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Effects of Golden Apple Snail (*Pomacea Canaliculata*, Lamarck) Shell Particle Size on Growth Performance, Carcass Quality, Bone Strength and Small Intestinal Histology in Thai Native Chickens (Pradu Hang Dum Chiangmai 1)

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Abstract

To study whether the particle size of golden apple snail (*Pomacea Canaliculata*, Lamarck) shell induces negative effects on the growth performance, carcass quality, bone strength and small intestinal histology in Thai native chickens (Pradu Hang Dum Chiangmai 1), 192 chickens, both male and female, were divided into 4 groups with 4 replicates of 12 chickens each at 5 weeks of age. The control group received limestone as a source of calcium. The experimental groups received diets containing golden apple snail shell particles, with sizes ranging from 0:50 to 1:00, from 1.00 to 1.70, and from 1.70 to 2.80 mm as a calcium source. Feed intake and weight were measured weekly, and carcass quality, tibial bone strength and small intestinal histology were observed at 16 weeks of age. The data of growth performance, carcass quality, tibial bone strength and small intestinal histology did not present negative results after feeding any of the golden apple snail shell sizes. On the contrary, a slightly higher weight gain was observed in the group consuming the 1.00 to 1.70 mm snail shell particles during the period from the 13th to the 16th week. Improved carcass quality and pectoralis major and tibial bone strength, as well as significantly increased duodenal villus surface and jejunal crypt cell numbers ($p < 0.05$) were observed in the group fed the 1.00 to 1.70 mm snail shell particles. These results suggest that golden apple snail shell particles between 1.00 and 1.70 mm can improve growth performance due to hypertrophied intestinal function.

Keywords: golden apple snail, growth performance, particle size, Pradu Hang Dum Chiangmai

1. Introduction

The golden apple snail (*Pomacea canaliculata*, Lamarck) was introduced from Argentina to South East Asian countries in 1980 (Naylor, 1996; Mochida, 1991) as a dietary protein supplement and source of income for the rural poor (Matienzo, 1984). However, as consumers disliked the taste of the snail (Ito, 2002), market demand for golden apple snail was poor (Teo, 2006). The golden apple snail egg contains a neurotoxin with a strong lethal effect on the neurons in the spinal cords of mice (Heras et al., 2008; Frassa et al., 2010). Consequently, as snail-farming projects were abandoned, the snail escaped through waterways and canals, and subsequently the snail became an invasive species pest of crop. The snail is now well known as a major and serious rice pest in many regions of Asia, as it damages young rice seedlings (Halwart, 1994; Naylor, 1996; Yusa and Wada, 1999). Though numerous studies dealing with control techniques such as biological (Halwart, 1994b; Teo, 2001), cultural (Teo, 2003) and chemical (Litsinger and Estano, 1993; Palis et al., 1994) have been reported, economic utilization of the snail should also be explored. For example, the golden apple snail was reported to be a useful alternative source of protein for tiger shrimp (Bombeo-Tuburan et al., 1995). In our previous studies, improved egg yolk color (Khotthong et al., 2014) and hypertrophied intestinal epithelial cells (Maneewan et al., 2015) were observed in hens fed dietary 1.00-1.70 mm particle-size golden apple snail shell. Thai native chickens (Pradu Hang Hum) are expensive, and the shells of the golden apple snail are thrown away without being effectively used, and are thus a contributor to environmental pollution. These facts suggest that the golden apple snail might be a useful alternative

source of calcium, one that is cheap and locally available as a native chicken feed ingredient if measured values of birds fed dietary golden apple snail shell do not show a significant decrease compared with those in the control.

The aim of the present study was to study the effects of the size of the particles of golden apple snail shell on the growth performance, carcass quality, tibial bone strength and small intestinal histology in Thai native chickens.

2. Materials and Methods

2.1 Birds and Experimental Design

One hundred and ninety–two Thai native chickens (Pradu Hang Dum Chiangmai 1), both male and female, allotted in completely randomized design (CRD), were divided into 4 groups with 4 replicates of 12 chickens each at 5 weeks of age. The control group received limestone as a source of calcium. Experimental groups received diets containing golden apple snail shell in particle sizes ranging from 0:50 to 1:00, from 1.00 to 1.70, and from 1.70 to 2.80 mm as a source of calcium. Each diet was formulated to contain equal amounts of calcium, protein and calories level from 0-6 weeks of age (Table 1(and from 7-16 weeks of age (Table 2). Birds had *ad libitum* access to feed and water. Weekly feed intake and weight were recorded to calculate aspects of growth performance, such weight gain, feed intake and feed efficiency.

2.2 Growth Performance

After 16 weeks of the trial, the total body weight of 12 chickens in each replicate was measured, and 4 birds showing mean body weight in each replicate were chosen from each of the 4 replicates. An average of these 4 means of body weight from each of the 4 replicates was expressed as a mean body weight per group (n=4). Then, these 4 birds were slaughtered by decapitation, and used to conduct carcass, bone strength and intestinal histological observations.

Table 1. Feed composition of control diet supplemented with limestone and experimental diets supplemented with golden apple snail shell particle size of 0.50-1.00, 1.00-1.70 and 1.70-2.80 mm (0-6 weeks of age)

Ingredients (kg)	Control	Particle size (mm)		
		0.50-1.00	1.00-1.70	1.70-2.80
Corn	58.75	58.40	58.40	58.40
Soybean meal	14.50	14.50	14.50	14.50
Fine rice bran	15.40	15.80	15.80	15.80
Fish meal (61%CP)	9.75	9.75	9.75	9.75
Limestone	0.40	0.00	0.00	0.00
Golden apple snail shell	0.00	0.35	0.35	0.35
Salt	0.50	0.50	0.50	0.50
Methionine	0.20	0.20	0.20	0.20
Premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Cost (bath)	15.19	15. 18	15.18	15.10
Calculated nutrient composition (% air dry)				
Protein	19.06	19.09	19.09	19.09
ME (Kcal ME/kg)	2,970	2,960	2,960	2,960
Fiber	3.65	3.68	3.68	3.68
Fat	5.67	5.72	5.72	5.72
Calcium	0.80	0.80	0.80	0.80
Phosphorus, available	0.39	0.4	0.4	0.4
Methionine	0.59	0.59	0.59	0.59
Lysine	1.04	1.04	1.04	1.04

Premix contains 4.80 million units Vitamins A, 0.96 million units vitamin D3; 3,200 IU vitamin E; 0.30 g vitamin K3; 0.40 mg vitamin B1; 1.60 g vitamin B2; 1.12 mg Vitamin B6; 0.004 g vitamin B 12; 3.76 g pantothenic acid; 6.00 g Nicotinic acid; 0.20 g folic acid; 0.036 g biotin, 24.00 g Fe; 12.40 grams copper, 24.00 g manganese; 16.00 grams zinc; 0.14 grams iodine, 0.04 grams selenium; 0.20 grams preservatives, 0.88 g feed additives; add feed media until 1.00 kg.

Table 2. Feed composition of control diet supplemented with limestone and experimental diets supplemented with golden apple snail shell particle size of 0.50-1.00, 1.00-1.70 and 1.70-2.80 mm (7-16 weeks of age)

Ingredients (kg)	Control	Particle size (mm)		
		0.50-1.00	1.00-1.70	1.70-2.80
Corn	64.00	64.00	64.00	64.00
Soybean meal	9.80	9.80	9.80	9.80
Fine rice bran	17.00	17.00	17.00	17.00
Fish meal (61%CP)	5.50	5.50	5.50	5.50
P14	1.80	1.80	1.80	1.80
Limestone	0.70	0.00	0.00	0.00
Golden apple snail shell	0.00	0.70	0.70	0.70
Salt	0.50	0.50	0.50	0.50
Methionine	0.20	0.20	0.20	0.20
Premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Cost (bath)	13.45	13.44	13.44	13.44
Calculated nutrient composition (% air dry)				
Protein	15.08	15.10	15.10	15.10
ME (Kcal ME/kg)	2,960	2,960	2,960	2,960
Fiber	3.58	3.59	3.59	3.59
Fat	5.70	5.70	5.70	5.70
Calcium	1.14	1.17	1.17	1.17
Phosphorus, available	0.49	0.49	0.49	0.49
Methionine	0.51	0.51	0.51	0.51
Lysine	0.75	0.75	0.75	0.75

Premix contains 4.80 million units Vitamins A, 0.96 million units vitamin D3; 3,200 IU vitamin E; 0.30 g vitamin K3; 0.40 mg vitamin B1; 1.60 g vitamin B2; 1.12 mg Vitamin B6; 0.004 g vitamin B 12; 3.76 g pantothenic acid; 6.00 g Nicotinic acid; 0.20 g folic acid; 0.036 g biotin, 24.00 g Fe; 12.40 grams copper, 24.00 g manganese; 16.00 grams zinc; 0.14 grams iodine, 0.04 grams selenium; 0.20 grams preservatives, 0.88 g feed additives; add feed media until 1.00 kg.

2.3 Carcass Quality and Bone Strength

Among these 4 birds in each replicate, 3 of the birds in each of the 4 replicates were used to measure carcass and bone strength. The weight of the carcass and each body part were recorded in order to calculate the whole carcass and carcass parts percentage. Tibia bone strength data were analyzed, according to Shafer et al. (2005). An average of these measured values from 3 birds in each of the 4 replicates was expressed as a mean value per group (n=4).

2.4 Tissue Sampling

Another single bird from each of the 4 replicates (a total of 4 birds per group) was used to conduct histological observations of the villi in each intestinal segment. The intestinal segments from the gizzard to pancreatic and bile ducts were regarded as duodenum, jejunum from the duct to Meckel's diverticulum and ileum from the diverticulum to the ileocecal-colonic junction. A 2-cm length of intestine was taken from the middle part of each intestinal segment as a tissue sample. After dehydration in graded alcohol, each intestinal segment was embedded in paraplast. Transverse sections were cut into 5-**µm** sections, and every 10^{th} section was collected. After staining with haematoxylin-eosin, the following values were measured using an image analyzer (Nikon Cosmozone IzS, Nikon Co., Tokyo, Japan).

2.5 Villi Number Analysis

From each of the 4 chickens in each group, 8 sections from the duodenum, jejunum and ileum of each chicken were taken, and all villi from these 8 sections were counted. An average of these 8 sections were expressed as a mean

villus number for each bird. Finally, the 4 mean villus numbers from the 4 birds were expressed as a mean villus number for one group (n=4).

2.6 Measurement of Villus Height

To measure villus height, 2 villi in one transversal section having a lamina propria were randomly selected from the duodenum, jejunum and ileum of each chicken. The length from the tip to the base, excluding the intestinal crypt, was measured. A total of 16 villi were counted from 8 different sections from each bird. An average of these 16 villi was expressed as the mean villus height for each bird. Finally, the mean villus heights from the 4 birds were expressed as a mean villus height for one treatment group (n=4).

2.7 Calculating the Villus Area

The villus width was measured at the basal and apical parts, and 2 villi from one transversal section were randomly selected from the duodenum, jejunum and ileum of each chicken. A total of 16 villus widths were counted from 8 different sections from each bird. The apparent villus area was calculated from the villus height, basal width and apical width (Iji et al., 2001). An average of these 16 villus widths was expressed as the mean villus width for each bird. Finally, the mean villus width from the 4 birds was expressed as a mean villus area for one group (n=4).

2.8 Measurement of Crypt Cell Number

From each of the 4 chickens in each group, 8 sections from the duodenum, jejunum and ileum were taken, and all cells within the crypt from these 8 sections were counted. An average of these 8 sections was expressed as a mean cell number for each bird. Finally, the 4 mean cell numbers from the 4 birds were expressed as a mean cell number for one group (n=4).

2.9 Measurement of Tibia Breaking Strengths

The tibias were dried at 105°C for 24 h and placed in a desiccator, after which bone weight was recorded. Tibia breaking strengths (breaking force divided by bone weight expressed as kilograms per gram) were measured using an Instron Model 3115 with a 50-kg-load cell at 50-kg-load range with a crosshead speed of 500mm/min with tibia supported on a 3.35-cm span (Shafer et al., 2001).

3. Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) using CRD. The average value of each experimental result was compared using Duncan's New Multiple Range Test by the SPSS (SPSS. Inc, Chicago, USA) software program, with 95% confidence.

All experimental and animal management procedures were performed according to the Ethics, Principles and Guidelines for the Use of Animals for Scientific Purposes, National Research Council of Thailand.

4. Results

4.1 Growth Performance

The feed intake, body weight gain and feed efficiency of all experimental groups did not significantly differ from those of control group at the ages of 5-8, 9-12, 13-16 and 5-16 weeks (Table 3(. However, most of the feed intake in the experimental groups showed better values than those of the control, and the group supplemented with 1.00 to 1.70 mm particle-size snail shell from weeks 13 to 16 showed a slightly higher weight gain than those of other groups, resulting in improved feed efficiency in the dietary 1.00 to 1.70 mm group during 13-16 weeks of age.

4.2 Carcass Quality and Tibia Bone Strength

The carcass percentage of carcass yield, shank and feet, skeleton, feathers, blood, thighs, superficial pectoral muscle, deep pectoral muscle, whole wings, liver, gizzard, proventriculus, heart, spleen, and head and neck, as well as tibial bone strength did not show a significant difference ($P > 0.05$) among the groups (Table 4). However, the meat parts percentage of carcass yield, thigh and pectoralis minor per live body weight were better in the 1.00 to 1.70 and 1.70-2.80 mm particle-size groups ($P > 0.05$). Live weight, and percentages of pectoralis major and tibia bone strength (kilogram-force) per live body weight tended to increase with increasing levels of snail shell particle size.

4.3 Histological Characteristics of the Small Intestine

Villus height, villus surface area, villus numbers and crypt cell numbers in the control chickens and in the chickens supplemented with golden apple snail shell particles from 0.50-1.00, 1.00-1.70 and 1.70-2.80 mm had no significant ($P> 0.05$) difference among the groups (Table 5). However, the villus surface area of the duodenum

in all experimental groups tended to be broader than in the control, and increased significantly in the 1.00-1.70 and 1.70-2.80 mm particle-size groups ($P < 0.05$). Intestinal crypt cell numbers of the jejunum in all experimental groups tended to be broader than in the control, and the 1.00-1.70 mm particle-size groups showed the highest values.

Table 3. Feed intake, body weight gain and feed efficiency in control chicken and in chickens supplemented with golden apple snail shell particle size of 0.50-1.00, 1.00-1.70 and 1.70-2.80 mm during 5-8, 9-12, 13-16 and 5-16 weeks old (mean ± SEM, n = 4)

Characteristics	Control	Particle size			P-value
		0.50-1.00	1.00-1.70	1.70-2.80	
Feed intake (g/bird)					
Week 5-8	1,125.33±24.08	1,125.14±24.58	1,155.15±32.31	1,150.46±9.91	0.727
Week 9-12	1,945.95±36.14	2,115.04±32.08	2,035.18±106.67	2,155.53±39.37	0.135
Week 13-16	2,747.08±98.61	2,763.15±77.31	2,836.41±65.04	2,879.74±106.29	0.690
Week 5-16	5,818.38±133.30	6,003.36±125.58	6,026.74±141.43	6,185.73±106.34	0.305
Weight gain (g/bird)					
Week 5-8	334.38±4.64	326.77±8.31	322.33±19.56	312.37±1.80	0.565
Week 9-12	472.92±17.35	455.56±17.20	455.02±29.25	454.11±12.54	0.893
Week 13-16	414.23±17.72	395.47±51.98	438.68±28.32	410.19±14.15	0.811
Week 5-16	1,221.53±27.58	1,177.81±34.17	1,216.04±46.01	1,176.67±15.90	0.664
Feed efficiency (%)					
Week 5-8	29.74±0.64	29.05±0.68	27.93±1.67	27.16±0.69	0.292
Week 9-12	24.31±0.85[a]	21.51±0.52[b]	22.39±1.03[ab]	21.07±0.51[b]	0.048
Week 13-16	15.13±0.83	14.49±2.21	15.48±0.98	14.29±0.59	0.711
Week 5-16	21.00±0.32	19.67±0.91	20.18±0.68	19.04±0.45	0.175

[a-c] in the same row show statistically differences ($P<0.05$).

Table 4. Carcass quality and tibia bone strength in control chicken and in chickens supplemented with golden apple snail shell particle size of 0.50-1.00, 1.00-1.70 and 1.70-2.80 mm at 16 weeks old (Mean ± SEM, n = 4)

Items	control	Treatment			P-Value
		0.50-1.00	1.00-1.70	1.70-2.80	
Carcass yield and part yields (%BW)					
Live weight (g)	1,456.70±91.50	1,471.66±38.42	1,513.33±49.21	1,514.16±61.26	0.337
Carcass yield	92.22±0.93	91.42±1.59	93.70±0.51	93.54±0.84	0.389
Shanks and feet	4.34±0.02	4.12±0.31	4.25±2.25	4.52±0.19	0.630
Skeleton	16.42±0.48	15.76±0.33	15.86±0.32	15.78±0.21	0.503
Feather	2.19±0.54	3.27±1.62	1.950.31	2.35±0.44	0.746
blood	5.59±0.83	5.32±0.33	4.36±0.22	4.11±0.46	0.173
Thighs	23.45±0.56	24.36±0.17	24.29±0.59	23.93±0.67	0.620
Pectoralis minor	11.17±0.51	11.94±0.45	12.07±0.35	11.35±0.25	0.356
Pectoralis major	3.70±0.12	3.88±0.05	3.91±0.14	4.03±0.17	0.385
Whole wings	9.35±0.28	9.79±0.21	9.69±0.22	10.69±0.33	0.680
Liver	2.18±0.08	2.07±0.09	2.07±0.13	2.250.18	0.721
Proventriculus	0.43±0.01	0.41±0.04	0.40±0.02	0.41±0.03	0.871
Gizzard	3.25±0.19	3.02±0.37	3.04±0.23	2.68±0.12	0.468
Heart	0.54±0.07	0.49±0.03	0.48±0.33	0.54±0.01	0.675
Spleen	0.34±0.07	0.32±0.03	0.36±0.04	0.44±0.10	0.650
Head and neck	8.42±0.86	8.66±0.24	8.40±0.41	8.72±0.30	0.805
Tibia bone strength (Kilogram-force)	14.23±0.92	16.16±1.96	16.43±1.34	16.48±0.94	0.615

Table 5. Villus height, villus surface area, villus number and crypt cells number in control chicken and in chickens supplemented with golden apple snail shell particle size of 0.50-1.00, 1.00-1.70 and 1.70-2.80 mm at 16 weeks old (Mean ± SEM, n =4)

Items	Treatment				
	control	0.50-1.00	1.00-1.70	1.70-2.80	P-Value
Intestinal villus morphology					
Villus height (mm)					
Duodenum	1.28±0.14	1.27±0.06	1.34±0.02	1.38±0.05	0.781
Jejunum	1.11±0.09	1.13±0.08	0.87±0.09	0.98±0.13	0.260
Ileum	0.68±0.05	0.72±0.08	0.62±0.04	0.66±0.09	0.783
Villus surface (mm^2)					
Duodenum	0.15^{b}±0.01	0.17^{ab}±0.01	0.20^{a}±0.00	0.20^{a}±0.02	0.050
Jejunum	0.13±0.01	0.12±0.00	0.10±0.02	0.13±0.02	0.464
Ileum	0.05±0.00	0.06±0.00	0.06±0.00	0.06±0.01	0.870
Villus numbers					
Duodenum	20.09±1.73	22.28±1.25	17.06±2.96	17.50±1.12	0.237
Jejunum	17.31±1.44	15.44±0.73	15.41±1.09	12.69±2.02	0.196
Ileum	12.75±1.36	15.06±2.06	17.44±1.94	14.66±1.32	0.327
Crypt cells number					
Duodenum	214.56±14.88	260.41±16.73	259.22±24.30	258.09±23.40	0.342
Jejunum	206.84±9.50	276.78±34.13	299.16±31.74	261.53±5.93	0.093
Ileum	209.31±25.67	223.94±32.36	187.41±24.11	231.03±18.56	0.649

$^{a-c}$ in the same row show statistically differences ($P<0.05$).

5. Discussion

Although most Thai native chickens are bred and raised for the sport of cock fighting, the Pradu Hang Dum Chiangmai 1 is also popular as a high-quality meat for human consumption (Kammongkun & Leotaragul, 2015) due to its high protein content, as well as its lower fat, cholesterol and triglyceride levels (Pongduang et al., 2013; Sanchai et al., 2011). As the Pradu Hang Dum is an expensive meat, and as the golden apple snail egg includes a neurotoxin that has a strong lethal effect on the neurons in the spinal cords of mice (Heras et al., 2008; Frassa et al., 2010), and golden apple snail shells are discarded without being effectively used, the aim of the present study was to determine whether conventional limestone could be replaced by golden apple snail shell particles as a source of calcium to economize the feed costs associated with raising the Pradu Hang Dum without a significant decrease in growth performance, carcass weight, and intestinal function. Fundamentally, physiological and nutritional studies on growth performance must be conducted using only males, because physiological functions are affected by egg production and follicular hormones, etc. However, breeders sell the meat of a male and a female together.n the case of Pradu Hang Dum chickens. In addition, as we demonstrated, 1.00-1.70 mm particle-size golden apple snail shell (0.4 % from 0-6 weeks old, 0.7 % from 7-16 weeks old) could be used as a calcium source in layer diets (Khotthong et al., 2014) due to the effect of hypertrophied intestinal epithelial cells (Maneewan et al., 2015); thus, as a fundamental study, these data were applied to actual feeding experiments using male and female chickens until 16 week of age. The size of the calcium particles is an important determining factor in the availability of the calcium source. As eggshell is formed during the night, coarser particles are more advantageous than finer particles due to their slower rate of passing through the digestive system even during night (Tunç & Cufadar, 2015).

In regard to growth performance, a significant decrease was not obtained in any particle size of snail shell compared with the control. On the contrary, the group supplemented with 1.00 to 1.70 mm particle size from the 13the to the 16th week demonstrated improvements in weight gain and feed efficiency. This fact corresponds to the results of other studies that a minimum particle size of limestone less than 1.0 mm did not sustain retention in the gizzard (Rao et al., 1992), and that there was improved egg yolk color (Khotthong et al., 2014) and hypertrophied intestinal epithelial cells (Maneewan et al., 2015) in hens fed dietary 1.00-1.70 mm particle-size snail shell. The reason for the decreased weight gain and feed efficiency from the 5th to the 8th week with

increasing snail shell particle size might be related to the fact that the intestinal function of chicks at this age was not sufficiently developed to digest larger particle sizes of hard snail shell. In fact, after fasting, the decreased villus height recovered quickly in refeeding a powdered diet than in a mash diet (Shamoto et al., 1999). Therefore, it is better to feed powdered snail shell to young birds from 5 to 8 weeks, because the powdered shell is more easily absorbed from the intestine. After 8 weeks old, it is better to feed a coarser particle size due to its slower rate of passing through the digestive system, increasing the chances of nutritional absorption.

The carcass quality and tibia bone strength did not show a decrease after feeding any of the snail shell particle sizes. However, the main meat parts showed greater values in the 1.00 to 1.70 and 1.70-2.80 mm-size groups. It seems that these large particle sizes also play the role of grit in the gizzard to crush other ingested feed ingredients. The finely crushed feed is more easily absorbed from the intestine (Shamoto et al., 1999), resulting in an increase in the live weight and the weights of the main meat parts. On the other hand, coarser particle sizes of fed snail shell pass through the intestine more slowly, increasing the chance of absorption of the snail shell (Rao et al., 1992), resulting in increased tibia bone strength with increasing sizes of snail shell particle.

These results on growth performance accord with increased villus surface area of the duodenum in the 1.00-1.70 and 1.70-2.80 mm golden apple snail shell particle-size groups. Crypt cell numbers in the jejunum in the 1.00-1.70 mm golden apple snail shell particle-size group showed the highest value. As broilers showing an increased body weight gain also had higher villus height (Sittiya et al., 2014), and increased cell mitosis numbers induced improved feed efficiency in broiler chickens (Khonyoung et al., 2015), the present increased villus surface area and crypt cell numbers might be hypertrophied by the coarser particle size of snail shell, inducing improved meat weight gain. These facts suggest that the dietary feeding of 1.00-1.70 mm golden apple snail shell particle size can allow Pradu Hang Hum chickens to be shipped earlier than 16 weeks of age.

In conclusion, the 1.00-1.70 mm golden apple snail shell particle size can improve growth performance due to hypertrophied intestinal function.

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