tang YF, Zhang JH, Zhang ML, Zeng HY (2009) Essential oil composition and antimicrobial activity of Citrus reticulate. *Chemistry of Natural Compounds* **32**, 1–2.

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**, 3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Villalba JJ, Bach A, Ipharraguerre IR (2011) Feeding behavior and performance of lambs are influenced by flavor diversity. *Journal of Animal Science* **89**, 2571–2581. doi:10.2527/jas.2010-3435

Wadhwa M, Bakshi MPS (2013) Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products. FAO RAP Publication, 2013/04. (Ed. HPS Makkar) Available at http://www.fao.org/docrep/018/i3273e/i3273e.pdf [Verified 2 September 2015]

WaziriMA,RibaduAY,SivachelvanN(2010)Changesintheserumproteins, hematological and some serum biochemical profiles in the gestation period in the Sahel goats. *Veterinary Archives* **80**, 215–224.

WelchJG,SmithAM(1971)Effectofbeetpulpandcitruspulponrumination activity. *Journal of Animal Science* **33**, 472–475.