EVALUATING NUTRITIVE VALUE OF POULTRY BY-PRODUCT MEAL USING *IN SITU* AND *IN VITRO* GAS PRODUCTION TECHNIQUES

Abolfazl Aghajanzadeh-Golshani, *Naser Maheri-Sis, Ramin Salamat Doust-Nobar, Yahya Ebrahimnezhad and Abolfazl Ghorbani

Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran
* Corresponding Author Email: nama1349@gmail.com

ABSTRACT: This experiment was designed to determine the chemical composition, rumen degradation kinetics using *in situ* method and fermentation characteristics and energetic value of poultry by-product meal (PBM) by *in vitro* gas production technique. In the *in situ* experiment, three ruminally cannulated Ghezel rams were used to determine degradation characteristics of dry matter (DM), organic matter (OM) and crude protein (CP) of PBM. Nylon bags were filled with 3 g dried and ground samples and then incubated in the rumen of rams for the periods of 0, 2, 4, 8, 12, 16, 24, 36 and 48 h. After the removal of bags from the rumen, bags were washed and dried at 60°C for 48 h and then remaining residues were analyzed and calculated for DM, OM and CP degradability's. In the *in vitro* experiment, rumen fluid was obtained from three cannulated rams. Approximately 200 mg samples (as DM basis) were placed into syringes contain rumen fluid-buffer and then incubated at 2, 4, 6, 8, 12, 16, 24, 36, 48, 60 and 72 hours. The results showed that DM, OM, CP and ether extract (EE) content of PBM was 87.64, 81.49, 53.71 and 22.35%, respectively. Effective degradability (at 5% out flow rate) for DM, OM and CP of PBM were 49.93, 52.10 and 49.73%, respectively. Gas production volume at 24 h incubation, estimated OM digestibility, short chain fatty acids (SCFA), metabolizable energy (ME) and net energy for lactation (NE_L) contents of PBM were 8.83 ml/200mg DM, 53.13%, 0.192 mmol, 10.38 MJ/kg DM and 5.69 MJ/kg DM, respectively. In conclusion about half of the PBM is degradable in the rumen and remaining escaped into intestine. Thus it may provide sufficient protein to maintain microbial protein synthesis in the rumen as well as un-degradable protein supplies amino acids to the small intestine.

Keywords: poultry by-product meal, *in situ*, gas production, metabolizable energy, nutritive value

INTRODUCTION

Poultry by-product meal (PBM) is one of the important sources of animal protein that can be used in animal nutrition [1]. It is a by-product of the poultry industry consisting of the ground, dry-rendered parts of the slaughtered poultry such as heads, feet, intestines, blood and feathers [2]. This by-product is a palatable and high quality feed ingredient due to higher content of essential amino acids, fatty acids, vitamins, and minerals [1]. Ewing [3] stated that it may be used 5 to 7.5 % PBM in ruminant diets. Sahraei *et al* [4] reviewed that PBM can be used as a protein source in diets of pig, poultry and as a rumen undergradable protein (RUP) source in ruminant rations. Bohnert *et al* [5] also stated that supplementing RUP in ruminant diets can increase the flow of protein to the small intestine and lead to improved growth and efficiency of nitrogen utilization. They are suggested that PBM is an alternative source of supplemental protein in formulation of diets for ruminant animals and may be a protein source capable of supplying RUP. Feeding trials with steers has indicated that PBM can be effectively utilized as a source of supplemental nitrogen. However, it is notable that RUP content of PBM is higher than that of soybean meal [6] and rapeseed meal [7] and lower than that of meat and bone meal [8], feather meal [9] and blood meal [10].

There is a higher variation in chemical composition and subsequently nutritive value of PBM in different studies, due to different raw materials and techniques used for its processing [11, 12]. Crude protein, ether extract (EE) and ash content of PBM in various researches varied between 52.12-69.63, 13.8-25.18 and 7.83-18.34%, respectively [4, 6, 13, 14, 15]. Therefore, the tabulated values related to nutritive value of PBM may not be accurate when used under different conditions and make the nutritionist to re-evaluate the nutritive value of PBM in order to improving the accuracy of formulation of animal diets [11].

The rate and extent of degradation of dry matter (DM), organic matter (OM) and crude protein (CP) in the rumen are very important tools to evaluate the nutritional value of feeds for ruminants. The nylon bag technique has been used for many years to provide *in situ* degradability of DM, OM and CP in feedstuffs [16, 17]. Alternatively, Menke *et al* [18] developed the *in vitro* gas production technique to evaluate the nutritive value of feedstuffs using fermentation characteristics for ruminant animals. Gas production technique is an economic method and therefore preferred for developing countries. This method also can predict OM digestibility, microbial nitrogen supply, short chain fatty acids production, metabolizable energy and net energy for lactation (NE_L) of feedstuffs [19, 20, 21].

Since, there is a little information available regarding the nutritional value, degradation kinetics and fermentation parameters of PBM, this experiment conducted to determine chemical composition and estimating nutritive value of PBM produced in Iran, using *in situ* and *in vitro* gas production techniques.

MATERIALS AND METHODS
Chemical Analysis
Poultry by-product meal sample was supplied from Nirosahand Animal Feed Processing Unit, Tabriz, East Azerbaijan province, Iran. The samples were milled through a 1 mm sieve for chemical analysis and *in vitro* gas production procedure and 3 mm for nylon bag method. Dry matter was determined by drying the samples at 105°C overnight and ash content by igniting the samples in muffle furnace at 525°C for 8 h. Ether extract (EE) and nitrogen (N) content of the samples were determined by soxhlet extraction and Kjeldahl method, respectively [22]. Crude protein (CP) was calculated as N*6.25.

IN SITU DEGRADATION PROCEDURES

Three ruminally cannulated Ghezel rams (about 55 kg BW) were used to determine *in situ* degradation characteristics. Rams were housed in individual stalls bedded with stubble. Rams fed diets containing alfalfa hay (65%) and concentrate mixture (35%) at the maintenance levels [27]. Diet offered to the animals twice daily at 08.00 and 16.00 in equal sized meals. The animals had freely access to fresh water. Dacron bags (14*7 cm; about 50 micron pore size) were filled with 3 g dried and ground samples and then incubated in the rumen of rams for the periods of 0, 2, 4, 8, 12, 16, 24, 36 and 48 h. After the removal of bags from the rumen, bags were automatically washed in cold tap water until rinse were clear and dried at 60°C for 48 h [17, 23].

Remaining residues were analyzed for OM and CP concentrations. Rumen degradation kinetics of DM, OM and CP were calculated by the equations described by Ørskov and McDonald [17] using FITCURVE software version 6 [24]:

$$P = a + b (1-e^{-ct})$$
$$ED = a + (b*c)/(c+k)$$

Where:

P   =   Percentage of degradability for response variables at t.

ED   =   Effective degradability for response variables (%)

a   =   Highly soluble and readily degradable fraction (%)

b   =   Insoluble and slowly degradable fraction (%)

c   =   Rate constant for degradation of the fraction b (/h)

e   =   2.7182 (Natural logarithm base)

t   =   Time relative to incubation (h)

k   =   Rate constant of passage(/h)

When calculating effective degradability, rate constant of passage was assumed to be 0.02, 0.05 and 0.08 per hour [24] so that the results could be extrapolated to other ruminants that differ in rumen capacity.

IN VITRO GAS PRODUCTION
Rumen fluid was obtained from three fistulated rams before the morning feeding. The samples were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass [25]. Approximately 200 mg samples (as DM basis) were weighed and placed into a 100 ml calibrated glass syringe.

The syringes were pre-warmed at 39°C before the injection of 30 ml rumen fluid-buffer mixture 1:2 (v/v) into each syringe followed by incubation in a water bath at 39°C. The samples were incubated in triplicate. Three syringes with only buffered rumen fluid (as blank) were incubated for each run. Amounts of gas production were recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48, 60 and 72 hours after incubation and then net gas production were computed. Data from cumulative gas production were fitted to the equation of Ørskov and McDonald [17] and gas production characteristics estimated by the FITCURVE software version 6 [24]:

$Y = a + b\ (1 - e^{-ct})$

Where:

Y	=	The gas produced at time t
a	=	The gas production from the immediately soluble fraction (ml)
b	=	The gas production from the insoluble fraction (ml)
c	=	The gas production rate constant for the insoluble fraction (/h)
a + b	=	Potential gas production (ml)
t	=	Incubation time (h)

The short chain fatty acids (SCFA), digestible organic matter (DOM), net energy for lactation (NE_L) and metabolizable energy (ME) values in experimental feed were calculated using equations as below:

SCFA (mmol) = 0.0222 Gas – 0.00425   [26]
DOM % = 0.9991 Gas + 0. 595 CP + 0.181 CA + 9   [25]
ME (MJ/kg DM) = 0.157Gas + 0.084 CP + 0.22 EE – 0.081 CA + 1.06 [25]
NE_L (MJ/kg DM) = 0.115Gas + 0.054 CP + 0. 14 EE - 0.054 CA - 0.36 [25]
Where:
Gas is gas production at 24 hours incubation (ml/200 mg DM), CP, EE, CA are crude protein, ether extract, crude ash (% DM), respectively.

RESULTS AND DISCUSSION

Chemical composition of PBM is presented in Table 1.

Table1: Chemical composition of poultry by-product meal (PBM) on dry matter basis (%

DM	OM	CP	EE
87.64	81.49	53.71	22.35

DM: dry matter, OM: organic matter, CP: crude protein, EE: ether extract

The CP contents of PBM is similar to those reported by Emre *et al* ([13]; 52.12%) and Marghazani *et al* ([15]; 54.45%) and lower than that of reported by Ewing ([3]; 58%), Sahraei *et al* ([4]; 69.63%), Janmohammadi *et al* ([14]; 62.12%) and NRC ([27]; 62%). The EE content of PBM is in range of values obtained by Emre *et al* ([13]; 23.47%) and Marghazani *et al* ([15]; 20.37%) and lower than that of reported by Janmohammadi *et al* ([14]; 25.18%) and higher than obtained by Ewing ([3]; 18%), Sahraei *et al* ([4]; 16.53%), Kamalak *et al* ([6]; 13.8%) and NRC ([27]; 14.5%).

Table 2: Ruminal dry matter (DM), organic matter (OM) and crude protein (CP) degradation of poultry by-product meal (PBM) at different incubation times

Incubation time (h)	DM disappearance (%)	OM disappearance (%)	CP disappearance (%)
0	26.61	25.69	33.36
2	41.25	40.52	40.59
4	42.61	44.62	42.66
8	47.32	50.03	45.91
12	49.09	50.75	49.43
16	51.45	54.39	50.86
24	55.50	58.33	56.33
36	58.10	60.98	ND
48	60.93	65.02	ND

ND: Not determined

The ash content of PBM (18.51) is in line with findings of Emre *et al* ([13]; 18.34%) but higher than reported by Ewing ([3]; 10%), Sahraei *et al* ([4]; 7.86%), Marghazani *et al* ([15]; 8.06%) and NRC ([27]; 17%). Variations in chemical composition of PBM can be due to the raw material that is being processed [11, 12] and different techniques used for feed processing in various factories and countries [15]. It is predictable that, different chemical composition can lead to different nutritive value [28].

Results for ruminal DM, OM and CP degradation of PBM at different incubation times are given in Table 2.

Dry matter, OM and CP disappearance from nylon bags incubated in the rumen, exponentially increased with increasing time. Obtained data illustrated that approximately 40% of DM, OM and CP of PBM have been degraded up to 2h incubation time. It seems that current data is nearly in line with findings of Bohnert *et al* [10], who reported that PBM crude protein have three distinct fractions of ruminal degradability; 1) disappeared at 0 h, 2) rapidly degraded portion (0 to 4 h) and 3) slowly degraded portion (4 to 24 h). Klemesrud *et al* [8] reported that after 12 h of ruminal incubation, escape protein values of PBM sources ranged from 32.0 to 39.8%. These workers were found negatively correlated between CP to ash content. Escape protein, however, was positively correlated with ash content.

Results for the DM, OM and CP degradation kinetics of PBM are presented in Table 3.

Table3: Ruminal dry matter (DM), organic matter (OM) and crude protein (CP) degradation parameters and effective degradability of poultry by-product meal (PBM)

Items	DM	OM	CP
a (%)	30.7	30.17	35.17
b (%)	27.87	32.67	24.1
a+b (%)	58.57	62.83	59.27
c (/h)	0.115	0.114	0.082
ED (%); Out flow rate 0.02 /h	54.3	57.43	54.23
ED (%); Out flow rate 0.05 /h	49.93	52.1	49.73
ED (%); Out flow rate 0.08 /h	46.93	48.63	47.03

a, washout fraction as measured by washing loss from nylon bags; b, potentially degradable fraction; c, rate of degradation of fraction b (/h). ED: effective degradability

The quickly degradable DM fraction "a" of PBM (30.7%) was higher than obtained by Kamalak *et al* ([6]; 26.4%) and the potentially degradable DM fraction "b" in current study (27.87%) was lower than that reported by Kamalak *et al* ([6]; 48.3%). The effective DM degradability was calculated by using rumen outflow rates of 2, 5 and 8%. The effective DM degradability values decreased with increased outflow rates. This value at 2 and 5% outflow rates (54.3 and 49.93%) were in agreement with those reported by Kamalak *et al* ([6]; 57.6 and 46.8%). The effective degradability of a feed is a measure of its disappearance in the rumen over time, while considering the rate at which it flows from the rumen to the small intestine.

The CP degradation parameters (a = 35.17%, b = 24.1%), in this study were different from findings of Kamalak *et al* [6] and Marghazani *et al* [15] who reported lower values for fraction a (28.2% and 25.93%) and higher values for fraction b (47.1% and 39.31%). The CP effective degradability of PBM at 2% out flow rate (maintenance feeding level) was similar to finding of Marghazani *et al* ([15]; 54.23 vs. 55.22%) whereas lower than that of reported by Kamalak *et al* ([6]; 54.23 vs. 58.8%). Effective degradability of the CP at 5% out flow rate also was similar to that reported by Kamalak *et al* [6] and Marghazani *et al* [15].

It can be proposed two important variation sources for the obtained data from different studies; the first variation source is related to tested feed (PBM) and the second is related to evaluation method. The main variation sources related to PBM are differences in chemical composition (particularly CP, EE and ash content), variation in raw materials and different processing methods such as heating condition and degree of Maillard reaction [11, 12, 29]. The major variation sources of *in situ* degradability of feeds are nylon bags pore size, sample size, washing method and procedure, grinding size, diet offered to the experimental animals, species of animal, sample preparation, incubation procedure and differences between laboratories methods and apparatuses [6, 15, 23]. Due to we can not find any study based on *in vitro* gas production method for evaluation of PBM, so we emphasized only on the obtained results from present study. The gas production pattern of PBM is shown in figure 1.
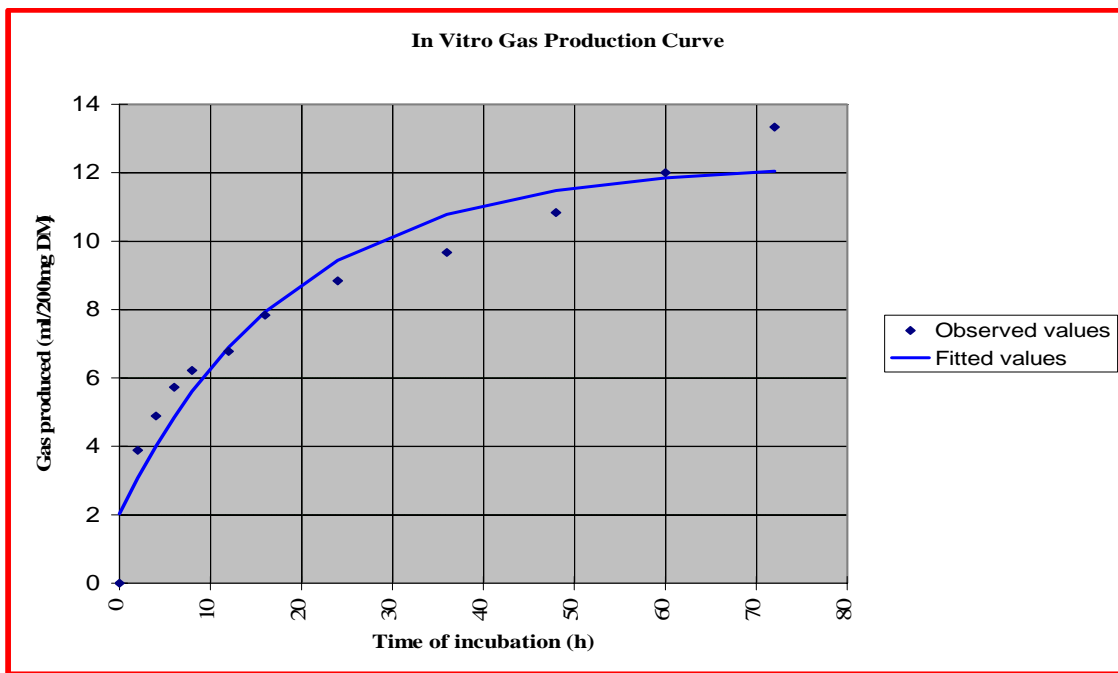
Figure1: Gas production volume at different incubation times of poultry by-product meal (PBM)

The gas production at 24 h incubation time obtained 8.83 (ml/200 mg). According to findings of Getachew *et al* [30] and Palizdar *et al* [31] plant origin source have higher gas production at 24 h incubation time than animal origin feeds. Robinson and Getachew [32] stated that gas produced by fermentation arises largely from the carbohydrate fraction of the feeds, since ash does not ferment, fat produces no gas, and protein produces very little gas. Cone and Van Gelder [33] also, reported that protein fermentation produces less gas compared with carbohydrates. Therefore it is predictable that, lower carbohydrate fraction and higher EE, ash and CP contents in PBM lead to a lower gas production. The gas production parameters, organic matter digestibility (OMD), short chain fatty acids (SCFA) and metabolizable energy (ME) and net energy for lactation (NE_L) contents of PBM are presented in Table 4.

Table4: The gas production parameters, organic matter digestibility (OMD), short chain fatty acids (SCFA) and metabolizable energy (ME) and net energy for lactation (NE_L) contents of poultry by-product meal (PBM)

Items	value
a (ml)	4.03
b (ml)	11.11
c (/h)	0.023
a+b (ml)	15.14
OMD (%)	53.13
SCFA (mmol)	0.192
ME (MJ / kg DM)	10.38
NE_L(MJ / kg DM)	5.69

a = the gas production from the immediately soluble fraction (ml); b = the gas production from the insoluble fraction (ml); c = the gas production rate constant for the insoluble fraction (/h).

The ME and NE_L contents of PBM in present study are 10.38 and 5.69 MJ/Kg DM, respectively, which are lower than that of reported by NRC ([27]; 12.12 and 7.52 MJ/Kg DM, respectively). Ewing [3] also reported the ME of poultry offal meal (14.5 MJ/Kg DM) higher than that of obtained in current research. Different results between current study and the NRC [27] and Ewing [3], may be due to different EE, ash and CP content of the PBM (as above mentioned) as well as measuring methods used for evaluation of energy content. According to related studies [18, 20, 25] OMD, ME and NE_L can be calculated by 24 h *in vitro* gas production volume and chemical composition of feed. If gas production be less, the energy value will be less.

CONCLUSION

In conclusion, about 50% of the poultry by-product meal is degradable in the rumen and remaining escaped into intestine. Thus it may provide sufficient protein to maintain microbial protein synthesis in the rumen as well as un-degradable protein that supplies amino acids to the small intestine. It is suggest the continuous evaluation of nutritive value of poultry by-product meals due to higher variation in its chemical composition. Based on the obtained results, it seems that *in vitro* gas production method under estimate the energy value of poultry by-product meal, and so may not be a reliable tool for such feeds contains high levels of EE, ash and CP.

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