Research Note: The comparative study of energy utilization in feedstuffs for Muscovy ducks between in vivo and in vitro

H. Wang, X. F. Zhang, S. S. Zhai, J. J. Yuan, W. C. Wang, Y. W. Zhu,[1] and L. Yang

Guangdong Provincial Key Laboratory of Animal Nutrition and Regulation, College of Animal Science, South China Agricultural University, Guangzhou 510000, China

ABSTRACT Our study was aimed to investigate the effects of dietary metabolizable energy (**ME**) and crude protein (**CP**) levels on the digestive physiology in the jejunal fluid of Muscovy ducks to provide digestive parameters for in vitro digestion. There were 6 ME levels (11.0, 11.5, 12.0, 12.5, 13.0, and 13.5 MJ/kg; Exp. 1) and six CP levels (140, 155, 170, 185, 200, and 215 g/kg; Exp. 2) and each treatment included 6 replicates with 3 ducks each replicate. In Exp. 3, the comparison of energy utilization was investigated between in vivo and in vitro using the digestion parameters obtained from Exp. 1 and 2. As dietary ME was increased, the chymotrypsin activity was increased linearly ($P < 0.05$), and the concentrations of Ca^{2+}, K^{+}, and Mg^{2+} were increased quadratically ($P < 0.05$) in the jejunal fluid. As dietary CP was increased, amylase activity was increased linearly ($P < 0.05$), whereas trypsin and chymotrypsin activities and Ca^{2+} concentration were increased quadratically ($P < 0.05$). The pH values were decreased quadratically with the increased dietary ME or CP levels ($P < 0.05$). The optimal digestion parameters for energy feedstuffs with 307.26 U/mL amylase, 54.68 U/mL trypsin, 24.90 U/mL chymotrypsin, 104.39 mmol/L Na^{+}, 51.25 mmol/L Cl^{-}, and pH 7.79; for protein feedstuffs with 381.88 U/mL amylase, 72.84 U/mL trypsin, 11.98 U/mL chymotrypsin, 93.53 mmol/L Na^{+}, 46.25 mmol/L Cl^{-}, and pH 7.80, respectively. Using the optimal digestion parameters for in vitro digestion, energy utilization in vitro reflected the degree of the apparent energy utilization of corn, sorghum, and barley as well as true energy utilization of soybean meal, rapeseed meal, and cottonseed meal in vivo and the variation of digestion was lower in vitro than in vivo.

Key words: metabolizable energy, crude protein, digestive enzyme, electrolyte ion concentration, energy utilization

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INTRODUCTION

The digestive capabilities of the gastrointestinal tract play a major role in dietary nutrient hydrolyzation for absorption, which mainly depended on the activity of the digestive enzyme, electrolyte concentration, and pH. Metabolic energy (**ME**) and crude protein (**CP**) as the main macronutrients have been proved to influence the digestive enzyme activity of intestinal fluid in Peking duck (Zhao et al., 2007). However, limited information was involved in the electrolyte ion concentrations as well as both digestive enzyme activities and electrolyte ion concentrations in response to the changes in dietary nutrient levels. And these in vivo digestive parameters such as digestive enzyme activities, electrolyte ion concentrations, and pH were necessary for simulated digestion method in vitro.

So far, the nutritional values of feedstuffs were evaluated by the traditional in vivo assay, which displayed the disadvantages of time-consuming, expensive, and laborious. It was necessary to obtain the optimal digestive parameters and then establish a quick, accurate, and inexpensive in vitro simulation method for evaluating the nutritional values of feed ingredients. Therefore, in our study, 2 experiments were conducted to determine the effects of various dietary levels of ME (Exp.1) and CP (Exp. 2) on digestive enzyme activity, electrolyte ion concentration, and pH values in the jejunal fluid of Muscovy duck. To verify the feasibility of in vitro method using the digestion parameters obtained from Exp. 1 and 2, a comparative study (Exp. 3) on the evaluation of energy utilization was investigated between in vivo and in vitro assays.

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[1]Corresponding author: 408034085@qq.com

MATERIALS AND METHODS

Animal Diet and Management

The animal care and use protocol were approved by the Institutional Animal Care and Use Committee of South China Agricultural University (SCAU-10564), and the study was performed following the Regulations for the Administration of Affairs Concerning Experimental Animals.

A total of 216 cannulated male Muscovy ducks of 20-week old were weighed individually (4.20 ± 0.24 kg) and randomly divided into 2 experiments to investigate the effects of dietary ME and CP levels on the physiological parameters in the jejunal fluid. Each experiment includes 6 treatments with 6 replicates per treatment and 3 ducks for each replicate (Exp. 1 and Exp. 2). The corn-soybean meal–based diets were formulated for Exp. 1 and 2 in accordance with China Agricultural Industry Standards (NY/T; NY/T, 2012). In Exp. 1, the nutrient levels of 6 experiment diets including grade ME levels (11.0, 11.5, 12.0, 12.5, 13.0, and 13.5 MJ/kg) were formulated to meet the nutrients requirements of Muscovy ducks (CP, 145 g/kg; Ca, 8.0 g/kg; P, 5.5 g/kg; lysine, 6.0 g/kg; methionine, 3.5 g/kg) except ME. In Exp. 2, the nutrient levels of 6 experiment diets including grade CP levels (140, 155, 170, 185, 200, and 215 g/kg) were formulated to meet the nutrients requirements of Muscovy ducks (ME, 11.3 MJ/kg; Ca, 8.0 g/kg; P, 5.5 g/kg; lysine, 6.0 g/kg; methionine, 3.5 g/kg) except CP.

All ducks were cannulated in accordance with the procedure described by Zhao (2006) to collect the jejunal fluid 3 times daily. Feed and water were provided ad libitum for cannulated ducks during the experimental period. In Exp 3, forty-two ducks were weighed and housed in individual cages (0.50 m length × 0.42 m width × 0.55 m height) and the cages were maintained in an environmentally controlled room (25°C) with 12 h of light per day (Zhao et al., 2007). Six ducks were assigned to determine the energy utilization of each feedstuff and the remaining 6 birds were assigned to receive dextrose for estimation of endogenous losses of nitrogen and energy. The energy feedstuff including corn, sorghum, and barley was fed as the only dietary ingredient, whereas the protein feedstuff including soybean meal, rapeseed meal, and cottonseed meal was tested as part of a complete diet (60% corn starch + 40% test ingredient) as described by Zhao et al. (2014).

In Vivo and In Vitro Digestible Methodology

The in vivo experiment was conducted in accordance with the force-feeding method, whereas the energy utilization in vitro was determined with 5 replicates of each sample using the procedure of the computer-controlled simulated digestion system described by Zhao et al. (2014). In Exp. 1 and 2, jejunal fluid from 3 ducks each replicate was mixed and then centrifuged at 3,000 × g for 10 min in a refrigerated centrifuge immediately. The supernatant was stored at −30°C for the determination of the enzyme activities, electrolyte ion concentrations, and pH values. In Exp.3, the total excreta samples of in vivo digestion were collected during the next 54 h after tube-fed and dried at 55°C for 48 h, after that the excreta samples were ground through a 0.5 mm screen before and then stored at −20°C for the determination of energy utilization in vivo. For the in vitro method, after the simulated digestion process, the undigested residues from each replicate were predried to constant weight and then extracted fat by ethanol; finally, the defatted residues were dried at 105°C to constant weight for the measurement of energy utilization in vitro.

Calculation and Statistical Analysis

The apparent and true energy utilization in vivo and energy utilization in vitro were calculated as follows:

$$\text{Apparent energy utilization} = (\text{EI} - \text{EO} / \text{EI});$$

$$\text{True energy utilization} = (\text{EI} - \text{EO} + \text{FEL} / \text{EI});$$

EI : gross energy intake; EO : gross energy output in the excreta;

FEL : fasting energy loss;

$$\text{Energy utilization in vitro} = [(\text{sample DM weight} \times \text{sample GE}) - (\text{defatted residue DM weight} \times \text{defatted residue GE})]/(\text{sample DM weight} \times \text{sample GE}).$$

All data were subjected to one-way ANOVA by using the General Linear Model procedure of SAS. Orthogonal polynomials contrasts were used to determine the effect (linear and quadratic) in response to increasing dietary ME and CP levels. Treatment means were separated for statistical significance ($P < 0.05$) by Duncan's difference test.

RESULTS AND DISCUSSION

In the present study, the chymotrypsin activity was increased linearly as dietary ME levels were increased (Table 1; $P < 0.01$). The results were agreed with those obtained in geese fitted with a cannula in the jejunum (Yang, 2015). However, Zhao et al. (2007) stated that no difference of chymotrypsin activity was observed in the jejunal fluid of Peking ducks fed diets between the low (11.72 MJ/kg) and high ME (12.76 MJ/kg) levels.

Table 1. Effect of dietary ME and CP levels on digestive enzyme activities and electrolyte ion concentration in the jejunal fluid of Muscovy ducks.

		Enzyme activity			Ionic concentration					
Items[1]		Amylase (U/mL)	Trypsin (U/mL)	Chymotrypsin (U/mL)	Ca^{2+} (umol/mL)	Cl^{-} (umol/mL)	Na^{+} (umol/mL)	K^{+} (umol/mL)	Mg^{2+} (umol/mL)	pH
Dietary ME levels, MJ/kg	11.0	256.40	40.03	6.16^{d}	4.17^{c}	46.50	101.00	12.30^{b}	$4.67^{b,c}$	7.86^{a}
	11.5	261.40	48.19	9.57^{d}	4.34^{c}	49.00	105.80	$11.90^{b,c}$	$4.93^{a,b}$	$7.78^{a,b}$
	12.0	307.30	54.68	14.00^{c}	8.49^{a}	49.70	95.20	14.20^{a}	5.36^{a}	7.73^{b}
	12.5	264.30	48.04	14.71^{c}	6.49^{b}	56.00	109.00	$10.80^{b,c,d}$	$4.77^{b,c}$	7.72^{b}
	13.0	256.40	46.33	18.44^{b}	$4.84^{b,c}$	53.50	110.00	$10.30^{c,d}$	4.45^{c}	7.85^{a}
	13.5	251.30	45.04	24.90^{a}	3.92^{c}	52.80	105.30	9.96^{d}	4.40^{c}	7.85^{a}
SEM		15.50	3.18	1.24	0.54	2.75	4.94	0.56	0.15	0.03
P value	Treatment	0.10	0.07	<0.01	<0.01	0.19	0.14	<0.01	<0.01	<0.01
	Linear	0.59	0.63	<0.01	0.71	0.03	0.01	<0.01	0.01	0.42
	Quadratic	0.15	0.01	0.06	<0.01	0.27	0.06	0.04	<0.01	<0.01
Dietary CP levels, g/kg	140	263.50^{b}	46.89^{c}	2.33^{b}	5.95^{c}	42.48	97.50	11.22	4.92	8.00^{a}
	155	291.60^{b}	$63.57^{a,b,c}$	9.18^{a}	$8.52^{b,c}$	48.35	93.33	12.83	5.28	7.79^{b}
	170	294.40^{b}	$66.53^{a,b}$	9.15^{a}	$11.41^{a,b}$	44.32	86.50	13.44	5.53	7.71^{b}
	185	381.90^{a}	72.84^{a}	8.40^{a}	12.26^{a}	52.98	94.50	14.23	5.48	7.73^{b}
	200	$343.20^{a,b}$	$54.86^{a,b,c}$	11.98^{a}	$11.11^{a,b}$	44.12	97.17	15.04	5.28	7.78^{b}
	215	$338.60^{a,b}$	$51.02^{b,c}$	9.04^{a}	$11.74^{a,b}$	45.22	92.17	15.46	5.33	7.70^{b}
SEM		24.63	5.74	1.44	1.06	2.92	5.32	1.00	0.17	0.03
P value	Treatment	0.03	0.03	0.01	<0.01	0.16	0.72	0.06	0.17	<0.01
	Linear	0.01	0.10	0.01	<0.01	0.70	0.87	<0.01	0.16	<0.01
	Quadratic	0.17	0.01	0.03	0.01	0.12	0.49	0.58	0.04	<0.01

[a-c]Means with different superscripts within the same column differ significantly ($P < 0.05$).
[1]Mean represents 6 observations of 9 samples collected for 1 h every 4 h in 12 h.

The inconsistent results probably due to the differences of the duck breeds (Peking duck vs Muscovy duck), diet composition (corn-soybean meal vs corn-soybean meal-rice hull), as well as ME levels (11.0–13.5 MJ/kg vs 11.72–12.76 MJ/kg). However, dietary ME levels did not affect the activities of amylase and trypsin in the jejunum of Muscovy ducks, which were consistent with the results from Cherry Valley ducks (Jiang et al., 2020) and Peking ducks (Zhao et al., 2007). It is implied that the activities of amylase and trypsin in poultry may display certain adaptability to dietary ME changes in a reasonable range. As with the increased dietary CP levels, the amylase activity was increased linearly (Table 1; $P = 0.01$), whereas the trypsin and chymotrypsin activities were increased quadratically (Table 1; $P < 0.05$). It is suggested that the dietary CP intake as the main factor could stimulate the synthesis of the digestive enzyme when dietary CP content was kept at a normal level. As reported by Zhao et al. (2007), the amylase, trypsin, and chymotrypsin activities in Peking ducks could make the adaptive changes in response to the dietary CP content variation.

In response to dietary ME levels, the concentrations of Ca^{2+}, K^{+}, and Mg^{2+} were increased quadratically as dietary ME levels increased (Table 1; $P < 0.05$). These results were partly agreed with the findings that Ca^{2+} and Mg^{2+} concentrations were increased in the intestine as dietary energy increases (Atinmo et al., 1985). As dietary CP levels were increased, the Ca^{2+} concentrations were increased quadratically (Table 1; $P = 0.01$), but no effects on the concentration of Na^{+} and Cl^{-} in the jejunal fluid. Hashimoto et al. (1996) indicated that the Ca^{2+} absorption tended to be lower in cat fed the

Table 2. Comparison of energy utilization of 6 feedstuffs between in vivo and in vitro digestion in Muscovy ducks.

	In vivo method[1]		In vivo method[1]		In vitro method[2]			
Items	Apparent energy utilization	CV	True energy utilization	CV	Energy utilization	CV	SEM	*P* Value
Corn	78.72^{b}	2.09	85.56^{a}	1.92	78.73^{b}	0.97	0.59	<0.01
Sorghum	80.84^{b}	5.87	88.74^{a}	5.34	81.17^{b}	1.75	0.77	<0.01
Barley	72.39^{b}	1.19	80.08^{a}	1.07	72.95^{b}	0.7	0.43	<0.01
SBM	50.26^{b}	5.45	69.39^{a}	4.08	72.51^{a}	1.47	1.42	<0.01
RSM	48.05^{b}	5.12	64.24^{a}	3.88	67.02^{a}	0.93	1.61	<0.01
CSM	47.54^{b}	2.28	66.02^{a}	1.64	66.61^{a}	1.47	0.47	<0.01

Means with different superscripts (a-b) within the same row differ significantly ($P < 0.05$).
Apparent energy utilization= (gross energy intake - gross energy output in the excreta)/gross energy intake.
True energy utilization= (gross energy intake - gross energy output in the excreta + fasting energy loss)/gross energy intake.
In vitro energy utilization = [(sample DM weight × sample GE) − (defatted residue DM weight × defatted residue GE)]/(sample DM weight × sample GE).
Abbreviations: CSM, cottonseed meal; CV, variable coefficient; RSM, rapeseed meal; SBM, soybean meal.
[1]The values represent the mean of 6 replicates per sample.
[2]The values represent the mean of 5 replicates per sample.

diets with higher CP levels. As reported in chicken previously (Ren et al., 2012), Na^+ and Cl^- as the major cation and anion in the intestine were quickly absorbed by intestinal and then were less affected by changes in dietary nutrient levels. Besides, the pH values were decreased quadratically with the increased dietary ME or CP levels, which was partially in agreement with the results reported by Tazzoli et al. (2013). Therefore, the optimal digestion parameters were screened for in vitro digestion method of energy feedstuffs (amylase 307.26 U/mL, trypsin 54.68 U/mL, chymotrypsin 24.90 U/mL, 104.39 mmol/L Na^+ and 51.25 mmol/L Cl^- and pH 7.79) and protein feedstuffs (amylase 381.88 U/mL, trypsin 72.84 U/mL, chymotrypsin 11.98 U/mL, 93.53 mmol/L Na^+, 46.25 mmol/L Cl^- concentrations are and, pH 7.80), respectively.

The comparative study of energy utilization between in vivo and in vitro was investigated to verify the feasibility and variability of in vitro method using the digestion parameters obtained from Exp. 1 and 2. For energy feedstuffs such as corn, sorghum, and barley, energy utilization in vitro was lower than true energy utilization in vivo (Table 2; $P < 0.01$), with no differences between energy utilization in vitro and apparent energy utilization in vivo. These results were agreed with those obtained in geese fitted with a cannula in the jejunum (Yang, 2015). However, Zhao (2006) reported that energy utilization in vitro using the conical flask system was lower than apparent and true energy utilization in vivo, which may be due to the mechanism of the feedback inhibition from the accumulating the products of enzymatic hydrolysis in a closed system. For protein feedstuffs such as soybean meal, rapeseed meal, and cottonseed meal, the energy utilization in vitro was higher than apparent energy utilization in vivo (Table 2; $P < 0.01$), which were confirmed in Peking ducks (Zhao et al., 2014). Besides, the CV of energy utilization in vitro was smaller than in vivo for each feedstuff, indicating that the degree of variation of energy utilization in vitro was smaller than that in vivo. Therefore, it is concluded that energy utilization in vitro could reflect the degree of apparent energy utilization in vivo for energy feedstuffs and true energy utilization in vivo for protein feedstuffs.

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DISCLOSURES

There is no conflict of interest with any financial organization regarding the material discussed in the article.

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