



Effects of L-tryptophan, Fructan, and Casein on Reducing Ammonia, Hydrogen Sulfide, and Skatole in Fermented Swine Manure

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ABSTRACT: The effects of daily dietary *Bacillus subtilis* (Bs), and adding L-tryptophan, fructan, or casein to fecal fermentation broths were investigated as means to reduce the production of noxious gas during manure fermentation caused by ammonia, hydrogen sulfide (H₂S), and 3-methylindole (skatole). Eighty swine (50.0±0.5 kg) were equally apportioned to an experimental group given Bs in daily feed, or a control group without Bs. After 6 weeks, fresh manure was collected from both groups for fermentation studies using a 3×3 orthogonal array, in which tryptophan, casein, and fructan were added at various concentrations. After fermentation, the ammonia, H₂S, L-tryptophan, skatole, and microflora were measured. In both groups, L-tryptophan was the principle additive increasing skatole production, with significant correlation ($r = 0.9992$). L-tryptophan had no effect on the production of ammonia, H₂S, or skatole in animals fed Bs. In both groups, fructan was the principle additive that reduced H₂S production ($r = 0.9981$). Fructan and Bs significantly interacted in H₂S production ($p = 0.014$). Casein was the principle additive affecting the concentration of ammonia, only in the control group. Casein and Bs significantly interacted in ammonia production ($p = 0.039$). The predominant bacteria were *Bacillus* spp. CWBI B1434 (26%) in the control group, and *Streptococcus alactolyticus* AF201899 (36%) in the experimental group. In summary, daily dietary Bs reduced ammonia production during fecal fermentation. Lessening L-tryptophan and increasing fructan in the fermentation broth reduced skatole and H₂S. (**Key Words:** Swine Manure, L-tryptophan, Fructan, Casein, Ammonia, H₂S, Skatole, *B. subtilis*)

INTRODUCTION

Environmental contamination caused by swine manure has provoked much public concern. More than 160 noxious substances have been discovered in swine waste (Le et al., 2005). Ammonia and hydrogen sulfide (H₂S) are routinely measured, and 3-methylindole (skatole), a byproduct of tryptophan degradation, also increases fecal odor. Deposition of skatole in adipose tissues can taint the meat, with harmful consequences for the beef and pork industries.

In efforts to reduce the production of ammonia, H₂S, and skatole, dietary additives have been investigated. These

have included probiotics (Wutzke et al., 2010), fructan (Xu et al., 2002), and enzymes or amino acids (Vhile et al., 2012). Castration and vaccination have also been used to control the production of noxious gases. Among the probiotics, effective microorganisms, *Bacillus subtilis* (Bs), and lactic acid bacteria reduce the production of ammonia by modifying the intestinal microflora (Jeon et al., 1996). Dietary fructan has been shown to selectively promote *Bifidobacterium* spp. in the gut, thereby reducing the production of skatole (Li, 2009), and there is evidence for the prebiotic effects of inulin-derived fructans in humans (Salazar et al., 2014). Amino acid-balanced diets (such as those containing tryptophan) were shown to reduce the protein ratio in daily feedings, which led to less ammonia production in swine manure (Powers et al., 2007). The purpose of the above methods was to reduce the production of foul-smelling gas from within the bodies of live swine. To our knowledge, there have been no investigations to reduce the unpleasant odor of fecal matter once outside the

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body, i.e., conducted *in vitro*.

The present study evaluated the effects of externally applied tryptophan, fructan, or casein on manure fermentation, and noted the interactions among these additives and dietary Bs. Our results should provide a solid reference for studies aimed at reducing the odor of fecal waste outside the live swine body.

MATERIALS AND METHODS

The Animal Welfare Committee of Shandong Academy of Agricultural Sciences reviewed and approved the study protocol.

Materials

Eighty Duchangda Sanyuan commercial hybrid pigs (50±0.5 kg) were purchased from Shangdong Lvan (Jinan, China). Bs (10⁹ CFU/g) was purchased from Shandong Huamu Tianyuan Nongmu (Jinan, China).

Experimental design

The 80 pigs were randomly divided into an experimental group and a control group (n = 40, each), constituting four replicates in each group and ten pigs in each replicate. Both groups were raised on a concrete floor. Pigs in the experimental group (Bs⁺) were given a corn-soybean basal diet supplemented with 0.2% Bs. The control group (Bs⁻) received the same diet, but without the bacteria. The basal diet was prepared in accordance with the China feeding standard for swine (NY/T65-2004).

After 6 weeks, fresh manure was quickly collected, before feeding in the morning, from 2 randomly selected pigs in each replicate for subsequent studies (Yokoyama and Carlson, 1974). In brief, 280 g of each fecal sample was first suspended in 2.8 L anaerobic mineral buffer (5.0 g/L NaHCO₃, 0.9 g/L NaCl, 0.9 g/L (NH₄)₂SO₄, 0.45 g/L KH₂PO₄, 0.45 g/L K₂HPO₄·3H₂O, 0.03 g/L CaCl₂·2H₂O, 0.02 g/L MgCl₂, 0.01 g/L MnSO₄·4H₂O, 0.01 g/L CoCl₂·6H₂O, 0.01 g/L FeSO₄·7H₂O, 0.1 g/L cysteine). The suspensions were subsequently transferred to sterile plastic bags filled with CO₂, thoroughly shaken for 5 min, and filtered through 6 layers of gauze to remove small particles. The filtrate from each suspension was distributed into 27 aliquots with 100 mL in each flask, and divided into 9 groups based on the amounts of L-tryptophan, fructan, or casein added to each flask (Table 1). The broths were incubated anaerobically for 24 h at 38°C. A 20-mL sample was taken from each flask to measure the gas composition. One milliliter of clear fermented broth was separately taken from both the Bs⁺ and Bs⁻ samples (the blank, containing no added L-tryptophan, fructan, or casein, broth 1 in Table 1), mixed, and stored at -20°C for microflora isolation and

Table 1. The doses of L-tryptophan, fructan and casein in pig manure fermentation broths

Group number	L-tryptophan	Casein (%)	Fructan (%)
1	0	0	0
2	0	0.125	0.75
3	0	0.25	1.5
4	0.05	0	0.75
5	0.05	0.125	1.5
6	0.05	0.25	0
7	0.1	0	1.5
8	0.1	0.125	0
9	0.1	0.25	0.75

identification.

Measurement of substances in the noxious gas

The fermented broth samples were measured after filtration through a 0.45-µm organic membrane. Ammonia was measured by the Nessler reagent colorimetric method. H₂S was quantified via sulfate-iodometric titration. L-tryptophan and skatole were measured using high-performance liquid chromatography, with reference to Li (2009), and conducted at the Center for Food Quality Supervision and Testing Ministry of Agriculture (Jinan, China).

Isolation and identification of fecal bacteria in the fermentation broth

Genomic DNA of fecal bacteria was purified using an OMEGA Stool DNA kit (Omega Bio-Tec, Norcross, GA, USA). Using V3-340F (5'-FAM-TCCTACGGGAGGCA GCAGT-3') and V3-532R (5'-TCCTACGGGAGGCAGC AGT-3') as universal primers, the V3 region of 16S rDNA was amplified through fluorescence-based polymerase chain reaction-single-strand conformation polymorphism-fragment length polymorphism (F-PCR-SSCP-FLP) methods, as described by Zhang et al. (2000). The 195-bp PCR products were analyzed with an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA, USA, 96-capillary array), in which the amount of each specific bacteria was quantified by the area size of their corresponding peaks. The 16S rDNA clone library (target fragment size 1.6 kb) was constructed by amplifying the 16S rDNA region of each bacteria using the V3 region fluorescent-labeled primers. The PCR products were purified and sequenced at the Biotechnology Research Center, Shandong Academy of Agricultural Sciences. All the sequences were BLASTed in the NCBI database to identify the bacterial species, and quantified using Mega6 software (<http://www.megasoftware.net>).

Statistical analyses

The amounts of ammonia, H₂S, and skatole of the Bs⁺

Table 2. Quantification of ammonia, H₂S and skatole after the manure fermentation

Group		1	2	3	4	5	6	7	8	9
NH ₃ (g/L)	Bs (-)	1.15±0.09	1.05±0.10	1.30±0.11	1.17±0.09	1.01±0.08	1.68±0.11	1.10±0.09	0.86±0.09	1.27±0.11
	Bs (+)	0.80±0.05 ^A	0.47±0.04 ^A	0.62±0.03 ^A	1.06±0.06 ^a	0.70±0.05 ^A	0.62±0.04 ^A	0.97±0.04 ^a	0.78±0.03 ^a	0.84±0.03 ^A
H ₂ S (mg/L)	Bs (-)	0.46±0.03	0.33±0.02	0.38±0.03	0.36±0.02	0.35±0.02	0.42±0.03	0.41±0.03	0.37±0.03	0.36±0.03
	Bs (+)	0.41±0.03 ^a	0.24±0.02 ^A	0.35±0.02 ^a	0.30±0.03 ^a	0.29±0.02 ^A	0.39±0.02 ^a	0.36±0.03 ^a	0.35±0.02 ^a	0.27±0.02 ^A
Trp (mg/L)	Bs (-)	30.70±1.12	17.25±0.96	27.92±1.16	27.33±1.12	36.74±1.33	30.04±1.19	28.56±1.05	36.01±1.10	32.53±0.97
	Bs (+)	18.46±1.18 ^A	14.90±1.14 ^a	20.49±0.97 ^a	13.03±1.08 ^A	17.16±1.15 ^A	14.62±1.16 ^A	19.37±1.17 ^A	19.52±1.06 ^A	23.99±1.21 ^A
Skatole (mg/L)	Bs (-)	83.94±1.25	95.67±1.42	93.83±1.03	99.96±1.07	114.24±1.25	101.88±1.36	90.15±1.21	93.10±0.94	112.19±1.36
	Bs (+)	52.91±1.21 ^A	71.41±1.44 ^A	84.30±1.31 ^a	87.50±1.04 ^a	89.86±1.56 ^A	94.57±1.33 ^a	83.96±1.35 ^a	82.7±1.25 ^a	72.41±1.05 ^A

^a Means bearing different superscripts in a column differ significantly (p<0.05).

^A Means bearing different superscripts in a column differ significantly (p<0.01); n = 8.

and Bs⁻ groups were compared using one-way analysis of variance and Student's *t*-test. A probability (p) value less than 0.05 was the standard for significance. The generalized linear model procedure (PROC GLM) was used in the 3×3 orthogonal experimental design, to analyze the average k values and the residual R values. Duncan's method was used for multi-comparisons (p = 0.05). SAS (V9.1) was used for statistical analyses. Data are presented as means± standard error of the mean.

RESULTS

Effect of different additives on ammonia, H₂S, and skatole production

The amounts of ammonia, H₂S, tryptophan, and skatole produced in the fermentation broths prepared from fecal samples from pigs given Bs (Bs⁺ group) were significantly lower than that of pigs of the control group (Bs⁻). This suggests that the addition of Bs in the diet was associated with reduced ammonia, H₂S, tryptophan, and skatole production in the fermentation broths (Table 2).

Effect of different added substances on the residual R-value of noxious gas

Casein showed the largest R-value to the ammonia in the fermentation broth in the Bs⁺ group, and L-tryptophan resulted in the largest R in the Bs⁻ group (Table 3). This indicated that the main experimental additives affecting the Bs⁺ and Bs⁻ groups were different. In the production of H₂S, fructan resulted in the largest R-value in both groups, which indicated that fructan was the main substance that affected the production of H₂S. In the result regarding tryptophan, L-tryptophan showed biggest R-value in both groups, indicating that external added tryptophan was the main cause for the tryptophan in the fermentation broth. For skatole, external L-tryptophan showed the biggest R-value, which indicated that external L-tryptophan was the main affecting element.

Regarding the effect of adding casein to the fermentation broth on ammonia production, there was no correlation ($r = -0.1551$) between Bs⁺ group (k1>k2>k3) and the Bs⁻ group (k1>k3>k2; Table 4). This shows that the effects of casein at different concentrations were different in the Bs⁺ group and the Bs⁻ group. Fructan at different

Table 3. Effect of different additional substances on the residual R-value of noxious gas

R-value	Bs(+)			Bs (-)		
	L-Trp	Casein	Fructan	L-Trp	Casein	Fructan
NH ₃	0.30±0.03 ^A	0.17±0.02	0.15±0.02	0.19±0.04	0.39±0.04 ^A	0.10±0.02
H ₂ S	0.01±0.03	0.06±0.01 ^A	0.08±0.01 ^A	0.01±0.01	0.04±0.01	0.07±0.02 ^A
Trp	6.02±2.09 ^A	2.51±1.01	1.47±0.77	7.08±2.32 ^A	1.30±0.81	6.54±1.80 ^A
Skatole	21.10±2.74 ^A	8.97±1.93	8.93±1.69	14.21±1.73 ^a	11.28±1.44	9.63±1.87

^a Means bearing different superscripts in a row differ significantly (p<0.05).

^A Means bearing different superscripts in a row differ significantly (p<0.01); n = 8.

Table 4. Effect of different additional substances on the k-value of noxious gas

k-value	Effect of casein on ammonia		Effect of fructan on H ₂ S		Effect of L-tryptophan on skatole	
	Bs(-)	Bs(+)	Bs(-)	Bs(+)	Bs(-)	Bs(+)
k1	1.13±0.14 ^a	0.87±0.11 ^A	0.42±0.08 ^A	0.38±0.07 ^A	91.15±1.36	69.54±1.01
k2	0.97±0.011	0.71±0.09	0.35±0.09	0.30±0.06	105.36±1.42 ^A	90.64±1.24 ^A
k3	1.36±0.15 ^A	0.70±0.08	0.38±0.08 ^a	0.33±0.06 ^a	98.48±1.16 ^a	79.69±0.99 ^a

^a Means bearing different superscripts in a column differ significantly (p<0.05).

^A Means bearing different superscripts in a column differ significantly (p<0.01); n = 8.

concentrations showed similar effects on H₂S, since the two effects (both k₁>k₃>k₂) were highly correlated ($r = 0.9981$); k₁ was higher than k₃ in both groups ($p < 0.05$), which indicated that the 1.5% added fructan reduced the H₂S levels in both the Bs⁺ and Bs⁻ groups. Tryptophan showed similar effects on skatole (both k₂>k₃>k₁, $r = 0.9992$), and k₁ was lower than k₃ in both groups ($p < 0.05$). This indicated that the 0.1% added tryptophan increased the concentrations of skatole in both the Bs⁺ and Bs⁻ groups.

Correlation between externally added substances and *Bacillus subtilis*

In the Bs⁺ group, there were no associated changes between externally added L-tryptophan and ammonia, H₂S, tryptophan, or skatole production ($p > 0.05$; Table 5). There was significant correlation between casein and Bs in the production of ammonia ($p = 0.0395$), as well as between fructan and Bs on the production of H₂S ($p = 0.0141$).

Microflora in then non-supplemented (blank) fermented manure broth

In the blank broth (i.e., with no added L-tryptophan, fructan, or casein) of the Bs⁺ group, the predominant bacteria were *Streptococcus alactolyticus* AF201899 (36%), *Lactobacillus amylovorus* (16%), *Bacteroidetes* bacterium (11%), and *B. asahii* strain MA001 (9%). In the Bs⁻ group, the dominate bacteria in the blank broth were *Bacillus* spp. CWBI B1434 (26%), *Bacteroidetes* spp. (18%), and *Lysinibacillus sphaericus* strain 13651V (6%). There were also uncultured bacteria without clear identification that

Table 5. Correlation of different additional substances and *Bacillus subtilis*

Interaction	NH ₃	H ₂ S	Trp	Skatole
p-value L-Trp	0.3591	0.9501	0.2559	0.7779
Casein	0.0395	0.9505	0.9244	0.9479
Fructan	0.8594	0.0141	0.5466	0.4016

constituted a high percentage in both groups. The differences in bacterial composition indicated that Bs significantly affected the species of microflora in the fermented manure broth (Table 6).

DISCUSSION

The ammonia, H₂S, and skatole produced during pig raising not only adversely affect the growth of pigs, but also contaminate the environment and harm human health. In this study, we found that incorporating Bs in the pigs' daily feed significantly reduced the concentrations of ammonia, H₂S, and skatole in the manure fermentation broth. Moreover, adding external L-tryptophan increased the production of skatole in the manure fermentation broths. Fructan inhibited the production of H₂S. The effect of casein to the ammonia production depended on the presence of Bs. These discoveries provide an important reference for the control of noxious gases.

Effects of *Bacillus subtilis* on the production of ammonia, H₂S and skatole in the fermented broth

Bs is routinely added to the daily swine diet to reduce the production of noxious gas. In the present study, ammonia production from fermentation broths prepared from the fecal matter of pigs given Bs was also reduced compared with that of pigs of the control group, which is consistent with the results of Kim et al. (2005). This may be because Bs secretes proteases that degrade proteins which otherwise transition to ammonia (Zhang et al., 2009). It is also possible that Bs promotes the presence of *Lactobacillus amylovorus* in the microflora (Su et al., 2006), which produces amylase (Eom et al., 2009) that facilitate the breakdown of ammonia.

Consistent with the discovery of Lee et al. (2009), in the present study dietary Bs was also associated with less H₂S production from the fermentation broths, compared with the

Table 6. The dominant bacteria in blank fermentation broths

	Num	Size	Strain name	Proportion (%)
Blank control group	1. PCR.fsa	170.52	Uncultured bacterium	15
	2. PCR.fsa	171.9	Uncultured bacterium	13
	3. PCR.fsa	172.88	<i>Lactobacillus</i> sp. DJF_RP24	5
	4. PCR.fsa	189.61	Uncultured bacterium	17
	5. PCR.fsa	192.43	<i>Lysinibacillus sphaericus</i> strain 13651V	6
	6. PCR.fsa	193.37	<i>Bacillus</i> sp. CWBI B1434	26
	7. PCR.fsa	194.5	Uncultured Bacteroidetes bacterium	18
Blank test group	1. PCR.fsa	168.22	Uncultured bacterium clone	12
	2. PCR.fsa	169.98	<i>Bacillus asahii</i> strain MA001	9
	3. PCR.fsa	187.49	Uncultured bacterium clone	16
	4. PCR.fsa	191.15	<i>Lactobacillus amylovorus</i>	16
	5. PCR.fsa	193.34	AF201899 <i>Streptococcus alactolyticus</i>	36
	6. PCR.fsa	194.3	Uncultured Bacteroidetes bacterium	11

control group. This reduction may be caused by metabolites of Bs that contain catalase and oxidase, which decrease the production of H₂S (Yumoto et al., 2004).

In the present study, dietary Bs was also associated with less skatole produced by the fermentation broths, compared with the control group. It was reported previously that bacteria could affect the pathway of skatole formation by way of tryptophan metabolism (Li, 2009; While et al., 2012), although the microorganisms tested were not Bs. The identity of the microorganisms responsible for the conversion of tryptophan to skatole has not been established. However, bacteria known to facilitate the degradation of skatole are: lactic acid bacteria 11201 (Yokoyama et al., 1983), *Clostridium drakei* and *C. scatologenes* (Whitehead et al., 2008), *C. disporicum* (Li, 2009), and *C. scatologenes* ATCC 25775 (Doerner et al., 2009).

The predominant bacteria discovered in the manure broth in this study were *Streptococcus alactolyticus* AF201899 and *Lactobacillus amylovorus*. No reports regarding the function of *Streptococcus alactolyticus* AF201899 have been published previously. Rinkinen et al. (2004) demonstrated that *S. alactolyticus* was the predominant lactic acid bacteria in the empty intestine and feces. *S. alactolyticus* could secrete β -glycoside hydrolase, α -galactosidase, amylase, urease, acidic galactose, and galactosidase.

It has also been reported that lactic acid in the daily diet could reduce skatole production in the gut (Nowak and Libudzisz, 2009). Investigated as a probiotic, *L. amylovorus* secreted lactic acid and amylase (Eom et al., 2009) and promoted the growth of lactobacilli and bifidobacteria when galactooligosaccharides and *Bifidobacterium* were added to an *in vitro* model of the large intestine (Martinez et al., 2013). *L. amylovorus* was not found to degrade skatole directly. Skatole could be degraded *in vitro* by *Streptococcus* 6020 (Li, 2011). The effect of *S. alactolyticus* AF201899, *L. amylovorus*, and *Bacillus* MA001 need further investigation.

In this study, the effect of Bs in reducing ammonia, H₂S, and skatole occurred in the swine gut, in the *in vitro* manure fermentation process, or both. Suárez-Estrella et al. (2013) reported that Bs could reduce changes in bacterial species during vegetable anaerobic composting, but there are no reports on the amount of ammonia, H₂S, or skatole. In the present study, some isolated bacteria could not be grown in culture, which is consistent with the general knowledge that there are many undiscovered bacteria in the swine intestine. Further investigations are needed to elucidate the effect of Bs on the production of ammonia, H₂S, and skatole.

Effect of added L-tryptophan, casein, and fructan on ammonia, H₂S and skatole production

In the present study, tryptophan as an additive was the

main variant that affected ammonia production in the Bs⁺ group, but not the Bs⁻ group. No significant interaction was observed between tryptophan and Bs, indicating that added tryptophan might affect the nitrogen level in feces through other bacteria. Pierce et al. (1931) discovered that yeast might enhance nitrogen and indole production; added tryptophan, cysteine, and phenylalanine inhibited ammonia production in feces. There has been no report regarding whether added tryptophan could affect the production of H₂S in swine manure. In our study, added tryptophan was not the main element affecting the production of H₂S.

We found that added tryptophan was the main variant affecting the amount of tryptophan and skatole produced in the fermented manure broth. This is consistent with the previous discovery that skatole in swine blood could be increased by injecting tryptophan into the swine appendix (Jensen, 2006). However, it was also found that addition of tryptophan into the daily diet had no significant effect on the level of skatole (Wesoly and Weiler, 2012). The differences might be caused by how the tryptophan was acquired by the swine or how it interacted with the manure. Wesoly and Weiler (2012) indicated that the insignificance of dietary tryptophan might be because tryptophan might be absorbed by the small intestine, instead of being utilized by bacteria in the colon. In the present study, added L-tryptophan showed no significant effect on skatole in either the Bs⁺ or Bs⁻ group (same k value, $r = 0.9992$), indicating that the fermentation of Bs in the intestine might not be simulated by the fermentation of tryptophan *in vitro*.

In this study, casein was the main factor affecting ammonia production in the control group, but was not significant for H₂S production, which is consistent with the reports of Powers et al. (2007). They showed that a diet balanced in amino acids with low crude proteins helped to reduce the ammonia level in feces, but exhibited no effect on H₂S. We found that the combination of dietary Bs with casein added to the fermentation broth was significantly associated with a reduction in ammonia ($p = 0.0395$), but not H₂S ($p = 0.9505$) produced from the fermentation broth. This was in accord with the various effects of different concentrations of casein on ammonia production in both the Bs⁺ and Bs⁻ groups ($r = -0.1551$). The effect of casein on ammonia production may be related to the protease secreted by Bs. Casein was not the main additive to affect skatole production. This is consistent with the conclusion of Lin et al. (1992) that daily diet had no effect on the production of skatole. Lundstrma et al. (1994) reported that an increase in the concentration of skatole did not correlate with an increase of protein. Casein or dietary proteins could result in changes in the microbiota in the intestine and therefore affect the fermentations (An et al., 2014).

Fructan, as a probiotic, modifies the microflora by increasing the amount of beneficial bacteria (Salazar et al.,

2014). In the present study, the addition of fructan to fermentation broths was associated with a reduction in H₂S, which correlated with the addition of dietary Bs. This is similar to a previous study (Zhao et al., 2013), which reported that a 1% addition of fructan had no effect on fertility, but lactic acid bacteria dramatically increased, with a significant decrease in *E. coli* ($p < 0.001$); the amount of ammonia, H₂S, and organosulfur significantly decreased ($p < 0.05$). Fructan had similar effects on H₂S in both the Bs⁺ and Bs⁻ groups of the present study ($r = 0.9981$), indicating that Bs did not affect the amount of H₂S. However, fructan did not significantly affect skatole, which is consistent with the conclusion drawn by Xu et al. (2002). They observed that 0.5% to 1% fructan increased the amount of indole, but not skatole, perhaps because the pH change resulted in a difference in the microbiota. In our research, although the microbiota of the Bs⁺ and Bs⁻ groups differed, we regret that the pH was not measured.

In this study, Bs was added to the diet, and not directly to the fecal fermentation broths. In fact, Bs can also be added directly to the broths. The production of crude proteins in fecal broth depended on the amount of casein added. Further study is required to determine the optimal means of introducing Bs to fermentations broths, and its interactions with different protein, tryptophan, and fructan concentrations.

CONCLUSION

Adding L-tryptophan to fecal fermentation broths increased production of skatole. Adding fructan to the broths reduced the production of H₂S. The effect of casein on ammonia depended on the addition of Bs in the daily diet. Daily dietary Bs reduced the production of ammonia, H₂S, and skatole in fecal fermentation broths.

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