Direct Research Journals

Vol.4 (7), pp. 176-181, July 2016
ISSN 2354-4147
Article Number: DRJA36074545

Direct Research Journal of Agriculture and Food Science
http://directresearchpublisher.org/aboutjournal/drjafs

Research Paper

Red (*Brassica oleracea* var. *capitata*) and White (*Brassica oleracea* var. *botrytis*) Cabbage Leaves Nutritional Value as Forage Feed: Comparison Study of *In Vitro* Gas Production and Determination of Chemical Composition

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Received 18 April 2016; Accepted 14 June, 2016

An *in vitro* rumen gas production technique was used to compare chemical composition and nutritive value of red (*Brassica oleracea* var. *capitata*) and white (*Brassica oleracea* var. *botrytis*) cabbage leaves (*Brassicaceae*). There were no significant differences in either extract concentration, but other chemical composition (such as Neutral detergent fiber: NDF; Acid detergent fiber: ADF; Ash, and Dry matter concentrations) was significantly higher for red cabbage leaves (RCL) and the crude protein (CP) concentration was higher ($p<0.05$) for white cabbage leaves (WCL). Total gas produced at 24, 48 h and B_{gas} (gas production from the insoluble but fermentable fraction; ml) was significantly highest for WCL than to RCL ($p<0.05$). The highest ($P<0.05$) estimated parameters from *in vitro* gas production values (Organic matter digestibility: OMD; Metabolisable energy: ME; Short chain fatty acid: SCFA, and microbial protein yield) were observed for WCL. *In vitro* rumen gas production can be used as a method to distinguish differences in nutritive value of forages like this study. A difference between two forage species in chemical composition is responsible for the fermentation pattern. Fresh agricultural waste, particularly leaves from RCL and WCL, should be a suitable feed for ruminants, although it seems that the nutritional value of WCL is better than RCL.

Key words: *Brassicaceae*, *in vitro*, chemical composition, gas production.

INTRODUCTION

The application of agro-industrial waste as animal feed has been a common practice for decades in industrialized country where many volumes of them are produced each year, but basically has not been considered in Iran. While some vegetable waste products have been successfully fed for decades in developed countries (e.g. sugar beet pulp), but others are not consistently used because of the uncertainty regarding availability, storage properties, poor handling characteristics, variation in nutrient composition, different digestibility, palatability or intake, and risk of toxic residues. A market scrutiny showed that waste of leaves from cabbage species can reach 30- 50% of total production (Nguyen and Ledin, 2005). Cabbage waste products can help reduce the dependence of livestock on grains which can be consumed by humans. Wadhwa et al. (2006) showed that cabbage leaves are good source of crude protein and have low levels of neutral detergent fiber (NDF) and lignin which indicate their potential for higher voluntary intake.

Many human studies have been conducted about nutritional value and anti-cancer characteristic (Cartea and Velasco, 2008; Fahey et al., 2001) of *Brassicaceae* family, but this studies about animal especially ruminant is scarce. The results from India researchers (Khan and Atreja, 2005; Wadhwa et al., 2006) and Vietnam (Nguyen and Ledin, 2005) have shown that vegetable waste can be excellent unconventional feedstuffs for ruminants. Reid et al. (1994) reported that the use of vegetable by-products in sheep diets significantly improved their growth performance.

Thus, the residues from agro-industrial may offer a solution to assist in improving the Iran smallholder farmers to improve animal's performance. Forage *Brassicas* are an essential component of the feedstuff for some Iran farms.

Unlike widespread application of these crops, performance by *brassica*-fed animals can be inconsistent. Ulyatt et al. (1980) reported the nutrient composition of *Brassica* species, but results were limited to metabolisable energy estimated and crude protein. A more extensive nutritional value of whole plant *Brassica* was described by De Ruiter et al. (2007) however data specific to leaf components of commercial Iran *Brassica* cultivars are unavailable. Nkosi, (2010) reported a higher intake and better feed conversion ratio of lambs fed on beetroot waste diets than those on a standard sheep diet.The objective of this research was to measure the chemical composition and nutritive value of red and white cabbage leaves (RCL and WCL) in field-grown commercially available cultivars by the usual *in vitro* techniques.

MATERIALS AND METHODS

Collection of red and white cabbage leaves

Red (*Brassica oleracea* var. *capitata*) and white (*Brassica oleracea* var. *botrytis*) cabbage leaves samples were collected at ferdowsi university of mashhad farming (khorasan-e razavi distinct, mashhad, Iran). The samples were harvested at November 2013 in farm Research Unit of Ferdowsi University of Mashhad about 999.2 m above sea level with rainfall mean of 262 mm per year in northeast of Iran. Leaves samples were mixed and sub-samples were taken from each mixture and then dried in a forced air oven (65°C) for 48 h. After drying, the samples were ground using a Wiley mill (2 mm screen) and reserved in the air dried place (nylon bags).

Chemical composition determination

Crude protein (CP), ether extract (EE), ash of samples were determined according to AOAC, (1990). The parameters of Acid detergent fiber (ADF) and Neutral detergent fiber (NDF) were measured according to Van Soest et al. (1991).

In vitro gas test

The rumen fluid for experiment was collected before the morning feeding from 4 Baluchi male sheep which have been nourished according to: animals were fed with 0.7 Kg DM alfalfa hay and 0.3 Kg DM concentrate (160 g CP/Kg DM/head/day (at maintenance). The sheep were equipped with permanent ruminal fistula approximately 150 days before use as donor of ruminal fluid. All Sheep were fed twice daily at 07:30 and 16:30 h. They had free access to water. Cumulative gas production was recorded by using the reading pressure technique ((PTB330, Env Company) according to Theodorou et al. (1994) procedure with the modification of Mauricio et al. (1999). Rumen fluid was picked up from multiple sites within the rumen of each animal separately in pre-warmed thermos flasks and transported immediately to the laboratory. Rumen fluid was strained through four layers of cheesecloth, and kept at 39 ◦C under a CO_2 atmosphere. Approximately 500 mg of plant sample were weighed into 120 ml serum bottles. Using an automatic dispenser (Jencons, Hemel Hemstead, England), 50 ml of buffer containing micro and macro-elements, a reducing agent and a reduction indicator of resazurin, was infused to each serum bottle. Bottles without sample (that is, 4 blanks) were also included to allow correction of 96 h degradability values for residual feed from rumen fluid and also 4 samples of alfalfa as slandered were incubated each run. Once filled up, all the bottles were closed with rubber stoppers, crimped with aluminum seals, shaken and placed in the incubator shaker at 39 ◦C. The gas production as cumulative was recorded at several incubation times (3, 6, 9, 12, 24, 32, 48, 72 and 96 h after inoculation time), using the transducer. The samples were incubated in quadruplicate.

Calculations and statistical analysis

The cumulative gas production data were fitted to the exponential equation $y = b\ (1 - e^{-ct})$ according to Ørskov and Mcdonald (1979), where b is the gas production from the insoluble but fermentable fraction (ml); c is the gas production rate constant for b (ml/h); t is the incubation time (h) and y is the gas production at time of t (ml). The metabolisable energy (ME) and organic matter digestibility (OMD) were calculated using equations of Menke et al. (1979) as: ME (MJ/kg DM) = 2.20 + 0.136 × Gp + 0.057 × CP; OMD (gkg of DM) =(14.88 + 0.889 ×

Gp + 0.45 × CP + 0.0651 × XA) ×10 , Where, CP is crude protein (%of DM), XA= ash (%of DM) and Gp is the net gas production (ml) from 200 mg(DM of sample) after 24 h of incubation. Short chain fatty acids (SCFA) is calculated using the equation of (Makkar, 2005) as: SCFA (mmol) = 0.0222×GP–0.00425 Where, GP is 24 h net gas production (ml/200mg DM). Microbial protein was calculated as 19.3 g microbial nitrogen per kg of OMD according to Czerkawski, (1986). A completely randomized design was applied for data analysis using the Statistical Analysis System (SAS) program General Linear Model procedure (SAS, 9.1). Significant means were compared; using the Duncan's multiple range tests. Mean differences were considered significant at $P < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition of red and white cabbage leaves is presented in (Table 1). There were observed variations in the chemical composition of WCL and RCL. The RCL was a significant (p<0.05) higher dry matter (15.42 vs. 13.82%), NDF (21.95 vs. 18.82%), ADF (15.35 vs. 14.01%) CP (16.2 vs. 14.1%) and Ash (11.5 vs. 10.85%) contents than WCL, but the crude protein content for WCL was highest (p<0.05). There was no significant difference between treatments for EE. The DM, Ash, NDF, CP and EE contents of cabbage waste products, were reported 10.2, 10, 20.9, 18.7 and 5.1 % respectively (Tobias et al., 2010), also Akinfemi, (2012) reported 50.86, 12.13, 46.90, 18.44 and 6.02 % values for mentioned parameters. The difference between researcher results may be due to several factors which can affect chemical composition of feed such as stage of growth, species or variety, soil types and growth environment (Chumpawadee et al., 2007). The CP values reported for the vegetables are within the range reported for vegetables elsewhere (Marimo et al., 2010). The forages contained above 50% NDF shows that they have high proportions of soluble carbohydrate, which is suitable source for proper rumen function (Oni et al., 2008), but the NDF content in this experiment for WCL and RCL was relatively low. The variation in the chemical composition of WCL and RCL contents may also be attributed to specie differences used for the study. The DM content of cabbage is below 120 g/kg of DM, and cabbage has a low content of water soluble carbohydrates (Freyman et al., 1991).

There was too much moisture in the WCL and in RCL relatively, so high humidity makes it difficult to conserve normally. The high water content in beetroot was reported to make it difficult to handle the product and also to affect the moisture content of the diet containing beetroot (Cassida et al., 1994). It was reported that the high moisture content in cabbage and beetroot made it difficult for these wastes to be stored in normal feed storage sheds before drying (Nkosi, 2010) that is agreed with our results. Cassida et al. (1994) reported that the high moisture content of vegetable waste products limits dry matter intake of *Brassica oleracea* forage via the gut fill effect of intercellular water. Similarly, Garcia et al. (2005) reported that the high moisture content in beetroot may make its handling difficult and leading to microbial contamination. The nutritive value of *Brassica* species is affected by the high water content of 93.4 % in cabbage and 89.9 % in cauliflower as reported by Nguyen and Ledin, (2005) which also negatively affects dry matter intake. According the (Table 1), the CP content of WCL and RCL is relatively high, although the CP content of WCL was significantly higher and it seems the CP content of them is comparable with alfalfa plant. Nitrogen balance and feed conversion ratio was significantly better (The efficiency of protein intake) on animals fed on diets containing vegetable residue compared to diets containing berseem and oat hay fed groups of lactating goats. According to Khattab et al. (1999) there was a positive effect of feeding rations containing vegetable residue on total dry matter intake and they proved to be more palatable than oat hay and berseem hay. In another study an analysis of major and trace minerals showed a positive balance thereby revealing an efficient utilization of minerals when vegetable-residue-based rations were fed to growing and milking goats (Khan and Atreja, 2005). Studies done by Wadhwa et al. (2006) reported that cabbage leaves, pea pods and cauliflower leaves are good sources for CP and have low levels of NDF and lignin which this potential promote the higher voluntary intake. The NDF and ADF contents in this experiment were relatively lower rather than other forages (such as straw and so on). The results of researcher showed that the chemical composition, digestibility of nutrients and efficient utilization of cauliflower and cabbage leaves and pea pods can be excellent uncommon feedstuffs for ruminants, equivalent to any conventional green fodder like green oats (El-Khoderi et al., 2007). According to

Table 1. Chemical composition of red and white cabbage leaves.

	Sample		
Item(%)	**Red cabbage leaves[1]**	**White cabbage leaves[2]**	**SEM**
DM	15.42[a]	13.82[b]	0.23
CP	14.1[b]	16.2[a]	0.34
NDF	21.95[a]	18.82[b]	0.43
ADF	15.35[a]	14.01[b]	0.32
EE	3.8[a]	3.5[a]	0.19
Ash	11.5[a]	10.85[b]	0.18

[a, b] Means in rows with differing superscripts differ ($P < 0.05$). DM=dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; OM = organic matter; EE = ether extract.[1]*Brassica oleracea* var. *capitata;* [2]*Brassica oleracea* var. *botrytis.*

Table 2. *In vitro* gas production parameters.

	Sample		
Item	**Red cabbage leaves[1]**	**White cabbage leaves[2]**	**SEM**
B_{gas}	88.75^b	103.78^a	1.38
C_{gas}	0.059^a	0.058^a	0. 007
$IVGP_{24}$	70.17^b	81.05^a	1.01
$IVGP_{48}$	83.26^b	97.52^a	1.04

[a, b]Means in rows with differing superscripts differ (P < 0.05). B_{gas} = the gas production from the insoluble but fermentable fraction (ml) (ml/500 mg of DM); C_{gas} = rate of gas production (ml/h/500 mg of DM) for b; IVGP24 = cumulative gas production after 24 h incubation (ml); IVGP48 = cumulative gas production after 48 h incubation (ml).[1]*Brassica oleracea* var. *capitata*; [2]*Brassica oleracea* var. *botrytis*.

Table 3. Estimated parameters from *in vitro* gas production.

	Sample		
Item	**Red cabbage leaves[1]**	**White cabbage leaves[2]**	**SEM**
OMD(g/kg DM)	469.47^b	517.24^a	4.40
ME(MJ/kg DM)	6.82^b	7.54^a	0. 06
SCFA (mmol)	0.62^b	0.71^a	0.009
Microbial protein yield(g/kg OMD)	9.06^b	9.98^a	0.08

[a, b]Means in rows with differing superscripts differ (P < 0.05). OMD = organic matter digestibility; ME = metabolisable energy; SCFA=short chain fatty acid.[1]*Brassica oleracea* var. *capitata*; [2]*Brassica oleracea* var. *botrytis*.

report of Hartnell et al. (2005) beetroot and other sugar beets are limited in 14 animals feeding because of glyphosate, which is a broad–spectrum non–selective herbicide used for inhibition of unwanted weeds and grasses in agricultural landscapes, decreases phosphoglyceric acid activity in sugar beet and beetroot. Overall, according to chemical composition and nutritive value of WCL and RCL, it is necessary to be careful in consumption of them.

In vitro gas production and estimated parameters

In vitro gas production parameters are presented in table 2. The total gas produced (24, 48 and B_{gas}) was greater for WCL (respectively 81.05, 97.52 and 103.78 ml/500 mg DM) followed by RCL (respectively 70.17, 83.26 and 88.75 ml/500 mg DM). There were no significant differences between treatments for rate of cumulative gas production (C_{gas}).

In comparison with *in vitro* gas production parameters for other forages, the estimated parameters for WCL and RCL are relatively acceptable. Evaluation of nutritive value of some vegetable wastes (cabbage leaves, cauliflower leaves and pea pods) indicated that they could serve as source of nutrients for ruminants (Wadhwa et al., 2006). The cumulative gas production for cabbage waste products in times of 24, 48 and 92 h was reported 51.4, 57 and 60.2 ml/200 mg of DM, respectively (Tobias et al., 2010) and Akinfemi, (2012) reported the values of 34 and 44 ml/200 mg of DM for 24 and 48 h post incubation time. The rate of gas production (C_{gas}) was reported by Akinfemi, (2012) about 0.0257 ml/h/200 mg of DM that is lower than to this experiment. The volume of gas production after *in vitro* incubation has been reported to be closely related to digestibility of feed for ruminants (Mebrahtu and Tenaye, 1997). In the other hand, the gas volume can be considered a good reflection of substrate fermentation to volatile fatty acids and an estimate of potential digestibility in the rumen. So the high volume of gas observed for WCL and RCL suggested a higher nutrient digestibility of these samples compared to other studies. The higher gas volume seems to be a reflection of a higher proportion of carbohydrate available for fermentation (Getachew et al., 1998). Estimated parameters from *in vitro* gas production are presented in (Table 3). The OMD, ME, SCFA and microbial protein yield (respectively 469.47 g/kg of DM, 6.82 MJ/kg of DM, 0.62 mmol and 9.06 g/kg of OMD) was significantly higher for WCL than to RCL (P<0.05). The ME and OMD contents for cabbage waste products were reported 10.1 MJ/kg of DM and 79% respectively by Tobias et al. (2010) that it is more estimated than our results. The values of estimated for OMD, ME and SCFA were 64.99%, 7.92 MJ/kg of DM and 0.753 µmol respectively (Akinfemi, 2012). Estimated ME is beneficial in the developing countries where estimation by *in vivo* is expensive. There is a good correlation between ME parameter measured *in vivo* and predicted from 24 h *in vitro* gas production and chemical composition of feed (Menke and Steingass, 1988). The ME *in vitro* estimation is cheap, less time consuming and allows for handling of large samples of feedstuff at the same time. Higher production of gas and SCFA in WCL, maybe result of an increased proportion of acetate and butyrate but may mean a decrease in propionate production as reported by Babayemi et al., (2004). Since the yield of SCFA for WCL was better than RCL, that suggests a potential to make energy available to the ruminants.

Conclusion

With the increased demand for food by the increasing human population, it is necessary to seek alternative cheap and available energy sources for animal nutrition. The high moisture content of almost all of the waste forage, relative to the nutrient composition, makes them costly to transport, difficult to handle and highly perishable. Lack of information on their composition and nutritive values however, limits their use. According to *in vitro* studies, nutritive value of WCL was significantly better than RCL. Cabbage leaves can be a superior feed compared to grass for farmers and a suitable low-cost alternative to other forage. So it recommands that farmers must be trained on the potential use of the vegetable waste products as feed ingredients and the different technologies that they can use. Also more research on the processing, harvesting time and use of vegetable waste products (such a cabbage leaves) should be done to support both the farmers and the retailers. Also *Brassicaceae* family such as cabbage have an anti-nutritional factors such as glucosinolates and S-methyl cysteine sulfoxide can affect animal thyroid gland function and reduce average weight gains in cattle and sheep, so the farmers should use the cabbage in the diet with caution.

ACKNOWLEDGEMENT

The author wishes to thank the Torbat-e jam Faculty of Agriculture and Animal Science for financial support of this research. We thank to ferdowsi university of mashhad farming for their sincere cooperation. We are grateful to Mr. Behzad Fahmideh for laboratory services.

AUTHOR`S DECLARATION

We declare that this study is an original research by our research team and we agree to publish it in the Journal.

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