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Original article

In vitro determination of ruminal dry matter and cell wall degradation, and production of fermentation end-products of various by-products

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Abstract — The samples used in the experiment were nine types of by-products: giant pumpkin, red pepper, melon, broccoli, brewer's grains, fresh and boiled artichoke, lemon peel and orange peel. The dry matter degradability of each by-product was determined by in vitro fermentation with ruminal fluid of the goat. The materials were incubated at 39 ºC for 12, 24, 48 and 72 h. At each time, pH, dry matter disappearance, neutral-detergent fibre disappearance, volatile fatty acids, lactic acid and ammonia productions were measured. Approximately 50% of the total dry matter loss of broccoli, melon and peels occurred at 12 h incubation. In addition, the total dry matter loss of the giant pumpkin, fresh and boiled artichoke, and brewer's grains was low (< 50% of dry matter loss) at 12 h. The molar proportion of acetate and propionate was influenced by the type of the feedstuffs. Thus, the acetate proportion was > 70% for all by-products, except for brewer's grains (63.8%), and the propionate proportion was < 20% for all by-products, except for the brewer's grains (21.3%). In conclusion, the ruminal dry matter and cell wall degradations, and production of fermentation end-products by in vitro determination offers a convenient method to compare the microbial digestion of the different by-products.

in vitro fermentation / dry matter degradation / cell wall degradation / by-product

Résumé — **Détermination de la dégradation ruminale in vitro, de la matière sèche, de la paroi cellulaire, et de la production des produits finaux de fermentation de divers sous-produits.** L'expérience a porté sur 9 types de sous-produits : citrouille, poivron, melon, brocoli, drèche de brasserie, artichaut cru ou bouilli, zeste de citron, et zeste d'orange. La dégradation de la matière sèche de chaque sous-produit a été déterminée par la fermentation in vitro dans du liquide ruminal de chèvre. Les échantillons ont été maintenus à 39 ºC pendant 12, 24, 48 et 72 heures. A tous ces temps, le pH, la disparition de la matière sèche, la disparition du NDF, les acides gras volatils, l'acide lactique et l'ammoniac ont été mesurés. Environ 50 % du total des pertes de matière sèche du brocoli, du melon et des zestes ont eu lieu après 12 heures d'incubation. De plus, la disparition de la matière sèche de la

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citrouille, de l'artichaut et de la drèche de brasserie a été faible (< 50 % des pertes de matière sèche) 12 heures après. La proportion molaire d'acétate et de propionate a été influencée par le type d'aliment utilisé. Ainsi, la proportion d'acétate a été supérieure à 70 % pour tous les sous-produits, sauf pour la drèche de brasserie (63,8 %), et la proportion de propionate a été inférieure à 20 % pour tous les sous-produits, sauf pour la drèche de brasserie. Ce travail permet de conclure que la détermination de la dégradation ruminale in vitro, de la matière sèche et du NDF, et la mesure des produits terminaux de fermentation de divers sous-produits offre une méthode appropriée qui permet de comparer la digestion microbienne de divers sous-produits.

fermentation in vitro / dégradation de la matière sèche / dégradation du NDF / sous-produits

1. INTRODUCTION

The effective use of by-products from agricultural industries as feedstuffs is dependent on several factors. These include the production, nutrient composition in relation to the nutrient requirements of the available animals, processing cost, the uniformity of supply of the by-product and the marked availability of competitively priced feedstuffs. Large quantities of by-products are used in the ruminant's diet in agro-industrial areas [10]. However, little is known about their fermentation pattern in the rumen and a better understanding of their digestion and products of fermentation is necessary in order to properly balance their introduction into the diets [7, 33]. In addition, the new systems to predict animal performance and milk production and composition include a nutritional model that requires digestion rates of the fibre and soluble carbohydrate fractions [6, 27].

The prediction of the extent of degradability using an in situ method has advantages and is now widely used and reported [16, 38]. This technique proposed by Ørskov et al. [26] can be used for the prediction of dry matter intake, digestible dry matter intake and animal performance. However, this technique has several disadvantages: it does not allow individual adaptation of micro-organisms to the solid substrates and fermentation end-products cannot be monitored.

Other methods have been developed to predict the extent of degradability. Thus, some studies conducted by Luchini et al. [18], Feng et al. [8] and Wilman et al. [39] propose in vitro methods for determining ruminal feed degradation because of a greater speed and lower expense than in vivo and in situ methods.

The by-products used in this work have been studied by Megías et al. [24] to determine the chemical composition and in vitro gas production. The objective of these experiments was to evaluate and compare the extent of digestion of DM and NDF and the production of fermentation end-products of by-products in standardised conditions in vitro.

2. MATERIALS AND METHODS

2.1. Samples

The samples studied in the experiment were the nine by-product types mainly used for the feeding of dairy cows feeding in the Murcia Region (Spain) and reported by Martínez-Teruel et al. [22]: giant pumpkin (*Curcubita ficifolia, Bouché*), red pepper (*Capsicum annuum, var. annuum*), whole melon (*Cucumis melo*), stem broccoli (*Brassica olareacea, var. italica*), brewer's grains, inflorescences of fresh artichoke and inflorescences of boiled artichoke (*Cynara scolymus*), lemon peel (*Citrus limon*) and orange peel (*Citrus aurantium*). The samples were oven-dried and ground through a 1 mm screen. Table I shows the chemical composition of the raw materials: DM

Table I. Nutrient composition of different by-products[a].

Sample	DM (%)	CP (%)	NDF (%)	ADF (%)	Lignin[b] (%)
Giant pumpkin	16.4	13.2	63.6	41.5	13.2
Red pepper	10.6	20.1	46.3	40.0	10.2
Melon	7.9	9.9	27.4	23.9	2.3
Stem broccoli	6.8	18.2	24.2	21.2	2.3
Brewer's grain	32.0	29.9	49.8	17.4	4.2
Fresh artichoke	16.6	13.4	57.4	39.9	8.2
Boiled artichoke	20.5	11.9	47.5	36.8	7.2
Lemon peel	17.7	11.4	29.0	26.8	3.4
Orange peel	15.2	8.6	27.8	24.5	4.2

DM: dry matter; CP: crude protein; NDF: neutral-detergent fibre; ADF: acid-detergent fibre.
[a] Values except DM are expressed on a DM basis.
[b] Permanganate lignin.

by drying at 60 ºC for 48 h, OM by ashing at 550 ºC for 3.5 h, CP by Kjeldahl × 6.25, fibre and lignin by Van Soest et al. [37].

2.2. Collection of ruminal fluid

The dry matter degradability of each by-product was determined by in vitro fermentation with ruminal fluid. Ruminal fluid was collected approximately 4 h after feeding from three mature Murciano-Granadina goats consuming alfalfa hay ad libitum. Ruminal fluid was transported to the laboratory in a sealed thermos and was immediately squeezed through four layers of cheesecloth. The resulting ruminal fluid was purged with deoxygenated CO_2 before use as the inoculum.

2.3. In vitro procedure

In vitro incubations were conducted as described by the first stage of the Tilley and Terry procedure [21]. Thus, 10 mL of ruminal inoculum and 40 mL of buffer solution were added to 0.5 g of the sample of each by-product, in bottles. The buffer solution [23] was used by Tilley and Terry [35]. The bottles were flushed with CO_2 and sealed. Duplicate bottles were incubated in a 39 ºC shaking water bath for each of two replicates for 12, 24, 48 and 72 h. At each time, pH, dry matter (DM) disappearance, neutral-detergent fibre (NDF) disappearance, volatile fatty acids (VFA), lactic acid and ammonia productions were measured. After each respective incubation interval, the fermentation medium was decanted into plastic tubes, and then the tubes were centrifuged at 3000 × *g* for 20 min. Supernatants were decanted into glass bottles, 1 mL of 50% sulphuric acid was added and they were frozen at –20 ºC until analysis. The residues of each sample after incubation were filtered through Whatman® 541 paper and were washed sequentially with water. The samples were dried and weighed to determine the in vitro DM disappearance. In vitro dry matter disappearance was calculated as follows: $(1 - ((\text{DM residue} - \text{blank}_{DM}) / \text{DM original})) \times 100$, where DM residue is the DM recovered after 12, 24, 48 and 72 h of fermentation, blank_{DM} is the DM recovered in the corresponding blank after the same fermentation time, and DM original is the DM of the substrate placed in the tube. Also,

NDF residues were determined on the DM residue by in vitro NDF disappearance using a modified method (neutral-detergent digestion with heat-stable α-amylase) described by Van Soest et al. [37].

The supernatants were analysed for VFA concentration by capillary gas chromatography described by Madrid et al. [20], using 4-methyl-n-valeric acid as the internal standard, for lactic acid and ammonia concentrations by spectrophotometric methods described by Madrid et al. [19] and Chaney and Marbach [4], respectively.

2.4. Mathematical model and statistical analysis

DM disappearance, NDF disappearance, pH, VFA, lactic acid and ammonia values were analysed using ANOVA for two-way comparison with interactions [32]. The model used was:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + \varepsilon$$

where A, B and AB are the effects of the incubation time, the by-product type and the incubation time × by-product type interaction, respectively.

3. RESULTS AND DISCUSSION

3.1. Dry matter degradation

The in vitro DM apparent disappearance of by-products is given in Table II. Approximately 50% of the total DM loss of broccoli, melon and peels occurred at 12 h incubation. The total DM loss of the giant pumpkin, fresh and boiled artichoke, and brewer's grains was, however, low (< 50% of DM loss) at 12 h.

The incubation time significantly ($P < 0.001$) influenced DM disappearance. The major part of the DM loss occurred at 48 h. Herbert and Thomson [12] used 48 h losses of DM to account for the disappearance of fractions of four barley straw genotypes using the nylon bag procedure. The by-products with a major percentage of DM loss at 48 h were orange and lemon peels (89.8 and 86.0%, respectively) and broccoli (86.1%). It is reasonable to expect a good efficiency in ruminant degradation when the citrus by-products are studied because these feeds are highly fermentable [5, 17]. Also melon, boiled and fresh artichoke had high values (77.5, 73.0 and 65.0%, respectively). These results were expected because Gasa et al. [10] reported high values of organic matter digestibility in sheep for by-products of the processing industry of the artichoke (75%). In addition, in our work the red pepper had intermediary degradability (60.9%) at 48 h incubation, and Gasa et al. [10] also indicated an intermediary digestibility value of 61.5% for pepper by-products.

The by-products with less DM loss at 48 h were the brewer's grains (48.1%) and giant pumpkin (40.0%). The low values of DM disappearance were similar to those observed by Kabatange and Shayo [14] for maize stover (42.2%) after 48 h incubation using an in situ method.

The variations in dry matter loss may be related to the differences in chemical composition [1] or to variations in physical structure, such as the distribution within the tissues of lignified cells [29]. Thus, the by-products with a high loss of dry matter (peels, broccoli and melon) had high levels of cell content (ranging from 70.9 to 75.7%) and low contents of lignin (ranging from 2.3 to 4.2%). Van Soest [36] indicated that the cell content is almost completely digestible and lignin is the main factor limiting the digestibility in forages.

In addition, the incubation time × feedstuff interaction was significant ($P < 0.001$) because an increased level of DM loss was the marker for the peels, broccoli and melon, followed by artichoke by-products. These results agreed with those of studies which showed that DM degradability is

Table II. In vitro DM and NDF disappearance (%) of by-products.

	Incubation time (h)					
	12	24	48	72	SE	Sig. level
DM disappearance						
Giant pumpkin	20.0[a]	38.5[b]	40.0[b]	43.7[b]	1.3	**
Red pepper	42.1[a]	54.1[b]	60.9[c]	61.5[c]	0.8	**
Melon	56.5[a]	68.5[ab]	77.5[bc]	83.7[c]	1.8	*
Stem broccoli	58.8[a]	76.1[b]	86.1[c]	87.3[c]	0.1	***
Brewer's grain	22.8[a]	34.5[b]	48.1[c]	51.0[c]	1.0	***
Fresh artichoke	29.3[a]	50.6[b]	65.0[c]	71.4[d]	0.3	***
Boiled artichoke	34.8[a]	53.7[b]	73.0[c]	72.8[c]	0.5	***
Lemon peel	50.1[a]	73.3[b]	86.0[c]	85.9[c]	0.9	***
Orange peel	56.9[a]	74.7[b]	89.8[c]	97.1[c]	0.8	***
NDF disappearance						
Giant pumpkin	12.0[a]	29.2[b]	34.3[b]	33.8[b]	1.5	*
Red pepper	5.3[a]	26.2[b]	37.0[c]	40.7[c]	0.5	***
Melon	3.8[a]	24.8[b]	53.5[c]	61.0[c]	1.3	***
Stem broccoli	37.7[a]	65.7[b]	70.9[b]	71.8[b]	0.9	***
Brewer's grain	0.0[a]	2.57[a]	23.9[b]	26.8[b]	0.7	***
Fresh artichoke	5.5[a]	33.3[b]	46.6[c]	61.4[c]	1.3	***
Boiled artichoke	2.5[a]	34.1[b]	59.0[c]	54.8[c]	0.4	***
Lemon peel	0.0[a]	51.5[b]	70.1[c]	67.7[c]	1.0	***
Orange peel	7.9[a]	40.7[b]	79.2[c]	93.0[c]	1.8	***
Bifactorial analysis	Incubation time (A)		By-products type (B)		A × B	SE
DM disappearance	***		***		***	0.2
NDF disappearance	***		***		***	0.3

[a,b,c,d] Means in the same row with different following letters are significantly different.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

generally influenced by incubation time and the type of incubated feed [9, 11, 25].

3.2. Neutral-detergent fibre degradation

The fermentation characteristics of the NDF of by-products are given in Table II. There were significant differences ($P < 0.001$) in NDF disappearance between the feedstuffs. Also, incubation time significantly ($P < 0.001$) influenced the NDF disappearance. The major part of the NDF loss of by-products occurred after 12 h incubation and there were no high differences in NDF disappearance between 48 and 72 h of incubation. The highest NDF loss at 48 h was observed in orange and lemon peels (79.2% and 70.1%, respectively) and broccoli (70.9%), and the lowest NDF loss at 48 h was observed for the red pepper (37.0%), giant pumpkin (34.3%) and brewer's grains (23.9%).

These results showed that citrus peels and broccoli by-products are digestible fibre sources. These characteristics and the high extent of their fibre digestion in the rumen support that they can be characterised as an energy feed, and have more in common with spring forage, sugar beet pulp or root crops than with cereal grains. In addition, Duran et al. [7] indicated that cell-wall constituents of citrus and beet pulp are more extensively degraded than the other by-products, such as wheat bran or straw.

The melon, and boiled and fresh artichoke had a medium-loss of NDF at 48 h (53.5, 59.0 and 46.6%, respectively). However, melon had a high percentage of DM loss at 48 h (> 75%) and it could also be considered as an energy feed with a very different carbohydrate profile than the usual raw materials used by stock feed manufacturers. On the contrary, boiled artichoke had higher NDF and DM losses (59.0 and 73.0%, respectively) than the fresh artichoke (46.6 and 65.0%, respectively). These results were probably due to the effect of boiled industrial processes on the chemical and physical structure of the artichoke.

In addition, the incubation time × by-product type interaction was significant ($P < 0.001$) because an increased level of NDF loss was a marker for peels and broccoli followed by melon and artichoke by-products more so than in the red pepper, giant pumpkin and brewer's grains.

3.3. Volatile fatty acids, lactic acid and ammonia production

The average concentration of volatile fatty acids and the molar proportions of each individual acid in the rumen liquor at 72 h of incubation are presented in Table III. The total concentrations of VFA at

Table III. Volatile fatty acid (VFA) concentrations at 72 h of incubation and influence of incubation time (12, 24, 48 and 72 h) on VFA concentration.

		Molar proportion (%)				
	Total VFA (mM)	Acetate	Propionate	Butyrate	Isobutyrate	Isovalerate
By-product type						
Giant pumpkin	52.9	71.6^{b}	17.7^{bc}	5.9	1.9	2.7^{cd}
Red pepper	62.5	72.5^{b}	16.5 ^{ab}	6.1	2.5	2.1^{bc}
Melon	85.4	76.2^{bc}	16.2^{ab}	5.4	1.1	1.9^{abc}
Stem broccoli	86.0	78.3^{bc}	13.7^{ab}	4.4	1.8	1.5^{ab}
Brewer's grain	57.1	63.8^{a}	21.3^{c}	4.9	3.8	3.4^{d}
Fresh artichoke	90.8	75.9^{bc}	15.5^{ab}	4.9	1.7	1.7^{ab}
Boiled artichoke	92.5	80.3^{c}	13.2^{a}	4.0	1.1	1.1^{a}
Lemon peel	96.8	73.5^{bc}	17.1^{ab}	5.9	1.8	1.5^{ab}
Orange peel	88.7	74.7^{bc}	16.5^{ab}	5.1	1.9	1.6^{ab}
SEM	3.6	0.7	0.4	0.1	0.1	0.0
Significance level	NS	**	*	NS	NS	**
Incubation time (h)						
12	45.6^{a}	74.9	19.2^{b}	4.0^{a}	1.3^{a}	0.3^{a}
24	54.9^{b}	74.0	18.8^{b}	4.5^{c}	1.6^{ab}	0.8^{b}
48	66.5^{b}	74.2	17.2^{a}	5.2^{b}	1.8^{ab}	1.3^{c}
72	79.2^{c}	74.1	16.4^{a}	5.2^{c}	2.0^{b}	1.9^{d}
Bifactorial analysis						
Incubation time (A)	***	***	**	***	*	***
By-product type (B)	***	*	***	***	***	***
A × B	NS	NS	NS	NS	NS	NS
SEM	1.6	0.4	0.2	0.0	0.2	0.0

^{a,b,c,d} Means in the same column with different following letters are significantly different.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS: not significant.

72 h did not significantly ($P > 0.05$) differ for the by-products. However, the by-product type significantly ($P < 0.001$) influenced the VFA concentration in bifactorial analysis. The values of VFA at 72 h incubation in the red pepper (62.5 mM), brewer's grains (57.1 mM) and giant pumpkin (52.9 mM) were lower than those of the other by-products (ranging from 85.4 to 96.8 mM), reflecting the lower DM and NDF degradabilities. Pitt et al. [27] confirmed the importance of these fermenting structural carbohydrates and fermenting non-structural carbohydrates on ruminal VFA. Also, Khandaker et al. [15] indicated that a higher concentration of VFA suggests an increased rumen microbial activity due to more quantities of organic matter being fermented in the rumen. Durand et al. [7] used the rumen simulation technique to compare various by-products with a wide range of fermentability which could be divided into 4 groups with decreasing values according to organic matter digestibility and total VFA production: pulp by-products (80%, 93 mM), cereal by-products (68%, 69 mM), NaOH-treated straw and hay (55%, 60 mM) and untreated and NH_3-treated straw (34%, 40 mM).

The molar proportion of acetate and propionate were influenced by the type of feedstuffs. Thus, the acetate proportion was > 70% for all by-products, except for the brewer's grains (63.8%), and the propionate proportion was < 20% for all by-products, except for the brewer's grains (21.3%). Durand et al. [7] reported that in comparison with other groups (citrus pulp, beet pulp, maize gluten meal and roughages), cereal by-products showed a particular VFA pattern with a high molar proportion of propionate and low proportion of acetate, probably because of their residual starch content. It is agreed with respect to the molar proportion of VFA that high levels of acetate usually occur in animals fed rations containing large amounts of roughage, whereas lower levels are associated with concentrates. Even though, the citrus peel is an energetic concentrate feed for ruminants, the effect of incubation citrus peel in the rumen liquor did not reduce the acetic acid as expected. Schaibly and Wing [31] reported that rations with citrus pulp increased the molar percent acetic acid. Ben-Ghedalia et al. [3] replaced barley with citrus pulp in diets for sheep and observed greater acetate: propionate ratios in the ruminal fluid of the sheep fed the citrus pulp. This effect may be explained by the higher pectin content of citrus by-products. These structural carbohydrates are rapidly fermentable and are related to acetic fermentations [34].

In addition, the isovalerate proportion was influenced by the type of by-products. The highest molar percentage of isovalerate was observed in the brewer's grains (3.4%). This is supported by the high level of crude protein of this by-product. Thus, Yan et al. [40] observed that supplements of soyabean meal and fish meal significantly increase the average molar proportions of isobutyrate and isovalerate in the rumen. This effect is probably derived from the increased deamination of branched-chain amino acids in the rumen. Thus, brewer's barley grains had the highest levels of ammonia concentration at 12 h incubation (5.3 mg·100 mL^{-1}) (Tab. IV). Barber and Lonsdale [2] however, reported that this by-product is scarcely degradable and its protein fraction is relatively insoluble in the rumen, because the grains are heated during malting and mashing.

Several in vitro studies have shown that maximum microbial growth occurs when the ammonia-N concentration ranges from 5 to 8 mg·100 mL^{-1} [30]. The major part of the by-products studied in our experiment had lower ammonia-N levels at 12 h incubation than the minimum concentration for the maximum efficiency of microbial synthesis.

Incubation time significantly ($P < 0.001$) influenced the ammonia concentration, but

Table IV. Lactic acid and ammonia concentration, and pH of by-products at different incubation times (12, 24, 48 and 72 h).

	Lactic acid (μg·mL^{-1})						Ammonia (mg·100 mL^{-1})						pH					
	12	24	48	72	SEM	Sig. level	12	24	48	72	SEM	Sig. level	12	24	48	72	SEM	Sig. level
Giant pumpkin	5.4^{a}	7.2^{a}	10.0^{b}	5.2^{a}	0.2	**	2.1^{a}	4.5^{ab}	3.2^{a}	8.4^{b}	0.5	*	7.2^{d}	7.0^{c}	6.8^{a}	7.0^{b}	0.0	***
Red pepper	7.7	8.3	5.5	7.1	0.4	NS	4.9^{a}	7.6^{ab}	9.4^{bc}	12.6^{c}	0.4	*	7.2	7.1	7.2	7.1	0.0	NS
Melon	11.2	10.7	9.9	12.1	0.3	NS	1.8^{a}	4.5^{ab}	7.3^{bc}	10.2^{c}	0.5	*	7.0^{b}	6.9^{a}	7.1^{b}	6.9^{a}	0.0	**
Stem broccoli	12.2	11.1	9.7	9.6	0.6	NS	3.7^{a}	5.6^{ab}	9.0^{b}	15.7^{c}	0.6	*	7.1^{b}	6.9^{a}	7.0^{b}	6.9^{a}	0.0	**
Brewer's grain	6.8^{b}	6.3^{b}	5.9^{ab}	5.7^{a}	0.0	*	5.3^{a}	8.7^{b}	15.8^{c}	16.2^{c}	0.3	**	7.2^{c}	7.0^{b}	7.2^{c}	6.9^{a}	0.0	**
Fresh artichoke	9.7	10.0	8.5	9.7	0.4	NS	1.6^{a}	3.5^{b}	5.4^{c}	7.7^{d}	0.1	***	7.1^{c}	6.9^{b}	6.9^{b}	6.7^{a}	0.0	**
Boiled artichoke	9.3^{a}	10.5^{a}	11.1^{a}	15.1^{b}	0.3	*	3.2	5.1	8.1	11.1	0.8	NS	7.2^{d}	7.1^{c}	7.0^{b}	6.8^{a}	0.0	***
Lemon peel	8.4	7.6	7.8	8.0	0.4	NS	1.9^{a}	3.3^{a}	6.0^{ab}	9.4^{b}	0.6	*	7.1	6.8	6.8	6.6	0.0	NS
Orange peel	11.2^{c}	9.5^{b}	8.5^{a}	8.0^{a}	0.1	**	1.6^{a}	2.7^{a}	5.3^{b}	6.2^{b}	0.2	*	7.0^{d}	6.7^{b}	6.8^{c}	6.6^{a}	0.0	*
Bifactorial analysis	Incubation time (A)						By-product type (B)						A × B				SE	
Lactic acid	NS						***						***				0.1	
Ammonia	***						***						NS				0.1	
pH	***						***						***				0.0	

^{a,b,c,d} Means in the same row with different following letters are significantly different.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS: not significant.

this effect was similar to that of Luchini et al. [18] and may have resulted from the catabolism of lysed cells because some nutrients become exhausted, some toxic waste products accumulate, balanced growth can no longer continue, and the bacteria enter a stationary phase during incubation in vitro.

Lactic acid is another product of carbohydrate fermentation [36]. In our case, the feedstuffs significantly ($P < 0.001$) influenced the lactic acid concentration (Tab. IV). The carbohydrates that can lead to the accumulation of lactic acid are starch, maltose, sucrose, lactose, cellobiose, fructose and glucose. The formation of lactic acid during fermentation is correlated with the fermentation rate, and the soluble sugars, sucrose, glucose and raffinose produce the highest lactic acid concentrations [5]. Thus, the by-products with the highest solubility (melon, broccoli and orange peel) had the highest levels of lactic acid at 12 h incubation.

In addition, the incubation time × feedstuff interaction was significant ($P < 0.001$) because the ranges of the levels of lactic acid at different times from in vitro fermentations were variable for each by-product. Thus, the lactic acid concentration of the giant pumpkin, orange peel and brewer's grain fermentations tended ($P < 0.05$) to decrease when the incubation time was increased. This result was similar to that of Piwonka and Firkins [28], which indicated that lactic acid could be partially fermented; they reported that the in vitro digestion trials contain bacteria that use lactic acid, as expected in a mixed culture from the rumen, and that lactic acid is fermented to propionate (30 to 35%) and butyrate (65 to 70%). Even so, the lactic acid of the fermentations of other by-products, such as red pepper, melon, broccoli, fresh artichoke and lemon peel were not affected ($P > 0.05$) by incubation time, except boiled artichoke.

In vitro pH decreased when the incubation time increased (Tab. IV). This effect could be caused by an accumulation of VFA and lactic acid in the in vitro medium. Hoover [13] reported that low ruminal pH lowers microbial growth efficiency and inhibits fibre fermentation. However, in vitro pH was not less than 6.0 in any case in our study.

4. CONCLUSIONS

In conclusion, the in vitro determination of ruminal dry matter and cell wall degradations, and production of fermentation end-products offers a convenient method to compare microbial digestion of various by-products. Thus, the extent of by-product digestion and their characteristics of ruminal fermentation support that broccoli, citrus peels and melon can be characterised as high-degradable feeds for ruminants followed by artichoke by-products more than the red pepper, giant pumpkin and brewer's grain.

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