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Short communication

Developing a silicon T-shaped cannula to sample intestinal digesta in ducks

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ABSTRACT

An experiment was conducted to develop and evaluate a T-shaped cannula for sampling intestinal digesta of ducks. Forty intestinal-cannulated drakes were selected to evaluate the survival rate for the cannulated ducks and body weight (BW) recovery after cannulation. After a recovery period of 30 days, 24 cannulated drakes and 24 intact drakes were selected by BW and divided into 8 replicates with 3 ducks per replication. The corn-soybean based diet 1 with a high metabolizable energy (ME) content and diet 2 with a low ME content were used to compare the digestible ability of cannulated drakes to intact drakes. Energy balance assays consisting of periods 1 (8 days), 2 (22 days) and 3 (22 days) were to determine the endogenous energy loss in 2 groups of ducks, and to determine the ME for the diets 1 and 2 with each using the same 2 groups of ducks, respectively. No significant difference was observed in the BW of the cannulated ducks on day 15 vs. day 0 after the surgical operation. The survival rate for the cannulated ducks was 75% on day 60 after the operation. In the energy balance assay, the BW loss of cannulated ducks was greater ($P < 0.05$) than that of intact ducks in the period 1, however, no significant difference of BW loss was observed between cannulated and intact ducks in the remaining 2 periods. Endogenous energy loss in the period 1, and the ME of diet 1 in the period 2 were not different between cannulated and intact ducks. However, in the period 3, less ($P < 0.01$) ME of diet 2 was observed in cannulated ducks than intact ducks. It indicated the cannulated ducks can be used for a period from 30 to 60 days after the cannulation.

1. Introduction

Intestinal digesta is an important material to study the digestive and absorptive abilities of animals. Cannulation techniques have been applied routinely to determine dietary nutrient digestibility or investigate activities of digestive enzymes in pigs (Mosenthin et al., 2007; Yang et al., 2010; Morales et al., 2012). However, postmortem removal of digestive content from the intestine is a more commonly used method for fowl (Weurding et al., 2001; Kadim et al., 2002; Fan, 2003; Kong and Adeola, 2014). Sampling at slaughter provides only 1 sample from each bird and volume of digesta collection is small. Consequently, a large number of animals are required to obtain sufficient quantities for analysis.

Abbreviations: AME, apparent metabolizable energy; BW, body weight; DM, dry matter; EEL, endogenous energy loss; ME, metabolizable energy; TME, true metabolizable energy.

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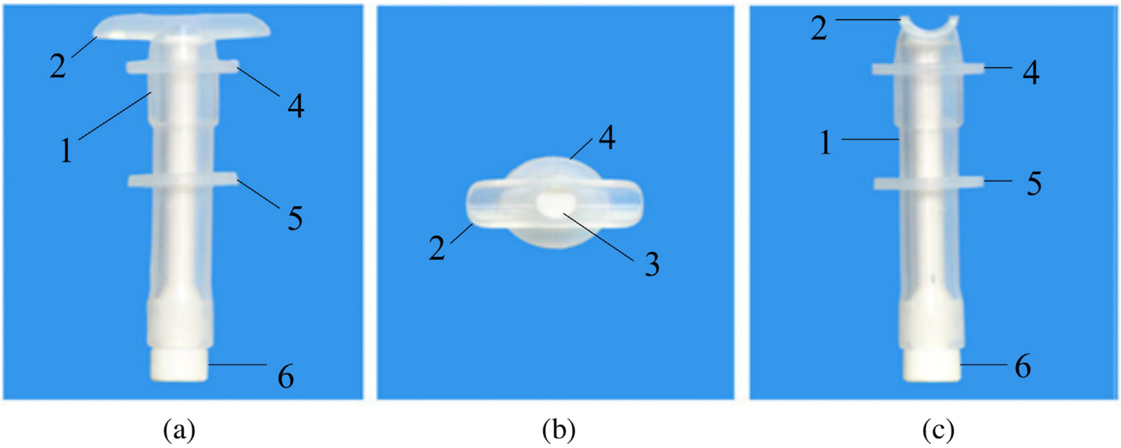

Fig. 1. Silicon T-shaped cannula for duck including 1) barrel, 2) flange, 3) central hole, 4) internal gasket, 5) external gasket and 6) rod. A, b, c are front, vertical, and lateral views of the cannula, respectively.

A surgical cannulation has been developed for roosters which addresses some of the disadvantages associated with postmortem collection (Raharjo and Farrell, 1984; Gurnsey et al., 1985; Van Leeuwen et al., 2000). The cannulated birds can recover in 2 weeks and survival rate through 60 days after surgery is more than 66% (Gurnsey et al., 1985; Van Leeuwen et al., 2000). However, surgical cannulation requires a large and deep incision to open the abdominal cavity. This procedure is associated with the risk of bowel adhesions and cannula rejection, which likely influences the ability of cannulated birds to digest dietary nutrients (Livingstone and Mcwilliam, 1985; Harmon and Richards, 1997). Recently, we developed a novel silicon cannula with a bigger inner diameter than that described by Gurnsey (1986) to repeatedly collect intestinal digesta for poultry. The objectives of the current study were to describe the preparation of a silicon T-shaped cannula for ducks, then investigate the effect of cannulation on the ME of corn-soybean meal-based diets to evaluate whether this cannula can be used to collect intestinal digesta in ducks.

2. Materials and methods

All procedures related to the use of live ducks were approved by the animal care and welfare committee of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China).

2.1. Cannula designation and surgical procedure

The cannula consisted of a barrel with a flange, an internal gasket, an external gasket and a polyethylene rod (Fig. 1). Six grams of medical-grade silicone elastomer (Daxiang Gaoke Co., Ltd, Beijing, China) were injected into a specific mold to prepare a T-shape tubing integrated the barrel, flange and internal gasket of cannula. The size of T-shape tubing was designed in accordance with the diameter of small intestine and the thickness of abdominal wall of adult Peking duck. The barrel (Fig. 1, Part #1) was 55 mm long with an internal diameter of 6 mm and an external diameter of 9 mm. The cannula flange (Fig. 1, Part #2) was a semi-cylinder with an internal diameter of 6 mm and a length of 20 mm. Its central hole (Fig. 1, Part #3) had an internal diameter of 6 mm and connected to the barrel. The internal gasket (Fig. 1, Part #4) was 15 mm in external diameter and 2 mm in thickness, located at 5 mm from the conjunction of barrel and flange. Its function was to prevent the T-shape tubing from pulling out the abdominal cavity of cannulated ducks. The external gasket (Fig. 1, Part #5) was 2 mm in thickness, with an external diameter of 15 mm and a central hole of 9 mm in diameter. It was rolled into the barrel and placed near to skin to prevent the T-shape tubing from sliding into the abdominal cavity of cannulated ducks. The rod (Fig. 1, Part #6) was made of polyethylene with a length of 58 mm. Its diameter was 6 mm and 6.2 mm at the top and bottom, respectively. Cannulas were soaked in sterile saline solution for 12 h then rinsed with 0.9% Nacl solution for use.

Before surgical intestinal cannulation, the ducks were fasted for 16–24 h, then administered 20 mg of sodium pentobarbital/kg of BW via hypodermic injection. Using a full aseptic technique, a 3 cm incision (parallel to the abdominal midline) was made through skin and subcutaneous fat tissues approximately 1.5 cm from the abdomen midline. Subsequently, the muscular layer and peritoneum were cut using blunt dissection. The intestine with the Meckel's diverticulum was elevated to the surface of the incision with a pair of forceps. Approximately 2 cm caudal to the Meckel's diverticulum, a 9 mm longitudinal incision was made on the antimesenteric side. The flange of the cannula was inserted into the lumen of the intestine through the incision. A Murphy purse-string suture (size 4 natural absorbable suture, China Pharmaceutical Group Beijing Medical Equipment Co., Beijing, China) was tightened slowly and gently to close the intestine around the barrel of the cannula. The cannulated intestine was humidified with damp gauze pad to prevent the tissue from drying, then returned to the abdominal cavity. The cannula was exteriorized through a stub incision made through the body wall on the abdomen midline. Then

Table 1
Ingredient and nutrient composition of diets (as-fed basis).

Item	CSM[a] diet 1	CSM diet 2
Ingredient, %		
Corn	71.64	74.03
Soybean meal	23.37	22.51
Soybean oil	1.42	–
Calcium phosphate	1.70	1.95
Limestone	1.23	0.94
Sodium chloride	0.30	0.30
DL-Methionine	0.07	0.04
L-lysine·HCL	0.04	–
Vitamin-mineral premix[b]	0.23	0.23
Analyzed nutrient content		
Dry matter, %	92.75	92.25
Crude protein, %	17.20	16.12
Gross energy, kcal/kg	4169	4025
Calculated nutrient content[c]		
AME, kcal/kg	3200	3120
TME, kcal/kg	3510	3430
Crude fiber, %	2.68	2.66
Ether extract, %	4.70	3.27
Lysine, %	0.88	0.83
Methionine, %	0.36	0.32
Calcium, %	1.02	0.96
Non-phytate phosphorus, %	0.46	0.51

[a] CSM, corn-soybean meal.
[b] Provided the following nutrients (per kg of air-dry diet): Vitamin: A, 2500 IU, D_3, 400 IU, E, 10 IU, K_3, 0.50 mg, B_1, 1.80 mg, B_2, 4 mg, B_6, 3 mg, B_{12}, 7 μg, calcium pantothenate, 11 mg, nicotinic acid, 55 mg, folate, 0.50 mg, biotin, 0.12 mg, choline chloride, 750 mg. Minerals: copper, 8 mg (as copper sulphate), iron, 80 mg (as ferrous sulphate), zinc, 40 mg (as zinc sulhpate), manganese, 60 mg (as manganese sulphate), selenium, 0.15 mg (as sodium selenite), iodine, 0.35 mg (as potassium iodine).
[c] The values are calculated according to the chemical composition of feedstuffs for duck (China Feed Database, 2014).

the peritoneum, muscular layer and skin of the main incision were closed separately with sutures. The external gasket was placed in a position of 20 mm from the bottom of the barrel, then the rod was plugged.

2.2. Ducks and experimental design

To investigate the survival rate and BW recovery of ducks after intestinal cannulation, eighty 14-week-old male white Peking ducks with an average BW of 3.5 kg were randomly assigned to 2 groups of 40 ducks in each and placed into individual cages (0.40 m × 0.38 m × 0.51 m). The cages were housed in a temperature-controlled room at 25 °C for a 12 L:12 D period. Forty ducks from 1 group were surgically cannulated near the Meckel's diverticulum. Each cannulated duck was fed 20 mL of a 10% (wt/vol) dextrose solution 3 times daily for 3 days after the operation. Then, they were provided ad libitum access to water and a corn-soybean meal-based diet 1 designed to satisfy the recommendations of the NRC (1994). The remaining 40 ducks were provided with a slightly restricted (90% of ad libitum amount) corn-soybean meal-based diet 1 and ad libitum access to water in order to keep their BW in accordance with the group of cannulated ducks. After a period of 30 days, 24 ducks from each group were selected according to the average BW of the group and divided into 8 replicates with 3 birds per replication. Water and the corn-soybean meal-based diet 1 were available ad libitum during the adaption and rest periods of bioassay.

To investigate the difference in the ability of cannulated vs. intact ducks to digest a diet, the bioassay method was adopted from that described by Zhang et al. (2007) and included the following: 5 day adaption, feed withdrawal of all ducks for 36 h prior to the feeding test samples, continued feed withdrawal to determine the endogenous energy loss (EEL) or 60 g test samples for force-feeding to determine the ME of diet, a 36 h period of excreta collection, weighed at 8:00 after the 36 h feed withdrawal and after excreta collection. In the current experiment, period 1 was to determine the EEL in 2 groups of ducks. Periods 2 and 3 were to determine the ME of the corn-soybean meal-based diets 1 and 2 with each using the same 2 groups of ducks, respectively. Between 2 periods, all ducks rested for 14 days to recover BW. So, there were 8, 22 and 22 days in the periods 1, 2 and 3, respectively. To avoid separation of ingredient in the test diets, 4 kg of each diet were ground through a 2 mm screen prior to pelleting. Pellets of 4 mm in diameter and 6 mm length were prepared by regulating the ratio of water to feedstuff with a laboratory non-steam press pellet mill (Model SKJ 120, Huaxiang machine Co., Zhangqiu, Shandong, China) and were air-dried until the water content was less than 14% before the bioassay (Table 1).

The excreta collection method was similar to that described by Zhang et al. (2007). After collection of excreta, all excreta samples were dried at 65 °C for 48 h, then re-equilibrated with air for 24 h and ground through a 0.42 mm screen before analysis. The diets and excreta were analyzed for DM (method 934.01) using the procedures of the AOAC (1990). The gross

Table 2
The BW and survival rate of cannulated ducks.

Days after operation	BW, g	Accumulative percentage, % (n = 40)		
		Cannula pull-out[a]	Death	Survival
0	3639	0	0	100
15	3612	0	0	100
30	–	7.5	0	92.5
45	–	12.5	0	87.5
60	–	20.0	5.0	75.0

[a] The cannula was pulled out of the body wall. BW, body weight.

Table 3
The endogenous energy loss and metabolizable energy of corn-soybean meal based diets 1 and 2 determined using cannulated vs. intact ducks.

Item	Duck	BW, g			Energy intake, kcal/bird	Energy output, kcal/bird	AME, kcal/kg	TME, kcal/kg
		Initial BW	Final BW	Weight loss				
Period 1								
Endogenous loss	Cannulated	3409	3119	290		20.99		
	Intact	3531	3268	263		19.10		
SEM		55	52	9		0.77		
P-value		0.123	0.047	0.028		0.114		
Period 2								
Diet 1	Cannulated	3345	3091	254	250.02	56.44	3480	3858
	Intact	3427	3203	224	250.02	55.29	3500	3844
SEM		51	48	12	0.03	0.68	12	12
P-value		0.265	0.105	0.082	0.983	0.250	0.254	0.451
Period 3								
Diet 2	Cannulated	3380	3151	229	241.20	54.65	3376	3756
	Intact	3374	3161	213	241.20	50.72	3447	3793
SEM		72	68	14	0.03	0.46	8	8
P-value		0.941	0.880	0.262	0.717	<0.001	<0.001	<0.001

BW, body weight; AME, apparent metabolizable energy; TME, true metabolizable energy; SEM, standard error of means.

energy contents of the feed and excreta were determined using an automatic adiabatic calorimeter (Model WZR-1TB, Bente Instrument Co., Changsha, Hunan, China); benzoic acid was used as the standard. All analyses were performed in duplicate.

The apparent metabolizable energy (AME) and true metabolizable energy (TME) were calculated as follows: AME (kcal/kg) = (Energy intake − Energy output)/Feed intake; TME (kcal/kg) = (Energy intake − Energy output + Endogenous energy output)/Feed intake (Zhang et al., 2007).

2.3. Statistical analysis

All statistical analysis were performed using the SAS program (SAS Software Release 9.0, SAS Institute Inc., Cary, NC. USA). The data of BW, energy intake, energy output, AME and TME from two treatments of ducks were analyzed using the *t*-test procedure.

3. Results and discussion

BW of cannulated ducks on day 15 after surgical operation were not significantly different from these of ducks before the operation (3612 ± 263 g vs. 3639 ± 298 g, $P > 0.05$) (Table 2). This result indicated that the BW of ducks can recover from the cannulation operation after 15 days. The estimate of 15 days was similar to estimates of 8–21 days for broilers reported by others (Van Leeuwen et al., 2000; Manangi et al., 2007). Of the 40 cannulated ducks, the cannula pulled out of the body wall in 8 ducks, and lameness was occurred in 2 ducks. The survival rate for the cannulated ducks was 75% on day 60 after the operation, which was greater than that reported by Gurnsey et al. (1985) in ileal-cannulated cockerels (66%) and Manangi et al. (2007) in ileal-cannulated hens (70%), but was less than that reported by Van Leeuwen et al. (2000) in ileal-cannulated roosters (greater than 90%). The differences in the survival rates of surgical cannulation between the present and previous studies might relate to the type of cannula, the position of the cannulation in the intestine, and the species of animal (Harmon and Richards, 1997). Our results suggest a surplus of 25% of experimental animal numbers is necessary to use intestinal-cannulated ducks.

The effect of cannulation on the ME of 2 corn-soybean meal-based diets were shown in Table 3. The initial BW of the ducks in each treatment were approximately the same at the start of period 1. However, when the collection of excreta was finished, the final BW of the cannulated ducks were significantly lower than these of the intact ducks ($P < 0.05$), which made the weight loss of the cannulated ducks greater than that of the intact ducks (290 g vs. 263 g; P < 0.05). After a 14-d rest period, the initial BW of the cannulated ducks and the intact ducks in period 2 were recovered to those of the ducks in period 1. When period 2 was finished, no significant differences were observed for the final BW and weight loss of the ducks in the 2 treatments (254 g vs. 224 g; P > 0.05). Similarly, after another 14-d rest period, the initial BW of the ducks from the 2 treatments in period 3 recovered to those of the ducks in period 2. No significant differences were observed for the final BW and weight loss of the ducks in two treatment at the end of period 3 (229 g vs. 213 g; P > 0.05). However, the accumulative mean BW loss in 3 periods for the cannulated ducks was greater than that of the intact ducks. This phenomenon was supported by the findings of Manangi et al. (2007), who reported that bodies of the cannulated animals had to overcome potential reactions to infections and rejections, which might require more nutrients to be consumed.

No significant differences were observed in the values of 20.99 kcal vs. 19.10 kcal of EEL for cannulated duck vs. intact duck. The values of EEL were consistent with the results reported by Adeola (2003); Fan (2003) and King et al. (1997). Therefore, intestinal cannulation had little effect on the endogenous excretions of digestive tract during experimental period 1. In the diet 1, two groups of ducks were fed the same amount of energy (250.02 kcal vs. 250.02 kcal). Values of 56.44 kcal vs. 55.29 kcal of energy outputs (P > 0.05) were observed for the cannulated and intact ducks, respectively. Dietary AME values (3480 kcal/kg vs. 3500 kcal/kg) and TME values (3858 kcal/kg vs. 3844 kcal/kg) did not differ for cannulated and intact ducks, respectively. However, in the diet 2, two groups of ducks were fed the same amount of energy (241.20 kcal vs. 241.20 kcal), but a greater energy outputs (54.65 kcal vs. 50.72 kcal, P < 0.01) were observed for cannulated ducks than intact ducks. Accordingly, AME (3376 kcal/kg vs. 3447 kcal/kg, P < 0.01) and TME (3756 kcal/kg vs. 3793 kcal/kg, P < 0.01) were reduced in cannulated ducks compared to intact ducks, respectively. The energy balance assay used in the present study assumed comparable ME values for a feed when the same group of intact adult male birds was repeatedly used in several experimental periods (China National Standard, 2010), suggesting no influence of experimental period on determined dietary ME values. Consequently, using intact adult ducks, the differences between the ME of diets 1 and 2 determined in the experimental periods 2 and 3, respectively, were only attributed to the diet formulation. In the current study, cannulated ducks had a comparable ability to digest the corn-soybean meal-based diet 1 in the experiment period 2 as intact ducks but less ability than intact ducks to digest the corn-soybean meal-based diet 2 in the experiment period 3. This means the cannulated ducks could have different digestible ability between these 2 experiment periods. Using the ME of feed ingredients for ducks published in the China Feed Database (2014), the values of 3450 and 3382 kcal AME/kg DM and 3784 and 3718 kcal TME/kg DM were estimated for the diets 1 and 2, respectively. The calculated differences between diets 1 and 2 were 68 kcal AME/kg DM and 66 kcal TME/kg DM. Because the ME values of feed ingredients in China Feed Database (2014) were determined with intact adult male Peking ducks using the same bioassay method as this experiment, these differences between the estimated ME of diets 1 and 2 should be similar to the differences between the determined ME of the 2 diets. Our results showed the differences of 53 kcal AME/kg DM and 51 kcal TME/kg DM were observed between the diets 1 and 2 using intact ducks, which was very close to the differences between the estimated ME of diets 1 and 2, indicating the digestive processes of intact ducks were functioning normally in the periods 2 and 3. However, differences of 104 kcal AME/kg DM and 102 kcal TME/kg DM were found between diets 1 and 2 using cannulated ducks. These values were approximately 50% greater than the differences between the estimated ME or the determined ME of diets 1 and 2 in intact ducks, indicating the digestive processes of cannulated ducks were functioning differently in periods 2 and 3. Because the digestive abilities of cannulated ducks were comparable to the intact ducks thus normal in period 2, abnormal digestion might have occurred in period 3. In consideration of 30 days for recovery period, 8 days for period 1, and 22 days for period 2, it deduces the cannulated ducks should be restricted to use for no more than 60 days after operation.

4. Conclusion

Using the novel silicon T-shaped cannula, 15 days for BW recovery and 2 months survival rate of 75% were observed for the cannulated ducks. Little detrimental effect of cannula on the ME of diet was found in the cannulated ducks during a period from 30 to 60 days after cannulation. Thus, this cannula is a potential tool to study of the digestive physiology for ducks, however, a surplus of 25% of experimental animal numbers is necessary and the duration for use of cannulated ducks should be considered.

Conflict of interest

In this study, Feng Zhao contributed to the design of the experiment, cannula preparation, surgical operation, animal experiment, data analysis and writing. Mulin Xie was responsible for the surgical operation, animal feeding, energy balance assay and chemical analysis. Hui Li contributed to the surgical operation and energy balance assay. Hongfu Zhang contributed to the design of the experiment. All authors have no conflicts of interest.

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