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Bacillus spp.-fermented mixture in goat starter diets on growth performance, blood, and carcass and gastrointestinal traits

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ABSTRACT - The aim of this study was to evaluate the effects of incorporating *Bacillus* spp.-fermented mixture (FM; 55% soybean meal and 45% feather meal) in the starter feed of goat kids, specifically its effects on growth performance, blood profile, carcass characteristics, and gastrointestinal traits; the FM protein profile was also evaluated. Seventy-five four-week-old male dairy goat kids were randomly assigned to three different starter diet groups containing B, D, or J strains of 5% *Bacillus* spp. FM (BG, DG, and JG), a commercial starter diet group (CG), and a starter diet group without FM (NG). During the starter phase (0-6 weeks), the average daily gain was higher in JG and BG goats than in the NG, with the average daily gain and feed conversion in BG found to be best at 6-8 weeks. Furthermore, the intestinal villi and papillae height in the ventral and dorsal sac of BG goats were higher than those in the other groups. The SDS-PAGE analysis of the FM samples further indicated that low molecular weight peptides in FM significantly increased after fermentation. Thus, using a suitable *Bacillus* sp. to ferment soybean and feather meals can increase the available nutrients. Taken together, these results indicate that adding 5% *Bacillus* spp.-fermented soybean and feather meal mixture to a starter feed is a feasible option to improve the growth performance of goat kids, without negatively impacting their health.

Keywords: feather meal, fermentation, soybean, small ruminant

1. Introduction

For ruminants, the starter diet is essential for development during the transition period from liquid (colostrum, milk, or milk replacer) to solid feed (forage or concentrate); hence, starter feeds are formulated to promote animal growth and health (Senevirathne et al., 2017). Soybean, the most abundant source of plant protein (Mukherjee et al., 2016), is extensively used due to its low cost and high nutritional value, including a good amino acid balance (Frias et al., 2008). However, the antinutritional factors (ANF) of soybean negatively impact the quality of animal feed (Liener, 1994), with particularly limited applications for young animals if untreated (Kim et al., 2010).

Additionally, animal protein, particularly feather meal, has been increasingly used in animal feeds. Globally, there were ~2.8 million tons of feathers produced in 2018 with more than 60,000 tons of poultry feathers produced each year in Taiwan alone (FAOSTAT, 2019). Meanwhile, although feather meal is a byproduct of the poultry industry, containing lipids (12-15%) and protein (58-65%), in Taiwan, it is only incorporated in ~5% of feed products (Dawood et al., 2020). This is largely due to its low digestibility and biological value, as well as deficiencies in essential amino acids, challenges that must be addressed prior to its use in animal feed (Onifade et al., 1998).

Feed processing conditions, including heat treatment, solvent extraction, and fermentation, can be employed to reduce soybean ANF (i.e., protease inhibitors, glycinin, and β-conglycinin). Fermentation of soybean with microbe, such as *Aspergillus oryzae* (Kwon et al., 2011), *Saccharomyces cerevisiae* (Song et al., 2008), or *Bacillus* spp. (Kader et al., 2012), can reduce the ANF level and improve its nutritional quality (Amadou et al., 2010). Hulse (2004) reported that microbial enzymes can also degrade keratin, the abundant and strongest protein in feathers.

Considering that the top priorities in the animal industry include minimizing the costs and improving overall value, the use of suitable microbes to ferment soybean and feather meal could prove advantageous. However, sufficient data on the effects of feeding fermented soybean meal and feather meal to goat kids is not available. Therefore, the aim of this study was to identify a suitable *Bacillus* spp. fermented mixture (FM), while evaluating the effect of these products as starter feed on the growth, blood profile, carcass characteristics, and gastrointestinal traits of kids.

2. Material and Methods

The experiments complied with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 2011). All animal treatments were conducted according to the Affidavit of Approval of Animal Use Protocol (IACUC 106001). The animal studies were performed in Chiayi, Taiwan (23°27'59" N and 120°29'9" E).

2.1. Preparation of fermented soybean meal and feather meal

Three different *Bacillus* spp. strains (Poultry Physiology Laboratory, Chiayi, Taiwan) were used to ferment the mixture. Soybean meal and feather meal were mixed at a 11:9 ratio on a dry matter basis. Dried soybean meal and feather meal were soaked in distilled water to maintain a moisture content of 65% overnight. Hydrated soybean meal and feather meal were sterilized in a steam tank at 65 °C for 1 h, cooled to 25 °C for 1 h, inoculated with three different strains of *Bacillus* spp. (B, D, and J at 10^9 cfu mL^{-1}) that are commonly used in processing soybean meal (Han et al., 2001) and feather meal (Lin et al., 1999; Suh and Lee, 2001), and subsequently mixed and fermented in a packed bed incubator (no-brand, Taiwan) at 35 °C for 48 h. The three selected strains exhibited superior performance to the other 254 strains according to the decomposition ratio on skin milk agar plates and production of gamma-glutamic acid (r-PGA; cfu mL^{-1}, a fermentation byproduct that is beneficial for animal growth). The fresh FM were then dried at 55 °C for 24 h and subsequently ground and refrigerated until use in the experimental starter diets.

2.2. Animal management

Seventy-five male goat kids (four weeks old) were randomly allocated to the following groups, which received one of the five starter diets: BG, DG, and JG received one of the three *Bacillus* spp. strain mixtures – B, D, and J; NG received no FM; and CG received a commercial calf starter diet, serving as the control group, as no commercial goat kid starter is available in Taiwan (Charoen Pokphand, Taiwan). The FM were added as 5% (as fed) to the basal diet. Starter diets were formulated according to NRC (2007) (Table 1). All goat kids were housed in individual pens (65 × 60 × 50 cm/pen), which were secured 95 cm from the ground. Owing to the unavailability of goat milk replacer in Taiwan, ruminant milk replacer powder (Table 2; Victoria Whole Milk Powder, Victoria, Australia) was mixed (1:7) with water at approximately 40 °C and cooled to 35-38 °C. Kids were fed 800 mL of milk replacer (12.5% as fed) per day over two meals, at 08:00 and 17:00 h, for eight weeks. Water and starter diets were available *ad libitum* during the trial.

Table 1 - Ingredient formulations in starter diets

Item	Diet[2]		
	BG, DG, JG	NG	CG
Ingredient (% fresh basis)			
Corn grain	61.4	57.1	50.1
Wheat bran	10	8	7.8
Molasses, cane	1.4	3.3	3
Soybeans, full-fat	6	13.4	10
Soybean meal, expelled	11	13	23
Mixture[1]	5	-	-
Dicalcium phosphate	1	1	1
Calcium carbonate	2.4	2.4	2.4
Salt	0.5	0.5	0.4
Mineral and vitamin premix	1.1	1.1	-
Antioxidants	0.2	0.2	0.2
Commercial product	-	-	2.1[3]
Chemical composition (as formulated)			
Crude protein	18.42	18.43	20.48
Metabolizable energy (kcal kg^{-1})	2923	2961	2989
Calcium	1.26	1.22	1.26
Phosphorus	0.70	0.67	0.7

[1] Fermented soybean meal and feather meal at a ratio of 11:9.
[2] BG, DG, and JG: feather meal and soybean meal mixture fermented by three strains (B, D, and J) of *Bacillus* spp. added at 5% to the basal starter diet; NG: unfermented starter diet; CG: commercial calf starter diet.
[3] Commercial product in CG contained mineral and vitamin premix and other feed additives.

Table 2 - Composition of ruminant milk replacer (Victoria Whole Milk Powder)

Chemical composition	(%)
Lactose	40.3
Fat	26.3
Protein	24.5
Minerals	5.8
Moisture	3.1

2.3. Growth performance and blood traits

Feed intake of the starter diets was recorded daily. The body weights, feed conversion ratio (FCR), and body weight gain (BWG) of goat kids were measured and calculated every two weeks. Jugular blood samples were collected during weight measurements for analysis of glucose (Glu), cholesterol (Cho), triglyceride (TG), total protein (TP), albumin (Alb), globulin (Glo), and the albumin-to-globulin ratio (A:G). All blood parameters were analyzed with a blood chemistry analyzer (LST008AS, Hitachi, Tokyo, Japan) to determine health status.

2.4. Carcass and gastrointestinal traits

To compare the effects of feeding fermented and unfermented mixture starter diets, with the same protein abundance, after eight weeks, 12 goat kids (three from each group, excluding the CG in

accordance with the reduction concept of 3R-replacement, reduction and refinement), were randomly selected and weighed after fasting for 12 h to determine the body weight before slaughter (BW, kg); BW after slaughter (SBW, kg) was also recorded. The dressed carcass (carcass weight [CW] in kg) comprised the body after removing the skin, head (at the atlanto-occipital joint), forefeet (at the carpal-metacarpal joint), hindfeet (at the tarsal-metatarsal joint), and viscera. Kidneys together with kidney and pelvic fat were retained in the carcass, whereas the testes and scrotal fat were removed. The total weight of visceral organs (VW, kg), heart (g), liver (g), kidney (g), stomach (g), reticulorumen (g), abomasum (g), omasum (g), and intestine (g) and intestine length (m) were recorded to calculate their percentage of CW.

Duodenum, ileum, jejunum, and rumen samples were collected to calculate the villus and ruminal papilla heights. One square centimeter of whole-thickness tissue samples from the duodenum, proximal, middle, and distal jejunum, ileum, as well as the front, post, ventral, and dorsal sacs of the rumen were collected for analyses and placed in 4% buffered formaldehyde (Sigma-Aldrich Corp., St. Louis, MO) for five days and subsequently stored in ethanol. Samples were then embedded in paraffin, and serial histological sections (5 µm thick) were stained with hematoxylin and eosin for histological analysis under a light microscope (Motic China Group CO., LTD, China). The lengths of intestinal villi and ruminal papillae were measured in 10 to 12 slides for each tissue sample with an optical binocular microscope (Olympus BX 61, Olympus, Warsaw, Poland) coupled with a digital camera to a PC equipped with Cell P (Olympus) software.

2.5. Protein profile of FM on SDS-PAGE

Based on the results of the abovementioned experiments, a FM of the suitable *Bacillus* spp. strain was selected. Next, 5 g of soybean and feather meal samples fermented for different durations (0, 12, 24, 36, and 48 h), as well as 5 g of the unfermented mixture, were prepared to observe changes in peptide profiles. A sample buffer containing 8 M urea, 2 M thiourea, 3% SDS, 75 mM DTT (DL-dithiothreitol), 25 mM Tris-HCl, 0.05% bromophenol blue, at pH 6.8 was added to the protein extract at a ratio of one part sample buffer to one part protein extract (v/v). The SDS-PAGE procedures for running proteins on 12% (or 15%) slab-gels (acrylamide: methylenebisacrylamide = 37.5:1, w/w) was performed according to that described by Laemmli (1970) with equal amounts of protein (150 µg) from each sample. A prestained marker, in the range of 9 to 170 kDa (GeneDireX® BlueRAY Prestained Protein Ladder; Cat No. PM006-0500, EGFIE LLC Company Herndon, VA, USA) was used as a protein standard. SE 400 slab-gel electrophoresis units (Hoefer Scientific Instruments, San Francisco, CA) were used to run all gels with 15 mA at 25 °C. The gels were then stained with Coomassie blue R-250 in methanol and acetic acid for 4 h and subsequently decolored with methanol and acetic acid. Image analysis was performed using the method of Chang (2019). There were two parts of peptide molecular weight which contained 25-75 kDa and <25 kDa. Soybean and feather meal obtained before fermentation were set at 100% to calculate the protein content in fermented samples.

2.6. Statistical analysis

Data pertaining to animal growth, blood parameters, and carcass traits were calculated using the GLM procedure of SAS (Statistical Analysis System, version 9.4), whereas protein molecular weight data were analyzed using the mixed model of SAS. Tukey's test was used to detect significant differences among groups with significance declared at $P<0.05$. Data were analyzed according to the following model:

$$Y_i = m + A_i + e_i,$$

in which Y_i represents the observed value for treatment; i is the addition of different *Bacillus* spp. strain FM; m represents the general mean; A_i is the effect of different diets; and e_i represents the random error associated with each observation.

3. Results

3.1. Growth performance and blood traits

The mean initial body weight was 7.36±0.34 kg in CG, 7.31±0.36 kg in BG, 7.04±0.33 kg in DG, 7.11±0.34 kg in JG, and 7.42±0.34 kg in NG, and final weight was 16.87±0.70 kg in CG, 16.55±0.73 kg in BG, 14.97±0.68 kg in DG, 15.77±0.70 kg in JG, and 15.87±0.70 kg in NG (P>0.05). The final weights in the BG and CG were higher than in the other groups (Table 3), indicating that the commercial calf

Table 3 - Effect of fermented soybean and feather meal mixture on the growth performance of male dairy goat kids

Time (week)	Diet[1]					P-value[2]
	CG	BG	DG	JG	NG	
			Body weight (kg)			
0 w	7.36±0.34	7.31±0.36	7.04±0.33	7.11±0.34	7.42±0.34	0.917
2 w	9.83±0.50	9.73±0.52	8.79±0.48	9.35±0.50	9.55±0.50	0.584
4 w	12.22±0.57	11.79±0.60	10.63±0.55	11.43±0.57	11.88±0.57	0.331
6 w	14.88±0.62	13.81±0.65	12.64±0.59	13.73±0.62	13.65±0.62	0.158
8 w	16.87±0.70	16.55±0.73	14.97±0.68	15.77±0.70	15.87±0.70	0.345
			Body weight gain (g day^{-1})			
0-2 w	177.5±19.1	172.7±20.0	125.4±18.4	160.8±19.1	152.5±19.1	0.319
2-4 w	171.7±15.6	148.2±16.3	131.5±15.0	148.3±15.6	166.7±15.6	0.365
0-4 w	174.2±13.3	158.2±13.9	128.5±12.8	152.5±13.3	160.8±13.3	0.169
4-6 w	190.0±13.3a	145.5±13.8bc	145.4±12.7bc	165.8±13.3ab	126.7±13.3c	0.017
0-6 w	180.0±10.6a	154.5±11.0ab	133.1±10.2b	157.5±10.6ab	147.5±10.6b	0.040
6-8 w	143.3±11.6b	196.4±12.2a	166.2±11.2ab	146.7±11.6b	158.3±11.6b	0.022
4-8 w	167.5±10.0	170.0±10.4	155.4±9.6	156.7±10.0	143.3±10.0	0.356
0-8 w	168.3±9.2	165.5±9.7	142.3±8.9	155.0±9.2	151.7±9.2	0.268
			Feed intake (g day^{-1})			
0-2 w	176.7±23.9	153.6±24.9	103.8±22.9	160.0±23.9	151.7±23.9	0.257
2-4 w	414.2±38.9	340.0±40.7	312.3±37.4	386.7±38.9	348.3±38.9	0.370
0-4 w	295.8±28.5	250.0±29.7	210.0±27.4	273.3±28.5	252.5±28.5	0.280
4-6 w	498.3±30.5	393.6±31.9	422.3±29.3	470.8±30.5	408.3±30.5	0.099
0-6 w	362.5±25.8	296.4±26.9	280.8±24.8	338.3±25.8	303.3±25.8	0.163
6-8 w	605.8±38.1	539.1±39.8	518.5±36.6	525.0±38.1	506.7±38.1	0.383
4-8 w	551.7±31.2	465.5±32.6	471.5±30.0	498.3±31.2	458.3±31.2	0.220
0-8 w	424.2±25.3	356.4±26.4	340.0±24.3	383.3±25.3	354.2±25.3	0.151
			Feed conversion ratio			
0-2 w	0.98±0.11	0.83±0.12	0.82±0.11	1.11±0.11	1.05±0.11	0.301
2-4 w	2.80±0.34	2.46±0.36	2.40±0.33	2.92±0.34	2.21±0.34	0.573
0-4 w	1.71±0.11	1.54±0.12	1.59±0.11	1.77±0.11	1.61±0.11	0.598
4-6 w	2.88±0.68	2.94±0.71	3.16±0.66	2.94±0.68	4.89±0.68	0.190
0-6 w	2.05±0.12	1.90±0.13	2.10±0.12	2.16±0.12	2.13±0.12	0.644
6-8 w	4.62±0.41c	2.81±0.43a	3.19±0.40ab	4.10±0.41bc	3.35±0.41ab	0.023
4-8 w	2.91±0.21	2.45±0.21	2.84±0.20	2.70±0.21	2.65±0.21	0.581
0-8 w	2.52±0.14	2.17±0.15	2.42±0.13	2.51±0.14	2.41±0.14	0.430

Data are presented as mean±SD.
[1] CG: commercial calf starter diet; BG, DG, and JG: feather meal and soybean meal mixture fermented by three strains (B, D, and J) of *Bacillus* spp. added at 5% to the basal starter diet; NG: unfermented starter diet.
[2] Groups with the same letter indicate no significant differences (P>0.05); groups with different letters indicate significant differences between groups in the row (P<0.05).

starter diet was suitable for goat kids and the FM of a specific *Bacillus* spp. strain would also benefit the growth of goat kids. The ADG in weeks 4-6 was highest in CG in the present animal trial (P<0.05). Moreover, the ADG during weeks 4-6 in the CG, JG, and BG was higher in the present animal trial (P<0.05). However, ADG and feed intake were not affected by the FM over the eight-week study. The FCR at weeks 6-8 indicated that BG had better performance than the other groups (P<0.05). Blood traits (Table 4), including Glu, Cho, TG, TP, Alb, Glo, and A:G, did not differ significantly among the goat kid groups (P>0.05).

Table 4 - Effect of feather and soybean-fermented mixtures on blood parameters in male dairy goat kids

Time (week)	Diet[1]					P-value
	CG	BG	DG	JG	NG	
			Glucose (mg dL^{-1})			
0 w	75.13±7.93	77.67±8.82	77.00±7.22	81.40±12.21	73.47±12.25	0.290
4 w	73.67±6.51	70.60±6.84	71.07±4.96	76.20±7.23	71.71±6.39	0.117
8 w	74.73±7.49	78.33±11.04	81.73±13.86	79.00±8.29	78.71±14.65	0.579
			Total cholesterol (mg dL^{-1})			
0 w	91.60±26.64	96.67±24.75	97.80±26.75	99.87±26.40	95.13±26.87	0.932
4 w	70.80±20.41	70.20±15.97	81.13±16.40	76.33±25.06	80.57±29.96	0.415
8 w	72.40±14.44	86.40±30.13	93.20±39.13	69.20±13.09	82.93±24.00	0.081
			Triglyceride (mg dL^{-1})			
0 w	43.80±22.37	31.13±15.70	39.47±27.53	36.27±18.86	34.20±13.79	0.503
4 w	27.07±14.53	31.60±12.80	28.13±13.28	30.53±10.82	27.29±12.64	0.830
8 w	29.87±14.50	27.40±15.75	31.40±12.73	25.20±10.02	29.50±13.75	0.752
			Total protein (g dL^{-1})			
0 w	6.03±0.88	5.80±0.31	5.95±0.61	5.75±0.84	5.87±0.48	0.756
4 w	6.01±0.75	5.46±0.43	5.69±0.32	5.66±0.54	5.93±0.72	0.075
8 w	6.17±0.75	5.89±0.56	6.31±1.03	6.01±0.41	6.15±0.68	0.552
			Albumin (g dL^{-1})			
0 w	3.03±0.55	3.23±0.32	3.17±0.27	3.19±0.77	2.96±0.34	0.525
4 w	3.25±0.47	3.26±0.29	3.37±0.26	3.18±0.32	3.22±0.36	0.666
8 w	3.45±0.38	3.53±0.38	3.61±0.36	3.43±0.21	3.44±0.41	0.583
			Globulin (g dL^{-1})			
0 w	3.01±1.02	2.57±0.36	2.77±0.58	2.97±0.73	2.91±0.59	0.402
4 w	2.77±1.03	2.20±0.43	2.33±0.36	2.48±0.72	2.63±0.92	0.241
8 w	2.73±0.96	2.35±0.36	2.69±0.77	2.57±0.58	2.71±0.84	0.611
			Albumin:globulin ratio			
0 w	1.11±0.38	1.27±0.27	1.20±0.28	1.12±0.34	1.07±0.28	0.416
4 w	1.31±0.43	1.55±0.44	1.48±0.31	1.41±0.50	1.29±0.39	0.370
8 w	1.40±0.42	1.52±0.25	1.39±0.24	1.41±0.41	1.39±0.37	0.819

Data are presented as mean±SD.
[1] CG: commercial calf starter diet; BG, DG, and JG: feather meal and soybean meal mixture fermented by three strains (B, D, and J) of *Bacillus* spp. added at 5% to the basal starter diet; NG: unfermented starter diet.

3.2. Carcass and gastrointestinal traits

No differences were observed in the weights or percent of carcass, total viscera, heart, liver, kidneys, stomach, reticulorumen, abomasum, omasum, or intestine among dietary treatments, except in the weight of the reticulorumen (Table 5). The weight of the reticulorumen in goat kids in the NG was the highest of the groups fed FM (P<0.05).

Moreover, the villi height in the duodenum and ileum in the BG were the highest (P<0.05), while the jejunum villi height was also greater in the BG; however, it did not differ significantly from that in the DG (P<0.05; Table 6). No difference was observed in the papilla heights within the proximal rumen; however,

Table 5 - Effect of feather and soybean-fermented mixtures on carcass traits in male dairy goat kids

Trait	Diet[1]				P-value[2]
	BG	DG	JG	NG	
BW (kg)	14.13±0.87	14.50±0.87	14.70±0.87	15.75±0.87	0.613
SBW (kg)	13.51±0.84	13.99±0.84	14.07±0.84	15.12±0.84	0.603
Carcass weight (kg)	5.84±0.40	6.05±0.40	6.07±0.40	6.13±0.40	0.960
Carcass (%)	41.43±1.29	41.64±1.29	41.26±1.29	38.94±1.29	0.455
VW (kg)	5.25±0.39	5.49±0.39	5.36±0.39	6.15±0.39	0.418
Visceral (%)	37.07±1.77	37.78±1.77	36.52±1.77	39.21±1.77	0.735
Heart weight (g)	69.67±6.22	73.53±6.22	75.87±6.22	71.27±6.22	0.901
Heart (%)	0.49±0.04	0.51±0.04	0.52±0.04	0.45±0.04	0.689
Liver (g)	333.27±25.88	317.07±25.88	346.40±25.88	366.33±25.88	0.606
Liver (%)	2.37±0.13	2.20±0.13	2.35±0.13	2.32±0.13	0.789
Kidney (g)	68.60±8.24	60.87±8.24	85.53±8.24	76.60±8.24	0.253
Kidney (%)	0.48±0.04	0.42±0.04	0.58±0.04	0.48±0.04	0.170
Stomach (g)	607.27±47.43	620.47±47.43	590.27±47.43	768.47±47.43	0.096
Stomach (%)	9.73±0.34	10.43±2.31	10.39±1.33	12.68±2.24	0.103
Reticulorumen (g)	467.33±37.19b	491.73±37.19b	452.53±37.19b	630.87±37.19a	0.033
Reticulorumen (%)	76.99±1.55	79.45±1.55	76.64±1.55	82.00±1.55	0.127
Abomasum (g)	107.87±20.28	103.87±20.28	133.13±20.28	102.47±20.28	0.691
Abomasum (%)	17.24±2.27	16.56±2.27	22.51±2.27	13.33±2.27	0.108
Omasum (g)	21.27±3.80	32.07±5.23	24.87±14.69	35.13±15.63	0.746
Omasum (%)	3.62±0.56	5.77±1.20	4.00±2.82	4.67±3.39	0.602
Intestine (g)	506.60±56.16	585.40±56.16	521.67±56.16	490.80±56.16	0.665
Intestine (%)	3.59±0.21	4.01±0.21	3.54±0.21	3.10±0.21	0.095
Intestine length (m)	16.86±1.91	10.12±1.91	9.92±1.91	12.65±1.91	0.105

BW - body weight before slaughter; SBW - slaughter body weight; VW - weight of visceral organs.
Data are presented as mean±SD.
[1] BG, DG, and JG: feather meal and soybean meal mixture fermented by three strains (B, D, and J) of *Bacillus* spp. added at 5% to the basal starter diet; NG: unfermented starter diet.
[2] Groups with the same letter indicate no significant differences (P>0.05); groups with different letters indicate significant differences between groups in the row (P<0.05).

Table 6 - Length of intestinal villi and ruminal papillae (μm) in male dairy goat kids fed fermented soybean and feather meal mixtures

	Diet[1]				P-value[2]
	BG	DG	JG	NG	
Duodenum	778.08±17.39a	593.88±19.19b	628.66±16.43b	602.19±23.34b	<0.001
Ileum	689.99±13.94a	640.10±14.30b	602.19±17.19b	548.28±15.98c	<0.001
Jejunum	636.19±12.92a	602.58±14.96ab	563.00±14.65b	584.55±15.16b	0.002
Rumen - F	2681.29±114.70	2372.61±116.97	2563.57±112.56	2824.81±151.01	0.090
Rumen - P	1928.45±74.90b	2071.03±72.52b	2003.92±77.99b	2550.89±76.18a	<0.001
Rumen - VS	2680.29±84.27ab	2470.48±88.72b	2884.13±77.07a	1865.77±81.24c	<0.001
Rumen - DS	2429.55±80.35a	1802.55±77.63c	1999.20±74.02bc	2144.03±84.48b	<0.001

F - front; P - post; VS - ventral sac; and DS - dorsal sac.
Data are presented as mean±SD.
[1] BG, DG, and JG: feather meal and soybean meal mixture fermented by three strains (B, D, and J) of *Bacillus* spp. added at 5% to the basal starter diet; NG: unfermented starter diet.
[2] Groups with the same letter indicate no significant differences (P>0.05); groups with different letters indicate significant differences between groups in the row (P<0.05).

significant differences were detected in the post, ventral, and dorsal sacs of the rumen (P<0.05). The papilla heights in the posterior rumen differed between NG and the FM groups. Meanwhile, the JG had the highest papillae height in the ventral sac of the rumen; however, this was not significantly different from that observed in the BG. Papillae height was the greatest in the dorsal sac of the rumen in the BG.

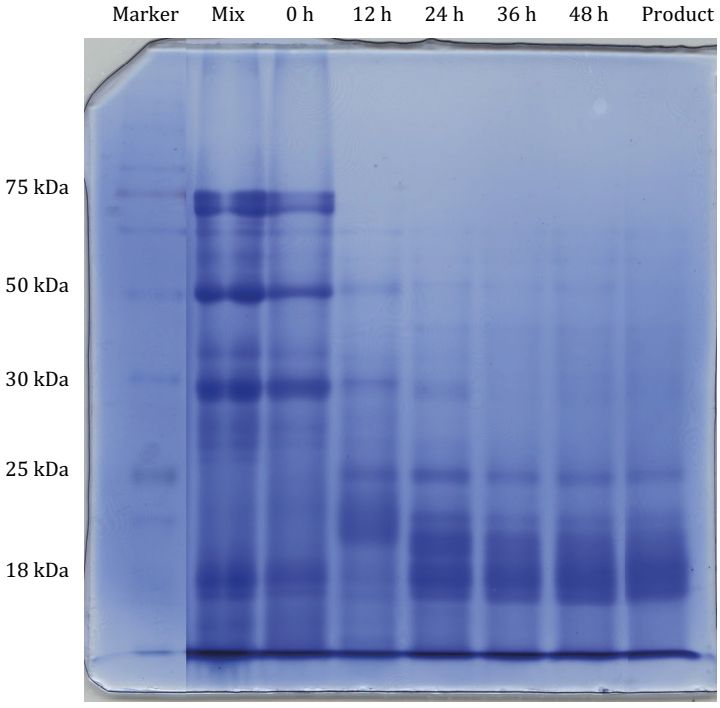
3.3. The protein profile of fermented mixtures

According to the above discussed results, we selected the FM generated with the B *Bacillus* spp. strain for SDS-PAGE analysis. Results showed that the proportion of high molecular weight peptides (HP, 25-75 kDa) or low molecular weight peptides (LP, <25 kDa) differed significantly throughout the course of the fermentation process (P<0.05; Table 7 and Figure 1). Specifically, the LP in samples collected after 48 h of fermentation was higher than in other samples (P<0.05). Meanwhile, the HP in samples collected after 36 h and 48 h of fermentation was lower than in other samples (P<0.05).

Table 7 - Peptide distribution (%) by size in soybean meal and feather meal mixtures throughout the fermentation process

Peptide size	Mixture[1]	Fermentation time[2]					Product[3]	P-value[4]
		0 h	12 h	24 h	36 h	48 h		
25-75 kDa	100a	66.88±0.97b	11.49±0.97c	6.85±0.97d	1.67±0.97e	1.67±0.97e	0.76±0.97e	<0.001
<25 kDa	100c	53.96±10.93d	92.77±10.93c	108.33±10.93c	110.99±10.93c	162.75±10.93b	203.92±10.93a	<0.001

Data are presented as mean±SD.
[1] Mixture of soybean meal and feather meal (11:9), no fermented fluid.
[2] Mixture added with fermented fluid and distilled water to maintain a 65% moisture content. The mixture was placed into the incubator, and samples were taken at 0, 12, 24, 36, and 48 h of fermentation.
[3] Mixture after 48 h of fermentation and dried under 45 °C to a moisture content lower 10%.
[4] Groups with the same letter indicate no significant differences (P>0.05); groups with different letters indicate significant differences between groups in the row (P<0.05).



Marker is GeneDirex®, BlueRAY Prestained Protein Ladder (EGFIE LLC Company Herndon, VA, USA).
Mix: mixture of soybean meal and feather meal (11:9) before fermentation.
0-48 h: fermentation time.
Product: mixture after 48 h of fermentation and dried under 45 °C to a moisture content lower than 10%.

Figure 1 - SDS-PAGE of fermented product during different fermentation times.

4. Discussion

Fermentation can reduce the level of antinutritional factors in soybean meal, while increasing the level of small peptides and improving the nutritional value for animals (Hong et al., 2004; Liu et al., 2007; Frias et al., 2008). As a result, supplying fermented soybean meal and hydrolyzed feather meal (*Bacillus* spp.-fermented) in feed can promote animal growth. However, according to the National Research Council (NRC, 2007), the optimal crude protein content in goat kid feed is 18-20%. Thus, although the results of the current study demonstrate that the growth performance in the CG was superior to that in the experimental fermentation groups, the higher crude protein abundance in the CG may have masked significant differences. On the other hand, growth performance, carcass traits, and intestinal villi height in the BG, which contained low crude protein content (18.42%) was superior to the other fermentation groups.

Although Hotz and Gibson (2007) hypothesized that fermentation is widely used to increase the bioavailability of nutrients, it did not improve the growth performance of all of the FM groups in our study; that is, the B, D, and J groups did not exhibit superior growth performance in relation to the goat kids fed the unfermented mixture. A similar result was observed with fermented soybean meal in calves (Kim et al., 2010). However, the mean ADG of goat kids in the current study was 125.4-196.4 g d^{-1}, which surpassed that reported by Soren et al. (2013). Moreover, the 9-16% increase in ADG and the 11-16% reduction in FCR in the BG compared with the other fermentation groups indicates a maximized gain in the DG, which is generally desirable in the animal agriculture industry. Similarly, Senevirathne et al. (2017) demonstrated that calves fed a starter ration with microbially enhanced soybean meal exhibited higher final weights with maintained growth performance following reduced intake, compared with calves fed raw soybean meal. Moreover, fermented soybean meal has demonstrated benefits for daily weight gain in piglets (Feng et al., 2007; Jones et al., 2010), and the addition of hydrolyzed feather meal to steer feed produces greater weight gain compared with feed lacking feather meal (Brown and Pate, 1997).

Blood glucose levels reflect the health and dietary nutrient intake of goat kids (Kaneko, 1997a). However, in the current study, glucose levels did not differ among the goat kids fed the various *Bacillus* spp.-fermented mixtures, which was supported by the dietary nutrient intake and utilization values. Normal blood glucose levels are maintained by a finely balanced system of hormonal interactions affecting the supply and removal of glucose from circulation (Kaneko, 1997a). As the result of the present study, it indicated the glucose circulation in goat kids was not affected by different diets. Additionally, Cho and TG levels, which are reflective of heart and blood health, did not differ significantly between the groups in this study. Similarly, TP, Alb, Glo concentration and A:G, which are used to evaluate protein utilization, did not differ between the experimental and control groups. These values are closely related to dietary protein intake, particularly TP, which reflects protein synthesis. Albumin is synthesized in the liver and accounts for 50 to 60% of the blood protein level. The total concentration of Alb decreases when its synthesis in the liver is too low or its gut concentration is low. Meanwhile, Glo is associated with immune function, and A:G reflects protein synthesis ability in the liver. Generally, the Alb value should be higher than that of Glo in healthy blood, and thus the A:G should be >1 (Kaneko, 1997b). Taken together, these results indicate that addition of the various *Bacillus* spp.-fermented mixtures to the starter diets of goat kids did not affect protein synthesis compared the the commercial calf starter diet, nor did the different *Bacillus* spp. strains impact protein intake or utilization in goat kids. Hence, the use of *Bacillus* spp.-fermented mixtures of soybean and feather meal in starter rations does not present health concerns to goat kids.

Visceral organ mass in ruminants increases with increased DM intake, energy density, and nutrient supply; however, it is not directly affected by the physiological state per se (Scheaffer et al., 2001; McLeod et al., 2007). Moreover, Drackley et al. (2014) reported that the total gastrointestinal tract and kidney mass were not significantly impacted by increased DM intake in cows. In line with these previous findings, the current study results show that the CW and VW did not differ significantly among the FM starter diets, which may indicate that the FM were sufficient, without being excessive, in the starter ration.

As Graham et al. (1984) indicated, shortened villi reduce the total surface area of the digestive tract, while also decreasing the secretion of digestive enzymes. Hence, long villi increase the surface area and produce more digestive enzymes. Prior to the full development of the rumen in goat kids, the intestinal villi play important roles in nutrient absorption from milk replacers or starters. The current study results indicate that the villi height was greater in goat kids fed the B strain of *Bacillus* spp.-fermented mixtures in the present trial, which may improve the absorption of milk or starter nutrients due to the increased abundance of small peptides and various fermented products, such as r-PGA that could improve the digestion of mineral, and reduced level of ANF, which could be benefit for villi growth and less diarrhea.

The ruminal epithelium also has a critical role in nutrient absorption in ruminants, and the papillae are used to assess the relative development of the rumen (Xiao et al., 2016). In goat kids, the *Bacillus* spp.-fermented mixture enhanced papillae height in the ventral and dorsal sac of the rumen, compared with the starter diet without the FM. A similar positive effect was reported by Lesmeister et al. (2004) and *Brewer* et al. (2014) by feeding *S. cerevisiae* fermentation products to dairy calves. Although it is unclear how these traits were improved by feeding *Bacillus* spp.-fermented mixtures, the results of this study suggest that *Bacillus* spp. additive has the potential to be efficacious in providing gastrointestinal stability and growth (Bampidis et al., 2019).

In addition, the SDS-PAGE analyses of the fermentation product samples indicated an increase in the abundance of low molecular weight peptides in feed after fermentation, with a corresponding decrease in high molecular weight proteins after 36 and 48 h of fermentation. Thus, fermentation could increase the abundance of smaller peptides by degrading high molecular weight proteins/peptides. In fact, Hirabayashi et al. (1998), Egounlety and Aworh (2003), Frias et al. (2008), Song et al. (2008), and Mukherjee et al. (2016) indicated that fermentation is capable of removing certain antinutritional factors from soybean meal, including trypsin inhibitors and allergens, while decreasing the level of high molecular weight peptides. Hence, the effect of nutrition utilization could be improved via fermentation of soybean meal (Hong et al., 2004). Besides, feathers contain high molecular weight proteins, such as keratin, that are difficult for animals to digest; thus, *Bacillus* spp.-fermented soybean and feather meal may improve nutrient utilization for goat kids.

5. Conclusions

Based on the results of this study, we infer that inclusion of the soybean meal and/or feather meal mixture fermented by the specific *Bacillus* spp. B strain in the starter ration is optimal for performance of goat kids. Specifically, these mixes would provide a nutrient source that can improve average daily gain by 9% and feed conversion ratio by 11% without adversely affecting the health or growth of the animals. Taken together, these findings suggest a cost-effective feed alternative for goat kid nutrition.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: H.-T. Hong and C.-P. Wu. Data curation: H.-T. Hong and C.-P. Wu. Formal analysis: H.-T. Hong and C.-P. Wu. Methodology: C.-P. Wu. Project administration: H.-T. Hong. Writing-original draft: H.-T. Hong. Writing-review & editing: C.-P. Wu.

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