

DIETARY INCLUSION OF FERMENTED GINGER STRAW EFFECT ON THE GROWTH PERFORMANCE, GASTROINTESTINAL TRACT DEVELOPMENT AND CAECAL FERMENTATION OF FATTENING RABBITS

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Abstract: This experiment was conducted to evaluate the effects of dietary inclusion of fermented ginger straw on the growth performance, gastrointestinal tract development and caecal fermentation of fattening rabbits. A total of 160 45-d-old Laiwu black rabbits were randomly divided into 4 groups and fed 0% (Control), 5, 10 or 15% fermented ginger straw in their diet as a replacement for peanut straw powder. The trial lasted for 7 d of adaptation and 43 d for testing. Growth performance was recorded from 52 to 95 d of age (n=5 per treatment with 30 rabbits, 3 males and 3 females per replicate), TTAD of nutrients from 91 to 95 d of age, and gastrointestinal tract development, caecum fermentation and carcass traits were determined at 95 d of age (n=5 per treatment with 10 rabbits, 1 males and 1 females in per replicate). The results showed that the average daily gain and final body weight in the experimental groups (5, 10 and 15% fermented ginger straw) were higher than in the control group ($P<0.05$). However, the average daily feed intake in the 15% group was higher than in the other groups, while the total tract apparent digestibility of crude protein, ether extract, neutral detergent fibre and acid detergent fibre were lower than in the control group ($P<0.05$), and the relative weights of the stomach, small intestine and caecum content in the 15% substitution group were higher than those in the control group ($P<0.05$). In addition, the thickness of the muscle layer in the 15% substitution group was higher than that in the other groups ($P<0.05$). Moreover, pH and total volatile fatty acids concentration in the caecal content were similar among the 4 groups ($P>0.05$). The current work shows that fermented ginger straw could be used as roughage material in fattening rabbit production up to a dietary dose of 10%.

Key Words: fermented ginger straw, rabbits, growth performance, gastrointestinal tract development, caecal fermentation.

INTRODUCTION

Rabbits are small monogastric herbivorous animals and fibre is one of the main constituents of diets for intensively reared rabbits, as the digestive physiology of the rabbit is well adapted to high intake of plant cell walls (De Blas *et al.*, 1999; Gidenne, 2003; Chen *et al.*, 2019). Peanut straw is widely used in rabbit production in China, and many studies have reported that its use as roughage raw materials in rabbit diets has no adverse effects on growth performance (Li *et al.*, 2014; Liu *et al.*, 2020); moreover, the proper level (15–30%) of peanut straw in the diet could improve the total tract apparent digestibility (TTAD) of nutrients, affect the balance of caecum microbiota and change

Correspondence: Liu G.Y., gongyanliu@foxmail.com. Received August 2021 - Accepted August 2022.
<https://doi.org/10.4995/wrs.2022.16093>

Cite as: Sun H.T., Wang Y., Bai L.Y., Liu C., Xu Y.H., Gao S.X., Jiang W.X., Yang L.P., Liu G.Y. 2022. Dietary inclusion of fermented ginger straw Effect on the growth performance, gastrointestinal tract development and caecal fermentation of fattening rabbits. *World Rabbit Sci.*, 30: 267-276. <https://doi.org/10.4995/wrs.2022.16093>

the proportions of some bacteria with important metabolic functions (Ma *et al.*, 2010; Wu and Guo, 2019). However, there has been a serious shortage of peanut straw in recent years. Therefore, it is important to develop and utilise unconventional feed resources for rabbits according to local conditions. Ginger (*Zingiber officinale*) is one such herb that belongs to the family Zingiberaceae. Research on ginger in recent years has mainly focused on its underground tubers, but there were few reports on above-ground ginger straw with a high fibre content. The annual output of ginger straw in China is nearly 10 million tons. In the main ginger-producing areas, the abandonment and burning of ginger straw is becoming increasingly serious, which brings about a series of environmental problems (Gao *et al.*, 2019). However, the high insoluble fibre (cellulose, hemicellulose and lignin) content (neutral detergent fibre, NDF 47~52%; acid detergent fibre, ADF 28~44%) makes ginger straw hardly digestible by animals (Liu *et al.*, 2021). Fermented feed refers to the fermentation of certain feed materials with beneficial microbial agents to feed animals and it can improve the quality of roughage and animal digestibility (Zhang *et al.*, 2018). Recently, fermented feed has attracted increasing attention because of its multiple benefits. However, no works have been conducted on the use of fermented ginger straw as a substitute for peanut straw powder in fattening rabbit feed. We therefore evaluated the use of fermented ginger straw in the diets of fattening rabbits by examining its effect on production performance, gastrointestinal tract development and caecal fermentation activity.

MATERIALS AND METHODS

Production of fermented ginger straw

After crushing the ginger straw, wheat bran was used to adjust the moisture content of the fermentation material to approximately 50%. Straw Starter (Ecobec Biotechnology Co., Ltd, China), a special fermentation bacteria preparation with *Enterococcus faecalis* ($>1.0 \times 10^9$ colony forming units per gram, CFU/g), *Bacillus subtilis* ($>1.0 \times 10^{10}$ CFU/g) and starch and 200 g Straw Starter was turned into 20 kg of clean water with 2 kg of brown sugar and added to straw. The bacteria could be activated successfully after 4-8 h at 30°C. The activated bacterial liquid was fermented in the appropriate proportion with 1000 kg ginger straw (fresh weight) in plastic bags lined with wool bags and used after compaction and anaerobic fermentation for 5 wk. The nutrient composition of fermented ginger straw is shown in Table 1.

Experimental design

All animal experiments were conducted in strict accordance with the guidelines of Shandong Academy of Agricultural Sciences (SAAS-2020-06) and performed according to the recommendations proposed by Directive 2010/63/EU (EU, 2010). A total of 160 45-day-old fattening Laiwu black rabbits (80 males and 80 females) with body weight of 1202.3 ± 25.9 g were randomly divided into 4 groups with 5 replicates in each group, and each replicate contained 8 rabbits (4 males and 4 females). The rabbits in the 4 groups were fed 0% (Control), 5, 10 or 15% fermented ginger straw (air-dried basis) as a replacement for peanut straw powder in their diets. The experimental diets are shown in Table 2 and were formulated according to the nutritional needs of fattening rabbits from De Blas and Wiseman (2020). The four experimental diets were fully mixed, pelleted and stored in a dark and dry place.

Experimental procedures

After the end of the 7-day pre-feeding period, the 43-day formal trial was started. Two experimental rabbits of the same gender were housed in cages (120×60×40 cm) and had *ad libitum* access to food and water. The rabbits

Table 1: Nutrient composition of fermented ginger straw and peanut straw powder (air-dry basis) %.

Raw material type	GE (MJ/kg)	DM	CP	EE	NDF	ADF	ADL	Ash	Ca	P
Fermented ginger straw	15.63	90.30	7.44	4.40	50.40	40.60	6.20	21.30	1.03	0.21
Peanut straw powder	15.15	89.24	14.50	1.60	42.00	28.30	11.00	16.80	1.64	0.16

GE: gross energy; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

Table 2: Composition and nutrient levels of experimental diets (air-dry basis) %.

Items	Proportion of fermented ginger straw in diet (%)			
	0 (Control)	5	10	15
Raw material composition				
Corn	15.0	15.0	15.0	15.0
Soybean meal	10.0	10.0	10.0	10.0
Wheat bran	20.0	20.0	20.0	20.0
Corn germ meal	15.0	15.0	15.0	15.0
Rice hull powder	7.0	7.0	7.0	7.0
Peanut straw powder	30.0	25.0	20.0	15.0
Fermented ginger straw	0.0	5.0	10.0	15.0
Premix ^a	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0
Nutrient levels ^b				
GE (MJ/kg)	16.20	16.25	16.24	16.29
DM	92.38	91.88	92.32	91.56
CP	16.70	16.20	15.95	15.60
EE	2.40	2.51	2.63	2.72
NDF	40.44	41.85	42.23	42.62
ADF	20.35	21.11	21.70	22.35
ADL	5.45	5.15	4.89	4.62
Ash	11.00	11.23	11.39	11.58
Ca	1.54	1.53	1.49	1.47
P	0.61	0.62	0.63	0.62

GE: gross energy; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin.

^aPremix provided the following per kg of diets: vitamin A 10000 IU, vitamin D₃ 2000 IU, vitamin E 50 mg, vitamin K₃ 2.5 mg, thiamine 5 mg, riboflavin 10 mg, nicotinic acid 20 mg, pantothenic acid 50 mg, folic acid 2.5 mg, vitamin B₁₂ 1 mg, choline chloride 400 mg, Fe 100 mg, Zn 50 mg, Cu 40 mg, Mn 30 mg, I 0.5 mg, Se 0.05 mg, CaHPO₄ 15g, NaCl 5g, Lysine 1.5 g, Methionine 1.5 g; the rest is miscellaneous meal carrier complement.

^bNutrient levels were measured values.

were housed in bicellular cages and in each replicate the 4 cages are side by side. During the experimental period, the rabbits were housed in a controlled closed building and the temperature was controlled between 18 and 25°C.

The weights of the fattening rabbits in each replicate were measured at the beginning and end of the trial and the average daily gain (ADG) was calculated from 52 to 95 d of age. The average daily feed intake (ADFI) was calculated according to total feed intake divided by the total number of experimental days in each replicate. The feed conversion ratio (FCR) was then calculated as the feed intake/weight gain. The ADG, ADFI and FCR calculations did not include the 7-d adaptation period.

The TTAD of dry matter (DM), gross energy (GE), crude protein (CP), ether extract (EE), NDF and ADF in the diets was measured in an *in vivo* digestibility assay carried out with 40 rabbits: 10 rabbits from each diet (5 replicates per diet and 2 rabbits with 1 male and 1 female per replicate) chosen individually as representatives of the live weight, mean and variability within the groups. The feed intake (*ad libitum* access) and total faecal output (caecotrophy was not prevented) were recorded for each rabbit housed in individual metabolic cages over a 4-d period from 91 to 95 d of age after a 3-d period of adaptation to the metabolism cages.

At the age of 95 d (the end of the experiment), the 40 rabbits for the digestion test were weighed and their slaughter weight (SW) and body length (BL) were measured before slaughtering. After bleeding, the pelts, stomachs, small intestines and caeca were removed immediately and weighed before and after emptying their contents. The pH value in each caecum was immediately measured. A sample of caecal contents was collected using a 4 mL cryopreservation

tube (Corning, USA) frozen in liquid nitrogen and stored at -80°C until analysis for caecal fermentation. Additionally, the lengths of the small intestines were measured with a measuring tape.

Measurements and analysis

Determination of nutritional components

The DM (934.01), CP (954.01), EE (920.39) and ash (942.05) contents in the diets and faeces were determined according to Association of Official Analytical Chemists (AOAC) International (2005) procedures. A Kjeldahl nitrogen analyser (Haineng, K9840, China) and a crude fat analyser (Xinjia, SZF-06A, China) were used to determine the CP content ($6.25 \times \text{N}$) and EE, respectively. The gross energy levels of the fermented ginger straw, peanut straw powder, diets and faeces were measured with an automatic oxygen bomb calorimeter (PARR 6300, NO. 4925315, USA). Analysis of fibre components was performed according to Van Soest *et al.* (1991). The calcium and phosphorus contents were determined by inductively coupled plasma atomic emission spectrometry (ICP-EMS; Optima 8000, Perkin Elmer, USA).

Morphological structure of the small intestine

Two-centimetre segments of the jejunum were cut and immediately fixed in 4% paraformaldehyde fix solution (Sangon Biotechnology, E672002, China). The samples were then dehydrated in a graded alcohol series (Aladdin, CAS No. 64-17-5, China), cleared with xylene (Aladdin, CAS No. 1330-20-7, China) and embedded in paraffin. The serial microtome sections ($6 \mu\text{m}$ thick) were stained with haematoxylin-eosin (HE, G1120, Solarbio, China). The villus height, crypt depth, villus width, mucosal layer thickness and muscle layer thickness of 10 slices of each tissue sample were measured at $40\times$ magnification under a light microscope (Nikon, Eclipse Ci-L, Japan) and the ratio of villus height/crypt depth was calculated.

Caecal fermentation

The pH value of the caecal content was determined from each rabbit using a portable pH meter (Electric ant, pH-10 No. 6971473048889, China) within 45 min after slaughter. The ammonia nitrogen concentration was measured using a spectrophotometer according to Weatherburn (1967). The volatile fatty acid (VFA) concentration in the caecal content was measured by 7890-5977 gas chromatography-mass spectrometry (GC-MS; Agilent Technologies Inc. CA, USA) detection. The chromatographic system adopted the Agilent Meteorological Chromatographic System (Agilent 7890, Agilent Technologies, USA), according to the properties of the compound. An HP-5 ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ mm}$) gas chromatography column was used, the injection volume was $1 \mu\text{L}$, the split ratio was 10:1, the carrier gas was high-purity helium and the flow rate was 1.0 mL/min . The initial temperature of the oven was 70°C for 5.0 min, and the temperature increased to 100°C at a rate of 6°C/min . The mass spectrometry system was a quadruple mass spectrometry detection system (Agilent 5977; Agilent Technologies, USA) equipped with an electron bombardment ion source (EI) and Mass Hunter Workstation, and the analytes were detected in selective ion test mode. The optimised conditions for mass spectrometry analysis were as follows: the inlet temperature was 260°C and the fourth stage temperature was 150°C .

Statistical analysis

Data on the growth performance ($n=5$ per treatment with 30 rabbits, 3 males and 3 females in per replicate), TTAD of the nutrients, gastrointestinal tract development, morphological structure of the small intestine and caecal fermentation ($n=5$ per treatment with 10 rabbits, 1 male and 1 female per replicate) were analysed. General linear models (GLMs) in SAS 9.1.3 statistical software (SAS Inst, Inc., Cary, NC, USA) were used to analyse the variance of the data and Duncan's test was used for multiple comparisons. The orthogonal polynomial contrast test was performed to determine the linear and quadratic effects of the inclusion level of fermented ginger straw in diets. The results are expressed as the mean and root mean square error (RMSE) and $P < 0.05$ was the significance level.

RESULTS AND DISCUSSION

Health conditions and growth performance

There was no rabbit weight loss, illness or death during the experiment. Different dietary supplementations with fermented ginger straw had linear or quadratic effects on the ADFI, ADG and FBW of fattening rabbits ($P < 0.05$); the ADG values in the experimental groups were higher than in the control group, and the ADFI in the highest ginger straw group (15% group) was greater than in the other groups ($P < 0.05$). There were no linear or quadratic effects on the FCR due to fermented ginger straw inclusion in the diets of fattening rabbits ($P > 0.05$; Table 3).

During fermentation, antinutritional substances undergo reduction, leading to an increase in the level of lactic acid, which exerts a beneficial effect by stimulating the immune system to maintain animal health (Bo'zena *et al.*, 2021). In previous research, the addition of fermented rapeseed meal to the diet of rabbits was shown to have a beneficial effect on their intestinal microbiota and health status (Wlazio *et al.*, 2021). The effects of dietary fibre on rabbit performance are largely dependent on the different hemicellulose constituents, cell wall complexity and highly variable lignification and particle size (Gidenne, 1992; Carabaño *et al.*, 2001; García *et al.*, 2002b; Romero *et al.*, 2011). Fermentation can change fibre structure and improve the nutritional value of plant materials (Shi *et al.*, 2017). Current research shows that the main constituent of fermented ginger straw is fibre (NDF > 50%), and the ADF was higher than that of peanut straw powder (40.60 vs. 28.30%; Table 1). Many studies have reported that fermented feed contributes to improving feed intake, nutrient utilisation and gut health (Mukherjee *et al.*, 2016; Wang *et al.*, 2018a, 2018b). Dietary energy level is one of the important factors that had a close relationship with animal feed intakes (Panda *et al.*, 2006; Wu *et al.*, 2017). In this study, the higher feed intake in the 15% ginger straw group was due to the increased fibre content of the fermented ginger straw and the lower DP/DE ratio in the diets (13.97 vs. 12.70 g/MJ in the control and 15% groups, respectively; Table 4).

TTAD of nutrients in the experimental diets

The TTAD of CP, EE, NDF and ADF of fattening rabbits were linearly and quadratically affected by different amounts of fermented ginger straw in diets ($P < 0.05$). The TTAD values for CP, EE, NDF and ADF in the higher replacement group (15% group) were lower than those of the control group ($P < 0.05$). However, the TTAD of the DM and GE of rabbits was not linearly or quadratically affected by dietary fermented ginger straw ($P > 0.05$; Table 4).

Fibre plays an important role in the regulation of intestinal transit, gut flora and intestinal mucosal integrity in rabbits (Romero *et al.*, 2011). Several studies have reported that the TTAD of energy, organic matter, DM, CP, EE, NDF and ADF is affected by different sources of fibre (De Blas *et al.*, 1999; Fortun-Lamothe and Boullier, 2007). With a decreasing fibre level in the diet, the improvements in the TTAD of nutrients and the nutritive value were confirmed (Gidenne and Bellier, 2000; Xiccato *et al.*, 2002). Fermentation can change fibre structure and improve the quality of roughage and animal digestibility (Zhang *et al.*, 2018). Current research shows that a higher content of fermented ginger straw in the

Table 3: Effects of dietary inclusion of fermented ginger straw on growth performance of fattening rabbits.

Items	Proportion of fermented ginger straw in diet (%)				RMSE	P-value		
	0 (Control)	5	10	15		Treatment	Linear	Quadratic
IBW ¹ (g)	1419.50	1459.75	1465.75	1483.25	45.055	0.190		
FBW ² (g)	2534.75 ^a	2629.50 ^b	2644.00 ^b	2666.00 ^b	70.442	0.045	0.007	0.013
ADG ³ (g/d)	25.94 ^a	27.20 ^b	27.40 ^b	27.50 ^b	1.328	0.045	0.057	0.009
ADFI ³ (g/d)	104.04 ^{ab}	103.09 ^a	106.59 ^b	111.23 ^c	2.034	<0.001	<0.001	<0.001
FCR ³	4.02	3.81	3.90	4.05	0.229	0.355	0.773	0.948

IBW: initial body weight; FBW: final body weight; ADFI: average daily feed intake; ADG: average daily gain; FCR: feed conversion ratio.

¹ 52 d of age, n=5 per treatment with 40 rabbits, 4 males and 4 females in per replicate.

² 95 d of age, n=5 per treatment with 30 rabbits, 3 males and 3 females in per replicate.

³ 52 to 95 d of age, n=5 per treatment with 30 rabbits, 3 males and 3 females in per replicate. Data were expressed as mean and root mean square error (RMSE), and different letters in the same row denote significant effect ($P < 0.05$).

Table 4: Effects of dietary inclusion of fermented ginger straw on total tract apparent digestibility of nutrients in fattening rabbits.¹

Items	Proportion of fermented ginger straw in diet (%)				RMSE	<i>P</i> -value		
	0 (Control)	5	10	15		Treatment	Linear	Quadratic
Total tract apparent digestibility								
GE	55.80	55.40	55.60	55.20	1.897	0.891	0.431	0.236
DM	54.00	54.20	53.00	53.80	1.061	0.766	0.597	0.438
CP	75.60 ^b	74.40 ^{ab}	74.20 ^{ab}	73.20 ^a	1.275	0.021	0.003	0.003
EE	87.00 ^b	85.40 ^{ab}	84.20 ^a	83.40 ^a	1.605	0.015	0.003	0.006
NDF	28.45 ^b	27.40 ^b	26.53 ^b	23.40 ^a	1.476	<0.001	<0.001	<0.001
ADF	18.04 ^c	16.19 ^{bc}	16.87 ^{ab}	15.27 ^a	0.878	0.001	0.001	0.004
Diet nutritive values (air-dry basis)								
DE (MJ/kg)	9.04	9.03	9.03	8.99				
DP (g/kg)	126.25	120.53	118.35	114.19				
DP to DE ratio (g/MJ)	13.97	13.35	13.11	12.70				

GE: gross energy; DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre; DE: digestible energy; DP: digestible protein.

¹91 to 95 d of age, n=5 per treatment with 10 rabbits, 1 males and 1 females in per replicate. Data were expressed as mean and root mean square error (RMSE), and different letters in the same row denote significant effect ($P<0.05$).

diet impairs the digestibility of CP, EE, NDF and ADF. This might be due to the higher ADF content in diets with increasing fermented ginger straw dose, and the high ADF content causes ginger straw to be poorly digested by rabbits (203.5, 211.1, 217.0 and 223.5 g ADF/kg in the 0%, 5%, 10% and 15% groups, respectively; Table 2).

Gastrointestinal tract development

Different amounts of fermented ginger straw linear or quadratic affected the relative weights of the stomach, small intestine and caecal contents ($P<0.05$), with the 15% substitution group showing higher contents than in the control group ($P<0.05$). In our study, different amounts of fermented ginger straw replacing peanut straw had no linear or quadratic effects on the relative weights of the stomach, small intestine and caecum or the relative length of the small intestine ($P>0.05$; Table 5).

Table 5: Effects of dietary inclusion of fermented ginger straw on gastrointestinal tract development of fattening rabbits.¹

Items	Proportion of fermented ginger straw in diet (%)				RMSE	<i>P</i> -value		
	0 (Control)	5	10	15		Treatment	Linear	Quadratic
Stomach relative weight (g/kg SW)	12.47	11.77	11.80	11.48	0.879	0.358	0.046	0.143
Stomach relative content weight (g/kg SW)	30.65 ^a	36.73 ^a	36.82 ^a	53.36 ^b	4.918	<0.001	<0.001	<0.001
Small intestine relative weight (g/kg SW)	23.48	23.92	24.10	24.87	2.209	0.794	0.392	0.677
Small intestine content relative weight (g/kg SW)	10.27 ^a	11.92 ^{ab}	10.35 ^a	14.56 ^b	2.305	0.031	0.068	0.064
Small intestine relative length (BL)	6.68	7.13	6.92	7.58	0.557	0.111	0.061	0.182
Caecum relative weight (g/kg SW)	25.91	25.33	26.17	24.74	1.865	0.636	0.595	0.471
Caecum content relative weight (g/kg SW)	46.77 ^a	49.22 ^{ab}	50.42 ^{ab}	53.09 ^b	4.345	0.042	0.036	0.639

SW, slaughter weight; BL, body length.

¹At the end of the trial (95 d of age), n=5 per treatment with 10 rabbits, 1 males and 1 females in per replicate. Data were expressed as mean and root mean square error (RMSE), and different letters in the same row denote significant effect ($P<0.05$).

According to Nath *et al.* (2016), knowledge of the biometry of the gastrointestinal tract is essential for management and treatment of digestive problems in New Zealand White rabbits. The fibre source in rabbit feed has important roles in the regulation of intestinal transit, gut flora, intestinal mucosa integrity and digestibility (De Blas *et al.*, 1999; Fortun-Lamothe and Boullier, 2007). There was a linear relationship between the retention time and NDF content in the diet (Fraga *et al.*, 1991). Different levels and types of dietary fibre play important roles in controlling gastrointestinal tract development and digestive content (Margüenda *et al.*, 2012). Generally, dietary fibre fractions change the rate of passage and mucosal functionality, and due to influence on caecal microbiota determine the performance and digestive health of animals (Gidenne, 2015). Furthermore, dietary fibre levels could affect the feed intake and retention time of the caecal contents, while a high-fibre diet can significantly increase the length of the gastrointestinal tract (De Blas *et al.*, 1999; García *et al.*, 1999; García *et al.*, 2002b). The increase in passage rate results in greater emptying of the caecum and stimulates higher feed intake (Vazquez *et al.*, 2018). In our study, the relative weight of the caecal content in the group fed 15% fermented ginger straw was the highest, which is consistent with the report that the caecal content weight was closely related to the ADFI (Liu *et al.*, 2018), and the ADFI level in the 15% group was higher than that in the other groups (104.04, 103.09, 106.59 vs. 111.23 g in the 0, 5, 10 and 15% groups, respectively; Table 3).

Histological evaluation of the small intestine

Fermented ginger straw replacing peanut straw in the rabbit diet had no linear or quadratic significant effect on the villus height, crypt depth, villus height/crypt depth ratio, villus width or mucosal layer thickness of fattening rabbits ($P>0.05$). However, the thickness of the muscle layer in the 15% substitution group was higher than in the other groups ($P<0.05$; Table 6).

The small intestine is the part of the digestive tract where most nutrient absorption occurs in rabbits. The level and type of dietary fibre can affect the morphological structure of the small intestine (Chiou *et al.*, 1994; Yu and Chiou, 1996; Romero *et al.*, 2011). The villus height and muscle layer thickness of the jejunum and colon and the crypt depth of the duodenum and ileum in domestic rabbits were affected by different fibre components in their diet (Chiou *et al.*, 1994). It has been reported that slight changes in jejunal villi were observed when growing rabbits were fed different levels of crude fibre (from 55 to 145 g/kg; Yu and Chiou, 1996). However, a high digestible fibre/starch ratio showed no differences in the villi length or crypt depth (Xiccato *et al.*, 2008). In the present study, the thickness of the muscle layer in the 15% substitution group was higher than that in the other groups, which may be due to the high level of dietary fibre supplied to this group compared with that in the other groups.

Caecal fermentation

The pH, ammoniacal nitrogen and total VFA concentration in the caecal content were similar among the 4 groups ($P>0.05$). In the 15% substitution group, the acetic acid concentration and acetic acid/(propionic acid+butyric acid) ratio were higher than those in the other groups ($P<0.05$). In addition, the propionic acid concentration in this same group was lower than that of the other groups ($P<0.05$). The butyric acid concentration was lower in the higher

Table 6: Effects of dietary inclusion of fermented ginger straw on morphological structure of small intestine of fattening rabbits.¹

Items	Proportion of fermented ginger straw in diet (%)				RMSE	P-value		
	0 (Control)	5	10	15		Treatment	Linear	Quadratic
Villus height (µm)	919.28	765.54	905.70	786.98	200.804	0.520	0.339	0.529
Crypt depth (µm)	123.01	152.41	132.22	134.22	23.659	0.293	0.906	0.928
Villus height/ Crypt depth	7.47	5.14	7.15	6.26	2.183	0.361	0.583	0.490
Villus width (µm)	146.38	183.21	138.83	123.05	31.888	0.054	0.088	0.051
Mucosal layer thickness (µm)	1065.81	900.28	1074.93	1007.53	214.989	0.567	0.652	0.781
Muscle layer thickness (µm)	94.44 ^a	107.48 ^a	113.61 ^a	127.08 ^b	26.809	0.015	0.027	0.234

¹At the end of the trial (95 d of age), n=5 per treatment with 10 rabbits, 1 males and 1 females in per replicate; Data were expressed as mean and root mean square error (RMSE), and different letters in the same row denote significant effect ($P<0.05$).

Table 7: Effects of dietary inclusion of fermented ginger straw on caecal fermentation of fattening rabbits (mg/g caecal contents).¹

Items	Proportion of fermented ginger straw in diet (%)					P-value		
	0 (Control)	5	10	15	RMSE	Treatment	Linear	Quadratic
pH value	7.20	6.87	7.10	6.89	0.310	0.293	0.117	0.050
Ammoniacal nitrogen	12.61	14.09	13.59	13.68	2.208	0.750	0.893	0.747
Total volatile fatty acid (VFA)	52.03	52.42	45.57	51.92	6.176	0.509	0.569	0.854
Acetic acid	31.86 ^a	30.44 ^a	29.75 ^a	39.80 ^b	5.635	0.044	0.032	0.145
Propionic acid	5.27 ^c	4.67 ^b	4.43 ^b	3.91 ^a	0.344	<0.001	<0.001	<0.001
Butyric acid	12.72 ^b	13.29 ^b	8.56 ^a	5.97 ^a	2.118	<0.001	<0.001	<0.001
Acetic acid/(Propionic acid+ Butyric acid)	1.78 ^a	1.73 ^a	2.35 ^a	4.12 ^b	0.520	<0.001	<0.001	<0.001

¹ At the end of the trial (95 days of age), n=5 per treatment with 10 rabbits, 1 male and 1 female per replicate. Data were expressed as mean and root mean square error (RMSE), and different letters in the same row denote significant effect ($P<0.05$).

substitution groups (10 and 15% groups) than in the lower substitution group (5% group) and the control group ($P<0.05$; Table 7).

Fermented feed, owing to its nutritional value and health-promoting properties, can beneficially affect production parameters, nutrient digestibility, gut microbiota and metabolic processes (Grela *et al.*, 2019). Intake of rapidly fermenting fibre has a beneficial effect on the activity and concentration of volatile fatty acids in the caecum and improves the functioning of the intestinal mucosa and the structure of the microbiota (Wlazlo *et al.*, 2021). In addition, ammoniacal nitrogen is an end product of protein fermentation, but it is used for bacteria, in combination with carbon chains produced from carbohydrates fermentation to synthesise new amino acids for bacterial growth (Bovera *et al.*, 2010). In the present trial, it is important to note that the ammonia level in the caecal content of the 4 different groups falls within the normal range indicated for rabbits fed balanced diets. The pH and VFA in the caecal contents are influenced by the nature of the fibre ingested, and they can be taken as indicators of fermentative activity (Carabaño *et al.*, 2009; Shang *et al.*, 2017). VFAs play an important role in the aetiology of digestive disturbances (Abu Hafsa *et al.*, 2021), and their levels are lower with high lignin fibre contents and higher with high pectin fibre contents (García *et al.*, 2000; García *et al.*, 2002a), with the latter also having a tendency to decrease the pH of the caecal contents (García *et al.*, 2002b). In addition, VFA concentrations in the caecum increased when rabbits were fed a high-fibre diet (Liu *et al.*, 2018). It has been reported that the acetate content increased and butyrate content decreased with increasing NDF levels in the diet, whereas propionate was positively correlated with the dietary content of uronic acids (García *et al.*, 2002b). The higher production of acetic acid with fermented ginger straw in diets indicates a more intense fermentation of structural carbohydrates (Bovera *et al.*, 2010). It is reported that acetic acid production is from the fermentation of cellulolytic bacteria, while butyrate acid and propionic acid are from fermentation of non-structural carbohydrates (Bovera *et al.*, 2012). In this study, we found that there were no effects on the caecal pH and total VFA contents in fattening rabbits fed different amounts of fermented ginger straw as a replacement for peanut straw, but the composition of VFAs (acetic acid, propionic acid and butyric acid concentrations and acetic acid/(propionic acid+ butyric acid) ratio) changed, which was caused by the differences in fibre components between the fermented ginger straw and peanut straw, especially ADF differences (40.60 vs. 28.30%, Table 1).

CONCLUSIONS

The use of 10% fermented ginger straw (air-dried basis) as a substitute for peanut straw powder in the diet (DE 9.03 MJ/kg, DP 118.35 g/kg, DP to DE ratio 13.11 g/MJ) of fattening rabbits had no adverse effects on production performance. Up to a dietary dose of 100 g/kg, fermented ginger straw could be considered a raw roughage material for fattening rabbits despite its high ADF content, which leads to a higher feed intake when the usage of fermented ginger straw is too high.

Conflict of interest: No potential conflict of interest was reported by the authors.

Funding: This study was supported by Earmarked Fund for Modern Agro-industry Technology Research System (CARS-43-G-7); Shandong Province Modern Agricultural Industry Technology System (SDAIT-21); Scientific and Technological Problems Project Unveiled by Shandong Academy of Agricultural Sciences (SHJB2021-43).

Acknowledgements: The authors thank the staff of Shandong Zhengyu Rabbit Industry Co., Ltd. for their excellent support during this experiment. Besides, the authors thank American Journal Experts (AJE, www.aje.cn) for English language editing.

REFERENCES

- Abu Hafsa S., Hassan, A., Sabek, A., Elghandour, M., Barbabosa-Pliego A., Alqaisi, Q., Salem, A. 2021. Extracted and characterized humic substances as feed supplement in rabbit feeding: effects on performance, blood metabolites and caecal fermentation activity. *Waste and Biomass Valorization*, 12: 5471-5479. <https://doi.org/10.1007/s12649-021-01392-3>
- Association of Official Analytical Chemists (AOAC). 2005. *Official methods of analyses. 18th ed. AOAC, Maryland, USA.*
- Bożena N., Łukasz, W., Anna, C., Dorota, K., Paweł, B., Małgorzata, R., Marcin, Ł., Mariusz, F. 2021. Effects of fermented rapeseed meal on gastrointestinal morphometry and meat quality of rabbits (*Oryctolagus cuniculus*). *Livest. Sci.*, 251: 104663. <https://doi.org/10.1016/j.livsci.2021.104663>
- Bovera, F., Marono, S., Di Meo C., Piccolo, G., Iannaccone, F., Nizza, A. 2010. Effect of mannan-oligosaccharides supplementation on caecal microbial activity of rabbits. *Animal*. 4: 522-1527. <https://doi.org/10.1017/S1751731110000558>
- Bovera, F., Lestingi, A., Marono, S., Iannaccone, F., Nizza, S., Mallardo, K., de Martino L., Tateo, A. 2012. Effect of dietary mannan-oligosaccharides on in vivo performance, nutrient digestibility and caecal content characteristics of growing rabbits. *J. Anim. Physiol. Anim. Nutr.*, 96: 130-136. <https://doi.org/10.1111/j.1439-0396.2011.01134.x>
- Carabaño, R., García, J., De Blas J.C. 2001. Effect of fibre source on ileal apparent digestibility of non-starch polysaccharides in rabbits. *Animal Sci.*, 72: 343-350. <https://doi.org/10.1017/S1357729800055843>
- Carabaño, R., Villamide, M.J., García, J., Nicodemus, N., Llorente, A., Chamorro, S., Menoyo, D., García-Rebollar P., García-Ruiz A.I., De Blas J.C. 2009. New concepts and objectives for protein amino acid nutrition in rabbits. *World Rabbit Sci.*, 17: 1-14. <https://doi.org/10.4995/wrs.2009.664>
- Chen, S., Deng, F., Jia, X., Liu, H., Zhang, G., Lai, S. 2019. Gut microbiota profiling with differential tolerance against the reduced dietary fibre level in rabbit. *Sci. Rep-UK*, 9: 288. <https://doi.org/10.1038/s41598-018-36534-6>
- Chiou, P.W.S., Yu, B., Lin, C. 1994. Effect of different components of dietary fibre on the intestinal morphology of domestic rabbits. *Comp. Biochem. Physiol. A.*, 108: 629-638. [https://doi.org/10.1016/0300-9629\(94\)90349-2](https://doi.org/10.1016/0300-9629(94)90349-2)
- De Blas C., García, J., Carabaño, R. 1999. Role of fibre in rabbit diets. *Ann. Zootech.*, 48: 3-13. <https://doi.org/10.1051/animres:19990101>
- De Blas C., Mateos, G. 2020. Feed formulation. In: C. De Blas and J. Wiseman, (ed), *Nutrition of the Rabbit*. CABI Publishing, New York, NY, USA. <https://doi.org/10.1079/9781789241273.0000>
- EU. 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off. J. Eur. Union L* 276, 33-79.
- Fortun-Lamothe L., Boullier, S. 2007. A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. *Livest. Prod. Sci.*, 107: 1-18. <https://doi.org/10.1016/j.livsci.2006.09.005>
- Fraga, M.J., Pérez De Ayala P., Carabaño, R., De Blas C. 1991. Effect of type of fibre on rate of passage and on the contribution of soft faeces to nutrient intake of fattening rabbits. *J. Anim. Sci.*, 69: 1566-1574. <https://doi.org/10.2527/1991.6941566x>
- Gao, Q., Sun, Z.M., Zhang, C.B. 2019. Current situation, countermeasures and technology prospect of ginger straw. *Agricultural Equipment and Vehicle Engineering*, 57: 178-181.
- García, J., Carabaño, R., De Blas J.C. 1999. Effect of fibre source on cell wall digestibility and rate of passage in rabbits. *J. Anim. Sci.*, 77: 898-905. <https://doi.org/10.2527/1999.774898x>
- García, J., Carabaño, R., Perez-Alba L., De Blas J.C. 2000. Effect of fibre source on caecal fermentation and nitrogen recycled through cecotrophy in rabbits. *J. Anim. Sci.*, 78: 638-646. <https://doi.org/10.2527/2000.783638x>
- García J., Gidenne T., Falcão-e-Cunha L., De Blas C. 2002a. Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Anim. Res.*, 51: 165-173. <https://doi.org/10.1051/animres:2002011>
- García J., Nicodemus N., Carabaño R., De Blas C. 2002b. Effect of inclusion of defatted grape seed meal in the diet on digestion and performance of growing rabbits. *J. Anim. Sci.*, 80: 162-170. <https://doi.org/10.2527/2002.801162x>
- Grela, E.R., Czech, A., Kiesz, M., Wlazło, Ł., Nowakowicz-Debek B. 2019. A fermented rapeseed meal additive: Effects on production performance, nutrient digestibility, colostrum immunoglobulin content and microbial flora in sows. *Anim. Nutr.*, 5: 373-379. <https://doi.org/10.1016/j.aninu.2019.05.004>
- Gidenne, T. 1992. Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. *Br. J. Nutr.*, 61: 133-146. <https://doi.org/10.1079/BJN19920015>
- Gidenne, T. 2003. Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. *Anim. Feed Sci. Technol.*, 81: 105-117. [https://doi.org/10.1016/S0301-6226\(02\)00301-9](https://doi.org/10.1016/S0301-6226(02)00301-9)

- Gidenne, T. 2015. Dietary fibres in the nutrition of the growing rabbit and recommendations to preserve digestive health: a review. *Animal*, 9: 227-242. <https://doi.org/10.1017/S1751731114002729>
- Gidenne, T., Bellier, R. 2000. Use of digestible fibre in replacement of available carbohydrates effect on digestion, rate of passage and caecal fermentation pattern during the growth of the rabbit. *Livest. Prod. Sci.*, 63: 141-152. [https://doi.org/10.1016/S0301-6226\(99\)00123-2](https://doi.org/10.1016/S0301-6226(99)00123-2)
- Li, H., Zhang, X., Qi, D., Chen, S., Liu, Y., Gu, Z. 2014. Effects of peanut seedlings with different substitution ratios on apparent digestibility of nutrients in growing Rex rabbits. *Chinese J. Anim Nutr.*, 26: 3676-3681.
- Liu, G.Y., Sun, C.R., Zhao, X.Y., Liu, H.L., Wu, Z.Y., Li, F.C. 2018. Effect of substituting guinea grass with sunflower hulls on production performance and digestion traits in fattening rabbits. *World Rabbit Sci.*, 26: 217-225. <https://doi.org/10.4995/wrs.2018.9375>
- Liu, G., Sun, H., Liu, C., Bai, L., Yang, P., Jiang, W., Wang, W., Gao, S. 2020. Effects of different roughage materials on slaughter performance and muscle quality of Minxinan black rabbits. *Chinese J. Anim. Nutr.*, 32: 4277-4284.
- Liu, G., Wang, Y., Zhao, G., Bai, L., Li, M., Sun, H., Xu, Y. 2021. Effects of different ratios of ginger straws replacing peanut seedlings in diets on growth performance, slaughter performance and meat quality of Laiwu black rabbits. *Chinese J. Anim Nutr.*, 33: 1633-1642.
- Ma, J., Guo, D., Tian, H., Li, J., Yang, G. 2010. Evaluation of apparent digestibility and digestibility of main nutrients of peanut straws in meat rabbits. *Feed Industry*, 31: 62-64.
- Margüenda, I., Nicodemus, N., Vadillo, S., Sevilla, L., García-Rebollar P., Villarreal, M., Romero, C., Carabaño, R. 2012. Effect of dietary type and level of fibre on rabbit carcass yield and its microbiological characteristics. *Livest. Sci.*, 145: 7-12. <https://doi.org/10.1016/j.livsci.2011.12.012>
- Mukherjee, R., Chakraborty, R., Dutta, A. 2016. Role of fermentation in improving nutritional quality of soybean meal-a review. *Asian-Austral. J. Anim. Sci.*, 29: 1523-1529. <https://doi.org/10.5713/ajas.15.0627>
- Nath, S.K., Das, S., Kar, J., Afrin, K., Kumar, A., Dash, A., Akter, S. 2016. Topographical and biometrical anatomy of the digestive tract of White New Zealand Rabbit (*Oryctolagus cuniculus*). *J. Adv. Vet. Anim.*, 3: 145-151. <https://doi.org/10.5455/javar.2016.c144>
- Panda, A., Niranjana, M., Reddy, B., Sharma, R. 2006. Influence of dietary energy on growth, immune competence and carcass characteristics of coloured broiler chickens. *Anim. Nutr. Feed Tech.*, 6: 115-121.
- Romero, C., Nicodemus, N., Rodríguez, J.D., García, A.I., de Blas C. 2011. Effect of type of grinding of barley and dehydrated alfalfa on performance, digestion, and crude mucin ileal concentration in growing rabbits. *J. Anim. Sci.*, 89: 2472-2484. <https://doi.org/10.2527/jas.2010-3226>
- Shang, S.M., Wu, Z.Y., Liu, G.Y., Sun, C.R., Ma, M.W., Li, F.C. 2017. Effect of substituting guinea grass with soybean hulls on production performance and digestion traits in fattening rabbits. *World Rabbit Sci.*, 25: 241-249. <https://doi.org/10.4995/wrs.2017.6654>
- Shi C., Zhang Y., Lu Z., Wang Y. (2017). Solid-state fermentation of corn soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* for degrading antinutritional factors and enhancing nutritional value. *J. Anim. Sci. Biotechnol.*, 8: 50. <https://doi.org/10.1186/s40104-017-0184-2>
- Van Soest P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Vazquez, Y., Valdivie, M., Berrios, I., Sosa, E. 2018. Morphometric analysis of the gastrointestinal tract of rabbits fed mulberry forage and sugarcane stems. *Cuban J. Agric. Sci.*, 52: 389-394.
- Wang C., Lin C., Su W., Zhang Y., Wang F., Wang Y., Shi C., Lu Z. 2018a. Effects of supplementing sow diets with fermented corn and soybean meal mixed feed during lactation on the performance of sows and progeny. *J. Anim. Sci.*, 96: 206-214. <https://doi.org/10.1093/jas/skx019>
- Wang C., Su W., Yu Z., Hao L., Wang Y. 2018b. Solid-state fermentation of distilled dried grain with solubles with probiotics for degrading lignocellulose and upgrading nutrient utilization. *Amb. Express.*, 8: 188. <https://doi.org/10.1186/s13568-018-0715-z>
- Weatherburn, M.W. 1967. Phenol hypochlorite reaction for determination of ammonia. *Anal. Chem.*, 39: 971-974. <https://doi.org/10.1021/ac60252a045>
- Wlazlo, Ł., Kowalska, D., Bielanski, P., Chmielowiec-Korzeniowska A., Ossowski, M., Łukaszewicz, M., Czech, A., Nowakowicz-Dębek B. 2021. Effect of fermented rapeseed meal on the gastrointestinal microbiota and immune status of rabbit (*Oryctolagus cuniculus*). *Animal*, 11: 716. <https://doi.org/10.3390/ani11030716>
- Wu, S., Guo, D. 2019. Effect of adding peanut seedling to diet on intestinal flora of rabbit. *Chinese J. Anim Nutr.*, 31: 2735-2744.
- Wu, S.J., Liu, L., Zhu, Y.L., Wang, C.Y., Li, F.C. 2017. Effect of varying the energy density on growth performance, meat quality, caecum fermentation and microbiota of growing Rex rabbits. *Anim. Prod. Sci.*, 57: 14933. <https://doi.org/10.1071/AN14933>
- Xiccato, G., Trocino, A., Sartori, A., Queaque, P.I. 2002. Effect of dietary starch level and source on performance, caecal fermentation and meat quality in growing rabbits. *World Rabbit Sci.*, 10: 147-157. <https://doi.org/10.4995/wrs.2002.487>
- Xiccato, G., Trocino, A., Carraro, L., Fragkiadakis, M., Majolini, D. 2008. Digestible fibre to starch ratio and antibiotic treatment time in growing rabbits affected by epizootic rabbit enteropathy. In *Proc.: 9th World Rabbit Congress, 10-13 June, Verona, Italy. 847-851.*
- Yu, B., Chiou, P.W.S. 1996. Effects of crude fibre level in the diet on the intestinal morphology of growing rabbits. *Lab Anim.*, 30: 143-148. <https://doi.org/10.1258/002367796780865826>
- Zhang, Y., Shi, C., Wang, C., Lu, Z., Wang, F., Feng, J., Wang, Y. 2018. Effect of soybean meal fermented with *Bacillus subtilis* BS12 on growth performance and small intestinal immune status of piglets. *Food Agr. Immunol.*, 29: 133-146. <https://doi.org/10.1080/09540105.2017.1360258>