In Vitro Gas Production Tests on Irradiated-Chicken Feathers to Estimate its Nutritive Value as Feed for Ruminants

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Chicken feathers are a highly abundant agro-waste product containing high amounts of protein from keratin. However, these are not practically utilized as animal feeds since they provide little, if any, nutritional value due to low digestibility in its natural state. Using an in vitro fermentation approach, the ruminant feed potential of chicken feathers treated with gamma-radiation was estimated. Gas production within an incubation period of 96 hours was monitored and values were fitted in the rumen degradability model by McDonald & Orskov (1981). Radiation treatment which could induce depolymerization of chicken feather keratin allowed for the improvement in the nutritive value for ruminants by liberating an additional 7.2% in metabolizable energy (ME) (P<0.005) for ruminant livestock. However, increasing the absorbed dose to 50 kGy resulted in significantly lower energy value for the feather substrate possibility accrued from the induced protein-protein cross-linking phenomenon.

Keywords: Hohenheim gas test, ruminant, rumen, fermentation, feed degradability

In the Philippines, the ruminant livestock productivity is low due to poor nutrition caused by inadequate supply of good quality feed. The situation is worsened by the fact that smallholder farmers have limited quality roughage in their locale available for feeding their ruminant livestock all year round. Unlike in developed countries, farmers here are also unable to select their basal diet according to the requirement of production since feed supplements, e.g., concentrates, are mostly imported and are considerably costly. In a study conducted by Alejandrino et al. (1999), smallhold farmers could barely meet the demands of nutritional stress during critical periods of the cattle’s reproductive life, i.e., ovulation and lactation. At a macroscale, this is reflected in the overall poor productivity performance, in terms of fertility and milk/meat production, of our ruminant livestock sector compared with counterparts in Latin America (http://www.fao.org). The strategy for improving production has therefore been to maximize the efficiency of utilization of the available potential feed resources in the rumen.

Chicken feathers are major waste products in poultry farming posing serious sewerage problems especially during rainy seasons. In farm waste management, chemical treatment of feathers is normally done to retard decay until this poultry garbage is disposed-off properly. Researches on economical processing methods to exploit this rich yet untapped protein resource from poultry feathers have been pursued.

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Some investigators propose hydrolysis at 130-145°C with steam pressure in the rendering of feathers, but then this process results to a non-profitable, low-grade feedstuff (Moritz & Latshaw, 2001).

The rate and extent of digestion of dry matter or organic matter in the rumen is regarded as one of the most important parameters to predict voluntary feed intake of ruminant (Khazaal et al., 1993). Since voluntary feeding behavior of the livestock has been responsible for 50% variation in its response to diet (Blummel, 1994), we evaluate alternative potential feed base that may be useful in improving animal nutrition with the least possible constraints to farmers while applying locally available technologies and biomass resources. Hence, this study explores radiolysis of chicken feathers as an alternative process to high-temperature hydrolysis in degrading the compact crystal structures of water-insoluble keratin into useable protein source for ruminant livestock.

Materials and Methods

Sample Preparation. Chicken feathers were collected from a poultry slaughterhouse in Blumentritt, Manila. The samples were washed with tap water and dried in a convection oven at 40°C. The feathers were double-packed in polyethylene bags and exposed to 25 and 50 kGy at the PNRI Pilot-Scale Co-60 Irradiation Facility. For another set-up, the dried chicken feathers were steam pressurized (autoclaved) for 30 min at 145°C, drained and oven-dried at 40°C. Prior to proximate and in vitro digestion experiments, the treated and untreated chicken feathers were hammer-milled through a 1.0-mm sieve.

Proximate Analyses. Samples were analyzed for moisture content, crude ash (XA) and total crude protein (CP) content using standard procedures (AOAC, 1990). Five grams of the samples were heated in a Sartorius moisture determination apparatus at 105°C until constant weight was achieved. Data of the moisture content served as basis for the dry matter (DM) estimation. Pre-weighted sample were ignited at 500-600°C in Thermolyne Type 1400 Furnace for 6-8 hours to determine XA. CP was analyzed from 1.0 gm of the ground sample digested with concentrated H2SO4 for 6-7 hours. Its hydrolyzate was analyzed by Kjeldahl method using Buchi distilling apparatus.

Hohenheim In Vitro Gas Production Technique. Duplicate samples (200.0 ± 0.1 g) were placed in 100-ml calibrated syringes and incubated with buffered rumen fluid at 39 ± 1°C in an anaerobic environment (Menke, et.al.,1988). Blanks (buffered rumen alone) and a reference IR-8 and concentrate standards (200.0 ± 0.1 g) were run in parallel serving as negative and positive controls, respectively. The rumen fluid was obtained from two goats fed with basal grass diet ad libitum (DM 25.6 %, crude protein 10.2 % DM). The gas volume was recorded after 2, 4, 8, 12, 24, 48, and 72 hours of incubation. The gas production data (mean of corrected duplicate runs) were fitted to the ruminal exponential degradation model (McDonald, 1981; McDonald & Orskov, 1970) with the Neway/Naway software donated by Dr. Orskov:

\[ P = a + b (1 - e^{-ct}) \]

where:
- \( P \) - cumulative gas production (ml) at a given time \( t \)
- \( a \) - produced gas from rapidly fermentable component
- \( b \) - gas volume from the insoluble but fermentable material
- \( c \) - rate of gas production \( h^{-1} \)
- \( (a + b) \) - potential gas volume or the asymptote of gas production
- \( t \) - time of fermentation (h)

Organic matter digestibility (DOM) and Total Metabolizable Energy (ME). Using net gas production (ml/200-mg DM) and proximate compositions: crude protein (CP) and ash content (XP), the estimate digestibility of organic matter and metabolizable energy was predicted using mathematical equations by Menke et al. (1988).

Statistical Analysis. The data collected were analyzed by one-way ANOVA and the difference between means was tested using Duncan's Multiple-Range Test (DMRT).

Results and Discussion

It is an accepted concept in ruminant nutrition that both ease in digestibility and degree of solubility of nutrients in the rumen and in other parts of the digestive tract of the farm animal have a major effect on dietary quality and digestive efficiency particularly for ruminants. Although these factors may not necessarily correlate with crystallinity and macromolecular structure, in the case of chicken feathers, its intermolecular packing and structural periodicity pose as major impediments in its usefulness as a feed component. Traditionally, farmers apply thermal energy by means of steam pressurization to sever the strong forces that bind tightly-packed keratin molecules, however, this process still has not been yielding to better-quality feed meals. In addition, the marginal differences in the nutrient content of autoclaved chicken feathers from lower crude protein content could probably be attributed to washing losses after draining the feathers (Table I). Since radiation...
energy can be exploited as a powerful and tractable approach in effectively modifying materials, the work presented here provides an alternative process to steam hydrolysis through protein radiolysis with the objective of altering ruminal feed degradation potential of chicken feathers. Radiation processing technology, in addition, may offer other important beneficial effects on the quality of the animal feed, such as pest control, extension of feed shelf-life and decreasing risk of aflatoxin via microbial decontamination (Urbain, 1978). Furthermore, it is emphasized that the investigation presented herein focuses more on understanding radiolytic effects on the feather substrate rather than on optimizing conditions for upgrading this agro-waste material for farm feeding.

Chicken feathers are a potentially abundant yet unexploited source of animal protein. The major component is keratin, a structural protein with very compact conformation composed of coiled coils of two or three \( \alpha \)-helices wound around each other forming a left-handed super-helix of repeat distance of 140 angstrom (McLachlan, 1978). Its macromolecular organization makes keratin very difficult for water molecules to solvate as well as an inaccessible site for proteolytic digestion.

Generally for biopolymers, the chemical changes that irradiation causes are fragmentation, cross-linking, aggregation and oxidation by oxygen radicals generated in the radiolysis of the water (Schuessler & Schilling, 1984; Garrison, 1987; Davies & Delsignore, 1987). It can be noted that the effect of oxygen radicals would be minor since keratin is immiscible in water and that the moisture content of the samples prior to irradiation were only within 11.1-12.05% (Table I). In general, radiation causes the irreversible changes at the molecular levels by breakage of covalent bonds of the polypeptide chain. Exposure of proteins to oxygen radicals results in both non-random and random fragmentation (Kemper, 1993).

### Table 1. Feed evaluation of processed feathers.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Gas Production (GP) (ml)</th>
<th>GP Rate Constant (hr(^{-1})) (c)</th>
<th>CP (in % DM)</th>
<th>XA (g/Kg DM)</th>
<th>DOM (g/Kg DM)</th>
<th>ME (MJ/Kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrate</td>
<td>74.9</td>
<td>0.2714</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td>IR-8</td>
<td>48.5</td>
<td>0.0597</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td><strong>Chicken Feathers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Irradiated</td>
<td>30.3(^a)</td>
<td>0.0023</td>
<td>93.45(^a)</td>
<td>1.14</td>
<td>73.64(^b)</td>
<td>11.76(^b)</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>39.1(^a)</td>
<td>0.0017</td>
<td>81.76(^c)</td>
<td>1.36</td>
<td>67.80(^d)</td>
<td>10.88(^d)</td>
</tr>
<tr>
<td>25 kGy-irradiated</td>
<td>28.2(^c)</td>
<td>0.0027</td>
<td>94.64(^c)</td>
<td>1.57</td>
<td>79.11(^c)</td>
<td>12.67(^c)</td>
</tr>
<tr>
<td>50 kGy-irradiated</td>
<td>26.6(^d)</td>
<td>0.0020</td>
<td>92.49(^d)</td>
<td>1.20</td>
<td>74.07(^d)</td>
<td>11.84(^d)</td>
</tr>
</tbody>
</table>

* Legend: n.a. = not analyzed; DM = dry matter; CP-total crude protein; XA = ash content; DOM = digestibility of organic matter; ME = metabolizable energy
** entries in columns without or have similar corresponding superscripts have variables that are not significantly different (\( r = 0.005 \) following DMRT)*** GP and GP rate constant are calculated from values of a, b, c and c of the degradation model of the Hohenheim in vitro gas production test (McDonald, 1981; McDonald & Orskov, 1981)
**** CP, XP, DOM and ME for concentrate and IR-8 rice straw were no longer determined as these materials served only as reference standards for the gas production experiment to ensure uniformity in the basal rumen microbial activity

### Table 2. Some proximate analyses performed on the chicken feathers after packing and irradiation.

<table>
<thead>
<tr>
<th>reference</th>
<th>Dry Matter (%)</th>
<th>Moisture Content (%)</th>
<th>pH (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Irradiated</td>
<td>88.65</td>
<td>11.35</td>
<td>7.15</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>87.95</td>
<td>12.05</td>
<td>7.39</td>
</tr>
<tr>
<td>25 kGy-irradiated</td>
<td>88.90</td>
<td>11.10</td>
<td>7.04</td>
</tr>
<tr>
<td>50 kGy-irradiated</td>
<td>88.30</td>
<td>11.70</td>
<td>6.62</td>
</tr>
</tbody>
</table>

Fragmentation involves reaction of \( \alpha \)-carbon radicals with oxygen to form peroxyl radicals that decompose to fragment the polypeptide chain at the \( \alpha \)-carbon. In substrates with high protein content, this fragmentation or depolymerization process is revealed from the drop in pH caused by the release of free amino acids and fixation of oxidative reactions. Radiation-treated chicken feathers at 25 kGy level registered modest improvement in metabolizable energy (+7.74%) and digestibility of organic matter (+7.43%).

Despite the two-fold increase in radiation dose (25 \( \rightarrow \) 50 kGy), the metabolizable energy and degradability of our chicken feather samples decreased by 6.55% and 6.37%, respectively. When non-treated feathers were compared to the set-ups irradiated at 50 kGy, we noted a very negligible improvement in degradability (<1% increase) in spite of the extensive radiolytic reactions that occurred with lower absorbed radiation dose of 25 kGy. Human hair keratin responded in a similar fashion in terms of ruminal degradability when irradiated at 50 kGy (manuscript in preparation).

There are could be two possible explanations for this observed trend in in vitro degradability. First, there may be an aberrant production of metabolic-inhibitory radiolytic products from keratin that affected the rate of degradation of the chicken feather meal. However, the documented metabolic inhibitors have been identified to
phenolic natural products such as tannins and saponins (Khazaal, Boza & Orskov, 1994). These compounds that interfere with microbial metabolism are highly complex and their free radical-mediated synthesis from amino acid precursors are not thermodynamically favorable.

Second, the structure of keratin may be altered at 50 kGy that might have rendered it less digestible to the rumen microbes. The culinary idea of increasing cooking time to improve texture and palatability of foodstuff may not straightforwardly work when we deal with radiation. At higher doses, proteins may be converted to higher molecular weight aggregates due to the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions and the formation of disulfide bonds (Davies & Delsignore, 1987; Moon & Song, 2000). Much so, covalent cross-links are formed between free amino acids and proteins and between peptides and proteins in solution after irradiation (Garrison, 1987; Puchala & Schessler, 1993). The further drop in pH from 7.15 (for non-irradiated feathers) to 6.62 (for 50 kGy-irradiated samples) indicated amino acid liberation, but this was not informative of cross-linking events. In the future, we intend to run the feathers samples in a thermogravitmetric analyzer that measures thermal degradation properties of its individual components.

Based on the present degradability potential of feathers irradiated at the two doses used for this experiment, it is recommended that optimization of radiolytic degradation may be done at doses below 25 kGy. Utilizing the digestibility data presented, theoretically, given a 300-kg beef cattle during its last 3 months of gestation, its metabolizable energy requirement for body weight maintenance is 58.88 MJ per day (PHILSAN, 1996). Farmers therefore need to collect 4.4-5.3 kg of chicken feathers on a dry matter basis daily to sustain a single cow. In a backyard farm setting of 5 heads, supplying 25 kilos of dried feather would be unprofitable and highly constrained. For the same cow model, if we look at its total protein requirement of 403 g per day, it would only require 874-gms chicken feather meal supplement to sustain the protein needs of the livestock ruminant if we are able to improve at least 50% keratin degradability through irradiation. A farmer would then only need to gather feathers from 3-4 medium-sized chickens to feed a cow!

Acknowledgments

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References


