Open Access
Asian-Australas J Anim Sci
Vol. 31, No. 4:564-568 April 2018
https://doi.org/10.5713/ajas.17.0785
pISSN 1011-2367 eISSN 1976-5517

AJAS
Asian-Australasian Journal of Animal Sciences

Effects of dietary β-mannanase supplementation on the additivity of true metabolizable energy values for broiler diets

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Submitted Oct 30, 2017; Revised Dec 17, 2017; Accepted Jan 24, 2018

Objective: This experiment was conducted to determine the effects of dietary β-mannanase on the additivity of true metabolizable energy (TME) and nitrogen-corrected true metabolizable energy (TME_n) for broiler diets.
Methods: A total of 144 21-day-old broilers were randomly allotted to 12 dietary treatments with 6 replicates. Five treatments consisted of 5 ingredients of corn, wheat, soybean meal, corn distillers dried grains with solubles, or corn gluten meal. One mixed diet containing 200 g/kg of those 5 ingredients also was prepared. Additional 6 treatments were prepared by mixing 0.5 g/kg dietary β-mannanase with those 5 ingredients and the mixed diet. Based on a precision-fed chicken assay, TME and TME_n values for 5 ingredients and the mixed diet as affected by dietary β-mannanase were determined.
Results: Results indicated that when β-mannanase was not added to the diet, measured TME and TME_n values for the diet did not differ from the predicted values for the diet, which validated the additivity. However, for the diet containing β-mannanase, measured TME_n value was greater ($p<0.05$) than predicted TME_n value, indicating that the additivity was not validated.
Conclusion: In conclusion, the additivity of energy values for the mixed diet may not be guaranteed if the diet contains β-mannanase.

Keywords: Additivity of Energy Values; Broiler Chicken; Dietary β-mannanase; True Metabolizable Energy

INTRODUCTION

When animal diets are formulated, there is a basic assumption that the total supply of available energy and nutrients in the mixed diet is equal to the sum of available energy and nutrients provided by each ingredient, which is often referred to the additivity [1]. Based on this fundamental assumption, animal nutritionists formulate a mixed diet with various sources and inclusion levels of feed ingredients. Thus, the additivity of energy and nutrient utilization is very important in diet formulation. The additivity for amino acid and phosphorus utilization in diet formulation has been validated for pigs [2,3] and poultry [4,5]. However, the information regarding the additivity of available energy in poultry diets is limited although energy ingredients are included at the highest levels and are the most expensive components in the diets [6].

The application of dietary enzymes targeting non-starch polysaccharides (NSPs) in diets is currently of major interest in the poultry industry because of their ability to improve energy and nutrient utilization in diets via both increased utilization of NSPs and decreased anti-nutritional effects of NSPs [7]. Dietary β-mannanase is an exogenous enzyme that hydrolyzes β-mannan, which accounts for 15% to 37% of the total concentration of NSPs in

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poultry diets [8]. There has been mounting evidence that dietary β-mannanase can increase energy and nutrient utilization, and thus, could decrease energy and nutrient supply in poultry diets [9-11]. However, the assumption that available energy values for ingredients are additive if dietary β-mannanase is added to the poultry diet has not been validated.

Therefore, the objective of the current experiment was to determine the effects of dietary β-mannanase supplementation on the additivity of true metabolizable energy values for the mixed diet fed to broiler chickens.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

Birds, diets, and experimental design

A total of 144 21-day-old Ross 308 broiler chickens (initial body weight = 0.95±0.01 kg) were randomly allotted to 1 of 12 dietary treatments with 6 replicates consisting of 2 birds per replicate. Two birds (1 male and 1 female) were raised together in a metabolic cage (35.2 cm×45.0 cm×55.3 cm = width×length×height). Room temperature was set at 23°C and the light was provided for 24 h during the experiment. Five treatments consisted of 5 ingredients of corn, wheat, soybean meal (SBM), corn distillers dried grains with solubles (DDGS), or corn gluten meal (CGM), which are the common ingredients for poultry diets. Those are prepared in a ground form. One mixed diet containing 200 g/kg of those 5 ingredients also was prepared. Analyzed nutrient and energy content in the 5 ingredients and the mixed diet were presented in Table 1. Additional 6 treatments were prepared by mixing 0.5 g/kg β-mannanase (CTCZYME; declared activity of 800,000 unit/kg, CTCbio, Inc., Seoul, Korea) with those 5 ingredients and the mixed diet.

A precision-fed chicken assay was conducted based on the method demonstrated by Kim et al [12]. In brief, broiler chicks were obtained at 1 day of age and were fed a commercial diet until 20 day of age. All birds were provided with diets and water *ad libitum* before the start of the precision-feeding. At the start of the experiment (21 day of age), all birds were fasted for 12 hours to empty their gastrointestinal tracts. After the 12-hour fasting, broiler chickens were fed 15 g of each ingredient or the mixed diet by a crop intubation. All excreta samples were collected continuously for 48 hours. Additional 16 birds were used to estimate endogenous losses of energy and nitrogen (N). Those birds also were fasted for 12 hours, and afterwards excreta were collected for 48 hours, which was similar to birds assigned to dietary treatments.

Collected excreta samples were dried and finely ground for the subsequent analysis. The samples for 5 ingredients and the mixed diet were analyzed for dry matter [13], ether extract [13], and crude ash [13]. The samples for 5 ingredients, the mixed diet and all excreta also were analyzed for N [13] and gross energy (GE) using bomb calorimetry (Model 6400; Parr Instruments Co., Moline, IL, USA) with benzoic acid used as the standard for calibration.

Table 1. Analyzed nutrient and energy contents of ingredients and a mixed diet (g/kg, as-fed basis)

Items	Corn	Wheat	SBM	DDGS	CGM	Mixed diet[1)]
Dry matter	913.0	910.0	923.0	924.0	960.0	935.0
GE (MJ/kg)	16.6	16.3	17.9	19.2	23.1	18.6
Crude protein	70.0	97.0	435.0	275.0	617.0	309.0
Crude ash	11.0	15.0	65.0	46.0	11.0	30.0
Ether extract	52.0	19.0	19.0	107.0	69.0	47.0

SBM, soybean meal; DDGS, corn distillers dried grains with solubles; CGM, corn gluten meal; GE, gross energy.
[1)] The mixed diet (1,000 g/kg) contained 200 g/kg of 5 individual ingredients including corn, wheat, SBM, DDGS, and CGM.

Calculations and statistical analysis

The values for true metabolizable energy (TME) and N-corrected true metabolizable energy (TME_n) of the ingredients and diets were calculated as followed [14]:

$$TME\ (MJ/kg) = \frac{(GEi - GEo + GEe)}{\text{feed intake}}$$

$$TME_n\ (MJ/kg) = \frac{\{GEi - [GEo + (Ni - No) \times 0.034] + [GEe + (Ni - No) \times 0.034]\}}{\text{feed intake}}$$

Where GEi represents the GE intake; GEo represents the GE output; Ni-No represents the gram N balance; GEe represents endogenous loss of energy; 0.034 (MJ/g) equals the N retained value [15].

The additivity of TME and TME_n values for the mixed diet was determined based on similarity between measured energy values for the diet and predicted energy values for the diet, which was calculated from the measured energy value for each ingredient [2].

All data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The replicate was considered as the experimental unit. Outlier data were identified according to the UNIVARIATE procedure of SAS, but no outliers were detected. The model included the main effects of dietary β-mannanase supplementation. The LSMEANS procedure was used to calculate mean values. For the determination of additivity, the differences in the values for TME and TME_n between measured and predicted values were estimated using the LSMEANS option in the MIXED procedure. The confidence intervals for the differences were estimated with an α-level of 0.05. If the confidence interval for the dif-

ference included zero, the difference between measured and predicted values was not considered significant, indicating that the assumption of additivity was warranted [2]. Significance for statistical tests was set at $p<0.05$.

RESULTS AND DISCUSSION

TME and TME_n

The analyzed concentrations of total nutrients and GE in each ingredient (Table 1) were comparable and were within the range of previously reported values [16-18]. In addition, the analyzed concentrations of total nutrients and GE in the mixed diet containing 200 g/kg of 5 ingredients were close to those calculated from total nutrients and GE in each ingredient, confirming that total nutrients and GE in the mixed diet were additive.

The measured TME_n values for corn and wheat when no β-mannanase enzyme was added (Table 2) were similar to the values for corn (14.5 and 14.6 MJ/kg) and wheat (13.3 and 13.1 MJ/kg) reported by NRC [16] and Rostagno et al [17], respectively. This result indicated that our experimental procedure of a precision-fed chicken assay was valid for measuring TME_n values for ingredients fed to broiler chickens. However, the measured TME_n values for SBM and CGM were greater than the values for SBM (10.4 and 10.8 MJ/kg) and CGM (15.9 and 16.2 MJ/kg) reported by NRC [16] and Rostagno et al [17], respectively. In addition, the measured TME_n value for DDGS was less than the value (13.0 MJ/kg) reported by NRC [16], but similar to the value (11.8 MJ/kg) reported by Batal and Dale [19]. Different origin and processing of those ingredients including SBM, CGM, and DDGS, and different experimental conditions among experiments may be the primary reason for this variation.

The addition of 0.5 g/kg β-mannanase had no effects on TME and TME_n values for all 5 ingredients (Table 2). Likewise, TME and TME_n values for the mixed diet containing 200 g/kg of those ingredients were not affected by dietary β-mannanase. These results may indicate that dietary β-mannanase has little effects on true energy metabolizability in ingredients and diets fed to broiler chickens. However, previous experiments reported that dietary β-mannanase increased apparent metabolizability of nutrients [9,11] and energy [20] in diets fed to broiler chickens. The reason for this discrepancy is not clear, but it may be related to the effect of dietary β-mannanase on endogenous energy losses because we measured true metabolizable energy but previous experiments measured apparent metabolizable energy as affected by dietary β-mannanase. However, no data regarding apparent and true energy metabolizability as affected by dietary supplementation of enzymes have been available in poultry.

Additivity validation

For the diet containing no β-mannanase, measured TME and TME_n values for the diet were very close to predicted TME and

Table 2. Effects of dietary β-mannanase supplementation on the values for true metabolizable energy (TME) and nitrogen-corrected true metabolizable energy (TME_n) of 5 ingredients and the mixed diet fed to broiler chickens[1)]

Items	TME (MJ/kg)				TME_n (MJ/kg)			
	β-mannanase (g/kg)		SEM	p-value	β-mannanase (g/kg)		SEM	p-value
	0	0.5			0	0.5		
Corn	14.7	14.7	0.36	0.96	14.6	14.6	0.34	0.95
Wheat	14.0	13.4	0.31	0.23	13.8	13.2	0.22	0.11
SBM	12.7	12.4	0.62	0.77	11.8	11.9	0.40	0.92
DDGS	12.5	12.5	0.46	0.94	11.4	11.2	0.42	0.70
CGM	19.8	19.8	0.56	1.00	18.0	18.1	0.30	0.80
Mixed diet[2)]	14.8	15.3	0.33	0.34	14.0	14.4	0.22	0.25

SEM, standard error of means; SBM, soybean meal; DDGS, corn distillers dried grains with solubles; CGM, corn gluten meal.
[1)] Data are least square means of 6 observations per treatment.
[2)] The mixed diet containing 200 g/kg corn, 200 g/kg wheat, 200 g/kg SBM, 200 g/kg DDGS, and 200 g/kg CGM.

Table 3. Measured and predicted values for true metabolizable energy (TME) and nitrogen-corrected true metabolizable energy (TME_n) in a mixed diet[1)]

Items	No β-mannanase				0.5 g/kg β-mannanase addition			
	Measured	Predicted	Difference	SE[2)]	Measured	Predicted	Difference	SE[2)]
TME (MJ/kg)	14.8	14.7	0.1	0.32	15.3	14.6	0.7	0.34
TME_n (MJ/kg)	14.0	13.9	0.1	0.24	14.4	13.8	0.6[3)]	0.20

[1)] Data are least square means of 6 observations per treatment. Measured values for a mixed diet were directly determined (Table 2), whereas predicted values were calculated from measured values for 5 ingredients (Table 2) with an additive assumption.
[2)] Standard error of the difference between measured and predicted values.
[3)] Measured and predicted values differ at $p<0.05$.

TME_n values, showing very high additivity (Table 3). However, when β-mannanase was added to the diet, measured TME and TME_n values for the diet were greater by 4.6% and 4.2%, respectively, than predicted TME and TME_n values; however, the difference was significant ($p<0.05$) only for the TME_n values. This result indicated that the additivity of TME_n values for the diet was not warranted if the diet contains β-mannanase. It may be speculated that energy values from each ingredient may not additive in mixed diets if dietary enzyme is included because possible interactions exist among energy utilization in ingredients by dietary enzymes. However, the clear reason why values were additive for TME, but not for TME_n of diets is difficult to explain. One possible reason is that the N-correction for TME values to determine TME_n values decreased the variation (i.e., SEM) of the data, and therefore, increased the probability of detecting the significance. A similar result was observed in our previous experiment reporting that dietary multi-enzymes increased both TME and TME_n values for diets fed to broiler chickens, but significance was observed only for TME_n values mainly due to lowered SEM values [21].

In conclusion, addition of dietary β-mannanase to ingredients and mixed diets has no effects on TME and TME_n values for the ingredients and mixed diets. For a diet containing no β-mannanase, there is a clear additivity of energy values for the mixed diet. However, when β-mannanase is added to the diet, the additivity of energy values for the diet may not be warranted.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

This research was carried out with the support of the Cooperative Research Program for Agriculture Science and Technology Development (ID: PJ01252804), Rural Development Administration, Republic of Korea. This research also was supported by the Chung-Ang University Research Scholarship Grants in 2018.

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