Scerra M, Rao R, Foti F, Caparra P, Cilione C, Chies L, 2021. Disposal of bergamot by-products by animal productions

Smart Innovation, Systems and Technologies, 3-10. ISSN: 2190-3018

which has been published in final doi: https://doi.org/10.1007/978-3-030-48279-4_1

Disposal of bergamot by-products by animal productions

Scerra Manuel* https://orcid.org/0000-0001-8288-5436, Rao Rosa, Foti Francesco, Caparra Pasquale, Cilione Caterina, Chies Luigi

Mediterranea University, Department of Agriculture, Reggio Calabria 89124 Italy, Division of Animal Production
*manuel.scerra@unirc.it

Abstract. The objective of this study was to investigate the utilization of bergamot by-product in monogastric and poligastric diets, as an interesting alternative to dispose this surplus. In ovine productions, 27 lambs were divided in three groups and fed for 90 days with 3 different diets: one group of lambs (C) was fed only concentrate while the other groups received concentrate and fresh bergamot pulp (FBP) at the level of 20% dry matter (DM) on the diet fed (BL20) and concentrate and FBP at the level of 35% DM on the diet fed (BL35); in pig production, for 120 days, 30 pigs were fed 3 dietary treatments formulated to contain: only concentrate (C), concentrate and ensiled bergamot pulp (EBP) at the level of 10% dry matter (DM) on the diet fed (BP10) and concentrate and EBP at the level of 15% DM on the diet fed (BP15). After 10 days of adaptation, the experimental animals of the two trials ingested all the bergamot by-products integrated in the diets, with a global utilization of 2412 kg and 5730 kg respectively for ovine and pig productions. No significant differences between treatments were found for final weight, average daily gain, dry matter intake, feed conversion ratio and carcass weight. The BL35 treatment tended to increase total polyunsaturated fatty acids (PUFA), mainly PUFA n-3, leading to a lower levels of PUFA n-6/n-3 ratio. Therefore, the utilization of FBP in animal productions is a valid alternative to dispose of it without detrimental effects on animal performances.

Keywords: Bergamot, By-products, animal.

1 Introduction

Increased disposal costs in many parts of the world have increased interest in utilization of citrus by-product feedstuffs as alternative feeds for ruminants. Bergamot (Citrus Bergamia Risso) is a natural hybrid fruit derived from bitter orange and lemon that is used mostly for juice and the extraction of its essential oil. The annual Italian production of bergamot amounts to 25500 tons and it is produced almost exclusively in province of Reggio Calabria (South Italy), where the cultivated area is about 1500 ha, contributing for more than 90% of world supply[1].

During the industrial processing of bergamot or in general of citrus fruits for juice and essential oil extractions, remains a solid residue which represents about 55-60% of the fruit. Some studies have investigated the chemical composition of bergamot derivatives and its industrial by-products and have been proved to exert important biological activities as protective agents on human cells exposed to tumour necrosis and antioxidants [2-3]. In fact the peel of bergamot fruit contain a significant amount of flavonoids, polyphenol compounds, found in lower levels in other Citrus peels [4]. Flavonoids are secondary metabolites well documented for their biological effects, including anticancer, antiviral, antimutagenic and anti-inflammatory activities [5-6]. For their content of polyphenols and others bioactive phytochemicals such as unsaturated fatty acids, sterols, tocopherols, carotenes, terpenes, several agro-industrial by-products can be considered as functional feedstuffs.

Obviously, if the solid residues resulting from the industrial processes of bergamot are not further processed or disposed, it can give rise to serious environmental pollution. In Calabria region these solid residues are not further processed due to the quantities produced. An interesting alternative to dispose this surplus, during the harvesting period of bergamot and subsequently of industrial processing, could be to use them for animal feeding.

In the literature there are some research on the use of dried citrus pulp in ruminant nutrition [7-8, 9]. However, citrus pulp can also be fed fresh or as silage. Both are generally very rapidly accepted by ruminants, but pulp and peels from lemons are somewhat more acceptable than those from oranges and grapefruit [10] or even more than those from bergamots for their strong acrid taste.

Few paper have been report on the utilization of fresh citrus pulp in diet for growing lambs and pigs and consequently of the disposal of these by-products by animal productions, and to the best our knowledge, just one paper reported some data on the possibility to use fresh bergamot by-product in lambs diet [11], while no studies investigated the utilization of the solid residue resulting from the industrial processes of bergamot in pig productions.
Therefore, the aim of this study was to evaluate the utilization of bergamot by-product in lambs and pigs diets as an alternative to dispose this surplus.

2 Material and Methods

2.1 Animal, and diets

For the first trial, a total of 27 Italian Merino male lambs, born within two weeks in September, were weaned at 60 d of age [average initial body weight 15.79 ± 0.50 (SD) kg] and individually straw-bedded pens. Lambs were randomly assigned to three dietary treatments adapted to the respective experimental diets for 10 days. The experimental period lasted 90 days. During this period lambs were fed on the following dietary treatments: only concentrate (group C), concentrate and fresh bergamot pulp at the level of 20% dry matter on the diet fed (BL20) and concentrate and fresh bergamot pulp at the level of 35% DM on the diet fed (BL35). The concentrate offered to the lambs of BL20 and BL35 groups had the same ingredients of the concentrate supplied to the lambs of C group but, in order to maintain a similar crude protein concentration between treatments, had a higher soybean meal and faba bean contents and consequently a lower percentage of cereal mix (table 1). The ingredients of each concentrate mixture were ground to make it decrease selective feeding and in BL20 and BL35 groups bergamot pulp was mixed with concentrate. The fresh bergamot pulp used in the study, consisting of peel, pulp, and seeds, was obtained after the cold extraction of bergamot juice and transferred to the farm once week from the start of the experiment.

The experimental diets were supplied at 0830 h and after 1900 h. The amounts of feed offered and refused were recorded every day in order to measure the daily voluntary feed intake and consequently the real quantitative of FBP disposed.

The lambs were weighed at weekly intervals prior to feeding in order to calculate average daily gain. At the end of the experimental period, following overnight fasting, the lambs were slaughtered at a commercial abattoir.

Table 1. Ingredients of concentrate (g/100g as fed) and chemical composition of diet constituents

	Concentrate group C	Concentrate group BL20	Concentrate group BL35	Fresh Bergamot pulp
Cereal mix	60	51	45	
soybean meal	10	15	20	
Faba bean	10	14	15	
bran	17	17	17	
Vitamin mineral premix[1]	3	3	3	
Chemical composition				
Dry matter (DM) g/Kg wet weight	892.5	892.7	891.1	146.8
Crude protein g/Kg DM	164.2	200.6	206.9	65.6
Ether extract g/Kg DM	25.3	24.6	24.4	11.4
Ash g/Kg DM	52.5	60.8	53.7	53.7
NDF g/Kg DM	317.4	296.3	304.8	347.8

[1]The mineral vitamin premix consisted of vitamina A=6750 UI; vitamin D3=1000UI; vitamin E 2 mg; vitamin B12 0,01 mg; vitamin B1 1mg; folic acid 0,2 mg; D-pantotenic acid 5 mg; Co 0,05 mg; Mn 12,5 mg; Zn 15 mg; Mo 0,5mg;

For the second trial, thirty barrows Apulo-Calabrese pigs were weighed, individually identified and assigned to three pens (10 pigs per pen). From 103.4±8.70 kg body weight until slaughter (150.4±9.97 kg) pigs were fed 3 dietary treatments formulated to contain: only concentrate (C), concentrate and ensiled bergamot pulp (EBP) at the level of 10 % dry matter (DM) on the diet fed (BP10) and concentrate and ensiled bergamot pulp at the level of 15% DM on the diet fed (BP15).

Diets with bergamot pulp (BP10 and BP15) were a mixture of C feed with the respective amount of ensiled bergamot pulp and different amounts of soya and lysine were added if necessary. The table 2 reports the ingredients and the chemical composition of the experimental diets, which were planned to provide similar levels of energy and nitrogen.

Bergamot by-product used in this trial was obtained from a juice citrus industry and ensiled for 90 days. Ensiled citrus pulp used contained 18.5% dry matter, 7.9% crude protein, 1.37% crude fat, 24.6% neutral detergent fibre and 13.6% acid detergent fibre.
All pigs were fed the experimental diets twice daily (0700 and 1600 h). The amounts of feed offered and refused were recorded every day in order to measure the daily voluntary feed intake and consequently the real quantitative of FBP disposed. The amount of feeds provided for the experimental pigs were 2.7 % of body weight as DM from 115 to 140 kg of body weight and 2% of body weight as DM from 140 kg to 150 kg. Water was continuously available. All pigs were weighed at weekly intervals prior to feeding in order to calculate average daily gain. At the time of submission of this paper, pigs were not slaughtered.

Table 2. Ingredients (g/100g as fed) and chemical composition of diet constituents

	Concentrate group C	Concentrate group BP10	Concentrate group BP15	Ensiled Bergamot pulp
Cereal mix	75	73	70	
soybean meal	8	10	13	
Faba bean	15	15	15	
Vitamin mineral premix[1]	2	2	2	
Chemical composition				
Dry matter (DM) g/Kg wet weight	897.2	896.3	892.1	185
Crude protein g/Kg DM	135.2	147.4	159.3	79.1
Ether extract g/Kg DM	31.3	30.6	29.4	13.7
Ash g/Kg DM	39.7	37.8	39.4	53.8
NDF g/Kg DM	432.6	421.2	420.4	246.7

[1]The mineral vitamin premix consisted of vitamina A=6750 UI; vitamin D3=1000UI; vitamin E 2 mg; vitamin B12 0,01 mg; vitamin B1 1mg; folic acid 0,2 mg; D-pantotenic acid 5 mg; Co 0,05 mg; Mn 12,5 mg; Zn 15 mg; Mo 0,5mg;

2.2 Analysis of feeds and meat samples

Samples of the feeds offered were collected 4 times during the trial, vacuum packaged and stored at -30 °C for analyses.
Feed samples were analyzed for neutral detergent fibre (NDF) [12], crude protein [13], crude fat [13] and ash [13].
The muscle longissimus thoracis et lumborum (LTL; approximately 80g and 400g, for lambs and pigs respectively) was removed from each carcass after slaughter and immediately transported, refrigerated, to the laboratory.
In samples of LTL, moisture (method no. 950.46), crude fat (method no. 991.36), ash (method no. 920.153) and protein (method no. 984.13) were assessed according to AOAC procedures [13], after 24 h thawing at 4 °C.

Intramuscular lipids were extracted from LTL samples according to the method used by Folch et al. [14]. Duplicates of 100 mg of lipid, were methylated adding 1 ml of hexane and 0.05 ml of 2 N methanolic KOH [15]. Gas chromatograph analysis was performed on a Varian model CP 3900 instrument equipped with a CP-Sil 88 capillary column (length 100 m, internal diameter 0.25 mm, film thickness 0.25 μm).

2.3 Statistical analysis

Data were analyzed using a one-way ANOVA to test the effect of the dietary treatment. Differences between means were assessed using Tukey's multiple-comparison test. Significance was declared at $P \leq 0.05$, whereas trends toward significance were considered when $0.05 < P \leq 0.10$. Statistical analyses were performed by the statistical software Minitab, version 14 (Minitab Inc, State College, PA).

3 Results and discussion

Feeding by-products of the crop and food processing industries to livestock could reduce the costs of expensive waste management programs and livestock dependence on grains that can be consumed by humans. However, some of these by-products, if not properly integrated, could reduce animal performance, with negative consequences from an economic point of view for the breeder; but, if properly managed, they could lead to a double advantage as previously emphasized. Furthermore, some of these by-products could have beneficial effect on the quality of meat for their high content of certain bioactive phytochemicals favourable for human health. In these experiments we fed two groups of lambs and two groups of pigs reducing the amount of dry matter from concentrate by replacing it with, in lambs groups, fresh bergamot pulp at the level of 20% and 35% dry matter on the diet fed, while, in pig groups, ensiled bergamot pulp at the level of 10% and 15% dry matter on the diet fed.
During the first trial, after 10-15 days of adaptation period, lambs consumed almost all the FBP offered, ingesting each lamb 1.1 kg/day and 1.88 kg/day for groups BL20 and BL35 respectively (Table 3).

Table 3. lambs performances *in vivo* and amount of n-6 and n-3 fatty acids (g/100 g of total fatty acids)

	Dietary treatments			SEM[6]	*P* value
	C	BL20	BL35		
No. Of lambs	9	9	9		
BW[1] at 70 days, kg	15,29	16,19	15,9	0.754	ns
BW[1] at 160 days, kg	31,83	32,5	32,6	0.643	ns
total DMI[2], g/d	823	897	844	22.04	ns
Concentrate DMI[2], g/d	823[a]	710[ab]	568[b]	24.30	<0.05
FBP[3] DMI g/d	0[a]	187,71[b]	276[c]	3.274	<0.001
FBP[3]intake kg/d	0[a]	1.1[b]	1.88[a]	0.113	<0.001
ADG[4], g/d	183	181	185	4.279	ns
FCR[5], g DMI[2]/g ADG[3]	4.49	4.95	4.65	0.198	ns
Carcass weight (kg)	14,41	14,63	14,71	0.312	ns
∑ n-6	13.58	11.55	12.66	0.848	ns
∑ n-3	1.99[b]	2.04[b]	3.76[a]	0.229	<0.001
n-6/n-3	6.84[c]	5.71[b]	3.27[a]	0.393	<0.001

[a,b,c] Within a row different superscript letters indicate significant differences ($P < 0.05$) tested using Tukey's adjustment for multiple comparisons.

[1]BW=Body weight; [2]DMI=dry matter intake; [3]FBP= fresh bergamot pulp; [4]ADG=averange daily gain; [5]FCR=feed conversion ratio; [6]SEM= standard error of means.

In the second trial, pigs of the two experimental groups also consumed all the EBP offered, ingesting each pig 1.91 kg/day and 2.87 kg/day for groups BP10 and BP15respectively (Tab.4).

Table 4. Pigs performances *in vivo*

	Dietary treatments			SEM[6]	*P* value
	C	BP10	BP15		
No. Of pigs	10	10	10		
Initial BW , kg	102.8±4.7	103.9±4.9	102.5±4.5	1.954	0.784
Final BW[1], kg	157.3±4.7	155.2±5.7	154.3±5.3	2.276	0.415
total DMI[2], kg/d	3.527	3.534	3.526	0.167	0.478
Concentrate DMI[2], kg/d	3.527[a]	3.180[b]	2.995[c]	0.149	0.041
EBP[3] DMI g/d	0[a]	354[b]	531[c]	8.721	0.001
EBP[3]intake kg/d	0[a]	1.913[b]	2.87[c]	0.142	0.001
ADG[4], g/d	454	427	431	8.231	0.692
FCR[5], g DMI[2]/g ADG[3]	7.76	8.27	8.18	0.392	0.285

[a,b,c] Within a row different superscript letters indicate significant differences (P < 0.05) tested using Tukey's adjustment for multiple comparisons.

[1]BW=Body weight; [2]DMI=dry matter intake;[3]EBP= ensiled bergamot pulp;[4]ADG=averange daily gain;[5]FCR=feed conversion ratio;[6]SEM= standard error of means.

At the end of experimental trials, the total amount of bergamot by-product ingested by each lamb was 99 kg, in BL20 group, and 169 kg, inBL35 group, with a total of by-products from the industrial processes of bergamot disposed in this trial of 2412 kg, while the total amount of bergamot by-product ingested by each pig was 229 kg and 344 kg in groups BP10 and BP15 respectively, with a total of by-products from the industrial processes of bergamot disposed of 5730 kg.

Although dietary treatments that included bergamot by-products obviously decreased the dry matter intake from concentrate (P<0.05), no significant differences were found between treatments for the main performance parameters. In fact, as shown in table 3 and 4, no significant differences were found for final weight, average daily gain, dry matter intake, feed conversion ratio and carcass weight (only in the trial with lambs). However, lambs from BL20 and BL35 groups and pigs from BP10 and BP15 groups consumed less (P<0.05) concentrate as compared to animals from control groups.

In the experiment with lambs, another important finding was the increase of concentration of desirable polyunsaturated fatty acids (PUFA) in meat consequently to the integration of fresh bergamot pulp in the diet (table 3). In this trial, the level of n-3 PUFA were higher in meat from lambs of BL35 group than in meat from lambs from BL20 and C groups. Instead, the levels of n-6 PUFA were comparable between treatments. Consequently, the level of PUFA n-6/n-3 ratio was significantly lower in meat from lambs fed with FBP-diets than in meat from lambs fed with only concentrate, especially in the group where fresh bergamot pulp was replaced at up to 35% of dietary DM. Nutritional guidelines recommend decreasing the PUFA n-6/n-3 ratio in food, which should not exceed a threshold value of 4 [16]. In our experiment the inclusion of 35% of FBP in the diet resulted in a PUFA n-6/n-3 ratio of 3.27, value lower compared to that observed in meat from animals fed with BL20 diet (inclusion of 20% of bergamot pulp in the diet) where the value was 5.71 and in meat from animals fed with control diet (only concentrate) where the ratio was almost 7. Some of these results could be explained considering that the dietary treatment affected the intake of fatty acids of the lambs.

4 Conclusion

the results showed that, through integration in animal diets, we disposed more than 8000 kg of bergamot by-products, without negatively influencing animals performances. Furthermore, the integration of fresh bergamot pulp in lambs diet increased the concentration of n-3 polyunsaturated fatty acids in meat, leading to reduce PUFA n-6/n-3 ratio, a value that showed nutritional importance in the human diet for its correlation with anticarcinogenic properties. In fact, dietary recommendations for humans promoting to keep this value below 4 to reduce the risk of coronary heart disease.

Therefore, present results suggest that inclusion of bergamot by-products in diets for fattening lambs and pigs might be an excellent strategy to reduce the costs of expensive waste management programs without compromising animal growth performances and meat quality.

References

1. ISMEA (The Institute for Services for the Agricultural and Food Market): Report Bergamotto. Roma, Italy (2012), ISMEA.
2. Leopoldini, M., Malaj, N., Toscano, M., Sindona, G., Russo, N.: On the inhibitor effects of bergamot juice flavonoids binding to the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) enzyme. Journal of agriculture and Food Chemistry 58(19), 10768-10773 (2010).
3. Trombetta, D., Cimino, F., Cristani, M., Mandalari, G., Saija, A., Ginestra, G., Speciale, A., Chirafisi, J., Bisignano, G., Waldron, K., Narbad, A., Faulds, C. B.: In vitro protective effects of two extracts from bergamot peels on human endothelial cells exposed to tumor necrosis factor-alpha (TNF-alpha). Journal of agriculture and Food Chemistry 58(14), 8430-8436 (2010).
4. Mandalari, G., Bennett, R. N., Bisignano, G., Saija, A., Dugo, G., Lo Curto, R. B., Faulds, C. B., Waldron, K. W.: Characterization of Flavonoids and Pectins from Bergamot (Citrus bergamia Risso) Peel, a Major Byproduct of Essential Oil Extraction. Journal of agriculture and Food Chemistry 54(1), 197–203 (2006).
5. Sommella, E., Pepe, G., Pagano, F., Tenore, G. C., Marzocco, S., Manfra, M., Calabrese, G., Aquino, R. P., Campiglia, P., 2014. UHPLC profiling and effects on LPS-stimulated J774A.1 macrophages of flavonoids from bergamot (Citrus bergamia) juice, an underestimated waste product with high anti-inflammatory potential. Journal of Functional Foods 7, 641–649.
6. Vuorela, S., Kreander, K., Karonen, M., Nieminen, R., Hamalainen, M., Galkin, A., Laitinen, L. and Salminen, et al.: Preclinical evaluation of rapeseed, raspberry, and pine bark phenolics for health related effects. Journal of Agriculture and Food Chemistry, 53, 5922–5931 (2005).
7. Bueno, M.S., Ferrari Jr., E., Bianchini, D., Leinz, F.F., Rodrigues, C.F.C.: Effect of replacing corn with dehydrated citrus pulp in diets of growing kids. Small Ruminant Research 46, 179–185 (2002).
8. Caparra, P., Foti, F., Scerra, M., Sinatra, M.C., Scerra, V.: Solar-dried citrus pulp as an alternative energy source in lamb diets: Effects on growth and carcass and meat quality. Small Ruminant Research 68, 303–311 (2007).
9. Lanza, M., Scerra, M., Bognanno, M., Buccioni, A., Cilione, C., Biondi, L., Priolo, A., Luciano, G.: Fatty acid metabolism in lambs fed citrus pulp. Journal of Animal Science 93, 3179-3188 (2015).
10. Bath, D.L., Dunbar, J.R., King, J.M., Berry, S.L., Leonard, R.O., Olbrich, S.E.: By-products and unsusual feedstuffs in livestock rations.Western Regional Extension Publication No. 39. USDA-ARS,Washington, DC, USA, (1980).
11. Scerra, M., Foti, F., Caparra, P., Cilione, C., Violi, L., Fiammingo, G., D'Agui', G., Chies, L.: Effects of feeding fresh bergamot (Citrus Bergamia Risso) pulp at up to 35% of dietary dry matter on growth performance and meat quality from lambs. Small Ruminant Research 169, 160-166 (2018).
12. Van Soest, P.J., Robertson, J.B., Lewis, B.A.: Methods for dietary fiber, neutral detergent fiber, and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583-3597 (1991).
13. AOAC (Association of Official Analytical Chemists). Official Methods of Analysis, Sixteenth Edition. AOAC, Washington, DC, USA (1995).
14. Folch, J., Lees, M., Stanley, G.H.S.: A simple method for the isolation and purification of lipids from animal tissue. Journal of Biological Chemistry 226, 497–509 (1957).

15. I.U.P.A.C.: Standard methods for the analysis of oils, fats and derivatives. Oxford: Pergamon Press (1987).
16. Department of Health. Nutritional aspects of cardiovascular disease. Report on health and social subject no. 46. London: Her Majesty's Stationery Office (1994).