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Research article

Effects of the fermented soya waste supplementation with various probiotic sources on growth performance of crossbred rabbits

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Abstract

The experiment on the growth of rabbits involved a completely randomized design with six treatments and six replications, with two rabbits with balanced sexual distribution per trial unit. The six treatments included different methods of treating soya waste, including cooking it (T1), and five commercial probiotics, namely the biotic probiotic (T2), garlic powder probiotic (T3), M-VS05 probiotic (T4), UV-Bacillus probiotic (T5), and the alive probiotic (T6). The soya waste was anaerobically fermentedin three days before feeding it to the experimental rabbits. The results showed that the use of probiotic treatments improved nutrient intake, nutrient digestibility, digestible nutrients, and nitrogen retention ($P>0.05$). The T3 treatment resulted in the highest daily weight gain ($P<0.05$) and economic returns for the experimental rabbits. There were no significant differences in the physiological and biochemical indicators of rabbit blood between treatments ($P>0.05$). The group of rabbits fed with T1 treatment (without probiotics) had a much higher density of *E. coli* in their fecesthan the other five groups. These findings indicated that treating anti-nutrients in soya waste with probiotics could replace heat treatment and result in better growth performance, improved daily weight gain, and increased profits for growing rabbits.

Keywords: Agricultural by-product, Feed utilization, Herbivore, Probiotic

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INTRODUCTION

Rabbit husbandry has the potential to be developed as a sector in the Mekong Delta region (Trung and Dong, 2016). Rabbits have the ability to digest fiber and other nutrients well through fermentation in the caecum (Leng, 2006), and are able to convert 20% of the protein in their feed into protein that accumulates in their body (Lebas et al., 1997; Dalle Zotte, 2014). This makes them ideal for taking advantage of natural vegetable sources. Additionally, the Mekong Delta region has an abundance of agro-industrial by-products, such as beer waste, soya waste, catfish fat, and coconut oil, that can be utilized as animal feed. Soya waste is a commonly used protein source with a high protein content (20.7%) that can be used to replace protein from commercial feed in rabbit diets (Dong, 2009). However, soya waste contains anti-trypsin, which inhibits the activity of trypsin and other anti-nutrients, such as lectin, saponin, and phytate, that affect animals' ability to digest and absorb nutrients (Li et al., 2014). People often use heat treatment to treat anti-nutrients (Anderson and Wolf, 1995). However, using this method, soya waste can only be used for a short time and requires both labor and fuel, making it more cost. Furthermore, high heat treatment can reduce the nutritional value of soya waste and does not completely remove the anti-nutrients (Li et al., 2014).

Fermentation is a widely used solution to increase nutrient absorption efficiency (Hotz and Gibson, 2007) and decrease the effects of soybean's anti-nutrient factors (Egounlety and Aworh, 2003). Some research (Frias et al., 2008; Song et al., 2008) has also shown that fermentation has beneficial effects on the degradation of anti-nutrient compounds in soya waste (S.W), thereby enhancing the utilization ability of S.W products (Frias et al., 2008). In addition, commercial yeast products that contain live microorganisms, when supplied in sufficient quantities, confer health benefits to animals by improving gut microorganisms (Bajagai et al., 2016). According to Papatsiros et al. (2014), organic acids, probiotics, prebiotics, phytogenics, and zeolite are effective options to antibiotics in livestock. Based on these benefits, this study was conducted to evaluate the effects of fermented soya waste as rabbit feed on digestibility, economic returns, disease resistance, and physiological and biochemical indices of rabbit blood.

MATERIALS AND METHODS

Site and duration

The experiment was conducted between June 2022 and November 2022 at the Cam Nhung rabbit farm located in the Can Tho city. Samples were analyzed at both the Center Lab Vietnam medical testing center and the laboratory of the Faculty of Animal Sciences, which is part of the College of Agriculture at Can Tho University.

Animals

Seventy-two weaned rabbits, comprising of 36 males and 36 females, were used in the experiment. The rabbits were one month old and had an average weight of 378.0 ± 17.32 g/per. They were housed in individual cages, in pairs consisting of 1 male and 1 female. They were fed twice daily at 7:00

and 18:00 and had free access to clean automatic drinking water. Prior to the experiment, the rabbits were given veterinary medicine and vaccinated against parasitic diseases and rabbit haemorrhagic disease. They were fed a basic diet for 7 days to determine the maximum amount of feed for the rabbits and to balance their diet.

The study was conducted with approval for animal care, housing, and sample collection under the Animal Welfare Assessments of the Scientific Committee Board, College of Agriculture (03.DTB/KNN).

Experimental design and diets

The experiment followed a completely randomized design with six treatments and six replications, and two rabbits per trial unit with balanced sexes. The rabbits were fed a basic diet consisting of *Operculina Turpethum* vine and a concentrate diet (which contained 54% soya waste, 10% broken rice, 32% soybean extraction meal, and 1% mineral (%DM)). The six treatments included different soya waste treatment methods, such as cooking soya waste (T1), and five commercial probiotics: the biotic probiotic (T2), the garlic powder probiotic (T3), the M-VS05 probiotic (T4), the UV-Bacillus probiotic (T5), and the alive probiotic (T6). The experimental diets' ingredients and chemical compositions are shown in Table 1.

The ingredients of probiotic products:

T2: Biotic (1kg product): *Lactobacillus acidophilus*: $2*10^{10}$CFU, *Bacillus subtilis*: $2*10^{10}$CFU, *Saccaharomyces cerevisiae*: $2*10^{10}$CFU, *Aspergillus oryzae*: $2*10^{10}$CFU.

T3: DR- Enzymes support digestion (Garlic powder) (1kg product): *Bacillus subtilis*: $5*10^{6}$CFU, Amylase: 15.000U, *Lactobacillus acidophilus*: $5*10^{9}$CFU, Protease: 5.000U.

T4: M. VS 05 (Active Probiotics) (1kg product): *Saccaharomyces cerevisiae*: $5,6.10^{6}$ CFU.

T5: UV- Bacillus (1kg product): *Bacillus subtilis*: $10*10^{9}$CFU, *Bacillus megaterium*: $10*10^{9}$CFU, *Bacillus polymyxa*: $10*10^{9}$CFU, *Bacillus licheniformis*: $10*10^{9}$CFU, *Bacillus mesentericus*: $10*10^{9}$CFU, *Lactobacillus acidophilus*: $5*10^{8}$CFU.

NT6: alive probiotic (1 liter solution): *Lactobacillus plantarum*: $10*10^{9}$CFU/ml, *Lactococus lactic*: $10*10^{9}$CFU/ml, *Bacillus megaterium*: $10*10^{9}$CFU/ml.

Table 1 The formula of the fermented concentrate diets

Feed (%)	T2	T3	T4	T5	T6
Soya waste	54	54	54	54	54
Soybean extraction meal	32	32	32	32	32
Broken rice	10	10	10	10	10
Mineral	1	1	1	1	1
Probiotics	3	3	3	3	3
Chemical composition					
% DM	20	20	20	20	20
% CP	24	24	24	24	24

DM: dry matter, CP: protein crude, mineral: Vitamin A, Vitamin D3, Vitamin E, ferrous sulfate, zinc oxide, copper sulfate, magnesium sulfate, manganese sulfate, Phytase, Bacillus subtilis, Pediococcus acidilactici, Calcium carbonate. T1: cooked soya waste , T2: fermented soya waste by biotic, T3: fermented soya waste by garlic powder, T4: fermented soya waste by M VS-05, T5: fermented soya waste by UV-Bacillus, T6: fermented soya waste by alive probiotic.

For the T1 treatment, cooked soya waste was mixed with soybean extraction meal (32%), broken rice (10%) and mineral (1%) before feeding for experimental rabbits. The soya waste was anaerobic fermented 3 days before feeding to experimental rabbits.

Table 2 Feed ingredients composition and chemical composition of diets in the experiment

Feed, g fresh form/day	Treatment					
	T1	T2	T3	T4	T5	T6
Operculina Turpethum vine	300	300	300	300	300	300
Treated soya waste	200	200	200	200	200	200
Chemical composition in diets (%DM)						
%CP	19.0	19.0	19.0	19.0	19.0	19.0
%NDF	34.8	34.8	34.8	34.8	34.8	34.8

CP: protein crude , NDF: Neutral detergent fiber, T1: cooked soya waste , T2: fermented soya waste by biotic, T3: fermented soya waste by garlic powder, T4: fermented soya waste by M VS-05, T5: fermented soya waste by UV-Bacillus, T6: fermented soya waste by alive probiotic.

Sampling, measurements, and chemical analysis

Feed offered and refusals were recorded daily in the morning during the experiment. The nutrient digestibility trial was conducted at the 10th week of the experiment and carried out for 6 days continuously. The feeds offered, refusals, feces and urine were collected constantly during 3 days for nutrient digestibility and nitrogen retention measurement. The samples were dried at 60^{0}C for 72 hours, and then crushed through a 1 mm mesh (Cutting Mill SM100, Retsch, Germany) to analyze nutrient components such as DM, OM, Ash and CP in accordance with AOAC (1991). Additionally, the NDF contents were determined by following the methods described by Van Soest et al. (1991). The nutrient digestibility of the diet was measured using the method outlined by McDonald (2010). The weight gain of rabbits was recorded weekly at a specific time.

Blood samples were collected at the end of the 16-week growth experiment. Rabbit blood was drawn from the ear vein in the early morning and before feeding. The blood was then divided into two test tubes - one containing EDTA and the other containing heparin. Immediately after collection, the blood samples were transferred to Center Lab Vietnam for analysis. The hematological parameters were analyzed by machine: Cell-DynR 1700 (manufacturer: Abort). The biochemical parameters were analyzed by machine: Humalyer 2000 (manufacturer: Humen).

The bacterial quantification of *E. coli* in the feces of the experimental rabbits was performed by counting the colonies using the Vietnamese standard method, TCVN 7924-1:2008. After counting the colonies on the plates, the next step was to calculate the number of *E. coli* in 1 gram of feces using the following formula:

$$N = \frac{\sum C}{Vd(n1 + 0{,}1 * n2)}$$

N: total *E. coli* in 1g feces (CFU/g), $\sum C$: the total number of colonies counted at the concentrations, V: volume of dilution used for sample culture (ml), d: countable starting concentration in the dilution series, n1: number of plates counted at concentration d và n2: number of plates counted at the next concentration.

Statistical analysis

Data were calculated the mean value and standard error by using Microsoft Excel 2010. The data of the experiment was analyzed by analysis of variance using the General Linear Model in Minitab 16.1.0 Software (Minitab, 2010). To compare the difference between mean values of treatments, Tukey's test was used (Minitab, 2010).

RESULTS

Feed and nutrients daily intake of experimental rabbits

Operculina turpethum vine and fermented soya waste were used to feed rabbits in the experiment. The *Operculina turpethum* vine was collected daily from areas surrounding the experimental farm. Table 3 shows the nutrient content of the feedstuffs used in the experiment. The values for DM, CP, and NDF were higher in the fermented soya waste that was treated with probiotics, compared to the cooked soya waste treatment. The DM content for the fermented soya waste treated with probiotics ranged from 22.1% to 25.8%, which was higher than the 20.2% observed for the cooked soya waste.

Table 3 Chemical composition of feeds used in the experiment (% DM, with the exception of DM as fed basic)

Feed	DM	OM	CP	NDF	Ash
Operculina Turpethum vine (*O.T*)	13.5	86.3	14.0	41.6	13.7
Cooked soya waste	20.2	94.6	24.5	28.6	5.44
Fermented soya waste by biotic	22.1	95.5	25.1	30.1	4.47
Fermented soya waste by garlic powder	25.7	94.4	24.9	29.2	5.65
Fermented soya waste by M. VS-05	23.9	94.7	25.4	30.4	5.28
Fermented soya waste by UV-Bacillus	25.8	94.6	26.3	33.0	5.45
Fermented soya waste by alive probiotic	23.8	95.3	26.2	37.3	4.65

DM: dry matter, OM: oranic matter, CP: crude protein, NDF: Neutral detergent fiber. Ash: the total mineral.

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Table 4 The feed and nutrient intakes of experimental rabbits

Item	Treatment						SEM	p
	T1	T2	T3	T4	T5	T6		
Nutrient intake daily, g/per/day								
DM *O.T* vine	26.7	25.6	27.1	26.8	27.2	27.9	2.929	0.997
DM treated soya waste	24.0	25.0	31.3	27.9	32.5	28.9	4.595	0.751
Total nutrient intake, g/head/day								
DM	50.7	50.7	58.4	54.7	59.7	56.8	2.618	0.084
OM	45.7	46.0	53.0	49.6	54.2	51.6	1.999	0.125
CP	9.60	9.90	10.5	10.8	12.4	11.5	0.855	0.210
NDF	18.0^{b}	18.2^{b}	20.4^{ab}	19.6^{ab}	22.0^{a}	22.1^{a}	0.799	0.002
Ash	4.96	4.63	5.49	5.14	5.50	5.17	0.237	0.120

a, b: Mean values with different superscripts within the same row are different (P<0.05). DM: dry matter, OM: oranic matter, CP: crude protein, NDF: Neutral detergent fiber. Ash: the total mineral. T1: cooked soya waste , T2: fermented soya waste by biotic, T3: fermented soya waste by garlic powder, T4: fermented soya waste by M VS-05, T5: fermented soya waste by UV-Bacillus, T6: fermented soya waste by alive probiotic.

The intakes of *Operculina turpethum* vine and treated soya waste by the experimental rabbits were similar (P>0.05) among all treatments. Therefore, there were no significant differences in nutrient intakes (P>0.05). However, nutrient intakes, such as DM, OM, CP, and Ash, tended to be higher (P>0.05) in the fermented soya waste treatments. The NDF intake of the fermented soya waste treatments (ranging from 18.2 to 22.1 g/day) was significantly higher (P<0.05) than that of cooked soya waste, which was only 18.0 g/day.

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The nutrient digestibility and digestible nutrient

In the digestibility trial, the digestibility of DM, OM, CP, and NDF, as well as the digestible DM, OM, and CP, were similar among all treatments (P>0.05). However, the T6 treatment had a significantly higher digestible NDF value (P<0.05) at 14.47 g/day compared to 9.98 g/day (T1), 9.79 g/day (T2), and 9.75 g/day (T4).

Table 5 The nutrient digestibility (%) and digestible nutrients (g/day) of experimental rabbits in the nutrient digestibility trial

			Treatment					
Item	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**SEM**	**P**
DMD	74.0	73.3	72.2	70.0	70.3	76.0	3.50	0.828
OMD	73.6	73.0	71.8	70.1	69.9	75.9	3.63	0.851
CPD	72.0	73.9	72.3	69.8	70.9	77.5	3.51	0.707
NDFD	62.0	59.1	59.1	56.1	57.1	69.8	4.85	0.402
The nutrient digestible, g/day								
DDM	35.0	35.9	37.8	36.1	36.7	40.2	3.28	0.900
DOM	32.6	33.5	34.7	34.0	34.2	37.8	3.21	0.905
DCP	6.77	7.44	8.08	7.38	7.67	8.71	0.93	0.764
DNDF	9.98^{b}	9.79^{b}	10.31^{ab}	9.75^{b}	10.54^{ab}	14.47^{a}	1.04	0.023

DMD: dry matter digestibility, OMD: oranic matter digestibility, CPD: crude protein digestibility, NDFD: Neutral detergent fiber digestibility, AshD: the total mineral digestibility. T1: cooked soya waste , T2: fermented soya waste by biotic, T3: fermented soya waste by garlic powder, T4: fermented soya waste by M VS-05, T5: fermented soya waste by UV-Bacillus, T6: fermented soya waste by alive probiotic..

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Table 6 Nitrogen balance, final live weight and daily weight gain of the experimental rabbits

Item	Treatment							
	T1	T2	T3	T4	T5	T6	SEM	P
Nitrogen balance								
N intake (NI), g/day	1.49	1.59	1.77	1.66	1.7	1.78	0.1424	0.710
N retention (NR), g/day	0.60	0.75	0.80	0.83	0.84	1.04	0.1391	0.405
%NR/NI	40.6	46.3	44.3	49.0	49.1	57.0	6.6939	0.639
$NI/W^{0.75}$	1.02	1.11	1.12	1.12	1.12	1.19	0.0609	0.541
$NR/W^{0.75}$	0.42	0.52	0.51	0.56	0.55	0.69	0.0847	0.377
Final live weight, daily weight gain (DWG)								
Initial live weight, g/head	378.0	385.9	386.8	391.0	403.1	407.2	17.32	0.859
Final live weight, g/head	1838	1801	2218	1952	2078	1957	98.7	0.056
Daily weight gain, (g/head/day)	17.4^{ab}	16.8^{b}	21.7^{a}	18.6^{ab}	20.0^{ab}	18.5^{ab}	1.02	0.026
FCR	2.94	3.05	2.53	2.95	3.00	3.09	0.13	0.488

Mean values with different superscripts within the same row are different (P<0.05). FCR: Feed conversion ratio. T1: cooked soya waste , T2: fermented soya waste by biotic, T3: fermented soya waste by garlic powder, T4: fermented soya waste by M VS-05, T5: fermented soya waste by UV-Bacillus, T6: fermented soya waste by alive probiotic.

Nitrogen balance and daily weight gain

The nitrogen intake and retention of the experimental rabbits tended to increase, with no significant differences observed (P>0.05) among the fermented soya waste treatments. Thus, the final live weight of the fermented soya waste treatments (except for T2) tended to be higher than that of the cooked soya waste (T1). However, this difference was not significantly different (P>0.05). The highest daily weight gain of the experimental rabbits was observed in the T3 treatment (21.7 g/head/day), and the lowest was observed in the T2 treatment (16.8 g/per/day) (P<0.05). No significant differences were observed in the feed conversion ratio between the treatments.

Table 7 Economic returns of experimental rabbits

Item (VND)	Treatment					
	T1	T2	T3	T4	T5	T6
Feed cost	36,070	35,417	37,929	36,608	38,891	45,036
Veterinary medicine	5,000	5,000	5,000	5,000	5,000	5,000
Breed cost	75,000	75,000	75,000	75,000	75,000	75,000
Total cost	116,070	115,417	117,929	116,608	118,891	125,036
Money of selling broiler rabbits	165,375	162,063	199,593	175,644	186,975	176,094
Differences	49,305	46,646	81,664	59,036	68,084	51,058

Operculina Turpethum: 500 VND/kg, soybean extraction meal: 15,000 VND/kg, soya waste: 1,000 VND/kg, Biotic: 90,000 VND/kg, Garlic powder: 120,000 VND/kg, M VS-05: 35,000 VND/0.5kg, UV-Bacillus: 110,000 VND/kg, Living Probiotic: 300,000 VND/liter, the price of broiler rabbits: 90,000 VND/kg. T1: cooked soya waste , T2: fermented soya waste by biotic, T3: fermented soya waste by garlic powder, T4: fermented soya waste by M VS-05, T5: fermented soya waste by UV-Bacillus, T6: fermented soya waste by alive probiotic.

Economic returns

The feed costs across T1 to T5 treatments varied between 35,000 to 39,000 VND. However, T6 treatment had the highest feed cost of 45,036 VND, while the cost of breeding and veterinary medicine remained the same across all treatments. Total income analysis revealed that the highest selling price of broiler rabbits was in T3 treatment, at 199,593 VND/head, while the lowest was in T2 treatment, at 162,063 VND/head.

Table 8 Physiological and biochemical in blood of experimental rabbits

Item	Treatment						SEM	P
	T1	T2	T3	T4	T5	T6		
WBC (10^3/µl)	4.18	4.01	3.05	4.09	4.64	4.33	0.10	0.909
LYM (%)	31.6	29.8	23.0	38.4	49.1	51.3	8.05	0.160
RBC (10^{12}/L)	4.60	5.14	5.05	4.75	5.02	4.60	0.29	0.673
HGB (g/dL)	10.3	11.1	11.3	10.3	11.4	10.4	0.62	0.650
HCT (%)	29.4	31.6	32.2	29.8	32.5	30.0	1.54	0.605
PLT (10^9/L)	293.1	298.0	321.6	350.4	169.8	296.9	43.0	0.139
Glu (mmol/L)	7.48	8.24	7.97	7.41	7.76	8.60	0.44	0.432
UrE (mmol/L)	4.67	5.90	5.43	4.77	4.73	5.23	0.59	0.644
AST (U/L)	44.6	34.7	80.3	25.5	30.7	35.9	21.4	0.535
ALT (U/L)	51.8	52.0	62.5	38.9	55.6	53.7	11.9	0.825
Protein (g/L)	55.0	56.3	56.6	56.3	54.3	54.0	1.82	0.840
Albumin (g/L)	32.6	31.9	32.6	32.1	32.4	32.2	0.52	0.887
GLO (g/L)	22.3	24.4	24.0	24.2	21.9	21.8	1.49	0.643

GLU: Glucose, Ure: Ure in plasma , AST: aspartate aminotransferase, ALT: Alanine Aminotransferase, Pro: protein in plasma, Glo: Globulin in plasma, Albumin: Albumin in plasma, WBC: white blood cell count , LYM: Lymphocytes, RBC: Red blood cell count , HGB: hemoglobin, HCT: RBC volume ratio, PLT: platelet count. T1: cooked soya waste , T2: fermented soya waste by biotic, T3: fermented soya waste by garlic powder, T4: fermented soya waste by M VS-05, T5: fermented soya waste by UV-Bacillus, T6: fermented soya waste by alive probiotic.

Physiological and biochemical of blood indicators

There were no significant differences in physiological and biochemical indicators of rabbit blood between treatments (P>0.05). However, the fermented soya waste treatments showed a higher red blood cell count (RBC), hemoglobin (HGB), and hematocrit (HCT) content compared to the cooked soya waste (T1) treatment. The number of erythrocytes in the fermented soya waste treatments increased from 4.06-5.14x10^{12}/L. In the T5 treatment, the HGB and the highest HCT rate were 11.4 g/dl and 32.5%, respectively. Blood biochemical indicators such as ALT, AST, protein, albumin, and GLO tended to be higher in the T3 treatment than in the other treatments.

Table 9 Quantitative results of *E. coli* on experimental rabbit feces

Treatments	Number of test samples	N × 10^5 ± SE (CFU/g)
T1	12	5.28^{a} ± 1,038
T2	12	1.73^{bc} ± 0,219
T3	12	2.82^{b} ± 0,688
T4	12	2.91^{b} ± 0,475
T5	12	2.05^{bc} ± 0,290
T6	12	0.42^{c} ± 0,070
Total	72	P=0.001

(N: average number of colonies per gram of sample)

$^{a.b.c}$ Values in the same row bearing at least one common symbol do not differ at P > 0.05.

Quantification of *E. coli* concentration in feces

The results showed that there was a significant difference in the density of *E. coli* in the feces of rabbits fed different diets (P<0.05). The group of rabbits fed with T1 treatment (without probiotics) had a much higher density of *E. coli* than the other five groups. Following this were T3 and T4 treatments (garlic powder and active microorganisms) which contained few types of microorganisms, specifically *Bacillus subtilis* and *Saccharomyces cerevisiae*. The two groups of rabbits in T2 and T5 treatments had a relatively low density of *E. coli* in their feces because the feed ingredients in these treatments contained a significant amount of beneficial microorganisms belonging to the probiotic family, such as *Bacillus subtilis, Bacillus megaterium, Bacillus polymyxa, Bacillus licheniformis, Bacillus mesentericus, Lactobacillus acidophilus, Saccharomyces cerevisiae*, etc. Lastly, the T6 treatment, consisting of alive probiotic solutions that were cultured in the laboratory from beneficial bacteria such as *Lactobacillus plantarum, Lactococcus lactis*, and *Bacillus megaterium*, had the best effect on reducing the presence of *E. coli* bacteria in the feces of the experimental group of rabbits.

DISCUSSION

The change in nutrient content in the fermented soya waste treatments, as compared to the initial one, could be attributed to the decrease in carbohydrate content in the soya waste, induced by the fermentation process utilized by microorganisms. This alteration in nutritional composition of the final mixture has been confirmed by the study conducted by Hong et al. (2004) which showed that fermentation with *Aspergillus Oryzae* improved the nutritional content of soya waste. Similarly, Feng et al. (2007) reported that the fermentation process not only removed anti-nutrients components in the soya waste but also enhanced DM, CP, and Ash. Zamora and Veum (1979) observed a CP content of more than 10% in comparison to before the fermentation process, without altering the essential amino acid components in the soya waste. During fermentation, long protein chains and anti-nutrient compounds as a protein were broken down leading to the growth of small-sized peptides (Hirabayashi et al., 1998). Conversely, the boost in microbial biomass during the fermentation process could also cause the rise in soya waste nutrient contents.

The digestibility of nutrients and digestible nutrient were not significantly different between the treatments. Although, the trial results indicated that the

amounts of DM, OM, and CP in the T5 treatment had improved, it was not significantly higher (P>0.05). This result is similar to the findings of Rotolo et al. (2014), which showed no change in the feed intake and digestibility of CP, OM, and DM when probiotics were added to diets. In some previous studies (Chaudary et al., 1995; Kimsé et al., 2012), the addition of probiotics (S. cerevisiae) also did not change the digestibility of nutrients in rabbits.The DM digestibility in this study was higher than the results of Kovitvadhi et al. (2019) being 55.7-68.4% but the CP digestibility values was similar (60.8-77.8%).
The results of Rotolo et al. (2014) recorded the change in NDF intake and NDF digestibility of the experiment. It was observed that increasing the rate of *S. cerevisiae* in the trial diet altered the digestibility of NDF in rabbits. However, in another study conducted in India, when probiotics containing relatives of *Lactobacillus acidophillus* were combined with *L. casei* and *S. cerevisiae*, there was no effect on the digestibility of NDF, ADF, hemicellulose, and cellulose in New Zealand White rabbits. Instead, the digestibility of CP and crude fiber increased when combined with the Lacto-Sacc group (El-Gaafary et al., 1992). In a separate study, using probiotics containing *Lactobacillus acidophillus* in the diet improved digestibility of DM, CP, and CF of the supplement group compared with the control (Amber et al., 2004). Thus, it is evident that variations in intake and nutrient digestibility of the experiment are dependent on the ability to combine probiotic groups in the diet, as well as the response of experimental animals to environmental conditions. Moreover, the association between probiotic groups and density in the rabbit diet is still a matter of controversy as the success of the experiments varies (Rabie et al., 2011).

According to the research conducted by Rotolo et al. (2014), growth parameters did not affect by diets supplemented with alive probiotics in the final experiment. However, the results showed that rabbits fed with fermented soya waste as probiotic supplementation experienced an improvement in daily weight gain. Although there was no significant difference in final live weight gain and FCR, it was observed that T3 treatment, which employed a combination of *Bacillus subtilis* and *Lactobacillus acidophilus* with the addition of protease and amylase enzymes, had the best effect on growth performance. This result was partly similar to the findings of Amber et al. (2004), reported a significant boost in FCR, daily weight gain, and final live weight of growing NZW rabbits that were fed diets containing *Lact-A-Bac* probiotics. No difference in feed intake was observed in this experiment. Additionally, it was noted that the growth performance of rabbits was positively improved when *Bacillus cereus* was added to their diet (Trocino et al., 2005).

Falcão-e-Cunha et al. (2007) demonstrated that including alive probiotics in the diet of rabbits led to a tendency towards weight gain. However, other studies, such as those by Ismail et al. (2004) and Zanato et al. (2008), did not find a positive effect of probiotics on various measures, including feed conversion ratio (FCR), final weight, intake, and daily weight gain. On the other hand, Phuoc and Jamikorn (2017) found that rabbits fed diets supplemented with *L. acidophilus* or mixed *B. subtilis* and *L. acidophilus* showed higher weight gain and lower FCR during the first two weeks post-weaning compared to the control group, but no difference was observed after that period. In contrast, Eiben et al. (2008) found no significant improvement in growth performance in rabbits aged 35-63 days when fed probiotics (BioPlus2B).

Experimental results have shown that the tendency to increase nitrogen intake and retention in the fermentation treatments was better than in the cooking treatment. This was especially evident in the combined treatments between the fermentation and control treatments, where *Bacillus* and *Lactobacillus* brought about more stable results. According to Phuoc and Jamikorn (2017), *L. acidophilus* enhanced the growth performance of weaned rabbits, indicating that nutrient digestibility and nitrogen retention were higher in supplemented rabbits with *B. subtilis* and *L. acidophilus*. Another study revealed that supplying *S. cerevisiae* at 1.5 and 3.0 g/kg improved the final live weight and feed intake with a better FCR than the control group (Onifade et al., 1999). This result was similar to the result obtained when only adding *Saccharomyces cerevisiae* (T4 treatment), but no positive effect was observed in the combined treatment of *Lactobacillus acidophilus, Bacillus subtilis, Saccaharomyces cerevisiae, Aspergillus oryzae* (T2 treatment). The lack of consistency in the results of studies with probiotic supplementation in the experiment was explained by Falcão-e-Cunha et al. (2007), who suggested that the different experimental and hygienic conditions, selection of probiotic strains, density of probiotics, and combination of different probiotic strains sometimes lead to competition instead of promoting their role, and that some strains need in combination to show their superiority. Similarly, there were no significant differences in blood parameters between the experimental treatments, despite the improvements reported by Mohamed et al. (2017) in the hematological parameters related to erythrocytes in the probiotic treatments compared with the control treatment; the highest results were obtained in the supplemented treatments with *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* (T2, T3, and T5 treatments). Attia et al. (2013) found that probiotic supplementation had a positive effect on the growth of HGB, RBC, and HCT%. Moreover, adding probiotics to the diet increased white blood cell counts, according to Attia et al. (2015). Atmaca et al. (2014) explained that the decrease in WBC and PLT counts was due to the harmful effects of the toxin on hematopoietic organs. However, the blood physiological indices of the experiment were within the normal physiological range, as recorded by previous hematological studies (Moore et al., 2015; Beshara et al., 2018).

Abdelhady and El-Abasy (2015) reported that there were no significant changes in albumin, ALT, AST, and urea levels with the addition of the probiotic mixture (Bio-Plus® 2B, *Bacillus subtilis*, and *Bacillus licheniformis*). Similarly, in the study by Chandra et al. (2015), no significant changes were observed between probiotic treatments on rabbit blood biochemical indices such as GLU, serum protein, albumin, globulin, and A/G ratio. The results of increased protein and globulin in T2, T3, and T4 were also not significant. This finding is similar to the study of Amber et al. (2014), where total serum protein, albumin, and globulin levels increased significantly when the diet contained *Bacillus subtilis* and *Bacillus licheniformis*.

A slight increase in rabbit blood glucose was noted in the T2 and T6 treatments during an earlier study by Ghoneim and Moselhy (2014). However, no significant increase in blood glucose levels was observed in the group of rabbits supplemented with *Lactobacilli*. Gilman and Cashman (2006) explained this change by showing that metabolites from *Lactobacilli* rapidly stimulated glucose transport, which could also be shared by other nutrients, resulting in an increase in blood glucose concentrations. On the other hand, *Saccharomyces* has been recognized for its ability to lower blood sugar and its potential application in controlling type II diabetes (Kadja et al., 2021). Accurately assessing blood sugar levels requires animals to fast for at least 12 hours (Lassen, 2004). However, rabbit digestive physiology is unique compared to other species due to their consumption of soft feces. Therefore, sugar levels in feed have a lesser impact on the test, and it is necessary for rabbits to fast for at least 96 hours to obtain accurate results (Kozma et al., 1974).

Hematology and blood biochemistry parameters are good indicators for determining nutrient deficiencies and animal health status (Etim et al., 2014). Blood protein and globulin levels are considered good indicators of the immune response, and albumin levels are reflective of liver function (Azoz and El-Kholy, 2006). Additionally, ALT and AST activity levels are diagnostic indicators of liver injury in animals (McGill, 2016). The hematological and blood biochemical parameter values obtained in the present study are within the range of physiological logic for rabbits as observed by other researchers. Therefore, it can be concluded that the use of probiotics to ferment soya waste can replace heat treatment without negatively affecting rabbit health while eliminating anti-nutrient factors.

The results of low *E. coli* density in fermented soya waste with probiotics show that probiotic strains added to a rabbit diet contribute to changes in the intestinal microflora, compete for binding sites on intestinal villi, and reduce the density of harmful bacteria in the gastrointestinal tract (Mattar et al., 2001). Previous research announced that rabbits supplemented with probiotics had a 25% reduction in *E. coli* in the small intestine (Lee et al., 2000). *Bacillus spp* stimulates the biosynthesis of strains of *Lactobacillus*, thereby increasing beneficial bacteria and reducing *Clostridium* and *E. coli* in the intestinal microbiota (Helal et al., 2021). It is possible that because of this mechanism, the treatment of group addition *Lactobacillus* (T6 treatment) gave the lowest *E. coli* density due to direct supply. La Ragione and Woodward (2003) pointed out that *B. subtilis PY79 hr* could inhibit the entry and survival of *Salmonella enteritidis, C. perfringens*, and *E. coli* in the intestinal tract, preventing the overgrowth of *E. coli*, improving resistance to *E. coli* invading the intestinal tract, and improving the disease process. As a result, a diet including *B. subtilis* can reduce the concentration of *E. coli in vivo*, and *B. subtilis* might be a method of competitive exclusion in the control of enteric pathogen infection (Guo et al., 2017). When supplied alone, *Saccharomyces cerevisiae* showed no positive effect on rabbit caecal microbiota (Belhassen et al., 2016).

CONCLUSIONS

Treating anti-nutrients in soya waste with probiotics for use as rabbit feed could potentially replace traditional heat treatment methods. When selecting microbial strains for fermentation, it is important to consider their density and competitiveness with each other to achieve optimal results. The use of probiotic sources containing *Bacillus subtilis* and *Lactobacillus acidophilus* strains resulted in higher growth performance, greater digestibility, and improved economic returns for growing rabbits.

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AUTHOR CONTRIBUTIONS

Trung Thanh Truong; Conceptualization and design of the experiment, investigation, supervision, editing, and finalization.
Hai Long Tran; Investigation, methodology, formal analysis, manuscript preparation, editing, and finalization.
Nghia Khoi Nguyen; Conceptualization and design of the experiment, investigation, editing, and finalization.
Tam Thu Nguyen; Conceptualization and design of the experiment, investigation, editing, and finalization.

CONFLICT OF INTEREST

We have no conflict of interest.

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