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EFFECTS OF MICROBIAL INOCULANT ON CHEMICAL COMPOSITIONS AND *IN VITRO* DIGESTIBILITY OF ELEPHANT GRASS-BASED SILAGE

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SUMMARY

Probiotic microbial inoculants have been used to improve fermentation quality of green forage feed in pig farming. This study aimed to formulate and evaluate effects of microbial inoculants on quality and digestibility of elephant grass-based silage. Green forage formula was made based on economical and agro ingredients resulting in 36% of cost reduction in comparison to the basal diet. The additive containing 1% microbial mixture of *Lactobacillus plantarum* LCN13, *Bacillus velezensis* VTX9 and *Saccharomyces cerevisiae* MCN9 as starter cultures significantly reduced crude fiber (12.39 ± 1.01% Dry Matter (DM)) and neutral detergent fiber concentrations (28.49 ± 0.93% DM) after 120 h (p < 0.05). In contrast, remarkable increases (p < *0.05*) were determined in metabolizable energy (2972 ± 18.18% DM) and crude protein (16.32±1.15% DM). These results indicated that metabolic activities by microbial inoculants contributed considerably to the nutritive value of the elephant grass-based diet. The experiment along ensiling time revealed that significant changes in nutritive compositions were observed at 120 h and 168 h (p < 0.05). In support of these results, *in vitro* digestibility indexes including dry matter, organic matter, and crude protein showed an increasing trend corresponding to ensiling times. Since there were no significant differences between 120 h and 168 h, fermentation time of 120 h was sufficient to obtain desired outcomes. These findings emphasized that elephant grass-based forage fermented with microbial inoculant could be a promising cost-effective and high-quality feed for pig production. Further investigations are required to evaluate safety, growth performance, and pig intestinal microbiota.

Keywords: digestibility, elephant grass, metabolizable energy, neutral detergent fiber, silage.

INTRODUCTION

Forage grasses are economical feed materials that play an important role in maintaining health, growth performance, and well-being of pigs (Rivero *et al*., 2019). Given that forage grasses are rich in fiber, supplementation of forage grasses in diets of livestock has had positive effects on immunity, antioxidant capacity, and intestinal microbiota (Huang *et al*., 2018; Hen *et al*., 2013). It was reported that the addition of the elephant grass *Pennisetum purpureum* effectively improved antioxidant indexes, immune parameters and intestinal microbiota composition of the pregnant sows (Huang *et al*., 2021). To reduce feeding cost and achieve higher quality of forage grasses for pig production, ensiling is an effective approach. The genus *Bacillus* is important in the initial phase of fermentation owing to its probiotic properties such as production of natural antibiotics and lactic acid, controlling of yeast growth, and hemicellulose and cellulose degradation (Junior *et al*., 2021). Importantly, with pH level remarkably reduced due to organic acid production of lactic acid bacteria, the growth of undesirable microorganisms was inhibited. The acidic pH also prevents plant enzymatic activities, protein hydrolysis and decreases dry matter (DM) loss. Moreover, the richness of proteins, organic acids, natural antimicrobial substances, minerals, amino acids, and satisfactory fibers have made forage grasses a valuable high-quality feed for pig farming.

Elephant grass (*Pennisetum purpureum*) is a popular fodder for rearing the livestock in the tropics. Due to its high productivity and excellent DM, elephant grass is widely used as an alternative for annual crops in ensiling processes (Guimaraes *et al*., 2018). However, the use of this forage compromised the quality of the ensiled material because of a high moisture content at the time of cutting and undesired natural fermentation. Elephant grass-based silage supplemented with wet brewery waste and buriti meal resulted in acidic pH, lower dry matter and fiber concentrations, and higher nutritional contents (Silva *et al*., 2020). A study showed that the addition of ground maize and fermented juice of epiphytic lactic acid bacteria improved the quality of elephant grass silage (Bezerra *et al*., 2019). Thus, it is necessary to optimize the formulation of elephant grass-based silage to avoid nutrient loss and enhance growth performance of pigs.

Besides main ingredients, probiotic microorganisms are crucial in the ensiling process. Living microorganisms with probiotic properties belonging to several genera including *Lactobacillus*, *Bacillus*, *Streptococcus*, *Bifidobacterium*, and *Saccharomyces*, maintain the intestinal microbial balance, lower cholesterol, inhibit pathogenic microbes, and stimulate immune responses (M. de Angelis *et al*., 2006; Konstantinov *et al*., 2004). In recent studies, we demonstrated that *Bacillus velezensis* VTX9 and *Lactobacillus plantarum* LCN13 were potential feed additives for pig farming (Quach *et al*., 2021; Nguyen *et al*., 2022). This study aimed to formulate and evaluate effects of microbial inoculants on quality and digestibility of elephant grass-based silage.

MATERIALS AND METHODS

Ensiling materials and silage preparation

Ingredients including cassava residues, soybean waste, corn starch, tapioca starch, fish meal, and NaCl were provided by Thien An Agricultural product company, Vietnam. Among them, the fish-meal is a high-protein feed supplement containing calcium, phosphorus and other minerals. The elephant grass was obtained from an experimental field at the Faculty of Animal Science and Aquaculture, Vietnam National University of Agriculture. The grass was harvested and then chopped into 1-2 cm in length. Forage feed for pigs was formulated based on the requirements for growing-fattening pigs following the recommendations of Vietnamese standard TCVN 1547:1994 on compound feed for pigs and the National Research Council (National Research Council., 1998). Three probiotic strains for swine farming including *Lactobacillus plantarum* LCN13, *Bacillus velezensis* VTX9 and yeast *Saccharomyces cerevisiae* MCN9 were used in silage preparation. The two strains LCN13 and VTX9 were reported previously (Quach *et al*., 2021 Nguyen *et al*., 2022). The MCN9 strain obtained from the microbial collection of VAST-Culture Collection of Microorganisms, was cultured in Hansen medium containing (g/L) glucose 50, peptone 10, KH_2PO_4 3, $MgSO_4.7H_2O$ 3, yeast extract 1, agar 20 and adjusted to pH 6, at 30°C and 200 rpm.

For silage preparation, 3 microbial strains were cultivated on appropriate medium (Quach *et al*., 2021; Nguyen *et al*., 2022) and then lyophilized to dried powder as previously described (Zhang *et al*., 2016). The microbial mixture powder made up of these 3 strains with each strain containing 10^8 CFU/g. Then, different additions of 0.5%, 1% or 1.5% of microbial inoculants based on the total mass were added to 500 g of green forage and mixed well. The prepared silage was immediately packed into plastic silo bags and kept at 37ºC for 120 hours. The experiment was done in triplicates for each percentage of inoculant.

Chemical analysis

Samples of diet ingredients were dried at 65°C for 24 h, milled, and sieved over a 1-mm screen before analysis. Crude protein (CP), crude fiber (CF), ether extract (EE), total mineral (Ash), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents were determined using AOAC International guidelines (Williams 1990). Digestible energy (DE) and metabolizable energy (ME) were calculated based on the following formulas (Spiehs *et al*., 2002):

DE kcal/kg DM = 4151 - (122 × % Ash) + (23 × % Crude Protein) + (38 × % Fat) - (64 × % Crude Fiber)

ME kcal/kg DM = DE × (1.2003 - (0.0021 × % Crude Protein))

The pH value was measured by using the digital pH meter (HANNA, Woonsocket, RI, USA).

Determination of *in vitro* digestibility

In vitro digestibility was performed according to the method of Dierick (1985) and Löwgren (1989). Briefly, the fermented green forage diet samples were treated with pepsin and 0.075 N HCl (pH 2.0) at 39°C with continuous stirring. After that, the samples were neutralized by 0.05 N NaOH followed by incubation with porcine pancreatic enzyme for 4 h. At the end, the mixtures were filtered, washed with solvents including ethanol and acetone, and then dried in oven at 65ºC. The dried residues were used to calculate *in vitro* digestibility values such as dry matter (DM), organic matter (OM) and crude protein (CP) according to TCVN-4326-2001 and TCVN-4328-2007, respectively.

Statistical analysis

The results were expressed as the mean ± standard deviation (SD). The data was analyzed using one-way analysis of variance (ANOVA), followed by Tukey HSD's least significant difference (LSD) comparison. P value ≤ 0.05 was statistically significant.

RESULTS AND DISCUSSION

Nutritional composition of green forage prior to ensiling

Based on the basal diet following the recommendations of Vietnamese Standard TCVN 1547:1994 on compound feed for pigs and the National Research Council (National Research Council., 1998), green forage was formulated. Main ingredients of the basal diet such as rice bran, tapioca starch, and soybean residues were 2.7-, 20-, and 3.4-fold higher than those in the feeding green fodder, respectively (Table 1). In compensating for these ingredients, elephant grass (25% DM) and water hyacinth (16% DM) were used to make liquid feed. In addition, agro by-products such as cassava residues were added to 13.3% DM in the green forage diet. This green forage diet was naturally rich in detergent fibers as represented by NDF (43.25 ± 0.71% DM) and CF (14.64 ± 0.78% DM) values. Chemical composition analysis revealed a lower level of ME in the green forage diet (2650 ± 73.33 kcal/kg DM) compared to that in the basal diet (3030 ± 38.42 kcal/kg DM). As a result, the cost was reduced by around 36% with 4800 ± 53.50 vnd/kg for the green fodder versus 7500 ± 81.46 vnd/kg for the basal diet, implying a remarkable cost savings for the green forage diet. A previous report of pigs fed with steamed potato-green forage illustrated significant longer-carcasses and increased backfat depth ($p \leq 0.05$) without affecting the meat parameters (Turyk *et al*., 2014). Moreover, another study showed that the use of silage juice did not cause gastrointestinal microbiota imbalance in pigs and no statistically differences detected for meat quality compared to control group (Keto *et al*., 2021). Therefore, elephant grass-based forage feed in this study potentially offers great benefits in swine farming. In addition, a recent report showed that the addition of elephant grass to gestational diets improved antioxidant capacity and immune function of sows through modulating intestinal microbiota (Huang *et al*., 2021). Moreover, elephant grass is rich in moisture with soluble carbohydrates contents, contributing to better fermentation and final products (Amaral *et al*., 2020).

Table 1. Ingredients and nutrient composition of the basal and green forage diets.

	The basal diet (% DM)	**Green forage diet (% DM)**
Ingredients		
Corn starch	10.6	16.2
Rice bran	45.4	16.5
Tapioca starch	20.0	1.0
Cassava residue	-	13.3
Soybean residue	21.2	6.2
Fish meal	2.0	5.0
NaCl	0.5	0.5
Premix	0.3	0.3
Elephant grass	-	25.0
Water hyacinth	-	16.0
Nutrient component		
ME (kcal/kg DM)	$3030^{b}\pm38.42$	$2650^{a}\pm73.33$
CP	$15.19^{a}\pm1.26$	$14.94^{a}\pm1.53$
EE	$6.15^{a}\pm0.94$	$5.49^{a}\pm0.74$
CF	$7.00^{a}\pm1.05$	$14.64^{b}\pm0.78$
NDF	$24.02^{a}\pm1.44$	$43.25^{b}\pm0.71$
ADF	$12.32^{a}\pm1.36$	$18.17^{b}\pm1.07$
Ash	$4.28^{a}\pm1.21$	$4.39^{a}\pm0.54$
Cost (vnd/kg 88% DM)	$7500^{b}\pm81.46$	$4800^{a}\pm53.50$

*Notes: Metabolizable energy: ME, Crude protein: CP, crude fiber: CF, ether extract: EE, total mineral: Ash, neutral detergent fiber: NDF, acid detergent fiber: ADF. Values with different letters are significantly different according to Tukey HSD test ($P < 0.05$).

Effect of microbial inoculants on fermentation characteristics

To determine the effect of inoculant on the quality of green silage, different microbial inoculants ranging from 0.5-1.5% DM were used. After 120 h, inoculation with the microbial mixture led to a significant increase ($p < 0.05$) of ME ranging from 2893-3018 kcal/kg DM compared to the control (2650 ± 73.33 kcal/kg DM) (Table 2). Among them, ME of 1% and 1.5% microbial mixtures showed no significant differences ($p > 0.05$) with 2972 ± 18.18 and 3018 ± 35.68 kcal/kg DM, respectively, which were comparable to the basal diet's ME (3030 ± 38.42 kcal/kg DM). These results implied a positive role of the microbial inoculant on the effective change of ME value. In contrast, CF and NDF concentrations were significantly reduced ($p < 0.05$) in green forage diet with inoculant, while ADF content did not significantly differ compared to control. Notably, the NDF value (27.9 2± 1.13 % DM) after 120 h of ensilage was reduced to approximately that of basal diet (24.02 ± 1.44 % DM). This might have occurred because the strain *L. plantarum* LCN13 and/or both *B. velezensis* VTX9 and *S. cerevisiae* MCN9 produced extracellular enzymes such as cellulase that decomposes cellulose and other biopolymer contents including hemicellulose, lignin, and pectin present in plants (Nguyen *et al*. 2022). The obtained results were also consistent with previous studies indicating the advantages of microbial inoculants over a single strain (Turyk *et al*., 2014; Luo *et al*., 2021). Luo's study (2021) showed that the content of main nutrients and the compositions of dietary fiber in the mixed fungi-fermented wheat bran (FWB) were improved compared to that with wheat bran (WB) alone. The results led to the *in vivo* digestibility of fiber in FWB-fed pigs which was also enhanced ($p < 0.05$) compared to the control and/or WB without affecting their growth performance (Luo *et al*., 2021).

Furthermore, CP content increased relatively after microbial fermentation with the addition of 1% and 1.5% microbial mixture (Table 2). These changes could be due to the presence of microorganisms in the elephant grass or other diet components as well as the added cultures which was not included in the control sample. These microbes could inhibit spoilage organisms, and especially prevented protein digestion and hydrolysis by undesired microorganisms, therefore increasing CP composition (Filya, 2002). Moreover, as expected, the addition of microbial mixtures accelerated production of organic acids such as lactic acid, while lowering silage pH. Nguyen's study (2022) determined that *L. plantarum* LCN13 strain had a strong capability to produce lactic acid up to 18.5 ± 0.31 g/L and resist silage pH 2 (Nguyen *et al*., 2022). In this study, a significant reduction of pH value was only observed in 1% and 1.5% microbial mixtures, at pH 4.45 and pH 4.18, respectively, comparable to that of control at pH 6.5. For all analyzed nutritional parameters, no significant differences were observed between 1% and 1.5% microbial mixtures. Taken together, the 1% microbial inoculant was selected for further studies.

Table 2. Effect of microbial inoculants on nutritive values (% DM) of elephant grass-based silage after 120 h fermentation.

Nutritive composition	**Control**	**Amount of microbial mixture (% DM)**		
		0.5	**1**	**1.5**
ME (kcal/kg DM)	2650^{a}±73.33	2893^{b}±41.40	2972^{c}±18.18	3018^{c}±35.68
CP	14.94^{a}±1.53	$15.12^{a,b}$±1.26	16.32^{b}±1.15	16.48^{b}±1.11
EE	5.49^{a}±0.74	5.53^{a}±0.35	5.63^{a}±0.12	5.73^{a}±1.31
CF	14.64^{c}±0.78	$13.60^{b,c}$±0.77	$12.39^{a,b}$±1.01	11.88^{a}±0.80
NDF	43.25^{c}±0.71	31.47^{b}±0.69	28.49^{a}±0.93	27.92^{a}±1.13
ADF	18.17^{b}±1.07	18.25^{b}±0.59	17.31^{a}±1.11	17.19^{a}±0.99
Ash	4.39^{a}±0.54	4.32^{a}±0.31	4.25^{a}±0.25	4.22^{a}±0.25
pH	6.45^{b}±0.36	6.05^{b}±0.17	4.45^{a}±0.34	4.18^{a}±0.13

$^{a-c}$ Differences of means in a row with different superscripts are significant ($p < 0.05$).

In order to determine the optimal fermentation time, nutritive values were determined for different silage fermentation periods at 72 h, 120 h and 168 h with 1% microbial mixture (Table 3). The results indicated a significant increase of ME at 120 h (2972 ± 18.18 kcal/kg DM) and 168 h (3064 ± 44.84 kcal/kg DM) compared to that at 0 h

(2552 ± 21.12 kcal/kg DM). Therefore, after more than 120 h of silage fermentation, the ME value of green forage diet was similar to that of the basal diet (3030 ± 38.42 kcal/kg DM). The CP value increased along with ensilage time and no significant differences was determined between 120 h and 168 h ($p > 0.05$). Of note, increasing ensilage time led to a significant decrease of the NDF, ADF, and CF concentrations, among which the highest reduction was found in the NDF values. Compared to the pH value before ensiling (pH 6.37), the pH value at 72 h showed no significant difference while pH at 168 h (pH 4.3) was significantly lower. Van Winsen *et al.* (2001) described the desirable characteristics for fermented liquid feed as having a pH below 4.5. Also, their study had shown that presence of strain *L. plantarum* in fermented feed lowered the pH in the stomach of swine to below 4.0 and might limit the infection caused by *Enterobacteriaceae* in the gastrointestinal tract of pig (van Winsen *et al*., 2001). A recent report demonstrated that the addition of starter cultures consisting of *L. plantarum*, *L. brevis*, and *Pediococcus pentosaceus* showed better fermentation quality and pH reduction in Napier grass (*Pennisetum purpureum*) silage, but not in whole-plant corn (*Zea mays* L.) after 72 h (Jaipolsaen *et al*., 2021). This implied that the desired microorganisms may require time to adapt and grow on elephant grass-based forage.

Table 3. The nutritive values (% DM) of green forage diet in different fermentation periods.

Nutritive composition	Ensilage time			
	0 h	72 h	120 h	168 h
ME (kcal/kg DM)	2552^{a}±21.12	2834^{b}±30.53	2972^{c}±18.18	3064^{d}±44.84
CP	14.37^{a}±1.53	15.46^{b}±0.98	16.32^{c}±1.15	16.62^{c}±1.27
EE	5.14^{a}±0.34	5.55^{a}±0.15	5.63^{a}±0.12	5.35^{a}±0.36
CF	14.93^{d}±0.45	13.21^{c}±0.97	12.39^{b}±1.01	11.77^{a}±1.18
NDF	44.56^{d}±0.89	31.66^{c}±1.07	28.49^{b}±0.93	23.76^{a}±0.97
ADF	18.48^{c}±1.71	17.76^{b}±0.74	17.31^{b}±1.11	16.65^{a}±1.20
Ash	4.24^{a}±1.25	4.35^{a}±0.12	4.25^{a}±0.25	4.18^{a}±0.18
pH	6.37^{b}±0.31	5.2^{b}±0.41	4.45^{a}±0.34	4.3^{a}±0.22

$^{a\text{-}d}$ Differences of means in a row with different superscripts are significant ($p < 0.05$).

In vitro digestibility of elephant grass-based silage

To further evaluate the quality of elephant grass-based silage, *in vitro* digestibility indexes including values of DM, OM, and CP were determined at ensilage time from 0-168 h. The obtained results showed that the *in vitro* digestibility increased along ensilage time (Table 4). The highest values for DM (75.48 ± 1.06%), OM (78.15 ± 0.95%) and CP (86.13 ± 1.24%) were found after 168 h, followed by 120 h and 72 h (Table 4). These results suggested a kinetic relationship between *in vitro* digestibility and the incubation time. In contrast, Ammar *et al*. (2008) observed a significant decrease in *in vitro* DM digestibility value after 144 h incubation of browse plants with sheep and goat ruminal fluid (Ammar *et al*., 2008). Based on the changes in chemical compositions of grass-based silage during ensiling, fermentation time at 120 h was sufficient to produce high-quality silage. Given that *in vitro* digestibility indexes represent nutrient digestibility of feed materials in animals (Tilley and Terry, 1963), these results provided important implications for high-quality silage production in organic pig farming. Overall, nutrient analysis showed that the green forage diet contained more fiber substrates including CF, NDF and ADF than the basal diet. More importantly, it was clear that the silage with the presence of microbial mixture and appropriate fermentation time could contribute to the better feed digestibility and higher nutrient supply.

Table 4. *In vitro* digestibility of fermented green forage diet at different period.

Fermentation time	*In vitro* digestibility (%)		
	DM	OM	CP
0 h	66.92^{a}±0.91	68.21^{a}±0.83	74.59^{a}±1.11
72 h	72.63^{b}±1.20	74.92^{b}±0.86	83.43^{b}±1.16
120 h	73.97^{b}±0.98	76.13^{b}±0.94	$84.98^{b,c}$±1.34
168 h	75.48^{c}±1.06	78.15^{c}±0.95	86.13^{c}±1.24

$^{a\text{-}c}$ Differences of means in a column with different superscripts are significant ($p < 0.05$).

CONCLUSION

In the present study, green forage was mainly formulated based on components including elephant grass, water hyacinth, corn starch, rice bran, and cassava residue, which reduced the cost by 36% in comparison to the basal diet. In addition, the supplement of 1% microbial inoculant containing *L. plantarum* LCN13, *B. velezensis* VTX9 and *S. cerevisiae* MCN9 improved the fermentation quality and nutritional composition of green silage after 120 h incubation. Thus, the elephant grass-based silage is a promising high-quality feed in swine farming which requires future investigations to evaluate its safety and efficiency in pig production.

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REFERENCES

Amaral RC, Carvalho BF, Costa DM, Morenz MJF, Schwan RF, da Silva Ávila CL (2020) Novel lactic acid bacteria strains enhance the conservation of elephant grass silage cv. BRS Capiaçu. *Anim Feed Sci Technol* 264, 114472.

Ammar H, López S, André S, Ranilla MJ, Bodas R, González JS (2008) In vitro digestibility and fermentation kinetics of some browse plants using sheep or goat ruminal fluid as the source of inoculum. *Anim Feed Sci Technol* 147(1-3): 90-104

Bezerra HFC, Santos EM, Oliveira JS, Carvalho GGP, Pinho RMA, Silva TC, Pereira GA, Cassuce MR, Zanine AM (2019) Fermentation characteristics and chemical composition of elephant grass silage with ground maize and fermented juice of epiphytic lactic acid bacteria. *S Afr J Anim Sci* 49(3): 522-533.

De Angelis M, Siragusa S, Berloco M, Caputo L, Settanni L, Alfonsi G, Gobbetti M (2006) Selection of potential probiotic lactobacilli from pig feces to be used as additives in pelleted feeding. *Res Microbiol* 157(8), 792-801.

Filya I (2002) The effects of lactic acid bacterial inoculants on the fermentation, aerobic stability and in situ rumen degradability characteristics of maize and sorghum silages. *Turk J Vet Anim Sci* 26(4): 815-823.

Guimarães CG, Bonfá CS, Evangelista AR, Santos ASD, Pantoja LDA, Castro GHDF (2018) Fermentation characteristics of elephant grass silages with macaúba cake. *Acta Sci, Anim Sci* 40.

Hen L, Zhang HF, Gao LX, Zhao F, Lu QP, Sa RN (2013) Effect of graded levels of fiber from alfalfa meal on intestinal nutrient and energy flow, and hindgut fermentation in growing pigs. *J Anim Sci* 91(10): 4757-4764.

Huang PF, Mou Q, Yang Y, Li JM, Xu ML, Huang J, Yin YL (2021) Effects of supplementing sow diets during late gestation with *Pennisetum purpureum* on antioxidant indices, immune parameters and faecal microbiota. *Vet Med Sci* 7(4): 1347-1358.

Huang Q, Holman DB, Alexander T, Hu T, Jin L, Xu Z, McAllister TA, Acharya S, Zhao G, Wang

Y (2018) Fecal microbiota of lambs fed purple prairie clover (*Dalea purpurea Vent*.) and alfalfa (*Medicago sativa*)". *Arch Microbiol* 200(1): 137–145.

Jaipolsaen N, Sangsritavong S, Uengwetwanit T, Angthong P, Plengvidhya V, Rungrassamee W, Yammuenart S (2021) Comparison of the Effects of Microbial Inoculants on Fermentation Quality and Microbiota in Napier Grass (*Pennisetum purpureum*) and Corn (*Zea mays* L.) Silage. *Front Microbiol* 12, 784535.

Junior VB, Horst EH, Guimarães VAP, Junior FM, Moraes GJ, Meza DAR, Galbeiro S (2021) Effect of microbial inoculants on the chemical composition and aerobic stability of Tanzania guinea grass silages. *S Afr J Anim Sci* 51, 1.

Keto L, Tsitko I, Perttilä S, Särkijärvi S, Immonen N, Kytölä K, Rinne M (2021) Effect of silage juice feeding on pig production performance, meat quality and gut microbiome. *Livest Science* 254, 104728.

Konstantinov SR, Awati A, Smidt H, Williams BA, Akkermans ADL, de Vos WM (2004) Specific response of a novel and abundant *Lactobacillus amylovorus*-like phylotype to dietary prebiotics in the guts of weaning piglets. *Appl Environ Microbiol* 70(7): 3821.

Luo Y, He J, Li H, Lan C, Cai J, Chen H, Yan H (2021) Wheat bran fermented by mixed fungal strains improves the digestibility of crude fiber and may benefit the gut health without impacting the growth performance in weaned pigs. *Food Funct* 12(7): 2962-2971.

National Research Council (1998) *Nutrient Requirements of Swine*, 10th revised edition. National Academy Press, Washington DC, 189 pp.

Nguyen VTH, Tung QN, Lien BT, Trang NH, Loi NTT, Ha CH, Tien PQ (2022) Efficacy of biosynthesizing folate, riboflavin and typical probiotic traits reveal the potential use of *Lactobacillus plantarum* LCN13 as a feed additive for swine farming. *Academia J Biol* 44(1): 73-82.

Quach NT, Vu THN, Nguyen NA, Bui TL, Ky SC, Le TL, Phi QT (2021) Phenotypic features and analysis of genes supporting probiotic action unravel underlying perspectives of *Bacillus velezensis* VTX9 as a potential feed additive for swine. *Ann Microbiol* 71(1): 1-14.

Rivero MJ, Rodríguez-Estévez V, Pietrosemoli S, Carballo C, Cooke AS, Kongsted AG (2019) Forage consumption and its effects on the performance of growing swine-discussed in relation to European wild boar (*Sus Scrofa* L.) in semi-extensive systems: A review. *Animals* 9*(*7): 457.

Silva ARPD, Dias FJ, Rufino JPF, Tanaka EDS, Lopes MM (2020) Effect of using inoculant on elephant grass silage with additives. *Acta Sci Anim Sci* 42.

Spiehs MJ, Whitney MH, Shurson GC (2002) Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J Anim Sci* 80(10): 2639-2645.

Tilley JMA and Terry RA (1963) A two-stage technique for the in vitro digestion of forage crops. *J Brit Grassl Soc* 18: 104-11.

Turyk Z, Osek M, Olkowski B, Janocha A (2014) Pig feeding under the potato-green forage base system with or without addition of herbs versus a concentrate based system: Effect on post-slaughter performance and pork characteristics. *Asian-Australas J Anim Sci* 27(5): 683.

Van Winsen RL, Urlings BA, Lipman LJ, Snijders JM, Keuzenkamp D, Verheijden JH, van Knapen F (2001) Effect of fermented feed on the microbial population of the gastrointestinal tracts of pigs. *Appl Environ Microbiol* 67(7): 3071-3076.

WILLIAMS, Sidney (1990) Official methods of analysis. Association of Official Analytical Chemists15th edn. *Arlington, VA.*

Zhang Q, Li X, Zhao M, Yu Z (2016) Lactic acid bacteria strains for enhancing the fermentation quality and aerobic stability of *Leymus chinensis* silage. *Grass Forage Sci* 71(3): 472-481.