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Full Length Research Paper

Effect of moist heat on *in vitro* gas production parameters of some of legume seeds

Jamal Seifdavati, Akbar Taghizadeh[1]

Department of Animal Science, Faculty of Agriculture, University of Tabriz, Iran

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Effect of moist heat autoclave at 127°C with a steam pressure of 117 k Pa for 20 min on *in vitro* gas production parameters of common vetch (CV), chickling vetch (CLV) and bitter vetch (BV) seeds were evaluated. Gas production volumes were recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation. The gas production data was fitted using $Y = A (1 – e^{-ct})$. This experiment was carried out according to a completely randomized design and the data were analyzed using factorial method and the Mixed Model procedure. The obtained data at 24 h after incubation were used for estimation of metabolizable energy (ME), net energy for lactation (NEL), short chain fatty acids (SCFA), digestible organic matter (DOM) and microbial protein (MP). Moist heat decreased (P<0.05) gas production of CV seed after 24 h, but the differences between CLV and BV seeds were not significant. Heating process resulted decreasing in gas production of CV seed after 6 h. The rate of gas production were for CLV (0.052/ h), CV (0.053/ h) and BV seed (0.063/ h) that decreased by moist heat autoclave for CV and BV seeds (P<0.05). Moist heat decreased (P<0.05) gas production of soluble and insoluble fraction (A) of CV seed, but did not affect on CLV and BV seeds. Moist heat of CV seed significantly decreased (P<0.05) values of ME, NEL, SCFA, DOM and MP, but did not affect significantly mentioned parameters in CLV and BV seeds. The rate of gas production of CV seed can be decreased by moist heat autoclave resulting improved rumen ecosystem and decreased risk of health problems due to decreasing of rate of fermentation. The similarity of gas production between CLV and BV seeds indicated the similar fermentation kinetics of their carbohydrate contents.

Keywords: Autoclaving, Bitter vetch, Chickling vetch, Common vetch seed, Gas production

INTRODUCTION

Local protein sources are likely higher degradability in the rumen due to 85–100% of protein is in albumins (water soluble) and globulins (salt soluble), none in prolamines (alcohol soluble) and 0–15% in glutelins (dilute alkali soluble) (McDonald, 1988; Van Straalen,1990). Therefore, the use of legume seeds in ruminant nutrition, particularly in high-producing, is limited and the utilization is inefficient under certain conditions. In addition, legume seeds contain anti-nutritional compounds that depending upon seed and phonological conditions have different effects on livestock body (Yu, 2002). Heat treatments of legume seeds can inactivate ANFs (Van Der Poel, 1991), it also effective in reducing the solubility of the protein in the rumen and increasing the amounts of protein entering the small intestine for absorption and digestion. Moist heat treatment (autoclaving) is a process where feeds are heated with steam under pressure. Several workers have found that moist heat treatment of protein meals such as seeds of pea, lupin, field bean, vetch, bitter vetch, canola and mustard meal reduces ruminal protein degradability and increases the concentrations of amino acids available for intestinal digestion (Mustafa, 1999; Aguilera, 1992).The effect of moist heat treatment (autoclaving) on ruminal degradation of protein of feedstuffs have been intensively studied, mostly *in situ*. This technique fractionates feed material into a washable fraction, a potentially degradable fraction and an undegradable fraction (Michalet-Doreau, 1992). Using an appropriate fractionation method to separate the washable fraction into an insoluble washable fraction and a soluble washable fraction along with an *in vitro* gas production technique is considered an appropriate method to characterize the degradative behaviour of the washable fraction (Azarfar, 2007). So

*Corresponding Author email: ataghius@yahoo.com
Tell: +98-4113392029 Fax: +98-4113356004.

that heat processing induce diminish the adverse effects of anti-nutritional factors in legume seeds fermentation and gas production in the closed space, and also heat processing make decrease soluble protein in washable fraction of feeds and accordingly make smaller ammonia nitrogen. The *in vitro* gas method is more efficient than the *in situ* method in evaluating the effects of tannins or other anti-nutritive factors. In the *in situ* method these factors are diluted in the rumen after getting released from the nylon bag and therefore do not affect rumen fermentation appreciably. In addition, the in vitro gas method can better monitor nutrient-antinutrient and antinutrient-antinutrient interactions (Makkar, 1995), Abreu et al (1998), Hanbury et al (2000) found that main anti-nutritional factors in CLV seed are ß-N-oxalyl-L-a, ß-diaminopropionoc acid (ODAP). As well as, Aguilera et al (1992) autoclaving decreased both the soluble fraction and the fractional rate of protein degradation of the slowly degraded fraction. Abreu et al (1998) showed gas production at 48 h CV seed was 119.9 ml/g DM. Therefore, they found that gas production in itself was not enough to assess OMD of legume seeds. Also the presence of ANFs substances may affect the OMD of these feeds. Razmazar et al (2009) showed the gas yield after 24 h incubation was 59.20 ml/200 mg of sample. The potential gas production obtained 72.17 ml the ME, OMD and SCFA CV seed were estimated 10.35 MJ/kg, 67.68%, and 1.313 m mol. The *in vitro* gas production system helps to better quantity the nutrient utilization and its accuracy in describing digestibility in animal has been validated in numerous experiments (Besharati, 2009). Although, gases produced during rumen fermentation are colossal waste products and of no nutritive value to the ruminant, but gas production technique are used routinely in feed research as gas volumes are related to both the extent and rate of substrates degradation (Blummel, 1997a). This quick and cheaper method have been used to evaluate feed resources before making them available to livestock (Larbi et al., 1998) and suitable for use in developing countries. There is a lack of information regarding the effect of heat treatment on *in vitro* gas production kinetics of protein sources. This research is done in order to investigate the effects of moist heat treatment (autoclaving) on protein composition and potential of *in vitro* gas production of bitter vetch (*Vicia ervilia*), common vetch (*Vicia sativa*) and chickling vetch (*Lathyrus sativus*) and to determine the nutritive value of these legume seeds with using data obtained from gas production technique.

MATERIALS AND METHODS

Legumes

Seeds of commercially available legume, BV, CV, and CLV were used and two or more districts each from the legume seeds producing cities of Ardabil province (Meshgin, Razei, Germi and Ardabil) in northwest Iran were randomly selected for the survey. Then, two peasant associations each were randomly selected from the identified districts. A systematic sampling choosing every second farmer was done in each of the selected peasant associations until total fifteen farmers were selected for the study which brings the number of farmers selected to thirty in every district. Seeds were autoclaved in 48×32×30cm^3 metal pans using a Consolidated Stills and Sterilizers (Boston, MA, USA) autoclave, with five times their weight of distilled water at 127°C with a steam pressure of 117 k Pa for 20 min (about 50% of the seeds were soft when felt between fingers). The heated materials were allowed to air cool for 1 h and then sealed in plastic bags. Subsamples of raw and heated legume seeds were grounded through a 1mm screen and defatting was done by extraction with petroleum ether for 6 h according to the AOAC procedure (AOAC, 2000). The defatted samples were then analysed for dry matter (DM), ash, crude protein (CP) (Kjeldahl N × 6.25) and acid detergent fibre(ADF), neutral detergent fibre (NDF) was determined without the use of sodium sulphite as described by Van Soest et al(1991) using the Ankom NDF/ADF fiber system (Ankom,2008). neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN), determined by measuring nitrogen contents of NDF and ADF, described above.

In Vitro Gas Production Trial

The DM degradability of raw and heated legume seeds was determined by *in vitro* fermentation with ruminal fluid. Ruminal fluid was collected approximately 2 h after morning feeding from 2 cannulated sheep consuming 400 g alfalfa hay, 200 g barley, and 200 g soybean meal. Ruminal fluid was immediately squeezed through 4 layers of cheesecloth and was transported to the laboratory in a sealed thermos. The resulting ruminal fluid was purged with deoxygenated CO_2 before use as the inoculum. Gas production was measured by Fedorak and Hurdy (1983) method. Approximately, 300 mg of dried and ground (2mm) raw and heated legume seeds samples were weighed and placed into serum bottles. Buffered rumen fluid with McDougall's buffer (20ml) was pipetted into each serum bottle (McDougall 1948). The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml /g DM. The gas production profiles in triplicate fitted in equation 1:

$$Y = A (1 - e^{-ct}) \quad (1)$$

Where: Y is the volume of gas production (ml/g DM) at time t, A is gas production from soluble and insoluble fraction, c is the gas production rate, and t is the incubation time (h). The ME contents of GP and DOM

Table 1. Chemical composition and in vitro digestibility of different legume seeds. (g / kg DM)

Item	Chickling Vetch	Common vetch	Bitter vetch	p-value	Root MSE	p-value		
						Feed	process	Feed×process
Dry matter (g/kg fresh matter)								
Non autoclaved	907^{A}	906^{A}	914^{A}	0.219	0.533	0.706	<0.001	0.234
Autoclaved	881^{B}	888^{B}	880^{B}	0.556	0.887			
Organic matter								
Non autoclaved	935^{a}	953^{a}	907^{b}	<0.001	0.321	<0.001	0.170	0.977
Autoclaved	939^{a}	959^{a}	911^{b}	<0.001	0.912			
Ether extract								
Non autoclaved	17.6	23.1	24.2	0.318	0.519	0.104	0.723	0.812
Autoclaved	17.9	26.5	23.5	0.288	0.609			
Crude protein								
Non autoclaved	259^{bA}	278^{aA}	255^{b}	<0.001	0.192	<0.001	<0.001	<0.006
Autoclaved	243^{bB}	267^{aB}	250^{b}	<0.001	0.142			
Neutral detergent fibre								
Non autoclaved	337	349	315	0.088	1.554	0.201	0.974	0.198
Autoclaved	325	339	336	0.646	1.819			
Acid detergent fibre								
Non autoclaved	61^{B}	52	62^{B}	0.148	0.608	0.001	0.008	0.055
Autoclaved	78^{aA}	52^{b}	67^{aA}	<0.001	0.401			
ADIN (%CP)								
Non autoclaved	8.9^{a}	8.2^{bB}	4.2^{c}	<0.001	0.183	<0.001	<0.001	<0.001
Autoclaved	7.5^{b}	12.0^{aA}	3.1^{c}	<0.001	0.072			
Gross energy (MJ/kg DM)								
Non autoclaved	17.1^{a}	17.4^{a}	16.8^{b}	0.027	0.197	<0.001	<0.001	<0.001
Autoclaved	17.1^{b}	17.8^{a}	17.4^{ab}	0.006	0.152			

Different lowercase letters within a same row indicate significant differences among feedstuffs ($p < 0.05$).
Different uppercase letters within a same column indicate significant differences ($p < 0.05$) because of autoclaving.

were calculated according to equations of Menke et al (1979) that are shown in equation 2 and 3 respectively:

ME (MJ/kg DM) = 1.06+0.1570 GP + 0.0084 CP+ 0.0220 EE – 0.0081 XA (2)

DOM, g/100 g DM = 9.00+0.9991 GP + 0.0595 CP+0.0181 XA (3)

Where: DOM = OM digestibility (g/100 g DM), XA = ash in g/100 g DM, and Gv = the net gas production (ml) at 24 h.

The SCFA and NE_L were calculated using equation 4 and 5.

SCFA, m mol = –0.00425 + 0.0222 Gv (4)

NE_L (MJ/kg DM) = -0.36 +0.1149 GP+ 0.0054CP + 0.0139 EE – 0.0054 XA (5)

Where: gas is 24 h net gas production (ml/g DM), CP is crude protein (%DM), and EE is crude fat (%DM).

As well as, the microbial protein (MP) was calculated according to Czerkawski (1986) formula that is shown in equation 6.

MP (g/kg DOM) =19.3 × (DOM) (6)

Statistical analysis

Data obtained from this study was subjected to ANOVA as a completely randomized design with 3 replicates by the GLM procedure (SAS, 2001), and treatment means were compared by the Duncan test.

RESULTS AND DISCUSSION

Chemical Composition

Table 1 shows the chemical composition and GE content of seeds of CLV, CV and BV before and after autoclaving. The legume seeds on tested showed high protein content (from 255 to 296 g/kg DM). Legumes generally contain low fat contents in the range of 1 - 2% with the exception of chickpea- 6.7% (Costa, 2006); soybean- 21% and peanut- 49% (Augustine, 1989). The EE of legume seeds on tested was within the range of (1.76–2.65 % DM). The chemical composition of the legume seeds experienced indicated that CLV is different to CV and BV, belonging to the same genus (*Vicia*). One of the most relevant characteristics of the legumes tested is their high protein content, as reported by others (Abreu, 1998; Gonzalez, 2003; Sadeghi, 2004/ 2009; Haddad, 2006; Ramos Morales, 2008; Rezayazdi, 2008; Razmazar, 2009; Abdullah, 2010),

which could make them adequate as protein supplement in animal nutrition for alternating with soybean meal.

CP of legume seeds in this study agree with by above mentioned researchers. The studied legume seeds showed values of CP and EE within the range of values observed by others (Goelema, 1998; Yu, 2005b). The NDF values found in this experiment were similar to those found by Hadjipanayiotou et al (1985); Abreu (1998); Goelema et al (1998); Amini et al (2001); Yu (2005b); Razmazar et al (2009) and higher than those reported by other authors Aletor et al (1994); Farhangi (1996), Abdullah et al (2010). In general, the differences of chemical composition of legume seeds has been discussed in thought of review articles, could be explained because of a higher content of kernel in the samples, where the fibre components are located (Van der Poel , 1991) and as well, differences between cultivars and associated with growth, harvest, processing and storage conditions (Dixon,1992).

Autoclaving affected the chemical composition of the protein supplements, the CP content of legume seeds were lost on autoclaving, which decreased from 6%, 4% and 2% for CLV, CV and BV, respectively, but ADF (Table 1), which increased 27% in CLV and 8% in BV ($P<0.001$). The losses in protein could be attributed to partial removal of certain amino acids, along with the leaching other soluble nitrogenous compounds and non-fibre material during the autoclaving procedure into the autoclave medium, on heating as has previously been reported by other authors (Clawson, 1993; Cros, 1992; Monica, 1992; Eugene, 2002; Zia-ur Rehman, 2005; Osman, 2007). The lowest ADIN values corresponded to BV but CV was highest ($p<0.001$) with autoclaving. These results suggest that the heat inputs used in the present study were not severe enough to damage the CLV and BV seed protein. It has also been found that the sensitivity of the variety or genus to heat was different and therefore the tissue inherent protein structure changes were different (Yu, 2007).However, heating (autoclaving) increased ADIN by 46% for CV.

In Vitro Digestibility

Cumulative gas volumes at 2, 6, 12, 24, 48 and 72 h after incubation are shown in Table 2. The result showed that cumulative gas volume at 2, 6, 12, 24, 48 and 72 h after incubation and Gas production characteristics(A and c) and estimated lag time differed significantly ($P<0.05$). Local protein sources are likely higher degradability in the rumen due to the nature of their leguminous like other legume seeds. This reason is important that the lag time of CV seed compared with CLV seed and BV seed is low. The lag phase is the required time for digestion of both the washable fraction into an insoluble washable fraction and a soluble washable fraction. Then, the feed by microorganisms have been identified and will begin to degradation. BV seed fermented faster and CLV seed and CV seed fermented slower in test feeds. CLV and BV seed had highest gas production volume; the reason of more gas production volume in both may be caused by present of low level of protein compared to CV. Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while contribution of fat to gas production is negligible (Wolin, 1960). The strong correlation between extent of gas production and chemical composition and the poor correlation between rate of gas production and chemical composition is consistent with Nsahlai et al (1994). Getachew et al (2004) reported that feed CP level was negatively correlated with gas production.

The moist heat of the autoclave affected the gas volume in the next 6 hours in CV seed and only in the next 12 hours in BV seed and lead to reduce the volume of gas ($P<0.05$).The A parameter of CV seed reduced by moist heat treatment ($P<0.05$). However, the other test feed have shown no effect of this parameter of gas production.

The rate of gas production CV and BV seeds reduced by moist heat treatment ($P<0.05$) which diminished from 0.053 to 0.041/ hour and from 0.062 to 0.053/hour in CV and BV seeds, respectively. The volume and rate of gas production CLV did not affect of moist heat treatment. First choice physical treatment is heat processing feeds. An objective of heat processing feeds is done in an effort to manipulate the digestive behaviour of the macronutrients of feed. For protein, in terms of ruminant nutrition, the objective of heat treating a feed is to increase the amount of dietary bypass crude protein or in other words the quantity of RUP, without negatively affecting the digestibility of the protein in terms of the whole gastrointestinal tract (Yu, 2002). As well as, heat processing cause a balance between feed protein breakdown and microbial protein synthesis, resulting reduce in unnecessary N loss from the rumen. Heat processing make also decrease soluble protein in washable fraction of feeds and accordingly make smaller ammonia nitrogen and lead to reduce degradation of structural protein to volatile fatty acids (VFA) such as acetic and valeric acids. Likewise, heat processing induce diminish the adverse effects of anti-nutritional factors in legume seeds fermentation and gas production in the closed space (Getachew, 2004).

The differences between result from this study and other reported values could be explained by the relative concentrations of different proteins and methodological difference. Among legume seeds, CV seed was seen highly damage regarding to smaller seed size and core that due to increased ADIN by 46% in it and non-enzymatic browning (Maillard reaction) between the reducing sugars from starch hydrolysis and the proteins as well as the thermal cross linking that occurred during heating as shown on table1. Heat treatment reduces the accessibility of the substrate and forms linkages resistant to enzyme attack, and will result indenaturation

Table 2. Gas volume and in vitro gas production characteristics of legume seeds

Item	Chickling Vetch	Common vetch	Bitter vetch	p-value	Root MSE	p-value		
						Feed	process	Feed× process
Gv2 (mL /g DM)								
Non autoclaved	23.23^{a}	19.68^{b}	23.78^{a}	0.003	1.72	0.022	0.024	0.68
Autoclaved	18.23	17.90	21.67	0.28	4.34			
Gv6 (mL /g DM)								
Non autoclaved	81.46^{b}	75.79^{bA}	93.33^{a}	<0.001	4.97	<0.001	<0.001	0.005
Autoclaved	74.35^{a}	47.82^{bB}	80.90^{a}	<0.001	9.27			
Gv12 (mL /g DM)								
Non autoclaved	153.43^{b}	138.89^{cA}	170.41^{aA}	<0.001	9.88	<0.001	<0.001	0.11
Autoclaved	142.99^{a}	106.59^{bB}	148.44^{aB}	<0.001	14.39			
Gv24 (mL /g DM)								
Non autoclaved	246.29^{a}	207. 33^{bA}	246.29^{a}	<0.001	12.24	<0.001	0.003	0.072
Autoclaved	239.08^{a}	161.93^{bB}	232.08^{a}	<0.001	26.68			
Gv48 (mL /g DM)								
Non autoclaved	307.72^{a}	261.09^{b}	295.39^{a}	<0.001	12.09	<0.001	0.114	0.392
Autoclaved	304.83^{a}	237.45^{b}	289.62^{a}	<0.001	25.28			
Gv72 (mL /g DM)								
Non autoclaved	332.51^{a}	282.34^{b}	321.86^{a}	<0.001	13.25	<0.001	0.114	0.526
Autoclaved	326.96^{a}	258.36^{b}	315.31^{a}	<0.001	28.40			
A (mL /g DM)								
Non autoclaved	343.39^{a}	291.02^{cA}	322.98^{b}	<0.001	5.15	<0.001	0.002	0.083
Autoclaved	337.80^{a}	279.35^{cB}	321.52^{b}	<0.001	5.65			
Lag time (hour)								
Non autoclaved	0.85^{aB}	0.59^{cB}	0.73^{b}	<0.001	0.034	<0.001	<0.001	<0.001
Autoclaved	1.24^{aA}	1.05^{bA}	0.76^{c}	<0.001	0.097			
c (hour)								
Non autoclaved	0.052^{b}	0.053^{bA}	0.062^{aA}	<0.001	0.004	<0.001	<0.001	0.002
Autoclaved	0.052^{a}	0.041^{bB}	0.053^{aB}	<0.001	0.003			

Different lowercase letters within a same row indicate significant differences among feedstuffs ($p < 0.05$).
Different uppercase letters within a same column indicate significant differences ($p < 0.05$) because of autoclaving.
A = Potential gas production (mL /g DM), c = Rate constant of gas production during incubation (mL /h), Gv = Gas volume during incubation hours.

of protein and probably transform the proteins to a more resistant structure (Van Soest, 1994). ADIN is considered a marker for heat damage and the Maillard reaction (Van Soest and Mason, 1991).On the other hand, ANFs of CV seed did not inhibit exactly as compared to BV and CLV seeds and leads to a decrease in gas volume. Farhangi (1996) incubated CLV seed in sheep rumen fluid and reported a rapid disappearance of ODAP (>90% in 4 h), supporting the idea that certain rumen microorganisms can destroy the toxin. In a separate sample autoclaving for 2 h decreased the concentration of ODAP from 0.24 to 0.11%. Aguilera et al (1992) showed autoclaved meals had lower protein degradability than non-heated meals, the extreme values being those of BV and pea seeds (78-72% and 69-62%, respectively), and found that autoclaving decreased degradation of DM and protein non-heated meals (BV and CV) in 48 h of rumen incubation 6.2%, 4.8 and 11.4,16.6 per cent respectively.

ME, NE_L, SCFA, DOM and MP of the feedstuffs are shown in Table 3. The values for the ME, NE_L, DOM, SCFA and MP ranged from in 7.82 CV to 8.99 in CLV, 4.56 in CV to 5.42 in CLV,0.92 in CV to 1.09 in BV,52.17 in CV to 59.89 in BV and 62.93 in CV to 72.25 in BV, respectively. Low determination of CV metabolizable energy can be resulted from its low rate of gas production and extent of gas production at 24 h. For gas volume and in vitro gas production characteristics, (Menke, 1988) suggested that gas volume at 24h after incubation is an indirect relationship with ME in feedstuffs. Gas production can be regarded as an indicator of carbohydrates degradation; (Steingass, 1986) suggested that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the in vitro system. Gas production is basically the result of

Table 3. Gas production estimated parameters of legume seeds

Item	Chickling Vetch	Common vetch	Bitter vetch	p-value	Root MSE	p-value		
						Feed	process	Feed×process
ME								
Non autoclaved	8.99^{a}	7.82^{Ab}	8.98^{a}	<.001	0.384	<.001	0.003	0.075
Autoclaved	8.76^{a}	6.40^{Ba}	8.54^{a}	<.001	0.840			
NE_L								
Non autoclaved	5.42^{a}	4.56^{Ab}	5.42^{a}	<.001	0.281	<.001	0.003	0.074
Autoclaved	5.26^{a}	3.52^{Ba}	5.09^{a}	<.001	0.610			
SCFA								
Non autoclaved	1.08^{a}	0.92^{Ab}	1.09^{a}	<.001	2.44	<.001	0.003	0.071
Autoclaved	1.06^{a}	0.72^{Ba}	1.03^{a}	<.001	0.12			
DOM								
Non autoclaved	59.87^{a}	52.17^{Ab}	59.89^{a}	<.001	0.054	<.001	0.002	0.072
Autoclaved	58.33^{a}	43.02^{Ba}	57.07^{a}	<.001	5.33			
MP								
Non autoclaved	72.22^{a}	62.93^{Ab}	72.25^{a}	<.001	2.95	<.001	0.003	0.073
Autoclaved	70.36^{a}	51.89^{Ba}	68.79^{a}	<.001	6.43			

Different lowercase letters within a same row indicate significant differences among feedstuffs (P< 0.05).
Different uppercase letters within a same column indicate significant differences (P< 0.05) because of autoclaving.
ME = Metabolizable energy (MJ/kg DM), NE_L = Net energy lactation, SCFA = Short chain fatty acid (mmoL), DOM = digestible Organic matter (%), MP = Microbial protein g/kg DOM

fermentation of carbohydrates to acetate, propionate and butyrate (Steingass, 1986) and substantial changes in carbohydrates fractions were reflected by total gas produced.

The high non-fiber carbohydrate content of CV leads to proportionally higher propionate production, thereby reducing the acetate to propionate ratio (Getachew, 2004). Highly significant correlation has been observed between SCFA and gas production (Beuvink, 1992). The molar proportions of different SCFA (acetate, propionate and butyrate) produced is dependent on the type of substrate (Beuvink, 1992). The high CP content of CV and its degradation leads to a proportionally smaller amount of SCFA. The extent of SCFA production from proteins is dependent upon on the amino acid composition of the feeds and the extent of rumen deamination of these amino acids. The carbon skeleton arising from deamination gives rise to a variety of VFA. For example, fermentation of glycine can lead to ammonia and acetic acid without the release of CO_2 and that of leucine, isoleucine and valine to isovaleric acid, 2-methyl butyric acid and isobutyric acids, respectively (Besharati, 2008).

In vitro gas production is a rapid, simple and less time consuming method, and mostly correlated with in vitro digestibility (IVD). Therefore, gas production method has been successfully used to evaluate the DM degradability, DOM or ME of feedstuff (Lee, 2000). In contrast with these points of view, according to Cone et al (1999) study because of accumulation of gas in syringe or serum bottle, the in vitro gas production is not a suitable method to estimate ME of rich protein sources, particularly in legume seeds contain ANFs. So another method is recommending for estimation of ME in legume seeds.

CONCLUSION

There were significant differences among the legume seeds in terms of chemical composition. The differences in chemical composition of legume seeds resulted in the differences in the in vitro gas production, gas production kinetics and the estimated parameters such as ME, DOM and MP. Moist heat of common vetch seed significantly decreased values of ME, NE_L, SCFA, DOM and MP, but did not affect significantly mentioned parameters in chickling vetch and bitter vetch seeds. The rate of gas production of common vetch seed can be decreased by moist heat autoclave resulting improved rumen ecosystem and decreased risk of health problems due to decreasing of rate of fermentation. The similarity of gas production between chickling vetch and bitter vetch seeds indicated the same effect of rumen environment for both.

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