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Determination of Energy and Nutrient Utilization of Enzyme-treated Rump Round Meat and Lotus Root Designed for Senior People with Young and Aged Hens as an Animal Model

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Abstract

This study aimed to examine the nutrient utilization of rump round meat and lotus root using young (32 wk) and aged hens (108 wk) as an animal model. Rump round meat and lotus root were prepared with or without enzymatic treatment. For each age group of laying hens, a total of 24 Hy-Line Brown laying hens were randomly allotted to one of two dietary treatments with six replicates. For rump round meat, the true total tract retention rate (TTTR) of dry matter (DM) and nitrogen (N) were unaffected by either enzymatic treatment or hen age. However, aged hens had greater ($p<0.01$) TTTR of energy and crude fat than young hens. Enzymatic treatment did not influence the TTTR of energy or crude fat. In addition, we did not observe any significant interaction between the TTTR of DM, energy, N, or crude fat in rump round meat and hen age or enzymatic treatment. The TTTR of DM remained unchanged between controls and enzyme-treated lotus root for young hens. However, enzyme-treated lotus root exhibited greater ($p<0.05$) TTTR of DM than control lotus root for aged hens, resulting in a significant interaction ($p<0.05$). The TTTR of energy and N in lotus roots were greater ($p<0.01$) for aged hens than for young hens. In conclusion, enzymatic treatment exerted beneficial effects on energy and nutrient utilization in aged hens, suggesting the aged hen model is practical for simulation of metabolism of elderly individuals.

Keywords: animal model, enzyme-treated food for the elderly, laying hen, true total tract retention rate

Introduction

Senior-friendly food products have gained widespread attention because the market of foods for elderly has grown rapidly with the growing elderly population (Kwak *et al*., 2013). Considerable efforts have been put into the development of senior-friendly food products to improve nutrient utilization because elderly individuals have decreased ability to digest and utilize nutrients. However, direct determination of the efficacy of food products designed for elderly individuals in terms of nutrient utilization has been limited.

Rump round meat is frequently used by *sous vide* cooking for soup or seasoned-steamed meat preparation due to its lower level of marbling, and may serve as a good protein source for elderly individuals. Lotus root is one of the favorite vegetables in the Korean diet; however, its unique hard texture and lower digestibility have limited its easy consumption for elderly individuals.

In vitro tests and animal models have been suggested as a useful means to test the efficacy of food products and their safety for humans (Hsu *et al*., 1977; Rowan *et al*., 1994). Animal models are considered better than *in vitro* tests due to their biological similarities to humans; rodents and pigs have been often chosen as models for the determination of food product utilization (Rowan *et al*., 1994; Sarwar *et al*., 1989). However, animal models that can be used for evaluating food products for elderly individuals have been limited because of the difficulties in finding appropriately aged animal models.

It has been reported that aged laying hens exhibit very low productive performance, possibly due to a decrease in digestion ability, absorption, and utilization of nutrients in the body (Kim *et al*., 2013). Therefore, a comparison

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of nutrient utilization of test foods in young hens compared to aged laying hens may be useful for evaluating food products designed for elderly individuals. However, this hypothesis has not yet been tested.

The objective of this study was to determine the nutrient utilization of rump round meat and lotus root designed for elderly individuals using young (32 wk) and aged hens (108 wk) as an animal model.

Materials and Methods

Enzymatic treatment of food samples

Rump round meat and lotus root were purchased from local markets and treated with an enzymatic process as follows. Briefly, frozen rump round meat was cut into 1.5 cm thick pieces and steamed for 10 min at 120°C, and then cooled down to room temperature for 30 min. Meat samples were placed into zippered bags with 250 mL 0.1% bromelain enzyme, and further treated for 4 h under 55 MPa of pressure at 55°C. Enzymes were inactivated by heating at 95°C for 15 min, and the enzyme-treated meat samples were freeze-dried for downstream analysis.

Lotus roots cut into 1.5 cm thickness were steamed for 30 min at 120°C, cooled down to room temperature for 30 min, and then frozen at -18°C for 24 h. Lotus roots were thawed for 3 h at room temperature, placed into zippered bags with 500 mL 0.5% Rohament CL enzyme (AB Enzymes, Germany), and placed under a vacuum for 5 min for efficient infusion of enzyme. The enzymatic reaction was then incubated for 1 h at 60°C. Enzymes were inactivated at 95°C for 15 min, and the enzyme-treated lotus root samples were freeze-dried for downstream analysis.

Experimental design and diets

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University (Korea). The experiment was performed using a completely randomized design with 2×2 factorial arrangements of two different ages of laying hens (32 wk and 108 wk) and two different dietary treatments (with or without enzymatic treatment) for each food material. Two food materials of rump round meat and lotus root were tested, and each food was treated with a specific enzyme to produce food materials that were favorably utilized for senior people. Within each age of laying hens, a total of 24 Hy-Line Brown laying hens were randomly allotted to one of two dietary treatments with six replicates. An additional 32 hens (16 young hens

and 16 aged hens) were prepared to estimate the endogenous loss of dry matter (DM), energy, crude fat, and nitrogen (N) of both young hens and aged hens. Birds were placed in a metabolic cage ($35 \times 45 \times 55$ cm, width $\times$ length $\times$ height) that was equipped with a feeder and a drinker, which allowed for the collection of total excreta. During the experiment, hens were provided with water *ad libitum* and exposed to a 16 h light/ 8 h dark lighting schedule. The ambient temperature was set to 24°C during the experiment.

A precision-fed method as described by Parsons *et al*. (1982) was used to determine the nutrient and energy retention of each food material. Briefly, following a 24 h fasting period, 20 g of each food material was fed by intubation to 48 birds (24 fed with raw food material, and 24 fed enzyme-treated food material). An additional 32 birds (16 young hens and 16 aged hens) were used to estimate the endogenous loss of DM, energy, crude fat, and N. After 24 h fasting, total excreta voided from all birds was collected for 48 h, dried in a force-air drying oven at 60°C for 72 h, and finely ground for subsequent analysis.

Chemical analysis and calculation

Diets and excreta samples were analyzed for gross energy (GE) using a bomb calorimeter (Parr Instrument Company, USA), and for DM, crude fat, and N according to methods 930.15, 920.39, and 990.03, respectively, of the Association of Official Analytical Chemists (AOAC) International (2007). The TTTR of DM, GE, N, and crude fat were calculated using the following formula from Prola *et al*. (2013):

$$X\ TTTR = (\text{total X ingested} - \text{total X excreted} + \text{endogenous loss of X}) / (\text{total X ingested})$$

where X represents DM, GE, crude fat, and N.

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) based on a completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., USA). Each replicate was considered the experimental unit for all measurements. The model analyzed the effect of hen age, enzymatic treatment, and their interaction. The LSMEANS procedure was used to calculate mean values, and the PDIFF option of SAS was used to separate means. Significance was considered at $p < 0.05$ for all analyses.

Table 1. Nutrient compositions of rump round meat and lotus root

Variable	Rump round meat		Lotus root	
	Enzyme treatment[1]			
	+	–	+	–
Dry matter (%)	97.2	97.7	95.8	95.8
Gross energy (kcal/kg)	5,754	5,735	4,060	4,056
Nitrogen (%)	13.8	13.9	0.9	09
Crude fat (%)	7.3	7.3	2.8	2.9

[1]Rump round meat and lotus root were treated with protease (bromelain) and cellulase (Rohament CL), respectively.

Results

The concentrations of DM, GE, N, and crude fat in rump round meat and lotus root are presented in Table 1. As expected, the concentrations of GE, N, and crude fat were greater for the rump round meat than for the lotus root. The concentrations of GE, N, and crude fat were very similar between the enzyme-treated and control groups, which may indicate that enzymatic treatment has no significant effect on the total amounts of energy and nutrients, especially for protein and fat.

The TTTR of DM, energy, N, and crude fat in the control and enzyme-treated rump round meat for young hens and aged hens are shown in Table 2. The TTTR of DM and N were unaffected by either enzymatic treatment or hen age. However, aged hens exhibited a greater ($p<0.01$) TTTR of energy and crude fat than did young hens. Enzymatic treatment did not appear to influence the TTTR of energy and crude fat. There were also no significant interactions for TTTR of DM, energy, N and crude fat between the age of hens and enzymatic treatment.

The TTTR of DM, energy, N, and crude fat in the control and enzyme-treated lotus roots for young and aged hens are presented in Table 3. The TTTR of DM did not differ between the control and enzyme-treated lotus root for young hens. However, enzyme-treated lotus roots exhibited greater ($p<0.05$) TTTR of DM than control lotus roots for aged hens, which resulted in a significant interaction ($p<0.05$). However, there were no significant interactions observed for the TTTR of energy, N and crude fat between enzymatic treatment and the age of hens. The TTTR of energy and N were greater ($p<0.01$) for aged hens than for young hens.

Discussion

It has been suggested that digestion ability and absorption of nutrients in diets is likely to be reduced in aged hens (Kim *et al*., 2013). Hens older than 70 wk of age are considered aged hens in commercial situations in South Korea. Therefore, we chose 108-wk-old hens as to serve

Table 2. True total tract retention rate (TTTR) of energy and nutrients in rump round meat for young (32-wk old) and aged hens (108-wk old)[1]

Variable	Young hens		Aged hens		SEM	P-value		
	–[2]	+	–	+		Age (A)	Enzyme (E)	A × E
TTTR of DM[3] (%)	73.7	77.0	79.7	79.5	3.19	0.20	0.64	0.58
TTTR of energy (%)	83.2	84.9	88.8	88.4	1.29	<0.01	0.60	0.41
TTTR of N[4] (%)	53.4	54.6	44.5	50.9	3.43	0.08	0.28	0.46
TTTR of crude fat (%)	80.5	86.5	97.6	101.9	3.50	<0.01	0.16	0.81

[1]Each value represents the least square means of six replicates per treatment.
[2]"-": untreated food; "+": enzyme-treated food.
[3]DM: dry matter.
[4]N: nitrogen.

Table 3. True total tract retention rate (TTTR) of energy and nutrients in lotus roots for young (32-wk old) and aged hens (108-wk old)[1]

Variable	Young hens		Aged hens		SEM	P-value		
	–[2]	+	–	+		Age (A)	Enzyme (E)	A × E
TTTR of DM[3], %	74.7	75.7	85.8	97.4	2.52	<0.01	0.02	<0.05
TTTR of energy, %	79.8	78.1	87.4	91.4	2.01	<0.01	0.59	0.17
TTTR of N[4], %	-11.2	41.3	97.6	185.3	40.66	<0.01	0.10	0.67
TTTR of crude fat, %	108.5	114.5	91.3	110.7	7.15	0.16	0.09	0.36

[1]Each value represents the least square means of six replicates per treatment.
[2] "-": untreated food; "+": enzyme-treated food.
[3]DM: dry matter.
[4]N: nitrogen.

as our aged animal model in this experiment. Before the start of the experiment, we hypothesized that enzyme-treated food materials designed for elderly individuals may exhibit greater energy and nutrient utilization in diets for aged hens than for young hens. However, this hypothesis was only valid for DM in lotus roots, but not for energy, N, or crude fat in lotus roots. No interactive changes were observed for DM, energy, N, and crude fat in rump round meat.

Surprisingly, we observed that the utilization of energy and crude fat in rump round meat, as well as that of DM, energy, and N in lotus roots were greater for aged hens than for young hens. The reason for this observation remains unknown because this is the first study to examine nutrient utilization in human food using young and aged hens. However, this result could be attributed to the fact that both food materials were highly digestible and that very small quantities were fed to the hens in a short time, which may be favorable for aged hens. It has been reported that aged hens have greater and longer gastrointestinal tracts than do young hens (Hudson *et al.*, 2004), which may help overcome their decreased digestive ability and nutrient absorption. In addition, age-dependent nutrient utilization may differ among food materials. In this experiment, the N utilization in rump round meat, which is high in N (i.e., protein), tended to be less ($p<0.10$) for aged hens than for young hens. On the other hand, fat utilization was greater for aged hens than for young hens. This result was not the case for lotus roots, which is comprised primarily of carbohydrates. More research is required to provide a clear explanation of these observations.

There were no significant improvements observed in energy and nutrient utilization when raw food materials were treated with enzymes, except for the DM utilization in lotus roots. The reason for this observation is also unclear; however, it may also be attributed to the fact that both food materials were highly digestible and that very small quantities were fed to hens in a short time, as described above.

In this experiment, some of the calculated values were not physiologically valid; retention rates greater than 100% (e.g., 185.3%) or negative (e.g., -11.2%) are theoretically impossible under normal physiological conditions. These values are likely artifacts as resulting from high endogenous losses of energy and nutrients from laying hens, and their subsequent correction in the calculation of TTTR from apparent total tract retention of nutrients. This may also be the reason why the TTTR of

nutrients for aged hens was greater than that for young hens, as the amount of endogenous loss of nutrients was greater for aged hens than for young hens. More research regarding the effect of animal age on endogenous nutrient loss is necessary.

In conclusion, enzymatic treatment exerted a few beneficial effects on energy and nutrient utilization in both young and aged hens. Aged hens are not a perfect model for simulating human diets; however, further studies could refine and improve this animal model for research focused on elderly individuals.

Acknowledgements

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