# Effects of Multi-Enzyme Preparation on Carcass Traits, Fat Deposition, and Apparent Nutrient Digestibility in Pekin Ducks (Postprint)

**Authors:** Li Fuhuang, Yang Peilong, Chen Yu, Xie Ming Hou Shuisheng, Wen Zhiguo

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## Abstract

This experiment aimed to investigate the effects of dietary complex enzyme preparation levels on carcass traits, body fat deposition, and nutrient apparent digestibility in Beijing force-fed ducks. Ninety-six healthy, uniform-sized male Beijing ducks at 35 days of age were selected and randomly divided into 4 groups, with 6 replicates per group and 4 ducks per replicate. The control group was fed a corn-soybean meal basal diet, while the diets of experimental groups 1, 2, and 3 were supplemented with 100, 200, and 300 mg/kg of complex enzyme preparation in the basal diet, respectively. The feed amounts for the experimental ducks during force-feeding were 260 g and 300 g at 35 and 36 days of age, respectively, and 400 g/d for all groups during the subsequent 5 days. The experimental period lasted 7 days. The results showed: 1) Dietary complex enzyme preparation levels had no significant effects ($P>0.05$) on slaughter weight, dressing percentage, eviscerated weight, eviscerated percentage, leg muscle weight, leg muscle percentage, keel length, and breast width of Beijing force-fed ducks, but had significant effects ($P<0.05$) on weight gain, breast muscle weight, breast muscle percentage, breast muscle thickness, gizzard weight, and gizzard percentage. The breast muscle weight and gizzard weight of Beijing force-fed ducks in experimental groups 2 and 3 were significantly increased by 7.88% and 7.60% compared with the control group ($P<0.05$), respectively. 2) Dietary complex enzyme preparation levels had no significant effects ($P>0.05$) on skin fat weight, skin fat percentage, abdominal fat weight, abdominal fat percentage, liver weight, liver weight percentage, and liver fat percentage of Beijing force-fed ducks. The skin fat thickness in experimental group 1 was significantly increased by 16.2% compared with the control group ($P<0.05$). 3) Dietary complex enzyme preparation levels had no significant effects ($P>0.05$) on dry matter and gross energy apparent digestibility of Beijing force-fed ducks, but had a significant effect ($P<0.05$) on crude protein apparent digestibility of

Beijing force-fed ducks. It can be concluded that dietary supplementation of complex enzyme preparation had no significant effect on body fat deposition in Beijing force-fed ducks, but could improve the apparent digestibility of crude protein in the diet and promote the growth and development of breast muscle and gizzard.

## Full Text

# Effects of Compound Enzyme Preparation on Carcass Traits, Body Fat Deposition and Nutrient Apparent Digestibility of Force-Feeding Pekin Ducks

**LI Fuhuang[1], YANG Peilong[2], CHEN Yu[1], XIE Ming[3], HOU Shuisheng[3], *WEN Zhiguo*[2]**

[1]Beijing Animal Husbandry Service Station, Beijing 100107, China
[2]Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[3]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193, China

### Abstract

This experiment was conducted to investigate the effects of dietary compound enzyme preparation levels on carcass traits, body fat deposition, and nutrient apparent digestibility in force-feeding Pekin ducks. Ninety-six 35-day-old healthy male Pekin ducks with uniform body size were randomly divided into 4 groups with 6 replicates per group and 4 ducks per replicate. The control group was fed a corn-soybean meal basal diet, while experimental groups 1, 2, and 3 were fed the basal diet supplemented with 100, 200, and 300 mg/kg compound enzyme preparation, respectively. Ducks were force-fed 260 g and 300 g of diet at 35 and 36 days of age, respectively, and all groups received 400 g/d during the subsequent 5 days. The experimental period lasted 7 days.

The results showed: 1) Dietary compound enzyme preparation level had no significant effects on dressing weight, dressing percentage, eviscerated weight, eviscerated percentage, leg muscle weight, leg muscle percentage, keel length, or breast width ($P>0.05$), but had significant effects on weight gain, breast muscle weight, breast muscle percentage, breast muscle thickness, gizzard weight, and gizzard percentage ($P<0.05$). Breast muscle weight and gizzard weight in experimental groups 2 and 3 were significantly increased by 7.88% and 7.60% compared with the control group ($P<0.05$). 2) Dietary compound enzyme preparation level had no significant effects on skin and subcutaneous fat weight, skin and subcutaneous fat percentage, abdominal fat weight, abdominal fat percentage, liver weight, liver percentage, or liver fat percentage ($P>0.05$). Skin and subcutaneous fat thickness in experimental group 1 was significantly increased

by 16.2% compared with the control group (P<0.05). 3) Dietary compound enzyme preparation level had no significant effects on apparent digestibility of dry matter and gross energy (P>0.05), but had a significant effect on crude protein apparent digestibility (P<0.05). In conclusion, dietary supplementation with compound enzyme preparation had no significant effect on body fat deposition in force-feeding Pekin ducks, but could improve crude protein apparent digestibility and promote the growth and development of breast muscle and gizzard.

**Key words:** force-feeding Pekin ducks; compound enzyme preparation; carcass traits; body fat deposition; nutrient apparent digestibility

## Introduction

Peking roast duck is renowned both in China and abroad as a typical representative of Beijing' s culinary culture, and its raw material comes from force-fed ducks. In force-feeding duck production, ducks rapidly deposit fat after being fed high-energy diets based on corn. The reason is that carbohydrates entering the animal body are converted into triglycerides through pathways such as the pentose phosphate pathway [1] and glycolysis, ultimately leading to fat deposition in the liver and other adipose tissues. In Pekin ducks, a force-feeding level of 480 g/d can increase skin and subcutaneous fat weight and abdominal fat weight by 18.7% and 39.6%, respectively, and increase liver fat content by 70.5% [2]; meanwhile, force-feeding can also lead to increased intramuscular fat content in Pekin ducks [4], with intramuscular fat content reaching 2.26%–5.57% [5]. Similar results have been found in mule ducks, where force-feeding significantly increased skin fat percentage, abdominal fat percentage, and liver-to-body ratio [3]. Abundant subcutaneous fat and rapid breast muscle growth are essential conditions for the crispy, golden-brown skin and tender, smooth meat of "Peking roast duck."

In force-feeding duck production, increasing feed intake is commonly practiced to enhance skin fat deposition, but weight gain in Pekin ducks is not proportional to feed intake. As feed intake increases, the utilization efficiency of dietary nutrients gradually declines, and some feed is not fully utilized but is directly excreted [2]. Previous studies have found that endogenous enzyme secretion in the digestive tract of force-feeding ducks is insufficient [6], and large amounts of anti-nutritional factors in corn-soybean meal diets also affect the digestion and absorption of nutrients such as proteins and carbohydrates [7]. It remains unclear whether excessive nutrient intake leads to insufficient endogenous enzymes in force-feeding ducks and whether excessive anti-nutritional factors in feed lead to decreased nutrient digestibility.

Since feed enzymes play significant roles in improving animal growth performance, promoting nutrient digestion, and supplementing intestinal endogenous enzymes [8-11], investigating whether adding enzyme preparations to force-

feeding duck diets can supplement digestive tract endogenous enzymes and degrade anti-nutritional factors in feed, thereby ultimately improving nutrient digestibility and reducing feed waste, is of great importance. Based on previous findings that exogenous enzyme supplementation can improve growth performance in force-feeding ducks [12], this study further measured carcass traits, body fat deposition, and nutrient apparent digestibility to explore whether adding compound enzyme preparations can achieve feed savings by improving nutrient digestibility in force-feeding ducks, which is of significant importance.

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## 1. Materials and Methods

**1.1 Enzyme Preparation** The single enzyme preparations were provided by Shandong Sinobite Biotechnology Co., Ltd. The compound enzyme preparation was formulated from various single enzyme preparations, containing per gram: xylanase 12,500 U, cellulase 600 U, amylase 1,200 U, acid protease 12,500 U, and glucoamylase 5,120 U.

**1.2 Experimental Design and Diets** Ninety-six Z4 male Pekin ducks (bred by the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences) were fed conventional diets from 1 to 35 days of age. At 35 days of age, 96 healthy ducks with uniform body size were randomly selected and divided into 4 groups with 6 replicates per group and 4 ducks per replicate. The control group was fed the basal diet, while experimental groups 1, 2, and 3 were fed the basal diet supplemented with 100, 200, and 300 mg/kg compound enzyme preparation, respectively. The composition and nutrient levels of the basal diet are shown in Table 1 . Ducks had free access to water. Force-feeding amounts were 260 and 300 g/d at 35 and 36 days of age, respectively, and all groups received 400 g/d during the subsequent 5 days. The experimental period lasted 7 days. Feed and water were mixed at a ratio of 1.0:1.2 and force-fed mechanically 4 times daily (06:00, 12:00, 18:00, and 23:00). The force-feeding machine was provided by Beijing Daying Hongguang Economic and Trade Co., Ltd.

**1.3 Management** The experiment adopted net rearing, with each replicate housed in a single pen and provided free access to water. During the force-feeding period, the duck house temperature was 22–25 °C, relative humidity was 60%–72%, and artificial lighting was provided with 24 h illumination. Other management practices followed conventional procedures.

**1.4 Sample Collection and Measurements** At 42 days of age, all experimental ducks were fasted (with free access to water) for 12 h and weighed to calculate weight gain (WG) during the force-feeding period. All ducks were then measured for breast muscle thickness (BMT) and skin and subcutaneous fat thickness (SSFT) using a B-ultrasound machine (veterinary, REF411281), and breast width (BW) and keel length (KL) were measured using vernier calipers.

After measurement, ducks were slaughtered by bleeding from the oral cavity. After feather removal and water drainage, carcass weight was recorded. Subsequently, skin with subcutaneous fat, abdominal fat (including fat around the gizzard), breast muscle, leg muscle, gizzard, and liver were collected.

**1.4.1 Carcass Traits** After sample collection, dressed weight (DW), eviscerated weight (EW), breast muscle weight (BMW), leg muscle weight (LMW), and gizzard weight (GW) were measured. Dressing percentage (DP), eviscerated percentage (EP), breast muscle percentage (BMP), leg muscle percentage (LMP), and gizzard percentage (GP) were calculated according to the method of Yang [13].

**1.4.2 Body Fat Deposition** After slaughter, skin and subcutaneous fat weight (SSFW), abdominal fat weight (AFW), and liver weight (LW) were measured, and skin and subcutaneous fat percentage (SSFP), abdominal fat percentage (AFP), and liver percentage (LP) were calculated. A small piece of frozen liver tissue was thawed, minced with ophthalmic scissors, dried at 65 °C to prepare powder, rehydrated at room temperature for 24 h, and reserved for determination of liver moisture and fat content to calculate liver fat percentage (LFP). Liver moisture and fat content were determined by the difference method and Soxhlet extraction method, respectively, following the procedures described by Wang [14].

Skin and subcutaneous fat percentage (%) = (skin and subcutaneous fat weight / eviscerated weight) × 100; Abdominal fat percentage (%) = [abdominal fat weight / (eviscerated weight + abdominal fat weight)] × 100; Liver percentage (%) = [liver weight / (eviscerated weight + liver weight)] × 100; Liver fat percentage (%) = (liver fat content / liver air-dry weight) × 100.

**1.4.3 Nutrient Apparent Digestibility** Nutrient apparent digestibility was determined using 0.5% chromium oxide (Cr2O3) as an external indicator added to the diet. The middle stage of the experimental period (day 5) was selected to determine nutrient apparent digestibility under different compound enzyme preparation levels. On day 5, excreta were collected per replicate every 6 h, immediately mixed with 10% hydrochloric acid (to prevent ammonia volatilization), thoroughly mixed, and 20% of the fresh fecal sample was taken, refrigerated at 4 °C, then dried to constant weight in a 65 °C oven, rehydrated at room temperature for 24 h, ground to pass through a 40-mesh sieve, and stored for analysis. During force-feeding, 100 g of feed samples were collected at different time periods, then mixed thoroughly, ground to pass through a 40-mesh sieve, and stored for analysis.

Determination of Cr2O3 content in feed and excreta: Cr2O3 was digested with a strong oxidant (a mixture of sodium molybdate, concentrated sulfuric acid, and perchloric acid prepared in a certain ratio) and measured at 460 nm wavelength using a spectrophotometer, following the method of Divakaran et al. [15]. Dry

matter (DM) (GB/T 6435–2014) and crude protein (CP) content (GB/T 6432 –1994) in feed and excreta were determined by conventional methods. Gross energy in feed and excreta was measured using an oxygen bomb calorimeter (Parr 6100 calorimeter, USA). The formula for calculating nutrient apparent digestibility was as follows:

Nutrient apparent digestibility (%) = 100 - 100 × [(Cr2O3 content in feed / Cr2O3 content in excreta) × (nutrient content in excreta / nutrient content in feed)].

**1.5 Data Processing** Experimental data were analyzed using SAS 8.0 for one-way ANOVA. Duncan' s multiple comparison test was used for significance testing of differences among group means. Orthogonal polynomials were used to analyze linear and quadratic effects.

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## 2. Results

**2.1 Effects of Compound Enzyme Preparation on Carcass Traits of Force-Feeding Pekin Ducks** As shown in Table 2 , dietary compound enzyme preparation level had significant effects on weight gain, breast muscle weight, breast muscle thickness, gizzard weight, and gizzard percentage of force-feeding Pekin ducks ($P<0.05$). Among these, breast muscle weight, gizzard weight, and gizzard percentage showed significant linear effects ($P<0.05$), while breast muscle thickness showed a significant quadratic effect ($P<0.05$). However, dietary compound enzyme preparation level had no significant effects on other carcass trait indicators ($P>0.05$). Breast muscle weight and gizzard weight reached maximum values in experimental groups 2 and 3, respectively, which were significantly increased by 7.88% and 7.60% compared with the control group ($P<0.05$). Breast muscle thickness showed a trend of increasing first and then decreasing with increasing dietary compound enzyme preparation level, reaching maximum value in experimental group 1, which was significantly higher than the control group ($P<0.05$). Gizzard weight in all experimental groups was significantly higher than in the control group ($P<0.05$), but there were no significant differences among the experimental groups ($P>0.05$). These results indicate that dietary supplementation with compound enzyme preparation can further promote the growth and development of breast muscle and gizzard in force-feeding Pekin ducks.

**Table 2** Effects of compound enzyme preparation on carcass traits of force-feeding Pekin ducks

*In the same row, values with different small letter superscripts mean significant difference ($P<0.05$), while with the same or no letter superscripts mean no significant difference ($P>0.05$). The same as below.*

**2.2 Effects of Compound Enzyme Preparation on Body Fat Deposition of Force-Feeding Pekin Ducks** As shown in Table 3 , dietary compound enzyme preparation level had a significant effect on skin and subcutaneous fat thickness of force-feeding Pekin ducks ($P<0.05$), showing a significant quadratic effect ($P<0.05$), but had no significant effects on skin and subcutaneous fat weight, skin and subcutaneous fat percentage, abdominal fat weight, abdominal fat percentage, liver weight, liver percentage, or liver fat percentage ($P>0.05$). Skin and subcutaneous fat thickness was highest in experimental group 1, which was significantly increased by 16.2% compared with the control group ($P<0.05$). Further addition of compound enzyme preparation (200 and 300 mg/kg) had no significant effect ($P>0.05$). These results indicate that dietary supplementation with compound enzyme preparation increased skin and subcutaneous fat thickness in force-feeding Pekin ducks, and abundant subcutaneous fat is an essential condition for the crispy, golden-brown skin and tender, smooth meat of "Peking roast duck."

**Table 3** Effects of compound enzyme preparation on body fat deposition of force-feeding Pekin ducks

**2.3 Effects of Compound Enzyme Preparation on Nutrient Apparent Digestibility of Force-Feeding Pekin Ducks** As shown in Table 4 , dietary compound enzyme preparation level had no significant effects on apparent digestibility of dry matter and gross energy in force-feeding Pekin ducks ($P>0.05$), but had a significant effect on crude protein apparent digestibility ($P<0.05$). Compared with the control group, experimental group 2 significantly improved crude protein apparent digestibility by 2.9% ($P<0.05$). Crude protein apparent digestibility in experimental groups 1 and 3 showed no significant differences from the control group ($P>0.05$), and there was no significant difference between experimental groups 2 and 3 ($P>0.05$). These results indicate that excessive addition of compound enzyme preparation (300 mg/kg) cannot further improve crude protein apparent digestibility in force-feeding Pekin ducks.

**Table 4** Effects of compound enzyme preparation on nutrient apparent digestibility of force-feeding Pekin ducks

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## 3. Discussion

**3.1 Effects of Compound Enzyme Preparation on Carcass Traits of Force-Feeding Pekin Ducks** Enzymes are active substances produced by living organisms, and the digestion of various nutrients in animals must occur under the action of digestive enzymes. When waterfowl are force-fed large amounts of feed, endogenous enzyme secretion becomes insufficient, pancreatic and digesta enzyme concentrations decrease, and anti-nutritional factors such as non-starch polysaccharides in the diet cannot be fully degraded, resulting in low nutrient utilization efficiency [2,16]. In force-feeding duck production, feed-

ing large amounts of corn-based carbohydrates to achieve rapid fat deposition leads to decreased digestibility. Therefore, supplementation with exogenous enzyme preparations may ameliorate problems encountered in force-feeding duck production.

Numerous studies have confirmed that dietary enzyme supplementation can significantly improve poultry growth rate and reduce feed conversion ratio [17-19]. Kang et al. [20] found that in meat ducks fed corn-rice-soybean meal diets, supplementation with compound enzyme preparation significantly increased daily weight gain and decreased feed intake and feed conversion ratio, which is consistent with our previous research results [12]. Based on previous experiments, this study further explored the effects of compound enzyme preparation on carcass traits of force-feeding Pekin ducks. The results showed that dietary supplementation with compound enzyme preparation could significantly increase breast muscle weight, breast muscle thickness, gizzard weight, and gizzard percentage. Breast muscle weight and gizzard weight reached maximum values in the 200 and 300 mg/kg compound enzyme preparation groups, respectively, which were increased by 7.88% and 7.60% compared with the control group. This is consistent with the research results of Rayan et al. [21] in broilers. Rayan et al. [21] found that dietary supplementation with 200 mg/kg compound enzyme preparation increased breast muscle percentage by 7.5% and significantly increased gizzard weight. The reason may be that adding compound enzyme preparation to force-feeding duck diets can supplement the insufficient endogenous enzymes in the digestive tract, further improve intestinal protein digestion and absorption, and promote protein synthesis in breast muscle and gizzard. Indeed, the significant increase in crude protein apparent digestibility after compound enzyme supplementation in this experiment also validates the conclusion that both breast muscle weight and gizzard weight were significantly increased.

Breast muscle thickness in force-feeding Pekin ducks showed a trend of increasing first and then decreasing with increasing dietary compound enzyme preparation level. Meanwhile, gizzard weight in compound enzyme supplementation groups was significantly higher than in the control group, but there were no significant differences among the compound enzyme supplementation groups, indicating that further increasing dietary compound enzyme preparation level had no obvious effect on force-feeding ducks. This is consistent with the research results of Tang et al. [22] in Cherry Valley ducks. The application effect of enzyme preparations is related not only to temperature and pH but also to enzyme dosage [23]. Within a certain range, as enzyme preparation level increases, its effect on animal growth performance and feed efficiency also increases, but when the addition level exceeds a certain point, animal growth performance does not show significant improvement.

**3.2 Effects of Compound Enzyme Preparation on Body Fat Deposition of Force-Feeding Pekin Ducks** In waterfowl, the main purposes of force-feeding are rapid fat deposition and fatty liver production. The mecha-

nism by which force-feeding induces body fat deposition in waterfowl has been studied in depth [2-3]. When hepatic fatty acid synthesis exceeds catabolism, de novo synthesized triglycerides are stored in the liver and adipose tissues. However, the effect of compound enzyme preparation on body fat deposition in force-feeding Pekin ducks has not been reported. The results of this experiment showed that dietary compound enzyme preparation level had a significant effect on skin and subcutaneous fat thickness of force-feeding Pekin ducks, but had no significant effects on skin and subcutaneous fat weight, skin and subcutaneous fat percentage, liver weight, liver percentage, or liver fat percentage. Abdominal fat percentage and abdominal fat weight showed an increasing trend, but the difference was not significant. Skin and subcutaneous fat thickness was highest in the 100 mg/kg compound enzyme preparation group, which was increased by 16.2% compared with the control group. Further addition of compound enzyme preparation (200 and 300 mg/kg) had little effect.

Within a certain range, as energy intake in poultry gradually increases and exceeds energy expenditure, excess energy accumulates as fat in the body. Meanwhile, fat deposition rate increases linearly with energy level, but when energy level increases to a certain extent, fat deposition remains basically stable [24-26]. Additionally, Wen et al. [2] reported that the appropriate force-feeding amount for Pekin ducks is 390–414 g/d, and excessive energy intake does not further increase duck body weight or body fat deposition. Therefore, the 400 g/d force-feeding amount in this experiment had already reached the maximum energy intake of Pekin ducks, and further addition of compound enzyme preparation may not improve energy utilization efficiency of feed, resulting in no significant differences in body fat deposition. Furthermore, the degree of fat development and deposition in poultry mainly depends on serum triglyceride and liver fat content [25]. Fat in abdominal fat and skin fat of force-feeding Pekin ducks mainly originates from excessive secretion of very low-density lipoprotein (VLDL) from the liver. VLDL is a lipoprotein synthesized in the liver from endogenously produced triglycerides, phospholipids, cholesterol, and apolipoproteins, which is then secreted into the blood. In blood, VLDL is broken down by lipoprotein lipase into triglycerides, which are then synthesized into fat in adipose tissues. In this experiment, compound enzyme preparation had little effect on liver fat content of force-feeding Pekin ducks, and previous experiments found that dietary compound enzyme supplementation did not affect serum triglyceride content. This may be the reason for the lack of significant differences in body fat deposition in this experiment.

**3.3 Effects of Compound Enzyme Preparation on Nutrient Apparent Digestibility of Force-Feeding Pekin Ducks** Numerous studies have shown that dietary enzyme supplementation can improve nutrient apparent digestibility in poultry. Research has found that adding compound enzyme preparation (xylanase, glucanase, amylase) to corn-soybean meal diets increased metabolizable energy by 2.28% and improved amino acid digestibility in broilers [18]. Tang et al. [27] obtained similar results in meat ducks, finding that adding

200 μL/kg compound enzyme preparation to low-energy diets could improve growth performance and ileal nutrient apparent digestibility. However, few studies have investigated the effects of compound enzyme preparation on nutrient apparent digestibility in force-feeding waterfowl. As force-feeding amount increases in Pekin ducks, apparent digestibility of crude protein and dry matter gradually decreases, while energy apparent digestibility remains unchanged [2]. Tafaj et al. [28] also reported that when feeding amounts for dairy cows and sheep were three times their ad libitum intake, nutrient utilization efficiency decreased in both species and body weight gain was not obvious. Generally, the more feed force-fed, the faster the feed passes through the digestive tract and the smaller the effective contact area between the intestine and food, inevitably leading to decreased feed digestibility [29].

Under the conditions of this experiment, with consistent force-feeding amounts, dietary supplementation with 200 mg/kg compound enzyme preparation improved crude protein apparent digestibility in force-feeding Pekin ducks by 2.9% compared with the control group, while dietary compound enzyme preparation had little effect on apparent digestibility of dry matter and gross energy. Excessive addition of compound enzyme preparation (300 mg/kg) could not further improve crude protein digestibility. Therefore, when the force-feeding amount for Pekin ducks is 400 g/d, adding compound enzyme preparation can improve dietary crude protein apparent digestibility and further reduce ammonia and nitrogen emissions. In this experiment, since fat content in feces was low, fat digestibility was not considered, which may have increased analytical error.

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## Conclusions

Dietary supplementation with compound enzyme preparation can increase breast muscle weight, breast muscle thickness, and gizzard weight in force-feeding Pekin ducks. Additionally, dietary compound enzyme preparation supplementation can improve crude protein apparent digestibility in force-feeding Pekin ducks, but has no significant effect on apparent digestibility of dry matter and gross energy.

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*Note: Figure translations are in progress. See original paper for figures.*